RESEARCH REPORT
PORK SAFETY

Title: The effects of water delivery of probiotics or organic acids on weanling pig performance, intestinal health and integrity, and immune status – **NPB #05-181**

Investigator: John Scott Radcliffe

Institution: Purdue University

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I. Industry Summary:

The swine industry experiences significant economic loss as a result of *Samonella* infection (Schwartz, 1990). Treatment of *Salmonella enterica* var Typhimurium infection in pigs with antibiotics has been reported to be unsuccessful at preventing subsequent shedding and further infection of young pigs (Roesler et al., 2005). The use of low doses of antibiotics for growth promotion in livestock is under continuous scrutiny due to the growing fear of antibiotic resistance (Barton, 2000). The removal of growth promotional antibiotics from livestock diets in Europe has sparked renewed interest into antibiotic alternatives. Probiotics, are usually defined as 'live microorganisms which when administered in adequate amounts, confer health benefits on the host' (FAO/WHO, 2001). Direct-fed microbials (DFMs) as they are known in animal nutrition, have been shown to improve growth performance (Simon et al., 2001), beneficially alter intestinal microbial balance (Fuller, 1989) and in general, positively impact gastrointestinal health. Certain DFMs selected based on their anti-*Salmonella* activity demonstrated the ability to reduce *Salmonella* shedding following a *Salmonella enterica* var Typhimurium challenge in pigs (Casey et al., 2007). Also, the addition of organic acids to water or feed seems to be promising for reduction of *Salmonella* in swine (Wingstrand et al., 1997). The objective of this study was to evaluate the potential of a water delivered *Enterococcus/Bacillus* combination or organic acid as a substitute for antibiotics following a *Salmonella* challenge in weanling pigs. Therefore, the ***objective*** of this experiment was to determine the effects of water deliverd DFMs or organic acids on nursery pig growth performance, gut health and integrity, and immune status prior to and following an oral *Salmonella* Typhimurium challenge in comparison to responses observed when subtherapeutic levels of antibiotics were included in the diet.

To accomplish this objective, 88 crossbred pigs (equal barrows and gilts) were weaned at an average of 19 d of age and used in a 14 d study to evaluate the potential of direct fed microbials (DFMs) or organic acids to replace antibiotics following a *Salmonella enterica* var Typhimurium challenge. At weaning, pigs were randomly assigned to 4 treatments:

1) Control
2) Control + water delivered DFM
3) Control + water delivered organic acid based blend
4) Control + 55 ppm carbadox in the feed

Diets were fed continuously from d 0 - 14. Pigs were allotted based on genetics, sex and initial BW (average = 6.2 kg). There were 4 pigs/pen and 6 pens/trt with the exception of diets 1 and 4 which had 5 pens/trt. All pigs had unlimited access to feed and water through a 5-hole self feeder and a single nipple waterer in each pen. Pigs were housed in an isolated room at the USDA Livestock Behavior Research Unit at Purdue University. Dietary treatments were administered for 14 consecutive days. All diets were formulated to meet or exceed the estimated nutrient requirements for pigs (NRC, 1998). Pigs were individually weighed and feed disappearance was recorded on d 0, 5, 8, 10 and 14 for calculation of growth rate, feed intake, and feed efficiency. Pigs were challenged with *Salmonella* 6 d post commencement of treatments. Serum samples were taken on d 6, 8, 10, and 14 for determination of TNFα concentration. Fecal samples were taken on d 0, 5, 7, and 11 for detection of *Salmonella* shedding and enumeration of *Enterobacteriace*. Pigs (n=22/d) were harvested on d 6, 8, 10, and 14. Intestinal and cecal tissue and contents and mesenteric lymph nodes were taken for *Salmonella* detection. Jejunum samples were taken for determination of nutrient uptake in modified Ussing Chambers. Duodenal, jejunal and ileal mucosal scrapings and tissue were taken for measurement of mucosal TNFα concentration and intestinal morphology, respectively. All treatments, in particular water administration of DFM improved growth rate from d 2 to 6 post-challenge compared to the negative control ($P < 0.05$). Duodenal villus height also tended to be improved (P=0.08) by DFM administration on d 4 post-challenge. *Salmonella* presence was reduced (P=0.001) in mesenteric lymph nodes, feces and ileal and cecal tissue over time. Antibiotic and DFM treatments tended to reduce (P=0.09) *Salmonella* presence in ileal contents. Serum TNFα tended to increase (P=0.10) up to d 4 post-challenge while TNFα concentrations in the duodenum tended to linearly decrease (P=0.10) post-challenge and TNFα in the ileum decreased (P=0.008) immediately post-challenge. *Salmonella* infection resulted in a linear decrease in phosphorus (P=0.001) active transport, and an increase (P=0.001) in glutamine uptake up to d 4 post-challenge. Glucose uptake was increased immediately post-challenge by in-feed antibiotics or water delivered DFM (P=0.009). Active ion transport was reduced by *Salmonella* infection, however water delivered DFM or organic acid treatments were most successful at attenuating this decline (P=0.001). Water acidification and antibiotic treatment tended to reduce carbachol induced chloride secretion (P=0.07).

In conclusion, water delivery of a DFM combination or organic acid appears to be a successful means of administering a potential antibiotic alternative to weanling pigs. Following a *Salmonella enterica* var Typhimurium challenge, both DFM and organic acid treated drinking water enhanced growth performance and gastrointestinal histology. *Salmonella* presence in ileal contents was reduced by DFM treatment and active glucose transport increased, while water acidification reduced induced chloride secretion. Current findings suggest that under a disease challenge situation water acidification and DFM treated drinking water performed similar or in some cases superior to in feed antibiotics and therefore must be considered as a viable alternative in weanling pig diets.

II. Scientific Abstract:

Pigs (n=88) weaned at ~ 19 d were used in a 14 d study to evaluate the effects of water delivered DFM (Bioplus DP, Chr. Hansen: *Enterococcus faecium, Bacillus subtillus and Bacillus licheniformis* - 10^9 cfu/L) or organic acids (2.58 mL/L of a propionic acid based blend; Kemin Americas) on immune status, nutrient uptake, *Salmonella* shedding, intestinal morphology, and intestinal microbial population following nasal inoculation of *Salmonella typhimurium* (10^9 cfu/pig). Pigs were challenged with *Salmonella* 6 d post commencement of water treatments. Serum samples were taken on d 6, 8, 10, and 14 for determination of TNFα concentration. Fecal samples were taken on d 0, 5, 7, 11 for detection of *Salmonella* shedding and enumeration of *Enterobacteriace*. Pigs (n=22/d) were harvested on d 6, 8, 10, and 14. Intestinal and cecal tissue and contents and mesenteric lymph nodes (MLN) were taken for *Salmonella* detection. Jejunum samples were taken for determination of nutrient uptake in modified Ussing Chambers. Duodenal, jejunal and ileal mucosal scrapings and tissue were taken for measurement of mucosal TNFα concentration and intestinal morphology, respectively. All treatments, in particular water administration of DFM improved ADG on d 2 to 6 post-challenge above the negative control

(P < 0.05). Duodenal villus height also tended to be improved (P=0.08) by DFM administration on d 4 post-challenge. *Salmonella* presence was reduced (P=0.001) in MLN, feces and ileal and cecal tissue over time. Antibiotic and DFM treatment tended to reduce (P=0.09) *Salmonella* presence in ileal contents. Serum TNFα tended to increase (P=0.10) up to d 4 post-challenge while TNFα concentrations in duodenum tended to linearly decrease (P=0.10) post-challenge and TNFα in the ileum decreased (P=0.008) immediately post-challenge. *Salmonella* infection resulted in a linear decrease in phosphorus (P=0.001) active transport, and an increase (P=0.001) in glutamine uptake up to d 4 post-challenge. Glucose uptake was increased immediately post-challenge by in-feed antibiotics or water delivered DFM (P=0.009). Active ion transport was reduced by *Salmonella* infection, however water delivered DFM or organic acid treatment were most successful at attenuating this decline (P=0.001). Water acidification and antibiotic treatment tended to reduce carbachol induced chloride secretion (P=0.07). In conclusion, water administration of DFM and water acidification, following a *Salmonella enterica* var Typhimurium challenge affected weanling pigs in a similar and in some cases more beneficial manner than low dose antibiotics, lending support for their use as an antibiotic alternatives.

