Feeding a Diet Containing a Fructooligosaccharide Mix Can Enhance Salmonella Vaccine Efficacy in Mice

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Abstract

Fructooligosaccharides (FOS) are considered prebiotics because of their ability to promote growth of specific beneficial gut bacteria, such as bifidobacteria. Some studies reported potential immune-modulating properties. The aim of this study was to investigate the effect of FOS:inulin mix on murine response to Salmonella vaccine and evaluate the relevance toward protection against Salmonella infection. Balb/c mice were fed a diet containing 5% FOS:inulin mix or a control diet 1 wk before oral immunization with a suboptimal dose of live attenuated Salmonella typhimurium vaccine. Four weeks after vaccination, mice were infected with LD100 of virulent S. typhimurium. Specific blood Salmonella immunoglobulin G and fecal immunoglobulin A significantly increased in mice fed the diet containing prebiotics compared with control mice 4 wk postimmunization. Peritoneal macrophage phagocytic activity also significantly increased in FOS:inulin-fed mice at 1 wk postimmunization compared with control mice. No detectable effects were observed on the percentage of lymphoid cell subsets in the spleen. However, production of cytokines, interferon-γ, interleukin-12, and tumor necrosis factor α, was numerically increased in spleen cell cultures stimulated with mitogens from FOS:inulin-fed mice 1 and 4 wk postimmunization. Salmonella translocation to lymphoid organs was not affected by feeding FOS:inulin. However, the improved response to Salmonella vaccine was concomitant with an increase in the survival rate of FOS:inulin-fed mice upon challenge with virulent Salmonella. No detectable effects were observed on the composition or the metabolic activity of the microbiota. Overall, the data suggest that a diet supplemented with FOS:inulin mix stimulates mucosal immunity and seems to improve efficacy of an oral vaccine.

Introduction

Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon and thus improve host health (1). Fructooligosaccharides (FOS) selectively promote the growth of bifidobacteria in the colon of humans and may decrease the count of potentially detrimental bacteria, thus being considered as prebiotics (1–3). Besides their bifidogenic effect, short-chain FOS and long-chain FOS (such as inulin) have additional effects, including reducing colonic pH and increasing stool weight and stool bulking, justifying their classification as dietary fibers (4). Furthermore, FOS and inulin are able to increase the bioavailability of calcium (5) and possibly inhibit hepatic lipogenesis (6).

Immune-modulating effects of FOS were reported by several research groups. FOS increase leukocyte numbers in the gut mucosa and in the blood (7) and decrease pro-inflammatory cytokines in human patients (8). Moreover, FOS were shown to indirectly enhance T cell functions, natural killer cells, and phagocytic activities through modulation of gastrointestinal tract lactic acid bacteria density, which protected mice from enteric and systemic pathogens and tumor inducers (9).

The importance of Salmonella infections (particularly in developing countries) (10,11), the relatively low efficacy of the commercially available Salmonella typhi vaccine (12,13), and the increasing appearance of antibiotic-multi-resistant strains prompted the scientific community to design new vaccine strains (14–16). The difficulty was to obtain a strain highly attenuated, safe, and sufficiently immunogenic to induce a significant protective response (12,17).

An alternative to improving the vaccine efficacy would be to use nutritional supplements that could stimulate the immune system as adjuvant. In that sense, several studies have shown that different nutritional interventions, including dietary nucleotides, probiotics, and prebiotics, could likely enhance immune responses to various vaccines in children and adults through a T helper 1 (Th1)-like-mediated adjuvant effect (7,18–21).
Salmonella has been suggested to preferentially cross the intestinal epithelial barrier through specialized M cells, found at the surface of the Peyer’s patches (PP), before disseminating to distant sites such as the liver and spleen (22,23).

It is well documented that host resistance to Salmonella relies initially on the production of inflammatory cytokines leading to the infiltration of activated inflammatory cells in the tissue, including interferon-γ (IFNγ), tumor necrosis factor-α (TNFα), and interleukin-12 (IL-12). Thereafter, both T and B cell-mediated immune responses are elicited to control primary infection and protection against secondary challenge (24,25). In that context, secretory immunoglobulin (Ig) A produced in the intestine play a major role in the defense mechanism against these bacteria (15,26).

The aim of this exploratory study was to investigate the capacity of a FOS:inulin mix to enhance murine immune response to Salmonella vaccine and evaluate its relevance for protection against Salmonella infection.

