Nutritional Immunology

Dietary Fructooligosaccharides Increase Intestinal Permeability in Rats

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ABSTRACT   We showed previously that fructooligosaccharides (FOS) decrease the resistance to salmonella infection in rats. However, the mechanism responsible for this effect is unclear. Therefore, we examined whether dietary FOS affects intestinal permeability before and after infection with *Salmonella enterica* serovar Enteritidis. Male Wistar rats were fed restricted quantities of a purified diet that mimicked the composition of a Western human diet. The diet was supplemented with 60 g/kg cellulose (control) or 60 g/kg FOS and with 4 mmol/kg of the intestinal permeability marker chromium EDTA (CrEDTA) ($n = 8$ or 10). After an adaptation period of 2 wk, rats were orally infected with 10^8 colony-forming units (cfu) of *S. enteritidis*. Mucin concentrations in intestinal contents and mucosa were measured fluorimetrically, as markers of mucosal irritation. Intestinal permeability was determined by measuring urinary CrEDTA excretion. Translocation of salmonella was quantified by analysis of urinary nitric oxide metabolites with time. Before infection, FOS increased mucosal lactobacilli and enterobacteria in cecum and colon, but not in the ileum. However, FOS increased cytotoxicity of fecal water and intestinal permeability. Moreover, FOS increased fecal mucin excretion and mucin concentrations in cecal and colonic contents, and in cecal mucosa before infection. After infection, mucin excretion and intestinal permeability in the FOS groups increased even further in contrast to the control group. In addition, FOS increased translocation of salmonella to extraintestinal sites. Thus, FOS impairs the intestinal barrier in rats, as indicated by higher intestinal permeability. Whether these results can be extrapolated to humans requires further investigation.   J. Nutr. 135: 837–842, 2005.

KEY WORDS: • *infection* • *microflora* • *mucin* • *prebiotics* • *salmonella*

The enteric pathogen *Salmonella enterica* serovar Enteritidis is one of the leading causes of gastrointestinal infections, ranging from mild, self-limiting inflammation of the intestinal mucosa to life-threatening systemic infection (1). To successfully colonize the host, salmonella must overcome the acidity of the stomach, the endogenous microflora and its bactericidal fermentation metabolites, and the intestinal barrier.

The epithelial barrier of the host can potentially be affected by dietary prebiotics. Prebiotics, which include fructooligosaccharides (FOS),[2] are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, which can improve host health (2). By stimulating the protective endogenous microflora (3) and increasing bactericidal organic acid concentrations (4,5) within the lower gut, FOS may potentially enhance resistance to intestinal pathogens. However, the effects of FOS on the resistance to intestinal bacterial infections have rarely been the subject of in vivo studies. Unexpectedly, we consistently found that FOS increased translocation of salmonella toward extraintes-

tinal sites in several strictly controlled rat infection studies (6–8). Moreover, FOS resulted in significant infection-induced growth impairment (6–8), gut inflammation (8), and diarrhea (6). At present, the mechanism responsible for these adverse effects of FOS is still unclear.

One possible explanation could be that rapid fermentation of FOS by the endogenous microflora results in overproduction of organic acids (5,6). This may subsequently lead to epithelial injury and increased intestinal permeability (9–11). Therefore, the aim of the present rat experiments was to study the effect of FOS on intestinal permeability before and after infection with the invasive pathogen *S. enteritidis*.

MATERIALS AND METHODS

Expt. 1

Experimental design.   The animal welfare committee of Wageningen University approved the experimental protocol. Specific pathogen–free 8-wk-old male Wistar rats, with a mean body weight of 226 g (WU, Harlan), were housed individually in metabolic cages. All rats were kept in a temperature- (22–24°C) and humidity- (50–60%) controlled environment with a 12-h light:dark cycle. To study the effects of dietary FOS on the main effect variables, intestinal permeability and salmonella translocation, rats were randomly assigned to either an experimental diet supplemented with 60 g/kg cellulose or FOS (purity 93 g/100 g; Raftilose P95, Orafti) ($n = 10$). After adaptation to the housing and dietary conditions for 2 wk, rats

were orally infected by gastric gavage of 2×10^8 colony-forming units (cfu) *S. enteritidis* (clinical isolate, phage type 4 according to international standards; B1241 culture of NIZO food research) suspended in 1 mL of saline containing 30 g sodium bicarbonate/L on d 0. Salmonella was cultured and stored as described earlier (12). On d 10 after infection, rats were killed by carbon dioxide inhalation.

