

1 **DIET COMPOSITION AND ILEITIS IN PIGS 2**

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3 **Effect of including distiller's dried grains with solubles in the diet, with or**
4 **without antimicrobial regimen, on the ability of growing pigs to resist**
5 **a *Lawsonia intracellularis* challenge¹**

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12 **ABSTRACT: A disease challenge experiment was conducted to determine if**
13 **including 10% DDGS in the diet, with or without antimicrobial supplementation,**
14 **reduces the incidence and/or severity of intestinal lesions in growing pigs after a *L.***
15 ***intracellularis* challenge. One hundred 17-d old weaned pigs were blocked by sex**
16 **and weight and randomly allotted to one of five treatment groups: negative control**
17 **(NC) - unchallenged, corn-soy diet; positive control (PC) - challenged, corn-soy diet;**
18 **10% DDGS diet (D) – challenged; positive control with antimicrobial regimen (PC +**
19 **A) – challenged; and 10% DDGS diet with antimicrobial regimen (D + A) -**
20 **challenged. For antimicrobial-supplemented treatments, diets contained 30 g/ton**

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1 **BMD[®] continuously, with Aureomycin[®] pulsed at 500 g/ton from d 3 pre-challenge**
2 **to d 11 post-challenge. Challenged pigs were orally inoculated with 8.0×10^8 L.**
3 **intracellularis after a 4-wk pre-challenge period. On d 21 post-challenge, pigs were**
4 **euthanized, lesions of intestinal mucosa was evaluated, and ileal tissue samples were**
5 **analyzed by immunohistochemistry to determine presence and proliferation of L.**
6 **intracellularis. Compared to other dietary treatments, feeding a diet containing**
7 **10% DDGS reduced ileum and colon lesion length and prevalence ($P < 0.05$), and**
8 **reduced severity of lesions in the ileum ($P < 0.05$) and colon ($P < 0.10$) in challenged**
9 **pigs. Pigs fed the diet containing the antimicrobial regimen had a lower prevalence**
10 **and severity of lesions in the jejunum ($P < 0.05$), and tended to have reduced total**
11 **tract lesion length ($P = 0.11$) compared to other challenged pigs. No differences in**
12 **length, severity, or prevalence of lesions were observed in D + A pigs ($P > 0.15$), but**
13 **fecal shedding of L. intracellularis was reduced on d 14 post-challenge ($P < 0.05$)**
14 **compared to other challenged pigs. No dietary effects on fecal shedding were**
15 **observed by d 20 post-challenge ($P < 0.10$). The proportion of cells infected with L.**
16 **intracellularis was reduced when DDGS ($P = 0.05$) or antimicrobial ($P = 0.10$) diets**
17 **were fed. Dietary inclusion of 10% DDGS appears to provide some benefit to**
18 **growing pigs subjected to a moderate ileitis challenge, similar to a currently**
19 **approved antimicrobial regimen.**

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21 **Key Words: Pig, Ileitis, Distiller's dried grains with solubles, Antimicrobial**

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Introduction

Ileitis, also known as porcine proliferative enteropathy (PPE), is an enteric disease in swine that can decrease feed intake, reduce growth rate, and increase mortality. The disease is caused by *Lawsonia intracellularis*, an intracellular, microaerophilic bacteria that infects the enterocytes of the intestine, causing cellular proliferation and thickening of the infected intestine. It has been estimated that PPE results in increased production costs ranging from \$3 to \$11 per pig, due to increases in feed cost and time to reach market weight (McOrist et al., 1997).

Prevention and (or) control of PPE has largely been focused on the use of antimicrobial agents. Tylosin phosphate (Tylan[®]), lincomycin (Lincomix[®]), tiamulin (Denagard[™]), and chlortetracycline (CTC) + bacitracin methylene disalicylate (BMD[®]) are the only FDA-approved antimicrobial regimens for prevention of PPE. Sub-therapeutic levels of these antibiotics improve pig performance but often fail to prevent the disease (Gebhart et al., 1998; Schwartz et al., 1998; Winkelman, 1998). Providing CTC strategically at therapeutic levels can positively affect growth performance and reduce the occurrence and severity of intestinal lesions caused by PPE (McOrist, 1998; Winkelman et al., 1998). Schultz et al. (1997) observed an additive and (or) synergistic effect when feeding the combination of BMD[®] and CTC for the treatment of ileitis.

Feeding therapeutic levels of feed-grade antibiotics is very expensive and can generally only be done for a limited period of time. Additionally, food safety concerns over potential residue violations in meat and the risk of antibiotic-

1 resistance in human strains of pathogenic organisms precludes continued use of
2 these drugs. Reports from informal field studies have suggested that including
3 distiller's dried grains with solubles (DDGS) in grow-finish diets in commercial
4 herds that have historically had recurring problems with ileitis, may reduce
5 dependence on antibiotics to combat this disease (Goihl, 2001). In several
6 commercial grow-finish herds, dietary levels of 5 – 15% DDGS have resulted in a
7 decrease or complete removal of antibiotics, while reducing the negative effects on
8 growth performance as well as reducing mortality rates in herds with recurring
9 problems with ileitis (Goihl, 2001). Distiller's dried grains with solubles is a co-
10 product of the dry mill fuel ethanol industry that contains approximately 10%
11 crude fiber, and the fiber composition is primarily insoluble (42.2%) versus soluble
12 (0.7%) in nature (Shurson et al., 2000). Feeding diets that are low in soluble non-
13 starch polysaccharides can reduce the proliferation of pathogenic organisms in the
14 gastrointestinal tract (Hampson et. al, 1999). Smith and Halls (1968) suggested that
15 fiber influences the secretory function of the epithelium, and this alteration may
16 impair bacterial adhesion. Fiber also has a "cleansing" effect in the gut as a result
17 of reducing the viscosity of digesta (Lawrence, 1972). The objective of this study
18 was to evaluate the effect of dietary inclusion of DDGS, with or without use of a
19 strategic antimicrobial regimen (CTC & BMD[®]), on the ability of growing pigs to
20 resist a *L. intracellularis* challenge.

