

Effect of including distillers dried grains with solubles in the diet, with or without antimicrobial regimen, on the ability of growing pigs to resist a *Lawsonia intracellularis* challenge¹

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ABSTRACT: A disease challenge experiment was conducted to determine if including 10% dried distillers grains with solubles (DDGS) in the diet, with or without antimicrobial supplementation, reduces the incidence or severity, or both, of intestinal lesions in growing pigs after an *Lawsonia intracellularis* challenge. One hundred 17-d-old weaned pigs were blocked by sex, ancestry, and BW and randomly allotted to 1 of 5 treatment groups: negative control, unchallenged, corn-soy diet; positive control, challenged, corn-soy diet; 10% DDGS diet, challenged; positive control with antimicrobial regimen, challenged; and 10% DDGS diet with antimicrobial regimen, challenged. For antimicrobial-supplemented treatments, diets contained 33 ppm bacitracin methylene disalicylate throughout the experiment, with chlortetracycline (Aureomycin) pulsed at 550 ppm from d 3 prechallenge to d 11 postchallenge. Challenged pigs were orally inoculated with 8.0×10^8 *L. intracellularis* organisms after a 4-wk prechallenge period. On d 21 postchallenge, pigs were euthanized, lesions of intestinal mucosa were evaluated, and ileal tissue samples were analyzed by immunohistochemistry to determine the presence and proliferation rate of *L. intracellularis*.

Compared with other dietary treatments, feeding a diet containing 10% DDGS reduced ileum and colon lesion length and prevalence ($P < 0.05$) and reduced severity of lesions in the ileum ($P < 0.05$) and colon ($P < 0.10$) in challenged pigs. Compared with other challenged pigs, those fed the diet containing the antimicrobial regimen had a lower prevalence and severity of lesions in the jejunum ($P < 0.05$) and tended to have reduced total tract lesion length ($P = 0.11$). Compared with other challenged pigs, pigs on the 10% DDGS diet with antimicrobial regimen exhibited no differences in length, severity, or prevalence of lesions ($P > 0.15$), but fecal shedding of *L. intracellularis* was reduced on d 14 postchallenge ($P < 0.05$). No dietary effects on fecal shedding were observed by d 20 postchallenge ($P > 0.10$). The proportion of cells infected with *L. intracellularis* was reduced when DDGS ($P = 0.05$) or antimicrobial ($P = 0.10$) diets were fed. Under the conditions of this experiment, dietary inclusion of 10% DDGS appears to provide some benefit to growing pigs subjected to a moderate *L. intracellularis* challenge, similar to those of a currently approved antimicrobial regimen.

Key words: antimicrobial, distillers dried grains with solubles, pig, ileitis

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INTRODUCTION

Ileitis, or porcine proliferative enteropathy (PPE), is an enteric disease in swine that can decrease feed intake and growth rate, and increase mortality. The disease is caused by *Lawsonia intracellularis*, which are intracellular bacteria that infect the enterocytes of the intestine and are estimated to cost between \$3 and 11 per pig (McOrist et al., 1997).

Prevention and control of PPE has focused on use of antimicrobial agents. Subtherapeutic levels of antibiotics improve pig performance but often fail to prevent the disease (Gebhart et al., 1998; Schwartz et al., 1998; Winkelman, 1998). Providing chlortetracycline (CTC) strategically at therapeutic levels can reduce the occurrence and severity of intestinal lesions caused by PPE (McOrist, 1998; Winkelman et al., 1998). Schultz et al. (1997) observed an additive effect when feeding a combination of bacitracin methylene disalicylate (BMD) and CTC for treating ileitis.

Field reports indicate that including distillers dried grains with solubles (DDGS) in grow-finish diets may aid in reducing severity of ileitis (J. Goihl, Agri-Nutrition Services, Shakopee, MN, personal communication).

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The fiber composition of DDGS is primarily insoluble (42.2%) vs. soluble (0.7%; Shurson et al., 2000). Feeding diets low in soluble nonstarch polysaccharides can reduce proliferation of pathogenic organisms in the intestine (Hampson et al., 1999). Fiber influences secretory function of the epithelium, impairing bacterial adhesion (Smith and Halls, 1968), and also has a cleansing effect in the gut as a result of reducing the viscosity of digesta (Lawrence, 1972). The objective of this study was to evaluate the effect of dietary inclusion of DDGS, with or without use of a strategic antimicrobial regimen (CTC and BMD), on the ability of growing pigs to resist a *L. intracellularis* challenge.

