Fermentable Fiber Reduces Recovery Time and Improves Intestinal Function in Piglets Following *Salmonella typhimurium* Infection[1,2]

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ABSTRACT Diarrhea is a leading cause of morbidity and mortality in infants. The addition of fiber to infant formulas reduces recovery time following pathogenic infection in infants >6 mo old, but effects on neonates are unknown. The hypothesis that fermentable fiber reduces infection-associated symptoms and enhances intestinal structure and function in neonates was examined. Piglets (2 d old) were randomly assigned to receive formula alone (control) or formula containing methylcellulose (MCEL), soy polysaccharides (SPS) or fructooligosaccharides (FOS) for 14 d. On d 7, piglets were further randomly assigned to receive an oral gavage of *Salmonella typhimurium* or serve as noninfected controls. *S. typhimurium* infection produced diarrhea in controls and MCEL groups, but not in the SPS and FOS groups. Postinfection physical activity was lower ($P = 0.0001$) in the controls than in all other groups. Ileal lactase activity was reduced ($P < 0.05$) following infection in the control group but not in the MCEL, SPS and FOS groups. Ileal mucosal barrier function, measured as resistance, was impaired by infection ($P < 0.05$) in the control and SPS groups, but was unaltered in the jejunum and colon. Total ion transport and basal short-circuit current were higher ($P < 0.05$) in jejunum than in ileum and colon, irrespective of diet or infection. SPS and FOS increased ($P < 0.05$) ileal glutamine transport relative to piglets fed MCEL, irrespective of infection. Because fermentable fiber enhances intestinal function and reduces the severity of *S. typhimurium* infection–associated symptoms, it may be a cost-effective way in which to reduce the severity of pathogenic infection–associated symptoms in infants. J. Nutr. 133: 1845–1852, 2003.

KEY WORDS: • *dietary fiber* • *diarrhea* • *intestine* • *piglets* • *Salmonella typhimurium*

The incidence of acute diarrhea in children <3 y old in the United States is on the order of 1.3–2.3 episodes per child per year (1). Diarrhea accounts for ~9% of all hospitalizations of children <5 y old (1). According to the Centers for Disease Control, ~300 children <5 y old die each year in the United States of diarrhea and dehydration (2). At highest risk of dying from diarrhea are children who were born prematurely (3), children of adolescent mothers, children of mothers who received poor prenatal care, and children who are poor and belong to minority groups (4). The combined cost of inpatient and outpatient care for pediatric diarrhea is >$2 billion/y (1).

Breast-fed infants have fewer episodes of acute diarrhea and intestinal infections than formula-fed infants (5). Human milk promotes the development of gastrointestinal (GI)[5] digestive and absorptive function, which may also be protective against diarrheal diseases (6). Despite the clear advantages of breast-feeding, recent surveys show that only ~55% of mothers in the United States nurse their infants in the hospital, and at 6 mo, only 20% are still breast-feeding their infants (4). Clearly, efforts must be made to optimize the composition of infant formula so that the majority of infants receive a diet that provides the advantages of breast milk.

There is physiologic rationale to support the addition of dietary fiber to infant formulas. Dietary fiber normalizes colonic function by increasing fecal weight and bowel frequency (7). Furthermore, the bacterial degradation of dietary fiber or other fermentable substrates, such as fructooligosaccharides, to SCFA, is essential in maintaining small bowel and colonic mucosal structure and function (8). Despite several clinical trials with fiber-supplemented liquid formulas in adult hospitalized patients (9–12), relatively little work has been done in pediatric populations. Soy polysaccharides reduced the duration of bacterial or viral diarrhea in developing countries (13). More recently, the use of soy polysaccharide–supplemented formula was reported to reduce the duration of diarrheal symptoms in U.S. infants >6 mo old with acute diarrhea (14).

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[5] Abbreviations used: cfu, colony forming units; CON, control formula; FOS, fructooligosaccharides; GI, gastrointestinal; MCEL, methylcellulose; SPS, soy polysaccharides.

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However, the effect of fermentable fiber on the gastrointestinal tract of healthy or acutely ill newborn infants is unknown. Therefore, the current study tested the hypothesis that fermentable fiber reduces infection-associated symptoms and enhances intestinal structure and function following *Salmonella typhimurium* infection in neonatal piglets.

The neonatal piglet model was chosen due to similarities in nutritional requirements, intestinal physiology and metabolism with the human infant (15,16). The full-term neonatal piglet has a body composition similar to that of the premature human infant (23–31 wk gestation), permitting more invasive methods of investigation while maintaining clinical relevance (17). A human infant doubles its birth weight by 4–6 mo, whereas a piglet doubles its birth weight in 7–10 d, thereby providing a rapid model of growth and development for focused investigations (15,17). On the basis of these data, the neonatal piglet model used for this study is a critical element of the experimental design and has yielded data with good clinical applicability.