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Materials and Methods

Animals and Allotment

Experimental protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Minnesota. One hundred crossbred pigs (50 gilts and 50 barrows, 1/4 Landrace x 1/4 Large White x 1/2 Duroc) were obtained and transported from a commercial farrowing unit to isolation barns located on the University of Minnesota (St. Paul) campus. The source herd had no history or recorded cases of proliferative enteropathy, and was serologically negative for *Lawsonia intracellularis*, porcine respiratory and reproductive syndrome (PRRS), and *Actinobacillus pleuropneumonia*. The source herd was also clinically negative for *Salmonella cholerasuis*, transmissible gastroenteritis (TGE), and pathogenic *Brachyspira* species. Pigs (approximately 17 d of age) were blocked by gender and weight, and blocks were randomly allotted to one of five treatment groups: negative control (NC) corn-soybean meal diet fed without disease challenge, positive control (PC) corn-soybean meal diet fed with disease challenge, 10% DDGS diet fed with disease challenge (D), control diet with antimicrobial regimen fed with disease challenge (PC + A), or 10% DDGS diet with antimicrobial regimen fed with disease challenge (D + A). The antimicrobial regimen consisted of continuous BMD[®] inclusion in the diet (33 g/tonne) along with

1 dietary pulsing of Aureomycin[®] (550 g/tonne) from d-3 pre-challenge to d-11 post-
2 challenge. The DDGS utilized for the study was obtained from Al-Corn Clean Fuel
3 (Claremont, MN). Animals were housed in isolation rooms, with 10 pigs per room
4 (7.25 m² per room, 10 rooms total) and 2 rooms per treatment group.

6 *Experimental Diets*

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8 All pigs were fed a similar commercial pelleted Phase I nursery diet for the first
9 4-d of the experiment to encourage feed intake prior to initiation of dietary
10 treatments. After the initial 4-d acclimation period, animals were fed their
11 respective experimental diets for the remainder of the 53 d study. Representative
12 samples of each diet were obtained and analyzed for dry matter, gross energy, crude
13 protein, ash, ether extract, crude fiber, calcium, phosphorus, and individual amino
14 acid composition. Additionally, samples of the medicated feed were submitted to the
15 Alpharma Analytical Laboratory for analysis of BMD and CTC levels.
16 Experimental diets were formulated to contain equivalent energy (3390 kcal/kg
17 ME), calcium (0.80%), total phosphorus (0.70%), and apparent ileal digestible
18 lysine (1.15%). Diets were formulated based on recently determined DDGS nutrient
19 values for energy (Spiehs et al., 1999), total amino acid and mineral levels (Spiehs et
20 al., 2002), and apparent ileal amino acid digestibility coefficients (Whitney et al.,
21 2000). The ME value used for DDGS was 3350 kcal/kg on an as-fed basis. All other
22 nutrients were provided to meet or exceed NRC (1998) recommendations.

1 **Digestible and metabolizable energy values were calculated based on proximate**
2 **analysis values using the following formulas from Noblet and Perez (1993):**

$$3 \quad \text{DE kcal/kg} = 4151 - (122 \times \% \text{ Ash}) + (23 \times \% \text{ CP}) + (38 \times \% \text{ EE}) - (64 \times$$

4 **Crude fiber)**

$$5 \quad \text{ME kcal/kg} = \text{DE} \times (1.003 - (0.0021 \times \% \text{ CP}))$$

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7 *Disease Challenge*

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9 **Four wks after experimental diets were initiated (d 32), pigs were manually**
10 **restrained and provided 40 ml of either saline (NC) or an inoculation of *L.***
11 ***intracellularis* (PC, D, PC + A, and D + A treatments) via stomach tube. The**
12 **inoculate was prepared as a mucosal homogenate collected from the small intestines**
13 **of pigs previously infected with *Lawsonia intracellularis* and exhibiting lesions**
14 **consistent with ileitis. Mucosal material was collected by scraping the lumen of the**
15 **infected intestine, and was then diluted with a sucrose-phosphate-glutamate buffer**
16 **with the goal of obtaining a dosage rate of 1×10^8 *L. intracellularis* per pig. A**
17 **representative sample of the harvested gut material was submitted to the University**
18 **of Minnesota Veterinary Diagnostic Lab, and actual dosage rate of *L. intracellularis***
19 **provided per pig was determined to be 8.0×10^8 . Additionally, the material was**
20 **screened and determined to be negative for other pathogens, including spirochetes,**
21 **viruses, parasite ova, B-hemolytic *E. coli* and *Salmonella* sp. Care was taken to**
22 **avoid cross-contaminating pigs from different rooms after the disease challenge.**
23 **Biosecurity procedures included use of separate coveralls, boots, and gloves for each**

1 room. In addition, cleaning and feeding schedules were developed and implemented
2 to ensure movement between rooms was conducted in order from non-infected (NC)
3 to infected groups.

4

5 *Data Collection*

6

7 Growth performance and feed intake data were collected for both the pre- and
8 post-inoculation periods. Clinical observations for alertness, gauntness, and
9 diarrhea were scored 3 times/week following challenge. Alertness was scored on
10 animal behavior characteristics, with 1 = normal, 2 = slightly depressed and/or
11 listless, and 3 = severely depressed or recumbent. Gauntness scores were based on
12 visual body condition, with 1 = normal, 2 = slightly to moderately gaunt, and 3 =
13 severely gaunt. Diarrhea was scored based on the following characteristics of feces:
14 1 = no diarrhea, 2 = semi-solid feces without blood, 3 = watery feces without blood, 4
15 = blood-tinged feces that was loose or formed, and 5 = profuse diarrhea with frank
16 blood or dark tarry feces. Fecal samples were collected on d 14 and d 20 post-
17 inoculation, and sent to the University of Minnesota Veterinary Diagnostic
18 Laboratory for polymerase chain reaction (PCR) evaluation of *L. intracellularis*
19 presence to determine shedding of the organism. Bacterial DNA was extracted from
20 fecal samples using a Qiagen extraction kit (Qiagen, Valencia, CA) prior to PCR
21 analysis using a Quantitect kit (Qiagen, Valencia, CA) and following the procedures
22 of Jones et al. (1993).

1 **On d 20 or d 21 post-challenge, all pigs were euthanized and necropsies were**
2 **performed. Weights of the heart, empty stomach, liver, and empty small and large**
3 **intestine were measured. Representative samples of digesta from the small and**
4 **large intestines were collected and pH was measured. Length of the small and large**
5 **intestine was also measured and visual evaluation of the length of observable lesions,**
6 **and location and severity in the intestinal tract of lesions were made. Density of**
7 **both the small and large intestines was calculated by dividing empty intestinal**
8 **weight by intestinal length. Lesions were scored for severity based on the following**
9 **characteristics: 0 = normal (no visual appearance of lesion), 1 = mild mesenteric**
10 **and intestinal wall edema and hyperemia, 2 = mild to moderate edema and**
11 **hyperemia of the mesentery and intestinal wall, and corrugated intestinal mucosa**
12 **(PIA), 3 = severe mesenteric and intestinal wall edema and hyperemia, and necrosis**
13 **of the mucosal surface with formation of pseudo-diphtheric membrane (necrotic**
14 **enteritis), and 4 = moderate to severe edema and hyperemia of the mesentery and**
15 **intestinal wall, thick and corrugated mucosa, and blood clots in the intestinal lumen**
16 **(PHE). A 10 cm tissue section of the distal ileum proximal to the ileal-cecal junction**
17 **was collected from each pig, along with adjacent lymph nodes, and were fixed by**
18 **immersion in 10% neutral buffered formalin, embedded in paraffin, and analyzed**
19 **by immunohistochemistry (IHC) using a monoclonal antibody specific for L.**
20 **intracellularis (McOrist et al., 1987). The reaction to L. intracellularis antigen was**
21 **graded from 0 (no L. intracellularis positive antigen labeled) to 4 (100% of epithelial**
22 **cells in the crypts with positive antigen labeling) (Guedes et al., 2002).**

