

Effect of dietary inclusion of distillers dried grains with solubles on the ability of growing pigs to resist a *Lawsonia intracellularis* challenge¹

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ABSTRACT: An experiment was conducted to determine if including distillers dried grains with solubles (DDGS) in the diet of growing pigs reduces the incidence or severity of infection after a *Lawsonia intracellularis* challenge. Eighty 17-d-old weaned pigs were blocked by sex, ancestry, and BW and randomly allotted to 1 of 4 treatment groups: negative control (NC), unchallenged, corn-soy diet; positive control (PC), challenged, corn-soy diet; 10% DDGS diet (10D), challenged; and 20% DDGS diet (20D), challenged. Challenged pigs were orally inoculated with 1.5×10^9 *L. intracellularis* organisms after a 4-wk prechallenge feeding 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 unchallenged pigs, challenging pigs with *L. intracellularis* reduced growth rate, feed intake, and efficiency of gain ($P < 0.01$) and increased gauntness ($P < 0.05$) and diarrhea ($P < 0.01$). Diet did not affect growth performance postchallenge ($P > 0.40$). Feeding 10 or 20% DDGS diets did not reduce lesion length, prevalence, proliferation of *L. intracellularis*, or severity of lesions ($P > 0.10$). Thus, dietary inclusion of DDGS did not reduce the incidence or severity of lesions under the conditions of a severe *L. intracellularis* challenge used in this study.

Key words: diet, disease, distillers dried grains with solubles, ileitis, pig

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INTRODUCTION

Ileitis, or porcine proliferative enteropathy (PPE), is an enteric disease in swine caused by *Lawsonia intracellularis*. The intracellular bacteria infect immature epithelial cells located in the crypts of the villi of the intestine. Cellular proliferation and thickening of the infected intestine occur and may result in necrosis, ulceration, or hemorrhaging of the epithelial surface, or all of these. *Lawsonia intracellularis* is present in approximately 75% of all US swine herds (Bronsvort et al., 2001), costing producers \$3 to 11 per pig (McOrist et al., 1997).

Antibiotics have been used effectively against acute outbreaks of *L. intracellularis*. Subtherapeutic levels often fail to prevent the disease, whereas therapeutic levels of feed-grade antibiotics can be very expensive. Increasing public pressure to decrease the use of antibiotics in livestock production also exists, signifying a

need to identify alternative approaches to disease control.

Including distillers dried grains with solubles (DDGS) in grow-finish diets may reduce or eliminate the dependence on antibiotics to combat PPE (J. Goihl, Agri-Nutrition Services, Shakopee, MN, personal communication). Distillers dried grains with solubles contain approximately 10% crude fiber, and the fiber composition is primarily insoluble (42.2%) vs. soluble (0.7%) in nature (Shurson et al., 2000). According to Hampson et al. (1999), feeding diets that are low in soluble non-starch polysaccharides can reduce the proliferation of pathogenic organisms in the gastrointestinal tract. Smith and Halls (1968) suggested that fiber influences the secretory function of the epithelium, and this alteration may impair bacterial adhesion. Fiber 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 on the ability of growing pigs to resist a challenge with *L. intracellularis*.

MATERIALS AND METHODS

Animals and Allotment

The experimental protocols used in this study were reviewed and approved by the Institutional Animal

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

Item	Dietary treatment ²			
	NC	PC	10D	20D
Ingredient, %				
Corn	61.91	61.91	52.77	43.62
Soybean meal, 47% CP	32.62	32.62	31.77	30.92
DDGS ³	0.00	0.00	10.00	20.00
Choice white grease	2.20	2.20	2.30	2.40
Dicalcium phosphate	1.67	1.67	1.37	1.07
Limestone	0.56	0.56	0.77	0.98
Vitamin/trace mineral premix ⁴	0.45	0.45	0.45	0.45
Salt	0.40	0.40	0.40	0.40
L-Lysine	0.15	0.15	0.15	0.15
DL-Methionine	0.04	0.04	0.02	0.01
Nutrient analysis				
CP, %	21.13	21.13	23.16	24.65
Lysine, ⁵ %	1.23	1.23	1.25	1.23
Methionine, %	0.33	0.33	0.35	0.36
Threonine, %	0.73	0.73	0.80	0.85
Tryptophan, %	0.23	0.23	0.24	0.26
ME, ⁶ kcal/kg	3,152	3,152	3,171	3,197
Ca, %	0.89	0.89	0.86	0.85
P, %	0.58	0.58	0.60	0.57

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; 10D = 10% DDGS; and 20D = 20% DDGS diet.

