Effect of dietary inclusion of distiller's dried grains with solubles, soybean hulls, or a polyclonal antibody product on the ability of growing pigs to resist a *Lawsonia intracellularis* challenge[1]

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ABSTRACT: An experiment was conducted to determine if dietary inclusion of distiller's dried grains with solubles (DDGS), soybean hulls, or soybean hulls sprayed with an egg-based, polyclonal antibody product reduces the incidence and/or severity of infection in growing pigs after a *L. intracellularis* challenge. One hundred 17-d old weaned pigs were blocked by sex and weight and randomly allotted to one of five treatment groups: negative control (NC) - unchallenged, corn-soy diet; positive control (PC) - challenged, corn-soy diet; 20% DDGS diet (D) – challenged; 5% soybean hulls diet (SH) – challenged; and SH sprayed with

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polyclonal antibody product diet (PA) - challenged. Challenged pigs were orally inoculated with 8×10^8 *L. intracellularis* after a 4-wk pre-challenge feeding period. On d 21 post-challenge, pigs were euthanized, lesions of intestinal mucosa were evaluated, and ileal tissue samples were analyzed by immunohistochemistry to determine presence and proliferation of *L. intracellularis*. Challenging pigs with *L. intracellularis* reduced growth rate, feed intake, and feed conversion ($P < 0.02$), and increased the proportion of internal organ weights relative to body weight ($P < 0.01$). Dietary treatment did not affect growth performance pre- or post-challenge ($P > 0.10$). Heart, empty stomach, and liver weights were similar among dietary treatments ($P > 0.10$). Weight of the small intestine as a percentage of body weight was increased in SH pigs compared to PA pigs, while weight of the large intestine as a percentage of body weight was increased in D and SH pigs compared to PC pigs ($P < 0.05$). Lesion length, prevalence, and severity and fecal shedding of *L. intracellularis* were minimally affected by dietary treatment ($P > 0.10$), although lesion length and severity observed in the ileum tended to be greater in PA vs. D pigs ($P < 0.10$). Diet composition may affect length, severity and prevalence of lesions caused by L. intracellularis in growing pigs subjected to a moderate ileitis challenge, but beneficial results were not observed by feeding the dietary treatments used in this study.

Key Words: Pig, Ileitis, Distiller's dried grains with solubles, Soybean hulls, Polyclonal antibody

Introduction

Ileitis, also known as porcine proliferative enteropathy, is an enteric disease that results in decreased feed intake, reduced growth rate, and increased mortality in swine. The causative organism, *L. intracellularis,* has been estimated to be present in approximately 75% of all U.S. swine herds (Broonsvoort et al., 2001). Strategic therapeutic use of antibiotic regimens, including tylosin phosphate, lincomycin, tiamulin, and chlortetracycline with bacitracin methylene disalicylate has been effective in treating acute cases of *L. intracellularis* (McOrist and Gebhart, 1999). Sub-therapeutic levels of these antibiotics have improved pig performance, but often fail to prevent the disease (Gebhart et al., 1998; Schwartz et al., 1998; Winkelman, 1998). Therapeutic levels of feed-grade antibiotics are very expensive and can generally be used for a limited period of time. Also, 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.

Reports from informal field studies suggest that including distiller's dried grains with solubles (DDGS) or soybean hulls in grow-finish diets may reduce dependence on antibiotics to combat ileitis. DDGS contains approximately 10% crude fiber, and the fiber composition is primarily insoluble (42.2%) versus soluble (0.7%) in nature (Shurson et al., 2000). Soybean hulls have higher crude fiber (40%), insoluble fiber

(75.5%), and soluble dietary fiber (8.4%) compared to DDGS (Shurson et al., 2000). Feeding diets low in soluble non-starch polysaccharides has been shown to reduce the proliferation of pathogenic organisms in the gastrointestinal tract (Hampson et al., 1999). Dietary fiber generally increases the secretory function of the epithelium, and this alteration may assist in impairing bacterial adhesion to the intestinal wall (Smith and Halls, 1968). Fiber also may provide a "cleansing" effect in the gut by reducing the viscosity of digesta (Lawrence, 1972).

