# Enrichment of Formula in Probiotics or Prebiotics and Risk of Infection and Allergic Diseases up to Age 5.5 Years in the Nationwide Etude Longitudinale Française depuis l'Enfance (ELFE) Cohort

Moufidath Adjibade, Camille Davisse-Paturet, Amandine Divaret-Chauveau, Karine Adel-Patient, Chantal Raherison, Marie-Noëlle Dufourg, Sandrine Lioret, Marie-Aline Charles, Blandine de Lauzon-Guillain

# Enrichment of formula in probiotics or prebiotics and risk of infection and allergic diseases up to age 5.5 years in the nationwide ELFE cohort

Moufidath Adjibade*[1], Camille Davisse-Paturet*[1], Amandine Divaret-Chauveau[2,3], Karine Adel-Patient[4], Chantal Raherison[5], Marie-Noëlle Dufourg[6], Sandrine Lioret[1], Marie-Aline Charles[1,6], Blandine de Lauzon-Guillain[1]

*The two first authors have equally contributed to this work

[1] Université de Paris, CRESS, Inserm, INRAE, 75004 Paris, France

[2] EA3450, Université de Lorraine, 54500 Vandoeuvre-lès-Nancy, France

[3] Unité d'allergologie pédiatrique, Hôpital d'Enfants, CHRU de Nancy, Vandoeuvre-lès-Nancy, France

[4] Université Paris-Saclay, CEA, INRAE, DMTS, 91191 Gif-sur-Yvette, France

[5] Bordeaux University, Inserm, Bordeaux Population Health Research Center, Team EPICENE, UMR 1219, Bordeaux, France

[6] Unité mixte Inserm-Ined-EFS Elfe, INED, Paris, France

**Corresponding author:**

Blandine de Lauzon-Guillain

Hôpital Paul Brousse, Bâtiment Leriche,
15-16 avenue Paul Vaillant Couturier
94807 Villejuif Cedex, France

Tel: +33145595019
E-mail: blandine.delauzon@inserm.fr

**Running title:** Probiotic-enriched formula and infection/allergy

**Sources of Support**:

The present study is funded by an ANR grant (InfaDiet project, grant number: ANR-19-CE36-0008).

The ELFE cohort is a joint project between INED (Institut National d'Etudes Démographiques), INSERM (Institut National de la Santé et de la Recherche Médicale), EFS (Etablissement Français du Sang), InVS (Institut de Veille Sanitaire), INSEE (Institut National de la Statistique et des Etudes Economiques), the Ministry of Health (DGS, Direction Générale de la Santé), the Ministry of Environment (DGPR, Direction Générale de la Prévention des Risques), the Ministry of Health and Employment (DREES, Direction de la Recherche, des Etudes, de l'Evaluation et des Statistiques), and the CNAF (Caisse Nationale des Allocations Familiales), with the support of the Ministry of Research and CCDSHS (Comité de Concertation pour les Données en Sciences Humaines et Sociales) and the Ministry of Culture (DEPS, Département des études, de la prospective et des statistiques). As part of the RECONAI platform, the study received state funding from the ANR within the framework of the "Future Investments" program (reference: ANR-11- EQPX-0038, ANR-19-COHO-0001).

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Conflict of interest**:

Amandine Divaret-Chauveau has been invited to allergology and pediatric congresses (inscription and accommodation) by Mead Johnson, Sodilac and Nutricia. Karine Adel-Patient has been invited by Stallergènes to speak at a conference, outside of the submitted work. None of the other authors declare any conflicts of interest.

**Abbreviations:**

FOS: fructo-oligosaccharides

GOS: galacto-oligosaccharides

GBTM: group-based trajectory modelling

LRTI: lower respiratory tract infection

PANDiet: Probability of Adequate Nutrient intake based Diet quality index

RCT: randomized controlled trials

URTI: upper respiratory tract infection

**ABSTRACT**

**Background**: An increasing number of infant and follow-on formulas are enriched with probiotics and/or prebiotics; however, evidence for health effects of such enrichment in early childhood remains inconclusive.

**Objective**: The present study aimed to assess, whether consumption of formula enriched with probiotics or prebiotics was associated with the risk of infection and allergic diseases in early childhood.

**Methods**: Analyses involved data for 8,389 formula-fed children from the *Etude Longitudinale Française depuis l'Enfanc*e (ELFE) cohort. Enrichment of the formula with probiotics or prebiotics that was consumed from age 2 to 10 months was identified by the formula ingredient list. Lower respiratory tract infection (LRTI), upper respiratory tract infection (URTI), gastrointestinal infection, wheezing, asthma, food allergy and itchy rash were prospectively reported by parents up to age 5.5 years. Adjusted logistic regression models were used to assess associations between consumption of enriched formula and risk of infection and allergic diseases.

**Results**: At age 2 months, more than half of formula-fed infants consumed probiotic-enriched formula and only one in 10 consumed prebiotic-enriched formula. Consumption of *Bifidobacterium lactis*-enriched formula at 2 months was associated with a lower risk of LRTI (OR[95%CI]=0.84[0.73-0.96]). Consumption of *Bifidobacterium breve*-enriched formula up to 6 months was associated with a higher risk of LRTI (OR[95%CI]=1.75[1.29-2.38]) and asthma (OR[95%CI]=1.95[1.28-2.97]), while its consumption from 6-10 months was associated with a lower risk of LRTI (OR[95%CI]=0.64[0.48-0.86]) and asthma (OR[95%CI]=0.59[0.40-0.88]). Moreover, consumption of *Streptococcus thermophilus* from 6-10 months was associated with a higher risk of asthma (OR[95%CI]=1.84[1.29-2.63]). No significant association was found for gastrointestinal infection, food allergy, and itchy rash.

Overall, consumption of prebiotic-enriched formula was not significantly associated with infection and allergy risk.

**Conclusions**: Associations between consumption of probiotic-enriched formula and risk of respiratory symptoms differ according to the strain considered and consumption period. Further well-designed studies are needed to confirm these results.

**Keywords**: Infancy, Enrichment, Infant formula, Prebiotic, Probiotic, Infection, Allergy, Birth cohort

## INTRODUCTION

There is increasing evidence that the microbiome could influence the infant's immune system development and that an imbalanced microbial composition and reduced diversity (dysbiosis) could promote susceptibility to metabolic and immune- related diseases (1, 2). Colonization of the digestive tract and formation of the newborn's microbiota begins mainly at birth, and both its composition and function are variable for the first 3 years of life, after which it stabilizes (2, 3). The infant gut microbiome composition is affected by several factors such as the maternal microbiota composition, mode of delivery, feeding practices, environmental exposures and antibiotics use (2, 4-7).

Breastfeeding is the preferred nutrition option for newborns because it provides all of their nutritional needs (8). Human breast milk is a complex biofluid that contains proteins, lipids, carbohydrates and various minerals and vitamins (7, 9). It also contains a wide range of non-nutritional bioactive components and a variety of both prebiotics and probiotics that ensure neonate gut colonization by microbes beneficial for metabolism, immune development and lifelong health (1, 5, 10). According to the Food and Agriculture Organization and the World Health Organization, probiotics can be considered "living microorganisms, which when administered in adequate amounts confer health benefits on the host", while prebiotics are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already established in the colon, and thus in effect improve host health" (11).