Diets and CrEDTA. Rats were fed restricted quantities of a purified diet. Restricted food intake was necessary to prevent FOS-induced differences in food consumption as observed earlier (8), and hence differences in vitamin, mineral, and CrEDTA intake. Energy restriction was mild because the energy intake of the rats was 286 kJ/d based on consumption of 14 g/d high-fat feed. Rats consuming 20 g/d of a low-fat AIN93-based diet received 302 kJ/d (13). Demineralized drinking water was supplied ad libitum. Compared with the AIN-93 recommendation for rat diets (13), the diets were low in calcium (30 mmol $CaHPO_4 \cdot 2H_2O$/kg) and had a high fat content (200 g fat/kg), to mimic the composition of a Western human diet. The exact composition of the diets was described earlier (6). In addition, the inert intestinal permeability marker chromium EDTA (CrEDTA) was added to the diets (14,15). CrEDTA was prepared as described elsewhere (16). The complete formation and stability of the CrEDTA complex were checked by passing the prepared CrEDTA solution through a cation exchange resin column (Chelex® 100 Resin; Bio-Rad). No uncomplexed Cr^{3+} ions were present in the final solution, which was added to the diets (final concentration CrEDTA as analyzed was 3.9 mmol/kg diet). Moreover, no uncomplexed Cr^{3+} ions were present in feces and urine samples (data not shown).

Microbiological analyses of feces. Total 24-h feces were collected on the last 4 d before and on 10 consecutive days after infection. Feces were freeze-dried, pooled per rat per 2 consecutive days, and subsequently ground to obtain homogeneous powdered samples. DNA was isolated from feces collected before infection using the QIAamp DNA stool mini kit (QIAgen) according to the manufacturer's instructions. After addition of the lysis buffer, the samples were shaken for 3×1.5 min at 5000 rpm together with glass beads in a Bead Beater. Real-time quantitative PCR, targeting a 110-bp transaldolase gene sequence, was used to specifically quantify bifidobacteria in fecal samples collected before infection, as described and validated earlier (6,17). For lactobacilli determination, primers and probe targeted the 16S rDNA sequences. Forward primer LAB1 (5′-GGCAGCAGTAGGGAATCTTCCA-3′) targets positions 350–371 of the Lactobacillus 16S rDNA molecule and is selective at the 3′ end of the molecule. The LAB probe (5′-TGGAGCAACGCCGCGT-GAGTGA-3′) recognizes positions 390–411 and is selective at the 5′ end. Reverse primer LAB2 (5′-GTATTACCGCGGCTGCTGGCAC-3′) is a semiuniversal primer targeting 16S rDNA positions 504–524 of most bacteria. The fluorogenic oligonucleotide probe was labeled at the 5′ end with the reporter dye 6-carboxyfluorescein (FAM) and at the 3′ end with the quencher dye 6-carboxytetramethylrhodamine (TAMRA). Universal Taqman® PCR Master Mix was purchased from PE Applied Biosystems. Real-time PCR was performed using the ABI Prism 7700® Sequence Detection System (PE Applied Biosystems) under the following conditions: 2 min at 50°C and 10 min at 95°C to activate the AmpliTaq Gold DNA polymerase, and 50 cycles of 15 s at 95°C and 60 s at 65°C for amplification. To quantify *E. coli*, primers and probe targeting the *Escherichia coli*–specific 16S rDNA gene were designed as described and validated earlier (18). The cycle threshold values generated by real-time PCR from DNA extracts of dilutions of a suspension of *Lactobacillus acidophilus* (culture B228 and B1836 of NIZO food research, the Netherlands) and *E. coli* (culture JM109 of ATCC, USA) were used to plot a standard curve from which the number of bacteria in feces could be calculated.

