Effects of two direct-fed microbials on the ability of pigs to resist an infection with *Salmonella enterica* serovar Typhimurium

Mindy J. Spiehs, MS, PhD; Gerald C. Shurson, MS, PhD; Lee J. Johnston, MS, PhD

Summary

Objective: To evaluate the effects of direct-fed microbials (DFMs) containing either *Bacillus licheniformis* and *Bacillus subtilis* or *Enterococcus faecium* on the ability of finisher pigs to resist a *Salmonella* serovar Typhimurium infection.

Materials and methods: Forty finishing pigs were used in a 12-day disease challenge study. Dietary treatments included no DFM (Control; 20 pigs), DFM provided in drinking water (*E faecium*), or DFM provided in feed (*Bacillus*). Ten Control pigs were not challenged with *Salmonella* Typhimurium; the remaining 30 pigs were inoculated on Day 0. Fecal samples were cultured for *Salmonella* Typhimurium on Days 0 to 5, 9, and 12. Serum haptoglobin (Hp), α_1-acid glycoprotein (AGP), IgG, and IgM concentrations were measured. Pigs were euthanized on Day 12, and tissue and digesta were tested for presence and numbers of salmonellae.

Results: Pigs challenged with *Salmonella* Typhimurium had looser stools ($P < .05$), greater fecal shedding of salmonellae on Days 2, 3, 4, and 5 ($P < .05$), and higher serum Hp concentrations on Day 7 ($P < .05$) than nonchallenged pigs. Serum AGP, IgG, and IgM concentrations were similar between challenged and nonchallenged pigs on all days. Fecal and tissue concentrations of *Salmonella* Typhimurium and serum Hp, AGP, IgG, and IgM concentrations were similar among challenged groups fed the three dietary treatments.

Implications: Under the conditions of this study, DFMs are not effective in reducing prevalence of *Salmonella* Typhimurium in feces, gastrointestinal contents, or tissues, or decreasing the number of salmonellae shed.

Keywords: swine, *Salmonella enterica* serovar Typhimurium, direct-fed microbials, food safety, acute phase proteins

Received: February 13, 2007
Accepted: September 14, 2007

Resumen – Efectos de dos microbianos suministrados directamente en la habilidad de los cerdos para resistir una infección con *Salmonella entérica* serovar Typhimurium

Objetivo: Evaluar los efectos de los microbianos alimentos directamente (DFMs por sus siglas en inglés) conteniendo o *Bacillus licheniformis* y *Bacillus subtilis* o *Enterococcus faecium* en la habilidad de cerdos de finalización para resistir una infección de *Salmonella* serovar Typhimurium.

Materiales y métodos: Se utilizaron cuarenta cerdos de finalización en un estudio de enfermedad de 12 días. Los tratamientos de dieta incluyeron no DFM (Control; 20 cerdos), DFM provisto en el agua de bebida (E faecium), o DFM provisto en alimento (Bacillus). Diez cerdos Control no fueron retados con *Salmonella* Typhimurium; los 30 cerdos restantes fueron inoculados en el Día 0. Se cultivaron muestras fecales en busca de *Salmonella* Typhimurium los Días 0 a 5, 9, y 12. Se midieron las concentraciones de haptoglobina sérica (Hp por sus siglas en inglés), glicoproteína ácida α_1 (AGP por sus siglas en inglés), IgG, e IgM. Los cerdos se sacrificaron el Día 12 y se analizaron tejido y digesta en busca de la presencia y números de salmonellae.

Resultados: Los cerdos retados con *Salmonella* Typhimurium presentaron el excremento más flojo ($P < .05$), mayor excreción fecal de salmonellae en los Días 2, 3, 4, y 5 ($P < .05$), y mayores concentraciones de Hp sérica en el Día 7 ($P < .05$) que los cerdos no retados. Las concentraciones de AGP, IgG, y IgM séricas fueron similares entre los cerdos retados y los no retados en todos los días de prueba. Las concentraciones fecales y de tejido de *Salmonella* Typhimurium y de Hp, AGP, IgG, e IgM en suero fueron similares entre los grupos retados que recibieron los diferentes tratamientos.

Implicaciones: Bajo las condiciones de este estudio, los DFMs no son efectivos en la reducción de la prevalencia de *Salmonella* Typhimurium en heces, contenido gastrointestinal o tejidos ni en la disminución del número de salmonellae excretada.

Résumé – Effets de l'administration directe de deux préparations bactériennes sur la capacité des porcs à résister à une infection par *Salmonella enterica* serovar Typhimurium

Objectif: Évaluer les effets de l'administration directe de préparations bactériennes (DFM) contenant soit *Bacillus licheniformis* et *Bacillus subtilis* ou *Enterococcus faecium* sur la capacité de porcs en période de finition à résister à une infection par *Salmonella* serovar Typhimurium.

MJS, GCS: University of Minnesota Department of Animal Science, St Paul, Minnesota.

LJJ: University of Minnesota West Central Research and Outreach Center, Morris, Minnesota.

Corresponding author: Dr Mindy J. Spiehs, USDA-ARS-NPA US, Meat Animal Research Center, PO Box 166, Clay Center, NE 68933; Tel: 402-762-4271; Fax: 402-762-4273; E-mail: mindy.spiehs@ars.usda.gov.

This article is available online at http://www.aasv.org/shap.html.

Spiehs MJ, Shurson GC, Johnston LJ. Effects of two direct-fed microbials on the ability of pigs to resist an infection with *Salmonella enterica* serovar Typhimurium. *J Swine Health Prod.* 2008;16(1):27–36.

Matériel et méthodes: Quarante porcs ont été utilisés pour une infection expérimentale d'une durée de 12 jours. Les traitements alimentaires incluaient aucun DFM (témoin; 20 porcs), DFM dans l'eau de boisson (E faecium), ou DFM dans la nourriture (Bacillus). Dix animaux témoins n'ont pas été inoculés avec *Salmonella* Typhimurium; les 30 autres animaux ont été inoculés au Jour 0. Des échantillons de fèces ont été cultivés aux Jours 0, 5, 9, et 12 pour vérifier la présence de *Salmonella* Typhimurium. Les concentrations sériques d'haptoglobine (Hp), de glycoprotéine α_1-acide (AGP), d'IgG, et d'IgM ont été mesurées. Les porcs ont été euthanasiés au Jour 12 et des tissus et du contenu intestinal analysés pour détecter la présence et dénombrer les salmonelles.

Résultats: Comparativement aux porcs non-inoculés, les porcs inoculés avec *Salmonella* Typhimurium avaient des fèces plus molles ($P < .05$), une excrétion fécale de salmonelles plus importante aux Jours 2, 3, 4, et 5 ($P < .05$), et des concentrations sériques de Hp plus élevées au Jour 7 ($P < .05$). Les concentrations sériques d'AGP, d'IgG, et d'IgM étaient similaires pour les animaux inoculés et non-inoculés et ce pour tous les jours de prélèvement. Parmi les groupes inoculés, les concentrations fécales et tissulaires de *Salmonella* Typhimurium et les concentrations sériques de Hp, d'AGP, d'IgG, et d'IgM étaient similaires pour les animaux des trois traitements alimentaires.

Implications: Dans les conditions expérimentales de la présente étude, les DFM étaient inefficaces à réduire la prévalence de *Salmonella* Typhimurium dans les fèces, le contenu intestinal, ou les tissus, ou à réduire la quantité de salmonellae excrétée.

Salmonella is one of the leading causes of foodborne illness in the United States,[1] and contaminated pork is a significant source of human salmonellosis.[2] Pigs exposed to *Salmonella* organisms early in life may not appear sick at market age, but can shed salmonellae when stressed and infect other market-aged pigs during transport or lairage.[3] Infected pigs can harbor *Salmonella* organisms in the gastrointestinal tract and lymph nodes.[4] Bacteria from tissues and organs of infected pigs have been shown to contaminate the carcass.[5,6] Therefore, any significant reduction in prevalence of *Salmonella*-positive pigs entering the slaughter facility may lessen the risk of carcass contamination.