Although exclusive breastfeeding for the first 6 months of life or at least 4 months is the nutritional recommendation for healthy term infants (8), several studies of infant feeding practices have shown a high level of non-compliance with these recommendations (12-14). When exclusive breastfeeding is not attainable, infant formula is the preferred option. To

reproduce some of the beneficial aspects of breastmilk, a large number of formulas are enriched with probiotics, prebiotics, or both (i.e., symbiotics) (15, 16).

Several randomized controlled trials (RCTs) have assessed the effect of probiotic-, prebiotic- or symbiotic-enriched formula on infant health. Some showed a beneficial effect of probiotics and prebiotics on respiratory or gastrointestinal infections, antibiotics use, atopic dermatitis or other types of allergies (17-22), but others did not report any association with incidence of diarrhoea episodes, respiratory illnesses and allergic manifestations (18-24). Moreover, systematic reviews concluded that evidence was not conclusive for recommending the routine use of probiotic-, prebiotic- or symbiotic-enriched formula in healthy term infants (25-28). The Committee on Nutrition from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition also concluded lack of data to recommend their routine use (29).

In this study, we aimed to describe the consumption of probiotic- or prebiotic-enriched formula and its association with risk of infection and allergic diseases in early childhood.

## SUBJECTS AND METHODS

### Study design

This analysis was based on data from the *Etude Longitudinale Française depuis l'Enfance* (ELFE) study, a multidisciplinary nationwide birth cohort including 18,329 children born in 2011 in 320 participating maternity units among a random sample of 349 maternities in mainland France (30). Inclusion began in April 2011 and took place during 25 selected recruitment days over four waves of 4 to 8days each that covered all four seasons. Inclusion criteria were singleton or twins born after 33 weeks' gestation, from mothers aged 18 years or older and not planning to move outside of metropolitan France in the next 3 years. Foreign families also participated in the study if mothers were able to read French, Arabic, Turkish or English.

Participating mothers had to provide written consent for their own and their child's participation. Fathers signed the consent form for the child's participation when present at inclusion or were informed about their rights to oppose it. The ELFE study was approved by the Advisory Committee for Treatment of Health Research Information (Comité Consultatif sur le Traitement des Informations pour la Recherche en Santé), the National Data Protection Authority (Commission Nationale Informatique et Libertés), and the National Statistics Council.

**Data collection**

Families were followed by phone interview at age 2 months, 1 year, 2 years, 3.5 years and 5.5 years. They also completed a monthly internet/paper-based questionnaire on infant feeding from age 3 to 10 months.

***Infant milk feeding***

At the 2-month interview, if relevant, the age at first introduction of infant formula was collected.

From age 2 to 10 months, the name and brand of the formula used was collected monthly for formula-fed infants. Classification of all reported infant formulas was based on their nutritional characteristics (ingredient list and nutritional composition). Enrichment of the formula with probiotics (*Bifidobacterium breve [BC50]*, *Bifidobacterium lactis [BB12]*, *Bifidobacterium infantis*, other Bifidobacterium, *Lactobacillus reuteri [DSM 17938]*, *Lactobacillus fermentum [CECT5716]*, *Lactobacillus rhamnosus*, other non-specified Lactobacillus, *Streptococcus thermophilus*) or with prebiotics (galacto-oligosaccharides [GOSs] and fructo-oligosaccharides [FOSs]) was identified from the ingredient list.

Infants receiving both breast milk and formula were classified according to the type of formula they received.

***Infections and allergies***

In this study, severe/frequent infections, as well as allergies from age 2 months to 5.5 years were assessed as follows:

- Ever gastrointestinal infection: parental report of at least one hospitalization for gastroenteritis/dehydration or emergency consultation for diarrhea/vomiting/dehydration
- Ever upper respiratory tract infection (URTI): parental report of at least one hospitalization for URTI or emergency consultation for otalgia, at least three otitis events from birth, at least three laryngitis events from birth, or at least three angina events from birth
- Ever lower respiratory tract infection (LRTI): parental report of at least one bronchiolitis event or one hospitalization for bronchitis/bronchiolitis/pneumopathy
- Ever wheezing, asthma, itchy rash or food allergy: at least one parental report of wheezing in the chest, medical diagnosis of asthma, itchy rash, or medical advice to avoid certain foods due to a food allergy.

***Other variables***

Parental socio-demographic characteristics considered in this study were: maternal age at delivery (18–24, 25–29, 30–34, ≥35 years), number of older children in the household (no sibling, one sibling, at least two siblings), maternal migration status ("majority population" which included women born to two French parents [inside or outside of France]), "descendants of migrants" including women born in France with at least one non-French parent, "migrants" including women not born in France and without French citizenship at birth), maternal education level (up to lower secondary, upper secondary, intermediate, 3-year university degree, at least 5-year university degree), employment status during pregnancy (employed, unemployed, not in the labour force [e.g., housewife, student, disabled, retired])

and monthly household income per consumption unit (<€750, €751–1,111, €1,112–1,500, €1,501–1,944, €1,945–2,500, >€2,500). From the postal code of residence, we determined the region of residence (Paris region, North, East, East Paris Basin, West Paris Basin, West, South-West, South-East, Mediterranean) and the city size (rural area, urban area; >2000 inhabitants for rural area).

Maternal health characteristics included self-reported height and pre-pregnancy weight used to calculate pre-pregnancy body mass index (BMI) (<18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m²), smoking status (never smoker, smoker only before pregnancy, smoker only in early pregnancy, smoker throughout pregnancy) and diet quality during pregnancy (measured by using the Probability of Adequate Nutrient intake based Diet quality index [PANDiet] score, which reflects nutrient-based reference guidelines adapted for pregnancy; total scores range from 0 to 100) (31).

Newborn characteristics were collected from the medical record: child sex, gestational age, birth weight and delivery mode (C-section, vaginal). Birth weight categories (small, adequate or large for gestational age) were defined according to Audipog reference curves (32). The 2-month questionnaire collected the type of the physician consulted for the first visit after delivery (paediatrician, other child doctor, general practitioner, other including emergency).

Parent and sibling history of allergy (hay fever, asthma, eczema) was collected at the 2-month interview. Children were considered to have a family history of allergy if at least one parent or sibling had a history of allergy.

**Study sample**

Children whose parents withdrew their consent within the first year and requested deletion of their data (n=57) were excluded from this study. We also randomly excluded one twin out of two (n=287) to avoid family clustering.

For the present analysis, children not followed at the 2-month interview (n=1,696), those exclusively breastfed at age 2 months (n=5,045), those without sufficient data on the infant formula consumed at age 2 months (n=667), those with a medical diagnosis of cow's milk protein allergy at age 2 months (n=193) or those without any data on infection and allergy from age 2 months to 5.5 years (n=1,995) were excluded. The main analyses then involved 8,389 children **(Figure 1)**.

## Statistical analyses

### *Descriptive statistics*

Differences between excluded children (n=9,596) and children included in the main analyses (n=8,389) were assessed with Student *t* test and chi-square test for continuous and categorical variables, respectively.

