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Association between diet and fecal microbiota along the first year of life

María Gómez-Martín [a,b], Silvia Saturio [b,c], Silvia Arboleya [b,c], David Herrero-Morín [d], Margot Calzón [e], Teresa López [e], Sonia González [a,b,*], Miguel Gueimonde [b,c]

[a] Department of Functional Biology, Faculty of Medicine, University of Oviedo, 33006 Oviedo, Asturias, Spain
[b] Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), 33011 Oviedo, Asturias, Spain
[c] Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), 33300 Villaviciosa, Asturias, Spain
[d] Pediatrics Service, Centro Atención Primaria Infiesto, SESPA, 33530 Piloña, Asturias, Spain
[e] Primary health care, Área sanitaria V, 33204 Gijón, Asturias, Spain

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ABSTRACT

Extensive work has established the importance of the gut microbiota during the first years of life. However, there are few longitudinal studies describing the role of infants' diet on the evolution of the fecal microbiota and their metabolic activity during this stage. The aim of this work was to explore the impact of diet on the composition of the major intestinal microorganisms and their main microbial metabolites from birth to 12 months. This is a longitudinal prospective study of diet and fecal microbiota. Bacterial groups levels were determined by qPCR and short-chain fatty acids (SCFAs) concentrations by gas chromatography. Information from self-administered questionnaires about general characteristics and food frequency were obtained from a cohort of 83, Spanish and full-term, infants at 15, 90, 180 and 365 days of age. Results revealed that Enterobacteriaceae decrease in weaning period contrary to *Bacteroides* group and *Clostridium* cluster IV.

Conclusion: our study supports weaning period as a key step for gut microbiota transition and suggests the importance of the consumption of dietary fiber with the increase of certain bacterial groups as *Clostridium* cluster IV, which could be beneficial for the host. Finally, studies specially designed to analyze the production and the excretion of SCFAs in children are needed to understand how diet could influence in this process.

1. Introduction

The human intestinal tract is the portion of the organism most densely populated by microorganisms that, under normal conditions, have a mutualistic relationship with the host (Thursby & Juge, 2017). In contrast to adulthood, with a relatively stable and diverse microbial ecosystem, in early life the composition of the microbiota is less diversified, with a high proportion of some genus such as *Bifidobacterium* until weaning (Saturio et al., 2021). Newborn digestive tract colonization begins with the delivery process and continues evolving in parallel with the maturation of the gastrointestinal tract and childhood development (Edwards & Parrett, 2002; Grölund, Lehtonen, Eerola, & Kero, 1999; Salminen, 2004). The first intestinal colonizers are facultative anaerobes and aerotolerant microorganisms from the phyla Pseudomonadota and Bacillota (former Proteobacteria and Firmicutes, respectively). These microorganisms degrade the existing oxygen favoring the subsequent development of strict anaerobic bacteria such as *Bacteroides*, *Bifidobacterium* or *Clostridium* (Bäckhed et al., 2015; Gotoh et al., 2018). Disturbances in the early gut microbiota, referred to as dysbiosis, have been linked to increased risk of altered immune responses and the development of immune-related pathologies, such as asthma or allergy, and metabolic disorders, including obesity or diabetes (Bäckhed et al., 2015; Gotoh et al., 2018). It has been suggested that this initial colonization is also associated with adult microbial composition, and subsequently with the development of several pathologies later in life (Eggesbø, Botten, Stigum, Nafstad, & Magnus, 2003; Goulet, 2015; Huh et al., 2012; Sevelsted, Stokholm, Bønnelykke, & Bisgaard, 2015).

Several environmental factors such as the type of delivery, the infant feeding mode, the use of antibiotics, pre- and probiotic consumption, or the maternal diet have a significant influence on the infant's gut colonization (Fouhy, Ross, Fitzgerald, Stanton, & Cotter, 2012). Among them, infants' diet plays a key role in microbiota modulation. Extensive

* Corresponding author at: Department of Functional Biology, Faculty of Medicine, University of Oviedo, 33006 Oviedo, Asturias, Spain.
E-mail addresses: gomezmarmaria@uniovi.es (M. Gómez-Martín), silvia.saturio@ipla.csic.es (S. Saturio), silvia.arboleya@ipla.csic.es (S. Arboleya), herrerojose@uniovi.es (D. Herrero-Morín), margotcalzon@gmail.com (M. Calzón), soniagsolares@uniovi.es, soniagsolares@uniovi.es (S. González), mgueimonde@ipla.csic.es (M. Gueimonde).

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research supports the World Health Organization (WHO) recommendation on the importance of exclusively breastfeeding up to 6 months, and to 1 year of age, as the gold standard approach that provides all the nutritional and growth requirements (World Health Organization (WHO), 2011). In addition to the nutritional components, human milk is a source of bioactive compounds (such as human milk oligosaccharides (HMOs)) and microorganisms that are transferred from the mother to the infant (van den Elsen & Verhasselt, 2021). Some studies have shown an increased concentration of bifidobacteria and decreased in *Bacteroides* in breastfed infants' gut compared to those receiving starter formulas (Fallani et al., 2011; Lind, Larnkjær, Mølgaard, & Michaelsen, 2018), which is consistent with the bifidogenic effect described for HMOs (van Esch et al., 2020). Thereafter, complementary feeding (the introduction of solid food between 17 and 26 weeks of age) (Agostoni et al., 2008) also influences on the infant's microbiota although its effects on microbiota development are not yet fully understood (Koenig et al., 2011). Recent research has revealed the existence of crucial time points at which the introduction of certain foods had a substantial impact on microbiota composition (Koenig et al., 2011). In a general way, it has been observed that *Bifidobacterium* is the most abundant genus in the gut until 6 months of age (Endo, Pärtty, Kalliomäki, Isolauri, & Salminen, 2014) and then the progressive incorporation of solid foods up to 12 months increases the concentrations of Bacteroidota and Bacillota. More specifically, at around 170–190 days of life the introduction of formula and peas to the diet of exclusive breastfed babies produced an increase in Bacteroidota (Koenig et al., 2011). These changes in the bacterial ecosystem result in increased concentrations of short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate acids, as a byproduct of the microbial catabolism of carbohydrates (Norin, Midtvedt, & Björkstén, 2004).

Based on this evidence, a deeper understanding of these interactions may lead to new dietary strategies for early microbiota modulation and deserves further exploration. Therefore, the purpose of this study is to evaluate the impact of the feeding received during the first year of life on the composition of the major intestinal microorganisms and their main microbial metabolites, with special emphasis on the progressive introduction of complementary diet.

2. Subjects and methods

2.1. Sample recruitment and study design

The analyses present in this work have been carried out in a subcohort from the longitudinal prospective project Early-MicroHealth "Impact of early life diet on microbiome development and later health". The sample population consisted on 83 full-term (37–40 gestational weeks) neonates of which samples from 79 infants were available at 15 days of age, from 83 at 60–90 days, from 78 at 180 days and from 68 at 365 days of life (Fig. 1). Newborns were recruited through the Primary Care Pediatrics Service at the first consultation visit, at 10–15 days after birth. Inclusion criteria was to be born healthy without any condition that would compromise the regular intake or involved the administration of drugs or special cares. In all cases, written informed consent was obtained prior to enrolment. Parents were notified in writing of the contents, procedures and objectives of the study, as well as the option to withdraw from the study at any time. The study has been evaluated and approved by the Regional Ethics Committee of Clinical Research of Asturias (Ref. 12/16, 03/02/2016) and to the Committee on Bioethics of CSIC (Ref. PCIN-2015–233). The procedures have been performed in accordance with the fundamental principles set out in the Declaration of Helsinki, the Oviedo Bioethics Convention, the Council of Europe Convention on Human Rights and Biomedicine and in the Spanish legislation on bioethics. The Directive 95/46/EC of the European Parliament and the Council of 24 October 1995, on the protection of individuals regarding the processing of personal data and on the free movement of such data, has been strictly followed.

2.2. General characteristics

Information on characteristics from the neonates (gender, date of birth), from the mothers (gestational age, pre-gestational weight (kg) and height (m), pre-pregnancy Body Mass Index (BMI)(kg/m^2), education level and living area) and relative to pregnancy factors (type of partum, use of antibiotics intrapartum, number of previous pregnancies, pregnancy diseases and smoking habit) were recorded. Fecal samples and questionnaires were collected at the recruitment and at 90, 180 and 365 days of age.

