Human Milk Probiotic *Lactobacillus fermentum* CECT5716 Reduces the Incidence of Gastrointestinal and Upper Respiratory Tract Infections in Infants

*José Maldonado, †Francisco Cañabate, ‡Luis Sempere, †Francisco Vela, †Ana R. Sánchez, §Eduardo Narbona, ||Eduardo López-Huertas, ‡Arjan Geerlings, ‡Antonio D. Valero, ‡Mónica Olivares, and ¶Federico Lara-Villoslada

See ''Probiotics: Fishing in the Ocean'' by Vandenplas and Veereman-Wauters on page 4.

ABSTRACT

Objectives: The aim of the study was to examine the effects of a follow-on formula containing *Lactobacillus fermentum* CECT5716 (*L fermentum*) on the incidence of infections in infants between the ages of 6 and 12 months.

Patients and Methods: A randomized double-blinded controlled study including infants at the age of 6 months was conducted. Infants were assigned randomly to either follow-on formula supplemented with *L fermentum* plus galactooligosaccharide (experimental group, EG), or the same formula supplemented with only galactooligosaccharide (control group, CG). The main outcome was the incidence of infections for the 6-month duration of the study.

Results: The EG showed a significant 46% reduction in the incidence rate (IR) of gastrointestinal infections (EG: 0.196 ± 0.51, CG: 0.363 ± 0.53, IR ratio 0.54, 95% confidence interval [CI] 0.307–0.950, $P = 0.032$), 27% reduction in the incidence of upper respiratory tract infections (EG: 0.969 ± 0.96, CG: 1.330 ± 1.23, IR ratio 0.729, 95% CI 0.46–1.38, $P = 0.026$), and 30% reduction in the total number of infections (EG: 1.464 ± 1.15, CG: 2.077 ± 1.59, IR ratio 0.70, 95% CI 0.46–1.38, $P = 0.003$), at the end of the study period compared with CG.

Conclusions: Administration of a follow-on formula with *L fermentum* CECT5716 may be useful for the prevention of community-acquired gastrointestinal and upper respiratory infections.

Key Words: follow-on formula, infants, infection, *Lactobacillus fermentum*, probiotics

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Infectious diseases are the most common type of illness for infants worldwide. In Spain, data from 2007 show that of all of the hospital admissions resulting from infectious diseases, the admission of infants younger than 1 year represents >50% of the total, with an average of 2174 cases per 100,000 people (1). Breast-fed children have a lower incidence of infections than formula-fed children, which could be mediated in part through modulation of the intestinal microflora by breast milk components (2). Indeed, breast-fed infants seem to develop a gut microflora richer in lactobacilli and bifidobacteria with reduced pathogenic bacteria compared with formula-fed infants (3). Because of its many benefits, exclusive breast-feeding for the first 6 months of life is the recommended way of feeding infants (4). When breast-feeding is not possible or insufficient, infant formula represents an alternative aimed to partially imitate the effects of breast milk. In the last decade, the manipulation of the intestinal microbiota of infants through the administration of probiotic strains has been recognized as a potential application for the treatment and prevention of infectious diseases. In a review by the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition, it was stated that a few probiotics supplemented to infant or follow-on formulas may be associated with a reduction in the risk of nonspecific gastrointestinal (GI) infections and a reduction in the risk of antibiotic use ((5) and references therein). However, the scientific evidence supporting the use of probiotics for the prevention of infectious diseases is only emerging. The efficacy of probiotics in the prevention of community-acquired infections in children has been investigated in several randomized controlled trials (RCTs) but the results shown are contradictory, showing that, apart from the variability seen between study populations, designs, and methodologies, the results suggest that effects observed by 1 probiotic strain cannot be extrapolated to others (6,7).

Prebiotics such as galactooligosaccharides (GOSs) are indigestible nutrients found in human breast milk that stimulate the growth and metabolic activity of beneficial bacteria in the gut flora, which may also produce a direct immunological effect (8,9).