To take into account the inclusion procedure and biases related to non- consent and attrition, the data were weighted (33) in sensitivity analyses. Weighting also included calibration on margins from the state register's statistical data and the 2010 French National Perinatal study (34) for the following variables: age, region, marital status, migration status, education level, and primiparity.

### *Main analyses*

Multiple imputations were performed to deal with missing data (covariates and longitudinal trajectories of the type of formula consumed from age 2 to 10 months) (35, 36). We assumed that data were missing at random, i.e. that missing values in the data set may depend on the value of other observed variables, but cannot depend on the values of unobserved variables (37). We generated five independent data sets by using the fully conditional specification method (MI procedure) and the calculated estimates of pooled effects (MIANALYSE procedure; SAS software). Missing data for categorical variables were imputed by using a

multinomial model, for ordinal or binary variables by using logistic regression and for the continuous variables by using linear regression (36).

Adjusted associations between consumption of enriched formula and risk of infection or allergic diseases were examined by logistic regression models adjusted for confounding factors. Potential confounding factors were identified first from the literature, then selected by using the directed acyclic graphs method (38). The main model was adjusted for maternal and household characteristics (maternal age, education level, migration status, employment during pregnancy, smoking status, city size, region of residence, maternal diet quality during pregnancy [PANDiet score], number of older children, household income and family history of allergy), child characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge, C-section delivery, age at infant formula introduction) and variables related to study design (maternity size and recruitment wave). A second model was performed to additionally account for the use of antibiotics up to age 1 year.

Formula enriched with probiotics or prebiotics was first examined at the 2-month follow-up (enriched vs not enriched). Then, to account for the temporal evolution in the consumption of prebiotic or probiotic-enriched formula between child age 2 and 10 months, we identified groups of children with similar longitudinal patterns of consumption by using the Nagin's method for group-based trajectory modelling (GBTM) (39) with the TRAJ procedure from SAS software. These trajectories were modeled for children who consumed formula for at least 2 follow-ups from age 2 to 10 months.

***Sensitivity analyses***

Sensitivity analyses were conducted for children whose parents did not report any eczema, wheezing, gastrointestinal infection or respiratory tract infection at age 2 months (n=6,502), to address potential reverse causation bias.

Additional sensitivity analyses involved exclusively formula-fed infants at age 2 months (n=6,251). Moreover, to deal with the issue of change in infant formula from birth to the 2-month interview, a sensitivity analysis involved children without any change in infant formula up to age 2 months (n=4,399). Finally, given our previous findings on the association between partially hydrolyzed formula and allergy (40), a sensitivity analysis was conducted among infants consuming non-hydrolyzed formula (n=7,805).

To deal with selection and attrition bias, a sensitivity analysis was conducted with weighted data according to the weighting described previously. Finally, as the main analyses used multiple imputation to deal with missing data, the main analyses were replicated on the complete-case sample.

All analyses were carried out with SAS 9.4 (SAS Institute, Cary, NC, USA).

## RESULTS

As compared with excluded children, included children frequently were the first born (46.6% vs 42.3%, p<0.0001), were born to older mothers (mean age 31.0 ± 4.9 vs 30.6 ± 5.2 years, p=0.00003), mothers who were employed during pregnancy (75.2% vs 67.3%, p<0.0001) and had a higher education level (40.8% vs. 35.9% with at least 3-year university degree, p<0.0001). The characteristics of included children are described in **Table 1**.

### Enrichment of formulas with prebiotics and probiotics

At the 2-month follow-up, more than half of formula-fed infants consumed probiotic-enriched formula. The enrichment with prebiotics usually using a mixture of GOS and FOS, concerned 9.1% (95% confidence interval=8.4%-9.8%) of infants.

The weighted prevalence of formula-fed infants consuming probiotic-enriched formula at the 2-month follow-up is presented in **Figure 2**. The main probiotics added to infant formula

were *Streptococcus thermophilus, Lactobacillus reuteri (DSM 17938),* and *Bifidobacterium breve (BC50),* followed by *Lactobacillus fermentum (CECT5716)* and *Bifidobacterium lactis (BB12)*. Enrichment with *S. thermophilus* was always associated with another enrichment (*B. breve, B. lactis* or *L. reuteri*). The combined enrichment with *Bifidobacterium infantis* and *Lactobacillus rhamnosus* was consumed by less than 3% of infants and was not further examined.

The longitudinal trajectories of consumption of formulas enriched with probiotics or prebiotics from age 2 to 10 months are presented in **Supplementary Figure 1**. For *B. breve* and *S. thermophilus*-enriched formula, a 4-group solution was identified with infants consuming 1) never-enriched formula, 2) enriched formula up to age 5-6 months, 3) enriched formula from age 6-7 months and 4) enriched formula throughout infancy. For *L. fermentum* and *L. reuteri*-enriched formula, a 3-group solution was identified with infants consuming 1) never-enriched formula, 2) occasionally-enriched formula and 3) enriched formula throughout infancy. For *B. lactis*-enriched formula, a 2-group solution was identified with infants consuming 1) never-enriched formula and 2) enriched formula throughout infancy. For GOS-enriched formulas, a 3-group solution was identified, with infants consuming 1) never-enriched formula, 2) early use of enriched formula and 3) delayed use of enriched formula.

**Probiotic-enriched formula and risk of infection or allergic diseases up to age 5.5 years**

The findings of unadjusted analyses are presented in **Supplemental tables 1 and 2**.

***Bifidobacterium enrichment***

In the main model, compared to infants consuming non-*Bifidobacterium*-enriched formula, those consuming *B. lactis*-enriched formula at age 2 months were at lower risk of LRTI and wheezing from age 2 months to 5.5 years (**Table 2**). We found no significant association between *Bifidobacterium* enrichment and URTI, asthma (**Table 2**), gastrointestinal infection,

food allergy or itchy rash up to age 5.5 years (**Table 3**). Further adjustment for antibiotic use up to age 1 year did not substantially modify the risk estimates, but the observed associations between consumption of *B. lactis*-enriched formula and wheezing were no longer significant (**data not shown**).

The observed associations for the consumption of *Bifidobacterium*-enriched formula and the risk of respiratory symptoms, gastrointestinal infection, food allergy and itchy rash were consistent in analyses among exclusively formula-fed infants (**Supplementary tables 3 and 4**), in weighted analyses and analyses in the complete-case sample (**Supplementary tables 5-8**). Similar findings were also observed in the other sensitivity analyses (analyses conducted in subsamples of infants without any symptoms up to 2 months, infants without any change in formula up to age 2 months (**Supplementary tables 9-12)**, and infants consuming non-hydrolyzed formula (**data not shown**).

According to the GBTM method, infants always consuming *B. lactis*-enriched formula from age 2 to 10 months were also at lower risk of URTI and asthma up to age 5.5 years (**Table 2**). Consumption from age 6 months of *B. breve*-enriched formula was related to a lower risk of LRTI and asthma up to age 5.5 years, whereas consumption in the first months only was related to a higher risk of LRTI and asthma up to age 5.5 years (**Table 2**). Similar findings were observed after further adjustment for antibiotic use (data not shown) and in sensitivity analyses (**Supplementary tables 3-12)**.