Production procedures aimed at reducing foodborne pathogens at the farm level may reduce contamination in pork slaughter facilities and throughout the food chain. Dietary manipulation is one pre-harvest management technique that has potential to reduce salmonellae contamination of pork. Altering the diet changes the microbial population in the gastrointestinal tract of the pig[7,8] and has been shown to decrease the numbers of salmonellae in the gastrointestinal tract[9,10] and feces[7,9] of pigs.

Direct-fed microbials (DFMs) are live, naturally occurring microorganisms, fed as supplements, which improve the balance between beneficial and pathogenic microorganisms in the host's intestinal bacterial ecosystem.[11,12] Past research on DFMs in swine nutrition focused on growth performance in early-weaned pigs and use of DFMs as alternatives to growth-promoting antimicrobials.[13] More recently, DFMs have been evaluated for their potential to improve pig health and food safety through competitive exclusion, immunomodulation, or production of antimicrobials which prevent the fecal shedding and horizontal transmission of *Salmonella* serovar Typhimurium[9,14,15] and *Salmonella* serovar Choleraesuis.[7]

Multiple *Bacillus* and *Enterococcus* strains may be effective in reducing shedding of salmonellae in swine. Diarrhea was significantly reduced in piglets supplemented with *Bacillus cereus,*[16-18] *Bacillus licheniformis*,[17] or *Enterococcus faecium*.[19] Preventative treatment with *E faecium* has been shown to prevent *Salmonella* serovar Pullorum infection in experimentally infected poultry.[20] Therefore, a study was conducted to evaluate the effect of DFMs containing either *B licheniformis* and *B subtilis* or *E faecium* strains SF-273 and SF-301 on the ability of finisher pigs to resist an infection with *Salmonella* Typhimurium, as measured by culture of *Salmonella* Typhimurium in feces of exposed pigs.

Materials and methods

Experimental design

The experimental protocol used in this study was approved by the University of Minnesota Institutional Animal Care and Use Committee. The study included a 14-day pre-trial period to allow pigs sufficient time to acclimate to diets, and a 12-day disease-challenge study. A total of 40 crossbred, mixed-gender, late-finishing pigs (initial bodyweight = 110 ± 0.5 kg) from the University of Minnesota's West Central Research and Outreach Center (WCROC) in Morris, Minnesota, were used in this study.

At the beginning of the 14-day pre-trial period, pigs were weighed individually on an electronic scale accurate to 0.1 kg and allotted randomly to three dietary treatments designated as Control, *E faecium*, and *Bacillus* (Table 1).[21] Twenty pigs were fed the Control diet, 10 pigs were fed the *E faecium* diet, and 10 pigs were fed the *Bacillus* diet. Pigs remained in the wean-to-finish barn for the 14-day pre-trial period.

At the end of the pre-trial period, all 40 pigs were transported to the University of Minnesota Veterinary Medicine isolation facility on the St Paul Campus for the disease-challenge portion of the study. Twelve hours before transport, all feed was removed from the feeders to simulate the commercial practice of withholding feed from slaughter pigs before transport. Immediately after arrival at the isolation facility (Day 0), 30 pigs (10 Control, 10 *E faecium*, and 10 *Bacillus*) were inoculated intranasally with a 2-mL suspension (10^9 colony forming units [CFU] per mL) of a *Salmonella* Typhimurium strain obtained from the University of Minnesota Veterinary PathoBiology Department. The remaining 10 pigs received a similar inoculation with sterile tryptic soy broth and served as the negative control group (NC).

Room temperature in the isolation facility was recorded once daily between 8:00 AM and 10:00 AM. Blood samples were collected on Days 0, 7, and 12. Beginning on Day 0, pigs were scored daily for behavior and abdominal distension-gauntness. Fecal scores were determined on samples collected on Days 0, 1, 2, 3, 4, 5, 9, and 12. Daily rectal temperatures were recorded for each pig as a measure of the nonspecific immune response to a *Salmonella* Typhimurium challenge.

Table 1: Composition and nutrient content (as-fed) of experimental diets fed to finishing pigs, with direct-fed microbials included either in the prepared feed or in the drinking water

	Control	*Enterococcus faecium*	*Bacillus*
Ingredients			
Corn (%)	81.96	81.96	81.92
Soybean meal (47% crude protein) (%)	13.31	13.31	13.30
Soybean oil (%)	2.24	2.24	2.24
Dicalcium phosphate (%)	1.54	1.54	1.54
Limestone (%)	0.55	0.55	0.55
Salt (%)	0.30	0.30	0.30
Vitamin trace-mineral premix (%)*	0.10	0.10	0.10
BioPlus 2B (%)†	0.00	0.00	0.05
Probios FS†	0.00	0.00	0.00
Total (%)	100.00	100.00	100.00
Laboratory analysis			
ME (Mcal/kg)‡	3.79	3.79	3.36
Crude protein (%)	13.62	13.62	14.54
Total lysine (%)	0.75	0.75	0.60
Total threonine (%)	0.54	0.54	0.48
Total tryptophan (%)	0.18	0.18	0.16
Total methionine (%)	0.25	0.25	0.21
Total Ca (%)	0.80	0.80	0.87
Total P (%)	0.66	0.66	0.63
Ca:P ratio	1.21	1.21	1.38

* Premix contained vitamin A, 3,528,000 IU/kg; vitamin D_3, 661,500 IU/kg; vitamin E, 13,230 IU/kg; vitamin K, 1764 mg/kg; riboflavin, 2646 mg/kg; pantothenic acid, 8820 mg/kg; vitamin B_{12}, 17.64 mg/kg; iodine, 441 mg/kg; selenium, 119 mg/kg; zinc, 24,553 mg/kg; iron, 14,553 mg/kg; manganese, 4851 mg/kg; niacin, 15,435 mg/kg; and copper, 1455 mg/kg

† Bioplus 2B (Chris Hansen Inc, Milwaukee, Wisconsin) contains *Bacillus licheniformis* and *Bacillus subtilis* and provided a total microbial activity of 6.1×10^5 colony forming units (CFU) (SD, 1.4×10^5 CFU) *Bacillus* organisms per g of feed. Probios FS (Chris Hansen Inc) contains *Enterococcus faecium* SF-273 and SF-301. Probios FS was included in the drinking water to provide total microbial activity of 4.2×10^5 CFU/mL (SD, 1.0×10^5 CFU/mL).

‡ Metabolizable energy (ME) was calculated using the method of Noblet and Perez.[21]

Pigs were euthanized with a barbiturate on Days 12 and 13, and necropsies were performed in the University of Minnesota Veterinary Medicine Diagnostic Laboratory. At necropsy, tonsil, mandibular lymph nodes, liver, spleen, kidney, ileal-cecal lymph nodes, cecum, and a section of the distal ileum were collected aseptically. Additionally, samples of digesta from the ileum and cecum, and fecal samples from the rectum, were collected.

To monitor potential *Salmonella* contamination of pigs from the feed source, random feed samples were collected from each feeder at the beginning of the pre-trial period and on Days 0 and 12. Samples were pooled by treatment, and 1-g subsamples were cultured for *Salmonella* at the University of Minnesota St Paul Campus.

Housing

Pigs were housed 10 per pen at the WCROC. Treatment groups were maintained separately during transport from the WCROC to the isolation facilities on the St Paul Campus. Upon arrival, pigs were assigned randomly within treatment to 20 isolation pens (two pigs per pen) located in 10 similar, individually ventilated rooms. Each pen provided 1.86 m^2 of floor space. Pens were constructed of concrete block and polyvinyl chloride (PVC) planking, and contained solid floors with a center drain in each room. To prevent cross-contamination between pens, the center drain was blocked off, and the space between the floor and PVC planking was sealed completely with water-proof caulk.