Human milk is a source of lactic acid bacteria for the infant gut, which may play an important role in infant protection against infectious agents (10,11). We identified and selected *Lactobacillus fermentum* CECT5716 from human breast milk and characterized

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From the *Hospital Universitario Virgen de las Nieves, Granada, the †Hospital Poniente de El Ejido, Carretera de Almerimar, El Ejido (Almería), the ‡Biosearch Life SA, Granada, the §Hospital Universitario San Cecilio, Granada, the ||Estación Experimental Zaidín, Consejo Superior Investigaciones Científicas, Granada, and the ¶Department of Food Safety and Nutrition, Puleva Food S.L. Granada 18004, Spain.

Address correspondence and reprint requests to Federico Lara-Villoslada, PhD, Department of Food Safety and Nutrition, Puleva Food S.L. 66, Camino de Purchil, Granada 18004, Spain (e-mail: flara@puleva.es).

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the safety and probiotic properties of the strain using in vitro animal models and RCT in human adults (12–15). Because this probiotic strain is naturally present in human breast milk, we hypothesized that its use in a follow-on formula could benefit the bottle-fed infant. In this RCT we evaluated the effect produced by a follow-on formula containing *L fermentum* and GOS in comparison to a control follow-on formula with only GOS on the incidence of infections in infants between the ages of 6 and 12 months.

PATIENTS AND METHODS

Study Design and Protocol

A randomized double-blind controlled study with 2 study groups was carried out in collaboration with the pediatrics departments of 3 Spanish hospitals, the University Hospital San Cecilio (Granada, Spain), University Hospital Virgen de las Nieves (Granada, Spain), and ''Poniente'' Hospital of El Ejido (Almería, Spain). Families that lived in proximity to the hospitals whose mothers had delivered their babies at the hospital and/or made regular visits to the pediatrician were considered for the study and contacted by the research nurse during scheduled visits to the hospital. Before the inclusion, infants received a physical examination and their clinical records were consulted for previous diseases and pharmacological treatments. Healthy 6-month-old infants who were exclusively formula fed were recruited into the study between May 2008 and July 2009 after informed written consent was obtained from the parents or tutors. The exclusion criteria included GI disorders (history of chronic diarrhea or constipation, gastroesophageal reflux), GI surgery, cow's-milk protein allergy, metabolic disorders (diabetes, lactose intolerance), immunodeficiency, antibiotic prescription 1 week before inclusion, and previous use of formula containing prebiotics or probiotics.

Sample size was estimated based upon the effects on GI infections. Based on previous data regarding incidence of diarrhea (16), the study was designed to have sufficient power (85%) to detect a 20% difference between the groups with a 0.05 significance level. The number of infants necessary in each group was 83 infants; however, we recruited 30% more infants to allow for dropouts.

A total of 215 infants were selected and distributed into 2 study groups according to a randomization list generated by a computer program (SIGESMU, Madrid, Spain). The formulas administered were standard powdered follow-on with nutritional composition in accordance with present European Union regulations, supplemented with GOS (0.4 g/100 mL) in the case of the control group (CG), and with the same amounts of GOS plus *L fermentum* CECT5716 (*L fermentum* Hereditum, Biosearch Life, Granada, Spain) at an average dose of 2×10^8 colony-forming units (CFU)/day in the case of the experimental group (EG). The concentration of the probiotic in the formula was analyzed and confirmed every 2 months. Both formulas were consumed during the 6-month intervention period. The follow-on formulas were provided by Puleva Food S.L. (Granada, Spain) in identical containers labeled in plain white with a code number that referred to the study groups. To ensure the blinding of the trial, both formulas were submitted to a sensorial test by an expert panel that finds both products to be identical. The pediatricians prescribed the amounts of formula per day to be administered to the infants. Parents received general guidelines for complementary feeding according to present ESPGHAN guidelines (17). Infants were scheduled to receive a clinical evaluation at baseline at age 6 months (T0), after 3 months at age 9 months (T3), and after 6 months at age 12 months (T6). Fecal samples were also collected at T0, T3, and T6. The formulas of the study were delivered to infants' homes every 2 months and the emptied containers were also collected. Exclusion criteria during the study were lack of compliance with the study protocol, adverse effects derived from the consumption of any of the formulas of the study, and do-not-attend scheduled visits to the hospital.