***Lactobacillus enrichment***

In the main model, compared to consumption of non-*Lactobacillus*-enriched formula, consumption of *L. reuteri*-enriched or *L. fermentum*-enriched formula at age 2 months was not associated with occurrence of infection or allergic diseases up to age 5.5 years (**Tables 2 and 3**). After further adjustment for antibiotic use, consumption of *L. reuteri*-enriched formula at

age 2 months was related to a lower risk of URTI up to age 5.5 years (OR=0.83 and 95% CI=0.72-0.97; data not shown).

According to the GBTM method, occasional, or regular consumption of *L. reuteri*-enriched formula from age 2 to 10 months was not associated with occurrence of infection or allergic diseases (**Tables 2 and 3**). Occasional consumption of *L. fermentum*-enriched formula from age 2 to 10 months was related to a higher risk of URTI up to age 5.5 years, but not regular consumption. Consumption of *L. fermentum*-enriched formula was not related to other infections or allergic diseases. Similar findings were observed after further adjustment for antibiotic use (data not shown) and in sensitivity analyses (**Supplementary tables 3-12)**.

***Streptococcus thermophilus enrichment***

In the main model, compared to consumption of non-*S. thermophilus*-enriched formula at age 2 months, consumption of *S. thermophilus*-enriched formula was not related to infections or allergic diseases up to age 5.5 years (**Tables 2 and 3**). Similar findings were observed after further adjustment for antibiotic use (data not shown).

According the GBTM method, consumption of *S. thermophilus*-enriched formula from age 2 to 10 months was related to a higher risk of URTI up to age 5.5 years. In addition, consumption of *S. thermophilus*-enriched formula up to age 6 months was related to a lower risk of asthma up to age 5.5 years, whereas consumption from 6 months was related to a higher risk of asthma (**Table 2**). Consumption of *S. thermophilus*-enriched formula was not related to other infections or allergic diseases (**Tables 2 and 3**). Similar findings were observed after further adjustment for antibiotic use (data not shown) and in sensitivity analyses (**Supplementary tables 3-12**).

**Prebiotic-enriched formula and risk of infection or allergic diseases up to age 5.5 years**

Consumption of prebiotic-enriched formula at age 2 months or between 2 and 10 months was not related to infection and allergic diseases, except for the association between early use of GOS-enriched formula and a lower risk of URTI (**Tables 2 and 3**). However, this association was no longer significant after further adjustment for antibiotic use up to age 1 year (data not shown). The observed findings for the consumption of prebiotic-enriched formula and the risk of infection and allergic diseases were similar in sensitivity analyses (**Supplementary tables 3-12**).

## DISCUSSION

Among children born in 2011, more than half of formula-fed infants had consumed probiotic-enriched formula at age 2 months. The infant formula consumed was often enriched with *L. reuteri (DSM 17938)* or *B. breve (BC50)* combined with *S. thermophilus*, followed by *L. fermentum (CECT5716)* and *B. lactis (BB12)* combined with *S. thermophilus*. Overall, the consumption of probiotic-enriched formula was related to the risk of respiratory diseases (respiratory tract infection, wheezing, asthma) up to age 5.5 years, but not with gastrointestinal infection, food allergy, and itchy rash. However, the observed associations for respiratory diseases were not consistent depending on the strains used for enrichment, as well as the consumption period of the formula. No significant association was found for the consumption of prebiotic-enriched formula.

As in our study, most RCTs highlighted null or beneficial effect of probiotic enrichment on respiratory diseases or respiratory infection-related outcomes (25, 29). However, when considering the strains, results are less constituent. Indeed, only one RCT including 80 healthy 6-month-old children in Spain showed a lower number of episodes of respiratory infections with consumption of formula enriched with *L. salivarius CECT5713*, compared with non-enriched formula (17). Another RCT involving 81 healthy infants in Finland who

consumed infant formula before age 2 months also showed a protective effect of formula enriched with *B. lactis BB12* combined with *L. rhamnosus* GG ATCC53103 on the incidence of recurrent respiratory infections during the first year of life (19). However, in this study, probiotics were not added to the formula during manufacturing, but rather as capsules (prebiotics/placebo) directly added to the formula. In addition, consumption of probiotic-enriched formula was not related to the incidence of respiratory infections.

In the ELFE study, consumption of probiotic-enriched formula was not associated with the risk of itchy rash and food allergy, which is consistent with the findings observed in other studies (29). The consumption of probiotic-enriched formula was also not associated with gastrointestinal infection in this study. Although some RCTs showed that consumption of probiotic-enriched formula (e.g. enrichment with *B. lactis BB12* alone or in combined with *S. thermophilus, L.s reuteri ATCC 55730* or *L. salivarius CECT5713*) had a protective effect on gastrointestinal infection outcomes (17, 18, 25), others examining the effect of formula enriched with *B. lactis BB12* combined with *L. rhamnosus GG ATCC53103, B. longum BL999* combined with *L. rhamnosus LPR,* or *B. breve C50* combined with *S. thermophilus* or with *L. johnsonii La1* found non-significant associations (19, 24, 25, 29).

In the present study, the effect of probiotic enrichment was evaluated for each probiotic strain, although some formulas were enriched with a combination of strains. This methodological approach does not consider the synergistic effect of probiotics strains; however, the different strains studied were adjusted for each other in all models. Interactions between strains were also tested and were not significant in any model.

Overall, studies that evaluated the effect of probiotic-enriched formula on childhood health showed inconsistent findings. This observation could be explained by the considerable heterogeneity in the RCT designs. Indeed, the types of probiotics and strains as well as the combinations used differed across studies, while the different probiotics strains have specific

properties. The measured outcomes and their definitions, duration and timing of intervention also differ across studies. Further studies with a long follow-up, large sample size and using similar methodology (same inclusion criteria, duration and timing of intervention, probiotics strains, combinations and dose used) are needed to establish the effects of probiotic-enriched formula on childhood health.

Regarding the effect of prebiotics-enriched formula consumption on infection and allergy in childhood, available studies are limited and the results are inconclusive (20-23, 26, 27, 29). We found no significant association between prebiotic-enriched formula consumption and the risk of gastrointestinal infection, respiratory symptoms and allergic diseases, consistent with the findings observed in some RCTs (20, 21, 23). In contrast, one RCT involving healthy term infants in Italy, from parents with a history of allergy (atopic eczema, allergic rhinitis, or asthma) found lower number of episodes of URTI during the 2-year follow-up among infants consuming hypoallergenic formula enriched with a mixture of short-chain GOS and long-chain FOS than those consuming the same formula without enrichment (21). In addition, one RCT found a significantly lower number of children with at least 1 episode of acute diarrhoea and lower number of diarrheal episodes during the 12-month follow-up in the group receiving GOS/FOS-enriched formula as compared with the control group (20), and another found that the cumulative incidences of allergic manifestations (atopic dermatitis, recurrent wheezing, and allergic urticaria) were lower in the intervention group, than in the control group (21).

To our knowledge, no other longitudinal cohort has assessed the association between probiotic or prebiotic formula enrichment and infection/allergic manifestations, which hinders international comparison of our results.

