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Lactobacillus fermentum CECT 5716 is safe and well tolerated in infants of 1–6 months of age: A Randomized Controlled Trial

Mercedes Gil-Campos^a, Miguel Ángel López^b, M^a Victoria Rodríguez-Benítez^a, Julio Romero^b, Inés Roncero^a, M^a Dolores Linares^b, Jose Maldonado^b, Eduardo López-Huertas^c, Regina Berwind^d, Kristin L. Ritzenthaler^d, Victor Navas^e, Carlos Sierra^e, Lluís Sempere^f, Arjan Geerlings^f, Jose A. Maldonado-Lobón^f, Antonio D. Valero^f, Federico Lara-Villoslada^g, Mónica Olivares^{f,*}

^a Hospital Universitario Reina Sofía, Avenida Menéndez Pidal, S/N, 14004 Córdoba, Spain

^b Hospital Universitario Virgen de las Nieves, Avenida de las Fuerzas Armadas 2, 18014 Granada, Spain

^c Estación Experimental Zaidín, Consejo Superior Investigaciones Científicas. Profesor Albareda 1, 18008 Granada, Spain

^d HiPP GmbH & Co Vertrieb KG, Georg-Hipp-Str. 7, D-85276 Pfaffenhofen, Germany

^e Hospital Universitario Carlos Haya, Avenida Carlos Haya, S/N, 29010 Málaga, Spain

^f Biosearch Life SA, 66, Camino de Purchil 66, 18004 Granada, Spain

^g Department of Food Safety and Nutrition, Puleva Food SL, Camino de Purchil 66, 18004 Granada, Spain

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ABSTRACT

The objective of the study was to evaluate the safety and tolerance of an infant formula supplemented with *Lactobacillus fermentum* CECT5716, a probiotic strain isolated from breast milk, in infants of 1–6 months of age. A randomized double blinded controlled study including healthy infants was conducted. One month aged infants received a prebiotic infant formula supplemented with *L. fermentum* (experimental group) or the same formula without the probiotic strain (control group) for 5 months. The primary outcome of the study was average daily weight gain between baseline and 4 months of age. Secondary outcomes were other anthropometric data (length and head circumference), formula consumption, and tolerance. Incidence of infections was also recorded by pediatricians.

No significant differences in weight gain were observed between both groups, neither at 4 months of age (29.0 ± 7.8 vs 28.9 ± 5.7 g/day) nor at 6 months (25.1 ± 6.1 vs 24.7 ± 5.2 g/day). There were no statistically significant differences in the consumption of the formulae or symptoms related to the tolerance of the formula. The incidence rate of gastrointestinal infections in infants of the control group was 3 times higher than in the probiotic group ($p = 0.018$).

Therefore, consumption of a prebiotic infant formula enriched with the human milk probiotic strain *L. fermentum* CECT5716 from 1 to 6 months of life is well tolerated and safe. Furthermore, the consumption of this formula may improve the health of the infants by reducing the incidence of gastrointestinal infections.

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1. Introduction

Most studies report that the stool microbiota of breast-fed infants differ from that of formula-fed infants [1] and could be responsible, at least in part, for some of the beneficial effects observed in these breast-fed infants [2,3]. In fact, microbiota plays

a variety of functions for the host and is even considered the “forgotten organ” [4]. These differences in microbiota are probably due to the presence of lactic acid bacteria in human milk, besides other bifidogenic compounds such as oligosaccharides which are transferred from the mother to the infants by lactation [5,6]. For these reasons infant formulae are increasingly being supplemented with probiotics, prebiotics or synbiotics with the objective to obtain in the formula-fed infants a similar intestinal microbiota to that of breast-fed children. The supplementation of infant formulae with probiotic strains naturally found in breast milk can be an interesting alternative since these strains are naturally supplied by the mother to their infants during lactation. In this context, the Committee on Nutrition of ESPGHAN concluded that the administration of the probiotic strains to healthy infants did not raise safety concerns

Abbreviations: RCT, Randomized Controlled Trial; GOS, galactooligosaccharides; CG, control group; EG, experimental group; SCFA, short-chain fatty acids; GI, gastrointestinal infections; PCR, Polymerase Chain Reaction; LRM, linear regression models; GLM, Generalized Linear Models; IR, incidence rate; NNT, infants needed to treat.

* Corresponding author. Tel.: +34 958 240152; fax: +34 958 240160.

E-mail address: molivares@biosearchlife.com (M. Olivares).

but data should not be extrapolated to other strains, thus, for each strain safety studies are needed [7]. Because of that, all strains used in infant nutrition, even those naturally found in human breast milk, need to demonstrate their safety in intervention studies.