III. Introduction:

Salmonellosis is generally accepted to be one of the most important zoonoses transmitted by meat in the developed world (Beran, 1995). In addition to the impact of salmonellosis on human health, the swine industry also experiences significant economic loss at the hands of this disease (Schwartz, 1990). Intestinal carriage of enteric pathogens such as *Salmonella* by swine may determine the degree of carcass contamination during slaughter. Treatment of *Salmonella enterica* var Typhimurium infection in pigs with antibiotics has been reported to be unsuccessful at preventing subsequent shedding and further infection of young pigs (Roesler et al., 2005). The use of low doses of antibiotics for growth promotion in livestock is under continuous scrutiny due to the growing fear of antibiotic resistance (Barton, 2000). The removal of growth promotional antibiotics from livestock diets in Europe has sparked renewed interest into antibiotic alternatives. Probiotics, are usually defined as 'live microorganisms which when administered in adequate amounts, confer health benefits on the host' (FAO/WHO, 2001). Direct-fed microbials (DFMs) as they are known in animal nutrition, have been shown to improve growth performance (Simon et al., 2001), beneficially alter intestinal microbial balance (Fuller, 1989) and in general, positively impact gastrointestinal health. Certain DFMs selected based on their anti-*Salmonella* activity demonstrated the ability to reduce *Salmonella* shedding following a *Salmonella enterica* var Typhimurium challenge in pigs (Casey et al., 2007). Also, the addition of organic acids to water or feed seems to be promising for reduction of *Salmonella* in swine (Wingstrand et al., 1997). The objective of this study was to evaluate the potential of a water delivered *Enterococcus/Bacillus* combination or organic acid as a substitute for antibiotics following a *Salmonella* challenge in weanling pigs.

IV. Objectives:

Determine the effects of water acidification, a direct-fed microbial (*Lactobacillus acidophilus*), and a subtherapeutic level of carbadox on nursery pig growth performance, gut health and integrity, and immune status prior to and following an oral *Salmonella* Typhimurium challenge.

V. Materials and Methods:

Eighty eight crossbred pigs (equal barrows and gilts) were weaned at an average of 19 d of age and used in a 14 d study to evaluate the potential of direct fed microbials (DFMs) or organic acids to replace antibiotics following a *Salmonella enterica* var Typhimurium challenge.

Experimental Diets and Animal Research

Pigs were randomly assigned to 4 dietary treatments: 1) control; 2) control + DFM (Bioplus DP) in drinking water at 10^9 cfu/L (Chr. Hansen: *Enterococcus faecium, Bacillus subtilis, Bacillus licheniformis*); 3)

control + an organic acid based blend in drinking water (2.58 mL/L of drinking water; KEM SAN^{TM}, predominantly propionic, acetic, and benzoic acid; Kemin Americas, Des Moines, IA); and 4) control + 55 ppm carbadox. Diets were fed continuously from d 0 - 14 (Table 1). Pigs were allotted based on genetics, sex and initial BW (average = 6.2 kg). There were 4 pigs/pen and 6 pens/trt with the exception of diets 1 and 4 which had 5 pens/trt. All pigs had unlimited access to feed and water through a 5-hole self feeder and a single nipple waterer in each pen. Pigs were housed in an isolated room at the USDA Livestock Behavior Research Unit at Purdue University. Dietary treatments were administered for 14 consecutive days. All diets were formulated to meet or exceed the estimated nutrient requirements for pigs (NRC, 1998). Pigs were individually weighed and feed disappearance was recorded on d 0, 5, 8, 10 and 14 for calculation of ADG, ADFI, and G:F. On d 6 post-weaning, one pig/pen was randomly selected, a venous blood sample was obtained and then the pig was euthanized by asphyxiation with CO_2 for collection of duodenal, jejunal, and ileal tissue. All remaining pigs were given an intra-nasal dose of 10^9 colony forming units (cfu) of *Salmonella enterica* var Typhimurium (Strain χ4232, Nalidixic acid resistant) per pig. Rectal temperatures were recorded on d 5, 7, 8, 10, and 14 to monitor changes in body temperature. Water samples were taken on d 0 and 13 for pH recording. All animals were cared for in accordance with Purdue University Animal Care and Use Committee regulations.

Tissue Collection and Preparation

Venous blood samples were obtained (Lawhorn, 1988) from one pig/pen prior to euthanasia on d 6, 8, 10 and 14 post weaning. Blood was collected from the anterior vena cava in serum separation vacutainer blood collection tubes (Becton Dickinson, Franklin Lakes, NJ). Blood samples were allowed to clot at room temperature for 2 h followed by centrifugation at 2000 × g for 20 mins. Serum was removed and frozen at – 80 ^{o}C for subsequent cytokine analysis. Pigs were euthanized by asphyxiation for collection of duodenal, jejunal, and ileal tissue. Duodenal, jejunal, and ileal mucosal scrapings were taken by removing the outer most mucosal layer using a microscope slide. Mucosal tissue was placed in Complet^{TM} protein extraction reagent (Apharma, Gaithersburg, MD) with added protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) and homogenized. Supernatant was removed following centrifugation at 27,000 × g for 15 mins and stored at -80 ^{o}C for subsequent cytokine analysis.

Microbial Analysis

A Nalidixic acid-resistant *Salmonella enterica* var Typhimurium (Strain χ4232) was used to challenge the study pigs. The inoculum (10^9 CFU per pig) was prepared as described by Hurd et al. (2001). All pigs were screened for the presence of *Salmonella* in fecal samples prior to weaning and at weaning (d 0) to allow for the elimination of *Salmonella* positive animals from the study. Fecal samples (2 per pen) were collected on d 5, 7 and 11 post-weaning for detection of *Salmonella*. Distal ileal tissue and content, cecal tissue and content, and ileocecal lymph nodes were collected from one pig per pen (total of 22 pigs/d) on days 6, 8, 10, and 14 post-weaning (d 0, 2, 4, and 8 post-challenge). All samples collected were processed for the isolation of *Salmonella enterica*, including a sequential enrichment in Tetrathionate broth (Neogen, Lansing, MI) and Rappaport-Vassiliadis broth (Neogen, Lansing, MI) containing novobocin (Sigma-Aldrich, St. Louis, MO), plating on XLT-4 agar (Neogen, Lansing, MI) containing Nalidixic acid (50 μg/mL), and identification on Rambach agar (VWR, Batavia, IL). Ileal and cecal contents from pigs euthanized on d 6, 8, 10 and 14 post-weaning were also serially diluted in buffered peptone water (VWR, Batavia, IL) and plated on MacConkey agar (Neogen, Lansing, MI) for enumeration of *Enterobacteriaceae*. *Enterococcus* were enumerated in fecal samples taken on d 5 (2 pigs/pen) and in cecal contents of pigs (n=22) harvested on d 14. Fecal samples were serially diluted in buffered peptone water (VWR, Batavia, IL), spread plated on Kanamycin Azide Aaesulin agar (KAA agar: Fisher, San Diego, CA) and incubated aerobically overnight at 37 ^{o}C. Water samples were taken on d 0, 7, and 14 for enumeration of *Enterococcus faecium* and *Bacillus* populations. Enumeration of *Enterococcus faecium* in water was conducted by serial dilution of water samples in buffered peptone water followed by spread plating on sterile Mitus Salivarus agar (Sigma-Aldrich, St. Louis, MO) with 100 μL sterile 1% potassium Tellurite (VWR, Batavia, IL) per 100 mL added after tempering at 48 ^{o}C in a water bath. Plates were then incubated for 2-3 days aerobically at 37 ^{o}C. Bacillus spores in water were enumerated by heating the water sample to 80 ^{o}C in a water bath for 10 min followed by cooling in cold water. Water samples were then serially diluted in peptone