Chemical analyses of feces. Before infection, total fecal lactic acid was measured using a colorimetric enzymatic kit (Boehringer Mannheim), as described elsewhere (12). Before and after infection, fecal mucin was extracted from freeze-dried feces and quantified fluorimetrically, as described earlier (12). Standard solutions of *N*-acetylgalactosamine (Sigma) were used to calculate the amount of oligosaccharide side-chains liberated from mucins. Therefore, fecal mucins are expressed as μmol oligosaccharide equivalents. Control experiments showed that interfering oligosaccharides of dietary origin were completely removed by the molecular filtration step in this

procedure (data not shown). No proper assessment of fecal wet weight excretion could be made because the pellets dried up during collection in the metabolic cages. Therefore fecal wet weight excretion was determined by the summed concentration of the fecal cations sodium and potassium before and after infection (19). To measure sodium and potassium, feces was treated with 50 g/L of trichloroacetic acid (1:1, v:v) and centrifuged for 2 min at $14,000 \times g$. The supernatants were diluted with 0.5 g/L of CsCl solution, and sodium and potassium were analyzed by inductively coupled plasma-atomic emission spectrophotometry (ICP-AES; Varian).

Analyses of fecal water. Before infection, fecal water was prepared by reconstituting freeze-dried feces with appropriate amounts of double-distilled water to obtain the physiologic osmolarity of 300 mOsmol/L. Samples were thoroughly mixed, incubated for 1 h at 37°C, and subsequently centrifuged for 1 h at $14,000 \times g$ (Hettich, Micro-rapid 1306). Supernatants (fecal water) were stored at −20°C until further analyses. The pH of the fecal water was measured at 37°C. Cytotoxicity of the fecal water was determined with an erythrocyte assay, as described previously (20) and validated earlier with intestinal epithelial cells (21). The incubations were of physiologic ionic strength (300 mOsmol/L) and buffered at pH 7.0 (final 100 mmol/L 3-*N*-morpholino-propanesulfonic acid, Sigma) to prevent acid-induced hemolysis.

Analyses of urine samples. Total 24-h urine samples were collected on the last 4 d before and on 10 consecutive days after infection. Oxytetracycline (1 mg) was added to the urine collection vessels of the metabolic cages to prevent bacterial deterioration. To measure CrEDTA, urine was treated with 50 g/L of trichloroacetic acid (1:1, v:v) and centrifuged for 2 min at $14,000 \times g$. The supernatants were diluted with 0.5 g/L of CsCl solution, and chromium was analyzed by ICP-AES (Varian). The concentration of NO_x (sum of nitrate and nitrite) was determined using a colorimetric enzymatic kit (Nr. 1746081; Roche Diagnostics). Briefly, urinary nitrate is reduced to nitrite by nitrate reductase. Subsequently, nitrite reacts with sulfanilamide and *N*-(1-naphthyl)-ethylene-diamine dihydrochloride, resulting in a red diazo dye, which was measured spectrophotometrically at 540 nm (22). Urinary NO_x is a sensitive and quantitative biomarker of intestinal bacterial translocation (23) that correlates with organ cultures (8,24) and the severity of systemic infectious disease in rats and humans (25–27).

Expt. 2

Diets and dissection of the rats. A second experiment was performed to study the effect of FOS on the luminal and mucosal microflora and mucin concentration throughout the intestinal tract. Specific pathogen–free male Wistar rats (8 wk old, mean body weight of 261 g, n = 8) were housed and fed the cellulose- or FOS-supplemented diets as described above for Expt. 1. After an adaptation period of 14 d, the distal ileum (last 12 cm proximal to the ileocecal valve), cecum, and the first 8 cm of the proximal colon were excised. Ileal, cecal, and colonic contents were sampled. Ileal contents were pooled per diet group to obtain enough sample for measurements. Subsequently, the intestinal segments were excised longitudinally, and washed in sterile saline. The mucosa was scraped off using a spatula and suspended in sterile saline.

Microbiological and biochemical analyses. Mucosal enterobacteria were quantified by plating 10-fold dilutions in saline on Levine EMB Agar (Difco Laboratories) and incubating aerobically overnight at 37°C. In addition, lactobacilli in intestinal mucosa were quantified by plating on Rogosa Agar (Oxoid) and incubating in anaerobic jars (MART microbiology) at 37°C for 3 d. The detection limit of this method was 10^2 cfu/g mucosal wet weight. Short-chain fatty acids (SCFA; acetate, propionate and butyrate) in cecal contents were determined by GC, as described elsewhere (28). Calcium and inorganic phosphate in cecal contents were analyzed by ICP-AES (Varian). Ileal, cecal, and colonic contents and mucosa were freeze-dried. Mucosal samples were sonicated for 1 min in PBS to liberate intracellular mucins. Mucins in ileal, cecal, and colonic contents and mucosa were isolated and quantified fluorimetrically, as described above.

