Dietary Fructo-Oligosaccharides Dose-Dependently Increase Translocation of Salmonella in Rats

Sandra J. M. Ten Bruggencate, Ingeborg M. J. Bovee-Oudenhoven,[1] Mischa L. G. Lettink-Wissink and Roelof Van der Meer

Nutrition and Health Program, Wageningen Center for Food Sciences/NIZO Food Research, Ede, The Netherlands

ABSTRACT Prebiotics, such as fructo-oligosaccharides (FOS), stimulate the protective gut microflora, resulting in an increased production of organic acids. This may result in increased luminal killing of acid-sensitive pathogens. However, host defense against invasive pathogens, like salmonella, also depends on the barrier function of the intestinal mucosa. Rapid fermentation of prebiotics leading to high concentrations of organic acids may impair the barrier function. Therefore, we determined the dose-dependent effect of dietary FOS on the resistance of rats to *Salmonella enteritidis*. Male Wistar rats were fed restricted quantities of a "humanized" purified diet supplemented with 0, 3 or 6 g/100 g of FOS ($n = 7$ in the 6% FOS group and $n = 8$ in the other diet groups). After an adaptation period of 2 wk, rats were orally infected with 1.7×10^{10} colony-forming units of *S. enteritidis*. Supplement-induced changes in the intestinal microflora and fecal cation excretion were determined before and after infection. Cytotoxicity of fecal water was determined with an in vitro bioassay, and fecal mucins were quantified fluorimetrically. Colonization of *S. enteritidis* was determined by quantification of salmonella in cecal contents and mucosa. Translocation of *S. enteritidis* was quantified by analysis of urinary nitric oxide metabolites in time. Before infection, FOS decreased cecal and fecal pH, increased fecal lactic acid concentration and increased bifidobacteria and enterobacteria. FOS also increased cytotoxicity of fecal water and fecal mucin excretion, indicating mucosal irritation. Remarkably, FOS dose-dependently increased salmonella numbers in cecal contents and mucosa and caused a major increase in infection-induced diarrhea. In addition, FOS enhanced translocation of salmonella. Thus, in contrast to most expectations, FOS dose-dependently impairs the resistance to salmonella infection in rats. These results await verification by other controlled animal and human studies. J. Nutr. 133: 2313–2318, 2003.

KEY WORDS: • *fermentation* • *fructo-oligosaccharides* • *infection* • *prebiotics* • *rats* • *salmonella*

Intestinal infections are still a major cause of disease in Western countries (1). Because of the large-scale use of antibiotics and hence the development of resistant bacterial strains, research strategy is now focusing on prevention (2). By influencing the composition of gastrointestinal contents, dietary nondigestible carbohydrates (i.e., oligosaccharides) could potentially modulate intestinal infections. Oligosaccharides affect the intestinal microflora (3,4) and the gastrointestinal survival of food-borne pathogens (5). Some studies have shown that oligosaccharides stimulate the growth of bifidobacteria and lactobacilli (3,4). These bacteria may reduce the survival of a pathogen by enhancing competition for nutrients and adhesion sites and production of organic acids (6). Organic acids, that is, lactic acid and SCFA, can inhibit growth of acid-sensitive pathogens like salmonella (5). Despite these potential beneficial effects, few studies show the effects of oligosaccharides on the gastrointestinal survival of food-borne pathogens (7–10). We previously showed that lactulose and fructo-oligosaccharides reduce the intestinal colonization of salmonella in rats (5, and I.M.J. Bovee-Oudenhoven, unpublished data). However, oligosaccharides have inconsistent effects on colonization of salmonella in studies with broiler chicks (7,8), mice (10) and swine (9).

Besides resistance to colonization, host defense against invasive pathogens like salmonella, also depends on the mucosal barrier. Bacterial translocation can occur when the ecological balance of the normal endogenous microflora is disrupted, when host immune defenses are impaired or during physical loss of the mucosal barrier (11). High concentrations of organic acids, arising from rapid fermentation of oligosaccharides, may potentially inhibit the colonization of acid-sensitive pathogens, but can also induce injury to the intestinal mucosa and hence impair its barrier function (12,13). Thus, inhibiting the intestinal colonization of an invasive pathogen under conditions of increased intestinal fermentation may not necessarily result in an overall resistance-enhancing effect.

Therefore, the aim of the present study was to determine the effect of increasing concentrations of dietary fructo-oligosaccharides (FOS)[2] on the resistance of rats to intestinal colonization and translocation of *Salmonella enteritidis*, which

[1] To whom correspondence should be addressed.
E-mail: Ingeborg.Bovee@NIZO.nl.

[2] Abbreviations used: CFU, colony-forming units; FOS, fructo-oligosaccharides; NO_x, sum of nitrate and nitrite.

Manuscript received 20 January 2003. Initial review completed 20 February 2003. Revision accepted 8 April 2003.

is a major cause of human food-borne infectious diarrhea in industrialized countries (1) and is sensitive to dietary modulation (5).