We identified and selected *Lactobacillus fermentum* CECT5716 from human breast milk and characterized its safety and probiotic properties by in vitro, in animal models and by Randomized Controlled Trials (RCT) in humans [8–13]. Its human milk origin and its probiotic properties encouraged us to test the safety and efficacy of this strain in infants between the age of 6 and 12 months. The administration of the strain during 6 months was safe and well-tolerated and was related to a significant reduction of the incidence of gastrointestinal and respiratory infections [14]. In this RCT we evaluate the safety of an infant formula containing *L. fermentum* CECT5716 on infants aged 1–6 months.

2. Material and methods

2.1. Study design and protocol

A randomized double blinded controlled study with two study groups was carried out in collaboration with the Pediatrics Department of three Spanish hospitals: Hospital Virgen de las Nieves (Granada, Spain), Hospital Reina Sofía (Córdoba, Spain) and Hospital Carlos Haya (Málaga, Spain). Healthy, one month old infants, who for reasons beyond the study, were exclusively formula-fed, were recruited into the study between May 2009 and September 2010 after informed written consent was obtained from the parents or caregivers. The exclusion criteria included history of mild or serious gastrointestinal disorders (history of chronic diarrhoea or constipation, gastroesophageal reflux), gastrointestinal surgery, cow's milk protein allergy, metabolic disorders (diabetes, lactose intolerance), immune deficiency and antibiotic prescription one-week prior to inclusion and previous use of formula containing probiotics. Exclusion criteria during the study were lack of compliance with the study protocol, adverse effects derived from the consumption of any of the formulae of the study, not attending scheduled visits to the hospital, and severe regurgitation and/or colic that, according to pediatricians, need prescription of a special formula.

Sample size was estimated upon the primary outcome that was average weight gain of infants between baseline and 120 ± 3 days of age. Based on previous publications where growth was the primary outcome variable as part of a safety study [15,16] and according to the Scientific Committee for Food Report the study was designed to have a power to detect a difference in weight gain equal to 0.5 standard deviations [17]. Thus, about 63 children would be needed in each formula group under the assumption of non-inferiority (one-sided test), with a significance level of 2.5% and a power of 80%. Drop outs were not included in the calculation.

One hundred and thirty seven infants were selected and distributed into two study groups, according to a randomization generated by a computer program (SIGESMU®). The formulae administered were standard powdered infant formula with a nutritional composition in accordance with current EU regulations, supplemented with galactooligosaccharides (GOS) (0.3 g/100 mL) in the case of control group (CG), and with the same amounts of galactooligosaccharide (GOS) plus *L. fermentum* CECT5716 (*Lactobacillus fermentum* Hereditum®) at a concentration dose of 10^7 cfu/g of formula in the case of the experimental group (EG). The concentration of the probiotic in the formula was analyzed and confirmed every two months. Both formulae were consumed by the infants until the age of 6 months (intervention period). The infant formulae were provided via Puleva Food SL (Granada, Spain) in identical plain white containers labeled with a code number that referred to

the study groups. In order to ensure the blinding of the trial, both formulas were submitted to a sensorial test by an expert panel that found both products to be identical. The pediatricians prescribed the amounts of formula per day to be administered to the infants and the guidelines for complementary feeding according to current ESPGHAN guidelines [18]. This 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). The trial was registered in the US Library of Medicine (www.clinicaltrial.gov) with the number NCT01346644.

2.2. Study outcomes and data collection

The primary outcome of the trial was average weight gain between baseline (T0) and 4 months of age (T2). Secondary outcomes were average length and head circumference gain, intestinal incidence of infections, feeding-related behavior, adverse effects associated with formula consumption, fecal microbiota, fecal concentration of short-chain fatty acids (SCFA), and fecal concentration of IgA. Infants were scheduled to receive four clinical evaluations during the intervention period: at baseline at the age of 1 month (T0), 2 months (T1), 4 months (T2), and 6 months (T3). Fecal samples were collected at T0, T2 and T3.

The diagnosis of infectious diseases was made by the pediatrician based on specific symptoms and standardized definitions. Gastrointestinal infection (GI infection) was defined as loose or watery stools at least three times per day with or without fever or vomiting [19] and respiratory tract infections as the presence of abundant mucosity and/or cough during two or more consecutive days with or without fever or the presence of wheezing and/or crepitations with or without fever. Infantile colic was defined as continuous crying that lasts for a period of more than three hours, occurring more than three days per week, and continuing for longer than three weeks [20]. Parents received a diary and 15-day questionnaires, in which information regarding daily number of depositions, daily amount of formula consumed, unscheduled visits to the doctor, behavior and gastrointestinal discomfort was recorded.

For fecal sample collection four simultaneous fresh fecal samples were collected from every volunteer at baseline (1 month of age), 4 and 6 months of age, preserved at -20°C and processed within 1 week. Three of the samples were used to evaluate the different parameters analyzed, and the remaining sample was stored at -80°C .

2.3. Fecal bacteria quantification

Fecal bacteria quantification was performed by classic microbiological techniques following the protocol described by Maldonado et al. [14].