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. Polyclonal antibody production is accomplished by exposing laying hens to specific antigens, and antibodies to these antigens are produced in the yolk of subsequent eggs. Improvements in growth performance and reduced mortality have been reported 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., LeCenter, 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 x 1/4 Large White x 1/2 Duroc) were obtained and transported from a commercial farrowing unit to isolation barns located on the University of Minnesota (St. Paul) campus. The source herd had no history or recorded cases of proliferative enteropathy, and was serologically negative for *Lawsonia intracellularis*, porcine respiratory and reproductive syndrome (PRRS), and *Actinobacillus pleuropneumonia*. The source herd was also clinically negative for *Salmonella cholerasuis*, transmissible gastroenteritis (TGE), and pathogenic *Brachyspira* species. Pigs, approximately 17 d of age, were blocked by gender and weight, and blocks randomly allotted to one of five 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 the resultant product was sprayed 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^2 per room, 10 rooms total) and 2 rooms per treatment group.

Experimental Diets

All pigs were fed a similar commercial pelleted Phase I nursery diet for the first 4 d of the experiment to encourage feed intake prior to initiation of dietary treatments. After the initial 4 d acclimation period, pigs were fed their respective experimental diets for the remainder of the 53 d study. Representative samples of each diet were obtained and analyzed for dry matter, gross energy, crude protein, ash, ether extract, crude fiber, calcium, phosphorus, and individual amino acids. 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 were fed under commercial conditions (Goihl, 2001). 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. Experimental diets were formulated to contain equivalent energy (3390 kcal/kg ME), 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 ME (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 3350 kcal/kg on an as-fed basis. All other nutrients were provided to meet or exceed NRC (1998) recommendations. Dietary digestible and metabolizable energy values

were calculated based on proximate analysis values using the following formulas from Noblet and Perez (1993):

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

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

Disease Challenge

Four wks after experimental diets were initiated (d 32), pigs were manually restrained and provided either 40 ml of either saline (NC) or an inoculation of *L. intracellularis* (PC, D, SH, and PA treatments) via stomach tube. The inoculate was prepared as a mucosal homogenate collected from the small intestines of pigs previously infected with *Lawsonia intracellularis* and exhibiting lesions consistent with ileitis. Mucosal material was collected by scraping the lumen of the infected intestine and diluting it with a sucrose-phosphate-glutamate buffer to obtain a dosage of 1×10^8 *L. intracellularis* per pig. A representative sample of the harvested intestinal mucosa was submitted to the University of Minnesota Veterinary Diagnostic Lab and dosage level determined to be 6.4×10^8 *L. intracellularis* / pig. Intestinal material was also screened and determined to be negative for the presence of other pathogens, including spirochetes, viruses, parasite ova, B-hemolytic *E. coli* and *Salmonella* sp. Care was taken to avoid cross-contaminating pigs from different rooms after the disease challenge. Biosecurity procedures included 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 non-infected (NC) to infected groups.

Data Collection

Growth rate and feed intake data were collected for both the pre- and post-inoculation periods. Clinical observations for alertness, gauntness, and diarrhea were scored 3 times/wk following challenge. Alertness was scored on animal behavior characteristics, with 1 = normal, 2 = slightly depressed and/or listless, and 3 = severely depressed or recumbent. Gauntness scores were based on visual body condition, with 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 = semi-solid feces without blood, 3 = watery feces without blood, 4 = blood-tinged feces that was loose or formed, and 5 = profuse diarrhea with frank blood or dark tarry feces. Fecal samples were collected on d 14 and d 20 post-inoculation, and sent to the University of Minnesota Veterinary Diagnostic Laboratory for polymerase chain reaction (PCR) evaluation of *L. intracellularis* presence in order to determine if the organism was being shed by animals. Bacterial DNA were extracted from fecal samples using a Qiagen extraction kit (Qiagen, Valencia, CA) prior to PCR analysis using a Quanitect kit (Qiagen, Valencia, CA) as outlined by Jones et al. (1993).

On d 20 or d 21 post-challenge, all pigs were euthanized and necropsies were performed. Weights of the heart, empty stomach, liver, and empty small and large

intestine were determined and recorded. Representative samples of digesta from the small and large intestines were collected and pH was measured. Length of the small and large intestine was also measured and visual evaluation of the general condition of the intestine, length of observable lesions, and location of lesions were made. Lesions were scored for severity based on the following characteristics: 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, and corrugated intestinal mucosa (PIA), 3 = severe mesenteric and intestinal wall edema and hyperemia, and necrosis of the mucosal surface with formation of pseudo-diphtheric membrane (necrotic enteritis), and 4 = moderate to severe edema and hyperemia of the mesentery and intestinal wall, thick and corrugated mucosa, and blood clots in the intestinal lumen (PHE). A 10 cm tissue section of the distal ileum proximal to the ileal-cecal junction was collected 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 L. intracellularis positive antigen labeled) to 4 (100% of epithelial cells in the crypts with positive antigen labeling) (Guedes et al., 2002).

