

# Effect of dietary inclusion of distillers dried grains with solubles, soybean hulls, or a polyclonal antibody product on the ability of growing pigs to resist a *Lawsonia intracellularis* challenge<sup>1</sup>

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**ABSTRACT:** An experiment was conducted to determine if dietary inclusion of distillers dried grains with solubles (DDGS), soybean hulls, or soybean hulls sprayed with an egg-based, polyclonal antibody product would reduce the incidence or severity of infection, or both, in growing pigs after a *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; 20% DDGS diet (D), challenged; 5% soybean hulls diet (SH), challenged; and SH sprayed with a polyclonal antibody product diet, challenged. Challenged pigs were orally inoculated with  $6.4 \times 10^8$  *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*. Challenging pigs with *L. intra-*

*cellularis* reduced growth rate, feed intake, and efficiency of gain ( $P < 0.02$ ) and increased the proportion of internal organ weights relative to BW ( $P < 0.01$ ). Dietary treatment did not affect growth performance pre- or postchallenge ( $P > 0.10$ ). Heart, empty stomach, and liver weights were similar among dietary treatments ( $P > 0.10$ ). Weight of the large intestine as a percentage of BW was increased in D and SH pigs compared with positive control pigs ( $P < 0.05$ ). Lesion length, prevalence, and severity, and fecal shedding of *L. intracellularis* were primarily unaffected by dietary treatment ( $P > 0.10$ ), although ileal lesion length and severity observed tended to be greater in the SH sprayed with polyclonal antibody product diet vs. the D pigs ( $P < 0.10$ ). Results from a previous study indicated that diet composition may affect length, severity, and prevalence of lesions caused by *L. intracellularis* in growing pigs subjected to a moderate challenge. However, beneficial results were not observed by feeding the dietary treatments used in this study.

**Key words:** distillers dried grain with solubles, ileitis, pig, polyclonal antibody, soybean hull

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J. Anim. Sci. 2006. 84:1880–1889  
doi:10.2527/jas.2004-578

## INTRODUCTION

Porcine proliferative enteropathy (PPE), or ileitis, is an enteric disease in swine caused by *Lawsonia intracellularis*. Strategic therapeutic use of antibiotics has been effective in treating acute cases of PPE (McOrist and Gebhart, 1999). Subtherapeutic levels have improved pig performance but often fail to prevent the disease (Gebhart et al., 1998; Schwartz et al., 1998; Winkelman, 1998). Food safety concerns over potential

residue violations in meat and the risk of antibiotic-resistance in human strains of pathogenic organisms may preclude continued use of many antimicrobials.

Including distillers dried grains with solubles (DDGS) or soybean hulls in grow-finish diets may assist in preventing ileitis. Both feed ingredients are dietary sources of insoluble fiber (Shurson et al., 2000). Diets low in soluble nonstarch polysaccharides reduce the proliferation of pathogenic organisms in the gastrointestinal tract (Hampson et al., 1999). Dietary fiber increases the secretory function of the epithelium and may assist in impairing bacterial adhesion to the intestinal wall (Smith and Halls, 1968).

Providing passive immune protection to pigs by dietary inclusion of egg-based polyclonal antibodies also has great potential for improving resistance or preventing specific diseases. Improvements in growth performance and reduced mortality have been reported

<sup>1</sup>We gratefully acknowledge the financial support of the Illinois Corn Marketing Board, Minnesota Corn Growers Association, and Camus Inc.

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Received October 26, 2004.

Accepted February 7, 2006.

with dietary inclusion of spray-dried egg protein products under commercial production conditions (Kichura, 1997; Shipp et al., 1999). The objective of this study was to evaluate the effect of dietary inclusion of DDGS, soybean hulls, or a newly developed polyclonal egg antibody product specific to *L. intracellularis* (Camas Inc., Le Center, MN) 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 in a companion report (Whitney et al., 2006a). 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 without disease challenge, positive control (PC) corn-soybean meal diet with disease challenge, 20% DDGS diet with disease challenge (D), 5% soybean hulls diet with disease challenge (SH), or a 5% soybean hulls diet containing a polyclonal antibody product (Camas Inc., Le Center, MN) with disease challenge (PA).

The polyclonal antibody product was produced by injecting laying hens several times with an antigen specific for *L. intracellularis*, collecting the eggs, harvesting and separating the yolk, and then spraying the resultant product onto soybean hulls. 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 m<sup>2</sup> per room, 10 rooms total) and 2 rooms per treatment group.