2.3. Fecal analyses

Families were instructed on the procedure for fecal sampling collection. For this purpose, fecal samples were obtained according to the standard procedures. Briefly, fecal samples were collected immediately after defecation in a sterile container by infant́s parents and right this moment frozen at −20 °C until delivery to the laboratory for further analyses. For DNA extraction, 1 g of sample was weighed, diluted 1:10 in

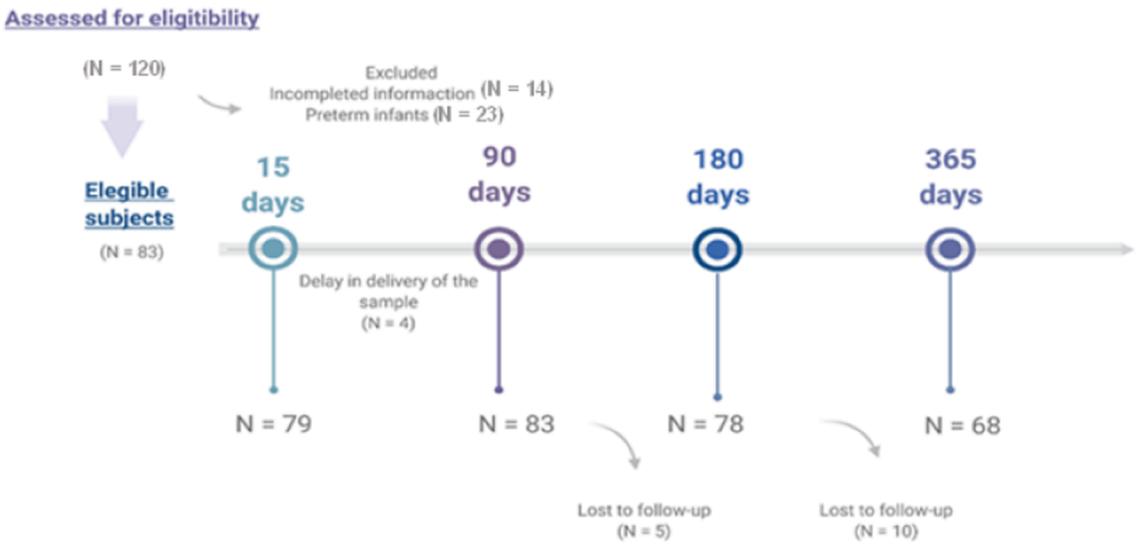

Fig. 1. Scheme of participant enrollment and study progress. Recruitment in the study and evolution of the sample size in each of the time periods analyzed.

sterile PBS solution, homogenized in a LabBlender 400 stomacher (Seward Medical, London, UK) at full speed for 3 min and centrifuged (10 000 g, 30 min, 4 °C). Then, supernatant and bacterial pellet were separated. Bacterial pellet was used for DNA extraction in accordance with the Q Protocol for DNA extraction of fecal samples defined by the International Human Microbiome Standards Consortium (Dore et al., 2015). Extracted DNA was kept frozen at − 80 °C until analysis.

The levels of *Bifidobacterium*, *Bacteroides* group, Enterobacteriaceae and *Clostridium* cluster IV were determined by qPCR using methods described elsewhere (Arboleya et al., 2012; Bartosch, Fite, Macfarlane, & McMurdo, 2004) (Supplementary Table 1).

The analysis of short-chain fatty acids (SCFAs) was performed by gas chromatography by using a chromatographic system composed of two 6890 N GC (Agilent Technologies Inc., Palo Alto, CA) connected to an FID and an MS 5973 N detector (Agilent). Cell-free supernatants from the homogenized feces were treated as described elsewhere (Arboleya et al., 2020) and used to determine the concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, caproate and valerate.

2.4. Dietary assessment and nutritional data analysis

Infants' dietary information has been compiled by means of a self-administered weekly food propensity questionnaire adapted from the Pilot study for Assessment of Nutrient intake and food Consumption Among Kids in Europe (PANCAKE) (Ocké et al., 2012) and adapted for Spanish population, thus including traditional regional recipes and typical foods. In addition, food questionnaires were developed using an online tool. This method has been used and described in previous studies (Gómez-Martín, Dominguez, Gueimonde, & González, 2021; Gómez-Martín et al., 2021). Foods were grouped into 11 food categories adapted from European Prospective Investigation Into Cancer (EPIC) classification (Slimani et al., 2002) and 2 additional for human breast milk and processed infant products as follows. Oils (vegetable oils and solid fats); vegetables (bulbs, mushrooms, roots, inflorescences, and stem and leaf vegetables); legumes (lentils, chickpeas, soya, beans and peas); fruits (fresh, dried, and canned fruits); potatoes and tubers (potato and sweet potato); cereals and cereal products (bread, pasta, flours and grains); meat and meat products (white meat (poultry, rabbit and pork loin), red meat (beef, lamb and pork chop), processed meat and others); fish (fish and fish products, crustaceans and mollusks); eggs; human breast milk, processed infant products (infant formulas: starter formulas, special starter formulas, follow-up formulas, special follow-up formulas, growing-up milk; infants cereals and infant purees: fruits, fruit and cereals, vegetables, legumes and pasta, meat, fish and others) (Gómez-Martín, Arboleya, Gueimonde, & González, 2019); milk and dairy products (milk, yogurt, fresh, mature, and processed cheeses) and sweet and desserts (sweets, cake, biscuits, chocolate, honey and others).

The questionnaires were filled in by the mother, father or caregiver of the infant who received them via email or mobile phone. Detailed directions for completing the food diary were included at the beginning of each category and the validated picture book developed by the PANCAKE consortium was used for portion size estimation according to EU-Menu recommendations (European Food Safety Authority (EFSA), 2014). As dietary information was collected over a prolonged period at different times from birth, adapted different version of the same was used at 15 days and 90 days, due to the absence of complementary feeding. Breastfeeding was collected at all-time points categorically.

For the categorical variable, the type of breastfeeding was classified as breastfeeding (including mixed-feeding), or infant formula. For the quantitative variables, the amount of breast milk consumed was estimated by using the mean values reported in the literature for each stage of age (780 ml for infants until 6 months and 600 ml for infants aged 7–12 months, in the cases of exclusive breastfeeding) (Devaney et al., 2004; Heinig, Nommsen, Peerson, Lonnerdal, & Dewey, 1993). For the infant formulas, the volume reported by the parents was used, assuming that the manufacturer's prescriptions regarding the weight of powdered milk to be dissolved per volume were respected. In mixed-fed infants, based on existing literature, the amount of formula consumed per day was measured and the remaining volume of formula consumed per day was assumed to be breastmilk up to 780 ml from start to 6 months and 600 ml at 12 months (Devaney et al., 2004). In addition, the online questionnaire included queries regarding the use of vitamin and mineral supplements. The consistency of diet, or adherence to a special diet were also recorded.

The consumption of foods was converted into energy and macronutrients using the food composition tables developed by the Centro de Enseñanza Superior de Nutrición Humana y Dietética (CESNID) (Centro de Enseñanza Superior de Nutricion Humana y Dietetica (CESNID), 2008). Nutritional composition data of human breast milk (Dalmau & Moreno, 2011), infant formula, cereal products and infant purees were completed from Gómez-Martín and collaborators' baby foods composition table (Gómez-Martín, Arboleya, Gueimonde, & González, 2019).

Additionally, detailed information regarding type of protein or carbohydrate consumed was extended from the food composition tables published by the United States Department of Agriculture (USDA) (United States Department of Agriculture (USDA), 2004), the major types of fiber (soluble and insoluble types) from Marlett et al. (Marlett & Cheung, 1997) and the content of the main classes and subclasses of

Table 1
General characteristics of the study sample.