MATERIALS AND METHODS

Animal research and experimental diets. Four piglets were selected from each litter and assigned randomly by weight to each of the four diet groups. Colostrum-fed piglets (2 d old; $n = 48$) were obtained from the University of Illinois Imported Swine Facility Laboratory. Piglets were assigned randomly to receive one of four diets for 14 d: *1*) a nutritionally complete nonmedicated sow's milk replacer formula (CON; Advance Baby Pig Liqui-Wean, Milk Specialties Company, Dundee, IL); *2*) control formula supplemented with 7.5 g/L methylcellulose (MCEL; nonfermentable); *3*) control formula supplemented with 7.5 g/L soy polysaccharide (SPS; moderately fermentable); or *4*) control formula supplemented with 7.5 g/L fructooligosaccharides (FOS; completely fermentable). Total daily formula intake was 15 mL/(kg · h), offered as an equal feeding every 12 h. Formula was provided to the nipple by gravity flow via tubing attached to a 1-L enteral nutrition bag (Flexiflow Easyfeed, Ross Laboratories, Columbus, OH). Unconsumed and spilled formula was quantified after each feeding period so that total consumed formula was estimated accurately.

On experimental d 7, piglets within each diet group were further randomized to receive an oral gavage of 10^{10} colony forming units (cfu) of *S. typhimurium* 798 or 9 g/L saline. *S. typhimurium* 798, a nalidixic acid resistant strain, was originally isolated from a pig and has been used extensively to create carrier piglets (18,19). All piglets continued to receive their assigned diets for an additional 7 d. Body weight, physical activity level and stool consistency were visually assessed daily. Rectal temperature was measured (CMATE Digital HT1856–1, Henry Schein, Denver, PA) before the morning meal every other day, except for the third day following infection when it was measured daily.

On experimental d 14, piglets were killed by an intracardiac injection of euthanasia-5 solution (72 mg sodium pentobarbital/kg body). The small intestine and colon were rapidly removed, flushed with ice-cold 9 g/L saline, weighed and measured. The small intestine was partitioned from the ligament of Treitz to the ileocecal valve and divided into thirds. The most proximal and distal third were used to obtain jejunal and ileal samples, respectively. Two 2-cm samples of jejunum, ileum and mid-colon were obtained immediately for electrophysiologic analysis in modified Ussing chambers, as described below. An additional 0.5-cm section from each segment was fixed in 10% formalin buffer for 24 h and then transferred to 70% ethanol for subsequent histomorphological analysis. The remaining portion of each segment was opened longitudinally, and the mucosa was scraped from the luminal surface using a glass microscope slide and snap-frozen for later analyses of enzyme activity. Colonic contents were frozen for subsequent measurement of SCFA concentration.

Approval was received for all animal procedures from the Institutional Animal Care and Use Committee of the University of Illinois, Urbana-Champaign, IL. Infected and noninfected piglets were housed individually within separate high efficiency particle air–filtered units of the containment area of the Edward R. Madigan Laboratory at the University of Illinois. Cage temperature was maintained at 30°C with a 12-h light:dark cycle. All personnel followed a one-way traffic flow inside the animal facility to ensure that noninfected piglets were not contacted after infected piglets. Equipment was treated with a 10 g/L bleach solution and personnel showered after each visit to the containment area. Personnel wore disposable coveralls, masks, eye shields and double gloves when entering the containment area.

Physical activity. Visual assessment of physical activity of each piglet was performed for 5 min by the same individual each morning and graded using the following scale: 1 = lethargic; 2 = weak; 3 = active. Piglets were evaluated according to their responses to a variety of stimuli such as feeding, handling during weighing and measuring of rectal temperature. The level of physical activity was quantified for each piglet using a scale that assigned a numerical value as follows: active piglets responded to all three events, weak piglets respond to two events and lethargic piglets were unresponsive during the entire observation period.

Stool consistency. Visual assessment of stool consistency was assessed by the same individual each morning using a modification of the method described by Zijlstra et al. (20). Recent excreta were graded using the following scale: 1 = solid; 2 = semisolid; 3 = loose; 4 = watery. The observations of stool consistency were quantified for each piglet using a scale that assigned a numerical value. Multiple piglets could receive the same score.

Histomorphology. Formalin-fixed samples of jejunum, ileum and colon were embedded in paraffin, sliced to ~5 μm with a microtome, and stained with hematoxylin and eosin. Villous height, villous width, crypt depth and muscularis thickness were measured using a Nikon microscope (Fryer, Carpentersville, IL) and Image-1 software (Universal Imaging, Westchester, PA) in 8–10 vertically well-orientated villi and crypts.

Disaccharidase activities. Jejunal and ileal mucosa were homogenized in a buffer containing protease inhibitors (0.45 mol/L sodium chloride, 0.001 mol/L phenylmethylsulfonyl fluoride, 0.002 mol/L iodoacetic acid). Analyses were completed on homogenates that did not undergo more than one freeze-thaw cycle. To reduce both intra- and interassay variability, all enzyme activity assays were performed in duplicate at one time. Sucrase and lactase specific activities were determined by the method of Dudley et al. (21). Enzyme activities were determined by incubating intestinal homogenates with the appropriate disaccharide (lactose or sucrose) for 30 min at 37°C. Liberated glucose was assayed by coupling the reaction to glucose-oxidase (Sigma, Chemical, St. Louis, MO).