broth and spread plated on Trypticase Soy Agar with 5% sheep blood (TSA II: Krackeler Scientific, Albany, NY). Plates were incubated overnight at 37 ^{o}C.

Cytokine Analysis

Concentrations of TNFα were determined in the supernatant of duodenal, jejunal, and ileal mucosal scrapings and blood serum using porcine specific cytokine kits (R&D Systems, Minnespolis, MN). Protein content of mucosal scrapings was determined using a BCA^{TM} Protein assay kit which has a working range of 20 - 2000 μg/mL (Pierce, Rockford, IL). Cytokine concentration in mucosal tissue was adjusted to an average protein yield of 0.066 g in duodenal mucosa, 0.049 g in jejunal mucosa and 0.050 g in ileal mucosa. The porcine specific TNFα ELISA kit has a range of 23.4 – 1500 pg/mL.

Nutrient Uptake

A 15 cm section of jejunum was removed immediately after each pig was euthanized, gently rinsed with ice-cold saline, and placed in an aerated buffer solution for use in modified Ussing chambers. Tissues were mounted in chambers within 40 minutes of collection. For intestinal tissue uptake studies using modified Ussing Chambers (Physiologic Instruments, San Diego, CA), the outer serosal layer of jejunal sections was removed and the lumen was exposed by cutting along the mesenteric border. Duplicate sections were mounted in modified Ussing chambers, with 1.0 cm^2 of tissue exposed. Tissue was bathed in 8 mL of a phosphorus-free modified Kreb's buffer that was oxygenated (95% O_2/5% CO_2) and maintained at 37 ^{o}C using a recirculating water bath. Chambers were connected to dual channel voltage/current clamps (VCC MC8, Physiologic Instruments), voltage was clamped and tissue was given 10-15 min to equilibrate prior to measurement of basal transmucosal short-circuit current, resistance and potential difference. Glucose, phosphate, and glutamine uptake were estimated based on the change in short circuit current resulting from the addition of 10 mM glucose, Na_2HPO_4, or glutamine, respectively, to the brush border membrane buffer solution, which was measured using a real-time computer interface and data acquisition software package (Acquire and Analyze software, Physiologic Instruments). Mannitol (10 m*M*) was added to the serosal buffer solution to balance ion flux and ensure that any change in Isc was due changes in nutrient uptake. In addition, chloride ion secretion on the mucosal side was determined following stimulation with carbachol and serotonin.

Gross Morphology

Duodenal, jejunal, and ileal tissue samples were rinsed in phosphate buffer and placed in No-Tox fixative (Scientific Device Lab, Des Plaines, IL – alcohol/aldehyde fixative) on a shaker for 48 h. Samples were then dehydrated through a graded alcohol series, cleared with Sub-X clearing agent (Surgipath, Richmond, IL), and embedded in paraffin. Tissue samples was sliced using a microtome, mounted on microsope slide and stained with Hematoxylin and Eosin (Sigma-Aldrich, St. Louis, MO) for light microscope examination. Determination of gross morphological parameters of intestinal structure (villus height, and crypt depth) were conducted according to Gao et al. (2000) and Applegate et al. (1999a).

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Inc, Cary, NC). Pen served as the experimental unit. The model included diet, replicate, and time post-challenge. Differences over time were determined using repeated measures. Treatments means were compared using the Duncan procedure of SAS if the overall treatment effect was significant ($P < 0.10$).

Table 1. Composition of basal nursery pig diets, as is basis.

Ingredients, %	Control Diet	Antibiotic Diet
Ground corn	40.70	40.42
Whey	25.00	25.00
SBM, 48% CP	17.20	17.23
Plasma protein	6.00	6.00
Soybean Oil	5.00	5.00
Fishmeal	4.00	4.00
Limestone	0.71	0.71
Dicalcium phosphate	0.59	0.59
Vitamin Premix[1]	0.25	0.25
Carbadox	----	0.25
Salt	0.20	0.20
TM Premix[2]	0.13	0.13
Lysine-HCL	0.10	0.10
Se premix[3]	0.05	0.05
DL-methionine	0.07	0.07
Calculated composition		
ME, kcal/kg	3473.00	3464.00
CP	24.00	24.00
Lys, %	1.55	1.55
Met, %	0.43	0.43
Met + Cys, %	0.89	0.89
Thr, %	1.08	1.08
Ca, %	0.90	0.90
P, %	0.74	0.74

[1] Vitamin premix provided per kg of diet: vitamin A, 6064 IU; vitamin $D_{3,}$ 606 IU; vitamin E, 44.1 IU; menadione, 2 mg; vitamin $B_{12,}$ 35 μg ; riboflavin, 7.1 mg ; d-pantothenic acid, 22 mg; niacin, 33 mg.

[2] Trace mineral premix provided per kg of diet: Iron, 121.2 mg; Zinc, 121.2 mg; Manganese, 15.0 mg; Copper, 11.2 mg; Iodine, .46 mg; Se, .30 mg.

[3] Se premix provided 0.3 mg Se per kilogram of diet.

VI. Results:

Water Analysis

At the beginning (d 0) and the end (d 13) of the study, pH readings were taken (Table 2). The average pH of drinking water for the acid treatment was 4.7 on d 0 and 4.8 on d 13. The average pH of drinking water for all other treatments was 7.6 on d 0 and 7.5 on d 13. Water samples were also taken on d 0, 7, and 14 for enumeration of administered DFM in drinking water (Table 3). *Enterococcus faecium* or Bacillus spp. were not detected in the drinking water of the water acidification, positive or negative control treatment groups. Throughout the study, the average colony count in the DFM treated drinking water for *Enterococcus faecium* was 4×10^3 cfu/L and Bacillus spp. was 1.5×10^4 cfu/L. The target count for each of these DFMs was 1×10^4 cfu/L.

Growth Performance

Prior to the *Salmonella* challenge (d 0 to 5; Table 4), the group of pigs administered DFMs in drinking water tended ($P < 0.10$) to have better feed efficiency than the negative control pigs, but were not different to other treatments (0.67 vs 0.54). Pre-challenge, ADG, ADFI and BW were not different between treatments. During the immediate pre- and post- challenge period (d 5 to 8), there were no differences in ADG, ADFI, G:F or d 8 BW between any of the four treatments. By d 2 to 4 post-challenge (d 8 to 10 post-weaning), pigs receiving drinking water treated with DFMs or organic acids, or in-feed antibiotics had significantly greater ($P < 0.05$) ADG than negative control pigs but were not different from each other (145, 73, 45 vs 2 g/d, respectively). There was no effect of treatment on d 10 BW, d 8 to 10 ADFI or G:F. By d 4 to 8 post-challenge (d 10 to 14 post-weaning), the earlier beneficial effects of the water treatments and antibiotic above the negative control treatment were no longer observed. On d 10 to 14 post-weaning, ADG, ADFI, G:F and d 14 BW were not different ($P > 0.10$) between any of the four treatments.

