Nutrient Interactions and Toxicity

Food Supplementation with Milk Fermented by *Lactobacillus casei* DN-114 001 Protects Suckling Rats from Rotavirus-Associated Diarrhea

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ABSTRACT Group A rotavirus is the leading cause of diarrhea among children aged 3–36 mo worldwide. Introducing fermented milk products into the infant diet has been proposed for the prevention or treatment of rotavirus diarrhea. The preventive effect of milk fermented by the *Lactobacillus casei* strain DN-114 001 was studied in a model of germfree suckling rats supplemented daily from d 2 of life and infected with SA11 rotavirus at d 5 (RF group). One group was supplemented with nonfermented milk (RM) and two uninfected groups (CM and CF) received either nonfermented or fermented milk. Frequency and severity of diarrhea were observed. Rats were killed at various times from 0 to 120 h postinfection (p.i.). Bacteria were measured in the intestine, and rotavirus antigens were detected by ELISA in fecal samples and in different parts of the intestine. Histologic observations were made, including vacuolation, morphology of intestinal villi and number of mucin cells. RM rats had diarrhea for 6 d; compared with the CM group, they had alterations of the intestinal mucosa characterized by cellular vacuolation at 48 and 72 h p.i. and a lower number of sulfated mucin cells 72 and 96 h p.i. (*P < 0.05*). Early supplementation with fermented milk significantly decreased the clinical signs of diarrhea from 24 to 144 h p.i. (*P < 0.05*) and prevented rotavirus infection in all sections of the intestine. Histologic lesions of the small intestine were greatly reduced (*P < 0.05*) and the number of mucin cells remained unchanged. The data are discussed with respect to the possibility of reducing rotavirus diarrhea in young children by consumption of fermented milk.

**KEY WORDS:** *Lactobacillus* • fermented milk • rotavirus • suckling rats • diarrhea • intestine

Group A rotavirus is the leading cause of diarrhea among children aged 3–36 mo worldwide. Rotavirus-associated diarrhea causes 870,000 deaths/y principally in developing countries (1). Symptoms are watery diarrhea, frequently associated with severe dehydration (2), and malabsorption of nutrients (3,4). Limited investigations by mucosal biopsy of infected infants have shown that rotavirus principally infects the cells of the small intestine.

Introduction of fermented milk products into the infant diet has been proposed for the prevention or treatment of acute diarrhea (5–8). These products contribute to a well-balanced diet and contain lactic acid bacteria (LAB),3 which are reputed for their healthful influence, especially in infants (9). Clinical and experimental studies have reported preventive and protective effects of LAB consumption on rotavirus diarrhea. The incidence of diarrhea and rotavirus shedding were reduced in infants receiving the bacterial association *Streptococcus thermophilus* and *Bifidobacterium bifidum* (10). After or during oral rehydration, a significant reduction of diarrheal symptoms was observed when infants consumed *Lactobacillus casei* strain GG (11–13), *Lactobacillus reuteri* (14) or a milk fermented by *Bifidobacterium longum* (15). In a previous study, we developed a germfree suckling rat model to study group A rotavirus–associated diarrhea (16). In this model, 5-d-old infected rats developed diarrhea that last for 6 d and was characterized by watery feces containing rotavirus antigens. Histologic analyses have demonstrated that rotavirus infects enterocytes and induces cellular vacuolation in the small intestine. The goal of this study was to shed light on how fermented milks protect against rotavirus-associated gastroenteritis. Clinical and histopathologic analyses were assessed in infected suckling rats supplemented for 3 d before being infected by a milk fermented by the *Lactobacillus casei* strain DN-114 001, which was found previously to have a beneficial effect on diarrhea in children (17).