The present study was carried out according to the Helsinki Declaration, and the protocol was approved by the regional ethics committee of the Sistema Andaluz de Salud based in Seville (Spain).

Study Outcomes and Data Collection

The primary outcome of the trial was the incidence of infections, including GI, respiratory, otitis, urinary, and other less common infections. Secondary outcomes were evolution of weight, length and head circumference, fever episodes, antibiotic prescriptions, and concentrations of short-chain fatty acids (SCFAs), immunoglobulin (Ig) A, and microbiota composition in feces. The incidence of recurrent (defined as $\geq$3 events) respiratory infections also were considered a secondary outcome.

The diagnosis of infectious diseases was made by the pediatrician at every visit based on specific symptoms and standardized definitions. GI infection was defined as loose or watery stools at least 4 times/day with or without fever or vomiting. Upper respiratory tract infections were defined as the presence of abundant mucosity and/or cough during $\geq$2 consecutive days with or without fever, including common cold, laryngitis, pharyngitis/tonsillitis, laringotracheitis, acute rhinitis, and acute rhinosinusitis. Lower respiratory tract infections were defined as mucosity and/or cough during 2 or more consecutive days with or without fever and presence of wheezing and/or crepitants, including acute bronchitis, bronchiolitis, and pneumonia. Otitis was defined by the following criteria: otalgia, expressed in the infant as unexplained irritability or rude awakening and recent otorrhea or a bulging eardrum with or without strong redness. Urinary tract infections were diagnosed by fever $\geq$38°C, pyuria (2 concordant consecutive test results with white cell counts $\geq$25/mL), and urine culture (2 concordant consecutive tests with growth of only 1 microorganism $\geq$100,000 CFU/mL). Under other infections we included chickenpox, conjunctivitis, oral candidiasis, Epstein-Barr virus, herpesvirus, and fever episodes of unknown origin.

Three types of questionnaires were used in the study: first, scheduled visit questionnaire, completed by pediatricians during the scheduled visits (T0, T3, T6), included study parameters and events related to the health behavior of the infant; second, parents diary and 15-day questionnaires, completed by the parents, in which information regarding daily number of depositions, respiratory symptoms, unscheduled visits to the doctor, diagnosed infections, sleeping and crying habits, changes in sleeping pattern, fever episodes, GI discomfort, and prescription of antibiotics were recorded. The diaries were used as a reference to fill the 15-day questionnaires in which the relevant information from the diaries was summarized. The parents were given instructions on completing the questionnaire and were encouraged to contact the research staff if necessary; third, occasional questionnaires, completed by pediatricians during unscheduled visits resulting from suspected infection or a health problem. In the case of an emergency department visit, a copy of the patient's report was sent to the pediatrics for inclusion in the research file.

Fecal Bacteria Quantitation

Fecal samples were homogenized individually in a peptone-saline solution (100 mg/mL). To estimate the concentration of bacterial groups, appropriate dilutions were spread in quadruplicate

onto plates of MRS agar for lactic acid bacteria, MRS agar supplemented with 0.5 mg/L dicloxacilin, 1 g/L LiCl, and 0.5 g/L L-cysteine hydrochloride for bifidobacteria, and reinforced clostridial agar containing 20 μg/mL of polimixina for clostridia and bile aesculin agar for bacteroides. All media were obtained from Oxoid (Basingstoke, UK), whereas antibiotics and other supplements were obtained from Sigma Chemical Co (St Louis, MO). Culture plates were incubated in anaerobiosis at 37°C for 24 to 48 hours. Similarly, 1 mL of each suitable dilution was spread onto specific Count Plates Petrifilm (3 mol/L; St Paul, MN) for total aerobes. Petrifilms were incubated in aerobiosis at 37°C for 24 hours. After incubation, colonies grown on the selective culture media were counted and the logarithm of the numbers of viable microorganisms per gram of feces (colony-forming units per gram) were calculated and represented as the average ± standard error of the mean.