Statistical analysis

Results are expressed as means ± SEM, $n = 8$ or 10. A commercially available package (Statistica 6.1, StatSoft) was used for all statistics. Differences between the FOS group and the control group, before or after infection, were tested for their significance using a Kruskal-Wallis ANOVA, followed by the nonparametric Mann-Whitney U test (two-sided) because several parameters were not distributed normally. Differences were considered significant when $P < 0.05$.

RESULTS

Expt. 1: Animal growth, food intake and fecal characteristics. Before and after infection, no significant differences in food intake or growth between the diet groups were observed. Before and after infection, all rats consumed the 14 g/d provided, as intended. Before infection, growth was 2.6 ± 0.8 and 2.6 ± 1.0 g/d in the control and FOS group, respectively. After infection, growth in the control and FOS group was 2.9 ± 1.5 and 2.7 ± 2.0 g/d, respectively. Before infection, FOS clearly stimulated fecal bifidobacteria, lactobacilli, and enterobacteria **(Table 1)**. In addition, fecal lactate concentration was higher and the pH of fecal water was lower in the FOS group (Table 1). FOS strongly increased the cytotoxicity of fecal water (Table 1). There were no infection-induced changes in fecal bacteria, pH, and cytotoxicity of fecal water (data not shown). FOS stimulated fecal mucin concentration, before infection **(Fig. 1A)**. After infection, FOS drastically increased fecal mucin concentration, whereas the control group still excreted preinfection levels. Daily mucin excretion before infection was 3.2 ± 0.4 μmol/d in the control group and 7.1 ± 0.8 μmol/d in the FOS group ($P < 0.05$). Peak mucin excretion after infection (d 8) in the control and FOS group was 4.0 ± 0.6 and 29.3 ± 4.1 μmol/d, respectively ($P < 0.05$). FOS increased diarrhea after infection, as indicated by the fecal cation excretion. Fecal cation excretion before infection was 85 ± 10 μmol/d in the control group and 139 ± 15 μmol/d in the FOS group. After infection, peak fecal cation excretion was 93 ± 11 and 188 ± 24 μmol/d in the FOS group ($P < 0.05$).

Expt. 2: Intestinal fermentation and mucin excretion. FOS stimulated the growth of both lactobacilli and enterobacteria on cecal and colonic mucosa **(Table 2)**. However, no enhanced growth of these bacterial genera occurred in the ileal mucosa. There were no differences in cecal SCFA concentrations between the diet groups. However, FOS increased the total cecal SCFA pool **(Table 3)**. In addition, soluble calcium

TABLE 1

Effect of dietary FOS on bacteria and lactate in feces, and pH and cytotoxicity of fecal water of rats, before infection[1]

	Control	FOS
Fecal bacteria		
Bifidobacteria, log_{10}/g wet	6.92 ± 0.07	9.45 ± 0.08*
Lactobacilli, log_{10}/g wet	8.90 ± 0.08	10.57 ± 0.11*
Escherichia coli, log_{10}/g wet	8.92 ± 0.16	9.44 ± 0.13*
Other fecal characteristics		
pH fecal water	6.63 ± 0.05	6.37 ± 0.09*
Lactate, μmol/g dry	2.93 ± 0.57	17.46 ± 5.20*
Cytotoxicity fecal water, % lysis	0.6 ± 0.1	71.2 ± 10.1*