**MATERIALS AND METHODS**

**Milk products.** Milk products were obtained from the VITAPOLE (Danone, Le Plessy Robinson, France) every 2 wk and were stored at 4°C throughout the study. The nonfermented, heat-treated (120°C, 15 s) milk was used as control and contained the following: 4.8% protein, 0.1% fat and 11.6% carbohydrates (7.5% lactose). After fermentation, the milk contained *L. casei* strain DN-114 001 [10^8 colony-forming units (cfu)/g] and 6.5% lactose. The products

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1 Supported in part by the VITAPOLE, Danone, France.
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3 Abbreviations used: cfu, colony-forming unit; LAB, lactic acid bacteria; MEM, modified Eagle’s medium; PFU, plaque-forming unit; p.i., postinfection.
were conditioned in sterile sealed tubes and were provided on a daily basis to isolators through a lock sterilized by peracetic acid (100 g/L).

**Virus inoculum preparation.** Rotavirus strain SA 11 was originally obtained from Dr. M. K. Estes (Baylor College of Medicine, Houston, TX) and prepared as described by Tournaud et al. (18). Briefly, the virus was propagated in fetal thymus monkey kidney cells (MA 104) that had grown under CO2 in modified Eagle’s medium (MEM; Gibco, Cergy Pontoise, France) containing 2.75 g/L NaHCO3. The infected cells were grown under CO2 in MEM containing 0.35 g/L NaHCO3 and supplemented with trypsin (0.5 mg/mL) and Hepes buffer (20 mmol/L, pH 7.6). Pools of rotavirus for administration to rats were prepared from clarified MA 104 cell lysate and stored at -80°C. The intra vitreous activity was determined using an agglutination plaque assay.

**Animal, feeding protocol and virus infection.** Pregnant germfree Fischer 344 rats, originating from the UEPDS breeding unit (INRA, Jouy en Josas, France), were reared in sterile Trelaxer type isolators (La Câhélene, Velizy, France) and consumed ad libitum an irradiated (45 kGy) commercial diet (UAR, Villemonaison/Orge, France). They were allowed to give birth naturally, and suckling rats remained with their dams throughout the study. Litters were maintained in sterile isolators until the age of inoculation and were housed in separate isolators depending on the treatment. Pups were infected by rotavirus and received the milk fermented by L. casei (RF group) or the nonfermented milk (RM group). Uninfected rats, the CF and CM groups, were used as controls for the supplementation matched groups, respectively. They were given 0.1 mL of the milk products by daily gavage from the age of 2 to 10 d and were inoculated at 5 days of age with a 0.1 mL single dose of either virus inoculum [1.6 × 1012 plaque-forming units (PFU)/L] or MEM as control [as previously described by Guérin-Danan et al. (16)]. Inoculation and gavage were performed with a plastic Pasteur pipette (Miliniquipette, Prolabo, France). After inoculation, infant rats were returned to their dams and allowed to suckle.

From 0 to 120 h postinfection (p.i.), pups were transferred into a laminar flux cabinet using a sterile container. They were killed with carbon dioxide; the entire intestinal tract was removed and divided into stomach, small intestine and colon. The intestinal compartments were either diluted immediately for Lactobacillus orography or each part of the intestine was opened, and the intestinal wall and contents were carefully separated, homogenized (Ultra-turrax, Bio-block, Paris, France) in 2 mL of sterile water and stored at -80°C before rotavirus antigen detection. For histologic observation, a 2-cm long segment was ligated in the midjejunum (2 ligatures before and after the selected anatomical site), and cooled ethanol was injected into the lumen of this selected segment using an insulin syringe. Then the sample was removed by sectioning between the two ligatures. The collected segments were prepared for histologic examination. All procedures were conducted in accordance with the Institute’s guide for the care and use of laboratory animals.

**Diarrhea examination.** Pups from 4–5 litters were checked daily for diarrhea by gentle massage of their abdomen (n = 55 in RF group, n = 39 in RM group, n = 34 in CF group, n = 40 in CM group). Diarrhea was defined when poorly formed yellow-green feces occurred immediately upon palpation. Control rats were treated identically to infected ones. Individual stool specimens were carefully collected in sterile plastic tubes on a weighed piece of plastic. Samples were stored at -80°C before rotavirus antigen detection.