Electrophysiological measurements in modified Ussing chambers. Techniques assessing gastrointestinal function through measurement of ion flux in modified Ussing chambers were described previously (22). Duplicate sections of jejunum, ileum and colon were cut longitudinally along the mesentery and mounted in modified Ussing chambers (Physiologic Instruments, San Diego, CA) to expose 0.5 cm^2 of tissue. The tissue was bathed in 8 mL of oxygenated (95% O_2/5% CO_2) modified Kreb's buffer maintained at 37°C with a circulating water bath (Fischer Scientific, Itasca, IL). Basal transmucosal short-circuit current, resistance and potential difference were measured after a 20- to 30-min equilibration period. Sodium-dependent nutrient transport was determined by measuring the change in short-circuit current induced by the addition of either 10 mmol/L glucose or glutamine to the medium in the mucosal reservoir. The modified Ussing chambers were connected to dual-channel voltage/current clamps (VCC MC2, Physiologic Instruments) with a computer interface that allowed for real time data acquisition and analysis (Acquire & Analyze software, Physiologic Instruments).

SCFA concentration in colonic contents. The concentrations of acetate, propionate and butyrate of colonic contents were determined by gas chromatography obtained at necropsy as an indicator of the degree of fiber fermentation (Hewlett-Packard 5890A Series II, Supelco, Bellefonte, PA). Duplicate samples of colonic contents (1 g) were frozen at −20°C immediately after collection. Samples were acidified and diluted (1:4) with 25% of metaphosphoric acid and distilled water for 30 min. After room-temperature centrifugation at

25,000 × *g* for 20 min, the supernatant was obtained and placed in microcentrifuged tubes and frozen at −20°C. After freezing, samples were further centrifuged at 12,000 × *g* for 10 min and placed in sealed vials to be processed in the gas chromatogram and glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200:1 mol/L H_3PO4 on 80/100 mesh Chromsorb WAW (Supelco). Nitrogen was used as the carrier gas with a flow rate of 75 mL/min. Oven (125°C), detector (175°C) and injector (180°C) temperatures were controlled (23).

Statistical methods. All data are expressed as means ± SEM. A completely randomized block two-way repeated-measures ANOVA was used to compare consecutive measurements of body weight, rectal temperature, physical activity and stool consistency. Sources of variation were block [experimental replicate (n = 6)], diet (n = 4), infection (n = 2) and diet interaction with infection. Analyses assessing intestinal structure and function were compared among treatment groups using a completely randomized block three-way ANOVA. Sources of variation were block [experimental replicate (n = 6)], diet (n = 4), infection (n = 2), intestinal segment (n = 3) and the appropriate interactions. Total SCFA concentration in the colonic contents was compared among treatments using a completely randomized block two-way ANOVA, blocking for experimental replicate with the main effects of diet and infection. Computations among main effects were performed using the general linear models procedure in SAS (Version 8e; SAS Institute, Cary, NC), whereas post-hoc analyses used protected multiple comparisons (pdiff) to identify experimental groups that differed significantly. Statistical significance was defined as $P \leq 0.05$ and $P \leq 0.0001$ as highly significant. Statistical trends were defined as $P \leq 0.1$.

RESULTS

Clinical measurements

Dietary intake and piglet growth. All piglets consumed the entire volume of formula provided each day. Weight gain over the entire study period was 2.7 ± 0.1 kg/piglet. Body weight did not differ among experimental groups at any time of the study, indicating the isoenergetic formulation of the diets as well as equivalent consumption among piglets, regardless of diet or infection (data not shown).

Body temperature. Rectal measurement of body temperature did not differ due to diet or infection at any time (data not shown). The body temperature of all piglets was maintained within the normal range for neonatal piglets (38.4–39.8°C) throughout the study.

Physical activity. Before infection, all piglets were active and displayed normal behavior. However, 48-h following *S. typhimurium* infection, activity was significantly reduced ($P \leq 0.02$) in piglets consuming the control formula compared with all other diet groups and noninfected controls (**Fig. 1**).

Stool consistency. Compared with noninfected controls, *S. typhimurium* infection resulted in significantly higher stool scores, indicative of diarrhea, for 72 h postinfection in the control group and at 72 h postinfection in the MCEL group (**Fig. 2**). The SPS-fed group had semisolid stools only at 24 h postinfection, with a recovery of normal stool consistency within 48 h postinfection. Stool consistency was not altered by infection in the FOS-fed group (Fig. 2).

Small intestinal structure

Small intestinal weight and length. Small intestinal weight (g/kg body) and length (cm/kg body) of the jejunum, ileum and colon did not differ among piglets due to infection or consumption of experimental diets (data not shown). Liver and spleen weights were not significantly affected by diet or infection (data not shown).

Histomorphology. Jejunal and colonic histomorphology was unaffected by diet and infection (data not shown). Ileal villous height tended to be greater (P = 0.06) following infection in piglets consuming the SPS and FOS supplemented formulas compared with the control formula group. However, villous width and crypt depth did not differ among the groups (data not shown).