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2 *Statistical Analysis*

3

4 **Growth performance data were statistically analyzed using the analysis of**
5 **variance GLM procedures of SAS (1985), with room as the experimental unit (two**
6 **replications per treatment). All other data were analyzed utilizing individual pig as**
7 **the experimental unit, resulting in 20 replications per treatment. Least squares**
8 **means were used to compare the negative and positive control groups, and thereby**
9 **evaluate the effects of infecting pigs on response criteria. Data involving pigs on the**
10 **disease challenge treatments were analyzed as a 2 x 2 factorial, with DDGS level (0**
11 **or 10%) and antimicrobial regimen (no antimicrobials or CTC/BMD) as the factors.**

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Results and Discussion

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16 *Diet Composition*

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18 **Experimental diet composition and nutrient analysis are provided in Table 1.**
19 **Calculated metabolizable energy (ME) concentration based on proximate analysis**
20 **tended to be lower in all diets compared to formulated levels (3145 vs. 3390 kcal/kg),**
21 **but was similar among experimental diets (range = 3097 – 3162 kcal/kg ME).**
22 **Calcium level tended to be higher in the corn-soybean meal diets (0.85% - 0.90%)**
23 **compared to DDGS diets (0.75% - 0.81%) and the calculated level (0.80%), but was**

1 within the permitted analytical range of 0.66% - 0.94% (AOAC, 1990). Total
2 dietary phosphorus concentration was similar among dietary treatments, but the
3 addition of DDGS to the diet increased crude protein level. The PC+A and D+A
4 diets contained 36 and 34.2 g/ton of BMD, respectively, which slightly exceeded the
5 target of 30 g/ton. Analyzed levels of CTC were 439 and 619 g/ton for the PC+A
6 and D+A diets, respectively, which were near the target of 500 g/ton.

7 8 *Growth Performance*

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10 Two pigs were removed from the experiment prior to completion due to health
11 reasons unrelated to the ileitis challenge. Body weights, growth rate, feed intake,
12 and feed conversion results are shown in Table 2. Average initial pig weight was
13 6.35 kg. During the pre-challenge period, growth, feed intake and feed efficiency
14 were similar across all treatments ($P \geq 0.23$). However, a DDGS x antimicrobial
15 interaction was observed in the pre-challenge period, with pigs in the D + A
16 treatment group tending to exhibit reduced feed intake ($P = 0.09$) compared to
17 providing DDGS or antimicrobial regimen alone in the diet.

18 Infecting pigs with *L. intracellularis* did not affect growth performance in the 3-
19 wk post-challenge period ($P \geq 0.29$), although a numerical reduction in growth rate
20 (16%) and feed intake (9%) was observed between the negative and positive control
21 groups. No DDGS, antimicrobial, or DDGS x antimicrobial interactions were
22 observed for ADG, ADFI, or G/F in the post-challenge period ($P \geq 0.25$). Including
23 the antimicrobial regimen in the DDGS diet numerically improved growth rate and

1 feed intake (33% and 22%, respectively) compared to providing no antimicrobial
2 regimen in the DDGS diet. Neither diet containing DDGS nor antibiotic regimen
3 affected growth performance of challenged pigs ($P \geq 0.25$). Body weight at the time
4 of necropsy was unaffected by *L. intracellularis* challenge and dietary treatment
5 within challenged groups ($P \geq 0.47$).

6 Previous research results by Whitney and Shurson (2004) have indicated that
7 dietary inclusion up to 25% DDGS provides similar growth performance of nursery
8 pigs when high-quality DDGS is used, diets are formulated on a digestible amino
9 acid basis, and pigs weigh at least 7 kg. Since only two replications per treatment
10 were used in the analysis of growth performance data, more replication would be
11 required to determine if the numerical trends observed for growth performance in
12 this experiment are dietary responses that could be expected on a consistent basis
13 under similar conditions. However, the main objective of this experiment was to
14 evaluate length, severity, and prevalence of lesions and fecal shedding of *L.*
15 *intracellularis* using PCR and immunohistochemistry techniques to determine
16 dietary effects during a *Lawsonia* infection.

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18 *Alertness, Gauntness, and Fecal Scores*

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20 Weekly gauntness and fecal scores are presented in Table 3. Pig behavior
21 appeared normal throughout the trial for all pigs, regardless of treatment.
22 Unchallenged pigs remained healthy throughout the post-challenge period, as
23 indicated by a lack of gauntness and normal fecal scores. Stools were of a looser

1 consistency (more watery) during wks 1, 2, and 3 post-challenge in PC pigs
2 compared to NC pigs ($P < 0.01$). Additionally, PC pigs were more gaunt during wks
3 1 and 3 post-challenge compared to NC pigs ($P < 0.01$), although no difference in
4 abdominal score was observed during wk 2 post-challenge.

5 Fecal looseness increased with increasing time post-challenge ($P = 0.001$). No
6 time x DDGS inclusion or time x antimicrobial regimen interactions were observed.
7 A time x DDGS inclusion x antimicrobial interaction was observed ($P = 0.02$, Fig. 2),
8 indicating that as fecal looseness increased over time, the combination of D + A
9 appeared to become more effective in reducing stool looseness compared to feeding
10 either D or A individually. Dietary treatment did not affect fecal consistency prior
11 to challenge ($P = 0.16$), although feeding the 10% DDGS diet tended to increase
12 looseness of stools ($P = 0.10$). During the first wk post-challenge, a DDGS inclusion
13 x antimicrobial regimen interaction was observed, with pigs fed the combination
14 tending to have increased fecal scores ($P = 0.08$). During wks 2 and 3 post-
15 challenge, however, no differences in fecal consistency due to dietary treatment were
16 observed ($P > 0.10$).