Each pen contained one nipple waterer and a two-hole self-feeder. Nipple waterers for pigs given *E faecium* in the drinking water were attached to two 19-L PVC water bags (The Coleman Company, Wichita, Kansas), with a flow rate of 0.9 L per minute. Bags were filled with water to which *E faecium* was added at the designated concentration. Water bags were drained and refilled with freshly treated water each morning. Pigs had ad libitum access to

water and their respective diets at all times while in the isolation facility.

Dietary treatments

Dietary treatments included a corn-soybean meal diet that contained no DFM (Control); the same corn-soybean meal diet with a DFM given via drinking water (5×10^9 CFU *E faecium* per pig per day, assuming water consumption of 10 L per day[22]) (*E faecium*); and the same corn-soybean meal diet with a DFM included in the feed (1×10^6 CFU of *Bacillus* organisms per gram of feed) (*Bacillus*). Probios FS (Chris Hansen Inc, Milwaukee, Wisconsin) was used to provide *E faecium* SF-273 and SF-301 in the drinking water of pigs on the *E faecium* treatment at sufficient levels to achieve the manufacturer's recommended dose of 5×10^9 CFU *E faecium* per pig per day. Inclusion of 0.05% Bioplus 2B (Chris Hansen Inc) in the feed supplied *B licheniformis* and *B subtilis* to pigs on the *Bacillus* treatment. Bioplus 2B, which is approved for use in pelleted diets, was included as per manufacturer's recommendations to achieve 1×10^6 CFU *Bacillus* organisms per g of feed. All diets were mixed and pelleted at a commercial feed mill and fed in pelleted form.

A 20-mL sample of *E faecium*-treated water from the WCROC and a representative sample of the feed containing *Bacillus* were collected and submitted to Chris Hansen Inc to test for microbial activity prior to initiation of the study. A sample of *E faecium*-treated water from the isolation facility was also collected on Day 12 and submitted to Chris Hansen Inc to test for microbial activity. *Enterococcus faecium*-treated water samples from each facility were tested because the source of the water differed between the two facilities. The source of the *Bacillus*-treated feed was the same for both facilities.

Diets were formulated to contain equal metabolizable energy (ME), apparent digestible lysine, total calcium, and available phosphorus. All diets were formulated to meet or exceed the National Research Council[23] recommendations for ME, total calcium, available phosphorus, and apparent digestible lysine, methionine, threonine, and tryptophan for mixed-gender pigs weighing 100 kg with an expected carcass lean-tissue gain of 300 g per day.

Inoculum

The strain of *Salmonella* Typhimurium used in this study had been used in previous swine disease-challenge studies to successfully infect nursery pigs, using an oral dose of 2×10^8 CFU per mL.[3,23] This strain's resistance to nalidixic acid was used as a marker to distinguish the challenge strain from "wild" strains that may have been present in the environment.

To prepare the inoculation suspension, colonies of *Salmonella* Typhimurium were grown on XLT-4 agar with nalidixic acid. Ten mL of sterile tryptic soy broth was inoculated with 10 µL of *Salmonella* Typhimurium and allowed to incubate at 37°C for 24 hours. To determine the number of salmonellae present in the inoculation suspension, subsamples (10 µL) of the inoculation suspension were diluted to 10^{-5} using sterile peptone water and plated directly on XLT-4 agar containing nalidixic acid (50 µg per mL). Plates were incubated at 37°C for 24 hours, and black *Salmonella* colonies were counted to estimate the number of salmonellae in the inoculation suspension. The suspension was then diluted using sterile tryptic soy broth to achieve the desired concentration of 10^9 CFU *Salmonella* Typhimurium per mL.

Each pig was inoculated with 2 mL of tryptic soy broth suspension. On Day 0, 1 mL of suspension was sprayed into each nostril of each pig receiving the challenge (n = 30). Control pigs received a similar inoculation with sterile tryptic soy broth. Pigs were returned to their respective diets immediately after inoculation. The inoculation suspension was refrigerated overnight. The following day, the inoculation was repeated using only 1 mL tryptic soy broth suspension per pig.

Behavior, abdominal distension, and fecal scoring

Pig behavior and abdominal distension-gauntness was monitored using a subjective scale of 1 to 3. A score of 1 in both categories indicated a healthy, alert pig that appeared to be eating, while a score of 3 in both categories indicated a pig that was not able to stand or appeared severely gaunt. Fecal scores ranged from 1 to 5, with a score of 1 indicating a normal stool and no diarrhea and a score of 5 representing profuse bloody diarrhea. The same person, not blinded to treatment, assessed behavior, abdominal distension-gauntness, and fecal scores each day measurements were recorded.

Serum collection and analyses

Blood from the jugular vein was collected into glass tubes and allowed to clot overnight at a temperature of 4°C. Serum was harvested by centrifugation the following day and assayed for haptoglobin (Hp), α_1-acid glycoprotein (AGP), IgM, and IgG concentrations. Serum Hp and AGP were measured via a radial immunodiffusion assay (Cardiotech Services, Louisville, Kentucky). Haptoglobin samples were diluted 1:2 with sterile distilled water before plating, while AGP samples were plated without dilution. Serum IgG concentrations were assayed using a Pig IgG ELISA Quantitation Kit (Bethyl Laboratories, Montgomery, Texas). Serum was diluted 1:100,000 with an assay buffer, and conjugate antibodies used in the ELISA were diluted 1:100,000. Serum IgM concentrations were analyzed using a Pig IgM ELISA Quantitation Kit (Bethyl Laboratories). Serum was diluted 1:100,000 with an assay buffer, and conjugate antibodies used in the ELISA were diluted 1:30,000.

Processing of fecal, feed, tissue, and digesta samples

Fecal samples were collected directly from the rectum of each pig before the initial inoculation on Day 0 and plated on XLT-4 agar plates to ensure that no pig was shedding *Salmonella* organisms at the onset of the disease challenge. Feed and fecal samples were plated for qualitative and quantitative analysis using 1-g samples. For the qualitative analysis, an enrichment method was used, similar to the procedure used by Isaacson et al[24] and Anderson et al,[10] except that XLT-4 agar containing nalidixic acid replaced brilliant green agar. Quantitative analysis was accomplished through serial dilutions in sterile peptone water. Samples were diluted to 10^{-5} and plated directly on XLT-4 agar containing nalidixic acid (50 µg per mL). Plates were incubated at 37°C for 24 hours and black *Salmonella* colonies were counted to estimate the number of salmonellae shed by each pig. Salmonellae were detectable at a level of $\geq$ 100 CFU per mL using this method.

Tissue samples were collected aseptically at necropsy, placed in plastic bags containing peptone water, and stored at 4°C for 24 hours before being cultured for *Salmonella* Typhimurium using a method similar to that described by Hurd et al[25] on XLT-4 agar containing naladixic acid (50 µg per mL).

Digesta samples from the ileum and cecum and fecal samples from the colon were also stored at 4°C for 24 hours and cultured for *Salmonella* Typhimurium. Quantitative and qualitative analysis were performed as described for feed and fecal samples, using approximately 1 mL for liquid samples or 1 g for solid samples.

Statistical analysis

All data were collected and analyzed for individual pigs. Results from each pig were then pooled by pen for statistical analysis, with pen the experimental unit. Means of the nonchallenged pens were compared to means of challenged pens (Control, *E faecium*, and *Bacillus*) using a Dunnett's contrast to determine the effect of disease challenge. Within challenged pigs, Hp, AGP, IgM, and IgG concentrations, quantitative fecal data, rectal temperature, fecal scores, behavior scores, and abdominal scores were analyzed using a completely randomized design with repeated measures in time using the general linear model procedure of SAS (SAS Institute Inc, Cary, North Carolina). Day post challenge was considered the time factor in the repeated measures analysis. Means separation at each time period was achieved using Fisher's least significant difference of all possible pairwise comparisons. Qualitative analysis of fecal salmonellae shedding and presence of *Salmonella* Typhimurium in tissue was analyzed using logistic regression (Statistical Program R; Free Software Foundation, Boston, Massachusetts).