Small intestinal function

Lactase activity. Lactase activity was not altered by diet or infection in the jejunal mucosa. Ileal lactase activity was reduced ($P \leq 0.05$) following infection in the control group but not the MCEL, SPS and FOS groups (**Table 1**).

Sucrase activity. Jejunal sucrase activity was not altered by diet or infection. However, ileal sucrase activity was increased ($P < 0.05$) following infection regardless of diet (Table 1).

Basal ion transport. Basal transmucosal short-circuit current, a measure of active ion transport, was not altered by infection or diet but was higher in the jejunum than all other

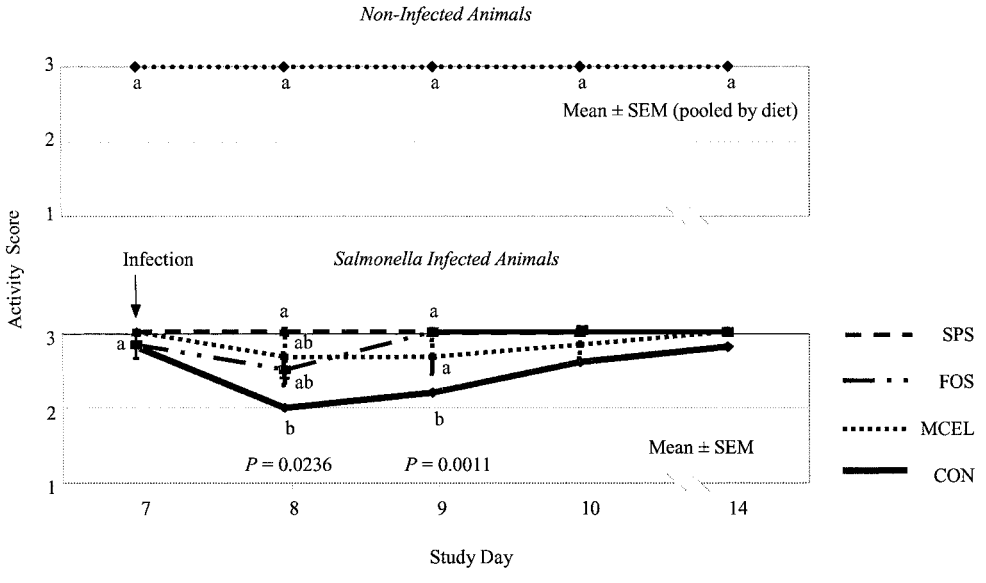

FIGURE 1 Physical activity of noninfected piglets (*upper panel*) and piglets infected (*lower panel*) with *Salmonella typhimurium* consuming control (CON), methylcellulose (MCEL), soy polysaccharide (SPS) or fructooligosaccharide (FOS) diets. Physical activity was scored as 1 = lethargic; 2 = weak; 3 = active, and was assessed before infection, at 24, 48 and 72 h postinfection, and at the end of the study (d 14). Activity in noninfected piglets was unaffected by diet so data were pooled. Data are means ± SEM (n = 6/group). Within each day, means with different letters differ, $P \leq 0.05$.