For detection of *L. fermentum* CECT5716 fecal samples were dispersed (100 mg/mL) in peptone saline buffer and spread in MRS agar. After a 48 h incubation period at 37°C and anaerobic conditions, colonies were recollected and suspended in distilled water. *L. fermentum* CECT5716, which total genome sequence is known [21], was detected in these bacterial suspensions following a nested PCR based strategy. Primers and probes were designed using Primer Express software (Applied Biosystems) coming from a species-specific genomic DNA sequence identified by genomic DNA subtractive hybridization (data not shown).

Briefly, the first standard amplification reaction was performed using the following oligonucleotides as primers HSL40.126D (5'-GCTTGCCGCTTCTCTGGT-3') and HSL40.126R (5'-CAACGACGATGAACACCACCT-3') at 500 nM in an Eppendorf Mastercycler Gradient equipment and Taq DNA polymerase (Roche). The PCR conditions were an initial denaturing step for

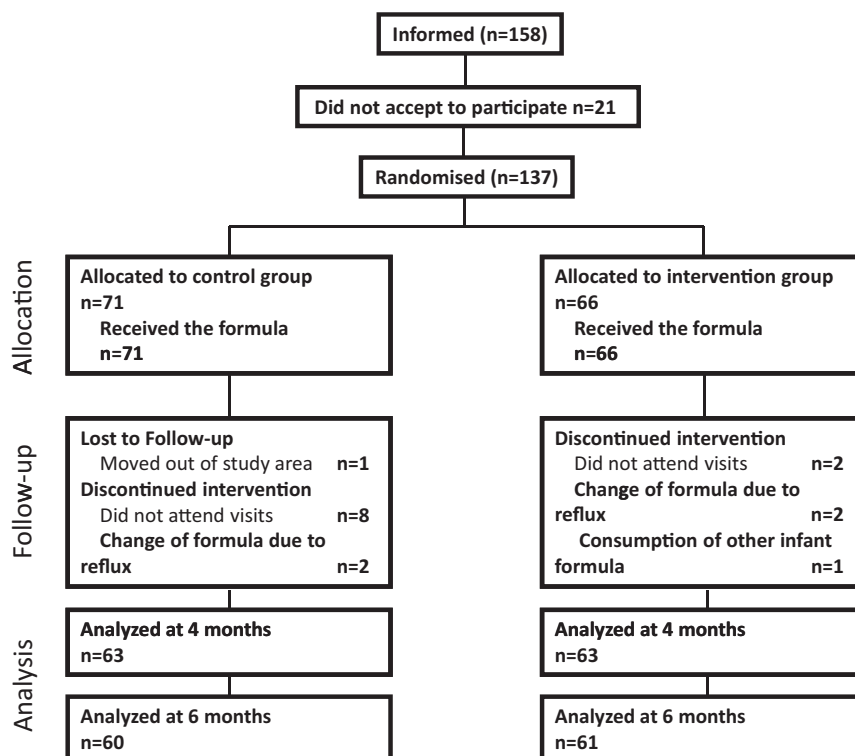


Fig. 1. Flow chart of participants.

5 min at 95 °C, followed by 40 cycles at 95 °C for 30 s, 46 °C for 30 s and 72 °C 30 s, and a final extension at 72 °C for 3 min. The result of the amplification and the amplicon size (222 bp) were confirmed by agarose gel electrophoresis.

The second amplification was a Taq-Man based PCR assay, which target sequence was located within the product of the first PCR. Primers (LC40C126.D 5'-TCAACGGCCCTTCAATACA-3', LC40C126.D 5'-GACCTAATTCACGTCAAACATATTTAC-3') were present in the reaction mixture at 500 nM and the probe (LC40C126.P 5'-AGTGGTGAGATGCCAGTGTTCCCG-3', JOE and BHQ1 labeled) at 250 nM. Amplification and detection were carried out in a Stratagene MX3005P thermocycler, using Taq DNA polymerase (Roche) and incubating 5 min at 95 °C followed by 40 cycle at 95 °C 30 for 15 s and 60 °C for 1 min.

2.4. Short chain fatty acids quantification

Fecal samples were homogenized with 150 mM NaHCO₃ (pH 7.8) (1:5, wt/v) in an argon atmosphere. Samples were incubated for fermentation during 24 h at 37 °C and stored at –80 °C until the extraction. The extraction of SCFA was performed by gas chromatography following the protocol described in Maldonado et al. [14].

2.5. Fecal IgA quantification

IgA concentration was measured in the supernatants of feces by an ELISA quantification kit, following manufacturer's instructions (Bethyl, Montgomery, TX).

2.6. Statistical analysis

The statistical software used to perform the analysis was R version 2.12.2. The statistical models applied to the primary and secondary outcomes were adjusted mainly by time, group of

treatment, gender and age at baseline in order to correct for differences at the starting age of the infant in the trial, differences between gender and hospital. Since there were no differences between hospitals the final analysis did not include hospital as a covariate and no adjustment for it had to be performed.