Short-chain Fatty Acids Quantitation

Fecal samples were homogenized with 150 mmol/L $NaHCO_3$ (pH 7.8) (1:5, wt/vol) in an argon atmosphere. Samples were incubated for 24 hours at 37°C and stored at −80°C until the extraction. To extract the SCFAs, 50 μL of 100 mol/L 2-methylvaleric acid (internal standard), 10 μL of sulfuric acid, and 0.3 mL of ethyl acetate were added to 1 mL of the homogenate. The mix was centrifuged at 10,000*g* for 5 minutes at 4°C. The supernatants were dehydrated with sodium sulfate (anhydrous) and centrifuged at 10,000*g* for 5 minutes at 4°C. Later, the sample (0.5 mL) was splitless inoculated into a gas chromatograph (mod. CP-3800; Varian, Lake Forest, CA) equipped with an inner diameter (CPWAX 52CB 60 m × 0.25 mm), and connected to a flame ionization detector (Varian). Helium was used as the carrier and the make-up gas, with a flow rate of 1.5 mL/minute. The injection temperature was 250°C. Acetate, propionate, and butyrate concentrations were automatically calculated from the areas of the resulting peaks using the Star Chromatography WorkStation program (version 5.5), which was connected online to the flame ionization detector.

Fecal IgA Quantitation

Fecal samples were homogenized individually in a peptone-saline solution (100 mg/mL) and centrifuged at 10,000*g* for 5 minutes at 4°C. IgA concentration was measured in the supernatants by enzyme-linked immunosorbent assay quantitation kits, following manufacturer's instructions (Bethyl, Montgomery, TX).

Statistical Analysis

Data were analyzed with statistical package STATA 11.1 (StataCorp LP, College Station, TX) by a blinded statistician. Poisson multilevel regression analysis used the scheme of fixed effects/random intercept considering 2 levels of data organization: the fixed effects factors were the group (control and experimental) and the period (0–3 months, 3–6 months) and the random effect factor were the child nested in the group and the measures of the period nested in each child. The incidence rate ratios (IRRs) were computed as measures of the effect when used number of events as the dependent variable. All of the data about infections occurring during the period 0 to 6 months were pooled and analyzed by classical Poisson regression and classical logistic regression were computed for the corresponding dependent variables. IRs were computed as the number of diagnoses (numerator) divided by the person-time contributed. The IRRs with 95% CI and *P* values were the outputs of the analysis.

When the variables were numeric the multilevel linear regression model was used as in the case of the previous one. Results coming from the different centers were also treated separately and compared and no statistically significant differences were found (data not shown).

RESULTS

Population

Of the 215 infants randomized in 2 study groups, 27 were excluded from the trial: 7 in the CG and 20 in the EG for the following reasons: 3 infants moved out of the study area (1 in CG and 2 in EG), 7 infants did not receive the probiotic experimental formula and the mistake was detected after they did not attend first medical visit, 7 infants were excluded after study termination because of incomplete data collection (2 in group CG and 5 in group EG), and 10 infants did not attend baseline medical visits (4 in group CG and 6 in group EG). Thus, the total number of volunteers analyzed (per protocol) was 188, of whom 91 were in the group CG and 97 in the group EG. Figure 1 is a flowchart of participants in the study. Before inclusion, none of the recruited children consumed a probiotic formula because at the moment of recruitment, there was no infant formula (from 0 to 6 months) supplemented with probiotics in the Spanish market. No statistically significant differences were observed between the groups in any of the baseline parameters analyzed (Table 1).

Five pediatricians participated in the recruitment and monitoring of the children (J.M., F.C., F.V., A.R.S., and E.N.) in representation of 3 centers, Hospital Virgen de las Nieves (Granada, Spain), Hospital Clínico (Granada, Spain), and Hospital de Poniente (Almería, Spain). The number of infants recruited and followed by each center was 68, 64, and 56, respectively.

Formula Intake and Tolerance and Growth

Both study formulas were well tolerated and compliance was good. No adverse effects related to the consumption of the formulas (including upper GI symptoms, such as spitting up) were reported and none of the volunteers abandoned the trial during the intervention period. No significant differences were found between the study groups regarding daily intake of formula (CG: 741 ± 146 mL/day vs EG: 732 ± 150 mL/day; Table 1). Baseline values showed significant differences between groups for length ($P = 0.047$) and head circumference ($P = 0.029$) as a result of the randomization. No differences were found for weight, length, head circumference, and growth rate between the study groups at times T3 and T6, indicating that the consumption of the study formula was safe (Table 2).