Body temperature

Rectal temperatures were taken prior to *Salmonella* challenge (d 5 post-weaning) and on d 1, 2, 4, and 8 post-challenge to determine the effect of treatment on body temperature (Table 5). There was a quadratic ($P < 0.05$) effect of day on body temperature. Body temperature increased on d 1 post-challenge compared to pre-challenge body temperature (39.6 vs 39.8 ^{o}C). However, body temperature returned to pre-challenge levels by d 2 post-challenge. On d 8 post-challenge, body temperature of pigs was further reduced to a body temperature that was lower than pre-challenge temperatures. Both DFM and organic acids water treatments resulted in a body temperature that was significantly lower ($P < 0.06$) than in-feed antibiotic treatment (39.4 and 39.4 vs 39.7 ^{o}C) while the body temperature of negative control pigs was not different to other treatments.

Gross Morphology

Villus height and crypth depth were measured in the duodenum, jejunum and ileum (Figure 1). On d 4 post-challenge, duodenal villi of pigs receiving DFMs in drinking water tended ($P < 0.08$) to be higher than villi of antibiotic and negative control treated pigs (549 vs 484, and 457 μm, respectively; Table 6). Pigs receiving acidified drinking water had duodenal villi that were not different in height to the other treatments. There was no effect of treatment on duodenal crypt depth, however, there was a linear increase in depth over the 8 day period post challenge ($P < 0.001$).

Jejunal villus height was not altered by treatment during the study, however there was a quadratic effect of day where *Salmonella* infection resulted in diminished height which returned to pre-challenge levels by d 8 post-challenge ($P < 0.008$). Pigs receiving water delivered DFM or organic acids had significantly deeper jejunal crypts than negative control pigs ($P < 0.004$). Jejunal crypt depth also linearly increased with time, with significantly shallower pre-challenge depths than d 8 post-challenge depths ($P < 0.001$). There was a quadratic effect of day ($P < 0.001$) on ileal crypt depth. *Salmonella* infection resulted in an increase in ileal crypt depth from a pre-challenge depth of 312 μm to a depth of 387 μm on d 4 post-challenge, however by d 8 post-challenge ileal crypt depth was reduced to 374 μm Ileal villus height was not affected by treatment or day post-infection.

Microbiology

Salmonella. Prior to intra-nasal *Salmonella* challenge, the presence of *Salmonella* in pig feces was negligible (Table 7). There was a quadratic effect ($P < 0.001$) of day on the presence of *Salmonella* in feces. On d 1 post-challenge, almost all pigs (97.8 %) were positive for *Salmonella* however, by d 5 post-challenge there was ~ 60 % reduction in fecal shedding of this pathogen. There was no effect ($P > 0.10$) of treatment on fecal shedding of *Salmonella* at any time point during the study. There was a quadratic effect ($P < 0.001$) of day on presence of *Salmonella* in mesenteric lymph nodes (MLN). *Salmonella* was detected in the MLN of 9.5 % of pigs prior to challenge and this was increased to 100 % of pigs by d 2 post-challenge. On d 4 post-challenge, *Salmonella* was detected in 95.3 % of MLN which was further reduced to 81.9 % by d 8 post-challenge.

There was a quadratic effect ($P < 0.001$) of day on the presence of *Salmonella* in ileal contents. *Salmonella* was detected in the ileal contents of 4.4 % of pigs prior to intranasal *Salmonella* challenge. *Salmonella* presence in ileal contents was increased to 90.3 % of pigs by d 2 post-challenge. On d 4 post-challenge, *Salmonella* was detected in the ileal contents of 93.2 % pigs, which was reduced to 78.1 % by d 8 post-challenge. Direct fed microbials in drinking water and antibiotics tended ($P < 0.09$) to decrease the presence of *Salmonella* in ileal contents compared to the water acidification treatment. There was a quadratic effect ($P < 0.001$) of day on *Salmonella* presence in ileal tissue. Prior to challenge, *Salmonella* was detected in ileal tissue of 9.7 % pigs. This detection level was increased to 100 % of pigs being positive for *Salmonella* on d 2 post-challenge. By d 4 post-challenge *Salmonella* was detected in the ileal tissue of 91.3 % of pigs which was further reduced to 77.2 % by d 8 post-challenge.

The administration of in feed antibiotics resulted in a 60 % reduction in the presence of *Salmonella* in cecal contents on d 8 post-challenge ($P < 0.001$). There was a quadratic effect ($P < 0.001$) of day on the *Salmonella* presence in cecal tissue. The detection of *Salmonella* in cecal tissue was negligible pre-challenge (0.6 %) but increased to 100 % by d 2 post-challenge. By d 8 post-challenge, *Salmonella* detection in ileal tissue was reduced below d 2 and d 4 post-challenge levels, but was still higher than pre-challenge levels. Treatment did not affect ($P > 0.10$) *Salmonella* presence in MLN, ileal tissue or contents and cecal tissue.

Enterobacteriaceae population. There was no effect of treatment on *Enterobacteriaceae* concentration in ileal contents ($P > 0.10$; Table 8). However, there was a quadratic effect ($P < 0.001$) of day on ileal *Enterobacteriaceae*. A reduction in *Enterobacteriaceae* concentration was observed on d 4 post-challenge (7.1 vs 5.9 $\log_{10}$ cfu/g) but by d 8 post-challenge, counts had returned to previous levels. There were significantly ($P < 0.05$) higher concentrations of *Enterobacteriaceae* in the cecal contents of pigs receiving DFM in drinking water compared to all other treatments. There was a trend for a quadratic effect ($P < 0.09$) of day on cecal *Enterobacteriaceae*. Similar to findings in the ileum, cecal *Enterobacteriaceae* were reduced on d 4 post-challenge but by d 8 post-challenge, concentrations had returned to previous levels.

Enterococcus populations. *Enterococcus* spp. were enumerated in feces pre-challenge and in cecal contents on d 8 post-challenge (Table 9). Pigs receiving DFM in drinking water tended to have higher counts in feces than antibiotic treated pigs prior to challenge but were not different to pigs administered the negative control or acidified water treatment ($P < 0.10$). On d 8 post-challenge, there was no difference ($P > 0.10$) in cecal *Enterococcus* population between treatments.

Cytokine responses

TNFα concentrations were measured in serum, duodenal, jejunal and ileal mucosa (Table 10). There was no effect ($P > 0.10$) of treatment on TNFα concentrations in serum or intestinal mucosa. However, there was a trend for a quadratic day effect ($P < 0.10$) whereby serum TNFα increased up to d 4 post-challenge (132.9 pg/mL) but by d 8 post-challenge serum concentration levels had decreased (124.2 pg/mL). In the duodenum, there was a trend for a linear decrease ($P < 0.10$) in TNFα concentration over time. TNFα concentration was lower on d 2 and 8 post-challenge than pre-challenge concentration (14.1, 18.6 vs 33.0 pg/g, respectively). TNFα concentration in the jejunum did not change over time. There was a quadratic effect ($P < 0.008$) of day on TNFα concentration in ileal mucosa. Pre-challenge concentrations of TNFα in the ileum were higher than post-challenge concentrations (110.0 vs 35.3, 42.2, and 17.0 pg/g for d 0, 2, 4, and 8 respectively).