### *Experimental Diets*

Pigs were allowed an acclimation period and then fed experimental diets that were formulated and analyzed as reported in the companion study (Whitney et al., 2006a). The dietary inclusion level of 5% soybean hulls was chosen because field reports indicated that clinical signs of ileitis were reduced when this level of soybean hulls was fed under commercial conditions (J. Goihl, Agri-Nutrition Services, Shakopee, MN, personal communication). The dietary crude fiber content provided by soybean hulls is equivalent to that contributed by 20% DDGS, and therefore, this level of DDGS was chosen (Table 1).

### *Disease Challenge*

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

either 40 mL of saline (NC treatment) or an inoculation of *Lawsonia intracellularis* (PC, D, SH, and PA treatments) via stomach tube, as described by Whitney et al. (2006a). Actual dosage level was determined to be  $6.4 \times 10^8$  *L. intracellularis* organisms per pig. Intestinal material was also screened and determined to be negative for the presence of other enteric pathogens, including *Brachyspira* species (by dark-field microscopy), viruses (by transmission electron microscopy), parasite ova (by flotation tests), *Yersinia* species,  $\beta$ -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 the companion paper (Whitney et al., 2006a).

### *Data Collection*

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

### *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, providing 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 pigs in order to evaluate the effects of infected vs. noninfected pigs for the various response criteria measured. Analysis of variance was used to compare response criteria among the disease challenge treatments (PC, D, SH, and PA). When treatment differences were detected, means were separated using the LSD method.

## RESULTS AND DISCUSSION

### *Diet Composition*

Calculated ME concentration based on proximate analysis of the diets tended to be lower in all diets compared with formulated levels (3,133 vs. 3,390 kcal/kg) but was similar among experimental diets (range = 3,102 to 3,151 kcal/kg of ME; Table 1). Addition of DDGS to the diet increased the CP level, threonine, and tryptophan concentration of the diet. Total lysine concentration was slightly greater in the corn-soybean meal diets compared with other dietary treatments.

**Table 1.** Composition of the experimental diets (as-fed basis)<sup>1</sup>

Item	Dietary treatment <sup>2</sup>				
	NC	PC	D	SH	PA
Ingredient, %					
Corn	61.91	61.91	43.62	55.61	55.61
Soybean meal, 47% CP	32.62	32.62	30.92	32.13	32.13
DDGS <sup>3</sup>	0.00	0.00	20.00	0.00	0.00
Soybean hulls	0.00	0.00	0.00	5.00	0.00
Soybean hulls + polyclonal antibody	0.00	0.00	0.00	0.00	5.00
Choice white grease	2.20	2.20	2.40	4.00	4.00
Dicalcium phosphate	1.67	1.67	1.07	1.73	1.73
Limestone	0.56	0.56	0.98	0.47	0.47
Vitamin/trace mineral premix <sup>4</sup>	0.45	0.45	0.45	0.45	0.45
Salt	0.40	0.40	0.40	0.40	0.40
L-Lysine	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.04	0.04	0.01	0.06	0.06
Analyzed composition					
CP, %	22.22	22.22	24.73	21.46	21.94
Lysine, <sup>5</sup> %	1.34	1.34	1.29	1.31	1.29
Methionine, %	0.36	0.36	0.37	0.37	0.37
Threonine, %	0.80	0.80	0.87	0.77	0.74
Tryptophan, %	0.24	0.24	0.27	0.24	0.24
ME, <sup>6</sup> kcal/kg	3,151	3,151	3,102	3,117	3,146
Ca, %	0.85	0.85	0.71	0.69	0.73
P, %	0.65	0.65	0.55	0.59	0.64

<sup>1</sup>Diets 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.

<sup>2</sup>NC = negative control, PC = positive control, D = distillers dried grains with solubles, SH = soybean hulls, and PA = polyclonal antibody provided in feed. Polyclonal antibody (Camas Inc., Le Center, MN) was produced with antigen specific to *L. intracellularis* and sprayed onto soybean hulls.

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

<sup>4</sup>Supplied per kilogram of premix: 1,466,667 IU of vitamin A as retinyl acetate, 246,400 IU of vitamin D<sub>3</sub>, 6,138 IU of vitamin E as DL- $\alpha$ -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 B<sub>12</sub>, 141 mg of iodine as EDDI (ethylenediamondihydroiodide), 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.

<sup>5</sup>Amino acids are expressed on a total basis.

<sup>6</sup>Calculated from equation by Noblet and Perez (1993):

DE (kcal/kg) = 4151 - (122 × %Ash) + (23 × % CP) + (38 × %EE) - (64 × %Crude fiber)

ME (kcal/kg) = DE × [1.003 - (0.0021 × % CP)]. EE = ether extract.