17 Gauntness, as measured by abdominal scores, did not increase appreciably
18 during the post-challenge period ($P > 0.10$), although time x DDGS inclusion ($P =$
19 0.02), time x antimicrobial regimen ($P = 0.04$), and time x DDGS inclusion x
20 antimicrobial regimen ($P = 0.01$) effects were observed (Fig. 1). Abdominal scores
21 tended to be affected by dietary treatment throughout the post-challenge period (P
22 < 0.10). Effects of DDGS inclusion, antimicrobial regimen, and DDGS inclusion x
23 antimicrobial regimen interaction were observed during wk 1 ($P = 0.06$) and wk 3 (P

1 = 0.03) post-challenge, with pigs fed the control corn-soybean meal diet exhibiting
2 more gauntness, although only 1 pig appeared gaunt each of the three wks post-
3 challenge. During the second wk post-challenge, only an interactive effect was
4 observed for abdominal score ($P = 0.05$), while no main effects were detected ($P =$
5 0.27).

6 Inclusion of some fiber sources in diets for growing pigs have increased the
7 viscosity of digesta and increased water content of feces, resulting in looser stools.
8 Feeding an 80% alfalfa meal diet decreased dry matter content of digesta in young
9 pigs (Pond et al., 1988), while similar results have been observed when including 4%
10 - 6% guar gum in the diet (Rainbird, 1986). Alfalfa contains 52.4% insoluble fiber
11 and 4.3% soluble fiber (Shurson et al., 2000), while the dietary fiber in guar gum is
12 soluble in nature (Grieshop et al., 2001). Including insoluble fiber in the form of
13 7.5% wheat bran or 30% oatmeal by-product also increased rate of passage of
14 digesta in the large intestine (Potkins et al., 1991). Cereal bran contains
15 approximately 28% insoluble fiber (Marlett, 1992), and therefore a 7.5% inclusion
16 rate, at the expense of corn, would result in an additional 1.75% insoluble fiber in
17 the complete diet. This is much less than the additional 3.1% insoluble fiber
18 contributed to the diet when including 10% DDGS, which contains 42.2% insoluble
19 fiber (Shurson et al., 2000), in the place of corn and soybean meal. Jorgensen et al.
20 (1996) observed a 5 – 6 fold increase in passage rate through the terminal ileum
21 when pigs were fed a high vs. low fiber diet (26.8% vs. 5.9% crude fiber), and
22 attributed this response to an increase in peristaltic action and increased transit
23 time.

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2 *Internal Organ Weights and Digesta Characteristics*

3

4 **Infecting pigs with *L. intracellularis* reduced stomach, liver, and small intestine**
5 **weight relative to body weight ($P < 0.05$), but did not affect other organ weights at**
6 **the time of necropsy (Table 4). No dietary effects were observed for heart or**
7 **stomach weights ($P \geq 0.17$), but antimicrobial regimen reduced liver weight relative**
8 **to body weight ($P < 0.001$). Feeding the DDGS diet increased weight of the large**
9 **intestine ($P < 0.01$), while antimicrobial regimen tended to increase total intestinal**
10 **tract weight ($P < 0.10$) relative to body weight. The combination of 10% DDGS and**
11 **antimicrobials in the diet, however, resulted in reduced weights of both the small**
12 **and large intestine, as a proportion of body weight, compared to intestine weights**
13 **when each was fed alone ($P < 0.02$). Intestinal length and density were unaffected**
14 **by disease challenge ($P \geq 0.22$) and diet ($P \geq 0.59$).**

15 **Challenging pigs with *L. intracellularis* resulted in more acidic digesta in the**
16 **large intestine ($P < 0.01$), but did not affect pH of digesta collected from the small**
17 **intestine ($P \geq 0.49$). Digesta dry matter was not affected by disease challenge ($P \geq$**
18 **0.19). Feeding DDGS increased the acidity of digesta collected from the large**
19 **intestine ($P < 0.02$), but did not affect digesta pH in the small intestine ($P \geq 0.25$).**
20 **Feeding the antimicrobial diets resulted in an increase in digesta pH collected from**
21 **the small intestine ($P < 0.03$), but did not alter digesta pH in the large intestine ($P \geq$**
22 **0.31). Feeding the combination of DDGS and antimicrobials tended to increase the**
23 **acidity of digesta collected from the small intestine ($P = 0.08$) compared to feeding**

1 the antimicrobial diet alone. No dietary effects or interactions were observed on dry
2 matter content of digesta from the small intestine, but pigs receiving the
3 antimicrobial regimen had increased dry matter content of digesta collected from
4 the large intestine ($P = 0.04$).

5 Changes in the mass of the gastro-intestinal tract and other internal body organs
6 have been shown to occur as a result of feeding diets high in insoluble fiber to pigs.
7 Ma et al. (2002) reported increased intestinal tract weight, relative to bodyweight,
8 when including 5% wheat bran, a source high in insoluble fiber. No differences in
9 intestinal tract weight were observed, however, when including 5% sugar beet pulp
10 as a source high in soluble fiber. Liver weights were reduced when feeding diets
11 containing either fiber source, but pancreas weight was reduced only when wheat
12 bran was included in the diet.

13 Pond et al. (1988), however, observed an increase in liver and kidney weights,
14 relative to body weight, when an 80% alfalfa diet was fed to market-age pigs.
15 Alfalfa contains high levels of both insoluble (52.4%) and soluble (4.3%) fiber
16 (Shurson et al., 2000). Jorgensen et al. (1996) also observed increases in stomach,
17 cecum, and colon mass when growing pigs were fed diets containing high levels of
18 insoluble fiber.

19 Research by Jin et al. (1994) indicated that insoluble fiber addition in the diet
20 increases the rate of cellular turnover in the intestine. The rate of cellular
21 proliferation in both the jejunum and colon was increased when feeding a diet
22 containing 10% wheat straw. Wheat straw is somewhat similar in dietary fiber
23 composition to DDGS, containing 71.0% insoluble fiber, but only 0.5% soluble fiber

1 (Shurson et al., 2000). Because *L. intracellularis* is an enteric pathogen that must
2 invade mucosa cells intracellularly for infection, increasing cell turnover in the
3 distal portion of the small intestine may shorten the time and reduce the ability of
4 the organism to successfully colonize in mucosa cells. A trend toward increased
5 small intestine weight by feeding diets containing DDGS or antimicrobials was
6 observed in the current study ($P \leq 0.15$), which may indirectly indicate an increase
7 in cell turnover. Additionally, research results reported by Zebrowska et al. (1983)
8 suggested that providing fiber (barley) in the diet increases endogenous secretion of
9 saliva, gastric juice, pancreatic juice, and bile. Because bactericidal enzymes and
10 antibacterial peptides are contained in these endogenous fluids, increasing secretion
11 by these organs may provide additional protection against infection by enteric
12 pathogens.