³Distillers dried grains with solubles (Al-Corn Clean Fuel, Claremont, MN).

⁴Amount 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.

⁵Amino acids are expressed on a total basis.

⁶Calculated from equation by Noblet and Perez (1993): DE, kcal/kg = 4,151 - (122 × %Ash) + (23 × %CP) + (38 × %EE) - (64 × %Crude fiber) ME, kcal/kg = DE × [1.003 - (0.0021 × %CP)]. EE = ether extract.

Care and Use Committee at the University of Minnesota. Eighty crossbred pigs (40 gilts and 40 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 had no history or recorded cases of proliferative enteropathy and was serologically negative for *Lawsonia intracellularis*, porcine respiratory and reproductive syndrome, and *Actinobacillus pleuropneumonia*. Pigs were also clinically negative for *Salmonella choleraesuis*, transmissible gastroenteritis, and pathogenic *Brachyspira* species.

Pigs, approximately 17 d of age, were blocked by sex, ancestry, and weight, and within each block were allotted randomly to 1 of 4 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 (10D), or 20% DDGS diet fed with disease challenge (20D). 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, 8 rooms total) and 2 rooms per treatment group.

Experimental Diets

All pigs were fed a common, commercial, pelleted phase I nursery diet for the first 4 d of the experiment to encourage feed intake before initiation of dietary treatments. After the initial 4-d acclimation period, animals were fed experimental diets for the remainder of the 53-d study (Table 1). Representative samples of each diet were obtained and analyzed for DM, GE, CP, ash, ether extract, crude fiber, calcium, phosphorus, and individual amino acid composition. Experimental diets were formulated to contain equivalent energy (3,390 kcal of ME/kg, as-fed), calcium (0.80%), total phosphorus (0.70%), and apparent ileal digestible lysine (1.15%). Diets were formulated based on recently determined DDGS nutrient values for energy (Spiehs et al., 1999), total amino acid and mineral levels (Spiehs et al., 2002), and apparent ileal amino acid digestibility coefficients (Whitney et al., 2000). The ME value used for DDGS was 3,350 kcal/kg on an as-fed basis, whereas apparent ileal digestible Lys, Met + Cys, Thr, and Trp levels were estimated at 0.39, 0.57, 0.55, and 0.13%, respectively. All other nutrients were provided to meet or exceed NRC (1998) recommendations.

Disease Challenge

Four weeks after the experimental diets were initiated (d 32), pigs were manually restrained and provided 60 mL of saline (NC) or an inoculation of *L. intracellularis* (PC, D10, and D20 treatments) via stomach tube (Winkelman, 1999). The inoculate was prepared as a mucosal homogenate collected from the small intestines of pigs previously infected with *L. intracellularis* and exhibiting lesions consistent with ileitis. Mucosal material was collected by scraping the lumen of the infected intestine and then diluting it with a sucrose-phosphate-glutamate buffer (pH = 7.0; 0.218 M sucrose, 0.0038 M monobasic potassium phosphate, 0.0072 M dipotassium phosphate, and 0.0047 M L-glutamic acid), with the goal of obtaining a dosage rate of 1×10^8 *L. intracellularis* organisms per pig.

A representative sample of the harvested intestinal material was submitted to the University of Minnesota Veterinary Diagnostic Lab, and the number of *L. intracellularis* was quantified by IPX staining (DAKO K675, Dako Corporation, Carpinteria, CA) using a monoclonal antibody specific for *L. intracellularis* (McOrist et al., 1987) in an indirect immunoperoxidase assay (Guedes et al., 2002a). Serial 1:10 dilutions of the inoculum were made in sterile phosphate-buffered saline. A 15-well glass slide was coated with 10 μ L of each dilution, dried at 37°C for 30 min, then fixed with cold acetone and stained by IPX. Numbers of *L. intracellularis* were counted using a light microscope. Actual dosage rate of *L. intracellularis* provided per pig was determined to be 1.56×10^9 . 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).

Care was taken to avoid cross-contaminating pigs from different rooms after the disease challenge. Biosecurity procedures included the use of separate coveralls, boots, and gloves for each room. In addition, cleaning and feeding schedules were developed and implemented to ensure that movement between rooms was conducted in order from noninfected (NC) to infected groups.