Infants' Health

During the 6-month intervention, 72.5% of the infants experienced respiratory infections, 15.7% from GI infections, 5.7% otitis, 1.8% urinary infections, and 4.2% from other infections as defined in the methods section (Table 3).

The EG showed a significant 46% reduction in the IR of GI infections (0.196 ± 0.51) compared with control (0.363 ± 0.53) at the end of the study period (IR ratio 0.54, 95% CI 0.307–0.950, $P = 0.032$). Regarding total respiratory infections, the EG showed a significant 26% reduction ($P = 0.022$) in the IR (1.093 ± 1.09) compared with the control (1.473 ± 1.31) at the end of the period (IR ratio 0.74, 95% CI 0.580–0.957, $P = 0.022$). 90% of the total respiratory infections were infections of the upper respiratory tract. We observed a 27% reduction in the incidence of upper respiratory

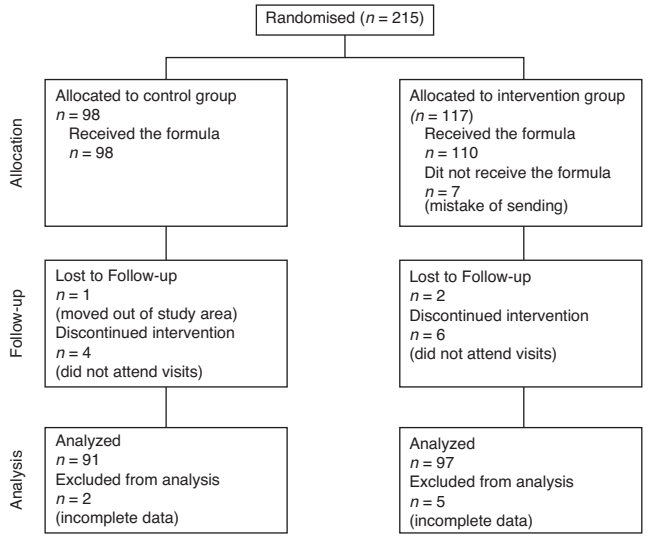

FIGURE 1. Flowchart of participants.

infections in the EG compared with controls (IR ratio 0.729, 95% CI 0.46–1.38, $P = 0.026$), with no significant difference regarding the rate of lower respiratory infections. The EG also reduced the incidence of recurrent respiratory infections by 72%.

No significant differences were found for the IRs of otitis, urinary tract, and other infections possibly because of the low number of events obtained in both study groups. When the total number of infections was analyzed, the EG showed a significant 30% reduction in the IR (1.464 ± 1.15) compared with the control (2.077 ± 1.59) at T6 (IR ratio 0.70, 95% CI 0.46–1.38, $P = 0.003$).

Regarding antibiotic treatment and the number of fever episodes, no significant differences between study groups were observed. Events of diarrhea associated with antibiotic treatments were detected in 17.5% of control infants versus 9.6% of infants in the EG (not significant, $P = 0.234$).

Fecal Parameters

Fecal samples from only 145 infants (80%) at T0, T3, and T6 reached the laboratory in good condition and were analyzed. Regarding the intestinal microbiota (Table 4), bacterial counts of both lactobacilli and bifidobacteria were significantly higher ($P = 0.008$ and $P = 0.022$, respectively) in the EG compared with controls at the end of the study. Within-group comparisons T0 versus T6 only showed increasing trends in the EG for both lactobacilli and bifidobacteria, and decreasing trends in the CG. No significant differences were observed for the other bacterial groups analyzed, and no significant differences were observed between both groups in fecal SCFAs such as butyric, propionic, and acetic acids. Finally, the concentration of IgA in fecal samples did not change throughout the study in either group.