13

14 *Clinical Lesion Evaluation*

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16 Clinical lesion evaluation results for the jejunum, ileum, cecum, and colon are
17 presented in Table 5. Two pigs in the NC group had lesions that were suspect for
18 ileitis. Overall, 59% of the pigs that were challenged exhibited lesions consistent
19 with ileitis. Lesion length, severity, and prevalence were greater in PC pigs
20 compared to NC pigs in the jejunum ($P = 0.02$), ileum, colon, and overall ($P < 0.01$).
21 Only one pig in each of the PC, D, and P+A groups was observed to have lesions
22 indicative of ileitis in the cecum.

1 Adding 10% DDGS to the diet reduced the proportion of pigs exhibiting lesions
2 in the gastro-intestinal tract on d 21 post-challenge ($P < 0.01$), with 40 and 50% of
3 the pigs on the D and D+A treatments exhibited lesions compared to 68 and 80% of
4 pigs receiving the PC and P+A treatments, respectively. Reductions in lesion
5 prevalence were observed in the ileum and colon ($P \leq 0.03$), but not jejunum or
6 cecum ($P > 0.60$) when pigs were fed DDGS in the diet. Lesion length and severity
7 were also reduced in the ileum ($P = 0.02$) and colon ($P < 0.10$), but not in the
8 jejunum or colon ($P > 0.10$) with dietary DDGS inclusion. Over the entire intestinal
9 tract, feeding the 10% DDGS diet did not significantly affect lesion length ($P = 0.14$),
10 although a numerical reduction of 42 and 68% was observed for the pigs on D and
11 D+A treatments, respectively, compared to PC pigs.

12 Providing BMD continuously in the diet, while strategically pulsing
13 chlortetracycline, resulted in a reduced prevalence of lesions observed in the
14 jejunum ($P = 0.04$), with 20 and 15% of pigs in the P+A and D+A groups exhibiting
15 lesions compared to 47 and 30% of pigs in the PC and D groups, respectively.
16 Lesion prevalence in the ileum, cecum, colon, and overall was unaffected by
17 antimicrobial regimen ($P > 0.20$). Lesion severity ($P = 0.03$), but not length ($P =$
18 0.18), was reduced in the jejunum of pigs on the antimicrobial regimen treatment,
19 while neither lesion length nor severity were affected by antimicrobial regimen in
20 the remaining portions of the gastro-intestinal tract ($P > 0.10$). A numerical, but
21 non-significant reduction ($P = 0.11$) in overall lesion length was observed in pigs
22 provided the antimicrobial regimen (45% and 68% for treatments P+A and D+A,
23 respectively), which was similar to the reduction observed with DDGS inclusion. No

1 DDGS x antimicrobial regimen interactions were observed for any of the lesion
2 parameters measured at the time of necropsy ($P > 0.15$), indicating no additive or
3 synergistic effect of combining both dietary treatments.

4 Length of lesions at necropsy is a useful quantitative measure of the severity of
5 ileitis in pigs and its impact on growth performance (Winkelman, 1999). In the
6 current study, DDGS inclusion in the diet reduced lesion length, severity, and
7 prevalence in both the ileum and colon. Fibrous diets have also been demonstrated
8 to have beneficial effects on the health of young pigs in relation to bacterial activity
9 and gastro-enteritis. Smith and Halls (1968) were unable to infect pigs with certain
10 types of *Escherichia coli* when fed a diet containing barley fiber. They suggested the
11 mode of action in preventing enteric disease was the ability of fiber to influence the
12 secretory or absorptive function of the epithelium, both of which are implicated in
13 bacterial adhesion. Drochner et al. (1978) also suggested that crude fiber in the diet,
14 especially lignin, can decrease bacterial activity in the gut of young pigs. Lawrence
15 (1970, 1972) suggested the suppression of certain bacteria in the intestine might be
16 associated with a change in transit time, fecal dry matter, and variations in bile
17 secretion and volatile fatty acid production.

18 The strategic use of chlortetracycline and BMD[®] resulted in reduced severity and
19 prevalence of lesions observed in the jejunum at the time of necropsy. These results
20 are similar to previous research results when evaluating the use of chlortetracycline
21 and (or) BMD[®] for ileitis prevention or control (Winkelman et al., 1997). In that
22 study, the authors observed improved growth performance, feed intake, and feed
23 conversion, with a concomitant reduction in diarrhea and gross intestinal lesions in

1 pigs challenged with ileitis when chlortetracycline (CTC) from Aureomycin[®] was
2 included in the diet from 4 d prior to the infection to 10 d after the disease challenge.
3 Feeding 500 g/ton of Aureomycin[®] appeared to provide some additional benefit over
4 the 100 g/ton level.

5 In comparative ileitis challenge studies, McOrist (1998) and Winkelman et al.
6 (1998) observed similar improvements in growth performance and presence of gross
7 intestinal lesions when pigs were fed CTC from Aureomycin[®] (300 – 600 g/ton fed
8 from 4 d pre-challenge to 10 d post-challenge) compared to pigs fed tylosin (100
9 ppm) and lincomycin (200 g/ton), respectively. Additive and (or) synergistic effects
10 have been observed when feeding the combination of BMD[®] and CTC for the
11 treatment of ileitis. In a BMD/CTC titration study, Schultz et al. (1997) observed a
12 78% improvement in growth of pigs, 21 d post-weaning, when 33 ppm BMD was
13 provided continuously in the diet, and CTC was provided at 110, 220, or 440 g/ton
14 from 4 d pre-challenge to 10 d post-challenge, compared to pigs fed a similar, but
15 non-medicated diet. All pigs in the positive control group developed proliferative
16 enteritis and visible lesions, with marked thickening of the mucosa at the terminal
17 ileum. However, there were no visible lesions observed in pigs fed the medicated
18 diets. Additionally, 63% of the positive control pigs developed looser stools
19 compared to 0% of the medicated pigs. The authors suggested that other
20 pathogenic organisms, such as Clostridium, bacteroides, and *E. coli* may exacerbate
21 the severity of ileitis, but BMD is effective in providing protection against these
22 pathogens.

1

2 *PCR and IHC Analysis*

3

4 All pigs tested negative for presence of *L. intracellularis* via the fecal PCR test
5 prior to being inoculated. Negative control pigs did not acquire ileitis, and
6 remained free of the organism as indicated by negative tests for fecal PCR on d 14
7 and d 21 post-challenge, and IHC from ileum collected at necropsy (Table 6). In
8 comparison, 60% of challenged pigs were shedding *L. intracellularis* by d 21 post-
9 challenge, while 97.5% of challenged pigs tested positive for the organism using
10 ileum tissue IHC.