**Lactobacilli enumeration.** The concentration of lactobacilli in the digestive tract was measured in 5-d-old rats supplemented with the fermented milk from 2 d of age (n = 6). Stomach, small intestine and colon were separately diluted in liquid casein yeast extract medium [casein enzymatic hydrolysate (U.S. Biochemical, Cleveland, OH), 2 g/L; yeast extract (Difco, Becton Dickinson, Le Pont de Clair, France), 2 g/L; NaCl, 5 g/L; KH2PO4, 1 g/L] and homogenized with an Ultra-turrax (Bio-block Scientific, Illkirch, France). Serial dilutions (10-2 to 10-5) were plated in 55 g/L MRS agar (Difco) and incubated at 30°C for 5 d in aerobic conditions as described previously (19).

**Rotavirus antigen detection.** Virus antigen was determined using a double sandwich ELISA. Microplates (Falcon 3915 probrind, Becton Dickinson, NJ) were coated overnight at 4°C with a 1/1000 dilution of anti-virus protein 6 monoclonal antibody (20) and saturated with 5% fetal calf serum. Samples were added to the plates and incubated for 1 h at room temperature. Rabbit antibodies to rotavirus were added to the washed plates, which were then incubated for 1 h at room temperature. The plates were washed again, and alkaline phosphatase-conjugated antibodies to rabbit immunoglobulin G were added. The plates were incubated for 1 h at room temperature and washed. The substrate p-nitrophenyl phosphate solution (1g/L) (Sigma Aldrich Chimie, St Quentin Fallarlin, France) in diethanolamine buffer (pH 9.8) was added. Absorbency was measured at 405 nm using a spectrophotometer (Labsystems Dynex, Cergy Pontoise, France). Negative and positive control tests were included in each plate. Negative tests consisted of PBS and positive ones of a serial dilution of the viral inoculum (from 1.6 × 1011 to 1.9 × 108 PFU/L). Each plate contained samples obtained from infected and uninfected pups of the same supplementation group. Assays were performed in duplicate. The viral antigen load in positive samples was determined relative to the standard curve in each plate and the dilution of samples at 405 nm.

**Histologic examination.** Examinations of histologic sections of the proximal small intestine were compared in three pups of each group. Diarrhea and the effect of fermented milk on the intestinal morphology were studied. Villi height, crypt depth, the number of mucus-containing cells and the presence of vacuoles were observed under light microscopy. Villi height, crypt depth, the number of mucus-containing cells and the presence of vacuoles were observed under light microscopy. Villi height, crypt depth, the number of mucus-containing cells and the presence of vacuoles were observed under light microscopy.

**Statistical analyses.** Data are expressed as mean ± SD. Statistical significance of differences was determined by ANOVA using StatView (Abacus Concepts, Berkeley, CA) with milk products and infection as factors. Statistical significance was set at P < 0.05. When differences were detected by ANOVA, differences between groups were determined using Fisher’s Protected Least Significant Difference test test when variances were equal and Scheffe’s F test when variances were unequal.

**RESULTS**

**Lactobacilli enumeration.** L. casei DN-114 001 survived throughout the intestinal transit as shown by the amount of bacteria recovered in the different parts of the digestive tract. Daily gavage of rats from 2 d of age maintained similar lactobacilli concentration in the stomach and the small intestine, 3.8 ± 0.5 and 3.5 ± 0.4 log (cfu/g), respectively. In the colon, the concentration of lactobacilli was significantly higher, 5.7 ± 0.6 log (cfu/g) (P < 0.05).