Statistical Analysis

Analysis of variance was conducted on all data utilizing the GLM procedures of SAS (1985). Growth performance data were analyzed by room using analysis of

variance (two replications per treatment). All other data were analyzed utilizing the individual pig as the experimental unit, providing 20 replications per treatment. Least squares means were used to compare the negative and positive control pigs, and thereby evaluate the effects of infected vs. non-infected 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). In addition, least squares means comparisons were conducted among challenged treatments to identify differences due to dietary treatments.

Results and Discussion

Diet Composition

Calculated metabolizable energy (ME) concentration based on proximate analysis of the diets tended to be lower in all diets compared to formulated levels (3133 vs. 3390 kcal/kg), but was similar among experimental diets (range = 3102 – 3151 kcal/kg ME; Table 1). Calcium level tended to be slightly higher in the corn-soybean meal diets (0.85%) compared to the formulated level (0.80%), while diets containing DDGS or soybean hulls were slightly below the formulation goal (0.71 and 0.69%, respectively). All analyzed calcium values, however, were within the permitted analytical range of 0.66% - 0.94% (AOAC, 1990). All diets contained total phosphorus levels below the formulation level of 0.70%, but only the D and SH

diets (0.55% and 0.59%, respectively) analyzed at less than the permitted analytical range of 0.61% - 0.79% (AOAC, 1990). Addition of DDGS to the diet increased the crude protein level, threonine, and tryptophan concentration of the diet. Total lysine concentration was slightly higher in the corn-soybean meal diets compared to other dietary treatments.

Growth Performance

One pig was removed from the experiment prior to completion due to health reasons unrelated to the ileitis challenge. Body weights, growth rate, feed intake, and feed conversion results are summarized in Table 2. Average initial pig weight was 5.6 kg. During the pre-challenge period, growth, feed intake and feed conversion 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 post-challenge 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 body weight gain ($P = 0.02$) compared to NC pigs. Similarly, NC pigs were 48% heavier at the time of necropsy. Dietary treatment did not affect post-challenge ADG, ADFI, G/F, or final bodyweight in challenged pigs ($P > 0.60$).

Alertness, Gauntness, and Fecal Scores

Pig behavior appeared normal throughout the trial for all pigs, regardless of treatment. Unchallenged pigs remained healthy throughout the post-challenge period, as indicated by a lack of gauntness and normal fecal scores (Table 3). Fecal consistency was similar among pigs prior to 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) post-challenge in challenged pigs (PC) compared to 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) post-challenge, compared to NC pigs. All pigs were in excellent body condition prior to challenge and during the first wk post-challenge.

Gauntness of pigs also increased during the post-challenge period (*P* < 0.05), but was similar among dietary treatments that were challenged (*P* ≥ 0.19). Fecal looseness also increased with increasing time post-challenge (*P* < 0.01). Dietary treatment did not affect fecal consistency prior to challenge (*P* = 0.17), nor did it affect fecal consistency during the post-challenge period (*P* ≥ 0.18). However, fecal consistency tended to be somewhat looser in D pigs compared to PC pigs during the first wk post-challenge (*P* < 0.10), and was looser in D pigs compared to SH pigs during the second wk post-challenge (*P* < 0.05). Including high-fiber ingredients has been shown to increase rate of passage through the gastro-intestinal 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 co-products in the diet, both of which are excellent sources of insoluble fiber (Grieshop et al., 2001). Therefore, including higher fiber ingredients in the diet would be expected to increase looseness of stools in pigs, as was observed when feeding diets containing DDGS in this study. During the final week of the study, PC pigs had looser stools than SH pigs (*P* < 0.05), with fecal scores of D and PA pigs being intermediate.

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 bodyweight (*P* < 0.001) at the time of necropsy. Diet did not affect heart, empty stomach, or liver weights relative to body weight in challenged pigs, however (*P* > 0.50). Weight of the small and large intestine, relative to body weight, was increased in challenged pigs (*P* > 0.005), while 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 in PA pigs compared to SH pigs (*P* < 0.05), while length of the small intestine tended to be reduced in PA and D pigs compared to PC pigs (*P* < 0.10). 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 body weight compared to challenged pigs fed the corn-soybean meal control diet (*P* < 0.05).