Nutrient uptake

There was a quadratic effect ($P < 0.009$) of day on basal Isc values (Table 11). Basal Isc which is indicative of active ion transport was decreased following *Salmonella* challenge from 31.7 pre-challenge to 26.3 and 19.4 $\mu A/cm^2$ on d 2 and 4 post-challenge, respectively. By d 8 post-challenge, basal Isc values had increased to 23.2 $\mu A/cm^2$. The only treatments that appeared to attenuate this decline in Isc were water delivered DFM or organic acids ($P < 0.001$). Active glucose transport was decreased following *Salmonella* challenge in pigs treated with acidified drinking water or the negative control diet. However, in-feed antibiotic and water delivered DFM resulted in increased glusoce uptake on d 2 post-challenge. Phosphorus transport linearly decreased from d 0 to 8 post-challenge ($P < 0.001$). There was a quadratic effect ($P < 0.001$) of day on active glutamine uptake. Active glutamine transport was increased on d 2 and 4 post-challenge, but by d 8, glutamine uptake had returned to pre-challenge levels. There was no effect of treatment on serotonin induced chloride secretion, however chloride secretion linearly decreased ($P < 0.05$) over time. The negative control treatment tended ($P < 0.066$) to have higher levels of carbachol induced chloride secretion than either the antibiotic or water acidification treatment.

Table 2. Drinking water pH.

	DFM	Acid	Pos. ctrl.	Neg. ctrl.
Average pH, d 0	7.54	4.70	7.62	7.60
Average pH, d 13	7.45	4.80	7.50	7.56

Table 3. Average direct fed microbial counts (cfu/L) in drinking water [a,b]

Days post-weaning	Enterococcus spp.	Bacillus spp.
0	1×10^3	---[c]
7	2×10^2	1×10^3
14	1×10^4	3×10^4

[a] Water samples taken from the acid, positive ctrl., and negative ctrl. pens were all negative for Enterococcus and Bacillus spp.

[b] Target DFM count is 1×10^4 cfu/L.

[c] Bacillus spp were not assayed on d 0 due to lack of appropriate media.

Table 4. Effects of antibiotics or antibiotic alternatives on weanling pig growth performance following a *Salmonella enterica* var Typhimurium challenge [1].

	Treatments				SEM	P, value
	DFM	Acid	Pos. ctrl.	Neg. ctrl		
Initial BW, kg	6.29	6.22	6.26	6.18	0.035	0.161
d 0 – 5						
Pigs/pen	4	4	4	4		
ADG, g/d	150	136	136	113	17.3	0.497
ADFI, g/d	222	204	222	186	12.3	0.555
G:F	0.67[a]	0.65[ab]	0.61[ab]	0.54[b]	0.052	0.311
d 5 BW, kg	7.14	6.93	6.94	6.94	0.115	0.461
d 5 – 8						
Pigs/pen	3	3	3	3		
ADG, g/d	136	177	159	173	20.4	0.479
ADFI, g/d	313	272	304	336	26.3	0.405
G:F	0.46	0.65	0.54	0.54	0.079	0.381
d 8 BW, kg	7.47	7.44	7.41	7.45	0.075	0.933
d 8 – 10						
Pigs/pen	2	2	2	2		
ADG, g/d	145[x]	73[xy]	45[xy]	2[z]	34.1	0.019
ADFI, g/d	304	241	272	222	35.9	0.377
G:F	0.39	0.30	0.18	0.007	0.132	0.143
d 10 BW, kg	16.74	16.16	16.80	15.84	0.535	0.519
d 10 – 14						
Pigs/pen	1	1	1	1		
ADG, g/d	200	236	282	150	68.1	0.599
ADFI, g/d	381	340	427	318	52.2	0.417
G:F	0.50	0.68	0.67	0.50	0.149	0.622
d 14 BW, kg	18.51	18.23	19.22	16.83	0.881	0.347

[1] Pigs were intra-nasally inoculated with *Salmonella enterica* var Typhimurium on d 6 post-weaning.

[xyz] Values with different superscripts differ by $P < 0.05$ by means separation using the Duncan multiple range test.

[ab] Values with different superscripts differ by $P < 0.10$ by means separation using the Duncan multiple range test.

Table 5. Effects of antibiotics or antibiotic alternatives on rectal temperature (^{o}C) of weanling pigs following a *Salmonella enterica* var Typhimurium challenge.

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad.	Linear	Trt.	Day	Trt.×Day
Rectal Temperature											
Pre-challenge	39.4	39.5	39.8	39.8	39.6	0.32	0.054	0.001	0.060	0.001	0.201
d 1 post-challenge	39.6	39.8	39.9	40.0	39.8						
d 2 post-challenge	39.5	39.4	39.8	39.8	39.6						
d 4 post-challenge	39.6	39.4	39.5	39.6	39.6						
d 8 post-challenge	39.3	38.9	39.3	38.8	39.1						
Mean	39.4^{y}	39.4^{y}	39.7^{x}	39.6^{xy}							

^{xy} Values with different superscripts differ by $P < 0.05$ by means separation using the Duncan multiple range test.

Table 6. Effects of antibiotics or antibiotic alternatives on villus height and crypt depth of weanling pigs following a *Salmonella enterica* var Typhimurium challenge.

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad	Linear	Trt.	Day	Trt.×Day
Duodenal villus height, μm											
0	483	543	667	564	564	44.8	0.001	0.184	0.199	0.003	0.081
2	496	494	502	535	507						
4	549	495	484	457	496						
8	624	538	649	539	588						
Mean	538	518	575	524							
Duodenal crypt depth, μm											
0	323	363	311	304	325	25.0	0.144	0.001	0.161	0.001	0.693
2	370	360	371	377	369						
4	427	428	407	377	410						
8	442	487	457	439	456						
Mean	390	409	387	375							
Jejunal villus height, μm											
0	590	591	571	515	567	46.4	0.008	0.043	0.464	0.013	0.975
2	526	516	545	506	523						
4	578	555	512	506	532						
8	633	584	626	598	611						
Mean	577	561	563	531							

Table 6 (cont'd). Effects of antibiotics or antibiotic alternatives on villus height and crypt depth of weanling pigs following a *Salmonella enterica* var Typhimurium challenge.

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad	Linear	Trt.	Day	Trt.×Day
Jejunal crypt depth, μm											
0	325	363	363	331	345	17.4	0.136	0.001	0.004	0.003	0.258
2	384	374	354	339	363						
4	400	401	353	356	378						
8	394	393	394	351	383						
Mean	376[x]	383[x]	366[xy]	344[y]							
Ileal villus height, μm											
0	435	506	537	463	485	38.9	0.889	0.709	0.802	0.872	0.214
2	502	496	446	429	468						
4	498	477	442	509	482						
8	504	452	481	449	472						
Mean	485	483	477	463							
Ileal crypt depth, μm											
0	320	321	328	380	312	24.1	0.001	0.001	0.321	0.001	0.552
2	374	348	401	337	365						
4	390	378	384	395	387						
8	399	357	359	381	374						
Mean	371	351	368	349							

[xy] Values with different superscripts differ by $P < 0.05$ by means separation using the Duncan multiple range test.