Our working hypothesis is that a prebiotic combination such as the association of FOS and inulin could exert immune-enhancing functions that could be useful to protect the host against pathogens and/or could act as adjuvant for vaccines.

Materials and Methods

Mice and diets. Conventional 6-wk-old female Balb/c mice (18–20 g) were used in all experiments (Ifaz-Credo). The mice were housed (5 mice per cage) under specific pathogen-free conditions at the Nestle research center animal facility. Mice were fed a commercial diet (Kliba 3434) supplemented with either 5% (wt:wt incorporated in the pellets) of a prebiotic mix (70% Raftilose P95–30% Raftiline HP wt:wt in the mixed powder preparation, both from Orafti) or cellulose, hereafter called FOS mix or control, respectively. The macronutrient content as fed to mice was 17.6% protein, 4.3% fat, 4.3% crude fiber, 51.3% carbohydrate, and 13.5 kcal/g metabolizable energy. The dose of prebiotics or cellulose corresponds to a daily intake of 8–10 mg/g of body weight. Such an amount of cellulose has never shown any effect on intestinal ecology or immunity in our previous animal studies, which is why it is commonly used as control for our prebiotic nutritional intervention studies (unpublished data).

Bacterial strains and cultures. The 2 bacterial strains used were derived from the same genetic background, Salmonella typhimurium SL1344, wild-type strain (14), used as infectious strain and its derivative, Salmonella typhimurium SL1479 Δaro A attenuated strain (ATCC-39183), was used as vaccine.

Bacteria were grown in Luria-Bertoni broth at 37°C for 18 h and then concentrated in 1 mL of PBS. The number of viable cells was determined by agar plate counting. Identification of Salmonella was conducted using the API 20E test (BioMérieux SA).

Vaccination and infection protocols. The first study aimed at evaluating the response to vaccine, 2 groups of mice were fed a control diet or a diet containing 5% FOS mix (n = 20 per group). One week after starting the feeding, the 2 groups of mice were immunized by oral gavage with 5 × 10⁷ colony-forming units (CFU) of attenuated S. typhimurium SL1479. This dose of Salmonella vaccine was established as conferring 50% protection against challenge with wild-type Salmonella strain in a previous study (unpublished data). The immune response was monitored during the 4 wk postimmunization that corresponds to the peak of antibody response (14–16,20) (see Fig. 1 for trial design). Two separate subgroups of mice from each treatment group (n = 5) were killed at 1 wk and 4 wk postvaccination to evaluate immune effects at the cellular level (peritoneal macrophages and splenocytes) and bacterial translocation vs. survival rate in lymphoid organs, respectively.

A second study aimed at evaluating the protection against Salmonella infection, mice were randomized into 4 groups (n = 20 per group). Group control were mice fed a control diet. Group control + vaccine were mice fed a control diet and vaccinated with a suboptimal dose of 5 × 10⁷ CFU of attenuated Salmonella SL1479 prior to infection (as described above). Group FOS consisted of mice fed a diet containing FOS mix, and Group FOS + vaccine consisted of mice fed a diet containing FOS mix and vaccinated prior infection (Fig. 1).

All groups of mice were infected 4 wk after vaccination or equivalent period (for unvaccinated mice) with 3 × 10⁷ CFU per mouse of S. typhimurium SL1344 by oral gavage. This dose of virulent Salmonella was established as the LD100 in a previous study (unpublished data). The

FIGURE 1 Trial design of control and treatment groups. Study 1: 2 groups were tested (mice fed a control diet or the same diet containing FOS-mix). Study 2: four groups were tested. Control, mice fed a control diet. Control + vaccine, mice fed a control diet and vaccinated. FOS, mice fed a diet containing FOS-mix. FOS + vaccine, mice fed a diet containing FOS-mix and vaccinated.

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infection was followed daily during 3 wk, including weighing and macroscopic examinations. Criteria justifying euthanasia of mice included bristled coat, loss of weight (>20%), and loss of mobility. Mice were killed by cervical dislocation under anesthesia with isofluorane.

Assessment of antibody responses. Blood samples were collected from the tail vein of mice of study 1 just before vaccination and at 4 wk postimmunization. Samples were incubated overnight at 4°C and centrifuged at 10,000 × g; 15 min. Serum were collected and frozen at −20°C until analysis for IgG.