A split-plot design was used to analyze the number of salmonellae in tissues and gastrointestinal-tract contents of challenged pigs. Diet served as the whole-plot effect and tissue was the split-plot effect. Whole-plot error was the diet-by-pen interaction. Tissue-by-diet interactions were also evaluated. All means are reported as least squares means. Numbers of salmonellae in feces, tissue, and gastrointestinal-tract contents were analyzed using logarithmic values in order that ANOVA assumptions would not be violated. Least squares means were transformed and reported as actual values; however, pooled standard errors are reported for the logarithmic values.

Results

Effect of disease challenge on clinical signs and mortality

Pigs inoculated with *Salmonella* Typhimurium developed acute signs of salmonellosis, as measured by higher fecal scores (Table 2) and increased levels of fecal shedding (Table 3) on Days 2, 3, 4, and 5, indicating a successful challenge. As the disease progressed, diarrhea subsided and fecal scores were once again similar between challenged and nonchallenged pigs on Days 9 and 12. Likewise, fecal shedding of *Salmonella* Typhimurium also decreased by Day 9.

Seven challenged pigs died suddenly during this study (one *Bacillus*, two *E faecium*, and four Control). No nonchallenged pigs died. Four pigs were found dead on the morning of Day 3 and an additional three pigs died on Day 4. All dead pigs were submitted to the University of Minnesota Veterinary Diagnostic Laboratory for postmortem examination. Because the pigs died during a weekend when diagnosticians were not available, postmortem condition of the first four pigs that died was unsuitable for complete examination. Samples were collected from rectum and colon content of all seven pigs. *Salmonella* Typhimurium was isolated from rectal and colon samples from all pigs at high concentrations (7.9×10^4 to 1.0×10^7 CFU per mL). Additionally, *Salmonella* Typhimurium was isolated from the lung, intestine, and colon of each of the three pigs that underwent complete postmortem examination. The histopathological

Table 2: Effect of *Salmonella* Typhimurium challenge and dietary treatment on fecal score from Day 0 to 12*

Study day	Treatment†				*P*‡	
	NC	Control	*E faecium*	*Bacillus*	NC vs challenged§	Control vs *E faecium* vs *Bacillus*¶
0	1.0	1.0	1.0	1.0	.70	.83
1	1.0	1.3	1.2	1.2	.40	.68
2	1.0	2.6[a]	2.2[ab]	1.6[b]	< .01	.03
3	1.0	2.7[a]	2.5[ab]	1.6[b]	< .01	.04
4	1.0	2.7[a]	1.9[b]	1.5[b]	< .01	.02
5	1.0	2.1	1.8	1.4	< .01	.14
9	1.0	1.2	1.0	1.0	.56	.28
12	1.0	1.1	1.0	1.0	.40	.50

* Late-finisher pigs were inoculated intranasally with *Salmonella* Typhimurium on Day 0. Data are least squares means (n = 5 pens/treatment, 2 pigs/pen). Fecal scores: 1 = firm stool, 2 = semi-solid feces with no blood in stool, 3 = watery diarrhea with no blood in stool, 4 = blood-tinged stool (either loose or formed), 5 = profuse bloody diarrhea.

† NC (negative control): fed control diet, not challenged with *Salmonella* serovar Typhimurium; Control: fed control diet, challenged with *Salmonella* Typhimurium; *E faecium*: fed Control diet, drinking water contained *E faecium* (approximately 5×10^9 CFU/pig/day), challenged with *Salmonella* Typhimurium; *Bacillus*: fed Control diet containing *Bacillus* organisms (1×10^6 CFU/g/feed), challenged with *Salmonella* Typhimurium. Dietary treatments were applied from Day -14 to Day 12.

‡ Determined by analysis of variance; $P < .05$ considered significant.

§ Pre-planned comparison of NC versus challenged pigs (ie, data pooled from the three challenged groups); pooled SEM = 0.47

¶ Pre-planned comparison of Control vs *E faecium* vs *Bacillus*; treatment ($P = .02$); time ($P < .001$); time × treatment ($P = .01$); pooled SEM = 0.03

[ab] Values with no common superscript within a row differ among challenged pigs ($P < .05$, analysis of variance).

Table 3: Effect of *Salmonella* Typhimurium challenge and dietary treatment on fecal shedding (CFU/g of feces/day) from Day 0 to 12*

Day	Treatment†				*P*‡	
	NC	Control	*E faecium*	*Bacillus*	NC vs challenged§	Control vs *E faecium* vs *Bacillus*¶
0	0.0	< 100	0	< 100	.47	.62
1	0.0	2.9×10^2	3.0×10^2	2.9×10^3	.08	.85
2	0.0	4.3×10^4	2.6×10^5	8.7×10^3	< .01	.50
3	0.0	1.9×10^5	8.7×10^4	5.2×10^2	< .01	.11
4	0.0	7.8×10^2	< 100	1.3×10^3	.02	.44
5	0.0	9.8×10^2	< 100	1.1×10^3	.03	.61
9	0.0	< 100	< 100	< 100	.13	.25
12	0.0	< 100	< 100	< 100	.26	.51

* Pigs were inoculated with *Salmonella* Typhimurium on Day 0. Data are least squares means (n = 5 pens/treatment, 2 pigs/pen); fecal shedding is the measure of the number of *Salmonella* serovar Typhimurium in a 1-g or 1-mL fecal sample collected directly from the rectum of the infected pigs. Laboratory techniques were accurate at > 100 colony forming units (CFU) /g of feces; therefore, samples testing positive for *Salmonella* organisms at < 100 organisms/g of feces are reported as < 100 CFU/g.

† Dietary treatments described in Table 2.

‡ Determined by analysis of variance using logarithmic values; $P < .05$ considered significant.

§ Pre-planned comparison of NC vs challenged pigs (ie, data pooled from the three challenged groups); pooled SEM = 0.04.

¶ Pre-planned comparison, control vs *E faecium* vs *Bacillus*; treatment ($P = .72$); time ($P < .001$); time × treatment ($P = .53$); pooled SEM = 0.05 CFU/g of feces/day.

diagnosis for all three pigs was septicemia caused by *Salmonella* Typhimurium.

Despite the high mortality rate of the challenged pigs, abdominal and behavior scores were similar between challenged and nonchallenged pigs (data not shown). Rectal temperatures were also similar between challenged and nonchallenged pigs except on Day 2, when the average temperatures of the challenged and nonchallenged pigs were 40°C and 39.2°C, respectively ($P < .05$; SEM = 0.01°C).

Serum immunoglobin concentrations and acute phase proteins

Serum IgM (pooled SEM = 0.73 g per L) and IgG (pooled SEM = 2.50 g per L) concentrations were similar between the challenged and nonchallenged pigs. Serum IgM concentrations of the nonchallenged pigs ranged from 8.69 to 11.33 g per L and serum IgM concentrations of the challenged pigs ranged from 9.43 to 12.29 g per L. Serum IgG concentrations were lowest on Day 7 (26.27 g per L) and highest on Day 12 (33.20 g per L) for the nonchallenged pigs. Average serum IgG concentrations for challenged pigs were lowest on Day 0 (29.99 g per L) and highest on Day 12 (41.72 g per L).

Serum Hp concentrations were higher ($P < .05$; pooled SEM = 0.168 g per L) for challenged pigs (2.64 g per L) than for nonchallenged pigs (1.74 g per L) on Day 7, but were similar on Day 0 (1.06 and 0.91 g per L, respectively, for challenged and nonchallenged pigs) and Day 12 (1.58 and 1.37 g per L, respectively, for challenged and nonchallenged pigs).