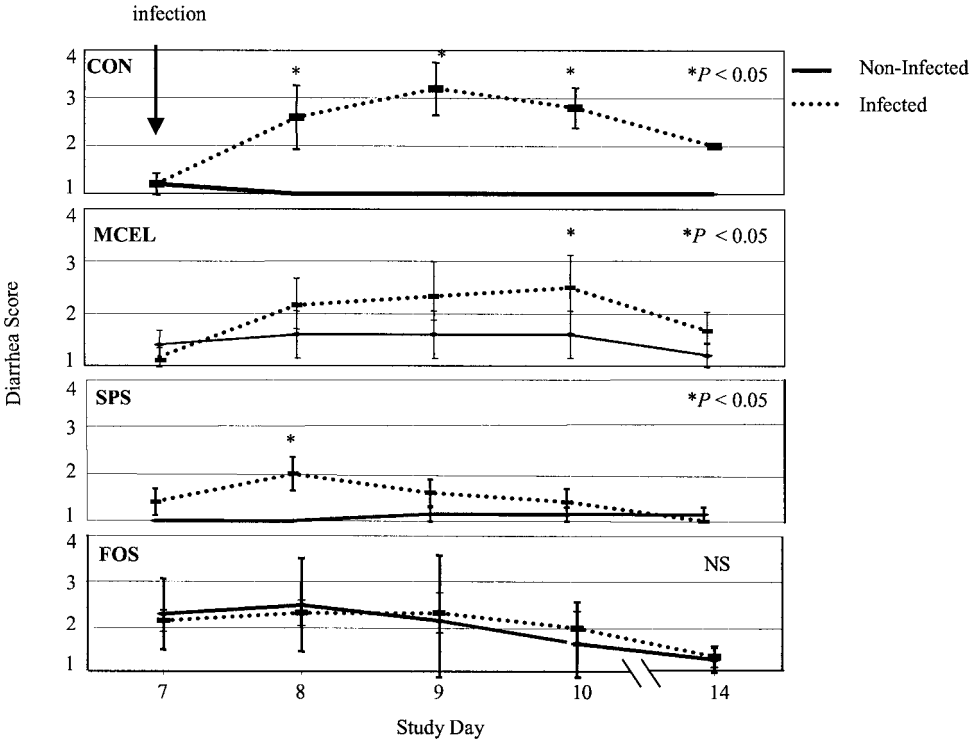

FIGURE 2 Stool consistency of noninfected piglets and piglets infected with *Salmonella typhimurium* consuming control (CON), methylcellulose (MCEL), soy polysaccharide (SPS) or fructooligosaccharide (FOS) diets. Stool was scored before infection, 24, 48 and 72 h postinfection, and at the end of the study (d 14). Stool consistency was evaluated (1 = solid; 2 = semisolid; 3 = loose; 4 = watery) to assess the effect of diet and infection. Diarrhea was present in the CON ($P < 0.05$) at 24, 48 and 72 h postinfection and in MCEL ($P < 0.05$) at 72 h postinfection. The SPS-fed group had semisolid stools 24 h postinfection but recovered by 48 h ($P < 0.05$), whereas the CON group had not recovered by 72 h after infection. Stool consistency in the infected FOS-fed piglets was not altered following infection. Data are means ± SEM (n = 6/group). Within each diet and day, means with different letters differ, $P \leq 0.05$.

intestinal segments. Transmucosal resistance, a measure of passive ion transport, was significantly lower ($P \leq 0.05$) postinfection in the ileum of control and SPS-fed groups compared with their noninfected counterparts (**Table 2**). Transmucosal resistance did not differ following infection in the MCEL and FOS groups (Table 2). No effects of diet or infection were observed in the jejunum or colon. Potential difference, a measure of total ion transport, was higher in jejunum than all other intestinal segments, irrespective of diet or infection.

Nutrient transporter activity. Jejunal, ileal and colonic transport of glucose by the brush border sodium-glucose cotransporter was not significantly altered by infection or experimental diet (Table 2). However, ileal sodium-dependent transport of glutamine was greater ($P \leq 0.05$) in the SPS and FOS groups compared with the MCEL group, irrespective of infection (**Fig. 3**). Diet did not affect nutrient transport activity in the jejunum and colon (data not shown).

SCFA concentration in colonic contents. Total SCFA concentration (sum of acetate, propionate and butyrate) was significantly greater ($P \leq 0.05$) in the colonic contents of the SPS and FOS groups compared with the control and MCEL groups, regardless of infection (**Fig. 4**).

DISCUSSION

Results from this study indicate that consumption of fermentable substrate reduces recovery time and improves infection-associated symptoms in piglets following *S. typhimurium* infection. *Salmonella* infection was selected as a model system for a bacterially induced diarrhea that causes sporadic, self-limiting, mild diarrhea during wk 1 postchallenge. A dose of 10^8 cfu is commonly used in 5- to 6-wk old piglets (19); however, data regarding the appropriate dose in neonatal piglets are lacking. A dose-response study, conducted in our laboratory, indicated that an oral gavage with 10^{10} cfu reliably

TABLE 1

Jejunal and ileal enzyme activities of noninfected piglets and piglets infected with Salmonella typhimurium consuming control (CON), methylcellulose (MCEL), soy polysaccharide (SPS) or fructooligosaccharide (FOS) diets[1,2]

	CON		MCEL		SPS		FOS	
	Noninfected	Infected	Noninfected	Infected	Noninfected	Infected	Noninfected	Infected
	μmol glucose/(min · g protein)							
Jejunum								
Lactase	182 ± 26	225 ± 28	347 ± 57	190 ± 37	275 ± 70	204 ± 39	223 ± 23	319 ± 44
Sucrase	95 ± 28	136 ± 59	102 ± 22	93 ± 29	86 ± 26	115 ± 11	96 ± 15	100 ± 18
Ileum								
Lactase	343 ± 88^{a}	90 ± 86^{b}	204 ± 39^{ab}	91 ± 30^{b}	357 ± 152^{a}	218 ± 66^{ab}	120 ± 31^{b}	221 ± 62^{ab}
Sucrase	28 ± 12^{b}	49 ± 13^{a}	29 ± 12^{b}	39 ± 11^{a}	26 ± 9^{b}	60 ± 21^{a}	29 ± 12^{b}	73 ± 42^{a}

[1] Values are means ± SEM, n = 6.
[2] Means in a row with different superscripts differ, $P \leq 0.05$.