DISCUSSION

We have shown that infants receiving a follow-on formula enriched with *L fermentum* demonstrated a reduction in GI and respiratory infections. The study of the effects of *L fermentum* administered to infants has been carried out for the first time. *L fermentum* was chosen because of its natural presence in human breast milk (10). This strain is also able to colonize the mammary gland when administered to lactating mothers in a capsule, compared with controls (15). Apart from the origin of *L fermentum* and its ability to colonize the human gut (14), this strain was selected because of its safety (13,18), anti-infectious (19), and immunomodulatory (20) properties. In addition, 2 RCTs in adults have shown the ability of *L fermentum* to reduce the incidence of episodes associated with influenza when administered before and after vaccination (14), and to be an efficient alternative to the use of antibiotics for the treatment of infectious mastitis during lactation (15). To investigate the health benefits of *L fermentum* in healthy children we designed this trial. A standard follow-on formula containing moderate amounts of GOS for both groups were used, aiming to theoretically enhance the effects of the probiotic strain, knowing that GOS has been described to stimulate the growth and activity of beneficial gut flora (8,9). Results indicate that the infants receiving the experimental formula containing *L fermentum* showed a reduced incidence of GI infections, respiratory infections including upper respiratory infections, and fewer infectious diseases overall at the completion of the study, compared with the infants fed the control formula.

The recruitment lasted 13 months, including seasons during which rates of respiratory and GI infections are high. The definition used to qualify for a GI infection (loose or watery stools $\geq$4 times/day) was stricter but in line with the World Health Organization

TABLE 1. Baseline characteristics of the subjects participating in the study

	Control group, n = 91	Experimental group, n = 97
Male/female, n (%)	40/51 (44/56)	54/43 (56/44)
Age at enrollment, mo, mean ± SD	6.5 ± 1.3	6.5 ± 1.2
Delivery by cesarean section, %	45	45
Gestational age, wk, mean ± SD	38.9 ± 1.4	38.8 ± 1.4
Breast-fed before the study period, %	71	69
Total duration exclusive breast-feeding, mo, (range)	2.8 (0.5–6.5)	3.0 (0.5–6.5)
Daily volume of follow-on formula prescribed, mL, mean ± SD	741 ± 146	732 ± 150
Complementary feeding, %	100	100
Rotavirus vaccination, %	75	74
Day care or child minder, %	25	27
Pets at home, %	13	16
Smoking during pregnancy or lactation, %	21	21
Smoking in the household, %	37	38

SD = standard deviation.

TABLE 2. Anthropometric measurements at baseline, T3 and T6 months, and gain T0–T6 of infants receiving either control or probiotic formula

	Control group				Experimental group			
Growth parameters	T0	T3 mo	T6 mo	Gain	T0	T3 mo	T6 mo	Gain
Weight, kg	7.8 ± 1.0	9.2 ± 1.58	9.9 ± 1.5	2.3 ± 0.7	7.4 ± 1.0	8.8 ± 1.1	9.7 ± 1.5	2.4 ± 0.7
Length, cm	66.6 ± 3.2	71.5 ± 2.8	75.2 ± 3.0	8.8 ± 2.4	65.6 ± 3.3*	70.2 ± 4.0	74.3 ± 3.8	8.7 ± 2.3
Head circumference, cm	43.7 ± 1.4	45.7 ± 1.4	47.1 ± 1.4	3.4 ± 0.9	43.2 ± 1.4*	45.4 ± 1.4	46.6 ± 1.6	3.5 ± 1.0

Values are mean ± SEM.
* $P < 0.05$ vs control.

definition of diarrhea (21) or the ESPGHAN definition of acute gastroenteritis (22) and has been used extensively in clinical trials investigating the prevention of diarrhea (23). Using this definition, the GI infection rates obtained in both study groups are in agreement with the IRs reported for infants of this age group in southern Spain, which vary between 0.4 and 0.6, with an average of 0.47 episodes per year (24). The rates obtained in the trial for respiratory infections were also in agreement with reported values for infants of this age (1).