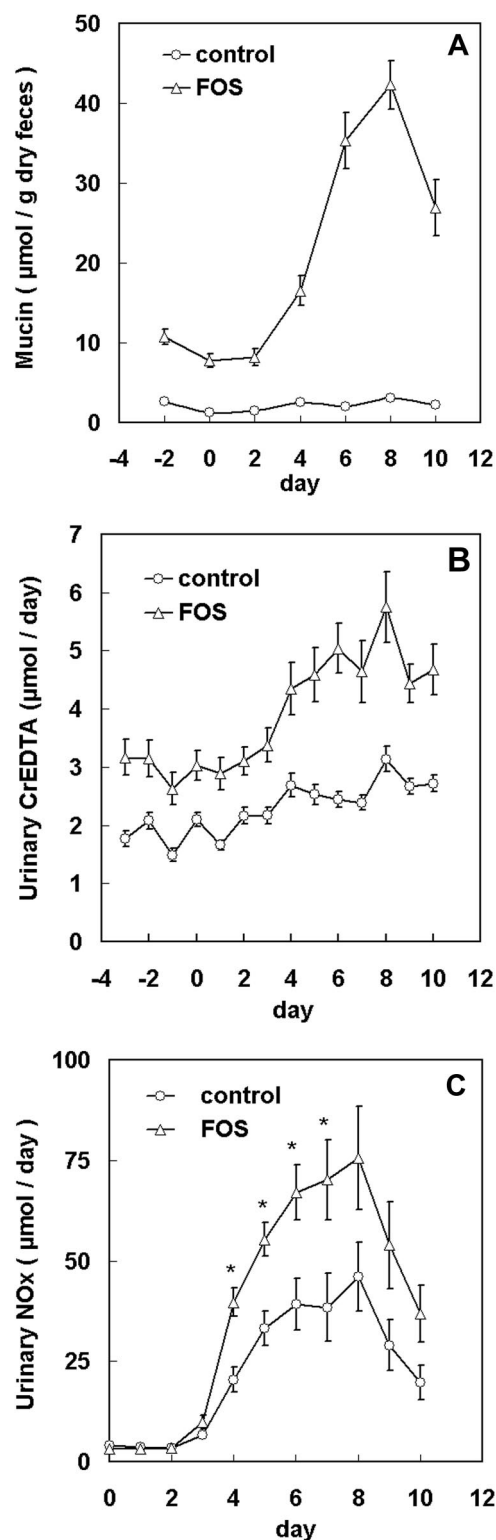[1] Values are means ± SEM, $n = 10$. * Different from control, $P < 0.05$.

FIGURE 1 Effects of dietary FOS on (A) fecal mucin concentrations, (B) urinary CrEDTA excretion, and (C) the kinetics of urinary NO_x excretion in rats. Results are means ± SEM, $n = 10$. (A,B) The FOS group differed from the control group at all time points ($P < 0.05$). (C) *Different from the control group at that time point, $P < 0.05$.

and phosphate concentrations within the cecum were increased in the FOS group (Table 3). FOS increased mucin concentrations in cecal and colonic contents, but not in ileal contents **(Table 4)**. Moreover, FOS increased mucin concen-

TABLE 2

Effect of dietary FOS on lactobacilli and enterobacteria on ileal, cecal, and colonic mucosa of uninfected rats[1]

	Control	FOS
Lactobacilli, log_{10} cfu/g wet		
Ileum	3.75 ± 0.26	3.82 ± 0.42
Cecum	4.00 ± 0.27	4.99 ± 0.32*
Colon	3.70 ± 0.29	4.69 ± 0.40*
Enterobacteria, log_{10} cfu/g wet		
Ileum	4.48 ± 0.19	4.21 ± 0.17
Cecum	4.90 ± 0.28	6.08 ± 0.14*
Colon	4.42 ± 0.23	5.68 ± 0.26*

[1] Values are means ± SEM, $n = 8$. * Different from control, $P < 0.05$.

TABLE 4

Effect of dietary FOS on mucin concentrations in ileal, cecal, and colonic contents and mucosa of uninfected rats[1]

	Control	FOS
Contents, $\mu mol/g$ dry		
Ileum[2]	31.2	32.0
Cecum	6.6 ± 1.3	31.3 ± 6.1*
Colon	1.9 ± 0.2	20.0 ± 5.6*
Mucosa, $\mu mol/g$ dry		
Ileum	15.9 ± 1.4	15.5 ± 1.0
Cecum	6.3 ± 0.7	10.5 ± 0.5*
Colon	15.3 ± 1.9	14.2 ± 1.6

[1] Values are means ± SEM, $n = 8$. * Different from control, $P < 0.05$.
[2] Mucin concentrations in ileal contents are presented without SEM because contents were pooled per diet group.

tration within the cecal mucosa. However, there was no FOS-induced change in mucin concentration within the ileal and colonic mucosa.