Challenging pigs had no effect on acidity of digesta collected from either the small (*P* = 0.45) or large (*P* = 0.66) intestine. Digesta dry matter tended to be reduced, however, in both the small and large intestine (*P* < 0.10) when pigs were challenged. Feeding soybean hulls increased the acidity of digesta collected from the small intestine (*P* < 0.05), and tended to decrease digesta pH in the large intestine (*P* < 0.13) compared to pigs fed either the control or DDGS diets. Dry matter concentration of digesta collected from challenged pigs did not differ between dietary treatments (*P* ≥ 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 body weight) 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 feed conversion, observed in the current study, would be expected due to 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 body weight) in young adult pigs fed a high-lucerne 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. DDGS 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 both insoluble (75.5%) and soluble (8.4%) fiber compared to 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, 1983; Dierick et al., 1989; Wenk, 2001).

Feeding a diet containing soybean hulls increased small intestine weights, while feeding soybean hull and DDGS diets increased large intestine weights, relative to overall bodyweight. These results support the observations of Kass et al. (1980), where increased small intestine, cecum, and colon weights (as a proportion of body weight) occurred when alfalfa meal (an excellent source of both 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 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 both 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, while slightly reducing 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 dry matter 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 – 15% of the diet on rate of passage, but noted an increase in feed transit time when dietary inclusion levels reached 22 – 30%. This would suggest that the levels utilized in the current study were perhaps too low to make any significant difference in digesta dry matter 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 midds, however, would be expected to contribute a substantial amount of dietary soluble fiber compared to 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 negative control (NC) group had a lesion that was suspect for ileitis, but IHC analysis indicated that it was negative. Overall, 81% percent of pigs that were challenged exhibited lesions consistent with ileitis, which was much greater than lesion prevalence (63% and 59%) observed in two previous ileitis challenge experiments (Whitney et al., 2004a; Whitney et al., 2004b). Challenging pigs with *L. intracellularis* resulted in significant increases in lesion length, severity, and prevalence in the jejunum, ileum, colon, and overall compared to unchallenged pigs ($P < 0.01$).

Lesion length, severity, and prevalence were unaffected by dietary treatment in the jejunum and ileum ($P > 0.10$). Pigs fed SH tended to have reduced lesion severity in the jejunum compared to pigs fed PA ($P < 0.10$). In the ileum, feeding SH or D tended to reduce lesion length and severity compared to feeding PA ($P < 0.10$), while lesion prevalence tended to be greater in the PA group compared to the SH group ($P < 0.10$). Lesion length tended to be reduced in the colon for the D, SH,

and PA groups compared to the PC group (*P* < 0.10). Lesion severity was unaffected by dietary treatment. Pigs fed SH or D, however, tended to have reduced lesion prevalence compared to PC pigs (*P* < 0.10). Overall, lesion parameters were minimally affected by dietary treatment, although feeding SH tended to reduce lesion length compared to the PC group and tended to reduce lesion severity compared to the PA group (*P* < 0.10).

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.

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 prior to challenge. Additionally, all NC pigs remained negative throughout the entire post-challenge period. Challenging pigs with *Lawsonia* resulted in a 98.7% detection rate of *L. intracellularis* 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 *Lawsonia intracellularis* in the ileum, as determined by IHC. Lesion severity tended to be somewhat greater in the PA group compared to the SH group, however ($P < 0.10$).

Dietary inclusion of DDGS, soybean hulls, or a polyclonal antibody product appeared to provide very little protective effects 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 *Lawsonia intracellularis* in the ileum. Actual dosage level of *Lawsonia intracellularis* provided to challenged pigs was much greater than the goal (6.4×10^8), and resulted in more severe lesions than would be anticipated in normal production conditions. Field observations have suggested that including either DDGS or soybean hulls as a dietary insoluble fiber source provides a protective effect on susceptibility or severity of the growing pig to ileitis (Goihl, 2001). 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 (Zebrowska, 1983; Smith and Halls, 1968), reducing available substrate for pathogens (Drochner et al., 1978), reducing digesta transit time (Kass et al., 1980; Low, 1985), or by 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 *Lawsonia intracellularis*, is limited.

Implications

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 higher 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 allow further understanding of the mechanisms involved with ileitis and to deterimine the effectiveness of possible alternative nutritional interventions.

Literature Cited

AOAC. 1990. Official Methods of Analysis, 15th Ed. Association of Official Analytical Chemists. Washington, D. C.

Bertschinger, H. U., U. Eggenberger, H. Jucker, and H. P. Pfirter. 1978. Evaluation of low nutrient, high fibre diets for the prevention of porcine *Escherichia coli* enterotoxaemia. Vet Microbiol., 3:281-290.