MATERIALS AND METHODS

Diets, infection and dissection of the rats. The animal welfare committee of Wageningen University (Wageningen, The Netherlands) approved the experimental protocol. Specific pathogen-free male Wistar rats (WU, 8 wk old, mean body weight = 239 ± 2 g; Harlan, Horst, The Netherlands) were housed individually in metabolic cages. All rats were kept in a temperature (22–24°C) and humidity (50–60%) controlled environment in a 12-h light/dark cycle. Rats (n = 8 per diet group) were fed restricted quantities (13 g/d; 266 kJ/d) of a purified diet. Restricted food intake was necessary to prevent differences in food consumption and hence differences in vitamin and mineral intake. The experimental diets were supplemented with 3 g of FOS/100 g (3%) or 6 g of FOS/100 g (6%) (purity 93 g/100 g; Raftilose P95; Orafti, Tienen, Belgium). FOS was added at the expense of glucose. (See **Table 1** for the exact composition of the diets.) Compared with the AIN-93 diet (14), diets were low in calcium (30 mmol of $CaHPO_4 \cdot 2H_2O$/kg) and had a high fat content (200 g fat/kg), to mimic the composition of a Western human diet. Demineralized drinking water was consumed ad libitum. Rats were acclimatized to the housing and dietary conditions for 2 wk. Subsequently, the rats were orally infected by gastric gavage of 1.7×10^{10} colony-forming units (CFU) *S. enteritidis* (clinical isolate, phage type 4 according to international standards; B1241 culture of NIZO Food Research, Ede, The Netherlands) suspended in 1 mL of saline containing 30 g of sodium bicarbonate/L. *S. enteritidis* was cultured and stored as described earlier (5). At d 9 after infection the rats were killed by carbon dioxide inhalation.

Microbiological analyses. Before infection, feces were quantitatively collected for 5 d. Feces were freeze-dried and subsequently ground to obtain homogeneous powdered samples. Real-time quantitative PCR targeting a 110-bp transaldolase gene sequence was used to specifically quantify bifidobacteria in fecal samples, as described and validated earlier (15). DNA was isolated from freeze-dried feces by use of the QIAamp DNA stool mini kit (QIAgen, Westburg, Leusden, The Netherlands). Real-time PCR was performed by use of the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). PCR primers were purchased from Amersham Pharmacia Biotech Custom DNA Synthesis Service (Roosendaal, The Netherlands). *Taq*man probes, containing a 5′ FAM fluorescent reporter dye (6-carboxyfluorosein) and a 3′ TAMRA quencher dye (6-carboxytetramethylrhodamine) were synthesized by PE Applied Biosytems Custom Oligonucleotide Synthesis Services (Nieuwerkerk a/d IJssel). Universal *Taq*man PCR Master Mix was purchased from PE Applied Biosystems. The cycle threshold values generated by real-time PCR from dilutions of DNA extracted from *Bifidobacterium infantis* and *Bifidobacterium breve* (cultures B651 and B655 of NIZO Food Research, respectively) were used to plot a standard curve from which the number of bifidobacteria in rat feces could be calculated.

Before infection, fresh fecal samples were collected directly from the anus of the rats and analyzed for the number of lactobacilli and enterobacteria. Lactobacilli were quantified by plating 10-fold dilutions in saline on rogosa agar (Oxoid, Basingstoke, UK) and incubating in an anaerobic jar (MART Microbiology, Lichtenvoorde, The Netherlands) at 37°C for 3 d. To determine fecal enterobacteria, 10-fold dilutions in saline were plated on Levine EMB Agar (Difco Laboratories, Detroit, MI) and incubated aerobically overnight at 37°C. The detection limit of this method was 10^2 CFU/g wet weight.

To determine salmonella numbers in cecal contents and mucosa, the cecum was excised and cecal contents were removed 9 d after infection. After extensive washing of the cecum in sterile saline, the cecal mucosa was scraped off by use of a sterile spatula. Mucosa samples were suspended in 1 mL of sterile saline and homogenized (Ultrathurrax Pro200; Pro Scientific, Monroe, CT). Tenfold dilutions in sterile saline were plated on modified brilliant green agar (Oxoid), and incubated aerobically overnight at 37°C. Sulfamandelate (Oxoid) was added to the agar plates to suppress swarming bacteria, like proteus species. The detection limit of this method was 10^2 CFU/g wet weight.

Chemical analyses of cecal contents and feces. The pH of cecal contents was measured at 37°C. Total lactic acid was measured in cecal contents, collected on d 9 after infection by use of a colorimetric enzymatic kit (Boehringer Mannheim, Germany), as described elsewhere (5). SCFA (acetate, propionate and butyrate) in cecal contents were determined by gas chromatography as described elsewhere (16). Feces were quantitatively collected and pooled per rat for 5 d before *S. enteritidis* infection and also on d 1–d 5 after infection for chemical analyses. Feces were freeze-dried and subsequently ground to obtain homogeneous powdered samples. To measure sodium and potassium, feces were treated with 50 g/L of trichloroacetic acid for 1 h at room temperature and centrifuged for 2 min at 14,000 × *g*. The supernatants were diluted with 0.5 g/L of CsCl, and sodium and potassium were analyzed by inductive coupled plasma–atomic emission spectrophotometry (ICP-AES; Varian, Mulgrave, Australia). Fecal ammonia was determined by use of a colorimetric kit (Procedure No. 640; Sigma Chemical Co., St. Louis, MO) as described earlier (5). Fecal mucin was extracted from freeze-dried feces and quantified fluorimetrically as described earlier (5). Standard solutions of *N*-acetylgalactosamine (Sigma) were used to calculate the amount of oligosaccharide side chains liberated from mucins. Interfering oligosaccharides of dietary origin were removed by filtration. Fecal mucins are expressed as μmol oligosaccharide equivalents.