To analyze repeated measures over time, and in order to taking into account the correlation of the responses within subjects, a Linear Mixed Model was applied. When the variables of the study were continuous responses as measures of the period of intervention, linear regression models (LRM) were applied to adjust the mean by the covariates of interest. For the outcome responses based on counts events or dichotomy values, Generalized Linear Models (GLMs) were applied. In particular for the number of events the Poisson regression model with the log link function was fitted, and for the occurrence a logistic regression model was used. The tests were performed at the two-sided 5% significance level and the 95% confidence intervals were obtained for the estimates.

3. Results

3.1. Population

One hundred and fifty eight parents were informed about the study. Finally, 137 infants were included in the study and randomized. Of the 137 infants, 16 dropped out from the trial: 11 in the control group and 5 in the experimental group. Dropouts during intervention were due to change of address out of the study area (1 in control group), change of formula due to reflux (2 in CG and 2 in EG), consumption of other infant formula (1 in EG), and loss during the intervention because of poor compliance and violation of the protocol (8 in CG and 2 in EG). The total number of volunteers analyzed at 4 months (per protocol) was 126, (63 per group), and at 6 months 121 infants (60 in the CG and 61 in the EG). A flow chart of participants is shown in Fig. 1. The baseline

Table 1
Baseline characteristics of the subjects that participated in the study.

	CG (n=60)	EG (n=61)
Male/female, n (%)	38/22 (63/37)	34/27 (56/44)
Age at enrolment (weeks), mean \pm SD	4.3 \pm 1.1	4.3 \pm 0.5
Birth weight (kg), mean \pm SD	3.15 \pm 0.6	3.24 \pm 0.6
Delivery by cesarean (%)	48	36
Gestational age (weeks) mean \pm SD	40 \pm 2.3	40.5 \pm 2.4
Age of mother at birth (years) mean \pm SD	30.6 \pm 4.9	28.2 \pm 5.4
Breast feeding (%)		
No breast feeding	74	73
<1 week	19	21
1–4 weeks	8	6
Smoking during pregnancy (%)	22	15
Smoking during lactation (%)	27	16
Smoking in the household (%)	52	48
Older siblings (%)	44	45
Attending day care before 6 months (%)	11	3
Weight of mother (kg) mean \pm SD	72.7 \pm 6.5	68.8 \pm 5.7
Family history of allergy (%)	32	23
Pets at home (%)	30	33
Rotavirus vaccination	68	69

characteristics of the 121 infants who completed the intervention period were comparable between the study groups (Table 1).

3.2. Percentiles and z-scores study

At the end of the trial the average percentiles of weight and head circumference for girls were around 75% and for boys between 50%

and 75% being similar in both groups (CG and EG). In the case of growing curves of length at the end of the trial in both groups the average percentiles for girls and boys were between the 50% and 75% (Fig. 2).

The z-scores of weight, length and head circumference for age were calculated based on the WHO Child Growth Standards [22]. The population of the study did not differ from the standard (Fig. 3). It was observed that the experimental formula effect was not significant for the weight for age z-scores ($p=0.061$) and neither for the head circumference z-scores ($p=0.453$). Regarding to the length for age z-scores, the treatment effect was significant ($p=0.021$) indicating that those infants in the treatment group had higher length for age z-scores compared to the control group.

3.3. Growth

Regarding to the weight, no significant differences were observed for weight and weight gain at 4 months of age (main outcome of the study) nor at the end of the intervention (6 months of age). Similar results were obtained for head circumference (Table 2).

No significant differences were observed between groups in the length of infants at 4 months of age, but at 6 months of age infants in EG were significantly taller than in CG ($p=0.038$). However, the length gain (cm/day) of the infants was equivalent and no significant differences were observed (Table 2).