**Clinical investigations.** The weight of 2-d-old suckling rats was 6.8 ± 0.3 g (n = 168). The milk product supplementation did not modify body weight; at 5 d, weights were 10.2 ± 0.9, 9.6 ± 0.8, 10.0 ± 1.2 and 9.6 ± 0.9 g in the CM, CF, RM and RF groups, respectively. Although a few pups of the RM group showed a relatively slower rate of growth during the 48 h p.i., the weight gain was not significantly different among the four groups (Fig. 1). Diarrhea occurred in the infected rats supplemented with the nonfermented milk, i.e., 60–80% of the pups in the RM group delivered poorly formed yellow-green feces, 5–60% in the RF group and 0% in the CM and CF groups.
green feces from 24 to 144 h p.i. (Fig. 2). Fermented milk consumption significantly decreased the percentages of rats delivering feces in the RF group compared with those obtained from the RM group. No significant difference was observed between the RF and CF rats fed the fermented milk. In these groups, the percentage of rats from which feces were obtained immediately was < 40% at each time point of the study. Even though the percentage of rats delivering feces was significantly higher in the RF group compared with the CM group at 24 and 72 h p.i., the consistency of the feces was similar, and the samples were small and of a dark color.

**Rotavirus detection.** Immediately after infection, rotavirus antigens were detected in the stomach contents of both infected RM and RF groups (Table 1). From 3 h p.i., rotavirus antigens were no longer detected in the stomach contents in the RM group. By contrast, they remained until 9 h p.i. in the RF rats. The stomach wall was never infected.

In the small intestine, rotavirus antigens were found from the time of inoculation to 120 h p.i. in the RM rats. In this group, the intestinal wall was infected from 3 h p.i. By contrast, in the RF group, no rotavirus antigen was found in the small intestine from 48 to 120 h p.i., except in one rat (120 h p.i.). In this group, the intestinal wall was infected from 3 to 24 h p.i.

In the colon, rotavirus antigens were detected in the contents and wall in the RM group, from 3 to 24 h p.i. In the RF group, viral antigens were found only in the colonic contents and with less frequency at 6, 24 and 72 h p.i.

In feces of pups tested for diarrhea (Fig. 2), rotavirus antigens were detected in 53 and 54% of the fecal samples in the RM group and 48 h p.i., respectively, and 37 and 27%, respectively, in the fecal samples of the RF group (Table 2). From 72 to 144 h p.i., the proportion of fecal samples shedding > 10^6 rotavirus/g decreased progressively in both the RM and RF groups. In the RF rats, the load of rotavirus antigens in the feces tended to be reduced (P = 0.001–0.15) from 24 to 120 h p.i. (Table 2). This difference was significant at 24 and 72 h p.i. (P < 0.05).

**Histological analyses.** In the midjejunum, the control section of the CM group had cells characterized by nuclei localized at their base and by a large supranuclear area occupying almost the whole apical cytoplasm (Fig. 3). In the RM group, cellular morphology of the villus basis was not affected by the virus. Mucus was not completely released in the intestinal lumen as suggested by the presence of stained goblet cells. The histologic changes associated with rotavirus infection were characterized by cellular vacuolation. In this group, 90, 70 and 16% of the villi presented vacuolation at 48, 72 and 96 h p.i., respectively, whereas in the CM group, vacuolation was observed in < 10% of the villi throughout the study (Fig. 4). The vacuolation rate of intestinal villi was significantly higher in the RM compared with the CM group (P < 0.05). An apparent migration of the vacuoles was observed from the basal to the apical area of the villi in the RM group. At 48 h p.i., the cellular vacuoles were located at the basal area of the villi. They were observed at the tip of the villi at 72 h p.i. (Fig. 3). Comparison of the two infected RF and RM groups showed that the vacuolation rate was significantly lower in the group receiving the fermented milk 48 and 72 h p.i. (P < 0.05). In both RF and CF groups receiving the fermented milk, the vacuolation rate was not significantly different. No significant difference was found between the control groups (CF and CM). In these groups, vacuoles were found principally at the basal area of the villi.