The rate of reductions in GI infection observed in the EG is comparable to other trials that reported a successful prevention of community-acquired GI infections or diarrhea episodes using a probiotic infant formula. For example, the administration of a formula containing either 10^7 CFU/g of *Bifidobacterium lactis* or 10^7 CFU/g of *Lactobacillus reuteri* to children 4 to 10 months old during a 3-month period reduced the numbers of diarrhea episodes compared to the control by 58% and 92%, respectively (7). In another study using a formula containing 2×10^7 CFU/g of *B lactis* and 14 mg/g of a prebiotic blend, the rate of diarrhea was reduced by 20% compared with the control (25). A recent large trial showed that the administration of 10^7 CFU/g of *B longum*, 10^6 CFU/g *Streptococcus thermophilus* and 28 mg/g FOS during a period of 3 months reduced the incidence of GI infections by 50% (26).

This trial showed a modest 26% reduction in the total number of respiratory infections. This finding applies only to upper respiratory tract infections, perhaps because of the low number of infections affecting the lower respiratory tract (12 in EG and 13 in CG).

Three studies have also shown reductions in respiratory infections with the administration of probiotic strains to infants. An RCT with 571 children between 1 and 6 years old, administration of *Lactobacillus GG* during a period of 7 months resulted in a 17% reduction in the number of children experiencing respiratory tract infections with complications (16). Another RCT with 281 children (age range 1–7 years), administration of *Lactobacillus GG* for 3 months, resulted in a 33% risk reduction in upper respiratory tract infections compared with the control (27). Results of a recent RCT with 69 newborn infants indicate that the administration of *Bifidobacterium animalis* BB-12 reduced the numbers of respiratory infections by approximately 30% compared with the control (6). Additional studies reported either trends (28) or no effects at all (7,16,26).

Fecal samples were analyzed to investigate the possible mechanism responsible for the reduction in GI infections. Although the production of SCFA and IgA did not change during the study, the intake of the experimental formula resulted in a 78% increase in lactobacillus and 70% in bifidobacteria at the end of the study. These changes in the gut flora could at least in part explain the reductions in the number of GI episodes observed in this group.

Interestingly, the population of bifidobacteria significantly increased in the EG in spite of the supplementation with lactobacilli

TABLE 3. Incidence of infectious disease, febrile episodes, and antibiotic treatment during the intervention period

	Control group, n = 91		Experimental group, n = 97				
	No. events	Incidence rate (SD)	No. events	Incidence rate (SD)	Incidence rate ratio (95% CI)	Incidence rate decrease, %	*P*
Gastrointestinal infections	33	0.363 (0.53)	19	0.196 (0.51)*	0.54 (0.31–0.95)	46	0.032
Respiratory infection	134	1.470 (1.31)	106	1.093 (1.00)*	0.74 (0.58–0.96)	26	0.022
Upper respiratory	121	1.330 (1.23)	94	0.969 (0.96)*	0.73 (0.56–0.95)	27	0.021
Lower respiratory	13	0.143 (0.35)	12	0.124 (0.33)	0.87 (0,40–1.90)	13	0.719
Otitis	12	0.132 (0.34)	7	0.072 (0.26)	0.55 (0.22–1.32)	45	0.177
Urinary tract infections	5	0.055 (0.22)	1	0.010 (0.10)	0.19 (0.02–1.56)	81	0.083
Other infections*	5	0.055 (0.22)	9	0.093 (0.29)	1.69 (0.50–1.85)	−69	0.326
Total infections	189	2.08 (1.59)	142	1.46 (1.16)*	0.70 (0.57–0.88)	30	0.002
Febrile episodes	78	0.857 (0.90)	67	0.690 (0.88)	0.81 (0.68–0.94)	19	0.203
Antibiotic treatments	57	0.626 (0.90)	52	0.536 (0.70)	0.86 (0.59–1.24)	14	0.445

CI = confidence interval; SD = standard deviation.
* Other infections include chickenpox, Epstein-Barr virus, herpesvirus, oral candidiasis, conjunctivitis, and febrile episodes of unknown origin.