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 × 1/4 Large White × 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 and herd health level were the same as described in a companion report (Whitney et al., 2006). Pigs (approximately 17 d of age) were blocked by sex, ancestry, and BW, and blocks were randomly allotted to 1 of 5 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 ppm, as-fed) along with dietary pulsing (providing a therapeutic level over a short period of time) of CTC (Aureomycin, 550 ppm) from d 3 prechallenge to d 11 postchallenge. The DDGS utilized for the study was obtained from Al-Corn Clean Fuel (Claremont, MN). Animals were housed in isolation rooms, with 10 pigs per room (7.25 m² per room, 10 rooms total) and 2 rooms per treatment group.

Experimental Diets

Pigs were allowed an acclimation period and then fed the experimental diets that were formulated and analyzed as in a companion report (Whitney et al., 2006; Table 1). Additionally, samples of the medicated feed were submitted to the Alpha Analytical Laboratory (Chicago Heights, IL) for analysis of BMD and CTC levels.

Disease Challenge

Four weeks after the experimental diets were initiated (d 32), pigs were manually restrained and were

provided 40 mL of saline (NC) or inoculated with *L. intracellularis* (PC, D, PC+A, and D+A treatments) via stomach tube, as described in a companion paper (Whitney et al., 2006). Actual dosage rate of *L. intracellularis* organisms provided per pig was determined to be 8.0×10^8 . Additionally, the material was screened and determined to be negative for other enteric pathogens, including *Brachyspira* species (by dark-field microscopy), viruses (by transmission electron microscopy), parasite ova (by flotation tests), *Yersinia* species, β -hemolytic *E. coli* species, and *Salmonella* (by routine culture). Biosecurity procedures and cleaning and feeding schedules were developed to reduce the risk of cross-contamination across rooms, as described in a companion paper (Whitney et al., 2006).

Data Collection

All growth performance, clinical scoring, evaluation of organism shedding, necropsies, internal organ measurements, lesion scoring, and organism quantification in intestinal tissue procedures were as reported in the companion study (Whitney et al., 2006).

Statistical Analysis

Growth performance data were statistically analyzed using the analysis of variance and were computed using GLM procedures of SAS (SAS Inst. Inc., Cary, NC), with room as the experimental unit (2 replications per treatment). All other data were analyzed utilizing individual pig as the experimental unit, resulting in 20 replications per treatment. Factors assessed for each model were treatment, room (treatment), and pig (treatment × room). Repeated measures analysis was conducted for alertness, gauntness, and diarrhea scores to account for differences over time postchallenge. Least squares means were used to compare the negative and positive control groups in order to evaluate the effects of infecting pigs on response criteria. Data involving pigs on the disease challenge treatments were analyzed as a 2 × 2 factorial, with DDGS level (0 or 10%) and antimicrobial regimen (no antimicrobials or CTC + BMD) as the factors. Differences were considered significant at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Diet Composition

Experimental diet composition and nutrient analysis are provided in Table 1. Calculated ME concentration based on proximate analysis tended to be lower in all diets compared with formulated levels (3,145 vs. 3,390 kcal/kg) but was similar among experimental diets (range = 3,097 to 3,162 kcal/kg of ME). Addition of DDGS to the diet increased CP concentration. The PC+A and D+A diets contained 39.6 and 37.6 ppm of BMD, respectively, which slightly exceeded the target

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

Item	Dietary treatment ²						
	NC	PC	D	PC + A ³	PC + A ⁴	D + A ³	D + A ⁴
Ingredient, %							
DDGS ⁵	0.00	0.00	10.00	0.00	0.00	10.00	10.00
Corn	61.91	61.91	52.77	61.86	61.58	52.72	52.44
Soybean meal, 47% CP	32.62	32.62	31.77	32.62	32.62	31.77	31.77
Choice white grease	2.20	2.20	2.30	2.20	2.20	2.30	2.30
Dicalcium phosphate	1.67	1.67	1.37	1.67	1.67	1.37	1.37
Limestone	0.56	0.56	0.77	0.56	0.56	0.77	0.77
Vitamin/trace mineral premix ⁶	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	0.04	0.02	0.02
BMD-30 ⁷	0.00	0.00	0.00	0.05	0.05	0.05	0.05
Aureo-90 ⁸	0.00	0.00	0.00	0.00	0.28	0.00	0.28
Nutrient analysis							
CP, %	21.00	21.00	22.66	21.39	21.66	22.88	22.69
Lysine, ⁹ %	1.21	1.21	1.26	1.24	1.27	1.28	1.26
Methionine, %	0.35	0.35	0.35	0.34	0.37	0.35	0.35
Threonine, %	0.73	0.73	0.78	0.73	0.77	0.79	0.77
Tryptophan, %	0.26	0.26	0.26	0.26	0.26	0.25	0.26
ME, kcal/kg	3,133	3,133	3,097	3,132	3,140	3,129	3,162
Ca, %	0.89	0.89	0.81	0.85	0.90	0.78	0.75
P, %	0.73	0.73	0.72	0.67	0.74	0.69	0.71

¹Diets were formulated to contain 3,390 kcal/kg of ME, 1.15% apparent digestible lysine, 0.65% apparent digestible methionine and cystine, 0.80% Ca, and 0.70% total P.