Serum AGP concentrations did not differ between the challenged and nonchallenged pigs ($P > .05$; pooled SEM = 20.0 µg per mL) on Day 0 (300 and 299 µg per mL, respectively), Day 7 (396 and 412 µg per mL, respectively), or Day 12 (330 and 420 µg per mL, respectively).

Serum IgM, IgG, Hp, and AGP concentrations of challenged and nonchallenged pigs increased between Day 0 and Day 12, resulting in a significant time effect ($P < .05$).

Effect of disease challenge on tissue and digesta cultures

No digesta or tissue samples from the nonchallenged pigs tested positive for *Salmonella* Typhimurium (data not shown). Overall prevalence of infection in challenged pigs was very high. Additionally, in 100% of challenged pigs, at least one tissue tested positive for *Salmonella* Typhimurium at necropsy. Among all treatments, tonsils averaged 7.6×10^4 CFU per g *Salmonella* Typhimurium, which was higher ($P < .01$) than the concentrations found in the cecum, ileal-cecal lymph nodes, ileum, kidney, liver, mandibular lymph, or spleen of challenged pigs (Table 4).

Microbial activity of *E faecium* and *Bacillus* treatments

The *E faecium*-treated water sample collected from the WCROC at the beginning of the pre-trial period contained both *E faecium* SF-273 and *E faecium* SF-301. Total microbial activity was $4.2 \pm 1.0 \times 10^5$ CFU per mL of drinking water. This was within the acceptable range of microbial activity according to the manufacturer's recommendations. However, the laboratory detected no significant microbial activity in the *E faecium*-treated water sample collected from the isolation facility on Day 12.

Total microbial activity in the *Bacillus*-treated feed sample was $6.1 \pm 1.4 \times 10^5$ CFU per g of feed, which adequately achieved the manufacturer's recommendations. Active cultures of *B licheniformis* and *B subtilis* were present in the feed sample. Microbial assays confirmed no cross-contamination between the dietary treatments. No detectable levels (ie, > 100 CFU per g) of *Salmonella* Typhimurium were found in feed samples from any dietary treatment.

Effects of direct-fed microbials on clinical signs and mortality of challenged pigs

Pigs fed the Control diet had higher fecal scores ($P < .05$) than pigs fed the *Bacillus* diet on Days 2 and 3, with pigs fed the *E faecium* diets being intermediate (Table 2). On Day 4, pigs fed diets containing either DFM had firmer stools than pigs fed the Control diet ($P < .05$). Fecal scores increased immediately after inoculation and returned to pre-inoculation levels by Day 12, resulting in a significant time effect ($P < .01$). Despite differences in fecal scores, dietary treatment had no effect on the numbers of salmonellae shed in feces of pigs inoculated with *Salmonella* Typhimurium (Table 3). The numbers of salmonellae shed in the feces increased following inoculation, and then declined by Day 12, resulting in a significant time effect ($P < .01$).

Average rectal temperatures of all challenged pigs were similar throughout the study, ranging from a low of 39.1°C on Day 1 to a high of 40°C on Day 2. There was no effect of dietary treatment on rectal temperature among the challenged pigs, except on Day 9, when pigs fed the Control diet had lower mean rectal temperature (38.9°C) than the pigs fed *E faecium* (39.7°C) ($P < .05$; pooled SEM = 0.02°C). Average temperature of pigs fed the *Bacillus* diet was 39.1°C on Day 9, similar to those of pigs fed the Control and *E faecium* diets. Throughout the course of the 12-day study, there was a significant time effect ($P < .01$), but no time-by-treatment interaction. Average rectal temperature peaked at Day 2 and returned to normal by Day 4.

Effect of DFM on acute phase protein and immunoglobin concentrations of challenged pigs

Serum concentrations of IgM (pooled SEM = 1.68 g per L) and IgG (pooled SEM = 5.53 g per L) were not affected by dietary treatment. Over the 12-day period, serum IgM concentrations were similar in Control pigs (7.94 to 11.35 g per L), in pigs on the *E faecium* treatment (9.14 to 13.15 g per L), and in pigs on the *Bacillus* treatment (10.14 to 12.29 g per L). During the same period, serum IgG concentrations were similar in Control pigs (28.30 to 45.25 g per L), in the *E faecium*-treated pigs (21.11 to 29.93 g per L), and in the *Bacillus*-treated pigs (30.50 to 42.65 g per L).

Table 4: Comparison of number of salmonellae in tissues at 12 days post challenge in pigs inoculated with *Salmonella* Typhimurium*

Tissue	*Salmonella* (CFU/g)
Tonsil	7.8×10^4 [a]
Ileum	4.9×10^3 [b]
Ileal-cecal LNs	1.8×10^3 [b]
Cecum	1.1×10^3 [b]
Mandibular LNs	3.7×10^2 [bc]
Kidney	1.4×10^2 [cd]
Liver	1.4×10^2 [cd]
Spleen	< 100 [d]

* Thirty late-finisher pigs challenged intranasally with *Salmonella* serovar Typhimurium, with treatments described in Table 2; treatment ($P = .13$); tissue ($P < .001$); treatment × tissue ($P = .97$); pooled SEM = 0.63 CFU/g.

[abcd] Values with no common superscript differ significantly ($P < .05$; analysis of variance performed on logarithmic values).

CFU = colony forming units; LN = lymph node.

Dietary treatment had no effect on serum Hp concentrations (pooled SEM = 0.30 g per L) or AGP concentrations (pooled SEM = 43.9 μg per L) in pigs challenged with *Salmonella* Typhimurium. Serum Hp concentrations were similar in pigs fed the Control diet (0.910 to 2.87 g per L), in pigs given the *E faecium* treatment (1.15 to 2.69 g per L), and in pigs fed the *Bacillus* treatment (1.11 to 2.43 g per L). Serum AGP concentrations were similar in pigs fed the Control diet (248 to 421 μg per mL), in pigs given the *E faecium* treatment (301 to 435 μg per mL), and in pigs fed the *Bacillus* diet (254 to 455 μg per mL).

Serum IgM, IgG, Hp, and AGP concentrations increased between Day 0 and Day 12 in all three challenged treatment groups. This resulted in a significant time effect ($P < .05$) for each blood parameter.

Effect of DFM on tissue and digesta cultures of challenged pigs

Overall concentration of *Salmonella* Typhimurium in digesta of pigs fed Control (111 CFU per mL), *E faecium* (182 CFU per mL), and *Bacillus* diets (161 CFU per mL) were similar ($P > .05$; SEM = 0.67 CFU per mL). Additionally, there were no differences in concentration of *Salmonella* Typhimurium among digesta collected from the ileum (< 100 CFU per mL), cecum (551 CFU per mL), or rectum (< 100 CFU per mL) of challenged pigs. Qualitative and quantitative analysis of tissue samples revealed no significant differences among finishing pigs fed the Control, *E faecium,* and *Bacillus* dietary treatments (data not shown).

Discussion

The challenge model used in this study resulted in a *Salmonella* Typhimurium infection. Higher fecal scores and increased fecal shedding on Days 2, 3, 4, and 5, a febrile response on Day 2, and increased serum Hp concentrations on Day 7 for pigs challenged with *Salmonella* Typhimurium compared to nonchallenged pigs are evidence of a successful challenge.