The villi length was 300 ± 45, 275 ± 35 and 285 ± 40 μm and the crypt depth 32 ± 3, 35 ± 2 and 35 ± 4 μm in 7-, 8- and 9-d-old CM rats, respectively. The villi length and the crypt depth were not affected by rotavirus infection, nor by fermented milk supplementation; thus, variables expressed relative to these criteria did not differ. The general morphology of intestinal villi determined by the villous length/crypt depth ratio was not different among groups at each time point (mean, 8.2 ± 1.3). The total number of mucus-containing...
cells/villous length ratio did not differ in the CM and CF groups (0.008 ± 0.001 n/μm); it was slightly but not significantly lower \( (P = 0.07) \) in the RM and RF groups at 48 h p.i. (0.006 ± 0.001 n/μm) with a mean of 0.009 ± 0.002 n/μm at 72 and 96 h p.i. in the four groups. Similar values were obtained for neutral and acid mucus-containing cells. The number of sulfated mucin-containing cells/villous length ratio was significantly lower in the RM group compared with the other groups 72 and 96 h p.i. \( (P < 0.05) \) (Fig. 5).

### DISCUSSION

This investigation confirmed that 5-d-old germfree rats infected with a group A rotavirus had a 6-d diarrhea and alterations of their intestinal mucosa (16). SA11 rotavirus infected the epithelium of both the small intestine and the colon, and dietary supplementation with milk fermented by *L. casei* DN-114 001 from the age of 2 d and infected at the age of 5 d with SA11 rotavirus \( (n = 3 \text{ or } 2 \text{ rats for each time}) \).

The damage intensities caused by rotavirus infection differ according to the strain of rotavirus and animal model (30). The dose of heterologous rotavirus required to induce diarrhea was usually 10^5 to 10^6 greater than the dose required for the homologous strain. A clear dose response has been demonstrated for SA11 rotavirus. In 7-d-old mice, intestinal replication of rotavirus could be induced with a dose as low as 10^5 PFU/mouse. Intestinal virus titers and severity of disease increased with virus dose; therefore, we chose to infect the pups with a high concentration of SA11 rotavirus. In mice inoculated with 8 × 10^6 PFU of virus, delay in weight gain, shortening of villi, and enterocytes containing vacuoles were observed. Group B rotavirus infection was accompanied in suckling rats by histologic changes defined as reduction of villous height from 18 to 72 h p.i. and subsequent increase in crypt depth, whereas villous height was progressively restored (25). Our results showed that the villous height and crypt depth were not altered. However, the number of cells containing sulfated mucins was significantly lower in RM rats than in the other groups at 72 and 96 h p.i. This relative decrease in sulfated mucins could be related to the large mucin release also observed in mice (29) and may contribute to fecal emission. The intestinal mucins represent an important barrier against rotavirus infection (31). The stimulation of mucus secretion might exceed the rate of biosynthesis and cause the lower staining of a specific transient bacteria. It was thus relevant to use the germfree model recently described (16).