Analyses of fecal water. Fecal water was prepared by reconstituting freeze-dried feces with appropriate amounts of double-distilled water to obtain the physiological osmolarity of 300 mOsmol/L. Samples were homogenized, incubated for 1 h at 37°C and subsequently centrifuged for 1 h at 14,000 *g* (Micro-rapid 1306; Hettich, Tuttlingen, Germany). Supernatants (fecal water) were stored at −20°C until further analyses. The pH of fecal water was measured at 37°C. The cytotoxicity of fecal water was determined with an erythrocyte assay as previously described (17), and validated earlier with intestinal epithelial cells (18). The incubations were of physiological 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.

Analysis of urine samples. Complete 24-h urine samples were collected starting 1 d before infection until 9 d after infection. Oxytetracycline (1 mg; Sigma) was added to the urine collection vessels of the metabolic cages to prevent bacterial deterioration. The concentration of NO_x (sum of nitrate and nitrite) was determined by automated flow-injection analysis. Briefly, diluted urine was passed over a cadmium column to reduce nitrate to nitrite, followed by reaction of nitrite with Griess reagent (19). The red azo-dye formed was measured spectrophotometrically at 538 nm.

TABLE 1

Composition of the experimental diets

Ingredient	Control	3% FOS	6% FOS
		g/kg	
Glucose	513	483	453
Acid casein	200	200	200
Palm oil	160	160	160
Corn oil	40	40	40
Cellulose	20	20	20
Fructo-oligosaccharide	0	30	60
Mineral mix[1]	48	48	48
$CaHPO_4 \cdot 2H_2O$	5.16	5.16	5.16
Vitamin mix[1]	14	14	14

[1] The composition of the vitamin and mineral mixtures was according to the recommendation of the American Institute of Nutrition 1993 (14), except that calcium was omitted. In addition, tripotassium citrate was added instead of KH_2PO_4 and choline chloride was added instead of choline tartrate. The concentrations of vitamins and minerals mixtures were increased to ensure adequate intake during restricted feeding. FOS, fructo-oligosaccharides.

Statistical analysis. Results are expressed as means ± SEM ($n = 7$ in the 6% FOS group and $n = 8$ in the other diet groups). A commercially available package (Statistica 5.5; StatSoft, Tulsa, OK) was used for all statistics. Not all data were normally distributed, as indicated by the Shapiro–Wilk's test. Therefore, the diet-induced differences within each period, either before or after infection, were tested for significance by use of a Kruskall–Wallis ANOVA. In the case of significant treatment effects, the nonparametric Mann–Whitney *U* test (two-sided) was used to test for differences between each treatment group and the control group. In all cases, Bonferroni correction was made for the number of comparisons ($n = 3$). Differences were considered significant at $P < 0.05$.

RESULTS

Growth and food intake of rats. One rat in the 6% FOS group was excluded from the study results because of oropharyngeal reflux of the salmonella suspension resulting in pneumonia. Before infection, growth among the diet groups did not differ; mean growth was 2.5 g/d. However, at wk 1 after infection FOS decreased growth: 3.1 ± 0.3 g/d in the control group, 2.4 ± 0.6 g/d in the 3% FOS group and 0.7 ± 1.4 g/d in the 6% FOS group ($P < 0.05$). Before infection, all rats consumed the provided 13 g/d. During wk 1 after infection, food intake in the control group was still 13 g/d. However, food intake in the 6% FOS group was less: 13.0 ± 0.05 g/d in the control group, 12.4 ± 0.3 g/d in the 3% FOS group and 11.6 ± 0.5 g/d in the 6% FOS group ($P < 0.05$). Thereafter, mean daily food intake was 13 g/d in all diet groups.

Intestinal microflora. Compared with the control group, 6% FOS increased the number of bifidobacteria in feces (**Fig. 1**). Fecal lactobacilli numbers between the diet groups did not differ. Both FOS groups increased by 100-fold the number of enterobacteria in feces (Fig. 1). Neither the bifidobacteria nor the lactobacilli and enterobacteria levels were affected by *S. enteritidis* infection, as determined in fecal samples collected after infection (data not shown).

Cecal and fecal parameters. Dietary FOS dose-dependently decreased cecal pH (**Table 2**). Moreover, FOS decreased the pH of fecal water. Fecal pH was 6.9 ± 0.1 in the control group, 6.5 ± 0.1 in the 3% FOS group and 6.3 ± 0.1 in the 6% FOS group ($P < 0.05$). No infection-induced change in cecal and fecal water pH was found for the diet groups (data not shown). In addition, 6% FOS resulted in higher lactic acid concentrations in cecal contents compared with the control group (Table 2). However, cecal SCFA concentration or cecal SCFA pool, expressed as μmol/cecum, did not differ among the diet groups (Table 2). Before infection, feces of both FOS groups were softened. After infection, feces of the 6% FOS group especially were watery, whereas the control group still had normal feces. In the FOS groups no proper assessment of fecal wet weight excretion could be made because of drying up of the pellets during collection in the metabolic cages. Therefore, fecal wet weight excretion was determined by the summed concentration of the fecal cations sodium, potassium and ammonia (20). Before infection, FOS increased the daily fecal cation excretion. This was largely attributable to a threefold increase in fecal sodium and a twofold increase in fecal ammonia. No changes in fecal potassium were observed. After infection, daily fecal cation excretion increased in all diet groups, especially in the 6% FOS group (**Fig. 2**). This was attributed to a sixfold increase in fecal sodium, a threefold increase in fecal ammonia and a twofold increase in fecal potassium, in the 6% FOS group compared with the control group. Both FOS groups strongly increased the cytotoxicity of fecal water before infection (**Fig. 3**). Cytotoxicity of fecal water was not affected by *S. enteritidis* infection. Moreover, 6% FOS stimulated daily fecal mucin excretion before infection (**Fig. 4**). Fecal mucin excretion increased even further after infection in the 6% FOS group.