## Growth Performance

One pig was 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 efficiency of gain results are summarized in Table 2. Average initial pig weight was 5.6 kg. During the prechallenge period, growth, feed intake, and efficiency of gain were similar across all treatments ( $P \geq 0.44$ ). At the time of challenge, pig weight averaged 18.4 kg and was similar among dietary treatment groups ( $P = 0.52$ ).

Infecting pigs with *L. intracellularis* significantly reduced growth performance during the 3-wk postchallenge period. Positive control pigs grew 68% slower ( $P = 0.01$ ), consumed 27% less feed ( $P < 0.001$ ), and were 55% less efficient in converting feed to BW gain ( $P = 0.02$ ) compared with NC pigs. Similarly, NC pigs were 48% heavier at the time of necropsy. Dietary treatment did not affect postchallenge ADG, ADFI, G:F, or final

BW in challenged pigs ( $P > 0.60$ ). These observations are similar to reductions in growth performance with ileitis challenge in the companion paper (Whitney et al., 2006a) but more severe than expected and previously reported using a similar challenge model (Winkelman, 1998).

## Alertness, Gauntness, and Fecal Scores

Pig behavior appeared normal throughout the trial for all pigs, regardless of treatment. Unchallenged pigs remained healthy throughout the postchallenge period, as indicated by a lack of gauntness and normal fecal scores (Table 3). Fecal consistency was similar among pigs before challenge ( $P > 0.10$ ), but stools were more watery during wk 1 ( $P = 0.08$ ), wk 2 ( $P = 0.03$ ), and wk 3 ( $P = 0.01$ ) postchallenge in challenged pigs (PC) compared with NC pigs ( $P < 0.01$ ), indirectly indicating clinical onset of ileitis. Additionally, PC pigs appeared to be more gaunt during wk 2 ( $P = 0.10$ ) and wk 3 ( $P = 0.06$ ) postchallenge compared with NC pigs. All pigs

**Table 2.** Effect of dietary inclusion of distillers dried grains with solubles, soybean hulls, or a polyclonal antibody product and ileitis challenge on growth rate, feed intake, and feed conversion efficiency in growing pigs

Item	Treatment <sup>1</sup>					Challenged treatment <sup>2</sup>	
	NC <sup>3</sup>	PC	D	SH	PA	SEM	<i>P</i>
Pretreatment (d 0 to 4)							
No. of pens	2	2	2	2	2	—	—
Initial wt, kg	5.61	5.65	5.61	5.59	5.60	0.02	0.86
Prechallenge (d 4 to 32)							
Initial wt, kg	6.90	6.99	6.83	6.75	6.87	0.04	0.78
ADG, g	427.7	431.0	390.2	395.3	418.5	10.4	0.54
ADFI, g	627.0	645.5	631.5	599.5	608.5	9.8	0.44
G:F	0.69	0.67	0.62	0.66	0.69	0.01	0.69
Postchallenge (d 32 to 53)							
Initial wt, kg	18.88	19.06	17.76	17.82	18.59	0.32	0.52
ADG, g	867.5	281.0	222.9	191.3	245.3	33.5	0.88
ADFI, g	1,357.7	985.3	967.8	944.2	913.6	18.1	0.64
G:F	0.64	0.29	0.23	0.20	0.26	0.02	0.89
Final wt, kg	37.09	25.10	22.55	22.02	23.81	0.84	0.68

<sup>1</sup>NC = negative control; PC = positive control; D = distillers dried grains with solubles; SH = soybean hulls; and PA = polyclonal antibody provided in feed. Polyclonal antibody (Camas Inc., Le Center, MN) was produced with antigen specific to *L. intracellularis* and sprayed onto soybean hulls.

<sup>2</sup>Comparison of PC, D, SH, and PA treatment groups.

<sup>3</sup>Significant difference between NC and PC groups for postchallenge ADG, ADFI, G/F, and final wt ( $P < 0.05$ ).

were in excellent body condition before challenge and during the first week postchallenge.

Gauntness of pigs also increased during the postchallenge period ( $P < 0.05$ ) but was similar among dietary treatments that were challenged ( $P \geq 0.19$ ). Fecal looseness also increased with increasing time postchallenge ( $P < 0.01$ ). Dietary treatment did not affect fecal consistency

before challenge ( $P = 0.17$ ), nor did it affect fecal consistency during the postchallenge period ( $P \geq 0.18$ ), although SH pigs appeared to have firmer stools, numerically, than challenged pigs on other dietary treatments during the last 2 wk of the study.