Bronsvoort, M., B. Norby, D. P. Bane, and I. A. Gardner. 2001. Management factors associated with seropositivity to Lawsonia intracellularis in U.S. swine herds. J. Swine Health Prod., 9(6):285-290.

Dersjant-Li, Y., M. W. A. Verstegen, H. Schulze, T. Zandstra, H. Boer, J. W. Schrama, and J. A. J. Verveth. 2001. Performance, digesta characteristics, nutrient flux, plasma composition, and organ weights in pigs as affected by dietary cation anion difference and nonstarch polysaccharide. J. Anim. Sci. 79:1840-1848.

Dierick, N. A., I. J. Vervaeke, D. I. Demeyer, and J. A. Decuypere. 1989. Approach to the energetic importance of fibre digestion in pigs. I. Importance of fermentation in the overall energy supply. Anim. Feed Sci. Technol. 23:141-167.

Drochner, W., A. S. Hazem, H. Meyer, and F. W. Rensmann. 1978. Praktische Versuche mit Modellstudien zur Pathogenese nutritive bedingter. Fortschr. Vetmed. 28:220-225.

Erhard, M. H., J. Bergmann, M. Renner, A. Hofmann, and K. Heinritzi. 1996.

Prophylaktische Wirkung von spezifischen Dotterantikorpern bei Escherichia coli K88 (F4)-bedingten Durchfallerkrankungen von Absatzferkeln. J. Vet. Med. A., 43:217-223.

Gebhart, C. J., N. Winkelman, and C. P. Cornell. 1998. Shedding of *Lawsonia intracellularis* in medicated and non-medicated pigs. Proc. Int. Pig Vet. Soc., 15th Cong., 2:61.

Goihl, J. 2001. Personal communication. Agri-Nutrition Services, Inc., Shakopee, MN.

Guedes, R. M., C. J. Gebhart, N. A. Winkelman, and R. Mackie-Nuss. 2002. Comparative study of an indirect immunofluorescent test and the immunoperoxidase monolayer assay for diagnosing porcine proliferative enteropathy. Vet. J. Diag. Inv., 14:420-423.

Hampson, D. J., D. W. Pethick and J. R. Pluske. 1999. Can diet be used as an alternative to antibiotics to help control enteric bacterial infections of pigs? Pages 210-219 in Manipulation of Pig Production VII. D. D. Cranwell, ed. APSA.

Jin, L., L. P. Reynolds, D. A. Redmer, J. S. Caton, and J. D. Crenshaw. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. J. Anim. Sci. 72:2270-2278.

Jones, G. F., G. E. Ward, M. P. Murtaugh, G. Lin, and C. J. Gebhart. 1993. Enhanced detection of intracellular organism of swine proliferative enteritis, *Ileal symbiont intracellularis,* in feces by polymerase chain reaction. J. Clin. Microbiol, 31:2611-2615.

Jorgensen, H., X. Q. Zhao, and B. Eggum. 1996. The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hing-gut and energy metabolism in pigs. Br. J. Nutr., 75:365.

Kass, M. L., P. J. Van Soest, W.l G. Pond, B. Lewis, and R. E. Mcdowell. 1980. Utilization of dietary fiber from alfalfa by growing swine. I. Apparent digestibility of diet components in specific segments of the gastrointestinal tract. J. Anim. Sci. 50:175-191.

Kichura, T.S. 1997. Commercial field trials evaluating pig performance with specialty egg protein. Proc. Am. Assoc. Swine Pract., 27:189-194.

Koong, L. J., C. L. Ferrell, and J. A. Nienaber. 1985. Assessment of interrelationships among levels of intake and production, organ size and fasting heat production in growing animals. J. Nutr. 115:1383-1390.

Lawrence, T.L.J. 1972. The effect of certain dietary factors on in vivo pH changes and pepsin activity in the stomach of the growing pig. Br. Vet. J. 128:402-411.

Low, A. G. 1985. Role of dietary fibre in pig diets. Pages 87-112 in Recent Advances in Animal Nutrition. W. Haresing and D. J. A. Cole, ed. Butterworths, London, UK.

Ma, Y., D. Li, S. Y. Qiao, C. H. Huang, and I. K. Han. 2002. The effects of fiber source on organ weight, digesta pH, specific activities of digestive enzymes and bacterial activity in the gastrointestinal tract of piglets. Asian-Aust. J. Anim. Sci., 15(10):1482-1488.

Marlett, J. A. 1992. Content and composition of dietary fiber in 117 frequently consumed foods. J. Am. Dietetic Assn. 92(2):175-186.