Data Collection

Growth rate and feed intake data were collected for the pre- and postinoculation periods. Clinical observations for alertness, gauntness, and diarrhea were scored 3 times/week after challenge. Alertness was scored on animal behavior characteristics (1 = normal; 2 = slightly depressed or listless; and 3 = severely depressed or recumbent). Gauntness scores were based on body condition (1 = normal; 2 = slightly to moderately gaunt; and 3 = severely gaunt). Diarrhea was scored based on the following characteristics of feces: 1 = no diarrhea; 2 = semisolid feces without blood; 3 = watery feces without

blood; 4 = blood-tinged feces that were loose or formed; and 5 = profuse diarrhea with frank blood or dark tarry feces.

Fecal samples were collected on d 14 and 20 postinoculation and sent to the University of Minnesota Veterinary Diagnostic Laboratory for PCR evaluation of *L. intracellularis* presence to determine if pigs were shedding the organism. Bacterial DNA were extracted from fecal samples using a Qiagen extraction kit (Qiagen, Valencia, CA) before PCR analysis using a Quantitect kit (Qiagen) and following the procedures of Jones et al. (1993).

On d 20 or 21 postchallenge, all pigs were euthanized and necropsies were performed. Internal organ weights of the heart, empty stomach, liver, and empty small and large intestine were determined. Representative samples of digesta from the small and large intestines were collected, and pH was measured. Length of the small and large intestines was also measured, and visual evaluation (scoring) of the general condition of the intestine (lesion severity), length of observable lesions, and location of lesions were recorded. Lesions were scored for severity based on the following criteria: 0 = normal (no visual appearance of lesion); 1 = mild mesenteric and intestinal wall edema and hyperemia; 2 = mild to moderate edema and hyperemia of the mesentery and intestinal wall along with corrugated intestinal mucosa; 3 = severe mesenteric and intestinal wall edema and hyperemia with necrosis of the mucosal surface and formation of pseudo-diphtheric membrane (necrotic enteritis); and 4 = moderate to severe edema and hyperemia of the mesentery and intestinal wall with thick and corrugated mucosa and blood clots in the intestinal lumen.

A 10-cm tissue section of the distal ileum cranial to the ileal-cecal junction was collected, along with adjacent lymph nodes, and fixed by immersion in 10% neutral buffered formalin, embedded in paraffin, and analyzed by immunohistochemistry (IHC) using a monoclonal antibody specific for *L. intracellularis* (McOrist et al., 1987). The reaction to *L. intracellularis* antigen was graded from 0 (no positive *L. intracellularis* antigen labeled) to 4 (100% of epithelial cells in the crypts with positive antigen labeling; Guedes et al., 2002a).

Statistical Analysis

Analysis of variance was conducted on all data utilizing the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Growth performance data were analyzed by room using ANOVA (2 replications per treatment). All other data were analyzed utilizing the individual pig as the experimental unit, which provided 20 replications per treatment. Factors assessed in each model were treatment, room (treatment), and pig (room \times treatment). 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 negative and positive control

Table 2. Effect of adding distillers dried grains with solubles (DDGS) to the diet and ileitis challenge on growth rate, feed intake, and feed conversion efficiency in growing pigs

Item	Dietary treatment ¹				Challenged treatment ²	
	NC ³	PC	10D	20D	SEM	P
Pretreatment, d 0 to 4						
No. of pens ⁴	2	2	2	2		
Start wt, kg	5.73	5.73	5.73	5.74	0.09	0.99
Prechallenge, d 4 to 32						
Start wt, kg	6.80	6.88	6.91	6.83	0.13	0.81
ADG, g	353.7	379.0	389.2	360.3	6.3	0.15
ADFI, g	567.0	594.5	592.5	588.5	7.4	0.97
G:F	0.62	0.64	0.66	0.61	0.012	0.43
Postchallenge, d 32 to 53						
Start wt, kg	16.70	17.49	17.81	16.92	0.31	0.51
ADG, g	599.5	311.0	258.9	245.3	25.9	0.67
ADFI, g	1,363.0	990.0	1,012.0	1,066.5	31.4	0.70
G:F	0.44	0.31	0.26	0.23	0.023	0.43
End wt, kg	29.91	24.46	23.66	22.57	0.54	0.36

¹NC = negative control; PC = positive control; 10D = 10% DDGS; and 20D = 20% DDGS diet.

²Comparison of PC, 10D, and 20D treatment groups.

³Significant difference between NC and PC groups for ADG, ADFI, and G:F during the postchallenge period ($P < 0.01$).

⁴Ten pigs per pen.

groups, to evaluate the effects of infecting pigs relative to the response criteria measured. Analysis of variance was conducted to compare response criteria among disease challenge treatments (PC, D10, and D20). When treatment differences were detected, means were separated using the least significant difference method.