Including high-fiber ingredients has been shown to increase rate of passage through the gastrointestinal

**Table 3.** Effect of dietary inclusion of distillers dried grains with solubles, soybean hulls, or a polyclonal antibody product and ileitis challenge on gauntness and fecal scores in growing pigs

Item	Treatment <sup>1</sup>					Challenged treatment <sup>2,3</sup>	
	NC <sup>4</sup>	PC	D	SH	PA	SEM	<i>P</i>
No. of pigs	20	20	20	20	19	—	—
Gauntness score <sup>5</sup> (1 to 3)							
Initial (d 32)	1.00	1.00	1.00	1.00	1.00	—	—
Wk 1 postchallenge	1.00	1.00	1.00	1.00	1.00	—	—
Wk 2 postchallenge	1.00	1.06	1.03	1.03	1.03	0.013	0.19
Wk 3 postchallenge	1.00	1.09	1.03	1.06	1.03	0.021	0.31
Fecal score <sup>6</sup> (1 to 5)							
Initial (d 32)	1.03	1.06	1.03	1.00	1.03	0.03	0.17
Wk 1 postchallenge	1.00	1.20	1.40	1.30	1.32	0.04	0.33
Wk 2 postchallenge	1.06	2.42	2.65	2.28	2.37	0.06	0.18
Wk 3 postchallenge	1.06	2.61	2.53	2.34	2.48	0.07	0.23

<sup>1</sup>NC = negative control; PC = positive control; D = distillers dried grains with solubles; SH = soybean hulls; and PA = polyclonal antibody provided in feed. Polyclonal antibody (Camas Inc., Le Center, MN) was produced with antigen specific to *L. intracellularis* and sprayed onto soybean hulls.

<sup>2</sup>Comparison of PC, D, SH, and PA treatment groups.

<sup>3</sup>Significant time effect for gauntness ( $P < 0.05$ ) and fecal scores ( $P < 0.01$ ).

<sup>4</sup>Significant difference between NC and PC groups for fecal scores wk 2 and 3 postchallenge ( $P < 0.05$ ).

<sup>5</sup>Gauntness scores: 1 = normal; 2 = slightly to moderately gaunt; and 3 = severely gaunt.

<sup>6</sup>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.

**Table 4.** Effect of dietary inclusion of distillers dried grains with solubles, soybean hulls, or a polyclonal antibody product and ileitis challenge in growing pigs on internal organ weight, intestinal length, and digesta dry matter and pH

Item	Treatment <sup>1</sup>					Challenged treatment <sup>2</sup>	
	NC <sup>3</sup>	PC	D	SH	PA	SEM	<i>P</i>
No. of pigs	20	20	20	20	19	—	—
Internal organ weight, % of BW							
Heart	0.43	0.51	0.52	0.55	0.51	0.011	0.51
Stomach	0.79	1.00	1.00	1.05	1.03	0.019	0.74
Liver	2.31	3.13	2.98	3.26	3.08	0.072	0.59
Small intestine	3.54	5.03	5.08	5.36	4.67	0.103	0.14
Large intestine	1.57	1.93 <sup>b</sup>	2.39 <sup>a</sup>	2.39 <sup>a</sup>	2.15 <sup>ab</sup>	0.059	0.01
Total intestine	5.11	6.96 <sup>b</sup>	7.48 <sup>ab</sup>	7.74 <sup>a</sup>	6.82 <sup>b</sup>	0.141	0.07
Intestinal length, cm							
Small intestine	1,552.0	1,487.8	1,366.9	1,410.0	1,353.4	27.8	0.28
Large intestine	397.4	318.2	336.9	334.6	325.7	7.0	0.74
Total intestine	1,949.3	1,806.0	1,703.7	1,744.6	1,679.1	30.1	0.48
Digesta dry matter, %							
Small intestine	10.75	9.29	8.83	8.98	9.30	0.25	0.68
Large intestine	20.10	18.75	18.87	19.04	18.83	0.21	0.54
Digesta pH							
Small intestine	6.42	6.30	6.25	5.93	6.18	0.06	0.11
Large intestine	5.67	5.73	5.74	5.52	5.65	0.05	0.33

<sup>a,b</sup>Within challenged treatment groups, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>NC = negative control; PC = positive control; D = distillers dried grains with solubles; SH = soybean hulls; and PA = polyclonal antibody provided in feed. Polyclonal antibody (Camas Inc., Le Center, MN) was produced with antigen specific to *L. intracellularis* and sprayed onto soybean hulls.

<sup>2</sup>Comparison of PC, D, SH, and PA treatment groups.