McOrist, S. and C. J. Gebhart. 1999. Porcine proliferative enteropathies. Pages 521-534 in Diseases of Swine. 8th Ed. B. E. Straw, S. D'Allaire, W. L. Mengeling and D. J. Taylor, ed. Iowa State Univ. Press, Ames.

McOrist, S., R. Boid, G. H. K. Lawson, and I. McConell. 1987. Monoclonal antibodies to intracellular *Campylobacter*-like organisms of the porcine proliferative enteropathies. Vet. Rec. 121:421-422.

National Research Council. 1998. Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, D. C.

Noblet, J. and J. M. Perez. 1993. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. J. Anim. Sci. 71:3389-3398.

Pond, W.G., H.G. Jung, and V.H. Varel. 1988. Effect of dietary fiber on young adult genetically lean, obese and contemporary pigs: Body weight, carcass measurements, organ weights and digesta content. J. Anim. Sci. 66:699-706.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, N.C.

Schwartz, K., D. Walter, J. Knittel, M. Roof, and M. Anderson. 1998. Pathogenesis of proliferative enteropathy infection and its control with antimicrobial feed medication. Proc. Int. Pig Vet. Soc., 15th Cong., 3:116.

Shipp, T. E., J. A. Godfredsen-Kisic, and T. S. Kichura. 1999. Hyperimmune spray dried eggs as a feed supplement for weanling pigs. Feed Mix. Elsevier International Business Information. Doetinchem, The Netherlands, 7(6):30-33.

Shurson, G.C., M.H. Whitney, M.J. Spiehs, S.K. Baidoo, and A. Renteria. 2000. The value of distiller's dried grains with solubles produced from newer ethanol plants in Minnesota and South Dakota. Pages 47-62 in Concepts in Pig Science. T.P. Lyons and D.J.A. Cole, ed. Nottingham University Press, Nottingham, U.K.

Smith, H.W. and S. Halls. 1968. The production of oedema disease and diarrhoea in weaned pigs by the oral administration of Escherichia coli: Factors that influence the course of the experimental disease. J. Med. Microbiol., 1:45-49.

Spiehs, M.J., G.C. Shurson, and M.H. Whitney. 1999. Energy, nitrogen, and phosphorus digestibility of growing and finishing swine diets containing distiller's dried grains with solubles. J. Anim. Sci. 77(Suppl. 1):188(Abstr.).

Spiehs, M. J., M. H. Whitney, and G.C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639-2645.

Stanogias, G. and G. R. Pearce. 1985. The digestion of fibre by pigs. 1. The effects of amount and type of fibre on apparent digestbility, nitrogen balance and rate of passage. Br. J. Nutr. 53(3):513-530.

Varel, V. H. and J. T. Yen. 1997. Microbial perspective on fiber utilization by swine. J. Anim. Sci. 75:2715-2722.

Wenk, C. 2001. The role of dietary fibre in the digestive physiology of the pig. An. Feed Sci. Tech. 90:21-33.

Whitney, M. H., M. J. Spiehs, G. C. Shurson, and S. K. Baidoo. 2000. Apparent ileal amino acid digestibilities of corn distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 78:185 (Suppl. 1).

Whitney, M. H., G. C. Shurson, R. C. Guedes, and N. L. Winkelman. 2004a. Effect of dietary inclusion of distiller's dried grains with solubles on the ability of growing pigs to resist a *Lawsonia intracellularis* challenge. To be submitted. J. Anim. Sci.

Whitney, M. H., G. C. Shurson, R. C. Guedes, and N. L. Winkelman. 2004b. Effect of including distiller's dried grains with solubles in the diet, with or without antimicrobial regimen, on the ability of growing pigs to resist a *Lawsonia intracellularis* challenge. To be submitted. J. Anim. Sci.

Winkelman, N. L. 1998. An evaluation of chlortetracycline feed additive for prevention or treatment of porcine proliferative enteropathy. Proc. Int. Pig Vet. Soc., 15th Cong., 3:112.

Zebrowska, T., A. G. Low, and H. Zebrowska. 1983. Studies on gastric digestion of protein and carbohydrate, gastric secretion and exocrine pancreatic in the growing pig. Br. J. Nutr. 49:401-410.

Table 1. Composition of the experimental diets (as-fed basis)[a]

	Dietary treatment [b]				
Item	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[c]	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[d]	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					
Crude protein, %	22.22	22.22	24.73	21.46	21.94
Lysine, %[e]	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, kcal/kg[f]	3151	3151	3102	3117	3146
Calcium, %	0.85	0.85	0.71	0.69	0.73
Phosphorus, %	0.65	0.65	0.55	0.59	0.64

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

[b] NC = negative control, PC = positive control, D = distiller's dried grains with solubles, SH = soybean hulls, and PA = polyclonal antibody provided in feed.