Table 7. Effects of antibiotics or antibiotic alternatives on *Salmonella* presence (%) in feces, mesenteric lymph nodes (MLN), and ileal and cecal contents and tissue of weanling pigs following a *Salmonella enterica* var Typhimurium challenge [1].

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad.	Linear	Trt.	Day	Trt.×Day
Fecal salmonella											
0	0	0	0.3	2.2	0.6	12.89	0.001	0.507	0.216	0.001	0.115
1	100	88.8	100	100	97.8						
5	0	50	60.3	42.2	38.1						
Mean	33.3	46.3	53.6	48.9							
MLN											
0	0	16.6	20.6	0.6	9.5	11.14	0.001	0.001	0.911	0.001	0.263
2	100	100	100	100	100						
4	100	100	100	80.6	95.3						
8	83.3	83.3	60.6	100	81.9						
Mean	70.8	75	70.6	70.6							
Ileal Contents											
0	0	16.6	0	1.5	4.4	14.83	0.001	0.001	0.089	0.001	0.548
2	100	100	79.5	81.5	90.3						
4	96.1	100	76.2	97	93.2						
8	50	100	79.5	81.5	78.1						
Mean	61.5^{b}	80.3^{a}	58.7^{b}	65.4^{ab}							
Ileal Tissue											
0	0	16.6	21.2	0.8	9.7	13.10	0.001	0.001	0.697	0.001	0.817
2	100	100	100	100	100						
4	83.3	100	100	80.8	91.3						
8	83.3	83.3	61.2	80.8	77.2						
Mean	66.7	75	71.1	65.8							

Table 7 (cont'd). Effects of antibiotics or antibiotic alternatives on *Salmonella* presence (%) in feces, mesenteric lymph nodes (MLN), and ileal and cecal contents and tissue of weanling pigs following a *Salmonella enterica* var Typhimurium challenge [1].

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad.	Linear	Trt.	Day	Trt.×Day
Cecal Contents											
0	16.6	0	0	0.7	4.3	7.43	0.001	0.001	0.002	0.001	0.001
2	100	100	99.6	100	100						
4	100	100	99.6	100	100						
8	100	100	39.6	100	85.1						
Mean	79.2	75	59.7	75.7							
Cecal Tissue											
0	0	0	1.1	1.4	0.6	10.59	0.001	0.001	0.834	0.001	0.303
2	100	100	100	100	100						
4	83.3	100	100	100	96.5						
8	100	66.6	61.1	81.4	77.3						
Mean	70.8	66.7	66.1	71.4							

[ab] Values with different superscripts differ by $P < 0.10$ by means separation using the Duncan multiple range test.

Table 8. Effects of antibiotics or antibiotic alternatives on *Enterobacteriaceae* counts in the ileum and cecum of weanling pigs following a *Salmonella enterica* var Typhimurium challenge [1].

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad.	Linear	Trt.	Day	Trt.×Day
Ileum, log_{10} cfu/g											
0	8.0	6.8	6.9	6.8	7.2	0.662	0.001	0.130	0.144	0.001	0.635
2	7.9	6.6	6.4	7.6	7.1						
4	6.0	6.3	6.2	5.2	5.9						
8	8.2	7.6	7.9	7.6	7.8						
Mean	7.5	6.8	6.9	6.8							
Cecum, log_{10} cfu/g											
0	8.2	6.7	7.1	6.7	7.2	0.553	0.088	0.303	0.015	0.165	0.860
2	8.2	6.6	7.2	6.9	7.2						
4	7.3	6.9	6.6	6.6	6.8						
8	7.9	7.8	7.3	7.4	7.6						
Mean	7.9^{x}	6.9^{y}	7.0^{y}	6.9^{y}							

[xy] Values with different superscripts differ by $P < 0.05$ by means separation using the Duncan multiple range test.

Table 9. Effects of antibiobics or antibiotic alternatives on fecal and cecal *Enterococcus* population of weanling pigs following a *Salmonella enterica* var Typhimurium challenge.

Enterococcus spp.	Treatments				SEM
	DFM	Acid	Pos. ctrl.	Neg. ctrl.	
Fecal, log_{10} cfu/g d -1 pre-challenge	7.02[a]	5.96[ab]	5.78[b]	6.06[ab]	0.562
Cecal contents, log_{10} cfu/g d 8 post-challenge	5.83	4.97	5.04	4.82	0.863

[ab] Values with different superscripts differ by $P < 0.10$ by means separation using the Duncan multiple range test.

Table 10. Effects of antibiotics or antibiotic alternatives on TNFα concentration in serum, and small intestinal mucosa of weanling pigs following a *Salmonella enterica* var Typhimurium challenge [1, 2].

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad.	Linear	Trt.	Day	Trt.×Day
Serum, pg/mL											
0	121.9	109.9	68.6	90.8	97.8	12.58	0.108	0.077	0.814	0.043	0.461
2	113.1	116.3	109.2	98.9	109.4						
4	118.7	124.9	156.8	130.9	132.9						
8	144.9	102.9	120.9	128.5	124.2						
Mean	124.7	113.5	113.8	112.3							
Duodenum, pg/g											
0	35.1	22.6	30.7	43.8	33.0	4.25	0.163	0.106	0.910	0.012	0.173
2	8.7	19.2	14.5	13.9	14.1						
4	30.8	16.8	29.9	20.5	24.5						
8	5.5	34.7	19.6	14.8	18.6						
Mean	20.0	23.3	23.7	23.3							
Jejunum, pg/g											
0	28.8	12.7	6.0	25.5	18.2	16.87	0.141	0.432	0.608	0.178	0.761
2	11.7	17.0	15.7	13.5	14.5						
4	22.3	9.74	33.1	56.1	30.3						
8	7.5	12.9	2.3	9.4	8.1						
Mean	17.6	13.1	14.3	26.1							
Ileum, pg/g											
0	69.6	96.9	135.9	137.7	110.0	11.52	0.008	0.001	0.284	0.001	0.630
2	21.1	40.5	51.1	28.5	35.3						
4	29.5	28.5	32.9	77.7	42.2						
8	21.7	20.3	13.5	12.5	17.0						
Mean	35.5	46.5	58.4	64.1							

[1] TNFα concentration in serum is reported as pg/mL of blood.

[2] TNFα concentration in duodenal, jejunal, and ileal mucosal tissue is reported as pg adjusted to an average protein yield of 0.066 g in duodenal mucosa, 0.049 g in jejunal mucosa, and 0.050 g in ileal mucosa.

Table 11. Effects of antibiotics or antibiotic alternatives on basal short circuit current, active glucose, phosphorus and glutamine transport, and serotonin and carbachol induced chloride ion secretion in the jejunum of weanling pigs following a *Salmonella enterica* var Typhimurium challenge.