<sup>3</sup>Significant difference between NC and PC groups for all internal organ weights, large intestine length, and large intestine digesta dry matter ( $P < 0.05$ ).

tract (Jorgensen et al., 1996), with increased peristaltic action attributed to the increase in passage rate. Potkins et al. (1991) also observed an increase in rate of passage of digesta by including wheat bran or oatmeal coproducts in the diet, both of which are excellent sources of insoluble fiber (Grieshop et al., 2001). Therefore, including greater fiber ingredients in the diet would be expected to increase looseness of stools in pigs.

### Internal Organ Weights and Digesta Characteristics

Internal organ weights and digesta characteristics are summarized in Table 4. Infecting pigs with *L. intracellularis* increased heart, empty stomach, and liver weight relative to BW ( $P < 0.001$ ) at the time of necropsy. Diet did not affect heart, empty stomach, or liver weights relative to BW in challenged pigs, however ( $P > 0.50$ ). Weight of the small and large intestine, relative to BW, was increased in challenged pigs ( $P < 0.005$ ), whereas length of the large ( $P < 0.001$ ) but not small ( $P = 0.35$ ) intestine was reduced by *L. intracellularis* challenge. Relative weight of the small intestine was decreased numerically in PA pigs compared with other challenged pigs, but diet did not significantly affect the relative weight ( $P = 0.14$ ) or length ( $P = 0.28$ ) of the small intestine in challenged pigs. No dietary treatment effects were observed for length of the large intestine ( $P = 0.78$ ), but pigs in the D and SH treatment groups had increased large intestine weights relative to BW

compared with challenged pigs fed the corn-soybean meal control diet ( $P < 0.05$ ).

Challenging pigs had no effect on acidity of digesta collected from the small ( $P = 0.45$ ) or large ( $P = 0.66$ ) intestine. Feeding soybean hulls appeared to decrease the pH of digesta collected from the small intestine, although dietary effects were nonsignificant ( $P = 0.11$ ). Diet did not appreciably affect digesta pH in the large intestine ( $P = 0.33$ ). Dry matter concentration of digesta collected from challenged pigs did not differ between dietary treatments ( $P \geq 0.54$ ).

The reduced length of the small intestine observed in challenged pigs would suggest a more limited ability to digest and absorb nutrients. Additionally, increased internal organ weights (relative to BW) were observed in challenged pigs. Koong et al. (1985) indicated that a high positive correlation between visceral organ weight and heat production exists. Therefore, a decline in efficiency of gain, observed in the current study, would be expected because of increased maintenance requirements and reduced nutrient digestibility in challenged pigs.

Feeding high-fiber ingredients in swine diets has produced variable effects on internal organ mass. Results from the current study indicated no dietary effects on heart, stomach, and liver weight. Pond et al. (1988) observed increased liver, heart, and empty stomach weights (relative to BW) in young adult pigs fed a high-alfalfa diet. However, Ma et al. (2002) reported reduced liver and pancreas weights from feeding a 5% wheat

bran diet. Cereal bran contains approximately 28% insoluble fiber and only 2.1% soluble fiber (Marlett, 1992), and therefore, a diet containing 5% wheat bran in replacement of corn could be expected to provide an additional 1.2% insoluble fiber but only 0.02% more soluble fiber. Distillers dried grain with solubles contains 42.2% insoluble fiber and 0.7% insoluble fiber (Shurson et al., 2000), and at a dietary inclusion level of 20% would be expected to contribute an additional 7.3% insoluble fiber, but would reduce dietary soluble fiber level by 0.2%. Soybean hulls contain greater concentrations of insoluble (75.5%) and soluble (8.4%) fiber compared with DDGS (Shurson et al., 2000) and would contribute an additional 2.7% insoluble fiber and 0.3% soluble fiber when included in the diet at a level of 5%, even though the crude fiber contribution of both DDGS and soybean hulls would be equivalent. Increases in endogenous secretion of saliva, gastric juice, pancreatic juice, and bile have been correlated with increasing fiber (soluble and insoluble) level of the diet, and may be associated with enlargement of these secretory organs (Zebrowska et al., 1983; Dierick et al., 1989; Wenk, 2001).

Feeding a diet containing soybean hulls increased small intestine weights, whereas feeding soybean hull and DDGS diets increased large intestine weights, relative to overall BW. These results support the observations of Kass et al. (1980), in which increased small intestine, cecum, and colon weights (as a proportion of BW) occurred when alfalfa meal (a source of insoluble and soluble fiber) was included in the growing pig diet. Ma et al. (2002) also reported an increase in intestinal tract weight when including 5% wheat bran in the diet as a source of insoluble fiber. Jorgensen et al. (1996) observed increased stomach, cecum, and colon weight when growing pigs were fed high fiber diets containing pea fiber and pectin. Dietary fiber of both sources would be primarily soluble vs. insoluble.