TABLE 2

Glucose and ion transport of noninfected piglets and piglets infected with Salmonella typhimurium consuming control (CON), methylcellulose (MCEL), soy polysaccharide (SPS) or fructooligosaccharide (FOS) diets[1,2,3]

	CON		MCEL		SPS		FOS	
	Noninfected	Infected	Noninfected	Infected	Noninfected	Infected	Noninfected	Infected
Jejunum								
Transmucosal resistance, $\Omega \cdot cm^2$	77.7 ± 5.3	70.8 ± 4.5	71.3 ± 15.9	78.2 ± 12.9	63.4 ± 15.5	71.7 ± 4.51	87.1 ± 14.2	115 ± 16.3
Basal Isc, $\mu A/cm^2$	25.3 ± 4.2	21.6 ± 4.4	18.8 ± 2.3	33.6 ± 1.0	30.4 ± 4.1	25.2 ± 4.0	16.5 ± 2.7	16.3 ± 4.5
Potential difference, *mV*	1.88 ± 0.24	1.49 ± 0.32	1.42 ± 0.46	2.40 ± 0.52	2.37 ± 1.01	1.79 ± 0.24	1.32 ± 0.23	1.52 ± 0.20
Glucose transport, $\Delta Isc/cm^2$	3.55 ± 1.45	4.74 ± 3.84	4.15 ± 1.67	3.01 ± 0.56	1.92 ± 0.84	3.54 ± 1.22	3.69 ± 1.23	1.88 ± 1.06
Ileum								
Transmucosal resistance, $\Omega \cdot cm^2$	206 ± 35^a	115 ± 24^b	179 ± 39^a	190 ± 32^a	202 ± 35^a	123 ± 20^b	118 ± 25^b	158 ± 36^{ab}
Basal Isc, $\mu A/cm^2$	3.92 ± 1.9	5.37 ± 1.91	1.25 ± 1.15	4.18 ± 2.64	3.57 ± 1.13	5.65 ± 1.50	8.23 ± 2.31	10.9 ± 3.7
Potential difference, *mV*	0.65 ± 0.25	0.64 ± 0.25	0.21 ± 0.23	0.83 ± 0.67	0.68 ± 0.31	0.63 ± 0.13	0.95 ± 0.36	1.13 ± 0.18
Glucose transport, $\Delta Isc/cm^2$	2.81 ± 1.56	4.92 ± 2.05	1.43 ± 0.76	2.84 ± 0.69	1.08 ± 0.50	4.29 ± 1.50	2.50 ± 0.35	4.82 ± 2.45
Colon								
Transmucosal resistance, $\Omega \cdot cm^2$	84.5 ± 36.2	79.1 ± 11.1	60.1 ± 12.2	66.5 ± 6.7	72.8 ± 3.5	63.3 ± 7.3	57.3 ± 13.2	62.9 ± 17.3
Basal Isc, $\mu A/cm^2$	14.1 ± 7.0	14.6 ± 1.9	17.9 ± 11.5	66.5 ± 6.8	17.4 ± 5.3	12.3 ± 2.5	25.9 ± 9.3	20.6 ± 4.6
Potential difference, *mV*	0.55 ± 0.27	1.12 ± 0.17	0.78 ± 0.25	66.5 ± 6.9	1.28 ± 0.36	0.84 ± 0.23	1.18 ± 0.27	0.94 ± 0.10
Glucose transport, $\Delta Isc/cm^2$	2.13 ± 0.68	1.76 ± 0.44	1.11 ± 0.30	66.5 ± 6.10	8.15 ± 6.71	1.35 ± 0.41	1.01 ± 0.31	2.21 ± 0.92

[1] Values are means ± SEM, $n = 6$.
[2] Means in a row with different superscripts differ, $P \leq 0.05$.
[3] Basal Isc (short-circuit current) and potential difference were higher ($P = 0.0001$) in jejunum than ileum and colon, regardless of diet or infection.

produced a bacterial challenge of the desired severity. The clinical data obtained in this study confirm the appropriateness of this *S. typhimurium* challenge because control piglets experienced both diarrhea and lethargy; however, changes in food intake, body weight gain, fever or any other symptoms of systemic infection were not observed.

The experimental diets were designed to deliver a range of fiber fermentability to the anaerobic bacteria residing in the distal GI tract. These substrates were added to the sow's milk replacer formula at a dose of 7.5 g/L based on our objective to provide diets resulting in a gradient of SCFA production while maximizing GI tolerance. Although many dietary fibers are well tolerated at doses as high as 30 g/100 g (24), reports of FOS-induced osmotic diarrhea and GI disturbances at 50 g/L (27 g/100 g) (8,25) prompted us to limit supplementation of all substrates to 7.5 g/L. In addition, these doses are physiologically relevant because they could be attained through consumption of a mixed diet. Howard et al. (26) reported 3 g FOS/L to be well tolerated in neonatal piglets, but no changes in SCFA production were noted. The appropriateness of the various substrates and the dose consumed was confirmed by the greater SCFA concentrations in the colonic contents of piglets consuming SPS- and FOS-containing formulas (Fig. 4).

Sow's milk replacer formula was used with the neonatal piglets in this study to meet their elevated protein requirement and support their accelerated growth (15). The energy composition of sow's milk replacer formula is 22% protein, 53% carbohydrates (primarily lactose) and 25% fat (Advance Baby Pig Liquiwean), whereas commercial infant formula provides an energy composition of 9% protein, 41–43% carbohydrates (primarily lactose) and 48–50% fat (27). In contrast to human breast milk, neither sow's milk replacer nor human formula contain appreciable amounts of fermentable carbohydrates sources; therefore, the fiber sources used in this project should have similar physiologic effects due to the absence of interacting carbohydrate sources in either sow or human-intended formula.