		Total sample (N = 83)	
Infant factors		**N**	
Gender	Male	48	57.8
	Female	35	42.2
Weight at birth (kg)	82	3.4 ± 0.4	
Height at birth (cm)	81	49.9 ± 1.7	
BMI z − score at birth	Underweight (−5.99 to < − 1)	15	18.5
	Normal weight (−0.99 to 0.99)	55	67.9
	Overweight risk (1 to 1.99)	10	12.3
	Overweight > 2	1	1.2
Maternal factors			
Age (y)	77	35 ± 5	
Weight pre-pregnancy (kg)	83	65.0 ± 12.6	
Height (m)	83	1.6 ± 0.1	
BMI pre-pregnancy (kg/m^2)	Underweight (<18.5)	2	2.4
	Normal weight (18.5 to 24.9)	55	66.3
	Overweight (25.0 to 29.9)	18	21.7
	Obese (>30)	8	9.6
Education level	Primary	4	4.8
	Secondary	13	15.7
	High school	24	28.9
	University	42	50.6
Living area	Urban	33	39.8
	Rural	50	60.2
Pregnancy factors			
Type of partum	Vaginal	65	78.3
	C-section	18	21.7
Antibiotics intrapartum	Yes	19	22.9
Parity, n	0	43	51.8
	1	34	41.0
	2	6	7.2
Pregnancy diseases	Preeclampsia (na = 2)	3	3.7
	HT (na = 3)	2	2.5
	Urinary infection (na = 3)	10	12.5
	Diabetes (na = 3)	14	17.5
Smoking habit	Smoke during pregnancy	9	10.8

Values are presented as mean ± standard deviation and number and % of the subjects. BMI, body mass index; C-section, caesarean section; HT, hypertension; na, no answer.

(poly)phenol from the Phenol-Explorer Database (Neveu et al., 2010).

2.5. Anthropometric measures

Children height (cm) and weight (kg) were registered to the nearest 0.1 cm and 0.1 kg, respectively, by pediatric nurse with calibrated and approved material at all-time points analyzed. Maternal BMI was calculated as weight, in kilograms, divided by the square of height, in meters and were classified as normal weighted (18.5–24.9 kg/m^2), overweighted (25.0–29.9 kg/m^2), and obese (≥30.0 kg/m^2), based on the Spanish Society for the Study of Obesity (SEEDO) criteria (Salas-Salvadó, Rubio, Barbany, Moreno, & Grupo colaborativo de la, 2007). BMI z-score were obtained relative to WHO Child Growth Standards, adjusted by gender and age, by using WHO ANTHRO, Software for Calculating Anthropometry, Version 3 (World Health Organization (WHO), 2008, 2019).

2.6. Statistical analyses

Results were analyzed using the IBM SPSS software version 25.0 (IBM SPSS, Inc., Chicago, IL, USA) and RStudio software version 1.4.1103. Goodness of fit to the normal distribution was checked by means of the Kolmogorov-Smirnov test. Therefore, non-parametric tests were used. Overall, categorical variables were summarized as percentages and continuous ones using medians and interquartile range or means and standard deviations for descriptive purposes. The Mann-Whitney *U* test and Kruskal-Wallis test were used to evaluate differences in continuous variables. In addition, multivariate analysis adjusted by gender, antibiotics intrapartum, maternal pre-pregnancy BMI, type of delivery and feeding and living area was used to investigate the association between the bacterial fecal groups and SCFAs by time. To explore deeper, the associations between fecal bacteria levels and SCFAs excretion with food groups and dietary components, Spearman correlation analyses were performed. A heatmap was generated under RStudio software version 1.4.1103 package corrplot. Also, a multivariate analysis between diet and microbiota and SCFAs has conducted. For this purpose, microbiota groups, acetate, propionate, butyrate and total SCFAs were introduced as dependent variables, dietary components as fixed factors and gender, maternal pre-pregnancy BMI, type of delivery, antibiotics intrapartum and living as covariates. At 180 and 365 days, volunteers were classified into consumers or non-consumers of each dietary group. At 365 days legumes, milk and dairy products, eggs, human breast milk, sweets and desserts, infant formula, infant cereals and infant puree were included. GraphPad Prism 8 was used for graphical representations.

Considering the mean level and standard deviation of the fecal bacteria and SCFAs in the sample we could reject the hypothesis with an estimated probability between and 0.88 to 1.00 (with the exception of isobutyrate and isovalerate from 90 to 180 days (0.76 and 0.60, respectively)) and a type I error probability of 0.05 for all significant models (Brant, 2022).

3. Results

3.1. Description of the sample

The basal characteristics of the study cohort are described in Table 1. The sample consisted of a total of 83 subjects, 48 males (57.8 %) and 35 (42.2 %) females. It was observed that most children were born vaginally (78.3 %), presented a normal birth weight (BMI z-score of −0.99 to 0.99) and 60.2 % live in rural areas. Concerning maternal factors, the average age of mothers was 35 ± 5 years old and the 66.3 % presented normal BMI pre-pregnancy.

3.2. Evolution of the microbial groups and short-chain fatty acids

Higher levels of *Bifidobacterium* and *Bacteroides* were observed in breastfed infants, of *Bacteroides* also in vaginally born infants and Enterobacteriaceae in male infants. Also, living area was related with differences in most bacterial groups and fecal SCFAs along time (Table 2 and Supplementary Table 2). *Bacteroides* group and *Clostridium* cluster IV increased progressively from 15 to 365 days (from 6.13 log n° cells/g feces to 8.88 and from 3.58 log n° cells/g feces to 8.39, p value < 0.001) while *Bifidobacterium* and Enterobacteriaceae only increased significantly from 15 to 180 days of life. In addition, it was observed a significantly decrease was observed from 180 to 365 days in the case of Enterobacteriaceae (Fig. 2).

To study the changes in fecal SCFAs along time the analyses presented in Fig. 3A and 3B were conducted. An increased in the median levels of all SCFAs from 15 to 365 days was observed (Fig. 3A). Relative proportions from the main SCFAs are represented in Fig. 3B. The percentage of acetate decreased from 90 days until 365 days (from 80.02 % to 67.02 %). In consonance, an increase of the proportion of propionate and butyrate was observed from 90 to 180 days (13.59 % to 16.46 %) and from 180 to 365 days (5.16 to 10.76 %), respectively.

3.3. Evolution of the diet

Regarding the evolution of the diet throughout the first year of age, as it was expected a pronounced increase in the mean consume of all food group, except for human breast milk and infant formulas was observed (Fig. 4A). At 6 months, the predominant food groups were fruits, vegetables, infant formula and human breast milk. However, at 12 months, some of those food groups as infant formula and human breast milk decreased while protein food groups such as meat, legumes, eggs and fish increased and the diet became more adult-like. In turn, energy and lipids increased along the study and the highest changes in macronutrients intake were observed between 180 days and 365 days. The pronounced increase in dietary fiber, soluble and insoluble at 90 to 180 days is noteworthy, as well as the increase in monounsaturated fatty acids and vegetable and animal protein at the same period of time (Fig. 4B).

3.4. Relationships among diet with bacterial groups and SCFAs

The linear relationship between microbial groups and SCFAs with diet has been explored through the Spearman correlations presented in Fig. 5. Breastfeeding has been inversely correlated to acetate, propionate, butyrate and total SCFAs. Moreover, propionate, butyrate and total SCFAs were directly associated with the intake of processed infant products at 180 days (Fig. 5A). Propionate and total SCFAs were also correlated positively with energy, lipids and total protein at 180 days (Fig. 5A). On the other hand, at 12 months, *Clostridium* cluster IV was found inversely correlated with the intake of some protein food groups such as fish and meat and meat products and also with infant processed products and sweets and desserts (Fig. 5B). From nutrients, isobutyrate and isovalerate were positively correlated with insoluble fiber at 12 months of age (Fig. 5B). Therefore, to deepen our understanding of how diet influences the intestinal microbiota and its metabolites, a multivariate analysis was performed (adjusted by gender, antibiotics intrapartum, maternal pre-pregnancy BMI, type of delivery and living area) (Table 3). From 15 to 180 days, lower mean levels of SCFAs were shown in breastfed children than in formula ones. At 180 days, the consumption of infant cereals or processed infant products was related with higher levels of fecal propionate (10.01 *vs* 16.19 and 5.42 *vs* 15.09, respectively). Also, those infant consuming sweets and dessert or infant formula at 12 months presented lower levels of *Bifidobacterium* and *Clostridium* cluster IV, respectively (Table 3).

Table 2
Differences in mean of microorganism levels (Log n° cells/g feces) by gender, type of delivery and feeding, living area, maternal pre-pregnancy BMI and antibiotics intrapartum from 15 days to 365 days.