Because increased intestinal weight indicates an increase in energy and nutrient needs of the organ, providing high fiber, and especially high insoluble fiber, ingredients in the diet may be viewed negatively because it indirectly increases maintenance energy requirements in swine (Koong et al., 1985). Similarly, Jin et al. (1994) observed an increase in the rate of cellular proliferation in the jejunum and colon when feeding a diet containing 10% wheat straw. Based on an insoluble fiber content of 71.0% for wheat straw (Shurson et al., 2000), the 10% wheat straw diet would be expected to contribute an additional 6.0% insoluble fiber and slightly reduce the soluble fiber content of the diet, similar to including 20% DDGS. These results indicated an increase in intestinal cell turnover with insoluble fiber addition in the diet. Because *L. intracellularis* is an enteric pathogen that must invade mucosa cells intracellularly for infection, increasing cell turnover (by dietary insoluble fiber addition) may thereby shorten the time and reduce the ability of the organism to successfully colonize in mucosa cells.

Although DM concentration of digesta was not affected greatly by diet in this experiment, inclusion of insoluble nonstarch polysaccharides in the diet has been implicated with decreasing intestinal transit time and enhancing water-holding capacity of digesta (Kass et al., 1980; Low, 1985). Stanogias and Pearce (1985) reported no effect of adding soybean hulls or wheat bran at 7 to 15% of the diet on rate of passage but noted a faster rate of passage when dietary inclusion levels reached 22 to 30%. This would suggest that the levels utilized in the current study were perhaps too low to make any significant difference in digesta DM content. The lack of response of digesta pH to diet fed in the current study supports previous research by Dersjant-Li et al. (2001) indicating that increasing the nonstarch polysaccharide level of the diet (15% wheat middlings inclusion) resulted in no significant changes in pH or buffering capacity of digesta in the stomach and small intestine. Wheat middlings, however, would be expected to contribute a substantial amount of dietary soluble fiber compared with insoluble fiber.

### Clinical Lesion Evaluation

Results for clinical lesion evaluation of the jejunum, ileum, cecum, and colon are presented in Table 5. One pig in the NC group had a lesion that was suspect for ileitis, but immunohistochemistry (IHC) analysis indicated that it was negative. Overall, 81% of pigs that were challenged exhibited lesions consistent with ileitis, which was much greater than lesion prevalence (63 and 59%) observed in 2 previous ileitis challenge experiments (Whitney et al., 2006a,b). Challenging pigs with *L. intracellularis* resulted in significant increases in lesion length, severity, and prevalence in the jejunum, ileum, colon, and overall compared with unchallenged pigs ( $P < 0.01$ ).

Lesion length, severity, and prevalence were unaffected by dietary treatment in the jejunum, ileum, and colon ( $P > 0.05$ ). Overall, lesion parameters were minimally affected by dietary treatment, although feeding SH appeared to numerically reduce lesion length compared with the PC group and reduce lesion severity compared with the PA group ( $P > 0.05$ ).

Antibody activity of the polyclonal antibody product at the time of feeding was not determined. However, based on clinical lesion scores, it appeared that the polyclonal antibody product was not active. The spraying process used would not be expected to damage or denature the protein in the antibody product. However, if the product was inactive, it would simply be a source of highly digestible amino acids and therefore may have encouraged pathogenic growth. This may have occurred, rather than deterring colonization of *L. intracellularis*. Similar polyclonal products have since been produced and marketed in a liquid form, providing more consistent positive results and maintaining antibody activity toward other enteric pathogens.

**Table 5.** Effect of dietary inclusion of distillers dried grains with solubles, soybean hulls, or a polyclonal antibody product and *L. intracellularis* challenge in growing pigs on lesion length, severity, and prevalence of ileitis