It has been suggested that cellular alterations in the small intestine play a role in rotavirus-associated diarrhea. The damage intensities caused by rotavirus infection differ according to the strain of rotavirus and animal model (30). The dose of heterologous rotavirus required to induce diarrhea was usually 10^5 to 10^6 greater than the dose required for the homologous strain. A clear dose response has been demonstrated for SA11 rotavirus. In 7-d-old mice, intestinal replication of rotavirus could be induced with a dose as low as 10^5 PFU/mouse. Intestinal virus titers and severity of disease increased with virus dose; therefore, we chose to infect the pups with a high concentration of SA11 rotavirus. In mice inoculated with 8 × 10^6 PFU of virus, delay in weight gain, shortening of villi, and enterocytes containing vacuoles were observed. Group B rotavirus infection was accompanied in suckling rats by histologic changes defined as reduction of villous height from 18 to 72 h p.i. and subsequent increase in crypt depth, whereas villous height was progressively restored (25). Our results showed that the villous height and crypt depth were not altered. However, the number of cells containing sulfated mucins was significantly lower in RM rats than in the other groups at 72 and 96 h p.i. This relative decrease in sulfated mucins could be related to the large mucin release also observed in mice (29) and may contribute to fecal emission. The intestinal mucins represent an important barrier against rotavirus infection (31). The stimulation of mucus secretion might exceed the rate of biosynthesis and cause the lower staining of
mucin cells. This effect was significant only with HID staining, suggesting that the rate of sulfation may be reduced. Cellular vacuolation has been associated with rotavirus gastroenteritis in several animal models (23,29,32). Rotavirus is thought to infect the differentiated cells at the tip of the intestinal villi. In our study, vacuoles were located at the basal area of the intestinal villi at 48 h.p.i.; they were observed 24 h later at the apical area of the villi. The presence of vacuoles in the basal area at the beginning of the period of diarrhea is consistent with the hypothesis recently proposed by Ball et al. (33). The authors described an enterotoxin-like effect of NSP4, one of the nonstructural proteins of rotavirus. According to this hypothesis, rotavirus particles would bind some cells, resulting in virus entry and gene expression at the tip of the villi. Then, NSP4 expressed in infected cells would be released into the lumen and would interact with a specific receptor on adjacent cells. This last interaction would increase the endogenous secretory pathway and induce diarrhea. The “migration” of the vacuoles from the basal to the apical area of the intestinal villi within 24 h is consistent with the enterocyte turnover time in the small intestine of suckling rats (34). The enterocyte migration may lead to the release of infected cells into the lumen. In our study, rotavirus antigens were detected in the small intestine of suckling rats (34). The enterocyte migration may lead to the release of infected cells into the lumen. This hypothesis is supported by the fact that rotavirus infection progresses from the proximal to the distal area of the small intestine (33).

Watery diarrhea associated with rotavirus infection has also been explained by malabsorption of nutrients after histologic lesions. A deficiency in intestinal lactase during rotavirus gastroenteritis has been described in mice (36,37) and in infants (38). In this study, rotavirus infection did not significantly modify the weight gain of suckling rats, suggesting that intestinal absorption was not greatly altered.

Early supplementation with milk fermented by L. casei DN-114 001 had a protective effect on both diarrhea symptoms and intestinal infection in suckling rats. The intensity and the duration of feces emission obtained immediately upon palpation were shorter in the RF group compared with the RM group. Furthermore, the amount, duration and incidence of rotavirus shedding were decreased in the RF group. In conventional mice, rotavirus-induced diarrhea did not modify the establishment of Lactobacillus spp., which was found in high levels in the intestine (10^7 bacteria/g contents) from the first days of life (39). This high level of lactobacilli did not improve the diarrhea symptoms, which were similar in conventional and germfree mice. In our experiment, although pups received 10^7 bacteria daily, the intestinal concentration of L. casei remained low in the small intestine (10^6 bacteria/g) compared with the high level of rotavirus found. This low shedding of...
Lactobacillles did not totally avoid infection in the enterocytes but significantly reduced the histologic changes. In the colon, 10^6 Lactobacillus/g totally suppressed infection of the colonic epithelium. This suggests a specific beneficial influence of Lactobacillus casei on intestinal mucosa. It is not known in what way lactobacilli may play a role in this protection. Lactobacillus consumption may reinforce the integrity of the mucosa and prevent the reinfection of the intestinal villi throughout the intestinal tract. Isolauri et al. (40) showed in 10-d-old rats inoculated with a group B rotavirus that the intestinal dysfunction characterized by increase of ionic conductance and macromolecule permeability was counteracted by daily gavage with Lactobacillus casei DN-114 001; they were inoculated with modified Eagle’s medium (MEM) at the age of 5 d. The infected groups, RM (n = 39) and RF (n = 55), were supplemented with milk or fermented milk from the age of 2 d and were inoculated at the age of 5 d with SA11 rotavirus. Results are expressed as the number of sulfated mucin-containing cells/villi length (n/µm). Values are means ± SD, n = 3. *Significantly lower in the RM group compared with the others at the same time postinoculation (ANOVA and subsequent Scheffe F test, P < 0.05). Abbreviation used: h p.i., hours postinfection.