Fresh fecal samples were collected just before vaccination and at 4 wk postimmunization. Mice were individually isolated in a clean cage and fecal samples were collected right after emission. Samples were homogenized with extraction buffer (50 mmol/L EDTA, 100 mmol/L soybean trypsin inhibitor in PBS; Sigma). After centrifugation at 10,000 × g; 15 min, the supernatants were given doses for protein contents by BCA protein assay (Pierce) and stored at −20°C until analysis for IgA.

Total antibody levels (IgA in fecal extract or IgG in sera) were measured by ELISA as previously described (27). A monoclonal mouse IgA or mouse IgG was used as a standard and results were then expressed as the means ± SEM of IgA or IgG in mg/L (the detection limits for both antibodies were 2 μg/L). The fecal IgA data were normalized to the protein content of each fecal extract.

The amounts of specific anti-Salmonella IgA and IgG in feces were determined using the same ELISA except that the plates were coated with either 5 μg per well of Salmonella-LPS (Sigma) or 10 μg per well of Salmonella-flagellin extracted as previously described (28,29). Briefly, bacteria were grown overnight and flagellin was isolated by acidic depolymerization for 30 min at room temperature. Supernatant containing depolymerized flagella was clarified by centrifugation at 100,000 × g; 1 h at 4°C and equilibrated at pH 8.0. Flagellin was identified by SDS-PAGE using the anti-flagellin monoclonal antibody 15D8 (igen International). The data were expressed as mean OD at 450 nm ± SEM.

Assessment of phagocytic activity. Subgroups of control and FOS mix-fed mice from study 1 were killed 1 wk and 4 wk postimmunization (n = 5 for each group at each time point). Peritoneal macrophages were collected in ice-cold sterile PBS. Macrophages were seeded into 48-well plates at a concentration of 5 × 10⁶ cells per well and mixed with fluorescent E. coli (E. coli: FITC) for 30 min (Phagotest-kit, Orpegen). Fluorescent macrophages, reflecting the phagocytic activity, were analyzed by flow cytometry according to the manufacturer’s recommendations.

Assessment of cell subsets, cell proliferation, and cytokine production. Spleen samples from subgroups of control and FOS mix-fed mice from study 1, killed at 1 and 4 wk postimmunization (as described in the section above), were analyzed (n = 5 for each group at each time point). Spleens were collected, homogenized through a cell strainer (70 μm) in 2 mL complete RPMI-1640 medium (Gibco), and centrifuged at 250 × g; 5 min. The pellets were rapidly lysed with 1 mL of sterile distilled water and centrifuged at 250 × g for 5 min. The cells pellets were then suspended in complete RPMI-1640 medium to obtain 10⁶ cells/L. Cell subsets were quantified by 2-color flow cytometry according to the method previously described (27). Spleen cell proliferation assay was performed according to the method described by Humen et al. (30). Proliferation rates were evaluated on unstimulated cells or cells stimulated with the following mitogens (all purchased from Sigma): 2 mg/L concanavalin A (T cell stimulant), 1 mg/L pokeweed mitogen (PWM; B cell stimulant), or 2.5 mg/L LPS from S. typhimurium (monocyte and B cell stimulant). After incubation at 37°C for 72 h, 0.15 mL of supernatant was collected for IL-12, INFγ, and TNFα cytokine content analysis using ELISA kits purchased from R&D Systems according to the manufacturer’s recommendations. As specified in the kit, the detection limits were 2.5 ng/L, 2 ng/L, and 5.1 ng/L, respectively.

Quantification of Salmonella in lymphoid organs. As indicated above in the section “Vaccination and infection protocols,” subgroups of control and FOS mix-fed mice from study 1 were killed at 1 and 4 wk postimmunization, which correspond to the peak of Salmonella colonization and survival limit, respectively (31).

PP, mesenteric lymph nodes (MLN), spleen, and liver were asceptically collected, weighted, and homogenized in 2 mL of sterile PBS. The number of viable bacteria in each lymphoid organ was determined by plating 0.1 mL of processed tissue on Luria-Bertoni agar. Salmonella O antisera (Becton Dickinson) was used for agglutination test to complete the identification of S. typhimurium. The minimal amount of Salmonella detectable is 20 CFU per total organ that corresponds to 1 colony per plate. Therefore, the limit of detection expressed in the results is log₁₀ 1.3.