The *Salmonella* Typhimurium infections resulting from our challenge model were more severe than anticipated. Seven challenged pigs died. The most difficult aspect of conducting a disease challenge study is perfecting the challenge model. A previous *Salmonella* Typhimurium challenge by our research group used oral inoculations of a strain of *Salmonella* Typhimurium obtained from the Minnesota Department of Health. Challenges using 1×10^6 and 1×10^9 CFU per mL of this organism were unsuccessful, as measured by a lack of salmonellae shed in the feces or any signs of salmonellosis in challenged pigs, eg, elevated body temperature or loose stools.[26] Intranasal inoculation elicits more severe clinical disease than intragastric inoculation.[27] Previous research[28] demonstrated that low and moderate doses of *Salmonella* Choleraesuis (10^3 and 10^6 CFU per mL, respectively) were

not sufficient to induce an acute *Salmonella* Choleraesuis infection in 2- to 8-week-old pigs, but that 10^9 CFU per mL would achieve clinical salmonellosis Therefore, a dose of 10^9 CFU per mL was administered via the intranasal route in the current study. A strain of *Salmonella* Typhimurium that had been previously used for oral inoculations of nursery pigs[3,24] was obtained from the Minnesota Department of Health to replace the less virulent strain. The combination of a high challenge dose (10^9 CFU per mL), intranasal inoculation, and a more virulent strain of *Salmonella* Typhimurium likely resulted in the more severe disease observed in this study than in other studies using the same strain,[3,24] route of administration,[27] or challenge dose.[28]

We expected to see a prolonged febrile response (eg, 3 to 4 days) similar to that noted by other researchers[29-32] who inoculated 4- to 5-week-old pigs with intragastric doses of *Salmonella* Typhimurium ranging from 5×10^8 to 10^9 CFU. Instead, rectal temperatures were higher for the challenged pigs than for the nonchallenged pigs only on Day 2. However, the pigs in this study were inoculated intranasally. Therefore, acute salmonellosis developed rapidly in these pigs. Because body temperatures were recorded only once daily, and pigs with systemic salmonellosis died suddenly, differences in body temperature and in abdominal and behavior scores were not detected.

The immune system involves two primary types of immunity: innate and acquired. Innate immunity is a nonspecific response that occurs immediately after detection of an immune stimulus. It acts as the first line of defense against pathogens and includes neutrophils, macrophages, natural killer cells, complement, interferons, and acute phase proteins.[33] Acquired immunity is a highly specific, inducible humoral and cell-mediated response dependent upon lymphocytes and antibodies.[33] In an effort to conduct a comprehensive evaluation of the immune response to *Salmonella* Typhimurium in the current study, we measured blood parameters that would indicate an innate response (ie, Hp and AGP) and an acquired response (ie, IgM and IgG) of finishing pigs to a challenge with *Salmonella* Typhimurium.

Serum Hp appears to be the best indicator of immune response in pigs challenged with *Salmonella* Typhimurium. The increase in serum Hp concentration at 7 days post challenge is consistent with the results of Turner et al,[30,31] who also reported a significant increase in serum Hp concentration 7 days after a *Salmonella* Typhimurium challenge, and a return to postchallenge concentrations 14 days after challenge. Previous studies have demonstrated a positive correlation between immune challenge and serum AGP concentrations.[30,31,34,35] However, Neiwold et al[36] reported that AGP was only a moderate indicator of immune response in pigs, which may explain why we did not see a difference in serum AGP concentration between challenged and nonchallenged pigs.

Given the higher number of salmonellae shed in feces of challenged pigs than in nonchallenged pigs on Days 2 to 5 and a febrile response in the challenged pigs on Day 2, we expected to see higher serum IgG and IgM concentrations in pigs inoculated with *Salmonella* Typhimurium than in nonchallenged pigs. Others have demonstrated that serum IgM concentrations increase to a peak level 7 to 21 days following a disease challenge with *Salmonella* Choleraesuis,[27,28] *Toxoplasma gondii,*[37] or *Salmonella* Typhimurium[38] and then begin a steady decline. Therefore, we expected to see a peak in serum IgM concentrations by Day 12. Serum IgG concentrations peak 21 to 49 days after infection with *Salmonella* Choleraesuis[27,28] or *Salmonella* Typhimurium[38] and remain elevated for as long as 3 to 4 weeks after exposure to bacterial pathogens.[37] Serum IgG is the most abundant antibody in the body;[39] therefore, it was expected that sufficient concentrations of IgG would be present in challenged pigs to detect differences in serum IgG concentrations between challenged and nonchallenged pigs even before the peak levels were reached at 21 days post challenge. Similarly, Turner et al[31] also found no response of serum IgG and IgM concentrations to a *Salmonella* Typhimurium challenge in a 14-day study. They concluded that the enteric infection was most likely contained within the gut via phagocytic cells in the intestinal wall or through an immunoglobulin A-mediated response, resulting in no detectable increase in serum IgG and IgM concentrations. In the current study, it appeared that serum IgG and IgM levels were continuing to increase and may not have peaked by Day 12, which may explain why we were not able to detect differences in serum IgG and IgM concentrations between challenged and nonchallenged pigs.

No tissue or ileal, cecal, or rectal content of nonchallenged pigs tested positive for *Salmonella*, indicating that biosecurity measures were effective in preventing transfer of the disease from challenged to nonchallenged pigs.

Concentrations of *Salmonella* organisms in the cecal contents of pigs in this study were higher than the value (< 1 CFU per g) reported at 2 weeks post challenge following an intranasal inoculation with 10^9 CFU per mL *Salmonella* Choleraesuis,[27] but similar to the concentration in cecal contents (501 CFU per g) of pigs orally infected with 10^8 *Salmonella* Cholerasuis.[40] Concentration of *Salmonella* organisms in ileal and rectal contents were not reported in either of these studies.

The overall high number of salmonellae in collected tissues of challenged pigs is consistent with the results of others,[4] who found high prevalence of positive cultures from tonsils and from mandibular lymph nodes, which receive lymph drainage from the tonsils. Concentrations of 1.3×10^4 and 5.9×10^4 CFU per g *Salmonella* Choleraesuis in tonsil have been reported.[27,28] High concentrations of *Salmonella* organisms in cecum, ileum, and ileocecal lymph nodes were also reported.[3,41-43] Two weeks after an intranasal inoculation with 10^9 CFU per mL *Salmonella* Choleraesuis, Gray et al[27] reported tissue concentrations of 2.5×10^5 CFU per g in the ileal-cecal lymph nodes, which was slightly higher than in our study (1.8×10^3 CFU per g). Concentrations of *Salmonella* Choleraesuis in tonsil ranged from too low to quantify to 1.3×10^4 CFU per g with an intranasal inoculation, but quantitative data was not presented for tissue from the spleen, liver, ileum, or cecum.[27] Gitter and Kidd[42] conducted necropsies on 4.5- to 7-month-old specific-pathogen-free pigs that were accidentally exposed to *Salmonella* Typhimurium at 4 weeks of age. *Salmonella* were isolated most often from the tonsil (10 of 12 pigs) and cecal contents (eight of 12 pigs), with isolation less frequently from small intestinal and rectal contents.

Presence of *Salmonella* Typhimurium in tissues and the gastrointestinal tract of the challenged pigs has implications for contamination at slaughter. High concentrations of *Salmonella* Typhimurium in the mesenteric lymph nodes indicate that the organism had spread beyond the gastrointestinal tract, resulting in systemic infection and potential contamination of multiple tissues throughout the body. Mandibular lymph nodes are often incised and inspected for gross lesions by federal meat inspectors at slaughter plants. When lesions are apparent, the lymph nodes are trimmed

from the carcass. However, mandibular lymph nodes infected with *Salmonella* Typhimurium may have a normal appearance;[4] therefore, they may not be trimmed from the carcass and could contaminate surfaces throughout the processing line. Preferential persistence of *Salmonella* Typhimurium in the gastrointestinal tract of swine might result in contamination during evisceration. Surface contamination during evisceration is well documented.[5] Bacteria present in the gastrointestinal tract, mouth, and tonsils have been shown to contaminate the carcass.[5,6]

Dietary treatment had no effect on incidence and level of *Salmonella* Typhimurium in the challenged pigs, as indicated by similar numbers of salmonellae shed in the feces and similar rectal temperatures. The initial increase in fecal shedding, fecal scores, and rectal temperature, followed by a decline in these parameters at Day 12, are a typical response to an acute *Salmonella* Typhimurium infection, and the febrile response (eg, 3 to 4 days) is similar to that observed by others.[29,32]