²NC = negative control; PC = positive control; D = distillers dried grains with solubles; and A = antimicrobial regimen provided in feed.

³Fed from d 4 to 29 and d 43 to 54.

⁴Fed from d 3 prechallenge to d 11 postchallenge.

⁵Distillers dried grains with solubles (Al-Corn Clean Fuel, Claremont, MN).

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

⁷Provided a final diet concentration of 33 ppm bacitracin methylene disalicylate.

⁸Provided a final diet concentration of 550 ppm chlortetracycline.

⁹Amino acids are expressed on a total analyzed basis.

of 33 ppm. Analyzed levels of CTC were 483 and 681 ppm for the PC+A and D+A diets, respectively, which were near the target of 550 ppm.

Growth Performance

Two pigs were removed from the experiment before completion because of health reasons unrelated to the ileitis challenge (diagnosed with *Mycoplasma hyopneumoniae* infection). Body weights, growth rate, feed intake, and feed conversion results are shown in Table 2. During the prechallenge period, growth, feed intake, and feed efficiency were similar across all treatments ($P \geq 0.23$). However, a DDGS \times antimicrobial interaction was observed in the prechallenge period, with pigs in the D + A treatment group tending to exhibit reduced feed intake ($P = 0.09$) compared with providing DDGS or antimicrobial regimen alone in the diet, indicating an increase in feed intake with DDGS inclusion in the absence of an antimicrobial.

Infecting pigs with *L. intracellularis* did not affect growth performance in the 3-wk postchallenge period

($P \geq 0.29$), although a numerical reduction in growth rate (16%) and feed intake (9%) was observed between the negative and positive control groups. No DDGS, antimicrobial, or DDGS \times antimicrobial interactions were observed for ADG, ADFI, or G:F in the postchallenge period ($P \geq 0.25$). Including the antimicrobial regimen in the DDGS diet numerically improved growth rate and feed intake (33 and 22%, respectively) compared with providing no antimicrobial regimen in the DDGS diet. Neither diet containing DDGS nor antibiotic regimen affected growth performance of challenged pigs ($P \geq 0.25$).

Previous research results by Whitney and Shurson (2004) indicated that dietary inclusion up to 25% DDGS provides similar growth performance of nursery pigs compared with conventional corn-soybean meal-based diets. Because only 2 replications per treatment were used in the analysis of growth performance data, more replication would be required to determine if the trends observed for growth performance in this experiment are actual dietary responses that could be expected on

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

Item	Treatment ¹					Effect (within challenged treatments) ²			SEM
	NC ³	PC	D	P + A	D + A	D	A	D × A	
Pretreatment (d 0 to 4)									
No. of pens ⁴	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
Prechallenge (d 4 to 32)									
Initial wt, kg	8.16	8.66	8.35	8.22	8.34	0.37	0.51	0.33	0.04
ADG, g	404	432	386	417	416	0.40	0.78	0.41	11.2
ADFI, g	695	645	726	731	692	0.47	0.39	0.09	16.6
G:F	0.58	0.67	0.53	0.57	0.60	0.34	0.80	0.17	0.03
Postchallenge (d 32 to 53)									
Initial wt, kg	19.47	20.76	19.16	19.90	19.99	0.40	0.98	0.35	0.37
ADG, g	799	672	542	642	720	0.75	0.39	0.25	38.0
ADFI, g	1,262	1,148	1,046	1,167	1,276	0.98	0.38	0.45	57.0
G:F	0.63	0.59	0.52	0.55	0.58	0.77	0.88	0.52	0.03
Final wt, kg	36.25	34.87	30.54	33.39	35.11	0.57	0.50	0.22	1.04
PreCTC pulse (d 4 to 29)									
ADG, g	411	437	382	421	414	0.29	0.76	0.39	12.1
ADFI, g	667	638	720	726	679	0.58	0.46	0.09	17.4
G:F	0.62	0.69	0.53	0.58	0.61	0.32	0.85	0.16	0.03
CTC pulse period (d 29 to 43)									
ADG, g	604	496	419	489	552	0.91	0.34	0.30	28.4
ADFI, g	1,071	920	902	952	1,036	0.73	0.41	0.60	39.2
G:F	0.56	0.54	0.47	0.51	0.54	0.75	0.70	0.47	0.03
PostCTC pulse (d 43 to 53)									
ADG, g	933	833	682	777	871	0.73	0.44	0.19	39.6
ADFI, g	1,454	1,349	1,209	1,379	1,488	0.92	0.33	0.42	64.5
G:F	0.64	0.62	0.56	0.56	0.60	0.84	0.85	0.51	0.02