Analysis of microbiota. Fecal samples were collected from mice in study 1 24 h and 1 wk after immunization to analyze specifically Salmonella content. In addition, fecal samples from the same mice were collected just before immunization (1 wk) and 4 wk after to evaluate microbiota composition. Freshly collected feces were homogenized in Ringer medium containing 10% glycerol, and enterobacteria, lactobacilli, bifidobacteria, and enterococci or Salmonella, when specifically evaluated, were immediately enumerated by platting on selective media following the method previously described (8).

Statistical analysis. Statistical analyses were performed to evaluate differences between treatment groups of mice (mean values for each group represent the experimental unit for this nutritional intervention study).

In study 1, differences among means (control vs. FOS) were evaluated for each variable within a sampling period using a 2-tailed Student’s t test. The antibody responses were further tested for changes over time using a 1-way ANOVA with repeated measures. Before each analysis, data were tested for normal distribution and homoscedasticity.

In study 2, differences among survival rates at 3 wk postinfection (control vs. FOS vs. control + vaccine vs. FOS + vaccine) were evaluated using a chi-square test for independence. A confidence level of 95% was applied for all tests. Values in the text are means ± SEM.

Results

Antibody responses (study 1). Neither total IgG levels in the sera nor IgA levels in the fecal contents were influenced by FOS mix supplementation (data not shown).

There were no differences in the specific anti-Salmonella antibody responses between the 2 groups at 1 wk postimmunization. The responses then significantly increased in both groups at 4 wk postimmunization. However, this increased anti-Salmonella response was much stronger in the FOS mix group compared with controls. Indeed, the specific-LPS-IgA levels in the fecal contents were greater in the FOS mix-fed group than in the control group (P = 0.04) 4 wk after vaccination (Fig. 2A). The amount of specific flagellin-IgG in the plasma was greater in the FOS mix-fed group than in the control group (P = 0.01) 4 wk after vaccination (Fig. 2B).

Stimulation of phagocytic activity (study 1). The phagocytic activity of peritoneal cells 1 wk postimmunization was higher in the FOS mix-fed group (52.5 ± 0.7%) than in the control group (38.1 ± 3.3%; P = 0.02). After 4 wk, phagocytic activity in the FOS mix-fed group (40.5 ± 0.4%) had returned to the level in the controls (39.3 ± 0.6%).

Spleen cell subsets, cell proliferation, and cytokine production (study 1). The percentage of cell subsets, including CD4⁺, CD8⁺, B220⁺, MHCI⁺, CD11b⁺, and CD11c⁺ cells, did not differ between FOS mix- and control mice. However, the mean fluorescent intensity of MHCI⁺ cells was greater in FOS mix-fed mice (135 ± 29.5) than in controls (89 ± 21.4; P = 0.03). Feeding the FOS mix did not significantly influence the splenoocyte proliferation rates (data not shown). However, IL-12, IFNγ, and TNFα cytokine concentrations in cell culture supernatants...
Recovery of Salmonella from lymphoid organs (study 1). The number of Salmonella recovered from lymphoid organs (PP, MLN, liver, and spleen) in both groups at 4 wk postimmunization was not significantly different between the 2 groups, with mean log_{10} CFU/g of feces of 3.3 ± 0.1 in control mice and 2.8 ± 0.1 in FOS mix group, and barely detectable numbers of salmonella decreased by more than 1 log in all organs in both groups at 4 wk postimmunization (data not shown).

Microbiota analysis (study 1). There was no significant effect of FOS mix on microbiota composition compared with controls. The fecal amounts of SCFA, including acetate, propionate, and butyrate, were not significantly different between the 2 groups (data not shown). Salmonella were rapidly cleared after immunization with a similar ~2 log bacterial loss in both groups at 24 h postimmunization (log_{10} CFU/g of feces of 5.4 ± 0.4 in control mice and 6.0 ± 0.1 in FOS mix group) and barely detectable levels at 4 wk postimmunization (log_{10} CFU/g of feces of 3.5 ± 0.1 in control mice and 2.8 ± 0.1 in the FOS mix group).

Protection against Salmonella infection (study 2). The survival rate in the control group was ~10% at 2 wk postinfection. This rate remained the same after 3 wk. FOS mix alone was not able to protect mice from Salmonella infection as observed in control mice at the same time point. As expected, vaccination of mice fed a control diet with a suboptimal dose of attenuated Salmonella led to 40% protection (Fig. 3). This protection rate improved to 73% upon feeding FOS mix (P = 0.04). In most cases, Salmonella infection leads to deaths between 7 and 15 d postinfection in nonprotected animals. During this period, the differences between the groups were in agreement with the ones described above at 3 wk postinfection.