Consumption of the SPS and FOS diets reduced the severity of the *S. typhimurium*–infection associated symptoms because visual assessment of stool consistency and piglet physical activity of piglets were largely unaffected postinfection. Consumption of these diets resulted in higher colonic SCFA

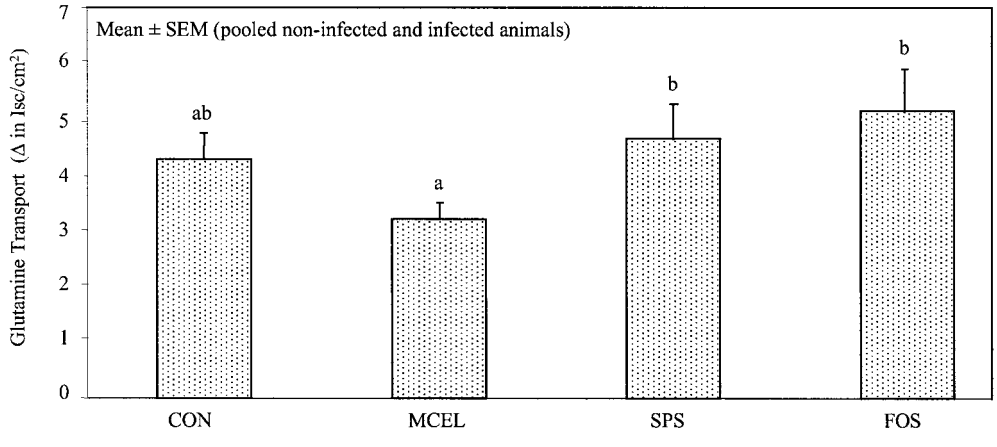

FIGURE 3 Ileal sodium-dependent glutamine transport [Δ short circuit current (Isc)/cm^2] of noninfected piglets and piglets infected with *Salmonella typhimurium* consuming control (CON), methylcellulose (MCEL), soy polysaccharide (SPS) or fructooligosaccharide (FOS) diets. Ileal glutamine sodium-dependent transport was greater in fiber-fed piglets compared with the CON group ($P < 0.05$). There was no significant effect of infection on glutamine transport; thus, data were pooled. Data are means ± SEM ($n = 6$/group). Means with different letters differ, $P \leq 0.05$.

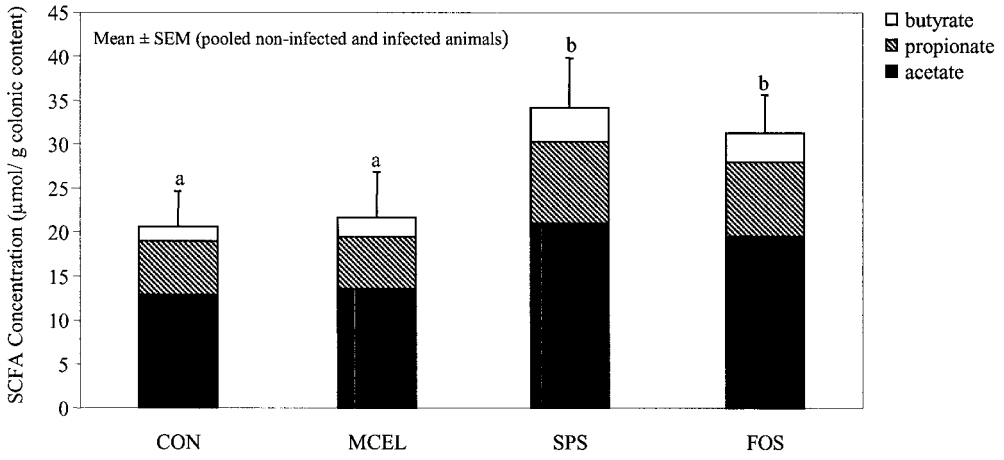

FIGURE 4 Total short-chain fatty acid (SCFA) concentration in colonic contents of noninfected piglets and piglets infected with *Salmonella typhimurium* consuming control (CON), methylcellulose (MCEL), soy polysaccharide (SPS) or fructooligosaccharide (FOS) diets. Total SCFA concentration in colonic contents was greater in SPS-fed and FOS-fed piglets compared with the CON and MCEL-fed groups ($P < 0.05$). There was no significant effect of infection on total SCFA concentration; thus, data were pooled. Data are means ± SEM ($n = 6$/group). Means with different letters differ, $P \leq 0.05$.

concentrations, which may have reduced the severity of the *S. typhimurium* infection for a variety of reasons. SCFA are one of the preferred fuels for the GI tract, promoting development of GI function, while improving its digestive and absorptive capacities (28–30). SCFA may also aid in preventing foreign bacteria from infiltrating the small bowel and colon because they maintain the mucosal barrier in piglets during challenges such as total parenteral nutrition (31).