RESULTS AND DISCUSSION

Diet Composition

Experimental diet composition and nutrient analysis are shown in Table 1. Calculated ME concentration was numerically lower in all diets compared with formulated levels (3,168 vs. 3,390 kcal/kg) but was similar among experimental diets (range = 3,152 to 3,197 kcal/kg of ME). Addition of DDGS to the diet increased CP level and tended to increase individual amino acid concentrations with the exception of lysine, which was relatively constant among dietary treatments. Nitrogen concentration and intake have been previously demonstrated to increase with DDGS inclusion in practical swine diets (Spiehs et al., 1999).

Growth Performance

A summary of the growth performance data for the pre- and postchallenge periods is provided in Table 2. Growth rate, feed intake, and feed conversion were similar among dietary treatments during the prechallenge period ($P \geq 0.15$). Challenging pigs resulted in a 55% decrease in growth rate, 25% decrease in feed consumption, and a 40% reduction in feed conversion ($P < 0.01$). Providing 10 or 20% DDGS in the diet did not significantly affect growth, feed intake, or efficiency of gain ($P > 0.40$).

The decrease in growth performance observed after challenging pigs with *Lawsonia intracellularis* was expected but was more severe than has typically been documented in field and disease challenge situations. Using a similar ileitis challenge model, but younger pigs (28 d of age at time of challenge), Winkelman et al. (1998) observed a 15% decrease in growth rate during the first 3 wk postinoculation. Growth performance was most greatly affected during the third week after challenge, with *L. intracellularis* inoculation reducing feed intake 14% and G:F 23%.

Alertness, Gauntness, and Fecal Scores

Pig behavior appeared normal throughout the experiment, with only one pig exhibiting slight depression during the final week of the study (data not shown). Clinical gauntness and fecal scores are presented in Tables 3 and 4, respectively. Unchallenged pigs remained healthy throughout the postchallenge period, as indicated by a lack of gauntness and near-normal fecal scores. Infecting pigs resulted in increased incidence of gauntness and diarrhea ($P < 0.05$). It appeared that increasing DDGS level in the diet might have reduced gauntness slightly, especially during wk 1 and 2 postchallenge. This difference, however, was not significant ($P > 0.10$) when evaluating the effect of dietary treatment on gauntness in infected pigs. Fecal scores were also similar among dietary treatments ($P > 0.10$) during the first 2 wk postchallenge. During the final week of the study, however, pigs fed the 10 or 20% DDGS diet tended to exhibit looser stools compared with positive control pigs ($P < 0.10$ for wk 3 average). On d 18 postchallenge, 10D pigs exhibited greater fecal scores than PC pigs, with 20D pigs intermediate ($P < 0.05$).

Table 3. Effect of adding distillers dried grains with solubles (DDGS) to the diet and ileitis challenge on gauntness scores, postchallenge, in growing pigs¹

Item	Dietary treatment ²				Challenged treatment ^{3,4}	
	NC ⁵	PC	10D	20D	SEM	P
Wk 1 postchallenge						
D 3	1.00	1.10	1.05	1.03	0.021	0.33
D 5	1.00	1.13	1.05	1.03	0.022	0.16
D 7	1.00	1.30	1.05	1.03	0.022	0.16
Average	1.00	1.10	1.05	1.03	0.023	0.21
Wk 2 postchallenge						
D 9	1.00	1.30	1.10	1.08	0.026	0.74
D 11	1.03	1.30	1.10	1.08	0.026	0.74
D 14	1.00	1.15	1.15	1.05	0.032	0.35
Average	1.01	1.25	1.12	1.07	0.028	0.63
Wk 3 postchallenge						
D 16	1.00	1.23	1.15	1.05	0.036	0.14
D 18	1.00	1.18	1.20	1.05	0.040	0.26
D 20	1.00	1.20	1.30	1.10	0.051	0.28
Average	1.00	1.20	1.22	1.07	0.044	0.21

¹Visual gauntness scoring: 1 = normal; 2 = moderately gaunt; and 3 = severely gaunt.

²NC = negative control; PC = positive control; 10D = 10% DDGS; and 20D = 20% DDGS diet. For each diet, number of pigs = 20.

³Comparison of PC, 10D, and 20D treatment groups.

⁴Indicates a significant time effect on gauntness in challenged treatments ($P < 0.05$).

⁵Significant difference between NC and PC groups for gauntness on d 9, 14, 16, 18, and 20, and wk 3 average postchallenge ($P < 0.05$).