Previous research evaluating the effects of DFMs on prevalence of *Salmonella* infection in pigs has produced variable results. Anderson et al[7] observed that fewer pigs challenged with *Salmonella* Choleraesuis and treated with cultures of microbes shed salmonellae in their feces (15% to 18%) than untreated, challenged control pigs (51%), and tonsils were culture-positive in fewer treated pigs (50%) than in controls (83%). Pigs in Anderson's study were inoculated orally with 10^6 to 10^7 CFU of *Salmonella* Choleraesuis. Nisbet et al[9] found that pigs challenged orally with 10^7 CFU of *Salmonella* Typhimurium and treated with competitive-exclusion microbes were less likely to shed salmonellae in feces (44%) than those not receiving a treatment (77%), and the cecum was culture-positive for *Salmonella* Typhimurium in fewer treated pigs (56%) than untreated pigs (100%).[9] However, in two studies by Letellier et al,[14,15] treatment with a combination of microorganisms in diets did not affect fecal shedding in 12-day old pigs inoculated with 10^7 CFU of *Salmonella* Typhimurium.[14,15] Only a slightly smaller proportion of carrier pigs, as determined by culture of salmonellae from tissues, was noted.[14,15]

Viability of the *E faecium* and *Bacillus* organisms would influence the ability of the DFMs to effectively compete with *Salmonella* organisms in the gastrointestinal tracts of the pigs. Lack of viable microorganisms in the *E faecium*-treated water sample from the isolation facility on Day 12 indicates a possible error in water preparation or laboratory procedures, or decreased viability of bacteria in the water sample, which may have contributed to the lack of response to the *E faecium* product. The water source for the isolation facility was chlorinated, which may have decreased the viability of the *E faecium*.[44] Viable *B licheniformis* and *B subtilis* were present in the *Bacillus*-treated feed sample, indicating that the heat of pelleting did not alter the viability of the *Bacillus* organisms. Therefore, it does not appear that a deficiency of viable organisms contributed to the lack of response to the *Bacillus* product. The overall high numbers of salmonellae in feces, gastrointestinal tract contents, and tissue samples, together with the high mortality rate, indicate a severe disease challenge. Direct-fed microbials must be able to compete with other microorganisms for nutrients and resist inhibition by pathogenic bacteria in the intestine to be effective against an invasion from a pathogen. It is possible that the DFMs were overwhelmed by *Salmonella* Typhimurium in the gastrointestinal tract. A dose-response study would be necessary to determine the maximum level of *Salmonella* Typhimurium exposure at which the DFMs would be effective.

Even though fecal shedding of *Salmonella* Typhimurium was not affected by dietary treatment, the use of DFMs appeared to reduce the severity of diarrhea, as indicated by fecal scores. This may indicate a quicker recovery from the disease challenge for pigs treated with a DFM. Pigs fed the *Bacillus* diet had lower fecal scores than pigs fed the control diet on Days 2 to 4, and pigs provided with drinking water containing *E faecium* had lower fecal scores than control pigs on Day 4. These results are consistent with previous research that has demonstrated less diarrhea when DFMs were fed to weaned pigs.[16-19] Pigs supplemented with 10^7 viable spores of *B licheniformis* per g of feed had significantly lower diarrhea scores for up to 28 days post weaning than pigs receiving no DFM.[17] Others[16] reported diarrhea in 36.2% of pigs given no DFM compared to only 18% of pigs receiving a DFM supplement containing 10^{12} viable spores of *B cereus* per kg of feed. Incidence of postweaning diarrhea was also lower in pigs supplemented with *B cereus* (27.4%) than in pigs receiving no DFM supplementation (67.5%)[18] and in pigs supplemented with *E faecium* (21%) than in pigs receiving no DFM supplementation (38%).[19]

It is not surprising that differences in serum IgM and IgG concentrations were not detected among challenged pigs fed the three dietary treatments, since no differences were detected in serum IgM and IgG concentrations between challenged and nonchallenged pigs. Serum IgM and IgG concentrations of pigs fed the three dietary treatments continued to increase throughout the study, and peaked at Day 12. The lack of a measurable response in serum IgM and IgG concentrations of challenged and unchallenged pigs in this study, the inability to detect differences in IgM and IgG concentrations among challenged pigs, and results reported by other researchers,[30] who found no difference in serum IgM and IgG concentrations in a *Salmonella* Typhimurium challenge model, suggest that serum IgM and IgG concentrations may not be good indicators of clinical *Salmonella* Typhimurium infection in a 12-day challenge study.

Serum AGP and Hp concentrations of pigs fed the dietary treatments were lowest before challenge, peaked at Day 7, and were beginning to decline by Day 12, which reflects the clinical signs of salmonellosis in our pigs. Fecal shedding and fecal scores also recovered to prechallenge levels by Days 9 to 12. Niewold et al[36] found serum Hp concentration to be a good indicator of an acute phase response in pigs. Therefore, it is likely that the pigs in our study were beginning to recover from the acute *Salmonella* Typhimurium infection by Day 12.

Implications

- Under the conditions of this study, treatment with the DFMs *Bacillus licheniformis*, *Bacillus subtilis,* and *Enterococcus faecium* SF-273 and SF-301 does not affect prevalence of *Salmonella* Typhimurium in feces, gastrointestinal contents, or tissues, or the numbers of organisms cultured from these sites.
- Under the conditions of this study, treatment with DFMs may lessen severity of diarrhea due to *Salmonella* Typhimurium.

- As tissues of apparently healthy pigs exposed to *Salmonella* Typhimurium are likely to contain high concentrations of bacteria, care should be taken to prevent exposure of edible lean tissue, equipment, or surfaces at the processing plant to tonsil, mandibular lymph nodes, and gastrointestinal tract contents.

Acknowledgment

The authors acknowledge financial support of this project by Chris Hansen, Inc.

References

1. White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD, Meng J. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *New Engl J Med*. 2001;345:1147–1154.

*2. Blaha T. Synopsis of the importance of *Salmonella* in swine and pork production. *Salmonella* control and research programs. *Proc AD Leman Conf*. Brooklyn Park, Minnesota 1998;1–4.

*3. Isaacson RE, Weigle RM, Firkins LD, Bahnson P. The effect of feed withdrawal on the shedding of *Salmonella* Typhimurium by swine. *Proc 3rd Int Symp of the Epidemiology and Control of Salmonella in Pork*. Washington, DC. 1999;296–298.

4. Wood RL, Pospischil A, Rose R. Distribution of persistent *Salmonella typhimurium* infection in internal organs of swine. *Am J Vet Res*. 1989;50:1015–1021.

5. Gill CO, Jones T. Control of contamination of pig carcasses by *Escherichia coli* from their mouths. *Int J Food Microbiol*. 1998;44:43–48.

6. Autio T, Sateri T, Fredriksson-Ahomaa M, Rahkio M, Lunden J, Korkeala H. *Listeria monocytogenes* contamination pattern in pig slaughterhouses. *J Food Protect*. 2000;63:1438–1442.

7. Anderson RC, Stanker LH, Young CR, Buckley SA, Genovese KJ, Harvey RB, DeLoach JR, Keith NK, Nisbet DJ. Effect of competitive exclusion treatment on colonization of early weaned pigs by *Salmonella* serovar Choleraesuis. *J Swine Health Prod*. 1999;7:155–160.

8. Anugwa FOI, Varel VH, Dickson JS, Pond WG, Krook LP. Effects of dietary fiber and protein concentration on growth, feed efficiency, visceral organ weights and large intestine microbial populations in swine. *J Nutr*. 1989;119:879–886.

*9. Nisbet DJ, Anderson RC, Harvey RB, Genovese KJ, DeLoach JR, Stanker LH. Competitive exclusion of *Salmonella* serovar Typhimurium from the gut of early weaned pigs. *Proc 3rd Int Symp of the Epidemiology and Control of Salmonella in Pork*. Washington, DC. 1999;80–82.