11 Although the combination of feeding the DDGS diet and antimicrobial regimen
12 appeared to increase fecal shedding on d 14 post-challenge ($P = 0.02$), there were no
13 dietary effects on fecal shedding of *L. intracellularis* by d 21 post-challenge ($P >$
14 0.20). Only 25% of pigs in the D and P+A groups were shedding *L. intracellularis* on
15 d 14 post-challenge, compared to 63% of PC pigs, but by d 21 post-challenge, 60%
16 and 65% of D and P+A pigs were shedding the organism, respectively. These
17 results may indicate an accelerated rate of progression of ileitis infection or recovery
18 when feeding diets containing both antimicrobials and DDGS, although further
19 research studies designed to examine fecal shedding at several different time periods
20 post-challenge are necessary to determine if such a response occurs.

21 Lesion prevalence, as determined by IHC, was unaffected by dietary treatment
22 ($P = 0.59$). With the exception of one pig in each of the D and D+A groups, all
23 challenged pigs tested positive for *L. intracellularis* by d 21 post-challenge. Lesion

1 severity was reduced by feeding the 10% DDGS diet ($P = 0.05$), and tended to be
2 reduced by feeding the antimicrobial regimen ($P = 0.10$). Pigs in the D, P+A, and
3 D+A groups had IHC scores of 1.95, 2.00, and 1.90, respectively, indicating that 25 –
4 50% of the mucosa was infected with *L. intracellularis*, compared to an IHC score of
5 2.58 in PC pigs, indicating that greater than 50% of the mucosa in these pigs was
6 infected with *L. intracellularis*.

9 Implications

10
11 Results from this study suggest that including 10% DDGS in growing pig diets
12 may provide some protection and aid the pig in resisting an ileitis challenge under a
13 moderate disease challenge situation. These results are consistent with field reports
14 suggesting that dietary DDGS inclusion results in reduced severity of clinical signs
15 caused during an ileitis outbreak. The beneficial effects (reduced severity and
16 prevalence of lesions in some parts of the intestinal tract) from feeding a diet
17 containing 10% DDGS in this study were similar to the results observed for an
18 approved antibiotic regimen (BMD[®] with 14-day Aureomycin[®] pulse). Although no
19 additive effects of feeding a diet containing DDGS and BMD/Aureomycin were
20 observed in this study, further investigation is needed to better understand the
21 interaction of diet and antimicrobials, and their application towards improving
22 gastro-intestinal health. The inoculum dosage rate used in this disease challenge

- 1 **study appeared to be an appropriate level for examining dietary effects on ileitis**
- 2 **infection.**
- 3

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Table 1. Composition and analyzed nutrient content of experimental diets (as-fed basis)^a

Item	Dietary treatment ^b						
	NC	PC	D	PC + A ^c	D + A ^c	PC + A ^d	D + A ^d
Ingredient, %							
DDGS ^e	0.00	0.00	10.00	0.00	10.00	0.00	10.00
Corn	61.91	61.91	52.77	61.86	52.72	61.58	52.44
Soybean meal (47% CP)	32.62	32.62	31.77	32.62	31.77	32.62	31.77
Choice white grease	2.20	2.20	2.30	2.20	2.30	2.20	2.30
Dicalcium phosphate	1.67	1.67	1.37	1.67	1.37	1.67	1.37
Limestone	0.56	0.56	0.77	0.56	0.77	0.56	0.77
Vitamin/trace mineral premix ^f	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
L-Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.04	0.04	0.02	0.04	0.02	0.04	0.02
BMD-30 (30 g/ton)	0.00	0.00	0.00	0.05	0.05	0.05	0.05
Aureo-90 (90 mg/ton)	0.00	0.00	0.00	0.00	0.00	0.28	0.28
Nutrient analysis							
Crude protein, %	21.00	21.00	22.66	21.39	22.88	21.66	22.69
Lysine, % ^g	1.21	1.21	1.26	1.24	1.28	1.27	1.26
Methionine, %	0.35	0.35	0.35	0.34	0.35	0.37	0.35
Threonine, %	0.73	0.73	0.78	0.73	0.79	0.77	0.77
Tryptophan, %	0.26	0.26	0.26	0.26	0.25	0.26	0.26
ME, kcal/kg	3133	3133	3097	3132	3129	3140	3162
Calcium, %	0.89	0.89	0.81	0.85	0.78	0.90	0.75
Phosphorus, %	0.73	0.73	0.72	0.67	0.69	0.74	0.71

^aDiets were formulated to contain 3390 kcal/kg of ME, 1.15% apparent digestible lysine, 0.65% apparent digestible methionine & cystine, 0.80% Ca, and 0.70% total P.

^bNC = negative control, PC = positive control, D = distiller's dried grains with solubles, and A = antimicrobial regimen provided in feed.

^cFed from d 4 to d 29 and d 43 to d 54.

^dFed from d 3 pre-challenge to d 11 post-challenge.

^eDistiller's dried grains with solubles (AI-Corn Clean Fuel, Claremont, MN).

^fAmount supplied per kg of premix: 1,466,667 IU vitamin A as retinyl acetate, 246,400 IU vitamin D₃, 6,138 IU vitamin E as dl- α -tocopherol acetate, 979 mg vitamin K as menadione dimethylpyrimidinol bisulfite, 1,467 mg riboflavin, 8,800 mg niacin, 5,867 mg pantothenic acid as d-calcium pantothenate, 6.6 mg vitamin B12, 141 mg iodine as EDDI, 99 mg selenium as sodium selenite, 59,840 mg zinc as zinc oxide, 59,840 mg iron as ferrous sulfate, 3,960 mg copper as copper sulfate, and 1,980 mg manganese as manganese oxide.

^gAmino acids are expressed on a total analyzed basis.

Table 2. Effect of dietary distiller's dried grains with solubles and antimicrobial regimen on growth performance, feed intake, and feed efficiency in pigs challenged with *L. intracellularis*.