		Bifidobacterium								***Bacteroides* group**							
		15 days N = 79		**90 days** N = 83		**180 days** N = 78		**365 days** N = 68		**15 days** N = 79		**90 days** N = 83		**180 days** N = 78		**365 days** N = 68	
		N	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**
Gender	Male	48	8.92 ± 1.53	48	9.63 ± 1.14	44	9.96 ± 0.53	41	9.48 ± 1.12	48	6.24 ± 1.84	48	6.46 ± 1.57	44	7.63 ± 1.63	41	8.89 ± 0.95
	Female	31	9.18 ± 1.24	35	9.79 ± 0.92	34	9.95 ± 0.70	27	9.41 ± 1.11	31	6.22 ± 1.92	35	6.91 ± 1.91	34	7.65 ± 1.80	27	8.48 ± 1.46
Delivery	Vaginal	63	9.12 ± 1.41	65	9.77 ± 0.85	62	9.97 ± 0.64	54	9.56 ± 0.97	63	6.71 ± 1.76*	65	7.01 ± 1.53*	62	7.93 ± 1.56*	54	8.82 ± 1.02
	C-section	16	8.65 ± 1.44	18	9.43 ± 1.58	16	9.87 ± 0.46	14	9.05 ± 1.50	16	4.36 ± 0.66	18	5.34 ± 1.80	16	6.51 ± 1.76	14	8.38 ± 1.72
Type of feeding	BF	51	9.17 ± 1.46*	41	9.49 ± 1.34	29	9.97 ± 0.67	16	9.55 ± 1.15	51	6.56 ± 1.88*	41	6.81 ± 1.97	29	7.71 ± 1.94	16	8.42 ± 1.18
	IF	28	8.76 ± 1.33	42	9.89 ± 0.61	49	9.94 ± 0.56	41	9.40 ± 1.21	28	5.63 ± 1.68	42	6.49 ± 1.44	49	7.59 ± 1.54	41	8.80 ± 1.29
	None[a]	0		0		0		11	9.51 ± 0.59	0		0		0		11	8.88 ± 0.72
Living area	Urban	30	8.73 ± 1.41	33	9.33 ± 1.35*	28	9.82 ± 0.68	22	9.07 ± 1.73*	30	5.54 ± 1.81*	33	5.83 ± 1.79*	28	7.03 ± 1.82*	22	8.13 ± 1.52*
	Rural	49	9.21 ± 1.41	50	9.94 ± 0.71	50	10.03 ± 0.55	46	9.64 ± 0.58	49	6.66 ± 1.77	50	7.20 ± 1.46	50	7.98 ± 1.53	46	9.01 ± 0.88
Maternal pre-pregnancy BMI	Underweight (<18.5 kg/m^2)	2	7.18 ± 3.99	2	9.93 ± 0.40	1	9.90	1	10.00	2	6.08 ± 2.81	2	7.19 ± 1.49	1	8.96	1	9.91
	Normal weight (18.5– 24.9 kg/m^2)	54	9.22 ± 1.27	55	9.84 ± 0.83	54	9.95 ± 0.57	44	9.48 ± 1.09	54	6.18 ± 1.77	55	6.74 ± 1.72	54	7.59 ± 1.59	44	8.55 ± 1.11
	Overweight (25.0–29.9 kg/m^2)	17	8.80 ± 1.37	18	9.58 ± 1.33	15	9.93 ± 0.54	15	9.34 ± 1.45	17	5.75 ± 2.01	18	6.17 ± 1.74	15	7.33 ± 1.93	15	9.02 ± 1.49
	Obese (>30.0 kg/m^2)	6	8.53 ± 1.71	8	8.91 ± 1.51	8	10.04 ± 0.99	8	9.47 ± 0.49	6	8.12 ± 1.07	8	6.99 ± 1.83	8	8.40 ± 1.94	8	9.01 ± 095
Antibiotics intrapartum	No	61	9.05 ± 1.42	64	9.69 ± 1.03	59	9.92 ± 0.64	52	9.51 ± 1.17	61	6.14 ± 1.94	64	6.58 ± 1.66	59	7.61 ± 1.70	52	8.66 ± 1.23
	Yes	18	8.93 ± 1.47	19	9.71 ± 1.12	19	10.04 ± 0.48	16	9.28 ± 0.91	18	6.53 ± 1.57	19	6.89 ± 1.94	19	7.71 ± 1.72	16	8.94 ± 1.05
		Enterobacteriaceae								***Clostridium* cluster IV**							
		15 days N = 79		**90 days** N = 83		**180 days** N = 78		**365 days** N = 68		**15 days** N = 79		**90 days** N = 83		**180 days** N = 78		**365 days** N = 68	
		N	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**
Gender	Male	48	7.85 ± 1.81*	48	7.80 ± 1.45	44	8.26 ± 0.93	41	7.45 ± 1.28	48	4.19 ± 1.27	48	5.53 ± 1.70	44	7.11 ± 1.34	41	8.02 ± 1.47
	Female	31	6.99 ± 1.58	35	7.90 ± 1.35	34	8.46 ± 0.82	27	7.67 ± 1.07	31	4.32 ± 1.18	35	5.45 ± 1.84	34	6.37 ± 1.78	27	8.12 ± 1.45
Delivery	Vaginal	63	7.53 ± 1.83	65	7.80 ± 1.37	62	8.39 ± 0.74	54	7.60 ± 1.03	63	4.35 ± 1.33	65	5.51 ± 1.71	62	6.79 ± 1.64	54	8.21 ± 1.19
	C-section	16	7.47 ± 1.53	18	8.01 ± 1.55	16	8.16 ± 1.30	14	7.28 ± 1.72	16	3.82 ± 0.57	18	5.44 ± 1.94	16	6.75 ± 1.36	14	7.50 ± 2.16
Type of feeding	BF	51	7.59 ± 1.70	41	7.73 ± 1.44	29	8.30 ± 1.04	16	7.69 ± 1.24	51	4.29 ± 1.21	41	5.26 ± 1.78	29	6.44 ± 1.77	16	8.43 ± 0.75
	IF	28	7.38 ± 1.89	42	7.95 ± 1.36	49	8.37 ± 0.77	41	7.51 ± 1.23	28	4.15 ± 1.28	42	5.73 ± 1.70	49	6.98 ± 1.43	41	7.76 ± 1.71
	None[a]	0		0		0		11	7.39 ± 1.08	0		0		0		11	8.64 ± 0.78
Living area	Urban	30	7.43 ± 1.86	33	7.45 ± 1.65	28	8.42 ± 0.99	22	7.60 ± 1.46	30	3.85 ± 0.58	33	4.41 ± 1.37*	28	6.13 ± 1.79*	22	7.77 ± 1.59
	Rural	49	7.57 ± 1.72	50	8.11 ± 1.16	50	8.30 ± 0.82	46	7.50 ± 1.06	49	4.48 ± 1.45	50	6.22 ± 1.61	50	7.15 ± 1.34	46	8.20 ± 1.38
Maternal pre-pregnancy BMI	Underweight (<18.5 kg/m^2)	2	6.42 ± 3.10	2	7.66 ± 1.18	1	8.52	1	7.35	2	3.43 ± 0.21	2	4.76 ± 1.66	1	7.91	1	9.55
	Normal weight (18.5– 24.9 kg/m^2)	54	7.38 ± 1.80	55	7.90 ± 1.44	54	8.31 ± 0.95	44	7.55 ± 1.15	54	4.27 ± 1.30	55	5.64 ± 1.81	54	6.70 ± 1.64	44	8.30 ± 1.07
	Overweight (25.0–29.9 kg/m^2)	17	7.92 ± 1.47	18	7.79 ± 1.31	15	8.40 ± 0.64	15	7.46 ± 1.60	17	4.17 ± 1.16	18	5.12 ± 1.69	15	6.40 ± 1.48	15	7.24 ± 2.19
	Obese (>30.0 kg/m^2)	6	7.95 ± 1.90	8	7.64 ± 1.59	8	8.46 ± 0.91	8	7.64 ± 0.69	6	4.44 ± 0.98	8	5.59 ± 1.64	8	7.92 ± 0.82	8	8.09 ± 1.23

(*continued on next page*)

Table 2 (*continued*)

		Enterobacteriaceae								*Clostridium* cluster IV							
		15 days N = 79		90 days N = 83		180 days N = 78		365 days N = 68		15 days N = 79		90 days N = 83		180 days N = 78		365 days N = 68	
		N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Antibiotics intrapartum	Yes	61	7.48 ± 1.68	64	7.95 ± 1.17	59	8.39 ± 0.93	52	7.56 ± 1.18	61	4.33 ± 1.25	64	5.36 ±1.68	59	6.70 ± 1.65	52	7.95 ± 1.55
	No	18	7.63 ± 2.05	19	7.48 ± 1.98	19	8.20 ± 0.72	16	7.45 ± 1.28	18	3.95 ± 1.13	19	5.95 ± 1.95	19	7.05 ± 1.37	16	8.41 ± 1.08

Values presented as mean ± standard deviation. Results derived from U-Mann Whitney or Kruskal-Wallis test. BF, breastfeeding; BMI, body mass index; IF, infant formula. [a] None of the above categories are taken. * p value < 0.05.