Colonization and translocation of salmonella. FOS dose-dependently increased salmonella numbers in cecal contents, 9 d after infection (**Fig. 5**). Moreover, 6% FOS increased salmonella numbers in cecal mucosa. In addition, major differences were observed in the effects of FOS on translocation of *S. enteritidis* as measured by the infection-induced urinary NO_x excretion in time (**Fig. 6**). After infection, urinary NO_x excretion of the 6% FOS group strongly increased to 202 μmol/d, a maximum reached at d 7. After d 7, urinary NO_x output gradually decreased to baseline levels again. Peak urinary NO_x excretion of the control and 3% FOS group was approximately half that of the 6% FOS group. However, the kinetics of urinary NO_x excretion were similar in all diet groups. The total infection-induced urinary NO_x excretion was higher in the 6% FOS group. The area under the curve corrected for baseline output was 332 ± 71, 452 ± 64 and 826 ± 51 μmol/9 d for the control, 3% and 6% FOS group, respectively.

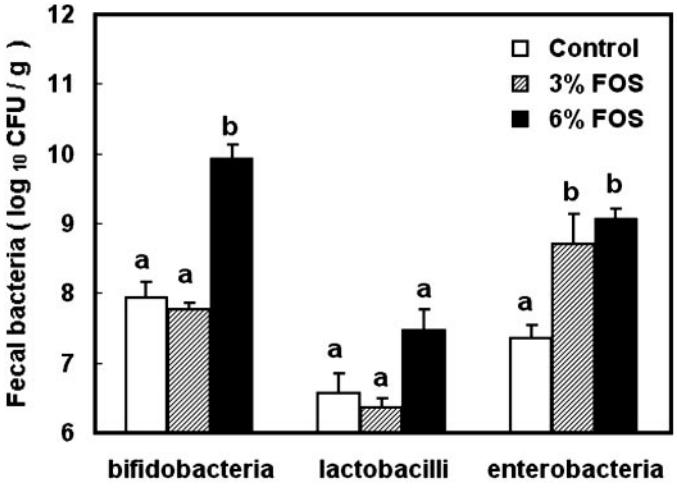

FIGURE 1 Effects of dietary fructo-oligosaccharides (FOS) on the number of bifidobacteria, lactobacilli and enterobacteria in fecal samples of rats, before infection. Bifidobacteria were specifically quantified by real-time quantitative PCR. Lactobacilli and enterobacteria were quantified by standard culturing on appropriate agar plates. Results are expressed as means ± SEM, $n = 7$ in the 6% FOS group and $n = 8$ in the other groups. Means without a common letter differ, $P < 0.05$.

TABLE 2

Effects of dietary fructo-oligosaccharides (FOS) on pH, short-chain fatty acids (SCFA) and lactic acid in cecal contents of rats[1]

Variable	Control	3% FOS	6% FOS
pH	7.6 ± 0.3[a]	6.7 ± 0.1[b]	5.8 ± 0.1[c]
SCFA, *μmol/g*	35.9 ± 5.5[a]	31.9 ± 4.3[a]	32.5 ± 5.9[a]
SCFA, *μmol/cecum*	33.4 ± 6.9[a]	53.0 ± 14.7[a]	82.8 ± 20.8[a]
Lactic acid, *μmol/g*	3.2 ± 0.2[a]	5.7 ± 1.4[a]	30.1 ± 5.6[b]
Lactic acid, *μmol/cecum*	2.7 ± 0.4[a]	7.7 ± 2.2[a]	76.4 ± 18.4[b]

[1] Results are expressed as means ± SEM, $n = 7$ in the 6% FOS group and $n = 8$ in the other groups. Means in the same row without a common letter differ, $P < 0.05$.

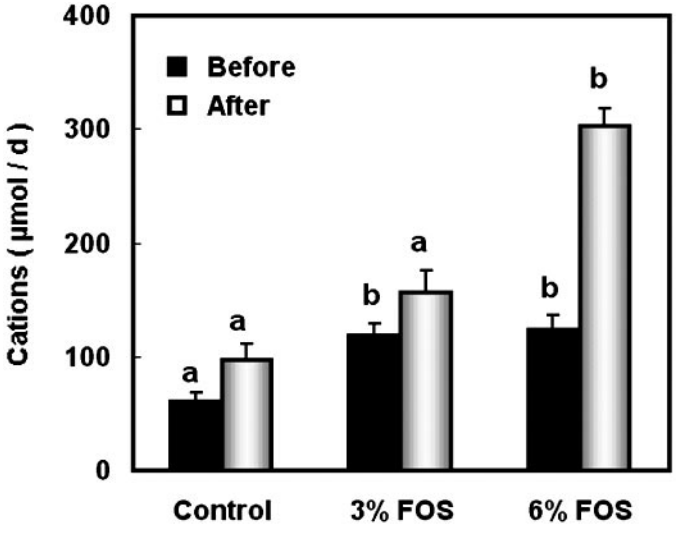

FIGURE 2 Effects of dietary fructo-oligosaccharides (FOS) on daily fecal cation excretion of rats, before and after infection. Sodium, potassium and ammonia were measured as the major fecal cations. Results are expressed as means ± SEM, $n = 7$ in the 6% FOS group and $n = 8$ in the other groups. Means, before or after infection, without a common letter differ, $P < 0.05$.