Expt. 1: Intestinal permeability. Due to restricted feeding, daily dietary intake of CrEDTA did not differ between the 2 diet groups (54 μmol CrEDTA/d). However, FOS increased urinary CrEDTA excretion before infection compared with the control group (Fig. 1B). Before infection, the control group excreted 3.4 ± 0.2% of the ingested CrEDTA in urine, whereas the FOS group excreted 6.0 ± 0.5% ($P < 0.05$). After infection, urinary CrEDTA excretion was increased even further, especially in the FOS group. The total infection-induced CrEDTA excretion (corrected for preinfection baseline output) was higher in the FOS group compared with the control group; 12.6 ± 2.3 vs. 3.6 ± 1.0 $\mu mol/10$ d, respectively ($P < 0.05$). In addition, similar results were obtained in control and FOS-fed rats that received a single oral dose of CrEDTA preinfection and on d 2 and d 6 postinfection (data not shown).

Expt. 1: Translocation of salmonella. Major differences were observed in the effects of FOS on intestinal translocation of salmonella, as measured by the infection-induced urinary NO_x excretion with time (Fig. 1C). After infection, urinary NO_x excretion of the FOS group strongly increased to 76 $\mu mol/d$, a maximum reached at d 8. After d 8, urinary NO_x output gradually decreased toward baseline levels. The total infection-induced urinary NO_x excretion was significantly higher in the FOS group. The area under the curve corrected for baseline output was 202 ± 36 for the control group and 471 ± 69 $\mu mol/10$ d for the FOS group ($P < 0.05$).

TABLE 3

Effect of dietary FOS on cecal SCFA, calcium, and phosphate concentrations and pH of uninfected rats[1]

	Control	FOS
SCFA, $\mu mol/g$ wet	37.5 ± 3.5	30.8 ± 1.7
SCFA, $\mu mol/cecum$	45.1 ± 5.9	140.4 ± 11.6*
Soluble Ca, $\mu mol/g$ wet	1.2 ± 0.2	2.1 ± 0.1*
Soluble P_i, $\mu mol/g$ wet	10.0 ± 1.0	14.6 ± 1.1*
pH	7.2 ± 0.2	6.9 ± 0.1

[1] Values are means ± SEM, $n = 8$. * Different from control, $P < 0.05$.

DISCUSSION

This study showed that FOS increased intestinal permeability and translocation of salmonella. Most previous studies focused on the effect of dietary prebiotics on the composition of the intestinal microflora and many of them assumed that improvements of host resistance to intestinal infections were likely. We contend that, irrespective of their prebiotic effect, the contribution of prebiotics in decreasing intestinal infections can be verified only in strictly controlled in vivo studies that include a challenge with a pathogen. The few previous studies that focused on the in vivo effects of fermentable fibers on pathogen survival in healthy animals showed either beneficial (29–32), inconsistent (33–35), or adverse effects (6,8,36). Most of these studies showed data on pathogen colonization, whereas translocation was not studied. However, in one study, the consumption of FOS seemed to increase salmonella numbers in liver and spleen of pigs, although it was not significant (35).

The mechanism behind the FOS-induced adverse effects on intestinal permeability and salmonella translocation is not clear. We have no reason to assume that these adverse effects are specific for our animal model and are not relevant for humans. Indeed, FOS is rapidly fermented in the rat cecum, whereas humans have a poorly defined cecal region (37). However, the number and general composition of bacteria in the rat cecum and human colon are largely comparable (37,38). Therefore, the physiologic effects of FOS (e.g., rapid generation and absorption of SCFA) in the cecum of rats will occur in the proximal colon in humans. Also, the relatively high dietary dose of FOS used in our animal studies does not explain the adverse effects. We showed earlier that lower dietary doses of 3–4% FOS induced comparable adverse effects (6,8). The latter concentrations are relevant for humans because these intakes can be reached when eating both FOS-rich foods (e.g., onions, artichokes, wheat-based products) and FOS-supplemented products, typically containing 3–10 g prebiotics per portion (39,40).