Guedes et al. (2002b) observed a high correlation between fecal consistency and body condition or gauntness on d 21 postchallenge in pigs dosed with 3.4×10^9 *L. intracellularis* from a mucosal homogenate chal-

lenge. In that study, gauntness was not observed until the second week postchallenge and peaked at around 3 wk. Fecal consistency began becoming semiloose 1 wk after challenge but was most profuse and noticeable

Table 4. Effect of adding distillers dried grains with solubles (DDGS) to the diet and ileitis challenge on fecal scores, postchallenge, in growing pigs¹

Item	Dietary treatment ²				Challenged treatment ^{3,4}	
	NC ⁵	PC	10D	20D	SEM	P
Wk 1 postchallenge						
D 3	1.33	1.80	1.90	1.78	0.12	0.90
D 5	1.20	1.85	2.03	1.88	0.09	0.69
D 7	1.15	1.80	1.88	1.83	0.08	0.93
Average	1.23	1.82	1.94	1.83	0.09	0.76
Wk 2 postchallenge						
D 9	1.15	1.88	2.10	1.95	0.08	0.51
D 11	1.15	2.00	2.10	2.10	0.09	0.88
D 14	1.03	1.98	2.28	2.25	0.09	0.35
Average	1.11	1.95	2.16	2.10	0.08	0.47
Wk 3 postchallenge						
D 16	1.05	2.03	2.45	2.33	0.07	0.05
D 18	1.03	2.10 ^a	2.53 ^b	2.38 ^{ab}	0.08	0.09
D 20	1.05	2.13	2.68	2.53	0.10	0.06
Average	1.06	2.09	2.55	2.41	0.08	0.06

^{a,b}Within challenged treatment groups, means without a common superscript differ ($P < 0.05$).

¹Fecal scoring: 1 = no diarrhea; 2 = semi-solid feces without blood; 3 = watery feces without blood; 4 = blood-tinged feces that is loose or formed; and 5 = profuse diarrhea with frank blood or dark tarry feces.

²NC = negative control; PC = positive control; 10D = 10% DDGS; and 20D = 20% DDGS diet. For each diet, number of pigs = 20.

³Comparison of PC, 10D, and 20D treatment groups.

⁴Indicates a significant time effect on fecal scores in challenged treatments ($P < 0.001$).

⁵Significant difference between NC and PC groups for fecal scores throughout the postchallenge period ($P < 0.05$).

around the third week postchallenge. In the current study, pigs had noticeably looser stools within 3 d after challenge, perhaps indicating a more severe disease challenge.

Although dietary treatment did not appear to greatly affect gauntness, dietary DDGS appeared to increase the incidence of diarrhea observed after challenge. The dietary fiber contained in DDGS is primarily insoluble (42.2%) vs. soluble (0.7%) in nature (Shurson et al., 2000), compared with corn and soybean meal, which contain much lower levels of insoluble fiber (4.7 and 13.2%) but are greater in soluble fiber content (1.7 and 1.6%), respectively. Soluble nonstarch polysaccharides have been shown to increase the viscosity of digesta by increasing retention of water, and thereby reducing the dry matter content of digesta, in the gastrointestinal tract (Rainbird, 1986). However, Pond et al. (1988) observed a 19% reduction in digesta DM content after feeding a diet containing 80% alfalfa meal. Alfalfa has approximately 4.3% soluble fiber but contains significantly more insoluble fiber (52.4%), indicating that insoluble fiber can also reduce digesta DM content in the pig.

Including high-fiber ingredients also has been shown to increase rate of passage through the gastro-intestinal tract. Jorgensen et al. (1996) indicated that passage rate was increased 5- to 6-fold through the terminal ileum when pigs were fed a diet high in insoluble fiber (268 vs. 59 g/kg). The authors attributed an increase in peristaltic action to the increased passage rate. Potkins et al. (1991) observed a 14 and 23% increase in rate of passage of digesta by including wheat bran or oatmeal coproducts in the diet. Wheat bran and oat fiber are excellent sources of insoluble fiber (Grieshop et al., 2001). Including greater fiber ingredients in the diet, regardless of solubility, would be expected to increase looseness of stools in pigs. The increased looseness of stools observed when feeding diets containing DDGS, therefore, may or may not be attributed to disease response.

Body and Internal Organ Weights

Body weights and internal organ data are summarized in Table 5. Initial BW and BW at the time of challenge were similar among dietary treatment groups ($P > 0.10$). Infecting pigs with *L. intracellularis* reduced BW at the time of necropsy ($P < 0.001$). Heart and stomach weights, relative to BW, were unaffected by challenge ($P > 0.10$). Liver and small intestinal weights, however, were increased relative to BW when pigs were challenged ($P < 0.01$), whereas large intestine weight also tended to be increased ($P < 0.10$). Intestinal length decreased in the small ($P < 0.10$) and large ($P < 0.01$) intestine when pigs were infected with *L. intracellularis*. Challenged pigs also had more acidic digesta collected from both the small and large intestines ($P < 0.05$).