[c] Distiller's dried grains with solubles (Al-Corn Clean Fuel, Claremont, MN).

[d] Supplied per kg of premix: 1,466,667 IU vitamin A as retinyl acetate, 246,400 IU vitamin D_3, 6,138 IU vitamin E as dl-a-tocopherol acetate, 979 mg vitamin K as menadione dimethylpyrimidinol bisulfite, 1,467 mg riboflavin, 8,800 mg niacin, 5,867 mg pantothenic acid as d-calcium pantothenate, 6.6 mg vitamin B12, 141 mg iodine as EDDI, 99 mg selenium as sodium selenite, 59,840 mg zinc as zinc oxide, 59,840 mg iron as ferrous sulfate, 3,960 mg copper as copper sulfate, and 1,980 mg manganese as manganese oxide.

[e] Amino acids are expressed on a total basis.

[f] Calculated from equation by Noblet and Perez (1993):
DE (kcal/kg) = 4151 – (122 x % Ash) + (23 x % CP) + (38 x % EE) – (64 x % Crude fiber)
ME (kcal/kg) = DE x (1.003 – (0.0021 x % CP)).

Table 2. Effect of dietary inclusion of distiller's dried grains with solubles, soybean hulls, or a polyclonal antibody product and ileitis challenge on growth rate, feed intake, and feed conversion efficiency

	Treatment [a]					Challenged treatments	
	NC[b]	PC	D	SH	PA	SEM	Pr>F
Pre-treatment (d 0 - 4)							
# of pens	2	2	2	2	2	.	.
Initial wt, kg	5.61	5.65	5.61	5.59	5.60	0.02	0.86
Pre-challenge (d 4 - 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
Post-challenge (d 32 - 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	1357.7	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

[a] NC = negative control, PC = positive control, D = distiller's dried grains with solubles, SH = soybean hulls, and PA = polyclonal antibody provided in feed.

[b] Significant difference between NC and PC groups for post-challenge ADG, ADFI, G/F, and final wt ($P < 0.05$).

Table 3. Effect of dietary inclusion of distiller's dried grains with solubles, soybean hulls, or a polyclonal antibody product and ileitis challenge on visual gauntness and fecal scores, post-challenge

	Treatment[a]					Challenged treatments[b]	
	NC[c]	PC	D	SH	PA	SEM	Pr>F
# of pigs	20	20	20	20	19	.	.
Abdominal score (1-3)[d]							
Initial (d 32)	1.00	1.00	1.00	1.00	1.00	.	.
Week 1 post-challenge	1.00	1.00	1.00	1.00	1.00	.	.
Week 2 post-challenge	1.00	1.06	1.03	1.03	1.03	0.013	0.19
Week 3 post-challenge	1.00	1.09	1.03	1.06	1.03	0.021	0.31
Fecal score (1-5)[e]							
Initial (d 32)	1.03	1.06	1.03	1.00	1.03	0.03	0.17
Week 1 post-challenge	1.00	1.20^{g}	1.40^{f}	$1.30^{f,g}$	$1.32^{f,g}$	0.04	0.33
Week 2 post-challenge	1.06	$2.42^{h,I}$	2.65^{h}	2.28^{i}	$2.37^{h,I}$	0.06	0.18
Week 3 post-challenge	1.06	2.61^{h}	$2.53^{h,I}$	2.34^{i}	$2.48^{h,i}$	0.07	0.23

[a] NC = negative control, PC = positive control, D = distiller's dried grains with solubles, SH = soybean hulls, and PA = polyclonal antibody provided in feed.

[b] Significant time effect for gauntness ($P < 0.05$) and fecal scores ($P < 0.01$).

[c] Significant difference between NC and PC groups for fecal scores week 2 and 3 post-challenge ($P < 0.05$).

[d] Abdominal scores: 1 = normal, 2 = slightly to moderately gaunt, and 3 = severely gaunt.

[e] 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.

[f,g] Within challenged treatment groups, means with different superscripts differ ($P < 0.10$).

[h,i] Within challenged treatment groups, means with different superscripts differ ($P < 0.05$).