Discussion

This study shows that a FOS mix (FOS:inulin, 70:30 wt:wt in the mixed powder preparation) is able to improve the immune response to a Salmonella vaccine that contributes to increased vaccine efficacy. The idea of using foods as booster and/or carrier for human vaccine has been proposed in several studies [reviewed by Korban et al. (32)]. However, these approaches mainly consist of using genetically modified products, the use of which may be subject to regulatory issues in some countries. In this work, the data support the concept of using nongenetically modified food supplements containing a FOS:inulin mix to enhance vaccine efficacy.

It was previously established that Salmonella strains mutated in genes involved in the aromatic biosynthetic pathway (aro mutants) were effective vaccines in several animal species (14,33,34). Immunization with Salmonella elicits a broad range of antigen-specific responses. In that respect, LPS and flagellin have gained particular interest as potential protective antigens (35–37). Therefore, both mucosal and systemic responses toward LPS and flagellin were investigated in this study.

We used a suboptimal dose of Salmonella vaccine to measure the potential effect of the dietary intervention. The low oral dose used in this study was still able to elicit detectable antibody titers, as observed in control mice. Specific anti-Salmonella antibody

| TABLE 1 | Cytokine concentrations in cell culture supernatants of splenocytes from mice fed FOS mix or controls stimulated with different mitogens (Study 1) |
|----------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Cytokines | Wk 1 | | | Wk 2 | | | | |
| | ConA | PWM | LPS | | ConA | PWM | LPS |
| IL-12 | | | | | | | |
| Control | 24 ± 5 | 18 ± 5 | 47 ± 9 | 42 ± 2 | 12 ± 5 | 25 ± 6 |
| FOS | 35 ± 10 | 25 ± 2 | 51 ± 7 | 64 ± 2* | 27 ± 9* | 26 ± 9 |
| TNFα | | | | | | | |
| Control | 174 ± 24 | 371 ± 40 | 1091 ± 189 | 286 ± 37 | 569 ± 63 | 843 ± 117 |
| FOS | 181 ± 43 | 337 ± 12 | 1209 ± 102 | 357 ± 34 | 722 ± 61 | 857 ± 114 |
| IFNγ | | | | | | | |
| Control | 3132 ± 228 | 5608 ± 476 | 404 ± 166 | 3919 ± 389 | 4822 ± 588 | 40 ± 19 |
| FOS | 4278 ± 729 | 6507 ± 39 | 935 ± 267* | 5130 ± 508 | 5785 ± 491 | 210 ± 55* |

*Values are means ± SEM, n = 5 triplicates. *Different from controls, P < 0.05.
responses were significantly enhanced in mice fed FOS mix compared with control mice, suggesting that a diet containing FOS mix might act as a mucosal adjuvant. One previous clinical trial has shown that the same FOS mix incorporated into cereals significantly improved the response to measles vaccine in infants (20). Nevertheless, these data should be confirmed and the capacity of this prebiotic to trigger and boost systemic responses to vaccines needs further investigation.

Uptake of *Salmonella* by phagocytic cells after the bacteria have crossed the epithelium represents a key feature of the innate response of the host to *Salmonella* infection (22,23). In this study, the percentage of peritoneal cell phagocytic activity was transiently but significantly enhanced in the FOS mix-fed group compared with controls ($P < 0.01$ at 1 wk postimmunization). Along with this observation, the phagocytic activity as previously tested in vitro was significantly enhanced after treatment of murine macrophage cell line J774 with 10 mg/L of FOS mix compared with nontreated cells ($P = 0.02$), reflecting the capacity of FOS mix per se to interact with immune cells (data not shown). The IL-12, IFN$\gamma$, and TNF$\alpha$ cytokines are known to play a key role in the regulation of both innate and acquired immunity to *Salmonella* (24,38). The relative increase in these cytokine levels, mainly IL-12 and IFN$\gamma$, seems to indicate an activation of T and B cells as well as macrophages. Moreover, the enhanced mean fluorescent intensity of MHCI$\alpha$ molecules upon feeding with FOS mix suggests a stimulation of antigen presentation in vivo. These results reflect the capacity of FOS mix to promote a Th1-like adjuvant effect that might have supported the strong specific humoral responses observed in FOS mix-fed mice.