10. Anderson RC, Buckley SA, Callaway TR, Genovese KJ, Kubena LF, Harvey RB, Nisbet DJ. Effect of sodium chlorate on *Salmonella* Typhimurium concentrations in weaned pig gut. *J Food Protect*. 2001;64:255–258.

11. Audisio MC, Oliver G, Apella MC. Effect of different complex carbon sources on growth and bacteriocin synthesis of *Enterococcus faecium*. *Int J Food Microbiol*. 2001;63:235–241.

12. Alexopoulos C, Georgoulakis IE, Tzivara A, Kritas SK, Siochu A, Kyriakis SC. Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores, on the health status and performance of sows and their litters. *J Anim Physiol Anim Nutr.* 2004;88:381–392.

13. Nousiainen J, Setala J. Lactic acid bacteria as animal probiotics. In: von Wright S, von Wright A, eds. *Lactic Acid Bacteria*. New York: Marcel Dekker; 1993:315–356.

14. Letellier A, Messier S, Lessard L, Quessy S. Assessment of different treatments to reduce carriage of *Salmonella* in swine. *Can J Vet Res*. 2000; 64:27–31.

*15. Letellier A, Messier S, Lessard L, Quessy S. Host response to different treatments to reduce *Salmonella* infection in swine. *Proc 3rd Int Symp Epidemiol Control Salm Pork*. Washington, DC. 1999;317–320.

16. Zani JL, Weykamp da Cruz F, Freitas dos Santos A, Gil-Turnes C. Effect of probiotic CenBiot on control of diarrhea and feed efficiency in pigs. *J Appl Microbiol* 1998;84:68–71.

17. Kyriakis SC, Tsiloyiannis VK, Vlemmas J, Sarris K, Tsinas AC, Alexopoulos C, Jansegers L. The effect of probiotic LSP 122 on the control of post-weaning diarrhea syndrome in piglets. *Res Vet Sci.* 1999;67:223–228.

18. Taras D, Vahjen W, Macha M, Simon O. Response of performance characteristics and fecal consistency to long-lasting supplementation with the probiotic strain *Bacillus cereus* var. toyoi to sows and piglets. *Arch Anim Nutr.* 2005;59:405–417.

19. Taras D, Vahjen W, Macha M, Simon O. Performance, diarrhea incidence, and occurrence of *Escherichia coli* virulence genes during long-term administration of a probiotic *Enterococcus faecium* strain to sows and piglets. *J Anim Sci.* 2006;84:608–617.

20. Audisio MC, Oliver G, Apella MC. Protective effect of *Enterococcus faecium* J96, a potential probiotic strain, on chicks infected with *Salmonella pullorum*. *J Food Protect*. 2000;63:1333–1337.

21. Noblet J, Perez JM. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. *J Anim Sci*. 1993;71:3389–3398.

22. Thacker PA. Water in swine nutrition. In: Lewis AJ, Southern LL, eds. *Swine Nutrition*. 2nd ed. Washington, DC: CRC Press; 2001;381–398.

23. National Research Council. *Nutrient Requirements of Swine*. 9th rev ed. Washington, DC: National Academy Press; 1988.

24. Isaacson RE, Firkins LD, Weigel RM, Zuckermann RZ, DiPietro JA. Effect of transportation and feed withdrawal on shedding of *Salmonella* Typhimurium among experimental infected pigs. *Am J Vet Res*. 1999;60:1155–1158.

25. Hurd HS, Gailey JK, McKean JD, Rostagno MH. Rapid infection in market-weight swine following exposure to a *Salmonella* Typhimurium-contaminated environment. *Am J Vet Res*. 2001;62:1194–1197.

*26. Spiehs M, Shurson G, Johnston L, Seifert K. Evaluation of corn distiller's dried grains with solubles and a polyclonal antibody on growth performance and the ability of pigs to resist an infection from *Salmonella* Typhimurium. *J Anim Sci*. 2005;83(suppl 2):32.

27. Gray JT, Fedorka-Cray PJ, Stabel TJ, Ackermann MR. Influence of inoculation route on carrier state of *Salmonella choleraesuis* in swine. *Vet Microbiol*. 1995;47:43–59.

28. Gray JT, Stabel TJ, Fedorka-Cray PJ. Effect of dose on immune response and persistence of *Salmonella choleraesuis* in swine. *Am J Vet Res*. 1996;57:313–319.

29. Balaji R, Wright KJ, Hill CM, Dritz SS, Knoppel EL, Minton JE. Acute phase responses of pigs challenged orally with *Salmonella typhimurium*. *J Anim Sci*. 2000;78:1885–1891.

30. Turner JL, Dritz SS, Higgins JJ, Herkelman KL, Minton JE. Effects of a *Quillaja saponaria* extract on growth performance and immune function of weanling pigs challenged with *Salmonella typhimurium*. *J Anim Sci*. 2002;80:1939–1946.

31. Turner JL, Dritz SS, Higgins JJ, Minton JE. Effects of *Ascophyllum nodosum* extract on growth performance and immune function of weanling pigs challenged with *Salmonella typhimurium*. *J Anim Sci*. 2002;80:1947–1953.

32. Burkey TE, Dritz SS, Nietfeld JC, Johnson BJ, Minton JE. Effect of dietary mannanoligosaccharide and sodium chlorate on the growth performance, acute phase response, and bacterial shedding of weaned pigs challenged with *Salmonella enterica* serotype Typhimurium. *J Anim Sci*. 2004;82:397–404.

33. Clancy J Jr. *Basic Concepts in Immunology: A Student's Survival Guide*. New York: McGraw-Hill; 1998.

34. Williams NH, Stahly TS, Zimmerman DR. Effect of chronic immune system activation on body nitrogen retention, partial efficiency of lysine utilization, and lysine needs of pigs. *J Anim Sci*. 1997;75:2472–2480.

35. Williams NH, Stahly TS, Zimmerman DR. Effect of chronic immune system activation on the rate, efficiency, composition of growth, and lysine needs of pigs fed from 6 to 27 kg. *J Anim Sci*. 1997;75:2463–2471.

*36. Niewold TA, Tousaint MJM, Gruys E. Monitoring health by acute phase proteins. *Proc 4th European Colloquium on Acute Phase Proteins*. Segovia, Spain. 2003;57–67.

37. Lind P, Haugegaard J, Wingstrand A, Henriksen SA. The time course of the specific antibody response by various ELISAs in pigs experimentally infected with *Toxoplasma gondii*. *Vet Parasit*. 1997;71:1–15.

38. Steinbach G, Methner U, Springer S, Linder T, Selbitz HJ. The humoral immune response of swine after experimental infection with *Salmonella* Typhimurium. *Berliner und Munchener Tierarztichel Wochenschrift*. 2003;116:124–129.

39. Co-Clough NC, Roth JC. *Understanding Immunology*. St. Louis: Mosby-Year Book; 1998.

40. Anderson RC, Nisbet DJ, Buckley SA, Genovese KJ, Harvey RB. Experimental and natural infection of early weaned pigs with *Salmonella choleraesuis*. *Res Vet Sci*. 1998;64:261–262.

41. Galton MM, Smith WV, McElrath HB. Salmonella in swine, cattle, and the environment of abattoirs. *J Infect Dis*. 1954;95:236–245.

42. Gitter M, Kidd ARM. Isolation of *Salmonella typhimurium* from carrier pigs. *Vet Rec*. 1967;81:358–359.

43. Newell KW, Williams LP Jr. The control of salmonellae affecting swine and man. *JAVMA*. 1971;158:89–98.

44. He J, Jiang S. Quantification of enterococci and human adenoviruses in environmental samples by real-time PCR. *Appl Environ Microbiol*. 2005;71:2250–2255.

* Non-refereed references.