The present prospective study involved children included in a large French birth cohort with detailed data on infant diet, which allowed us to examine among formula-fed infants and

368 under real conditions of use, the effect of formula enrichment with probiotics or prebiotics on
369 infection and allergic diseases in childhood. In this cohort, the number of infants exposed to
370 probiotic-enriched formula was higher than that observed in the trials (mostly small sample
371 sizes). This high proportion could possibly be attributable to popular beliefs about the health
372 benefits of probiotics. However, a previous study using the ELFE cohort data showed few
373 social and health-related factors associated with the use of prebiotic- or probiotic-enriched
374 formula (41), which suggests that reverse causality bias is limited in the present study. Indeed,
375 the authors found that only family income and breastfeeding duration were related to the use
376 of prebiotic- or probiotic-enriched formula at age 2 months and the type of infant formula
377 used was fairly stable between 2 and 10 months.

378 Although a wide range of socio-demographic and economic data was considered in the
379 present study, other unmeasured factors such as dietary supplements containing probiotics or
380 prebiotics consumed by mothers or infants and intake of prebiotics from complementary
381 foods might have led to potential residual confounding. In this cohort, health data were
382 reported by parents and not validated by medical records, which could lead to a potential
383 measurement bias. However, the items used were derived from international ones (42) to limit
384 this bias. In addition, repeated data on health-related outcomes in childhood limited memory
385 bias. The various sensitivity analyses performed to examine the robustness of our results and
386 address selection and reverse causation bias showed very similar findings, which suggests that
387 if there were any potential biases, they should have a limited impact on our findings.

388 In conclusion, this observational study showed that probiotic-enriched formulas are often
389 consumed, but few show a real health benefit. The consumption of prebiotic-enriched formula
390 does not have convincing effects on health. Replication in other observational studies and
391 larger well-designed RCTs are needed to confirm these results and possibly identify the most

interesting strains and optimal “window of opportunity” for prebiotic and probiotic-enriched formula consumption.

**Acknowledgments**:

We thank the scientific coordinators (B Geay, H Léridon, C Bois, JL Lanoé, X Thierry, C Zaros), IT and data managers, statisticians (M Cheminat, C Ricourt, A Candea, S de Visme), administrative and family communication staff, study technicians (C Guevel, M Zoubiri, L G L Gravier, I, Milan, R Popa) of the ELFE coordination team, as well as the families that gave their time for the study.

**Author’s contributions**: CR, M-ND, M-A C, SL and BL-G were responsible for the development of the design and protocol of the study; MA, CD-P and BL-G performed the statistical analyses; MA wrote the paper; BLG provided methodological guidance; MA, CD-P, AD-C, KA-P, CR, M-ND, SL, M-AC and BL-G were involved in interpreting the results and editing the manuscript for important intellectual content. All authors read and approved the final manuscript.

**References**


1. Mortensen MS, Rasmussen MA, Stokholm J, Brejnrod AD, Balle C, Thorsen J, Krogfelt KA, Bisgaard H, Sørensen SJ. Modeling transfer of vaginal microbiota from mother to infant in early life. Elife 2021;10. doi: 10.7554/eLife.57051.
2. van Best N, Hornef MW, Savelkoul PH, Penders J. On the origin of species: Factors shaping the establishment of infant's gut microbiota. Birth Defects Res C Embryo Today 2015;105(4):240-51. doi: 10.1002/bdrc.21113.
3. Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, Ross MC, Lloyd RE, Doddapaneni H, Metcalf GA, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature 2018;562(7728):583-8. doi: 10.1038/s41586-018-0617-x.
4. Cukrowska B, Bierła JB, Zakrzewska M, Klukowski M, Maciorkowska E. The Relationship between the Infant Gut Microbiota and Allergy. The Role of Bifidobacterium breve and Prebiotic Oligosaccharides in the Activation of Anti-Allergic Mechanisms in Early Life. Nutrients 2020;12(4). doi: 10.3390/nu12040946.
5. Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, Adisetiyo H, Zabih S, Lincez PJ, Bittinger K, et al. Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. JAMA Pediatr 2017;171(7):647-54. doi: 10.1001/jamapediatrics.2017.0378.
6. Thompson AL, Monteagudo-Mera A, Cadenas MB, Lampl ML, Azcarate-Peril MA. Milk- and solid-feeding practices and daycare attendance are associated with differences in bacterial diversity, predominant communities, and metabolic and immune function of the infant gut microbiome. Front Cell Infect Microbiol 2015;5:3. doi: 10.3389/fcimb.2015.00003.
7. Moore RE, Townsend SD. Temporal development of the infant gut microbiome. Open Biol 2019;9(9):190128. doi: 10.1098/rsob.190128.
8. WHO. Feeding and nutrition of infants and young children, guidelines for the WHO European region, with emphasis on the former Soviet countries. Geneva, 2003.
9. Richard C, Lewis ED, Field CJ. Evidence for the essentiality of arachidonic and docosahexaenoic acid in the postnatal maternal and infant diet for the development of the infant’s immune system early in life. Applied Physiology, Nutrition, and Metabolism 2016;41(5):461-75. doi: 10.1139/apnm-2015-0660.
10. Le Doare K, Holder B, Bassett A, Pannaraj PS. Mother's Milk: A Purposeful Contribution to the Development of the Infant Microbiota and Immunity. Front Immunol 2018;9:361. doi: 10.3389/fimmu.2018.00361.
11. FAO/WHO. Probiotics in food: Health and nutrition properties of probiotics in food including powder milk with live lactic acid bacteria and guidelines for the evaluation. Rome, Ilaly: FAO food and nutrition paper, 2006.

12. Victora CG, Bahl R, Barros AJD, França GVA, Horton S, Krasevec J, Murch S, Sankar MJ, Walker N, Rollins NC. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. The Lancet 2016;387(10017):475-90. doi: 10.1016/s0140-6736(15)01024-7.

13. Schiess S, Grote V, Scaglioni S, Luque V, Martin F, Stolarczyk A, Vecchi F, Koletzko B. Introduction of complementary feeding in 5 European countries. J Pediatr Gastroenterol Nutr 2010;50(1):92-8. doi: 10.1097/MPG.0b013e31819f1ddc.

14. Cai X, Wardlaw T, Brown DW. Global trends in exclusive breastfeeding. Int Breastfeed J 2012;7(1):12. doi: 10.1186/1746-4358-7-12.

15. Vandenplas Y, De Greef E, Veereman G. Prebiotics in infant formula. Gut Microbes 2014;5(6):681-7. doi: 10.4161/19490976.2014.972237.

16. Federik M. Use of probiotic, prebiotic and symbiotic in infant formulas. J Nutr Hum Health 2019 2019;3(1):12-8.

17. Maldonado J, Lara-Villoslada F, Sierra S, Sempere L, Gómez M, Rodriguez JM, Boza J, Xaus J, Olivares M. Safety and tolerance of the human milk probiotic strain Lactobacillus salivarius CECT5713 in 6-month-old children. Nutrition 2010;26(11-12):1082-7. doi: 10.1016/j.nut.2009.08.023.

18. Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. Pediatrics 2005;115(1):5-9. doi: 10.1542/peds.2004-1815.

19. Rautava S, Salminen S, Isolauri E. Specific probiotics in reducing the risk of acute infections in infancy--a randomised, double-blind, placebo-controlled study. Br J Nutr 2009;101(11):1722-6. doi: 10.1017/s0007114508116282.

20. Bruzzese E, Volpicelli M, Squeglia V, Bruzzese D, Salvini F, Bisceglia M, Lionetti P, Cinquetti M, Iacono G, Amarri S, et al. A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: an observational study. Clin Nutr 2009;28(2):156-61. doi: 10.1016/j.clnu.2009.01.008.

21. Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. J Nutr 2008;138(6):1091-5. doi: 10.1093/jn/138.6.1091.

22. Cuello-Garcia C, Fiocchi A, Pawankar R, Yepes-Nuñez JJ, Morgano GP, Zhang Y, Agarwal A, Gandhi S, Terracciano L, Schünemann HJ, et al. Prebiotics for the prevention of allergies: A systematic review and meta-analysis of randomized controlled trials. Clin Exp Allergy 2017;47(11):1468-77. doi: 10.1111/cea.13042.

23. Sierra C, Bernal MJ, Blasco J, Martínez R, Dalmau J, Ortuño I, Espín B, Vasallo MI, Gil D, Vidal ML, et al. Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: a multicentre, randomised, double-blind and placebo-controlled trial. Eur J Nutr 2015;54(1):89-99. doi: 10.1007/s00394-014-0689-9.

24. Thibault H, Aubert-Jacquin C, Goulet O. Effects of long-term consumption of a fermented infant formula (with Bifidobacterium breve c50 and Streptococcus thermophilus 065) on acute diarrhea in healthy infants. J Pediatr Gastroenterol Nutr 2004;39(2):147-52. doi: 10.1097/00005176-200408000-00004.

25. Skórka A, Pieścik-Lech M, Kołodziej M, Szajewska H. To add or not to add probiotics to infant formulae? An updated systematic review. Benef Microbes 2017;8(5):717-25. doi: 10.3920/bm2016.0233.

26. Skórka A, Pieścik-Lech M, Kołodziej M, Szajewska H. Infant formulae supplemented with prebiotics: Are they better than unsupplemented formulae? An updated systematic review. Br J Nutr 2018;119(7):810-25. doi: 10.1017/s0007114518000120.

27. Mugambi MN, Musekiwa A, Lombard M, Young T, Blaauw R. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. Nutr J 2012;11:81. doi: 10.1186/1475-2891-11-81.

28. de Silva D, Halken S, Singh C, Muraro A, Angier E, Arasi S, Arshad H, Beyer K, Boyle R, du Toit G, et al. Preventing food allergy in infancy and childhood: Systematic review of randomised controlled trials. Pediatr Allergy Immunol 2020;31(7):813-26. doi: 10.1111/pai.13273.

29. Braegger C, Chmielewska A, Decsi T, Kolacek S, Mihatsch W, Moreno L, Pieścik M, Puntis J, Shamir R, Szajewska H, et al. Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr 2011;52(2):238-50. doi: 10.1097/MPG.0b013e3181fb9e80.

30. Charles MA, Thierry X, Lanoe JL, Bois C, Dufourg MN, Popa R, Cheminat M, Zaros C, Geay B. Cohort Profile: The French national cohort of children (ELFE): birth to 5 years. Int J Epidemiol 2020;49(2):368-9j. doi: 10.1093/ije/dyz227.

31. Bianchi CM, Mariotti F, Verger EO, Huneau JF. Pregnancy Requires Major Changes in the Quality of the Diet for Nutritional Adequacy: Simulations in the French and the United States Populations. PLoS One 2016;11(3):e0149858. doi: 10.1371/journal.pone.0149858.

32. Mamelle N, Munoz F, Grandjean H. [Fetal growth from the AUDIPOG study. I. Establishment of reference curves]. J Gynécologie Obstétrique Biol Reprod 1996;25(1):61-70.

33. Juillard H. Weighting of Elfe survey data at time 0. pandora.vjf.inserm.fr/public/, 2015.

34. Blondel B, Lelong N, Kermarrec M, Goffinet F, National Coordination Group of the National Perinatal Surveys. Trends in perinatal health in France from 1995 to 2010. Results from the French National Perinatal Surveys. J Gynecol Obstet Biol Reprod (Paris) 2012;41(4):e1-e15. doi: 10.1016/j.jgyn.2012.04.014.

35. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, Wood AM, Carpenter JR. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. Bmj 2009;338:b2393. doi: 10.1136/bmj.b2393.

36. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. Stat Methods Med Res 2007;16(3):219-42. doi: 10.1177/0962280206074463.

37. Briggs A, Clark T, Wolstenholme J, Clarke P. Missing... presumed at random: cost-analysis of incomplete data. Health Econ 2003;12(5):377-92. doi: 10.1002/hec.766.

38. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. BMC Med Res Methodol 2008;8:70. doi: 10.1186/1471-2288-8-70.

39. Nagin D. Group-Based Modeling of Development. Cambridge, MA: Harvard Univ. Press, 2005.

40. Davisse-Paturet C, Raherison C, Adel-Patient K, Divaret-Chauveau A, Bois C, Dufourg MN, Lioret S, Charles MA, de Lauzon-Guillain B. Use of partially hydrolysed formula in infancy and incidence of eczema, respiratory symptoms or food allergies in toddlers from the ELFE cohort. Pediatr Allergy Immunol 2019;30(6):614-23. doi: 10.1111/pai.13094.

41. de Lauzon-Guillain B, Davisse-Paturet C, Lioret S, Ksiazek E, Bois C, Dufourg MN, Bournez M, Nicklaus S, Wagner S, Charles MA. Use of infant formula in the ELFE study: The association with social and health-related factors. Matern Child Nutr 2018;14(1). doi: 10.1111/mcn.12477.

42. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 2006;368(9537):733-43. doi: 10.1016/s0140-6736(06)69283-0.