The results obtained in animal models are in agreement with studies conducted in infants suffering rotavirus diarrhea and supplemented with Lactobacillus strains. The mean duration of diarrhea was decreased in infants supplemented daily with Lactobacillus GG as a fermented milk (13,42) or a freeze-dried powder (12,43). Similar results were obtained in diarrheal infants supplemented with L. reuteri (14). The duration and incidence of rotavirus shedding were decreased in infants given the bacterial association B. bifidum and S. thermophilus (10). The mechanisms proposed to explain the fermented milk properties involve improvement of the lactose digestion and of the intestinal ecology by their antibacterial and immunostimulating effects (9). Previous studies have reported an adjuvant effect of Lactobacillus rhamnosus GG on the immune response in infants infected with rotavirus during the convalescent period, whereas the symptoms were reduced during the acute phase of diarrhea (44,45). Isolauri et al. (46) demonstrated in healthy infants that Lactobacillus GG administration has an immunostimulating effect on oral rotavirus vaccination 8 d postvaccination. However, it is not well established in what way Lactobacillus consumption may play a protective role immediately during the diarrheal period. In infants, the Lactobacllus population is usually low (47). The survival of the ingested bacterial strains in the digestive tract may be an important factor in producing its effect in vivo (13,14,42). We observed previously that food supplementation with milk fermented by L. casei DN-114 001 and the yogurt ferments (S. thermophilus and L. bulgaricus) significantly increased the amount of Lactobacillus in feces compared with infants consuming yogurt or a nonfermented gelled milk (19).

This study suggests that regular consumption of milk fermented with L. casei DN-114 001 helps to protect against rotavirus diarrhea. In the intestine, L. casei DN-114 001 may reinforce mucosa integrity and reduce intestinal villi infection.

**LITERATURE CITED.**


![Figure 4](image-url)  
**FIGURE 4** Cellular vacuoles in jejunal villi of suckling rats infected by SA11 rotavirus, showing the influence of fermented milk supplementation. The control groups, CM (n = 34) and CF (n = 40), were supplemented daily from 2 d of age with milk or with milk fermented by Lactobacillus casei-114 001; they were inoculated with modified Eagle’s medium (MEM) at the age of 5 d. The infected groups, RM (n = 39) and RF (n = 55), were supplemented with milk or fermented milk from the age of 2 d and were inoculated at the age of 5 d with SA11 rotavirus. Results are expressed as the percentage of villi with vacuoles. Values are means ± SD, n = 3. *Significantly higher in the RM group compared with the others at the same time postinoculation (ANOVA and subsequent Scheffe F test, P < 0.05). Abbreviation used: h p.i., hours postinfection.

![Figure 5](image-url)  
**FIGURE 5** Sulfated mucin-containing cells in the jejunum of suckling rats infected by SA11 rotavirus, showing the influence of fermented milk supplementation. The control groups, CM (n = 34) and CF (n = 40), were supplemented daily from 2 d of age with milk or with milk fermented by Lactobacillus casei-114 001; they were inoculated with modified Eagle’s medium (MEM) at the age of 5 d. The infected groups, RM (n = 39) and RF (n = 55), were supplemented with milk or fermented milk from the age of 2 d and were inoculated at the age of 5 d with SA11 rotavirus. Results are expressed as the number of sulfated mucin-containing cells/villi length (n/µm). Values are means ± SD, n = 3. *Significantly lower in the RM group compared with the others at the same time postinoculation (ANOVA and subsequent Scheffe F test, P < 0.05). Abbreviation used: h p.i., hours postinfection.