¹NC = negative control; PC = positive control; D = distillers dried grains with solubles; and A = antimicrobial regimen provided in feed. Antimicrobial regimen provided 33 ppm bacitracin methylene disalicylate continuously while pulsing 550 ppm chlortetracycline (CTC) in diets from 3 d prior to 11 d postchallenge.

²*P*-values presented for main factors, along with overall model SEM and *P*-value.

³No significant difference between NC and PC groups (*P* > 0.10).

⁴Ten pigs per pen.

a consistent basis under similar conditions. However, the main objective of this experiment was to evaluate length, severity, and prevalence of lesions and fecal shedding of *L. intracellularis* using PCR and immunohistochemistry techniques to determine dietary effects during a *L. intracellularis* infection.

Alertness, Gauntness, and Fecal Scores

Pig behavior appeared normal throughout the trial for all pigs, regardless of treatment (data not shown). Weekly gauntness and fecal scores are presented in Table 3. Unchallenged pigs remained healthy throughout the postchallenge period, as indicated by a lack of gauntness and normal fecal scores. Positive control pigs were more gaunt during wk 1 and 3 postchallenge compared with NC pigs (*P* < 0.01), although no difference in gauntness score was observed during wk 2 postchallenge. Stools were of a looser consistency (more watery) during wk 1, 2, and 3 postchallenge in PC pigs compared with NC pigs (*P* < 0.01).

Gauntness did not increase appreciably during the postchallenge period (*P* > 0.10), although time × DDGS inclusion (*P* = 0.02), time × antimicrobial regimen (*P* =

0.04), and time × DDGS inclusion × antimicrobial regimen (*P* = 0.01) effects were observed (Table 3). Gauntness scores tended to be affected by dietary treatment throughout most of the postchallenge period (*P* < 0.10). Effects of DDGS inclusion, antimicrobial regimen, and DDGS inclusion × antimicrobial regimen interaction were observed during wk 1 (*P* = 0.06) and wk 3 (*P* = 0.03) postchallenge, with pigs fed the control corn-soybean meal diet exhibiting more gauntness, although only one pig appeared gaunt in each of the 3 wk postchallenge. During the second week postchallenge, only an interactive effect was observed for gauntness score (*P* = 0.05), whereas no main effects were detected (*P* = 0.27).

Fecal looseness increased with increasing time postchallenge (*P* = 0.001). No time × DDGS inclusion or time × antimicrobial regimen interactions were observed. A time × DDGS inclusion × antimicrobial interaction was observed, indicating that as fecal looseness increased over time, the combination of D+A appeared to become more effective in reducing stool looseness compared with feeding D or A individually. Dietary treatment did not affect fecal consistency before challenge (*P* = 0.16), although feeding the 10% DDGS diet tended to increase

Table 3. Effect of dietary distillers dried grains with solubles and antimicrobial regimen on visual gauntness and fecal scores in growing pigs after challenge with *Lawsonia intracellularis*¹

Item	Treatment ²					Effect (within challenged treatments) ³			SEM
	NC ⁴	PC	D	P + A	D + A	D	A	D × A	
No. of pigs	19	19	20	20	20	40	40	20	
Gauntness score ⁵ (1 to 3)									
Initial (d 32)	1.00	1.00	1.00	1.00	1.00	—	—	—	—
Wk 1 postchallenge	1.00	1.08	1.00	1.00	1.00	0.06	0.06	0.06	0.01
Wk 2 postchallenge	1.00	1.00	1.03	1.10	1.00	0.27	0.27	0.05	0.01
Wk 3 postchallenge	1.00	1.08	1.00	1.00	1.00	0.03	0.03	0.03	0.01
Fecal score ⁶ (1 to 5)									
Initial (d 32)	1.00	1.24	1.25	1.15	1.43	0.10	0.61	0.14	0.04
Wk 1 postchallenge	1.00	1.39	1.14	1.20	1.48	0.13	0.57	0.08	0.04
Wk 2 postchallenge	1.14	1.93	1.68	1.48	1.48	0.39	0.14	0.39	0.04
Wk 3 postchallenge	1.13	1.52	1.90	1.66	1.64	0.14	0.63	0.11	0.06

¹Gauntness 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.