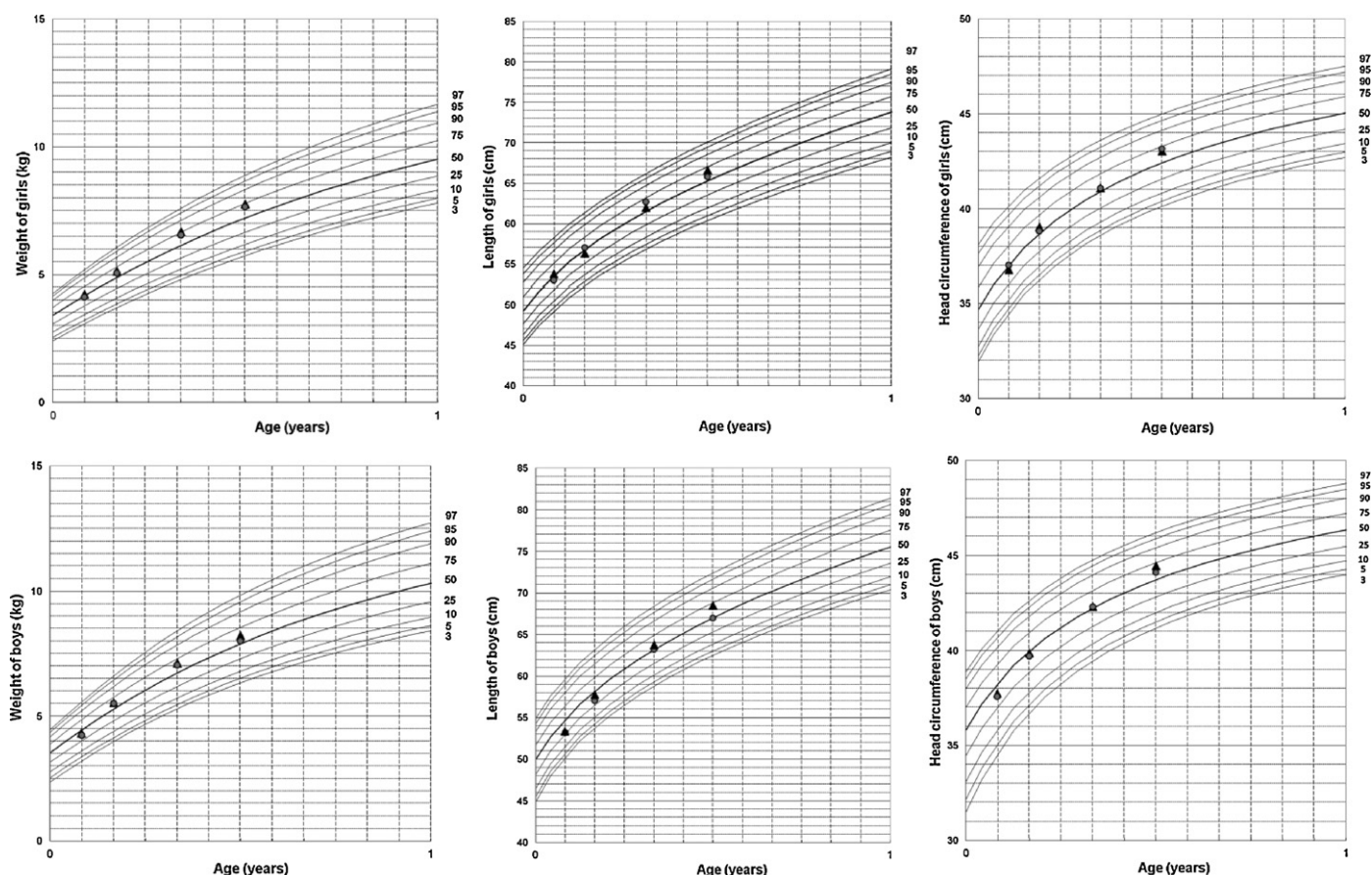


Fig. 2. Based on the mean, by gender and group, of the weights, lengths and head circumferences of infants, the corresponding percentile for the mean by boys and girls for each group over time are represented with respect to the standard curves. Dots show the growing curve in average for CG and triangles represent the growing curve in average for EG. Each of the curves (black lines) is the standard percentile at each point of age of children.

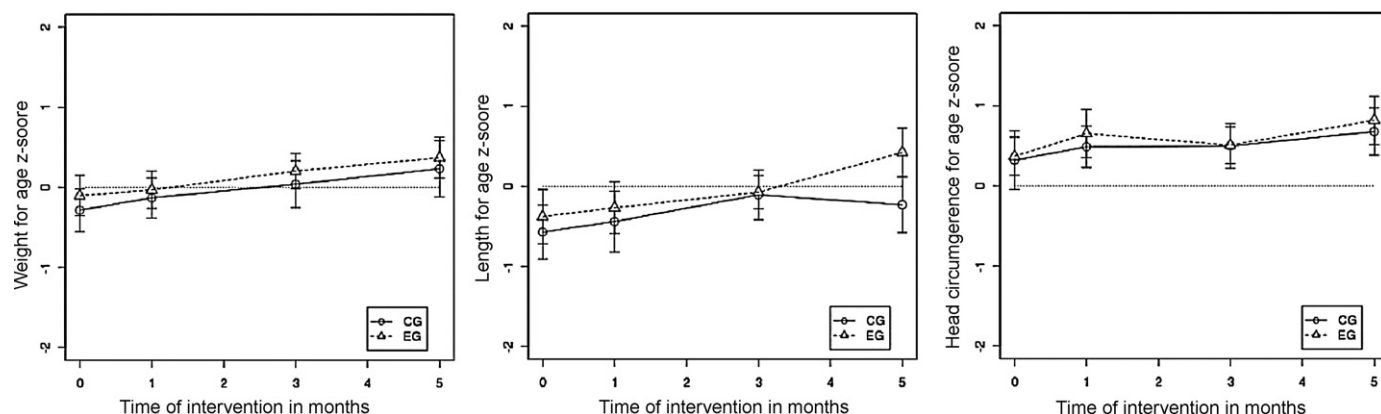


Fig. 3. The z-scores for weight for age, length for age and head circumference (mean and 95% CI by group), relative to WHO standards.