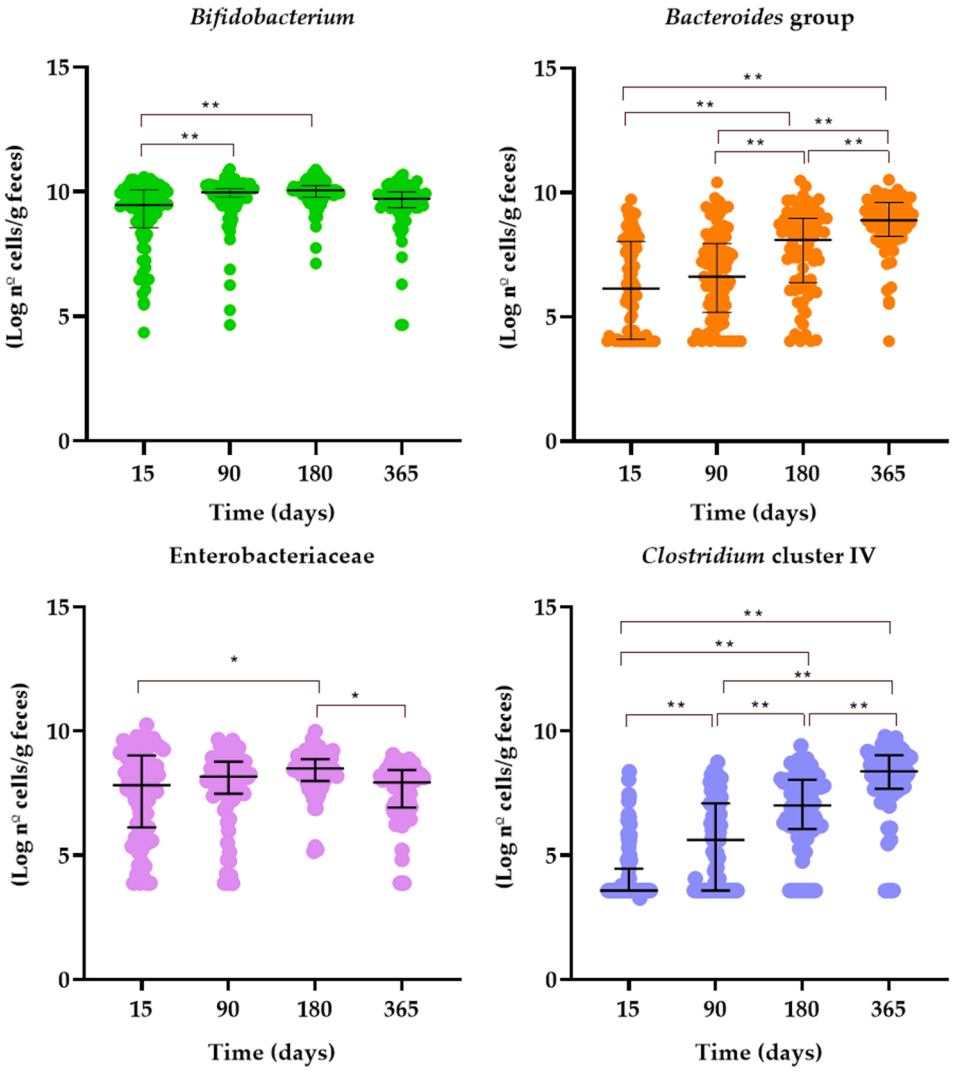

Fig. 2. Differences in the median levels of microorganisms (log n° cells/g feces) along the study. Statistical results derived from a multivariate analysis adjusted by gender, antibiotics intrapartum, maternal pre-pregnancy BMI, type of delivery and feeding and living area are shown. * p value < 0.05; ** p value ≤ 0.001.

4. Discussion

Gut microbiota and SCFAs have been shown to have a wide range of beneficial physiological effects on the host throughout the lifespan (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016; Morrison & Preston, 2016). Even though several studies have characterized the microbial profile in adults, there is scarce literature describing the evolution of the bacterial ecosystem during early life beyond weaning period. This work provides an image of how the bacterial composition and excretion of SCFAs changes upon the introduction of complementary feeding within the first year of life, supporting the importance of breastfeeding in the modulation of the infant's microbiota composition and activity.

From birth, the gut microbiota develops rapidly into a succession of bacterial strains (Endo et al., 2014) in a transition to an adult microbiota. Throughout this process different factors, related to pregnancy and delivery and the infant's environment, influence the establishment of the different bacterial groups. Among them type of delivery, gestational age, gender, or type of early feeding are known to have an influenced and, then, they should be considered when interpreting diet-microbiota relationships (Kumbhare, Patangia, Patil, Shouche, & Patil,

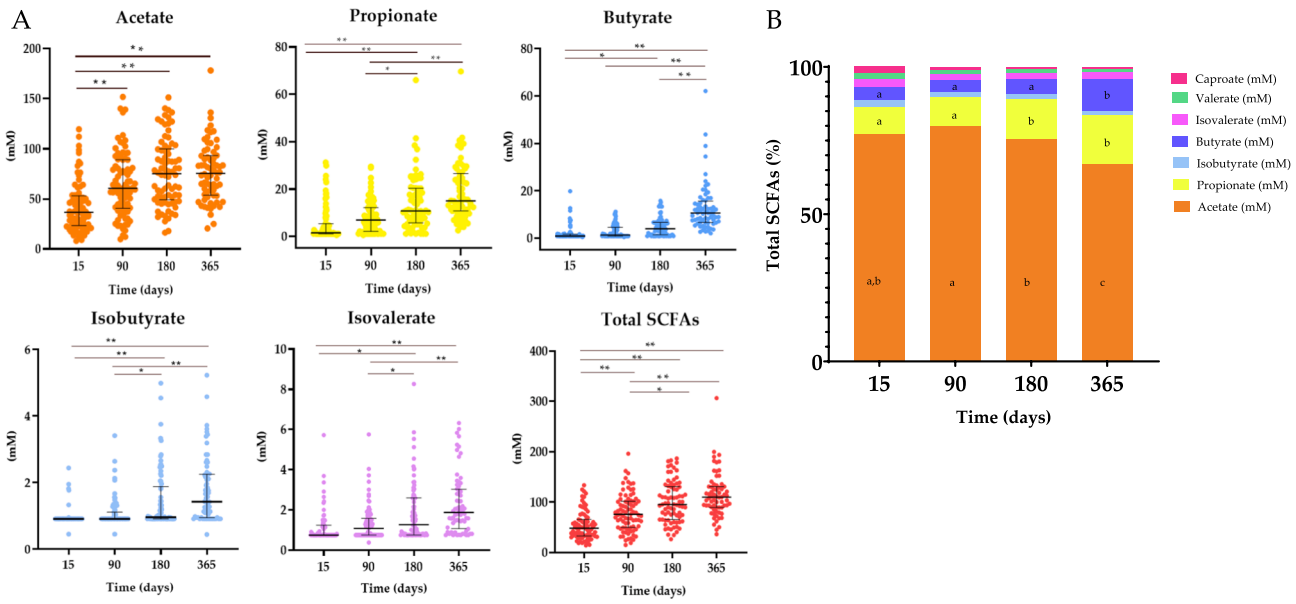

Fig. 3. (A) Changes in the medians of excreted short-chain fatty acids across time. Statistical results derived from a multivariate analysis adjusted by gender, antibiotics intrapartum, maternal pre-pregnancy BMI, type of delivery and feeding and living area are shown.* p value < 0.05; ** p value ≤ 0.001. (B) Evolution in the proportion of SCFAs presented in feces. Kruskal-Wallis test was used for comparison (different letters indicate significant differences between groups (p value < 0.05)).