²NC = negative control; PC = positive control; D = distillers dried grains with solubles; and A = antimicrobial regimen provided in feed. Antimicrobial regimen provided 33 ppm bacitracin methylene disalicylate continuously while pulsing 550 ppm chlortetracycline (CTC) in diets from 3 d prior to 11 d postchallenge.

³P-values presented for main factors, along with overall model SEM.

⁴Significant difference between NC and PC groups for abdominal score wk 1 and 3 postchallenge and fecal score at the time of challenge and during wk 1, 2, and 3 postchallenge ($P < 0.01$).

⁵Significant effect of time × D ($P = 0.02$), time × A ($P = 0.04$), and time × D × A ($P = 0.01$).

⁶Significant effect of time ($P = 0.001$) and time × D × A ($P = 0.02$).

looseness of stools ($P = 0.10$). During the first week postchallenge, a DDGS inclusion × antimicrobial regimen interaction was observed, with pigs fed the combination tending to have increased fecal scores ($P = 0.08$). During wk 2 and 3 postchallenge, however, no differences in fecal consistency due to dietary treatment were observed ($P > 0.10$).

Inclusion of some fiber sources in diets for growing pigs have increased the viscosity of digesta and increased water content of feces, resulting in looser stools. Feeding an 80% alfalfa meal diet decreased DM content of digesta in young pigs (Pond et al., 1988), whereas similar results have been observed when including 4 to 6% guar gum in the diet (Rainbird, 1986). Alfalfa contains 52.4% insoluble fiber and 4.3% soluble fiber (Shurson et al., 2000), whereas the dietary fiber in guar gum is soluble in nature (Grieshop et al., 2001). Including insoluble fiber in the form of 7.5% wheat bran or 30% oatmeal by-product also increased rate of passage of digesta in the large intestine (Potkins et al., 1991). Cereal bran contains approximately 28% insoluble fiber (Marlett, 1992), and therefore a 7.5% inclusion rate, at the expense of corn, would result in an additional 1.75% insoluble fiber in a typical swine finisher diet. This is much less than the additional 3.1% insoluble fiber contributed to the diet when including 10% DDGS, which contains 42.2% insoluble fiber (Shurson et al., 2000), in the place of corn and soybean meal. Jorgensen et al. (1996) observed a 5- to 6-fold increase in passage rate through the terminal ileum when pigs were fed a high vs. low fiber diet (26.8 vs. 5.9% crude fiber) and

attributed this response to an increase in peristaltic action and decreased transit time.

Internal Organ Weights and Digesta Characteristics

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

Challenging pigs with *L. intracellularis* resulted in more acidic digesta in the large intestine ($P < 0.01$) but did not affect pH of digesta collected from the small intestine ($P \geq 0.49$). Digesta DM was not affected by disease challenge ($P \geq 0.19$). Feeding DDGS decreased

Table 4. Effect of dietary distillers dried grains with solubles and bacitracin methylene disalicylate (BMD)/chlortetracycline (CTC) inclusion after a *Lawsonia intracellularis* challenge on internal organ weight, intestinal length, and digesta dry matter and pH in growing pigs

Item	Treatment ¹					Effect (within challenged treatments) ²			SEM
	NC ³	PC	D	P + A	D + A	D	A	D × A	
No. of pigs	19	19	20	20	20	40	40	20	
Internal organ weight, % of BW									
Heart	0.468	0.449	0.461	0.456	0.449	0.84	0.85	0.45	0.006
Stomach	0.753	0.803	0.842	0.841	0.810	0.88	0.92	0.17	0.013
Liver	2.604	2.591	2.664	2.349	2.397	0.30	0.001	0.83	0.032
Small intestine	3.363	3.806	4.266	3.921	3.809	0.14	0.15	0.02	0.061
Large intestine	1.611	1.602	1.987	1.697	1.680	0.01	0.12	0.01	0.037
Total intestine	4.974	5.408	6.252	5.618	5.489	0.03	0.09	0.01	0.088
Intestinal length, cm									
Small intestine	1,517.0	1,583.4	1,530.7	1,549.2	1,576.0	0.75	0.88	0.31	19.2
Large intestine	393.2	373.9	378.8	371.2	375.0	0.69	0.77	0.96	5.3
Total intestine	1,910.2	1,957.3	1,909.4	1,920.4	1,951.0	0.85	0.95	0.37	21.5
Intestinal density, g/cm									
Small intestine	0.82	0.83	0.84	0.84	0.84	0.98	0.68	0.84	0.01
Large intestine	1.48	1.48	1.57	1.54	1.56	0.25	0.64	0.55	0.02
Digesta DM, %									
Small intestine	10.49	9.46	8.85	8.11	9.66	0.41	0.66	0.07	0.29
Large intestine	19.89	19.00	18.34	21.26	19.84	0.25	0.04	0.68	0.46
Digesta pH									
Small intestine	6.40	6.30	6.37	6.72	6.41	0.25	0.03	0.08	0.05
Large intestine	6.23	5.82	5.72	5.94	5.74	0.02	0.31	0.46	0.03