Table 2

Anthropometric measurements at baseline (1 month), 4 and 6 months of age. Gain/day corresponding to difference among values at 6 months of age and baseline. Values are means \pm SEM. *, $p < 0.05$ versus control.

Growth parameters	Control group				Experimental group			
	1 month	4 months	6 months	Gain/day ^a	1 month	4 months	6 months	Gain/day ^a
Weight (kg)	4.2 \pm 0.6	6.8 \pm 0.8	7.9 \pm 1.0	25.3 \pm 6.0	4.3 \pm 0.5	6.9 \pm 0.7	8.0 \pm 0.9	24.8 \pm 5.1
Length (cm)	53.1 \pm 2.6	62.8 \pm 3.5	66.6 \pm 2.5	0.90 \pm 0.2	53.6 \pm 2.1	63.0 \pm 1.8	68.1 \pm 3.4*	0.96 \pm 0.3
Head circumference (cm)	37.4 \pm 1.6	42.1 \pm 2.7	43.7 \pm 1.3	0.421 \pm 0.1	37.3 \pm 1.1	41.1 \pm 4.7	43.7 \pm 1.6	0.43 \pm 0.1

^a For weight: g/day; for length and head circumference: mm/day.

3.4. Formula intake, tolerance and adverse effects

Both study formulae were well tolerated and compliance was good. No significant differences were found between the study groups with regard to daily intake of formula (630.9 ± 197.7 mL/day in CG vs 587.8 ± 201.3 mL/day in EG). Two infants in the CG and two infants in the EG discontinued the intervention due to reflux and had to change to an anti-reflux infant formula, but there were no significant differences in the dropout rates between both groups. Feeding-related behavior (fecal depositions/day, feces color, consistency, flatulence, regurgitation, sleeping hours and behavior) was similar in both groups (Table 3). No adverse effects associated to probiotic supplementation were detected during the study.

3.5. Infant's health

During the intervention, 51% of the infants suffered from respiratory infections and 16% from GI infections. Most of the infectious diseases were respiratory infections (76% of the total infections) (Table 4). No difference was found in the incidence rates of respiratory infections between both groups, although it was lower in the EG than in the CG. Regarding to GI infections, the EG showed a significant (71%) reduction in the incidence rate (IR: 0.082 ± 0.037) compared to the CG (IR: 0.283 ± 0.068) (Table 4). The incidence rate ratio for GI infections (IRR = 0.289 95% CI: 0.085 – 0.831) was significant ($p = 0.018$), indicating that in the CG the incidence rate of diarrhoea was higher than in the EG. The number of infants needed to treat (NNT) to reduce one event of diarrhoea was 5. The odds ratio of having at least one GI infection or respiratory infection was 0.36 (95% CI: 0.08 – 0.97) and 0.77 (95% CI: 0.36 – 1.66), respectively, but the difference was only significant ($p = 0.025$) for GI infections. In particular the odd of having an occurrence of GI infection in the treatment group was almost 3 times lower than in the control group.

Only 5 events (3 in CG and 2 in EG) of other infections (candidiasis, urinary infection, conjunctivitis, chickenpox or otitis) were

Table 3

Feeding related behavior.

TIME	1 month	4 months	6 months
Fecal depositions/day^a			
CG	1.88 \pm 0.6	1.84 \pm 0.5	1.98 \pm 0.2
EG	1.94 \pm 0.6	1.80 \pm 0.7	1.93 \pm 0.4
Feces color^b			
CG	1.8 \pm 0.8	2.10 \pm 1.0	2.49 \pm 0.8
EG	1.81 \pm 0.7	2.07 \pm 0.9	3.10 \pm 1.3
Consistency^c			
CG	2.92 \pm 0.6	3.08 \pm 0.5	2.63 \pm 0.6
EG	3.04 \pm 0.4	2.98 \pm 0.5	2.54 \pm 0.7
Flatulence^d			
CG	1.74 \pm 0.9	1.18 \pm 0.4	1.12 \pm 0.3
EG	1.64 \pm 0.7	1.33 \pm 0.8	1.20 \pm 0.7
Regurgitation^e			
CG	1.90 \pm 0.8	1.78 \pm 0.8	1.57 \pm 0.7
EG	1.77 \pm 0.7	1.89 \pm 0.9	1.68 \pm 1.0
Hour sleeping^f			
CG	2.56 \pm 0.8	3.14 \pm 0.7	3.47 \pm 0.7
EG	2.66 \pm 1.0	3.44 \pm 0.8	3.73 \pm 0.8
Total sum of sleeping^g			
CG	3.14 \pm 0.9	2.84 \pm 0.7	2.51 \pm 0.9
EG	3.26 \pm 0.7	2.82 \pm 0.9	2.78 \pm 1.0
Gender temper^h			
CG	2.62 \pm 0.7	2.44 \pm 0.5	2.47 \pm 0.5
EG	2.43 \pm 0.6	2.40 \pm 0.5	2.39 \pm 0.5

^a Fecal depositions/day: 1 = <1 time, 2 = 1–3, 3 = 4–6, 4 = 7–10, and 5 = >10.