**Table 1** Characteristics of children and families included in the main analyses (n=8,389)

| | Missing values (n) | % (n) or mean (SD) |
|---|---|---|
| **Family characteristics** | | |
| **Maternal age at delivery (years)** | 0 | 31 (4.9) |
| **Maternal education level** | 294 | |
| Up to lower secondary | | 3.4% (279) |
| Upper secondary | | 36% (2,911) |
| Intermediate | | 24.7% (1,999) |
| 3-year university degree | | 17.4% (1,411) |
| At least 5-year university degree | | 18.5% (1,495) |
| **Maternal employement during pregnancy** | 2 | |
| Employed | | 75.2% (6,306) |
| Unemployed | | 11.3% (949) |
| Out the labor force | | 13.5% (1,132) |
| **Household income (€/month/consumption unit)** | 247 | 1661.2 (974.8) |
| **Residence in rural area** | 2 | 23.3% (1,951) |
| **Smoking during pregnancy** | 100 | |
| Never smoker | | 54.3% (4,503) |
| Smoker only before pregnancy | | 24.7% (2,047) |
| Smoker only in early pregnancy | | 4.1% (336) |
| Smoker throughout pregnancy | | 16.9% (1,403) |
| **Maternal pre-pregnancy BMI (kg/m2)** | 127 | 23.7 (5) |
| **Children in household** | 0 | |
| No sibling | | 46.6% (3,907) |
| One sibling | | 36.2% (3,040) |
| At least 2 siblings | | 17.2% (1,442) |
| **Maternal diet quality during pregnancy (PANDiet 0-100 score)** | 978 | 54.9 (9) |
| **Child characteristics** | | |
| **Boys** | 0 | 51% (4,282) |
| **Gestational age (weeks)** | 132 | 39.2 (1.5) |
| **Child birth weight** | 241 | |
| Small weight for gestational age | | 10% (811) |
| Adequate weight for gestational age | | 80.1% (6,528) |
| Large weight for gestational age | | 9.9% (809) |
| **Age at infant formula introduction (months)** | 3 | 0.4 (0.5) |
| **Ever infection from age 2 months to 5.5 years** | | |
| Gastrointestinal infection | 0 | 13.4% (1,122) |
| Upper respiratory tract infection | 0 | 32.4% (2,716) |
| Lower respiratory tract infection | 0 | 37.8% (3,172) |
| **Ever allergic diseases from age 2 months to 5.5 years** | | |
| Wheezing | 0 | 42.4% (3,556) |
| Medical diagnosis of asthma | 0 | 14.1% (1,179) |
| Itchy rash | 0 | 45.9% (3,849) |
| Food allergy | 0 | 6.3% (525) |

*BMI* body mass index; *PANDiet* Probability of Adequate Nutrient intake-based Diet quality index

**Table 2** Adjusted associations between consumption of enriched formula at 2 months and from 2 to 10 months and the risk of respiratory diseases up to 5.5 years (n=8,389)

| | *n* | **Upper respiratory tract infection** | | **Lower respiratory tract infection** | | **Wheezing** | | **Asthma** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **OR [95% CI]** | **P** | **OR [95% CI]** | **P** | **OR [95% CI]** | **P** | **OR [95% CI]** | **P** |
| **Consumption at 2 months** | | | | | | | | | |
| **Enrichment in Bifidobacterium** | | | 0.1 | | 0.03 | | 0.2 | | 0.3 |
| No Bifidobacterium | 5,805 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| *Bifidobacterium breve (BC50)* | 1,372 | 1.19 [1.00; 1.41] | | 1.09 [0.93; 1.29] | | 1.06 [0.90; 1.24] | | 0.97 [0.77; 1.21] | |
| *Bifidobacterium lactis (BB12)* | 732 | 0.95 [0.82; 1.10] | | 0.84 [0.73; 0.96] | | 0.87 [0.75; 0.99] | | 0.83 [0.68; 1.01] | |
| Other Bifidobacterium or unspecified strain | 480 | 0.87 [0.71; 1.08] | | 1.15 [0.95; 1.40] | | 1.10 [0.91; 1.34] | | 1.25 [0.96; 1.62] | |
| **Enrichment in Lactobacillus** | | | 0.2 | | 0.5 | | 0.8 | | 0.1 |
| No Lactobacillus | 6,043 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| *Lactobacillus reuteri (DSM 17938)* | 1,679 | 0.86 [0.75; 1.00] | | 1.09 [0.95; 1.25] | | 1.00 [0.87; 1.14] | | 0.85 [0.70; 1.02] | |
| *Lactobacillus fermentum (CECT5716)* | 480 | 1.04 [0.87; 1.24] | | 0.96 [0.81; 1.15] | | 1.08 [0.91; 1.28] | | 1.19 [0.95; 1.50] | |
| Other Lactobacillus or unspecified strain | 187 | 1.18 [0.87; 1.60] | | 0.89 [0.67; 1.20] | | 0.95 [0.71; 1.26] | | 1.06 [0.72; 1.55] | |
| **Enrichment in Streptococcus** | | | 0.5 | | 0.6 | | 0.3 | | 0.3 |
| No Streptococcus | 6,295 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| *Streptococcus thermophilus* | 2,094 | 0.96 [0.86; 1.07] | | 0.97 [0.87; 1.08] | | 1.06 [0.95; 1.17] | | 1.08 [0.94; 1.26] | |
| **Enrichment in prebiotics** | | | 0.6 | | 0.9 | | 0.5 | | 0.7 |
| No prebiotics | 7,456 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| FOS/GOS or GOS only | 933 | 0.98 [0.91; 1.06] | | 1.00 [0.93; 1.08] | | 1.03 [0.96; 1.11] | | 0.98 [0.89; 1.09] | |
| **Consumption between 2 and 10 months** | | | | | | | | | |
| **Enrichment in *B. breve (BC50)*** | | | 0.4 | | 0.002 | | 0.4 | | 0.003 |
| Never | 6,053 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Up to 6 months | 284 | 1.16 [0.85; 1.60] | | 1.75 [1.29; 2.38] | | 1.28 [0.96; 1.72] | | 1.95 [1.28; 2.97] | |
| From 6 months | 748 | 1.01 [0.74; 1.40] | | 0.64 [0.48; 0.86] | | 0.90 [0.67; 1.20] | | 0.59 [0.40; 0.88] | |
| Always | 1,304 | 0.79 [0.62; 1.02] | | 0.90 [0.70; 1.15] | | 0.90 [0.71; 1.15] | | 0.74 [0.52; 1.05] | |
| **Enrichment in *B. lactis (BB12)*** | | | 0.01 | | 0.2 | | 0.1 | | 0.04 |
| Never | 7,598 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Always | 791 | 0.80 [0.68; 0.95] | | 0.89 [0.76; 1.05] | | 0.89 [0.76; 1.04] | | 0.78 [0.62; 0.99] | |
| **Enrichment in *L. fermentum (CECT5716)*** | | | 0.05 | | 0.3 | | 0.03 | | 0.3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Never | 7,717 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Sometimes | 329 | 1.21 [1.02; 1.44] | | 1.13 [0.96; 1.34] | | 1.13 [0.95; 1.34] | | 1.15 [0.92; 1.44] | |
| Always | 343 | 0.92 [0.78; 1.10] | | 0.90 [0.76; 1.06] | | 1.03 [0.87; 1.22] | | 0.95 [0.75; 1.20] | |
| **Enrichment in *L. reuteri (DSM 17938)*** | | | 0.2 | | 0.5 | | 0.9 | | 0.6 |
| Never | 6,468 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Sometimes | 590 | 1.00 [0.88; 1.14] | | 1.07 [0.94; 1.22] | | 1.02 [0.90; 1.15] | | 0.98 [0.82; 1.17] | |
| Always | 1,331 | 0.94 [0.84; 1.05] | | 0.94 [0.85; 1.05] | | 1.00 [0.90; 1.10] | | 0.97 [0.84; 1.12] | |
| **Enrichment in *S. thermophilus*** | | | 0.08 | | 0.4 | | 0.8 | | 0.003 |
| Never | 5,218 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Up to 6 months | 471 | 0.97 [0.76; 1.25] | | 0.81 [0.64; 1.03] | | 0.97 [0.77; 1.22] | | 0.66 [0.47; 0.94] | |
| From 6 months | 812 | 0.81 [0.61; 1.09] | | 1.13 [0.86; 1.50] | | 0.99 [0.75; 1.29] | | 1.84 [1.29; 2.63] | |
| Always | 1,888 | 1.34 [1.06; 1.69] | | 1.10 [0.88; 1.37] | | 1.10 [0.89; 1.36] | | 1.15 [0.85; 1.57] | |
| **Enrichment in GOS** | | | 0.1 | | 0.5 | | 0.2 | | 0.3 |
| Never | 7,205 | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Early use | 487 | 0.87 [0.76; 0.99] | | 0.96 [0.85; 1.10] | | 1.08 [0.95; 1.22] | | 1.06 [0.88; 1.28] | |
| Delayed use | 697 | 1.13 [0.97; 1.32] | | 0.99 [0.84; 1.16] | | 0.87 [0.75; 1.01] | | 0.88 [0.69; 1.12] | |