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad.	Linear	Trt.	Day	Trt.×Day
Basal Isc, μA/cm^2											
0	36.8	31.1	35.6	23.2	31.7	4.84	0.009	0.013	0.001	0.004	0.296
2	36.3	32.4	16.9	19.7	26.3						
4	20.5	24.7	17.0	15.7	19.4						
8	36.8	24.7	15.8	15.6	23.2						
Mean	32.6^x	28.2^x	21.2^y	18.6^y							
Glucose, ΔIsc, μA/cm^2											
0	34.6	72.1	9.9	61.3	44.5	11.96	0.099	0.016	0.022	0.033	0.009
2	38.9	36.9	48.3	30.3	38.6						
4	29.3	42.8	4.3	22.0	24.6						
8	30.2	24.5	26.0	36.8	29.4						
Mean	33.3	44.1	22.2	37.6							
Phosphorus, ΔIsc, μA/cm^2											
0	19.1	27.9	9.2	28.2	21.1	5.03	0.627	0.001	0.117	0.004	0.050
2	19.5	17.0	26.4	19.2	20.5						
4	14.2	24.3	12.2	17.9	17.2						
8	10.9	9.9	9.2	16.7	11.7						
Mean	15.9	19.8	14.3	20.5							
Glutamine, ΔIsc, μA/cm^2											
0	8.8	16.5	1.6	15.0	10.5	5.98	0.001	0.121	0.610	0.001	0.322
2	21.4	21.8	23.3	15.6	20.5						
4	11.2	20.2	26.0	18.2	18.9						
8	6.0	5.6	5.1	14.1	7.7						
Mean	11.8	16.0	14.0	15.7							

Table 11 (cont'd). Effects of antibiotics or antibiotic alternatives on basal short circuit current, active glucose, phosphorus and glutamine transport, and serotonin and carbachol induced chloride ion secretion in the jejunum of weanling pigs following a *Salmonella enterica* var Typhimurium challenge.

Day	Treatment						Effect of Day		P-value		
Post-challenge	DFM	Acid	Pos. ctrl.	Neg. ctrl.	Mean	SEM	Quad.	Linear	Trt.	Day	Trt.×Day
Serotonin, ΔIsc, μA/cm^2											
0	26.2	28.8	1.3	2.6	14.8	11.6	0.324	0.054	0.693	0.171	0.715
2	3.3	2.4	4.2	6.8	4.2						
4	2.7	4.9	2.6	4.1	3.6						
8	0	0.1	0.1	2.5	0.6						
Mean	8.0	9.1	2.0	4.0							
Carbachol, ΔIsc, μA/cm^2											
0	13.5	10.1	8.2	24.6	14.1	4.89	0.993	0.474	0.066	0.875	0.153
2	22.5	10.5	10.8	13.8	14.4						
4	7.0	13.5	10.6	20.2	12.8						
8	10.5	9.9	14.7	14.3	12.4						
Mean	13.4^{ab}	10.9^{b}	11.1^{b}	18.2^{a}							

^{xy} Values with different superscripts differ by $P < 0.05$ by means separation using the Duncan multiple range test.
^{ab} Values with different superscripts differ by $P < 0.10$ by means separation using the Duncan multiple range test.

VII. Discussion

Salmonella species can persist for long periods of time in swine, often resulting in carriage of the foodborne pathogen through to slaughter (Cray et al., 1995; Wood et al., 1989). Pigs are most susceptible to infection by pathogenic bacteria during the immediate post-weaning period (Aumaître et al., 1995) which may have important long term economic and health implications. Early intervention may not only interrupt carriage, resulting in a carcass with reduced *Salmonella* contamination but also improve the health and growth performance of the weaned pig. The objective of the current study was to determine the efficacy of water delivery of a DFM combination or water acidification as alternatives to low dose dietary antibiotics following a *Salmonella enterica* var Typhimurium challenge.

Water administration of a *Bacillus/Enterococcus* combination in the current study, resulted in improved feed efficiency pre-*Salmonella* challenge and substantially greater ADG post-challenge. In agreement with our findings, a study administering a five strain probiotic combination comprised primarily of *Lactobacillus* reported a similar improvement in ADG above the negative control in weanling pigs following a *Salmonella* challenge (Casey et al., 2007). Low dose in feed antibiotics and water acidification also resulted in better ADG than the negative control during the first four days post-challenge. The advantages of low-dose antibiotics (particularly those included in diets for weanling pigs) have been well documented and include improvements in average daily gain and feed efficiency (Dritz et al., 2002; Gaskins et al., 2002). Cole et al. (1968) reported improvements in BW gain and feed efficiency of weanling pigs with the addition of lactic acid (0.8%) in the drinking water. Additionally, Daniels (1983) observed that drinking water acidified with 20% propionic acid resulted in 2 kg heavier pigs than the control pigs following the nursery phase. Based on current findings, it appears that water acidification functions to enhance growth performance under a disease challenge situation.

Body temperatures were recorded pre- and post-challenge as a clinical sign of *Salmonella* infection. On d 1 post-challenge, body temperature significantly increased above pre-challenge levels. Normal body temperature for a pig ranges between 38.7 – 39.8 ^{o}C. *Salmonella* infection in pigs has been reported to induce an acute phase response including an increase in normal body temperature (Balaji et al., 2000). Water delivery of DFM and organic acids resulted in a reduction in body temperature compared to antibiotic treated pigs. Casey et al. (2007) reported no differences in body temperature between treatments, however, pigs in this study were orally inoculated with 10^8 cfu serovar Typhimurium on three consecutive days compared with one intra-nasal inoculation of the same serovar at 10^9 cfu in the current study.

Carbadox treatment reduced the number of pigs with ileal contents positive for *Salmonella* post-challenge. On d 2 post-challenge, only 79.5 % of ileal samples were positive compared to 100 % of samples from the water acidification and DFM treatments. There was also a reduction in number of pigs containing *Salmonella* in their cecum on d 8 post-challenge as a result of antibiotic treatment while *Salmonella* in the cecum of pigs on all other treatments remained at 100 %. The sub-therapeutic use of antibiotics is an effective means of reducing fecal *Salmonella* shedding and the clinical signs of infection in swine, calves, and chickens (Girard et al., 1976). Ebner and Mattew, (2000) reported a reduction in the incidence of *Salmonella* shedding in pigs receiving apramycin-oxytetracycline treatment compared to control pigs. A reduction in the number of *Salmonella* positive ileal contents was also observed in pigs receiving water delivered DFMs in the current study. A number of reports have been published describing probiotic-mediated reduction in intestinal *Salmonella* numbers (Genovese et al., 2003; Fedorka-

Cray et al., 1999). Both of these studies challenged pigs with serovar Choleraesuis and reported a reduction in *Salmonella* in cecal contents and ileocolic junction contents as a result of administration of a competitive exclusion culture originally isolated from pig cecum. The culture used consisted of a large mixture of bacterial species including *Enterococcus faecium*. Neonatal pigs as well as weaned pigs were used in this study and the competitive exclusion culture was given once orally compared to a continuous water administration of the *Bacillus/Enterococcus* mixture in the current study. Another study reported the ability a five strain *Lactobacillus/Pediococcus* mixture to reduce fecal shedding of *Salmonella* in weaned pigs following a *Salmonella typhimurium* challenge (Casey et al., 2007). Likewise, Pascual et al. (1999) found that *Lactobacillus salivarus*, when administered through drinking water or in feed reduced *Salmonella* presence in bird cecum from 100 % on d 1 to 0 % on d 21.

Water acidification had no effect on the prevalence of *Salmonella* positive samples throughout the present study. *Salmonella* enumeration was not conducted in the current study so therefore it is impossible to decipher if *Salmonella* number was decreased by treatment. The use of organic acids in drinking water is reported to reduce *Salmonella* carriage in poultry (Van Immerseel et al., 2006). However, similar to our findings, Letellier et al. (1999) failed to observe reduced infection by *Salmonella* as a consequence of acidified drinking water but *Salmonella* was not quantified in this study either. There was a significant decrease in the presence of *Salmonella* in MLN, ileal and cecal contents and tissue from d 2 to 8 post-challenge. These findings are in accordance with another report of reduced *Salmonella* shedding from d 1 to d 15 post-infection (Casey et al., 2007).