^b Feces color: 1 = yellow, 2 = mustard, 3 = brown, 4 = grey, and 5 = green.

^c Consistency: 1 = hard lumps, 2 = sausage with cracks, 3 = soft sausage, 4 = mushy (like porridge), and 5 = watery.

^d Flatulence: 1 = 0 h, 2 = <3 h, 3 = 3–6 h, 4 = 6–12 h, and 5 = >12 h.

^e Regurgitation: 1 = not at all, 2 = regurgitation of small amounts during or shortly after feeding, 3 = larger regurgitation during or shortly after feeding, 4 = minor vomiting with time-lag to prior feeding, and 5 = severe vomiting with considerable loss of fluid.

^f Hour sleeping (during the past 2 nights, the continuous night sleep was on average): 1 = <4 h, 2 = 4–6 h, 3 = 6–9, 4 = 9–12, and 5 = >12 h.

^g During the past 2 days the total sum of sleeping hours in 24 h was on average: 1 = <11 h, 2 = 11–14 h, 3 = 14–17, 4 = 17–20, and 5 = >20 h.

^h Gender temper: the infant's behavior when awake during the last 2 days is best described as: 1 = tired, passive, 2 = quiet, watching, 3 = well-balanced, active, 4 = bubbly, fidgety, excited, and 5 = disturbed, agitate.

Table 4

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

	Control group		Experimental group		Incidence rate ratio	IR decrease (%)	NNT	p-Value IRR
	No. events	Incidence rate (SE)	No. events	Incidence rate (SE)				
GI infections	17	0.283 (0.07)	5	0.082 (0.04)	0.289 (0.085–0.831)*	71.1	5	0.018
Respiratory infection	43	0.716 (0.11)	42	0.689 (0.11)	0.977 (0.623–1.530)	3.9	61	0.933
Total infections	63	1.050 (0.13)	49	0.803 (0.11)	0.778 (0.524–1.148)	23.5	4	0.339
Febrile episodes	13	0.220 (0.06)	13	0.213 (0.06)	0.967 (0.427–2.341)	3.3	–	–
Antibiotic treatments	7	0.115 (0.04)	8	0.131 (0.05)	1.105 (0.362–3.702)	–10.5	–61	0.807

* $p < 0.05$ versus control.

reported. The incidence rate of total infections was lower in EG but this difference was not significant. For febrile episodes or antibiotic treatments no significant differences were observed.

3.6. Fecal parameters

The observed mean of fecal counts of Lactobacilli, Bifidobacteria, Clostridia, Bacteroidaceae at each time point between both group was similar, although it was observed a significant increase in these bacterial groups with time (Table 5) was observed.

L. fermentum CECT5716 was detected alive in the fecal samples of 53% of the infants in the EG. Samples of two infants in the control group were found positive for *L. fermentum*. The capability of fecal microbiota of infants to produce short chain fatty acids (butyric, propionic and acetic) and concentration of IgA in feces were similar in both groups (Table 5).

4. Discussion

L. fermentum CECT5716 is a probiotic strain originally isolated from four-day postpartum human milk [2,8], and its safety and probiotic potential was demonstrated in animal models and in human studies including infants from 6 to 12 months of age [11,14,23]. In the present study, the safety and tolerance of *L. fermentum* in an infant formula in healthy infants aged 1–6 months of life was studied.

Determination of rate of gain in weight (main outcome of the study) is the single most valuable component of the clinical evaluation of an infant formula [24–26]. In this study weight for age and length for age z-scores indicated growth rates comparable to growth standards in both groups. Because these standards are based on healthy infants these results are a valuable indication of the nutritional sufficiency and safety of the experimental formula. No differences were observed in the weight, length and head circumference gain between EG and CG. However, at the end of the intervention it was observed that infants in the experimental group were significantly longer compared to the infants in the control group. There are previous reports about similar studies carried

out with other probiotic strains in which an effect of the probiotic intervention on the weight and length of the infants was observed [27,28]. The explanation for this effect it is not clear, but perhaps, the activity of the bacteria on mucosal physiology may influence the digestion and absorption of nutrients. Future studies designed to detect these differences in growth will have to be performed in order to corroborate this result and investigate the mechanisms involved.