¹NC = negative control; PC = positive control; D = distillers dried grains with solubles; and A = antimicrobial regimen provided in feed. Antimicrobial regimen provided 33 ppm BMD continuously while pulsing 550 ppm CTC in diets from 3 d prior to 11 d postchallenge.

²P-values presented for main factors, along with overall model SEM.

³Significant difference between NC and PC groups for weight of the stomach, liver, small intestine, total intestine, and pH of large intestine pH ($P < 0.05$).

the pH of digesta collected from the large intestine ($P < 0.02$) but did not affect digesta pH in the small intestine ($P \geq 0.25$). Feeding the antimicrobial diets resulted in an increase in digesta pH collected from the small intestine ($P < 0.03$) but did not alter digesta pH in the large intestine ($P \geq 0.31$). Feeding the combination of DDGS and antimicrobials tended to decrease the pH of digesta collected from the small intestine ($P = 0.08$) compared with feeding the antimicrobial diet alone. No dietary effects or interactions were observed on dry matter content of digesta from the small intestine, but pigs receiving the antimicrobial regimen had increased dry matter content of digesta collected from the large intestine ($P = 0.04$).

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

Pond et al. (1988), however, observed an increase in liver and kidney weights, relative to BW, when an 80%

alfalfa diet was fed to market-age pigs. Alfalfa contains high levels of both insoluble (52.4%) and soluble (4.3%) fiber (Shurson et al., 2000). Jorgensen et al. (1996) also observed increases in stomach, cecum, and colon mass when growing pigs were fed diets containing high levels of insoluble fiber.

Research by Jin et al. (1994) indicated that insoluble fiber addition in the diet increases the rate of cellular turnover in the intestine. The rates of cellular proliferation in the jejunum and colon were increased when feeding a diet containing 10% wheat straw. Wheat straw is somewhat similar in dietary fiber composition to DDGS, containing 71.0% insoluble fiber but only 0.5% soluble fiber (Shurson et al., 2000). Because *L. intracellularis* is an enteric pathogen that must invade mucosa cells intracellularly for infection, increasing cell turnover in the distal portion of the small intestine may shorten the time and reduce the ability of the organism to successfully colonize in mucosa cells. A trend toward increased small intestine weight by feeding diets containing DDGS or antimicrobials was observed in the current study ($P \leq 0.15$), which may indirectly indicate an increase in cell turnover. Additionally, research results reported by Zebrowska et al. (1983) suggested that providing fiber (barley) in the diet increases endogenous secretion of saliva, gastric juice, pancreatic juice, and bile. Because bactericidal enzymes and antibacterial

Table 5. Effect of dietary distillers dried grains with solubles and antimicrobial inclusion after a *Lawsonia intracellularis* challenge on lesion length, severity, and prevalence of ileitis in the intestinal tract of growing pigs

Item	Treatment ¹					Effect (within challenged treatments) ²			SEM
	NC ³	PC	D	P + A	D + A	D	A	D × A	
No. of pigs	19	19	20	20	20	40	40	20	
Jejunum									
Length, cm	1.26	22.16	14.65	8.6	10.2	0.68	0.18	0.50	3.32
Score ⁴ (0 to 4)	0.05	0.90	0.38	0.28	0.25	0.11	0.03	0.16	0.09
Prevalence, %	5.3	47.4	30.0	20.0	15.0	0.28	0.04	0.54	5.08
Ileum									
Length, cm	0.37	10.58	5.50	9.75	6.40	0.02	0.98	0.62	0.88
Score (0 to 4)	0.05	1.54	0.75	1.43	1.05	0.02	0.70	0.40	0.13
Prevalence, %	5.3	68.4	40.0	80.0	55.0	0.02	0.22	0.87	5.53
Cecum									
Length, cm	0.00	0.16	0.25	0.30	0.00	0.62	0.79	0.36	0.10
Score (0 to 4)	0.00	0.05	0.05	0.05	0.00	0.55	0.55	0.59	0.02
Prevalence, %	0.0	5.3	5.0	5.0	0.0	0.55	0.55	0.59	2.16
Colon									
Length, cm	0.00	2.11	0.30	1.20	0.50	0.02	0.51	0.30	0.27
Score (0 to 4)	0.00	0.47	0.10	0.20	0.15	0.09	0.37	0.19	0.06
Prevalence, %	0.0	31.6	5.0	20.0	10.0	0.03	0.70	0.32	4.20
Total									
Length, cm	1.63	35.05	20.40	19.45	11.35	0.14	0.11	0.67	1.03
Prevalence, %	10.5	68.4	40.0	80.0	50.0	0.01	0.32	0.94	5.86