Item	Treatment <sup>1</sup>					Challenged treatment <sup>2</sup>	
	NC <sup>3</sup>	PC	D	SH	PA	SEM	P
No. of pigs	20	20	20	20	19	—	—
Jejunum							
Length, cm	0.0	78.2	53.6	26.8	68.0	11.8	0.45
Score <sup>4</sup> (0 to 4)	0.00	1.20	1.41	1.09	1.80	0.15	0.37
Prevalence, %	0.0	55.0	55.0	50.0	68.0	5.6	0.70
Ileum							
Length, cm	0.7	15.9	13.5	13.2	19.6	1.2	0.21
Score (0 to 4)	0.05	1.85	1.65	1.50	2.32	0.14	0.21
Prevalence, %	5.0	85.0	75.0	65.0	89.5	4.7	0.25
Cecum							
Length, cm	0.0	1.3	0.0	0.0	0.5	0.3	0.55
Score (0 to 4)	0.00	0.15	0.00	0.00	0.05	0.04	0.58
Prevalence, %	0.0	10.0	0.0	0.0	5.2	2.2	0.29
Colon							
Length, cm	0.0	2.7	0.8	0.7	1.0	0.4	0.17
Score (0 to 4)	0.00	0.40	0.20	0.15	0.16	0.06	0.45
Prevalence, %	0.0	30.0	10.0	10.0	15.8	4.2	0.28
Total							
Length, cm	0.7	98.1	68.6	40.7	89.1	12.5	0.37
Score (0 to 4)	0.01	0.90	0.84	0.68	1.08	0.08	0.33
Prevalence, %	5.0	85.0	80.0	70.0	89.5	4.4	0.46

<sup>1</sup>NC = negative control; PC = positive control; D = distillers dried grains with solubles; SH = soybean hulls; and PA = polyclonal antibody provided in feed. Polyclonal antibody (Camas Inc., Le Center, MN) was produced with antigen specific to *L. intracellularis* and sprayed onto soybean hulls.

<sup>2</sup>Comparison of PC, D, SH, and PA treatment groups.

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

<sup>4</sup>Visual 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.

### PCR and IHC Analysis

Laboratory results are summarized for fecal PCR and ileal tissue IHC in Table 6. All pigs tested negative for *Lawsonia intracellularis* by PCR and IHC before challenge. Additionally, all NC pigs remained negative throughout the entire postchallenge period. Challenging pigs with *L. intracellularis* resulted in a 98.7% detection rate of the organism in ileal tissue, indicating that nearly all pigs were successfully infected with ileitis. Diet did not affect fecal shedding, as determined by fecal PCR, or presence of *L. intracellularis* in the ileum, as determined by IHC. Lesion severity tended to be somewhat greater in the PA group compared with the SH group, however ( $P < 0.10$ ).

Dietary inclusion of DDGS, soybean hulls, or a polyclonal antibody product appeared to provide very little protective effect in the gut to improve the ability of pigs to resist an ileitis infection, as determined by clinical lesion scoring, fecal shedding, or presence of *L. intracellularis* in the ileum. Actual dosage level of *L. intracellularis* provided to challenged pigs was much greater than the goal ( $6.4 \times 10^8$ ), and inoculation resulted in more severe lesions than would be anticipated in normal pro-

duction conditions. Field observations have suggested that including DDGS or soybean hulls as a dietary insoluble fiber source provides a protective effect on susceptibility or severity of the growing pig to ileitis (J. Goihl, Agri-Nutrition Services, Shakopee, MN, personal communication). Including fibrous ingredients in the diet has been shown to provide beneficial effects on enteric health, including resistance to *E. coli* infection (Smith and Halls, 1968; Bertschinger et al., 1978). Providing insoluble fiber in the diet may improve the pig's ability to resist bacterial enteric infection by increasing mucosal secretion (Smith and Halls, 1968; Zebrowska et al., 1983), reducing available substrate for pathogens (Drochner et al., 1978), reducing digesta transit time (Kass et al., 1980; Low, 1985), or increasing intestinal mucosal cell turnover rate through mechanical erosion of the mucosal surface (Jin et al., 1994; Varel and Yen, 1997). Immune protection against colonization with pathogenic *E. coli* has also been achieved by feeding pigs egg-based products produced by vaccinated hens (Erhard et al., 1996). Previous research examining dietary effects on colonization by other enteric pathogens, including *L. intracellularis*, is limited.

**Table 6.** Effect of dietary distillers dried grains with solubles, soybean hulls, or a polyclonal antibody product and 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 <sup>1</sup>					Challenged treatment <sup>2</sup>	
	NC <sup>3</sup>	PC	D	SH	PA	SEM	P
No. of pigs	20	20	20	20	19	—	—
Fecal PCR, %							
D 0	0.0	0.0	0.0	0.0	0.0	—	—
D 14	0.0	90.0	78.9	75.0	84.2	4.4	0.65
D 21	0.0	77.8	85.0	90.0	94.7	3.9	0.47
IHC							
Score <sup>4</sup> (0 to 4)	0.00	2.58	2.47	2.37	2.95	0.12	0.34
Prevalence, %	0.0	100.0	100.0	95.0	100.0	1.3	0.42

<sup>1</sup>NC = negative control; PC = positive control; D = distillers dried grains with solubles; SH = soybean hulls; and PA = polyclonal antibody provided in feed. Polyclonal antibody (Camas Inc., Le Center, MN) was produced with antigen specific to *L. intracellularis* and sprayed onto soy hulls.