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

	Treatment [a]					Challenged treatments	
	NC[b]	PC	D	SH	PA	SEM	Pr>F
# of pigs	20	20	20	20	19	.	.
Internal organ weights, % of body weight							
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[e,f]	5.08[e,f]	5.36[e]	4.67[f]	0.103	0.14
Large intestine	1.57	1.93[f]	2.39[e]	2.39[e]	2.15[e,f]	0.059	0.01
Total intestine	5.11	6.96[f]	7.48[e,f]	7.74[e]	6.82[f]	0.141	0.07
Intestinal lengths, cm							
Small intestine	1552.0	1487.8[c]	1366.9[d]	1410.0[c,d]	1353.4[c,d]	27.8	0.28
Large intestine	397.4	318.2	336.9	334.6	325.7	7.0	0.74
Total intestine	1949.3	1806.0	1703.7	1744.6	1679.1	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[e]	6.25[e]	5.93[f]	6.18[e,f]	0.06	0.11
Large intestine	5.67	5.73[c,d]	5.74[c]	5.52[d]	5.65[c,d]	0.05	0.33

[a] NC = negative control, PC = positive control, D = distiller's dried grains with solubles, SH = soybean hulls, and PA = polyclonal antibody provided in feed.

[b] Significant difference between NC and PC groups for all internal organ weights, large intestine length, and large intestine digesta dry matter ($P < 0.05$).

[c,d] Within challenged treatment groups, means with different superscripts differ ($P < 0.10$).

[e,f] Within challenged treatment groups, means with different superscripts differ ($P < 0.05$).

Table 5. Effect of dietary inclusion of distiller's dried grains with solubles, soybean hulls, or a polyclonal antibody product and ileitis challenge on lesion length, severity, and prevalence.

	Treatment [a]					Challenged treatments	
	NC[b]	PC	D	SH	PA	SEM	Pr>F
# 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 (0-4)	0.00	1.20[c,d]	1.41[c,d]	1.09[d]	1.80[c]	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[c,d]	13.5[d]	13.2[d]	19.6[c]	1.2	0.21
Score (0-4)	0.05	1.85[c,d]	1.65[d]	1.50[d]	2.32[c]	0.14	0.21
Prevalence, %	5.0	85.0[c,d]	75.0[c,d]	65.0[d]	89.5[c]	4.7	0.25
Cecum							
Length, cm	0.0	1.3	0.0	0.0	0.5	0.3	0.55
Score (0-4)	0.00	0.15	0.00	0.00	0.05	0.04	0.58
Prevalence, %	0.0	10.0[c]	0.0[d]	0.0[d]	5.2[c,d]	2.2	0.29
Colon							
Length, cm	0.0	2.7[c]	0.8[d]	0.7[d]	1.0[d]	0.4	0.17
Score (0-4)	0.00	0.40	0.20	0.15	0.16	0.06	0.45
Prevalence, %	0.0	30.0[c]	10.0[d]	10.0[d]	15.8[c,d]	4.2	0.28
Total							
Length, cm	0.7	98.1[c]	68.6[c,d]	40.7[d]	89.1[c,d]	12.5	0.37
Score (0-4)	0.01	0.90[c,d]	0.84[c,d]	0.68[d]	1.08[c]	0.08	0.33
Prevalence, %	5.0	85.0	80.0	70.0	89.5	4.4	0.46

[a] NC = negative control, PC = positive control, D = distiller's dried grains with solubles, SH = soybean hulls, and PA = polyclonal antibody provided in feed.

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

[c,d] Within challenged treatment groups, means with different superscripts differ ($P < 0.10$).

Table 6. Effect of dietary distiller's dried grains with solubles, soybean hulls, or a polyclonal antibody product and ileitis challenge on fecal PCR and ileal tissue IHC scores

	Treatment [a]					Challenged treatments	
	NC[b]	PC	D	SH	PA	SEM	Pr>F
# of pigs	20	20	20	20	19	.	.
Fecal PCR, %							
Day 0	0.0	0.0	0.0	0.0	0.0	.	.
Day 14	0.0	90.0	78.9	75.0	84.2	4.4	0.65
Day 21	0.0	77.8	85.0	90.0	94.7	3.9	0.47
IHC							
Score (0-4)	0.00	$2.58^{c,d}$	$2.47^{c,d}$	2.37^{d}	2.95^{c}	0.12	0.34
Prevalence, %	0.0	100.0	100.0	95.0	100.0	1.3	0.42

[a] NC = negative control, PC = positive control, D = distiller's dried grains with solubles, SH = soybean hulls, and PA = polyclonal antibody provided in feed.

[b] Significant difference between NC and PC groups for all PCR and IHC measures ($P < 0.01$).

[c,d] Within challenged treatment groups, means with different superscripts differ ($P < 0.10$).