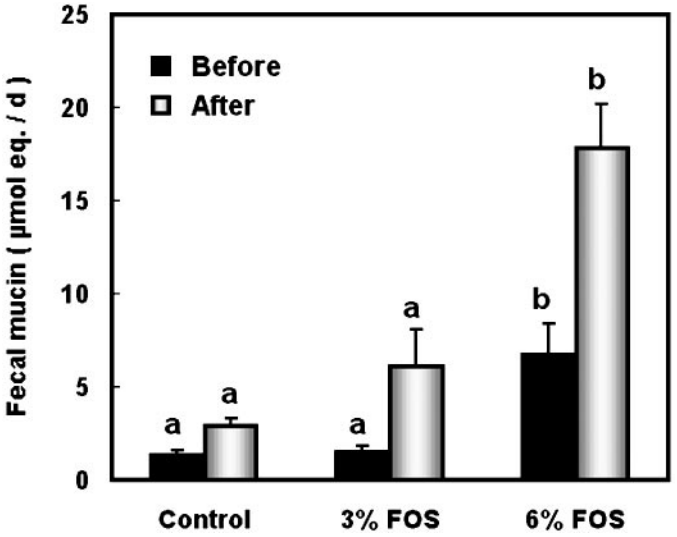

FIGURE 4 Effects of dietary fructo-oligosaccharides (FOS) on daily fecal mucin excretion of rats, before and after infection. Mucins were measured fluorimetrically and expressed as µmol oligosaccharide equivalents. Results are expressed as means ± SEM, $n = 7$ in the 6% FOS group and $n = 8$ in the other groups. Means, before or after infection, without a common letter differ, $P < 0.05$.

DISCUSSION

In contrast to most expectations, dietary FOS impaired the resistance to intestinal infections, as it dose-dependently increased colonization and translocation of *S. enteritidis* in the present rat study.

FOS is resistant to degradation by host digestive enzymes in the proximal small intestine, given that it is almost completely recovered from human (21) and rat ileum (22). Subsequently, FOS is rapidly fermented by the microflora of the lower gut. Fermentation results in the production of lactic acid and SCFA. This results in a lower intestinal pH, which can inhibit growth of acid-sensitive pathogens like salmonella (5). Indeed, FOS dose-dependently decreased cecal and fecal pH in the present study.

The intestinal microflora increases the resistance to pathogen colonization by competing for nutrients and mucosal adhesion sites and possibly by producing antimicrobial substances (6). Previous human and rat studies showed that FOS increases densities of bifidobacteria and lactobacilli, both of

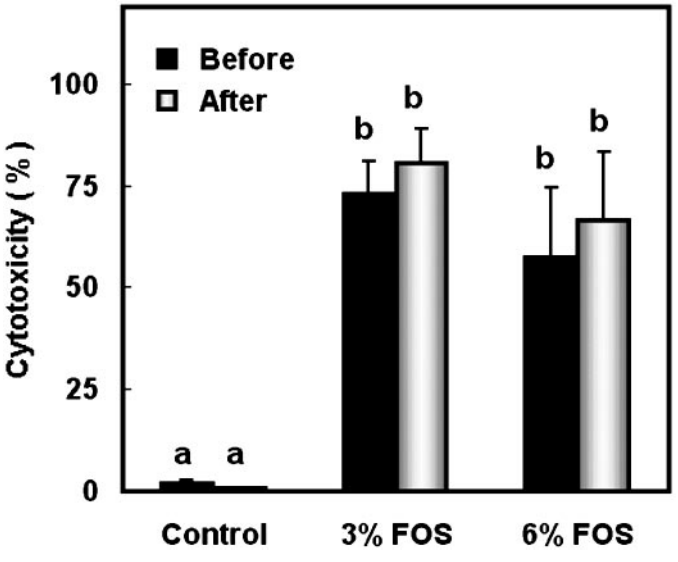

FIGURE 3 Effect of dietary fructo-oligosaccharides (FOS) on the cytotoxicity of fecal water of rats, before and after infection. Cytotoxicity was determined with a hemolysis assay. Results are expressed as means ± SEM, $n = 7$ in the 6% FOS group and $n = 8$ in the other groups. Means, before or after infection, without a common letter differ, $P < 0.05$.

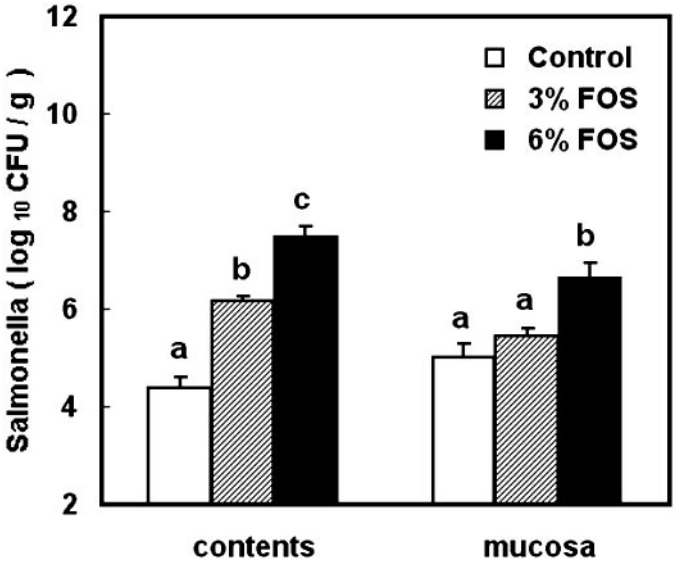

FIGURE 5 Effects of dietary fructo-oligosaccharides (FOS) on salmonella numbers in cecal contents and mucosa of rats, 9 d after oral administration of 1.7×10^{10} colony-forming units (CFU) of *S. enteritidis*. Salmonella was cultured aerobically on modified brilliant green agar. Results are expressed as means ± SEM, $n = 7$ in the 6% FOS group and $n = 8$ in the other groups. Means without a common letter differ, $P < 0.05$.