Despite the apparent insensitivity of intestinal structure to dietary fiber in the current study, the feeding of fiber-free diets to adult rodents results in atrophy of the small intestine and colon, whereas the addition of dietary fiber reverses these effects (7,32). Although most fiber sources have a trophic effect on the colon, it is primarily the highly fermented types, which produce higher amounts of SCFA, (e.g., FOS), that have an effect on the small bowel (33). This observation led to the conclusion that fermentation products, specifically SCFA, mediate this effect. Indeed, infusions of butyrate or SCFA mixtures are clearly trophic to the GI mucosa without the mechanical effects induced by the physiochemical characteristics of including fiber in the diet (34–38). The observed insensitivity of the neonatal piglet intestinal structure to dietary fiber within the current study may be multifactorial. First, the effects of dietary fiber and/or SCFA on the neonatal intestine are largely unknown and the intestinotrophic stimuli of the developmental process may indeed result in maximal intestinal growth that cannot be affected further by the consumption of modest amounts of dietary fiber. This premise is well supported by recent reports that insulin-like growth factor-I, another intestinotrophic stimulus, had a modest (39) or no effect (40) on intestinal mucosal weight or brush border morphology of neonatal piglets, although changes in lactase activity and nutrient transport were observed. Second, this study was designed to examine the full postinfection period to observe diet-induced changes on the course of the infection; therefore effects of fermentable fiber sources on the immediate postinfection period on intestinal structure may have been missed.

Disaccharidase activity was assessed as an index of brush border digestive function. Ileal sucrase activity was not affected by dietary treatments, but increased following *S. typhimurium* infection in the ileum of all piglets studied. An oral challenge of *S. typhimurium* increases the acute phase response, with plasma cortisol peaking 2.5-fold above control levels 24–48 h following infection in pigs (41). Cortisone results in a precocious induction of sucrase-isomaltase in the neonatal intestine (42–46); thus, we hypothesize that an acute phase–induced increase in plasma cortisol concentration results in the induction of sucrase observed in the current study.

Although well-controlled, focused investigations are lacking, *S. typhimurium* infection has long been associated with secondary intestinal lactase insufficiency (47,48). The results of the current study are particularly intriguing because only the control group exhibited decreased ileal lactase activity postinfection, whereas the fiber-consuming groups were unaffected. Soy polysaccharide–supplemented formula has been reported previously to increase mucosal lactase activity following massive small intestinal resection in rats (49), but the regulation is unknown. Currently, investigations in our laboratory are focusing on the physiochemical effects of the various fermentable substrates and the direct induction by SCFA of this important brush border hydrolase.

The observation that the consumption of fermentable substrates increases glutamine transport is very important because this amino acid is preferentially oxidized by both enterocytes and lymphocytes (50). It is of interest, however, that the SPS group exhibited reduced ileal barrier function following infection because this observation does not correspond with the healthy clinical measurements or the enhanced ileal function measured in this group. This alteration in barrier function may indeed be related to the enhanced glutamine transport in the SPS group because there is a simultaneous decrease in transmucosal resistance associated with sodium-dependent transport of nutrients across the brush border (51–53). This potential interaction between sodium-dependent glutamine transport and transmucosal resistance did not occur in the FOS groups. Therefore, investigations focusing on specific effects of purified, highly fermentable prebiotics, such as fructooligosaccharides, on the molecular regulation of tight junction physiology are warranted.

Basal short-circuit current and potential difference were higher in the jejunum than the ileum and colon, irrespective of diet or infection. This segmental difference was also reported in food-deprived piglets by Ferraris and Carey (54) who suggested that the diet composition and metabolic stress were potentially inducing the shift in basal ion transport from a net absorptive flux to one that was secretory in nature. Nutrient transport and enzyme activity were most responsive to infection in the ileum, the intestinal segment primarily attacked in other species (55,56). Furthermore, the ability of the ileum to undergo functional and structural adaptations is greater than that of jejunum (57), and may therefore explain the segmental responses observed in the current study. Colonic glucose transport was not altered by dietary treatment, intestinal segment

measured or infection. However, glutamine transport was significantly increased by the fermentable fiber diets compared with MCEL regardless of intestinal segment. This is a very interesting phenomenon because it indicates that even the colon can be stimulated by diet to increase its nutrient uptake capacity. Similarly, a recent report indicates that mRNA expression of PepT1, a peptide transporter, increases fivefold in patients with short bowel syndrome and may adaptively improve uptake of malabsorbed di- and tripeptides (58).

In conclusion, the maintenance of physical activity and stool consistency indicate that consumption of fermentable substrate reduces the severity of *S. typhimurium* infection-associated symptoms. Even at 7 d postinfection, when diarrhea had resolved, significant improvements in intestinal digestive and absorptive indices were apparent in piglets consuming fermentable fibers. Thus, we hypothesize that the effect of fermentable fiber on components of intestinal function would be magnified if measured immediately postinfection. Therefore, future efforts will focus on the cellular and molecular mechanisms underlying the diet-induced changes during the early phase of the infection and on the role of these fibers in both preventative and therapeutic protocols involving pathogenic infection. These outcomes will help nutritionists to design better formulas that can mimic the beneficial effects of breast milk in a cost-effective alternative that may reduce the susceptibility to and the severity of pathogenic infection–associated symptoms in infant populations.

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