<sup>2</sup>Comparison of PC, D, SH, and PA treatment groups.

<sup>3</sup>Significant difference between NC and PC groups for all PCR and IHC measures ( $P < 0.01$ ).

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

### Comparison of 3 Disease Challenge Studies

Pig responses to ileitis challenge in this study and the 2 previous studies presented (Whitney et al., 2006a; Whitney et al., 2006b) differed considerably, regardless of dietary treatment imposed. Positive responses to dietary manipulation were observed in Study 2 (Whitney et al., 2006b; DDGS or antimicrobial regimen) but not Study 1 (Whitney et al., 2006a; DDGS inclusion) or the present trial (Study 3; DDGS, soybean hulls, or polyclonal antibody inclusion).

Ability of the pig to resist disease challenge via dietary manipulation appeared to be directly related to severity of the challenge, as reflected by growth performance. When evaluating pigs that were challenged and fed the control diet (PC) in all 3 trials, pigs from Study 2 grew 116 and 139% faster and consumed 16 and 16.5% more feed compared with pigs from Studies 1 and 3, respectively, during the postchallenge period. Challenged pigs from all 3 studies appeared to be successfully infected by the mucosal homogenate challenge model, as indicated by a nearly 100% detection level of *L. intracellularis* in ileal cells from challenged pigs.

Part of the difference observed in disease severity may be explained by differences in dosage level. Because actual quantification of dosage level was not possible before inoculation, dosage levels differed considerably, and all were above the goal dosage level of  $1 \times 10^8$ . Dosage level was closest to the goal for Study 2 ( $6 \times 10^8$ ) and Study 3 ( $6.4 \times 10^8$ ) and was approximately 2× greater in Study 1 ( $1.5 \times 10^9$ ). Work from Guedes et al. (2003) indicates that increasing the dosage level from  $5.4 \times 10^8$  to  $5.4 \times 10^9$ , using a similar challenge model, increases mortality (0 vs. 6.3%,  $P < 0.05$ ) and numerically (but not statistically) reduces ADG 28% and ADFI 14%.

Although dosage discrepancies between trials may explain a portion of the differences observed in disease

severity between Study 1 and Study 2, it does not explain the increased severity observed in Study 3 compared with Study 2, when dosage levels for both trials were somewhat similar. Numerous other factors may contribute to development of ileitis. Transportation, overcrowding, extreme changes in weather conditions, and commingling of pigs from different sources have been identified as risk factors for clinical outbreaks of ileitis (McOrist, 1997; Bane et al., 1998; Dufresne, 1999). Schultz et al. (1997) suggested that other pathogenic organisms, such as *Clostridium*, bacteriodes, and *E. coli*, may exacerbate the severity of ileitis.

The inoculum used for these studies was screened and determined to be devoid of many common enteric pathogens, but effect of interaction with other organisms is unknown. Using a similar mucosal homogenate challenge model and dosage level, Winkelman et al. (2000a,b) observed mortality in untreated control groups ranging from 6.7 to 32%, considerably greater than mortality in most field outbreaks of ileitis. Actual dosage level provided appears to explain part but not all of the differences in mortality levels observed. Pure culture ileitis challenge models have been used (McOrist et al., 1993) and generally provide a more controlled and predictable infection but also reduce infection rate.

Results from this study suggest that minimal benefits of including DDGS or soybean hulls may be achieved for improving the pig's ability to resist an ileitis challenge. Use of a polyclonal antibody product provided no benefit. These results are consistent with a previous experiment, which also resulted in more severe lesions, and therefore may indicate that diet composition has a minimal positive effect on gut health during a severe *L. intracellularis* infection. The inoculation dosage rate used for this study was quite successful in infecting most pigs but appeared to be greater than what may be considered a typical level during an ileitis outbreak

under commercial conditions. Use of the mucosal homogenate model for challenging pigs does not provide the precision necessary to achieve a desired inoculation dosage as using a pure culture when studying dietary modification and enteric disease. Further research is necessary to refine the disease challenge model utilized for this study and previous studies and to allow further understanding of the mechanisms involved with ileitis and to determine the effectiveness of possible alternative nutritional interventions.

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