We speculate that the FOS-induced adverse effects can be explained by an accumulation of organic acids and other fermentation metabolites in the distal gut. In the present study and in a previous study (9), FOS did not affect cecal SCFA concentrations, whereas it increased the cecal pool of SCFA in the present study. The subsequent acidification of gut contents is counteracted by solubilization of the calcium phosphate complex. Indeed, FOS increased concentrations of sol-

uble calcium and phosphate within cecal contents. However, when the dietary calcium phosphate supply is limited, the amount of insoluble calcium phosphate may be insufficient to counteract acidification (41). The accumulation of fermentation metabolites and the subsequent acidification of gut contents may subsequently result in irritation of the mucosal barrier (9–11,42). To protect the intestinal mucosa, goblet cells respond to irritating components by increasing mucus excretion (43–45). Indeed, before infection, FOS increased fecal mucin excretion in the present study. Moreover, FOS increased mucin concentrations within cecal and colonic contents and within the cecal mucosa, whereas no effect was observed in the ileal contents and mucosa. In accordance with these results, Kleesen et al. (46) found a thicker mucous layer and more goblet cells per crypt in response to FOS. Moreover, a study of Tsukuhara et al. (47) showed that consumption of a high dose of FOS (100 g/kg) resulted in a very high mucous concentration in cecal contents. Mucins can be degraded by the intestinal microflora (48). Thus, one could speculate that an increase in fermentable FOS in the large intestine reduces fermentation of gastrointestinal mucins, due to substrate competition. This could subsequently result in a potential increase in the recovery of mucins in feces. However, we showed earlier that dietary resistant starch does not increase mucin secretion in rats, (8) although it is also completely fermented by the endogenous microflora of rats and humans (49). Therefore, the increase in fecal mucin excretion induced by FOS indicates increased secretion and not decreased mucin degradation. We do not dispute that mucin secretion itself is an important and beneficial host defense response. However, mucin production and secretion is enhanced by irritating components such as bacterial pathogens (50), endotoxins (51), bile acids (52), or SCFA (43,44) Thus, the mucin secretion stimulated indicates the need for the epithelial layer to protect itself against harmful substances.

In addition to mucin excretion, the higher intestinal permeability as measured by urinary CrEDTA excretion also indicates that fermentation of FOS induced mucosal damage. CrEDTA is a stable and inert molecule that has a very low permeability through an intact intestinal epithelium due to its size and hydrophilicity. Intestinal permeability to CrEDTA occurs passively by the paracellular route via tight junctions (53). Enhanced intestinal fermentation and production of organic acids may result in mucosal injury as measured by the increased intestinal permeability (10,54,55). Thus, the increased permeability might have facilitated salmonella translocation through the paracellular pathway. Indeed, it was shown that salmonella is able to use multiple pathways to reach extraintestinal sites (56–58).

In the present study, the FOS-induced increase in mucin excretion and intestinal permeability was more pronounced after infection. Indeed, several other studies showed that intestinal inflammation increases intestinal absorption of CrEDTA (14,59,60). Apparently, salmonella infection and the subsequent intestinal inflammation resulted in further impairment of the mucosal barrier in FOS-fed rats in contrast to the controls.

The enhanced translocation of salmonella in the FOS groups might also be the result of increased virulence of salmonella. Salmonella requires a large number of general stress response systems as well as specific virulence factors to successfully colonize the host, to translocate, and to avoid clearance by the host immune system. Virulence gene expression in salmonella is regulated by environmental factors. For example, changes in nutrient availability, osmolarity, pH, and organic acid concentrations can alter the virulence of salmo-

nella (61–63). Although rapid fermentation of FOS results in the production of organic acids (4,5,11), which inhibit the growth of salmonella in vitro, alterations in organic acid concentrations and profile might also increase translocation to extraintestinal sites by affecting salmonella virulence (61,62). The FOS-induced alterations in SCFA might increase salmonella virulence by providing a signal for expression of invasion genes (61,62). We are currently investigating whether fermentation of FOS affects salmonella virulence and thus risk of infection in vivo.

In conclusion, the present study shows that rapid fermentation of FOS by the endogenous microflora impairs the gut mucosal barrier, indicated by the increased intestinal permeability before infection. This could have resulted in increased translocation of salmonella in the distal gut. However, caution must be exercised when extrapolating findings from these animal experiments to humans because the intestinal permeability of rats is higher than that of humans (64). Therefore, we are now performing a human intervention study to verify the effects of FOS on mucosal irritation and intestinal permeability. These data and that of other controlled in vivo studies are required to evaluate the effects of prebiotics on intestinal host resistance.

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