The *Enterobacteriaceae* population encompasses an array of gram-negative micro-organisms which are generally accepted as being pathogenic including *Salmonella* and *E. coli* (Dowd et al., 2007). Enumeration of ileal *Enterobacteriaceae* in the current study observed no effect of treatment on population number even though antibiotic and water administered DFM treatments tended to reduce the presence of *Salmonella* in ileal contents. It is worth noting that *Salmonella* makes up just one species in this large group which includes, among others, Citrobacter, Shigella, Proteus and Eubacteria. It is possible that a decrease in one species may create the opportunity for proliferation of another species while still maintaining similar total *Enterobacteriaceae* counts. This observation may also lend some explanation as to the return of ileal and cecal *Enterobacteriaceae* counts to d 2 post-challenge levels on d 8 post-challenge after a significant decrease on d 4 post-challenge. Cecal contents of DFM treated pigs had greater counts of *Enterobacteriaceae* than all other treatments. This correlates to some degree to the numerically higher number of positive *Salmonella* samples from the same pigs. However, because *Salmonella* was not quantified it is not possible to definitively state that the higher *Enterobacteriaceae* counts are a function of higher *Salmonella* counts in the cecum.

Animals infected with *Salmonella* have been reported to have areas on their intestinal mucosal cells devoid of microvilli or with microvilli shortened by loss of their tips (Bayer et al., 1977; Wallis et al., 1986). Our observations suggest an ability of water administered DFMs to attenuate this decline in duodenal villus height on d 4 post-challenge. There are numerous reports of the beneficial effects of probiotics on gut architecture including two individual studies evaluating the effects of different probiotic strains on epithelial structure. These studies observed longer villus height in the jejunum of pigs supplemented with *Bacillus cereus* (Klein and Schmidt, 1997) and *Bacillus toyoi* or *Saccharomyces boulardii* (Goerke, 2000). Shirkey et al. (2003) also found that oral inoculation of pigs with *Lactobacillus fermentum* resulted in a greater

villus height:crypt depth than pigs inoculated with non-pathogenic *E. coli* K88 or adult porcine feces.

There are, however, no reports that we are aware of documenting changes in gut architecture in response to probiotics or water acidification following a *Salmonella* challenge. Throughout the current study, *Salmonella* induced deepening of crypts was reported in the duodenum, jejunum and ileum over an 8 day period. Increased crypt depth indicates that the proliferative activity in the crypts is augumented (Hedemann et al., 2005). Increased proliferative rates in crypts may also give rise to increased rates of cell loss and villus shortening (Pluske, 2001). The increase in crypt depth observed in the present study is most likely due to an attempt to repair mucosal cell damage cause by the *Salmonella* infection (Jepson and Clark, 2001).

In the early stages of *Salmonella* infection, macrophage activation by cytokines such as TNFα seems to be the prerequisite for the destruction of bacteria (Gulig et al., 1997). In the current study, serum TNFα was elevated above pre-challenge levels by d 4 post-*Salmonella* infection, but by d 8 post-challenge TNFα levels were not different. There are a number of reports of increased plasma levels of TNFα in pigs following i.p. LPS injection (Warren et al., 1997; Webel et al., 1997). However, Balaji et al. (2000) failed to detect an increase in plasma TNFα in pigs following an oral *Salmonella typhimurium* challenge. In this study, TNFα was only measured until d 5 post-infection, since we did not detect an increase in blood TNFα until d 4 post-infection, it is possible that the increase in TNFα had yet to happen.

TNFα levels in jejunal mucosa in the present study following *Salmonella* infection, were similar to pre-infection levels while TNFα levels in duodenal and ileal mucosa were significantly lower than pre-infection levels. These findings are contrary to a number of reports of elevated TNFα in pig intestine following a *Salmonella typhimurium* challenge (Šplichal et al., 2002, 2005). Skjolaas et al. (2007) also observed an increase in TNFα mRNA expression following *Salmonella* infection of a swine jejunal epithelial cell line. However, the increase in intestinal TNFα levels in these studies were all observed within 24 h of infection. In our study, intestinal TNFα was first measured 48 h post-infection and our failure to detect an increase in TNFα may be explained by a very fast turnover rate of TNFα as observed by Jesmock et al. (1992) in *E. coli* infected pigs. There was no affect of treatment on TNFα levels in serum or mucosal tissue in the present study. Shirkey et al. (2003) reported an increase in pro-inflammatory cytokines in distal sections of the small intestine when pigs were treated with a non-pathogenic strain of *E. coli* or adult porcine feces. However, similar to our findings with a *Bacillus/Enterococcus* combination, these pro-inflammatory cytokines failed to increase when pigs were treated with *Lactobacillus fermentum*. *Lactobacillus reuteri* (LR) has demonstrated direct anti-inflammatory activity in human epithelial cells in that live, but not heat-killed, or gamma-irradiated LR were able to inhibit *Salmonella enterica* serovar Typhimurium induced TNFα and IL-8 secretion (Ma et al., 2004). There is evidence to suggest that probiotics may play an immunomodulatory role, however, data documenting their effect during a *Salmonella* challenge is very scarce.

Phosphorus active transport were linearly decreased from d 0 to d 8 post-infection. This may be explained by the observed decrease in jejunal villus height which would lead to a reduction in absorptive surface area. Glucose uptake however, was increased immediately post-challenge by in-feed antibiotics and water delivered DFM. Interesingly, *Salmonella* infection has been shown to alter glucose metabolism in the ileal mucosa of rats. Rodenburg et al. (2007) reported a 3 fold increase in expression of glucose transporters SGLT-1, GLUT-2 and GLUT-5, which suggests a greater need for glucose by infected cells but this does not correspond with current findings. There are a number of studies reporting an enhancement of glucose uptake by

probiotics in non-pathogen challenge pigs (Lodemann et al., 2006; Breves et al., 2000). However, under a disease challenge situation, probiotics may also be successful at improving nutrient uptake. Active glutamine transport was increased on d 2 to 4 post-challenge but returned to pre-challenge levels by d 8 post-challenge. Glutamine is one of the most important fuels of intestinal epithelial cells (Lodemann et al., 2006) and this increased demand for glutamine post-challenge may be required to repair damage to intestinal epithelium caused by *Salmonella*.

Salmonella, by nature of its pathogenesis, stimulates chloride ion secretion (Groisman and Ochman, 2000). Water acidification and antibiotic treatments successfully attenuated this chloride ion secretion in the current study compared to the control. Antibiotic and water delivered DFM treatment also appeared to attenuate the decline in active ion transport seen in falling basal Isc values. Some antibiotics are reported to manipulate Na^+/K^+ ATPase activity and Na^+ mediated active transport (Parker, 1990). While there is no direct evidence to definitively prove this hypothesis, it has become apparent in this study that antibiotics function in a manner that promotes blunting of the adverse effects of *Salmonella* pathogenesis on intestinal membrane nutrient transport and function.

In conclusion, water delivery of a DFM combination or organic acid appears to be a successful means of administering a potential antibiotic alternative to weanling pigs. Following a *Salmonella enterica* var Typhimurium challenge, both DFM and organic acid treated drinking water enhanced growth performance and gastrointestinal histology. *Salmonella* presence in ileal contents was reduced by DFM treatment and active glucose transport increased, while water acidification reduced induced chloride secretion. Current findings suggest that under a disease challenge situation water acidification and DFM treated drinking water performed similar or in some cases superior to in feed antibiotics and therefore must be considered as a viable alternative in weanling pig diets.

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