*CI* confidence interval; *FOS* fructo-oligosaccharides; *GOS* galacto-oligosaccharides; *OR* odd ratio

Logistic regressions adjusted for maternal and household characteristics (maternal age, education level, migration status, employment during pregnancy, smoking status, pre-pregnancy BMI, diet quality during pregnancy, city size, region of residence, household income, family history of allergy and number of older children), child characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge, C-section delivery and age at infant formula introduction) and ELFE study design variables (maternity size and recruitment wave). For a given period, all enrichments were considered simultaneously.

**Table 3** Associations between consumption of enriched formula at 2 months and from 2 to 10 months and the risk of gastrointestinal infections, food allergies or itchy rash up to 5.5 years (n=8,389)

| | **Gastrointestinal infection** | | **Food allergy** | | **Itchy rash** | |
|---|---|---|---|---|---|---|
| | **OR [95% CI]** | **P** | **OR [95% CI]** | **P** | **OR [95% CI]** | **P** |
| **Consumption at 2 months** | | | | | | |
| **Enrichment in Bifidobacterium** | | 0.3 | | 0.4 | | 0.7 |
| No Bifidobacterium | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| *Bifidobacterium breve (BC50)* | 0.93 [0.75; 1.17] | | 0.87 [0.64; 1.18] | | 1.07 [0.91; 1.25] | |
| *Bifidobacterium lactis (BB12)* | 0.92 [0.76; 1.12] | | 0.91 [0.68; 1.21] | | 1.00 [0.88; 1.15] | |
| Other Bifidobacterium or unspecified strain | 1.27 [0.98; 1.64] | | 1.01 [0.67; 1.52] | | 1.00 [0.82; 1.21] | |
| **Enrichment in Lactobacillus** | | 0.5 | | 0.2 | | 0.8 |
| No Lactobacillus | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| *Lactobacillus reuteri (DSM 17938)* | 1.15 [0.95; 1.39] | | 0.78 [0.59; 1.02] | | 0.95 [0.83; 1.08] | |
| *Lactobacillus fermentum (CECT5716)* | 0.94 [0.72; 1.21] | | 0.79 [0.55; 1.12] | | 1.00 [0.84; 1.19] | |
| Other Lactobacillus or unspecified strain | 0.87 [0.58; 1.29] | | 1.74 [1.01; 3.01] | | 1.11 [0.83; 1.47] | |
| **Enrichment in Streptococcus** | | 0.9 | | 0.06 | | 0.3 |
| No Streptococcus | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| *Streptococcus thermophilus* | 1.01 [0.88; 1.17] | | 1.21 [0.99; 1.49] | | 0.94 [0.85; 1.04] | |
| **Enrichment in prebiotics** | | 0.3 | | 0.1 | | 0.5 |
| No prebiotics | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| FOS/GOS or GOS only | 1.05 [0.95; 1.17] | | 0.89 [0.76; 1.04] | | 0.98 [0.91; 1.05] | |
| **Consumption between 2 and 10 months** | | | | | | |
| **Enrichment in *B. breve (BC50)*** | | 0.6 | | 0.7 | | 0.2 |
| Never | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Up to 6 months | 1.21 [0.78; 1.88] | | 1.20 [0.69; 2.08] | | 1.29 [0.96; 1.73] | |
| From 6 months | 1.06 [0.70; 1.62] | | 1.02 [0.56; 1.87] | | 0.91 [0.69; 1.20] | |
| Always | 0.87 [0.62; 1.24] | | 1.00 [0.62; 1.63] | | 0.84 [0.66; 1.06] | |
| **Enrichment in *B. lactis (BB12)*** | | 0.8 | | 0.6 | | 0.5 |
| Never | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Always | 1.03 [0.82; 1.28] | | 1.08 [0.81; 1.44] | | 0.95 [0.81; 1.10] | |
| **Enrichment in *L. fermentum (CECT5716)*** | | 0.5 | | 0.1 | | 0.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Never | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Sometimes | 1.14 [0.89; 1.46] | | 0.79 [0.54; 1.16] | | 0.98 [0.83; 1.16] | |
| Always | 0.86 [0.67; 1.11] | | 1.00 [0.69; 1.44] | | 1.03 [0.87; 1.21] | |
| **Enrichment in *L. reuteri (DSM 17938)*** | | 0.7 | | 0.1 | | 0.2 |
| Never | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Sometimes | 1.08 [0.90; 1.30] | | 1.15 [0.90; 1.47] | | 1.02 [0.90; 1.16] | |
| Always | 0.96 [0.83; 1.13] | | 0.82 [0.66; 1.01] | | 0.93 [0.84; 1.03] | |
| **Enrichment in *S. thermophilus*** | | 0.5 | | 0.4 | | 0.3 |
| Never | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Up to 6 months | 0.84 [0.59; 1.20] | | 1.27 [0.83; 1.96] | | 0.83 [0.66; 1.04] | |
| From 6 months | 0.89 [0.60; 1.33] | | 0.73 [0.41; 1.31] | | 1.08 [0.83; 1.41] | |
| Always | 1.17 [0.86; 1.59] | | 0.82 [0.54; 1.26] | | 1.14 [0.92; 1.40] | |
| **Enrichment in GOS** | | 0.1 | | 0.2 | | 0.6 |
| Never | 1.00 [Ref] | | 1.00 [Ref] | | 1.00 [Ref] | |
| Early use | 1.18 [0.99; 1.42] | | 0.83 [0.62; 1.10] | | 0.95 [0.83; 1.07] | |
| Delayed use | 0.80 [0.63; 1.02] | | 1.03 [0.72; 1.49] | | 1.08 [0.93; 1.26] | |

*CI* confidence interval; *FOS* fructo-oligosaccharides; *GOS* galacto-oligosaccharides; *OR* odd ratio

Logistic regressions adjusted for maternal and household characteristics (maternal age, education level, migration status, employment during pregnancy, smoking status, pre-pregnancy BMI, diet quality during pregnancy, city size, region of residence, household income, family history of allergy and number of older children), child characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge, C-section delivery and age at infant formula introduction) and ELFE study design variables (maternity size and recruitment wave). For a given period, all enrichments were considered simultaneously.