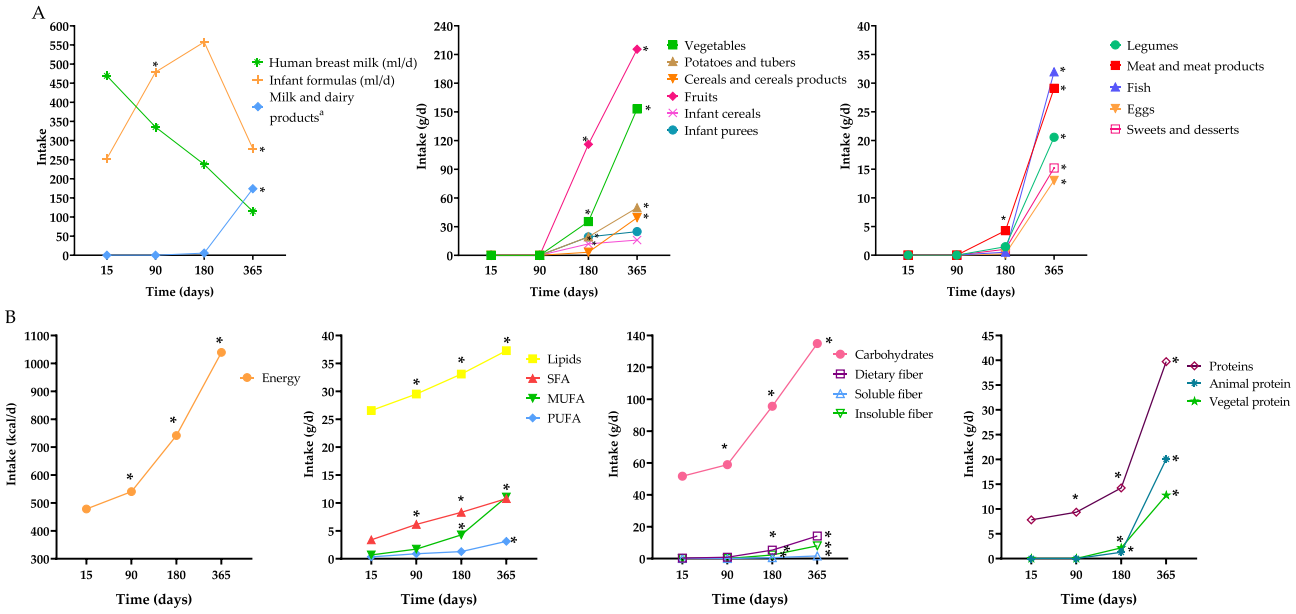

Fig. 4. Evolution of (A) diet and (B) energy and macronutrients along the study. Results derived from Kruskal-Wallis tests. [a] Milk and dairy products included: milk (ml/d), yogurt and cheese (g/d). MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. SFA, saturated fatty acids. * Differences obtained by comparing with the time-point previous category (p value < 0.05).

2019; Salminen, 2004).

The difference observed in the *Bifidobacterium* mean level between breastfed and formula-fed infants at 15 days of age (9.17 ± 1.46 *vs* 8.76 ± 1.33) is consistent with the ability of this bacteria to degrade some HMOs from human milk such as lacto-*N*-biose (Milani et al., 2017; Rubio-del-Campo, Alcántara, Collado, Rodríguez-Díaz, & Yebra, 2020). The first colonizers of the newborn's gastrointestinal tract are aerotolerant microorganisms which reduce oxygen levels facilitating an optimal environment for the proliferation of anaerobic bacteria such as *Bifidobacterium* (Del Chierico et al., 2015; Milani et al., 2017). According with that, bifidobacteria have been shown as the most abundant genus in the sample up to 6 months of age (Turroni et al., 2012) and declined during the weaning process, when diet become more diverse (Dewey, 2003). In line with previous studies in Spanish children the first food groups introduced in the sample during the weaning period were infant cereals (64.1 % of the sample), fruits (55.1 %), vegetables (44.9 %) and tubers (43.6 %) (Klerks, Roman, Bernal, Haro-Vicente, & Sanchez-Siles, 2021).

The reason for the changes on the microbiota following complementary feeding are not totally understood yet. Some authors argue that cessation of breastfeeding may play a more important role than the introduction of solid foods (Bäckhed et al., 2015; Stewart et al., 2018; Tsukuda et al., 2021), but based on our results it seems reasonable to speculate that the incorporation of complex polysaccharides in the

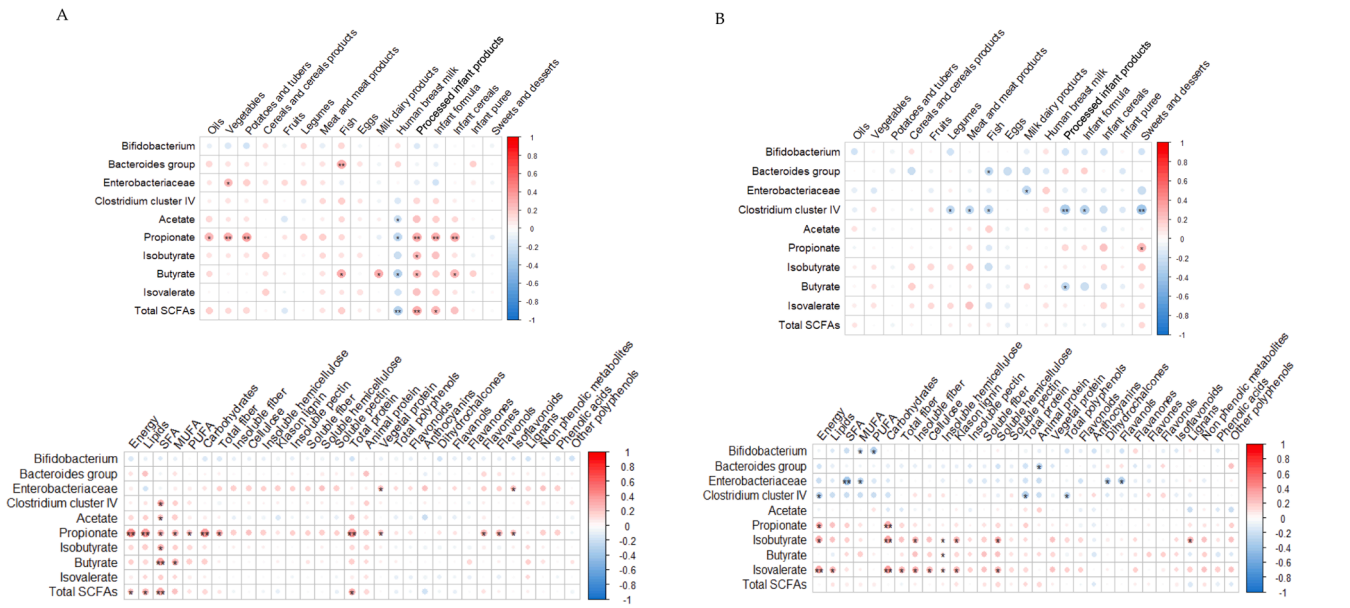

Fig. 5. Spearman correlation between fecal bacteria levels and short-chain fatty acids excretion with food groups and dietary components at (A) 180 days (B) and 365 days. Columns correspond to food groups and energy, macronutrients and bioactive compounds; rows correspond to bacteria groups and short-chain fatty acids. Blue and red colors denote negative and positive association, respectively. The size of the circle and the intensity of the colors represents the degree of association between these food groups or dietary compounds and the microbiota matrix. Valerate and caproate were excluded for the analysis. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCFAs, short-chain fatty acids; SFA, saturated fatty acids. * p value < 0.05; ** p value < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

infant́s diet, may also contribute by favoring the growth of some bacteria groups, such as *Bacteroides* and *Clostridium* cluster IV, which are specialized in the breakdown of fiber (Guo, Zhang, Ma, & He, 2020; Kaoutari, Armougom, Gordon, Raoult, & Henrissat, 2013; Xu et al., 2003). In this line, although given the nature of the study we are unable to establish the directionality, our results pointed to a direct association between *Clostridium* cluster IV levels and dietary fiber throughout the first year of life. Some authors have reported that diets enriched in different type of fiber, like inulin, oligofructose, guar gum, arabinoxylan and resistant starch, induce *Clostridium* cluster IV enrichment (Guo, Zhang, Ma, & He, 2020). Also, the decrease in Enterobacteriaceae and *Bifidobacterium* observed during the first months of life contrary to what occurs with *Bacteroides* and *Clostridium* cluster IV at 12 are in agreement with previous longitudinal studies (Yassour et al., 2016).