¹NC = negative control; PC = positive control; D = distillers dried grains with solubles; and A = antimicrobial regimen provided in feed. Antimicrobial regimen provided 33 ppm bacitracin methylene disalicylate continuously while pulsing 550 ppm chlortetracycline (CTC) in diets from 3 d prior to 11 d postchallenge.

²P-values presented for main factors, along with overall model SEM.

³Significant difference between NC and PC groups for lesion length, score, and prevalence in the jejunum, ileum, colon, and overall ($P < 0.05$).

⁴Visual lesion scoring: 1 = mild mesenteric and intestinal wall edema; 2 = mild to moderate edema of the mesentery and intestinal wall, and corrugated intestinal mucosa; 3 = severe mesenteric and intestinal wall edema and necrosis of mucosal surface; and 4 = moderate to severe edema of mesentery and intestinal wall, thick corrugated mucosa, and blood clots in lumen.

peptides are contained in these endogenous fluids, increasing secretion by these organs may provide additional protection against infection by enteric pathogens.

Clinical Lesion Evaluation

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

Adding 10% DDGS to the diet reduced the proportion of pigs exhibiting lesions in the gastro-intestinal tract on d 21 postchallenge ($P < 0.01$); 40 and 50% of the pigs on the D and D+A treatments exhibited lesions compared with 68 and 80% of pigs that received the PC and P+A treatments, respectively. Reductions in lesion prevalence were observed in the ileum and colon ($P \leq 0.03$) but not jejunum or cecum ($P > 0.60$) when pigs

were fed DDGS in the diet. Lesion length and severity were also reduced in the ileum ($P = 0.02$) and colon ($P < 0.10$) but not in the jejunum or colon ($P > 0.10$) with dietary DDGS inclusion. Over the entire intestinal tract, feeding the 10% DDGS diet did not significantly affect lesion length ($P = 0.14$), although a numerical reduction of 42 and 68% was observed for the pigs on D and D+A treatments, respectively, compared with PC pigs.

Providing BMD in the diet throughout the experiment, and strategically pulsing chlortetracycline, resulted in a reduced prevalence of lesions observed in the jejunum ($P = 0.04$); 20 and 15% of pigs in the P+A and D+A groups exhibited lesions compared with 47 and 30% of pigs in the PC and D groups, respectively. Lesion prevalence in the ileum, cecum, colon, and overall was unaffected by antimicrobial regimen ($P > 0.20$). Lesion severity ($P = 0.03$), but not length ($P = 0.18$), was reduced in the jejunum of pigs on the antimicrobial regimen treatment, whereas neither lesion length nor severity were affected by antimicrobial regimen in the remaining portions of the gastrointestinal tract ($P > 0.10$). A numerical but nonsignificant reduction ($P = 0.11$) in overall lesion length was observed in pigs provided the antimicrobial regimen (45 and 68% for treat-

ments P+A and D+A, respectively), which was similar to the reduction observed with DDGS inclusion. No DDGS \times antimicrobial regimen interactions were observed for any of the lesion parameters measured at the time of necropsy ($P > 0.15$), indicating no synergistic effect of combining both dietary treatments.

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

The strategic use of chlortetracycline and BMD resulted in reduced severity and prevalence of lesions observed in the jejunum at the time of necropsy. These results are similar to previous research results when evaluating the use of chlortetracycline or BMD or both for ileitis prevention or control (Winkelman et al., 1997). In that study, the authors observed improved growth performance, feed intake, and feed conversion with a concomitant reduction in diarrhea and gross intestinal lesions in pigs challenged with ileitis when CTC from Aureomycin was included in the diet from 4 d before the infection to 10 d after the disease challenge. Feeding 550 ppm of CTC appeared to provide some additional benefit over the 110 ppm level.