No adverse effects related to the consumption of the experimental formula were detected. Mild gastrointestinal disorders such as colic, regurgitation, soft feces and constipation are symptoms indicative of the tolerance of an infant formula. There were no differences in these symptoms between both intervention groups evidencing that the experimental formula containing the probiotic strain was well tolerated. In addition to this, the number of infants who discontinued the study by regurgitation problems was very low and similar in both groups. The rates or incidence values of infant colic, spitting up and constipation were within the range of Spanish infants at this age [29].

Although no significant differences in the fecal microbiota were detected between both groups, the probiotic strain *L. fermentum* CECT5716 could be detected alive in the feces of 53% of infants of the EG, demonstrating that this strain is able to survive the conditions of the gastrointestinal tract of the infants. The specific oligonucleotides used to detect the strain CECT5716 also recognized as positive two samples of the CG probably due to the natural presence of strains of *L. fermentum* very similar to CECT5716 in feces of these two infants.

Regarding to the health status of the infants, no significant differences between both groups were observed in the incidence rates of respiratory infections, the most common infectious problem in childhood. In a previous study in infants aged 6–12 months, the consumption of a prebiotic containing follow-on formula with the same strain of *L. fermentum* induced a significant reduction of 26% in the incidence of respiratory infections [14]. Noteworthy, in that study the reduction in the incidence of respiratory infections was mainly due to an effect on recurrent infection. During the first 6 months of life the incidence of recurrent infections is really low

Table 5

Intestinal microbiota counts in fecal samples of infants (as logarithm of cfu/g), fecal concentration of short chain fatty acids (SCFA, as mg/g feces) and IgA (as mg/g feces) at baseline (1 month of age), 4 and 6 months of age.

Bacterial group	Control group			Experimental group		
	1 month	4 months	6 months	1 month	4 months	6 months
<i>Lactobacillus</i> spp.	6.99 ± 0.2	7.35 ± 0.2	7.80 ± 0.2	6.79 ± 0.2	7.05 ± 0.2	7.88 ± 0.2
<i>Bifidobacterium</i> spp.	6.70 ± 0.2	7.18 ± 0.1	7.81 ± 0.2	6.65 ± 0.2	6.90 ± 0.2	7.83 ± 0.2
<i>Clostridium</i> spp.	6.80 ± 0.2	6.98 ± 0.1	7.43 ± 0.2	6.48 ± 0.2	6.81 ± 0.2	7.66 ± 0.2
<i>Bacteroides</i> spp.	6.66 ± 0.2	6.94 ± 0.1	7.52 ± 0.2	6.38 ± 0.2	6.84 ± 0.2	7.57 ± 0.2
SCFA						
Acetate	14.5 ± 1.1	13.4 ± 1.5	12.4 ± 1.1	12.8 ± 1.1	11.5 ± 0.9	14.4 ± 1.4
Propionate	2.75 ± 0.5	2.15 ± 0.2	2.71 ± 0.3	2.27 ± 0.2	1.89 ± 0.1	3.08 ± 0.5
Butyrate	2.31 ± 0.5	2.32 ± 0.4	2.38 ± 0.3	1.60 ± 0.3	1.73 ± 0.2	2.94 ± 0.4
Immunoglobulin A	1.16 ± 1.2	1.19 ± 0.9	0.89 ± 0.8	1.13 ± 1.0	1.09 ± 1.0	0.998 ± 1.0

and this might explain the lack of effect on respiratory infections observed in this study.

The consumption of the experimental formula containing *L. fermentum* was related to a significant reduction of 71% in the incidence of gastrointestinal infections compared to the infants fed with the control formula. This result is in line with previous results observed in infants from 6 to 12 months of age in which a reduction of 46% in the incidence of gastrointestinal infections was detected during the period of intervention using the same strain of *L. fermentum* [14]. This study was designed to detect differences in growth of infants, taking into account the low rates of gastrointestinal infections in the infants during the first 6 months of life, the statistical power of this study would be lower than 80%. Therefore, future studies with significantly more subjects will be needed to corroborate these results.

The rate of reduction in gastrointestinal infections observed in the EG is also comparable to other trials that reported a successful prevention of community-acquired gastrointestinal infections of diarrhoea episodes using a probiotic formula [30–34].

Both formulae, control and experimental, contained GOS (0.3 g/100 mL). The beneficial effects of prebiotics mainly rely on their influence on the gut microbiota composition and their ability to generate fermentation products (short-chain fatty acids) with diverse biological roles [35]. The presence of GOS in experimental formula may have a synergistic effect with *L. fermentum* improving the beneficial effect of the probiotic strain. However, since the control formula also contained the same concentration of GOS it is not possible to draw a conclusion.

In conclusion, consumption of a prebiotic containing infant formula enriched with *L. fermentum* CECT5716 is safe and well tolerated in infants from 1 up to 6 months of age. Moreover, the consumption of this formula may improve the health of the infants reducing the incidence of gastrointestinal infections.

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