Acetate is the main SCFAs in the sample, representing more than half of the total SCFAs found in feces, as it has been previously described in adults and infant (Arboleya et al., 2012; Rowland et al., 2018). The increase of *Bacteroides* and *Clostridium* groups with the weaning process may probably determine the increment in butyrate observed from 6 to 12 months (Guo, Zhang, Ma, & He, 2020; Koenig et al., 2011) since some strains of *Clostridium* genus are able to convert acetate into butyrate (Duncan, Hold, Harmsen, Stewart, & Flint, 2002; Mahowald et al., 2009).

The existence of higher levels of propionate and butyrate acids, observed during the first stage of life, in formula-fed infants is in agreement with previous studies (Edwards, Parrett, Balmer, & Wharton, 1994). With independence of breastfeeding, it is possible that, undigested proteins and amino acids can reach the large intestine, being fermented by some bacteria strains into bacterial metabolites, such as SCFAs (Kok et al., 2019; Zhao, Zhang, Liu, Brown, & Qiao, 2018).

Some authors have hypothesized that SCFAs represent the key molecular link between diet, gut microbiota and health playing an important role throughout the lifespan in protecting the body against deteriorating metabolic control and inflammatory status (Morrison & Preston, 2016). Further research is needed to determine the normal range of SCFAs levels and the threshold above which they may have an impact on health.

The study has some limitations related to its observational nature and the collection of dietary data. In interpreting this information, it should be noted that the energy and nutrient content of processed infant foods has been taken into account, a factor that has hitherto been underestimated in the literature. Regarding the quantification of breast milk energy, it is necessary to mention a limitation of the study. Since it was not possible to record the exact volume of milk produced by the mother, an indirect estimation was made using the mean amounts established in the literature for each age range (Devaney et al., 2004; Heinig, Nommsen, Peerson, Lonnerdal, & Dewey, 1993). While the quality of the FFQ depends on the respondent's memory, its ability to accurately classify energy and all nutrient intakes in children is enhanced by the fact that the questionnaires are adapted from the PANCAKE study and have photographs that make them easier to interpret. In addition, it allows comparison with other studies on the European infant population.

5. Conclusions

This work is one of the first to study how diet could influence microbiota composition in the first year of life. Our results support weaning period as a key step for gut microbiota transition. In turn, this study also suggests the importance of the consumption of dietary fiber with the increase of certain bacterial groups as *Clostridium* cluster IV, which could be beneficial for the host. Finally, studies specially designed to analyze the production and the excretion of SCFAs in children are needed to understand how diet could influence in this process.

CRediT authorship contribution statement

María Gómez-Martín: Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Silvia Saturio:** Investigation, Writing – review & editing. **Silvia Arboleya:** Investigation, Writing – review & editing. **David Herrero-Morín:** Writing – review & editing. **Margot Calzón:** Writing – review & editing. **Teresa López:** Writing – review & editing. **Sonia González:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Miguel**

Table 3
Multivariate analysis on the impact of diet on fecal microbiota levels (Log n° cell/g feces) and fecal short-chain fatty acids (SCFAs) (mM).

Fecal microbiota group and SCFAs	N	Item	Group	Mean ± SD	*P* value
15 days					
Bacteroides group	51	Type of lactation	BF	6.52 ± 0.21	0.025
	28		IF	5.71 ± 0.28	
Propionate	51	Type of lactation	BF	3.38 ± 0.90	<0.001
	28		IF	9.28 ± 1.23	
90 days					
Acetate	41	Type of lactation	BF	50.59 ± 4.69	<0.001
	42		IF	77.85 ± 4.63	
Propionate	41	Type of lactation	BF	5.03 ± 0.97	<0.001
	42		IF	11.03 ± 0.96	
Butyrate	41	Type of lactation	BF	1.98 ± 0.42	0.004
	42		IF	3.83 ± 0.41	
Total SCFAs	41	Type of lactation	BF	61.29 ± 5.30	<0.001
	42		IF	97.29 ± 5.23	
180 days					
Bacteroides group	63	Infant puree	NC	7.44 ± 0.19	0.025
	15		(11.43–217.14 g/d)	8.48 ± 0.40	
	50	Oils	NC	7.36 ± 0.22	0.040
	28		(10.00 ml/d)	8.14 ± 0.30	
Clostridium cluster IV	43	Vegetables	NC	6.46 ± 0.23	0.042
	35		(6.86–168.75 g/d)	7.18 ± 0.25	
Acetate	29	Type of lactation	BF	62.48 ± 6.49	0.012
	49		IF	84.50 ± 4.85	
Propionate	29	Type of lactation	BF	9.10 ± 2.09	0.006
	49		IF	16.85 ± 1.56	
	44	Potatoes and tubers	NC	11.46 ± 1.64	0.026
	34		(7.11–153.0 g/d)	17.22 ± 1.87	
	28	Infant cereals	NC	10.01 ± 2.06	0.021
	50		C (4.50–90.0 g/d)	16.19 ± 1.53	
	9	Infant processed products	NC	5.42 ± 3.59	0.014
	69		(4.5–1151.43 [a])	15.09 ± 1.28	
Butyrate	74	Milk and dairy products	NC	4.66 ± 0.43	0.012
	4		(53.57–160.71[b])	9.60 ± 1.87	
	72	Fish	NC	4.50 ± 0.43	0.001
	6		(2.86–12.86 g/d)	9.91 ± 1.56	
Total SCFAs	29		BF		0.003
	49	Type of lactation	IF	80.76 ± 7.67 / 112.08 ± 5.74	
	44	Potatoes and tubers	NC	92.00 ± 6.10	0.044
	34		(7.11–153.0 g/d)	111.36 ± 6.98	
365 days					
Bifidobacterium	11	Sweets and desserts	NC	10.14 ± 0.33	0.032
	57		(3.00–68.0 g/d)	9.32 ± 0.14	
Clostridium cluster IV	23	Infant formula	NC	8.60 ± 0.30	0.039
	45		(120.00–750.0 ml/d)	7.79 ± 0.21	

Values presented as mean ± standard deviation. Results derived from a multivariate analysis in which fecal gut microbiota groups and acetate, propionate, butyrate and total SCFAs were dependent variables. Diet was introduced as fixed factor while gender, maternal pre-pregnancy BMI, type of delivery, antibiotics intrapartum and living area were introduced as covariates. At 180 and 365 days, dietary groups were classified into consumers or non-consumers. BF, breast-feeding; D, day; IF, infant formula; NC, non-consumer; SCFAs, short-chain fatty acids. [a] Infant products included: infant formula (ml/d), infant cereals and infant puree (g/d). [b] Milk and dairy products included: milk (ml/d), yogurt and cheese (g/d). Only significant differences are shown.

Gueimonde: Conceptualization, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111994.

References

Agostoni, C., Decsi, T., Fewtrell, M., Goulet, O., Kolacek, S., Koletzko, B., … van Goudoever, J. (2008). Complementary Feeding: A Commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology & Nutrition, 46*(1), 99–110. https://doi.org/10.1097/01.mpg.0000304464.60788.bd

Arboleya, S., Binetti, A., Salazar, N., Fernández, N., Solís, G., Hernández-Barranco, A., … Gueimonde, M. (2012). Establishment and development of intestinal microbiota in preterm neonates. *FEMS Microbiology Ecology, 79*(3), 763–772. https://doi.org/10.1111/j.1574-6941.2011.01261.x

Arboleya, S., Saturio, S., Suárez, M., Fernández, N., Mancabelli, L., De Los Reyes-Gavilán, C. G., … Gueimonde, M. (2020). Donated human milk as a determinant factor for the gut bifidobacterial ecology in premature babies. *Microorganisms, 8*(5), 1–12. https://doi.org/10.3390/microorganisms8050760

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., … Jun, W. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host and Microbe, 17*(5), 690–703. https://doi.org/10.1016/j.chom.2015.04.004

Bartosch, S., Fite, A., Macfarlane, G. T., & McMurdo, M. E. T. (2004). Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Applied and Environmental Microbiology, 70*(6), 3575–3581. https://doi.org/10.1128/AEM.70.6.3575-3581.2004

Brant, R. (2022). Inference for means: Comparing two independent samples. Retrieved September 9, 2022, from https://www.stat.ubc.ca/~rollin/stats/ssize/n2.