TABLE 4. Intestinal microbiota counts in fecal samples of infants (as logarithm of CFUs/g), fecal concentration of SCFA, and IgA

	Control group, n = 70			Experimental group, n = 75		
	T0	T3 mo	T6 mo	T0	T3 mo	T6 mo
Bacterial group						
Lactobacillus spp	7.85 ± 0.1	7.72 ± 0.1	7.68 ± 0.1	7.81 ± 0.1	7.86 ± 0.1	8.06 ± 0.1*
Bifidobacterium spp	8.07 ± 0.1	7.84 ± 0.1	7.81 ± 0.1	7.93 ± 0.1	8.06 ± 0.1	8.16 ± 0.1*
Clostridium spp	7.77 ± 0.1	7.57 ± 0.1	7.54 ± 0.1	7.74 ± 0.1	7.64 ± 0.1	7.61 ± 0.1
Bacteroides spp	7.64 ± 0.1	7.65 ± 0.1	7.61 ± 0.1	7.86 ± 0.1	7.86 ± 0.1	7.65 ± 0.1
SCFA, mg/g feces						
Acetate	10.7 ± 0.8	10.0 ± 1,3	10.1 ± 0.8	9.9 ± 1.03	9.6 ± 0.5	11.3 ± 1.1
Propionate	1.85 ± 0.1	2.17 ± 0.2	2.17 ± 0.2	2.20 ± 0.4	2.30 ± 0.4	2.35 ± 0.3
Butyrate	2.15 ± 0.2	2.76 ± 0.4	2.94 ± 0.2	2.53 ± 0.5	3.05 ± 0.3	2.92 ± 0.3
IgA, μg/g feces	328 ± 244	ND	322 ± 212	329 ± 170	ND	316 ± 242

Values are mean ± SEM. IgA = immunoglobulin A; ND = not determined; SCFA = short-chain fatty acid.
* $P < 0.05$ vs control.

only. This phenomenon has been observed for other probiotic strains (29,30). It has been suggested that an increase in bifidobacteria may be the result of the metabolic activity, nutrient competition, and gut cell adhesion rates of lactobacilli, which may favor the growth of bifidobacteria. Using in vitro and in vivo studies, we have shown that *L fermentum* presents a high cellular adhesion rate, produces a number of antimicrobial substrates, releases significant amounts of the antioxidant glutathione, and is able to synthesize bifidogenic carbohydrates under certain conditions (11,19). In addition, in vitro studies have shown that *L fermentum* inhibit the adhesion of certain pathogenic bacteria to intestinal mucus and increase the expression of mucins, which could also be involved in the anti-infective effect shown by this probiotic strain (19). Although previous studies have demonstrated that *L fermentum* is able to colonize the gut (14), *L fermentum* was not quantified during this trial, thus limiting the interpretation of the results. Another limitation was that the feces of the infants diagnosed as having GI infections were not analyzed for single pathogens.

Regarding the mechanism for the effects on respiratory infections, a previous RCT in adults carried out with *L fermentum* in combination with an influenza vaccine showed a significant reduction in influenza-like illness (including respiratory symptoms) in the EG. The reduction was explained by significant increases in the proportions of natural killer cells, T-helper, and T-cytotoxic lymphocytes measured in the EG compared with the control (14). In the present study we did not observe differences in the fecal concentration of IgA. Because no blood samples were obtained from the infants in the study, we can only speculate stimulation of the immune response as a possible mechanism responsible for reductions in the respiratory infections. Furthermore, another limitation of the present study was the absence of a group exclusively breast-fed for comparison.

It has been reported that human milk contains oligosaccharides having anti-inflammatory and anti-infectious properties (31). Because both formulas contained GOS (0.5 g/100 kcal), the effects observed in the present study cannot be attributed to its GOS content. *L fermentum* and GOS together may have a synergic effect that could be superior to the health benefits of the individual components. It was, however, outside the scope of the present study to investigate the potential synergistic effects of the combination of *L fermentum* and GOS, which should be investigated in future studies.

In conclusion, considering the significant decrease in the number of infections, the administration of a follow-on formula enriched with *L fermentum* may be useful for the prevention of community-acquired GI and upper respiratory infections in infants. Additional controlled clinical studies investigating the effects of *L fermentum*, alone or in combination with other strains, in different settings, using biomarkers and infants of different age groups are needed before recommendations can be made.

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