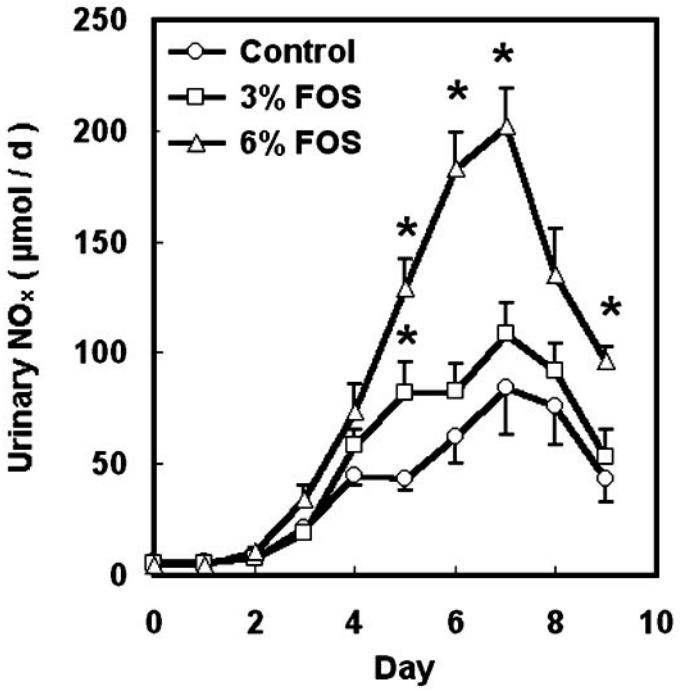

FIGURE 6 Effect of dietary fructo-oligosaccharides (FOS) on the kinetics of urinary NO_x (sum of nitrate and nitrite) excretion of rats after an oral challenge with 1.7×10^{10} colony-forming units of *S. enteritidis* on d 0. Results are expressed as means ± SEM, $n = 7$ in the 6% FOS group and $n = 8$ in the other groups. *Significantly different from the control group at that time point, $P < 0.05$.

which are considered beneficial for gut health (4,23). Indeed, in the present study, FOS stimulated the growth of bifidobacteria and enterobacteria, which suggests that the effect of FOS is fairly nonspecific because it stimulates the growth not only of potentially beneficial bacterial species but also that of potentially noxious species. The general opinion is that enterobacteria are not beneficial because they are linked to gut-derived sepsis (24). Previous animal and human studies showed that FOS either stimulates growth of enterobacteria (25,26) or has no effect (4,27).

Although FOS decreased intestinal pH and stimulated the endogenous bifidobacteria, it increased colonization of *S. enteritidis* as measured by increased salmonella numbers in cecal contents and mucosa. Obviously, FOS facilitates adhesion and multiplication of salmonella in the intestinal tract. Moreover, our in vitro study showed that salmonella can use FOS as a substrate for growth (data not shown). Accordingly, FOS increased infection-induced diarrhea as measured by the increased fecal cation excretion. The few infection studies present in the literature show inconsistent effects of oligosaccharides on salmonella colonization in broiler chicks (7,8), mice (10) and swine (9). For example, Lettelier et al. (9) showed that 1% FOS in water decreases colonization of salmonella, whereas FOS in feed increases colonization. Moreover, Chambers et al. (8) showed that 5% refined FOS decreases colonization, whereas artichoke flour (containing 5% FOS) increases colonization. Other studies showed no effect of oligosaccharides on colonization of salmonella (7,10). In addition, in a study with human volunteers FOS had no significant effect on travelers' diarrhea, which is mainly caused by the noninvasive enterotoxigenic *E. coli* (28).

Besides multiplication in the intestine and adhesion to the intestinal mucosa (colonization), salmonella is able to penetrate the intestinal mucosa and reach extraintestinal sites (translocation). Strikingly, we also found that FOS stimulated salmonella translocation, considering the strong increase in infection-induced urinary NO_x excretion. In contrast to organ cultures, which notably underestimate translocation resulting from the rapid immune-mediated killing of translocated salmonella, urinary NO_x is a sensitive and quantitative biomarker of intestinal bacterial translocation (29). We previously showed that urinary NO_x correlates with organ cultures (30,31). Several other animal (31) and human (32,33) studies have shown that serum or urinary NO_x correlates with the severity of systemic infectious disease. In summary, the FOS-induced increase in cecal colonization and intestinal translocation of salmonella, the dramatic increase in infection-induced diarrhea and the inhibition of growth in rats indicate that the infection was worse in FOS-supplemented rats. The FOS-induced stimulation of translocation could be attributed to its rapid fermentation, and hence production of organic acids. High concentrations of organic acids can induce colonic mucosal damage or inflammation (12). The intestinal mucosa responds to these irritants (and other irritants like bile acids and bacterial toxins) by increasing mucin excretion (34). In agreement, in the present study FOS strongly increased the cytotoxicity of fecal water and fecal mucin excretion. Considering that the major fermentation effects of FOS are seen within the distal gut (35), the mucosal barrier would be impaired at this site (36). Under normal circumstances, penetration of the mucosal barrier by salmonella is supposed to occur through specialized epithelial cells (called M-cells) that are located in ileal Peyer's patches (37), although this issue is still debated (38). Salmonella can also translocate to extraintestinal sites by dendritic cells, present in the mucosa throughout the entire intestine (39). In addition, salmonella is able to cross enterocytes para- and transcellularly (40). Thus, it is tempting to speculate that rapid fermentation of FOS expands the possibilities for salmonella to translocate even in the distal gut.