The ability of *S. typhimurium* to translocate into lymphoid organs is known to be one of the major steps of its pathogenicity (39). In agreement with a previous publication (31), the ΔaroA mutant strain SL1479 was not impaired in its ability to colonize and survive in lymphoid organs. The number of *Salmonella* recovered from PP, MLN, liver, and spleen at 1 wk after immunization did not differ between the 2 groups of mice, suggesting that FOS mix per se did not influence either the translocation or the survival rates of *Salmonella*. These data are in contradiction with results previously published by Bruggencate et al. (40) and Bovee-Oudenhoven et al. (41). The authors suggest that feeding FOS (Raftilose P95) could increase *Salmonella* translocation in rats in a dose-dependent manner. Whether these discrepancies could be attributed to a difference in the doses of prebiotics or a difference in *Salmonella* strains used in the 2 studies could be questioned. We cannot exclude that differences in macronutrient and micronutrient contents of the diets used in the 2 studies could have differently impacted the prebiotic effect on the host. We did not observe any signs of immune suppression or other potential side effects in our study upon feeding with FOS mix. The dose used in this study corresponds well with the assumed dose of nondigestible carbohydrates consumed by humans, which is $\sim 3$–5% of daily dry food intake (42).

The safety of FOS and the FOS:inulin mix is further supported by a few clinical trials in infants where no adverse effects have been observed. Importantly, no *Salmonellosis* has been reported so far upon feeding with prebiotics such as FOS and/or inulin in humans (4,20,43).

We wanted to evaluate if feeding the FOS mix could either have a direct protective effect or confer indirect protection through an improved response to vaccine. Unvaccinated mice were not protected regardless of diet, suggesting that the FOS mix alone was not able to protect mice from *Salmonella* infection. These results do not corroborate previous data reported by Buddington et al. (9) showing that feeding Raftilose P95 or inulin protected mice from systemic *Salmonella* infection and prevented *C. albicans* translocation. This difference in outcomes might be explained by the differences in the doses of prebiotics used. However, differences in the diet composition and the routes of infections could also explain the results in the 2 studies.

Our results showed that there was an improvement in the vaccine protection rate upon feeding with the FOS mix (from 40 to 73%). These results correlate well with the relative increase in the Th1 cytokine production and the high vaccine-specific immune response observed in FOS mix-fed mice at the time of challenge, i.e. 4 wk postimmunization, and are in agreement with the well-documented important role played by mucosal LPS-IgA and potentially systemic flagellin-IgG in the prevention against *Salmonella* infection (35–37,44,45).

It is postulated that clinical benefits from consumption of prebiotics are obtained through their effect on the colonic microbiota. Improved growth of lactic acid bacteria upon prebiotic feeding leads to a decrease in the pH that could contribute to the protection against pathogens (1,2,46). It is also assumed that modifications in gut microbiota induced by prebiotics such as FOS may ultimately affect immune functions [reviewed by Schley and Field (7)].

We attempted to highlight the potential mechanism by which the FOS mix could influence the immune system. No detectable effects were observed on microbiota composition or on SCFA fecal concentrations (data not shown). The prebiotic effect of the FOS:inulin mix, well studied in humans and other animals as indicated above (1,2,4,7,20,46), could not be featured in this model; however, because of limitations of the methods used in this study, we could not exclude it. Indeed, analysis of SCFA in the fecal content only partially reflects the real colonic metabolism situation and the bacterial count on semiselective agar media presents some limitations in sensitivity. Thus, evaluation of the microbial ecology with other techniques such as fluorescent in situ hybridization or denaturing gradient gel electrophoresis might help to better study the effect of FOS mix.

**FIGURE 3** Survival rate of mice that were fed a diet containing FOS mix or a control diet and were vaccinated or not with an attenuated *Salmonella* prior to an infection with virulent *Salmonella* at 4 wk after immunization or equivalent period for unvaccinated mice (study 2). Data are expressed as the percentage of survival, $n = 20$ for each group.

*Different from control + vaccine at that time, $P < 0.05$. #Different from nonvaccinated mice, $P < 0.05$. 

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The authors thank M. Marchesini, J-L. Sanchez, M-A. Rochat, and P. Sarran for technical assistance.

Literature Cited