	Treatment ^a					Effects (within challenged treatments)				
	NC ^b	PC	D	P+A	D+A	D	A	D x A	SEM	Pr>F
<i>Pre-treatment (d 0 - 4)</i>										
# of pens	2	2	2	2	2	4	4	2		
Initial wt, kg	6.38	6.37	6.33	6.33	6.33	0.31	0.31	0.31	0.01	0.38
<i>Pre-challenge (d 4 - 32)</i>										
Initial wt, kg	8.16	8.66	8.35	8.22	8.34	0.37	0.51	0.33	0.04	0.44
ADG, g	404	432	386	417	416	0.40	0.78	0.41	11.2	0.65
ADFI, g	695	645	726	731	692	0.47	0.39	0.09	16.6	0.23
G/F	0.58	0.67	0.53	0.57	0.60	0.34	0.80	0.17	0.03	0.38
<i>Post-challenge (d 32 - 53)</i>										
Initial wt, kg	19.47	20.76	19.16	19.90	19.99	0.40	0.98	0.35	0.37	0.61
ADG, g	799	672	542	642	720	0.75	0.39	0.25	38.0	0.49
ADFI, g	1262	1148	1046	1167	1276	0.98	0.38	0.45	57.0	0.67
G/F	0.63	0.59	0.52	0.55	0.58	0.77	0.88	0.52	0.03	0.89
Final wt, kg	36.25	34.87	30.54	33.39	35.11	0.57	0.50	0.22	1.04	0.47
<i>Pre-CTC pulse (d 4 - 29)</i>										
ADG, g	411	437	382	421	414	0.29	0.76	0.39	12.1	0.54
ADFI, g	667	638	720	726	679	0.58	0.46	0.09	17.4	0.26
G/F	0.62	0.69	0.53	0.58	0.61	0.32	0.85	0.16	0.03	0.36
<i>CTC pulse period (d 29 - 43)</i>										
ADG, g	604	496	419	489	552	0.91	0.34	0.30	28.4	0.52
ADFI, g	1071	920	902	952	1036	0.73	0.41	0.60	39.2	0.74
G/F	0.56	0.54	0.47	0.51	0.54	0.75	0.70	0.47	0.03	0.82
<i>Post-CTC pulse (d 43 - 53)</i>										
ADG, g	933	833	682	777	871	0.73	0.44	0.19	39.6	0.44
ADFI, g	1454	1349	1209	1379	1488	0.92	0.33	0.42	64.5	0.61
G/F	0.64	0.62	0.56	0.56	0.60	0.84	0.85	0.51	0.02	0.89

^aNC = negative control, PC = positive control, D = distiller's dried grains with solubles, and A = antimicrobial regimen provided in feed.

^bNo significant difference between NC and PC groups ($P > 0.10$).

Table 3. Effect of dietary distiller's dried grains with solubles and antimicrobial regimen on visual abdominal and fecal scores after challenge with *L. intracellularis*.^a

	Treatment ^b					Effects (within challenged treatments)				
	NC ^c	PC	D	P+A	D+A	D	A	D x A	SEM	Pr>F
# of pigs	19	19	20	20	20	40	40	20		
<i>Fecal score (1-5)^d</i>										
Initial (d 32)	1.00	1.24	1.25	1.15	1.43	0.10	0.61	0.14	0.04	0.16
Week 1 post-challenge	1.00	1.39	1.14	1.20	1.48	0.13	0.57	0.08	0.04	0.13
Week 2 post-challenge	1.14	1.93	1.68	1.48	1.48	0.39	0.14	0.39	0.04	0.30
Week 3 post-challenge	1.13	1.52	1.90	1.66	1.64	0.14	0.63	0.11	0.06	0.17
<i>Abdominal score (1-3)^e</i>										
Initial (d 32)	1.00	1.00	1.00	1.00	1.00
Week 1 post-challenge	1.00	1.08	1.00	1.00	1.00	0.06	0.06	0.06	0.01	0.02
Week 2 post-challenge	1.00	1.00	1.03	1.10	1.00	0.27	0.27	0.05	0.01	0.09
Week 3 post-challenge	1.00	1.08	1.00	1.00	1.00	0.03	0.03	0.03	0.01	0.01

^a Abdominal scores: 1 = normal, 2 = slightly to moderately gaunt, and 3 = severely gaunt.

Fecal scores: 1 = no diarrhea, 2 = semi-solid feces, 3 = watery feces, 4 = blood-tinged feces that are loose or formed, and 5 = profuse diarrhea with frank blood or dark tarry feces.

^b NC = negative control, PC = positive control, D = distiller's dried grains with solubles, and A = antimicrobial regimen provided in feed.

^c Significant difference between NC and PC groups for abdominal score week 1 and 3 post-challenge and fecal score at the time of challenge and during weeks 1, 2, and 3 post-challenge ($P < 0.01$).

^d Significant effect of time ($P = 0.001$) and time x D x A ($P = 0.02$).

^e Significant effect of time x D ($P = 0.02$), time x A ($P = 0.04$), and time x D x A ($P = 0.01$).

Table 4. Effect of dietary distiller's dried grains with solubles and BMD/CTC inclusion after a *L. intracellularis* challenge on internal organ weight, intestinal length, and digesta dry matter and pH.

	Treatment ^a					Effects (within challenged treatments)				
	NC ^b	PC	D	P+A	D+A	D	A	D x A	SEM	Pr>F
# of pigs	19	19	20	20	20	40	40	20		
<i>Internal organ weights, % of body weight</i>										
Heart	0.468	0.449	0.461	0.456	0.449	0.84	0.85	0.45	0.006	0.89
Stomach	0.753	0.803	0.842	0.841	0.810	0.88	0.92	0.17	0.013	0.59
Liver	2.604	2.591	2.664	2.349	2.397	0.30	0.001	0.83	0.032	0.001
Small intestine	3.363	3.806	4.266	3.921	3.809	0.14	0.15	0.02	0.061	0.02
Large intestine	1.611	1.602	1.987	1.697	1.680	0.01	0.12	0.01	0.037	0.001
Total intestine	4.974	5.408	6.252	5.618	5.489	0.03	0.09	0.01	0.088	0.01
<i>Intestinal lengths, cm</i>										
Small intestine	1517.0	1583.4	1530.7	1549.2	1576.0	0.75	0.88	0.31	19.2	0.76
Large intestine	393.2	373.9	378.8	371.2	375.0	0.69	0.77	0.96	5.3	0.97
Total intestine	1910.2	1957.3	1909.4	1920.4	1951.0	0.85	0.95	0.37	21.5	0.84
<i>Intestinal density, g/cm</i>										
Small intestine	0.82	0.83	0.84	0.84	0.84	0.98	0.68	0.84	0.01	0.98
Large intestine	1.48	1.48	1.57	1.54	1.56	0.25	0.64	0.55	0.02	0.59
<i>Digesta dry matter, %</i>										
Small intestine	10.49	9.46	8.85	8.11	9.66	0.41	0.66	0.07	0.29	0.24
Large intestine	19.89	19.00	18.34	21.26	19.84	0.25	0.04	0.68	0.46	0.13
<i>Digesta pH</i>										
Small intestine	6.40	6.30	6.37	6.72	6.41	0.25	0.03	0.08	0.05	0.03
Large intestine	6.23	5.82	5.72	5.94	5.74	0.02	0.31	0.46	0.03	0.07

^a NC = negative control, PC = positive control, D = distiller's dried grains with solubles, and A = antimicrobial regimen provided in feed.

^b Significant difference between NC and PC groups for weight of the stomach, liver, small intestine, total intestine, and pH of large intestine pH ($P < 0.05$).