Providing DDGS in the diet did not affect ending BW ($P > 0.10$), although increasing DDGS level in the diet numerically decreased final BW. Heart and liver weight, relative to BW, was numerically greater in 20D pigs compared with PC and 10D pigs. However, dietary treatment did not significantly affect internal organ weights, relative to BW, in challenged pigs ($P > 0.10$). Large intestine weight followed a similar numerical, but nonsignificant, trend ($P > 0.10$). Stomach and small intestine weights were unaffected by dietary treatment. Providing 10 or 20% DDGS decreased small intestine and total intestine length ($P < 0.05$) but did not appreciably alter length of the large intestine ($P > 0.10$). Digesta pH was unaffected by dietary treatment. No differences in BW or internal organ weights, when evaluated individually, were observed for pigs fed diets containing 10 and 20% DDGS.

Results from several studies indicate that feeding diets high in insoluble fiber increases the mass of the gastrointestinal tract relative to overall BW. Ma et al. (2002) observed a 21 and 25% increase in small and large intestine weights, respectively, relative to BW when an insoluble fiber source (5% wheat bran) was included in a corn-soybean meal-based grower pig diet. Including a soluble fiber source (5% sugar beet pulp), however, did not affect gastrointestinal weight. Jorgensen et al. (1996) noted an increase in stomach, cecum, and colon weights in growing pigs fed high insoluble fiber diets. Experiments by Zebrowska et al. (1983) indicated that feeding high fiber diets significantly increases the secretion of endogenous fluids, including saliva, gastric juice, pancreatic juice, and bile. The increase in dietary fiber content was accomplished mainly by increasing the soluble fiber content by feeding barley and soybean meal. Wenk (2001) suggested that the increased secretion of these fluids is associated with greater activity of secretory organs and therefore may result in their enlargement. Because bactericidal enzymes and antibacterial peptides are contained in endogenous fluids, increasing secretion by these organs due to feeding diets containing significant amounts of fiber may thereby provide additional protection against infection by enteric pathogens.

Feeding diets high in fiber has also been shown to affect other internal body organ weights. Ma et al. (2002) observed a reduction in liver weight with inclusion of wheat bran or sugar beet pulp. Pancreas weight was also reduced by adding 18% wheat bran to the diet. Other studies, however, have indicated an increase in internal organ weights in response to dietary fiber addition. Pond et al. (1988) observed an increase in the liver and kidney weights relative to BW when an 80% alfalfa meal diet was fed to mature barrows. In that study, feeding the 80% alfalfa meal diet, which was high in insoluble fiber, increased organ weight and was correlated with an increase in basal metabolic rate. In the current study, inclusion of DDGS in the diet as a source of insoluble fiber appeared to primarily increase visceral organ weights relative to BW and may therefore

Table 5. Effect of adding dietary distillers dried grains with solubles (DDGS) to the diet and ileitis challenge on BW, internal organ weight (relative to BW), intestinal length, and digesta pH in the growing pig

Item	Dietary treatment ¹				Challenged treatment ²	
	NC ³	PC	10D	20D	SEM	P
BW, kg						
Start wt	5.73	5.73	5.73	5.74	0.09	0.99
Challenge wt	16.71	17.49	17.81	16.92	0.31	0.51
End wt	29.91	24.46	23.66	22.57	0.54	0.36
Internal organ weight, % of BW						
Heart	0.500	0.480	0.499	0.533	0.007	0.79
Stomach	0.839	0.860	0.844	0.865	0.013	0.11
Liver	2.666	2.995	2.908	3.159	0.049	0.40
Small intestine	3.961	4.709	4.336	4.571	0.113	0.17
Large intestine	2.191	2.459	2.435	2.742	0.074	0.32
Total intestine	6.152	7.169	6.771	7.313	0.151	0.29
Intestinal length, cm						
Small intestine	1,501.3	1,497.2 ^a	1,357.9 ^b	1,363.2 ^b	32.2	0.04
Large intestine	384.7	343.6	333.2	337.7	7.4	0.69
Total intestine	1,886.0	1,840.8 ^a	1,691.1 ^b	1,700.9 ^b	34.8	0.05
Digesta pH						
Small intestine	6.57	6.21	6.30	6.14	0.07	0.68
Large intestine	6.37	6.04	6.06	5.91	0.05	0.44

^{a,b}Within challenged treatment groups, means without a common superscript differ ($P < 0.05$).