The dose of FOS used in the present study is realistic for the human situation. Daily intake of fermentable fibers for the United States and Europe has been estimated to be up to 10 g (41). This estimation does not take into account consumption of specific meals and products supplemented with inulin or FOS, typically 3–10 g per portion (42). Assuming a food intake of 400–500 g dry weight/d, this corresponds to 3–5% in the diet.

In summary, we found that FOS dose-dependently increased the colonization and translocation of *S. enteritidis* in rats. The effect may be attributed to the rapid production of acidic fermentation metabolites and hence impairment of the mucosal barrier. The results of the present study await verification by other controlled animal and human studies.

ACKNOWLEDGMENTS

The authors thank the biotechnicians at the Small Animal Center of Wageningen University (Wageningen, The Netherlands) for expert biotechnical assistance and Martijn Katan (Wageningen Center for Food Sciences) for stimulating discussions.

LITERATURE CITED

1. Olsen, S. J., Bishop, R., Brenner, F. W., Roels, T. H., Bean, N., Tauxe, R. V. & Slutsker, L. (2001) The changing epidemiology of salmonella: trends in serotypes isolated from humans in the United States, 1987–1997. J. Infect. Dis. 183: 753–761.

2. Kasper, H. (1998) Protection against gastrointestinal diseases—present facts and future developments. Int. J. Food Microbiol. 41: 127–131.

3. Gibson, G. R., Beatty, E. R., Wang, X. & Cummings, J. H. (1995)

Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 108: 975–982.

4. Kleessen, B., Hartmann, L. & Blaut, M. (2001) Oligofructose and long-chain inulin: influence on the gut microbial ecology of rats associated with a human faecal flora. Br. J. Nutr. 86: 291–300.

5. Bovee-Oudenhoven, I. M., Termont, D. S., Heidt, P. J. & Van der Meer, R. (1997) Increasing the intestinal resistance of rats to the invasive pathogen *Salmonella enteritidis*: additive effects of dietary lactulose and calcium. Gut 40: 497–504.

6. Orrhage, K. & Nord, C. E. (2000) Bifidobacteria and lactobacilli in human health. Drugs Exp. Clin. Res. 26: 95–111.

7. Oyarzabal, O. A. & Conner, D. E. (1996) Application of direct-fed microbial bacteria and fructooligosaccharides for salmonella control in broilers during feed withdrawal. Poult. Sci. 75: 186–190.

8. Chambers, J. R., Spencer, J. L. & Modler, H. W. (1997) The influence of complex carbohydrates on *Salmonella typhimurium* colonization, pH, and density of broiler ceca. Poult. Sci. 76: 445–451.

9. Letellier, A., Messier, S., Lessard, L. & Quessy, S. (2000) Assessment of various treatments to reduce carriage of Salmonella in swine. Can. J. Vet. Res. 64: 27–31.

10. Asahara, T., Nomoto, K., Shimizu, M., Watanuki, M. & Tanaka, R. (2001) Increased resistance of mice to Salmonella enterica serovar Typhimurium infection by synbiotic administration of Bifidobacteria and trangalactosylated oligosaccharides. J. Appl. Microbiol. 91: 985–996.

11. Deitch, E. A. (1994) Bacterial translocation: the influence of dietary variables. Gut 35: S23–S27.

12. Argenzio, R. A. & Meuten, D. J. (1991) Short-chain fatty acids induce reversible injury of porcine colon. Dig. Dis. Sci. 36: 1459–1468.

13. Remesy, C., Levrat, M. A., Gamet, L. & Demigne, C. (1993) Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. Am. J. Physiol. Gastrointest. Liver Physiol. 264: G855–G862.

14. Reeves, P. G., Nielsen, F. H. & Fahey, G. C., Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123: 1939–1951.

15. Requena, T., Burton, J., Matsuki, T., Munro, K., Simon, M. A., Tanaka, R., Watanabe, K. & Tannock, G. W. (2002) Identification, detection, and enumeration of human bifidobacterium species by PCR targeting the transaldolase gene. Appl. Environ. Microbiol. 68: 2420–2427.

16. Tangerman, A. (1996) A gas chromatographic analysis of fecal short-chain fatty acids, using the direct injection method. Anal. Biochem. 236: 1–8.

17. Bovee-Oudenhoven, I., Termont, D., Dekker, R. & Van der Meer, R. (1996) Calcium in milk and fermentation by yoghurt bacteria increase the resistance of rats to Salmonella infection. Gut 38: 59–65.