In comparative ileitis challenge studies, McOrist (1998) and Winkelman et al. (1998) observed similar improvements in growth performance and presence of gross intestinal lesions when pigs were fed CTC from Aureomycin (330 to 660 ppm fed from 4 d prechallenge to 10 d postchallenge) compared with pigs fed tylosin (100 ppm) and lincomycin (220 ppm), respectively. Additive or synergistic effects, or both, have been observed when feeding the combination of BMD and CTC for the treatment of ileitis. In a BMD/CTC titration study, Schultz et al. (1997) observed a 78% improvement in growth of pigs, 21 d postweaning, when 33 ppm BMD was provided in the diet throughout the experiment, and CTC was provided at 110, 220, or 440 ppm from 4 d prechallenge to 10 d postchallenge, compared with pigs fed a similar nonmedicated diet. All pigs in the positive control group developed proliferative enteritis and visible lesions with marked thickening of the mu-

cosa at the terminal ileum. However, there were no visible lesions observed in pigs fed the medicated diets. Additionally, 63% of the positive control pigs developed looser stools compared with 0% of the medicated pigs. The authors suggested that other pathogenic organisms, such as *Clostridium*, *bacteroides*, and *E. coli*, may exacerbate the severity of ileitis, but BMD is effective in providing protection against these pathogens.

PCR and IHC Analysis

All pigs tested negative for presence of *L. intracellularis* via the fecal PCR test before being inoculated. Negative control pigs did not acquire ileitis and remained free of the organism as indicated by negative tests for fecal PCR on d 14 and 21 postchallenge, and IHC from ileum collected at necropsy (Table 6). In comparison, 60% of challenged pigs were shedding *L. intracellularis* by d 21 postchallenge, whereas 97.5% of challenged pigs tested positive for the organism using ileum tissue IHC.

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

Lesion prevalence, as determined by IHC, was unaffected by dietary treatment ($P = 0.59$). With the exception of one pig in each of the D and D+A groups, all challenged pigs tested positive for *L. intracellularis* by d 21 postchallenge. Lesion severity was reduced by feeding the 10% DDGS diet ($P = 0.05$) and tended to be reduced by feeding the antimicrobial regimen ($P = 0.10$). Pigs in the D, P+A, and D+A groups had IHC scores of 1.95, 2.00, and 1.90, respectively, indicating that 25 to 50% of the mucosa was infected with *L. intracellularis*, compared with an IHC score of 2.58 in PC pigs, indicating that greater than 50% of the mucosa in these pigs was infected with *L. intracellularis*.

Results from this study suggest that including 10% DDGS in growing pig diets may provide some protection and aid the pig in resisting an ileitis challenge under a moderate disease challenge situation. These results are consistent with field reports suggesting that dietary DDGS inclusion results in reduced severity of clinical signs caused during an ileitis outbreak. The beneficial effects (reduced severity and prevalence of lesions in some parts of the intestinal tract) from feeding a diet containing 10% DDGS in this study were similar to

Table 6. Effect of dietary distillers dried grains with solubles and antimicrobial regimen after an ileitis challenge on percentage of pigs shedding *Lawsonia intracellularis* (as determined by fecal PCR) and proportion of ileal cells infected as determined by immunohistochemistry (IHC)

Item	Treatment ¹					Effect (within challenged treatments) ²			
	NC ³	PC	D	P + A	D + A	D	A	D × A	SEM
No. of pigs	19	19	20	20	20	40	40	20	
IHC									
Score ⁴ (0 to 4)	0.00	2.58	1.95	2.00	1.90	0.05	0.10	0.16	0.10
Prevalence, %	0.0	100.0	95.0	100.0	95.0	0.17	1.00	1.00	1.78
Fecal PCR, %									
Initial (d 32)	0.0	0.0	0.0	0.0	0.0	—	—	—	—
d 14 postchallenge	0.0	63.2	25.0	25.0	40.0	0.28	0.28	0.02	5.50
d 21 postchallenge	0.0	68.4	60.0	65.0	45.0	0.21	0.41	0.61	5.56

¹NC = negative control; PC = positive control; D = distillers dried grains with solubles; and A = antimicrobial regimen provided in feed. Antimicrobial regimen provided 33 ppm bacitracin methylene disalicylate continuously while pulsing 550 ppm chlortetracycline (CTC) in diets from 3 d prior to 11 d postchallenge.

²P-values presented for main factors, along with overall model SEM.

³Significant difference between NC and PC groups for all IHC and PCR values ($P < 0.01$).

⁴IHC scoring: 0 to 4 indicates 0 to 100% of epithelial cells positively labeled for *L. intracellularis*.

the results observed for an approved antibiotic regimen (BMD with 14-d CTC pulse). Although no additive effects of feeding a diet containing DDGS and BMD plus CTC were observed in this study, further investigation is needed to better understand the interaction of diet and antimicrobials and their application toward improving gastrointestinal health. The inoculum dosage rate used in this disease challenge study appeared to be an appropriate level for examining dietary effects on ileitis infection.

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