¹NC = negative control; PC = positive control; 10D = 10% DDGS; and 20D = 20% DDGS diet. For each diet, number of pigs = 20.

²Comparison of PC, 10D, and 20D treatment groups.

³Significant difference between NC and PC groups for end wt, liver wt, small intestine wt, total intestine weight, large intestine length, total intestine length, small intestine digesta pH, and large intestine digesta pH ($P < 0.05$).

indirectly increase the maintenance energy requirement of those pigs.

Clinical Lesion Evaluation

Clinical lesion evaluation results for the jejunum, ileum, cecum, and colon are presented in Table 6. No lesions were observed in the intestinal tract of NC pigs, whereas lesions were observed in 63% of challenged pigs. Lesion length and prevalence in the jejunum were not significantly affected by dietary treatment, although DDGS inclusion appeared to numerically increase both parameters. Feeding the 10 or 20% DDGS diets tended to increase the severity (as indicated by score) of lesions observed in the jejunum ($P < 0.10$). Prevalence, severity, and length of lesions appeared unaffected by diet in the ileum ($P > 0.10$). Pigs fed the 10% DDGS diet had increased prevalence, length, and severity of lesions in the cecum compared with pigs fed the 0 or 20% DDGS diets ($P < 0.05$). Lesion severity also tended to be greater in the colon for pigs fed the 10% DDGS diet ($P < 0.10$), whereas lesion prevalence and length were unaffected by dietary treatment. Overall, lesions were longer ($P < 0.05$) in pigs fed the 10% DDGS diet compared with PC pigs, whereas lesion length in pigs fed the 20% DDGS diet was intermediate. Number of pigs exhibiting lesions was unaffected by dietary treatment ($P > 0.10$).

Winkelman (1999) reported that the length of lesions at necropsy is a useful quantitative measure of the severity of ileitis infection in the pig and its impact on growth performance. In that study, using a similar mucosal homogenate challenge model, lesion length was highly correlated with growth rate in a linear fashion ($r^2 = 0.97$). Use of the current challenge model was highly successful in initiating disease, with lesions consistent with ileitis infection observed in the majority of challenged animals at the time of necropsy. Lesion prevalence in challenged pigs was greatest in the ileum (58%), as expected, but was also quite high in the jejunum (38%) and colon (18%). Lesions indicating proliferative enteropathy most commonly occur in the ileum and distal jejunum and less commonly in the proximal colon (McOrist and Gebhart, 1999).

The severity of disease in this study appeared to be much greater than that typically observed in commercial situations (N. Winkelman, Swine Services Unlimited, Morris, MN, personal communication). Quantification of actual dosage rate indicated an inoculation level of 1.56×10^9 *L. intracellularis* per pig was much greater than the goal of 1×10^8 as originally planned. Therefore, the results of this study may not be directly applicable to most situations typically observed for ileitis in commercial swine operations. The target dosage level was difficult to achieve because the inoculum was a mucosal homogenate harvested from infected tissues

Table 6. Effect of adding distillers dried grains with solubles (DDGS) to the diet and ileitis challenge on lesion length, severity, and prevalence of ileitis in growing pigs

Item	Dietary treatment ¹				Challenged treatment ²	
	NC ³	PC	10D	20D	SEM	P
Jejunum						
Length, cm	0.00	14.95	54.40	31.90	8.47	0.16
Score ⁴ (0 to 4)	0.00	0.40	1.10	1.20	0.16	0.08
Prevalence, %	0.00	20.00	50.00	45.00	6.33	0.12
Ileum						
Length, cm	0.00	7.45	11.75	11.05	1.37	0.39
Score (0 to 4)	0.00	0.85	1.45	1.50	0.17	0.22
Prevalence, %	0.00	50.00	65.00	60.00	6.41	0.63
Cecum						
Length, cm	0.00	0.00 ^a	1.45 ^b	0.15 ^a	0.27	0.05
Score (0 to 4)	0.00	0.00 ^a	0.50 ^b	0.05 ^a	0.08	0.03
Prevalence, %	0.00	0.00 ^a	20.00 ^b	5.00 ^a	3.60	0.06
Colon						
Length, cm	0.00	1.00	6.20	0.60	1.43	0.20
Score (0 to 4)	0.00	0.25	0.70	0.15	0.11	0.10
Prevalence, %	0.00	20.00	25.00	10.00	5.04	0.47
Total						
Length, cm	0.00	23.40 ^a	73.80 ^b	43.70 ^{ab}	9.50	0.09
Prevalence, %	0.00	55.00	70.00	65.00	6.27	0.62

^{a,b}Within challenged treatment groups, means without a common superscript differ ($P < 0.05$).