Table 5. Effect of dietary distiller's dried grains with solubles and antimicrobial inclusion after a *L. intracellularis* challenge on lesion length, severity, and prevalence in the gastrointestinal tract.

	Treatment ^a					Effects (within challenged treatments)				
	NC ^b	PC	D	P+A	D+A	D	A	D x A	SEM	Pr>F
# of pigs	19	19	20	20	20	40	40	20		
<i>Jejunum</i>										
Length, cm	1.26	22.16	14.65	8.6	10.2	0.68	0.18	0.50	3.32	0.49
Score (0-4)	0.05	0.90	0.38	0.28	0.25	0.11	0.03	0.16	0.09	0.03
Prevalence, %	5.3	47.4	30.0	20.0	15.0	0.28	0.04	0.54	5.08	0.12
<i>Ileum</i>										
Length, cm	0.37	10.58	5.50	9.75	6.40	0.02	0.98	0.62	0.88	0.11
Score (0-4)	0.05	1.54	0.75	1.43	1.05	0.02	0.70	0.40	0.13	0.10
Prevalence, %	5.3	68.4	40.0	80.0	55.0	0.02	0.22	0.87	5.53	0.06
<i>Cecum</i>										
Length, cm	0.00	0.16	0.25	0.30	0.00	0.62	0.79	0.36	0.10	0.76
Score (0-4)	0.00	0.05	0.05	0.05	0.00	0.55	0.55	0.59	0.02	0.80
Prevalence, %	0.0	5.3	5.0	5.0	0.0	0.55	0.55	0.59	2.16	0.80
<i>Colon</i>										
Length, cm	0.00	2.11	0.30	1.20	0.50	0.02	0.51	0.30	0.27	0.08
Score (0-4)	0.00	0.47	0.10	0.20	0.15	0.09	0.37	0.19	0.06	0.15
Prevalence, %	0.0	31.6	5.0	20.0	10.0	0.03	0.70	0.32	4.20	0.12
<i>Total</i>										
Length, cm	1.63	35.05	20.40	19.45	11.35	0.14	0.11	0.67	1.03	0.19
Prevalence, %	10.5	68.4	40.0	80.0	50.0	0.01	0.32	0.94	5.86	0.04

^a NC = negative control, PC = positive control, D = distiller's dried grains with solubles, and A = antimicrobial regimen provided in feed.

^b Significant difference between NC and PC groups for lesion length, score, and prevalence in the jejunum, ileum, colon, and overall (P < 0.05).

Table 6. Effect of dietary distiller's dried grains with solubles and antimicrobial regimen after a *L. intracellularis* challenge on fecal PCR and ileal tissue IHC scores.

	Treatment ^a					Effects (within challenged treatments)				
	NC ^b	PC	D	P+A	D+A	D	A	D x A	SEM	Pr>F
# of pigs	19	19	20	20	20	40	40	20		
<i>IHC</i> ^b										
Score (0-4)	0.00	2.58	1.95	2.00	1.90	0.05	0.10	0.16	0.10	0.05
Prevalence, %	0.0	100.0	95.0	100.0	95.0	0.17	1.00	1.00	1.78	0.59
<i>Fecal PCR</i> ^d , %										
Initial (d 32)	0.0	0.0	0.0	0.0	0.0
d 14 post-challenge	0.0	63.2	25.0	25.0	40.0	0.28	0.28	0.02	5.50	0.04
d 21 post-challenge	0.0	68.4	60.0	65.0	45.0	0.21	0.41	0.61	5.56	0.47

^a NC = negative control, PC = positive control, D = distiller's dried grains with solubles, and A = antimicrobial regimen provided in feed.

^b Significant difference between NC and PC groups for all IHC and PCR values ($P < 0.01$).

^c IHC = immunohistochemistry.

^d PCR = polymerase chain reaction.

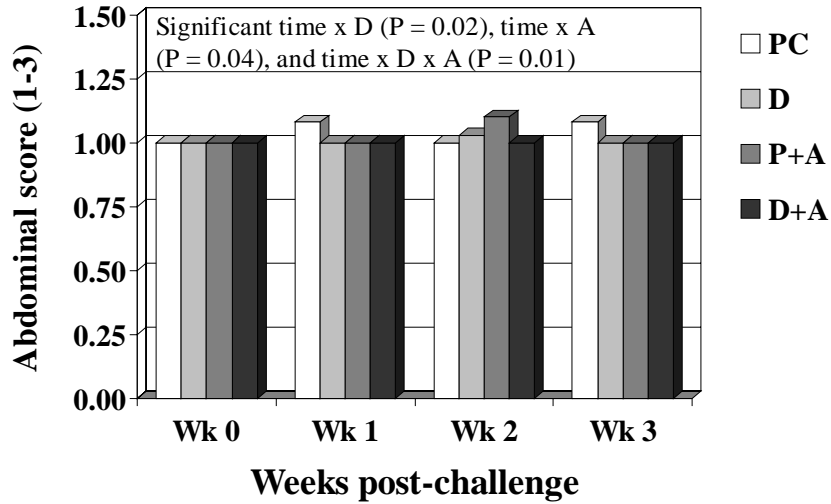


Fig. 1. Interactive effect of time (phase) and treatment on abdominal scores during the post-challenge period.

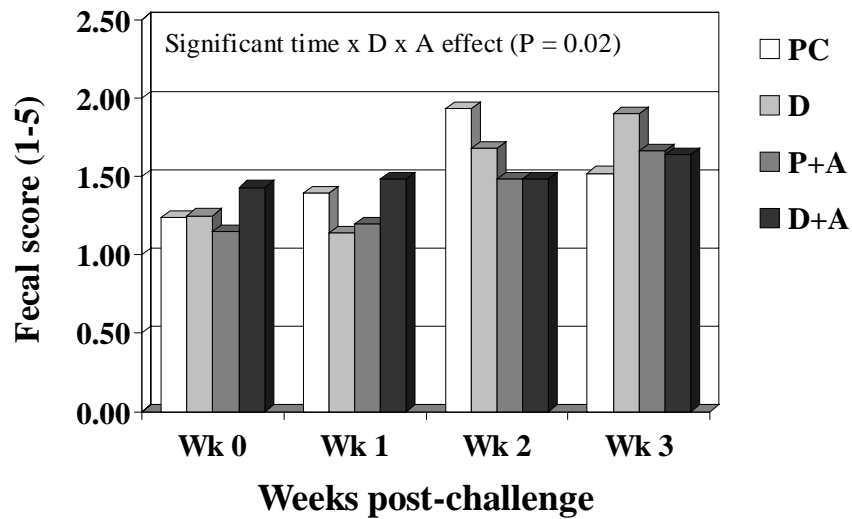


Fig. 2. Interactive effect of time (phase) and treatment on fecal scores during the post-challenge period.