18. Lapre, J. A., Termont, D. S., Groen, A. K. & Van der Meer, R. (1992) Lytic effects of mixed micelles of fatty acids and bile acids. Am. J. Physiol. Gastrointest. Liver Physiol. 263: G333–G337.

19. Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. & Tannenbaum, S. R. (1982) Analysis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. Anal. Biochem. 126: 131–138.

20. Fine, K. D., Krejs, G. J. & Fordtran, J. S. (1993) Diarrhea. In: Gastrointestinal Disease (Sleisinger, M. H. & Fordtran, J. S., eds.), pp. 1043–1072. W. B. Saunders, London, UK.

21. Ellegard, L., Andersson, H. & Bosaeus, I. (1997) Inulin and oligofructose do not influence the absorption of cholesterol, or the excretion of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. Eur. J. Clin. Nutr. 51: 1–5.

22. Nilsson, U., Oste, R., Jagerstad, M. & Birkhed, D. (1988) Cereal fructans: in vitro and in vivo studies on availability in rats and humans. J. Nutr. 118: 1325–1330.

23. Le Blay, G., Michel, C., Blottiere, H. M. & Cherbut, C. (1999) Prolonged intake of fructo-oligosaccharides induces a short-term elevation of lactic acid-producing bacteria and a persistent increase in cecal butyrate in rats. J. Nutr. 129: 2231–2235.

24. Kayama, S., Mitsuyama, M., Sato, N. & Hatakeyama, K. (2000) Overgrowth and translocation of Escherichia coli from intestine during prolonged enteral feeding in rats. J. Gastroenterol. 35: 15–19.

25. Oli, M. W., Petschow, B. W. & Buddington, R. K. (1998) Evaluation of fructooligosaccharide supplementation of oral electrolyte solutions for treatment of diarrhea: recovery of the intestinal bacteria. Dig. Dis. Sci. 43: 138–147.

26. Sakai, K., Aramaki, K., Takasaki, M., Inaba, H., Tokunaga, T. & Ohta, A. (2001) Effect of dietary short-chain fructooligosaccharides on the cecal microflora in gastrectomized rats. Biosci. Biotechnol. Biochem. 65: 264–269.

27. Kleessen, B., Sykura, B., Zunft, H. J. & Blaut, M. (1997) Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. Am. J. Clin. Nutr. 65: 1397–1402.

28. Cummings, J. H., Christie, S. & Cole, T. J. (2001) A study of fructo oligosaccharides in the prevention of travellers' diarrhoea. Aliment. Pharmacol. Ther. 15: 1139–1145.

29. Oudenhoven, I. M., Klaasen, H. L., Lapre, J. A., Weerkamp, A. H. & Van der Meer, R. (1994) Nitric oxide-derived urinary nitrate as a marker of intestinal bacterial translocation in rats. Gastroenterology 107: 47–53.

30. Bovee-Oudenhoven, I. M., Termont, D. S., Weerkamp, A. H., Faassen-Peters, M. A. & Van der Meer, R. (1997) Dietary calcium inhibits the intestinal colonization and translocation of Salmonella in rats. Gastroenterology 113: 550–557.

31. Sprong, R. C., Hulstein, M. F. & Van der Meer, R. (2000) Quantifying translocation of Listeria monocytogenes in rats by using urinary nitric oxide-derived metabolites. Appl. Environ. Microbiol. 66: 5301–5305.

32. Forte, P., Dykhuizen, R. S., Milne, E., McKenzie, A., Smith, C. C. & Benjamin, N. (1999) Nitric oxide synthesis in patients with infective gastroenteritis. Gut 45: 355–361.

33. Charmandari, E., Meadows, N., Patel, M., Johnston, A. & Benjamin, N. (2001) Plasma nitrate concentrations in children with infectious and noninfectious diarrhea. J. Pediatr. Gastroenterol. Nutr. 32: 423–427.

34. Barcelo, A., Claustre, J., Moro, F., Chayvialle, J. A., Cuber, J. C. & Plaisancie, P. (2000) Mucin secretion is modulated by luminal factors in the isolated vascularly perfused rat colon. Gut 46: 218–224.

35. Campbell, J. M., Fahey, G. C., Jr. & Wolf, B. W. (1997) Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J. Nutr. 127: 130–136.

36. Tsukuhara, T. (2002) An improved technique for the histological evaluation of the mucus-secreting status in rat cecum. J. Nutr. Sci. Vitaminol. 48: 311–314.

37. Jensen, V. B., Harty, J. T. & Jones, B. D. (1998) Interactions of the invasive pathogens *Salmonella typhimurium*, Listeria monocytogenes, and *Shigella flexneri* with M cells and murine Peyer's patches. Infect. Immun. 66: 3758–3766.

38. Hughes, E. A. & Galan, J. E. (2002) Immune response to Salmonella: location, location, location? Immunity 16: 325–328.

39. Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J. P. & Ricciardi-Castagnoli, P. (2001) Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. 2: 361–367.

40. Kops, S. K., Lowe, D. K., Bement, W. M. & West, A. B. (1996) Migration of Salmonella typhi through intestinal epithelial monolayers: an in vitro study. Microbiol. Immunol. 40: 799–811.

41. Coussement, P. A. (1999) Inulin and oligofructose: safe intakes and legal status. J. Nutr. 129: 1412S–1417S.

42. Van Loo, J., Coussement, P., de Leenheer, L., Hoebregs, H. & Smits, G. (1995) On the presence of inulin and oligofructose as natural ingredients in the Western diet. Crit. Rev. Food Sci. Nutr. 35: 525–552.