¹NC = negative control; PC = positive control; 10D = 10% DDGS; and 20D = 20% DDGS diet. For each diet, number of pigs = 20.

²Comparison of PC, 10D, and 20D treatment groups.

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

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.

on the day that pigs were challenged, and therefore, quantification of actual dosage level was not possible before the disease challenge.

PCR and IHC Analysis

Laboratory results are summarized for fecal PCR and ileal tissue IHC in Table 7. All pigs tested negative for presence of *L. intracellularis* via the fecal PCR test before being inoculated. Twenty percent of NC pigs tested positive for fecal shedding of the organism on d 14 postchallenge, and this value doubled by d 20 postchallenge but was still less than challenged pigs ($P < 0.01$). This indicates that cross-contamination occurred, and the NC group was in the early stages of an ileitis infection by the end of the study. Percentage of challenged pigs shedding *L. intracellularis* increased from 83% on d 14 to 92% on d 20 postchallenge. Feeding DDGS diets tended to increase percentage of pigs shedding on d 20 compared with PC pigs ($P < 0.10$). A similar but nonsignificant effect of dietary DDGS was observed on d 14 ($P > 0.10$).

Immunohistochemistry results showed that 30% of NC pigs were infected with *L. intracellularis*, but this was much lower than the 95% of challenged pigs testing positive ($P < 0.01$). Prevalence and proliferation of the

organism in ileal tissue, however, appeared unaffected by dietary treatment ($P > 0.10$).

Because a variety of other enteric diseases can appear grossly similar to the chronic or acute forms of PPE, diagnostic confirmation must be conducted to conclusively establish presence of PPE. Culturing of *L. intracellularis* has been extremely difficult, and therefore a PCR assay has been developed to detect presence of the organism in feces. Collection and analysis of fecal samples from infected pigs allows monitoring of the prevalence of PPE in a herd without sacrificing animals. However, the sensitivity of the assay does not generally allow for diagnosis of all infections and is likely due to the presence of inhibitors in feces that reduce the ability to detect *L. intracellularis* (Lawson and Gebhart, 2000). Microscopic examination of affected intestinal segments that have been fixed and stained by immunohistochemical techniques results in a more definitive measure of PPE infection. However, this procedure is expensive because animals must be killed to obtain the tissue samples (Guedes et al., 2002b).

Results from this study showed no beneficial effect of feeding diets containing DDGS on ability of growing pigs to resist an ileitis challenge. However, the severity of the challenge induced in this study was much greater than is commonly observed in commercial production

Table 7. Effect of adding distillers dried grains with solubles (DDGS) to the diet and ileitis challenge on percentage of pigs shedding *Lawsonia intracellularis* (as determined by fecal PCR) and proportion of ileum infected as determined by immunohistochemistry (IHC)

Item	Dietary treatment ¹				Challenged treatment ²	
	NC ³	PC	10D	20D	SEM	P
Fecal PCR						
D 0	0.0	0.0	0.0	0.0	0.0	—
D 14	20.0	70.0	90.0	90.0	4.9	0.15
D 20	40.0	80.0	95.0	100.0	3.6	0.06
IHC						
Score ⁴ (0 to 4)	0.55	2.00	2.15	2.25	0.12	0.71
Prevalence, %	30.0	100.0	90.0	95.0	2.8	0.36
Lamina propria, %	0.0	35.0	15.0	10.0	5.2	0.12
Ileocecal lymph node, %	5.0	50.0	55.0	65.0	6.5	0.63

¹NC = negative control; PC = positive control; 10D = 10% DDGS; and 20D = 20% DDGS diet. For each diet, number of pigs = 20.

²Comparison of PC, 10D, and 20D treatment groups.

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

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

situations and therefore may not be reflective of typical conditions when an ileitis outbreak occurs. Ileitis infection severely reduces feed intake, growth rate, and feed conversion. Feeding young growing pigs a 10 or 20% DDGS diet results in similar growth performance compared with feeding pigs a corn-soybean meal diet devoid of DDGS. Because of the severity of infection observed in this study and beneficial effects of DDGS inclusion that have been observed in commercial feeding situations, further evaluation is needed to determine if dietary DDGS inclusion during a more moderate ileitis challenge is effective.

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