Centro de Enseñanza Superior de Nutricion Humana y Dietética (CESNID). (2008). *Tablas de Composición de Alimentos por Medidas Caseras de Consumo Habitual en España.* Barcelona.

Dalmau, J., & Moreno, J. M. (2011). Leches de crecimiento en la alimentación infantil. *Acta Pediátrica Española, 69*(9), 373–378.

Del Chierico, F., Vernocchi, P., Petrucca, A., Paci, P., Fuentes, S., Praticò, G., … Putignani, L. (2015). Phylogenetic and metabolic tracking of gut microbiota during perinatal development. *PLOS ONE, 10*(9), e0137347–e0137373. https://doi.org/10.1371/journal.pone.0137347

Devaney, B., Kalb, L., Briefel, R., Zavitsky-Novak, T., Clusen, N., & Ziegler, P. (2004). Feeding infants and toddlers study: Overview of the study design. *Journal of the American Dietetic Association, 104*(SUPPL. 1), 8–13. https://doi.org/10.1016/j.jada.2003.10.023

Dewey, K. (2003). Guiding principles for complementary feeding of the breastfed child.

Dore, J., Ehrlich, S. D., Levenez, F., Pelletier, E., Alberti, A., Bertrand, L., … Consortium, I. (2015). IHMS SOP 06 V1: standard operating protocol for fecal sample DNA extraction. Protocol Q. *International Human Microbiome Standards*.

Duncan, S. H., Hold, G. L., Harmsen, H. J. M., Stewart, C. S., & Flint, H. J. (2002). Growth requirements and fermentation products of *Fusobacterium prausnitzii,* and a proposal to reclassify it as *Faecalibacterium prausnitzii* gen. nov., comb. *Nov. International Journal of Systematic and Evolutionary Microbiology, 52*(6), 2141–2146. https://doi.org/10.1099/00207713-52-6-2141

Edwards, C. A., & Parrett, A. M. (2002). Intestinal flora during the first months of life: New perspectives. *British Journal of Nutrition, 88*(S1), s11–s18. https://doi.org/10.1079/BJN2002625

Edwards, C. A., Parrett, A. M., Balmer, S. E., & Wharton, B. A. (1994). Faecal short chain fatty acids in breast-fed and formula-fed babies. *Acta Paediatrica, International Journal of Paediatrics, 83*(5), 459–462. https://doi.org/10.1111/j.1651-2227.1994.tb13059.x

Eggesbø, M., Botten, G., Stigum, H., Nafstad, P., & Magnus, P. (2003). Is delivery by cesarean section a risk factor for food allergy? *Journal of Allergy and Clinical Immunology, 112*(2), 420–426. https://doi.org/10.1067/mai.2003.1610

Endo, A., Pärtty, A., Kalliomäki, M., Isolauri, E., & Salminen, S. (2014). Long-term monitoring of the human intestinal microbiota from the 2nd week to 13 years of age. *Anaerobe, 28*, 149–156. https://doi.org/10.1016/j.anaerobe.2014.06.006

European Food Safety Authority (EFSA). (2014). Guidance on the EU Menu methodology. *EFSA Journal*, *12*(12),3944-4021. https://doi.org/10.2903/j.efsa.2014.3944.

Fallani, M., Amarri, S., Uusijarvi, A., Adam, R., Khanna, S., Aguilera, M., … Edwards, C. A. (2011). Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centers. *Microbiology, 157*(5), 1385–1392. https://doi.org/10.1099/mic.0.042143-0

Fouhy, F., Ross, R. P., Fitzgerald, G. F., Stanton, C., & Cotter, P. D. (2012). Composition of the early intestinal microbiota. *Gut Microbes, 3*(3), 203–220. https://doi.org/10.4161/gmic.20169

Gómez-Martín, M., Arboleya, S., Gueimonde, M., & González, S. (2019). Nutritional composition of processed baby foods targeted at infants from 0–12 months. *Journal of Food Composition and Analysis, 79*, 55–62. https://doi.org/10.1016/j.jfca.2019.03.009

Gómez-Martín, M., Domínguez, B., Gueimonde, M., & González, S. (2021). Identification of nutritional targets in Spanish children belonging to the LayDI cohort for the development of health promotion strategies in the first two years of life. *International Journal of Environmental Research and Public Health, 18*(3), 939–953. https://doi.org/10.3390/ijerph18030939

Gómez-Martín, M., Herrero-Morín, D., Solís, G., Suarez, M., Fernández, N., Arboleya, S., … González, S. (2021). Longitudinal study depicting differences in complementary feeding and anthropometric parameters in late preterm infants up to 2 years of age. *Nutrients, 13*(3), 982–997. https://doi.org/10.3390/nu13030982

Gotoh, A., Katoh, T., Sakanaka, M., Ling, Y., Yamada, C., Asakuma, S., … Katayama, T. (2018). Sharing of human milk oligosaccharides degradants within bifidobacterial communities in faecal cultures supplemented with *Bifidobacterium* bifidum. *Scientific Reports, 8*(1), 13958–13972. https://doi.org/10.1038/s41598-018-32080-3

Goulet, O. (2015). Potential role of the intestinal microbiota in programming health and disease. *Nutrition Reviews, 73*(suppl 1), 32–40. https://doi.org/10.1093/nutrit/nuv039

Grölund, M.-M., Lehtonen, O.-P., Eerola, E., & Kero, P. (1999). Fecal microflora in healthy infants born by different methods of delivery: Permanent changes in intestinal flora after cesarean delivery. *Journal of Pediatric Gastroenterology & Nutrition, 28*(1), 19–25. https://doi.org/10.1097/00005176-199901000-00007

Guo, P., Zhang, K., Ma, X., & He, P. (2020). Clostridium species as probiotics: Potentials and challenges. *Journal of Animal Science and Biotechnology, 11*(1), 1–10. https://doi.org/10.1186/s40104-019-0402-1

Heinig, M. J., Nommsen, L. A., Peerson, J. M., Lonnerdal, B., & Dewey, K. G. (1993). Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: The DARLING Study. *American Journal of Clinical Nutrition, 58*(2), 152–161. https://doi.org/10.1093/ajcn/58.2.152

Huh, S. Y., Rifas-Shiman, S. L., Zera, C. A., Edwards, J. W. R., Oken, E., Weiss, S. T., & Gillman, M. W. (2012). Delivery by caesarean section and risk of obesity in preschool age children: A prospective cohort study. *Archives of Disease in Childhood, 97*(7), 610–616. https://doi.org/10.1136/archdischild-2011-301141

Kaoutari, A., Armougom, F., Gordon, J. I., Raoult, D., & Henrissat, B. (2013). The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nature Reviews Microbiology, 11*(7), 497–504. https://doi.org/10.1038/nrmicro3050

Klerks, M., Roman, S., Bernal, M. J., Haro-Vicente, J. F., & Sanchez-Siles, L. M. (2021). Complementary feeding practices and parental pressure to eat among spanish infants and toddlers: A cross-sectional study. *International Journal of Environmental Research and Public Health, 18*(4), 1–17. https://doi.org/10.3390/ijerph18041982

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., … Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences*, *108*(Supplement_1), 4578–4585. https://doi.org/10.1073/pnas.1000081107.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell, 165*(6), 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041

Kok, C. R., Brabec, B., Chichlowski, M., Harris, C., Moore, N., Wampler, J., … Hutkins, R. (2019). Stool pH and short/branched chain fatty acids in infants receiving extensively hydrolyzed formula, amino acid formula, or human milk through two months of age (P11–076-19). *Current Developments. Nutrition, 3*(Supplement_1), 1–15. https://doi.org/10.1093/cdn/nzz048.p11-076-19

Kumbhare, S. V., Patangia, D. V., Patil, R. H., Shouche, Y. S., & Patil, N. P. (2019). Factors influencing the gut microbiome in children: From infancy to childhood. *Journal of Biosciences, 44*(2), 49–68. https://doi.org/10.1007/s12038-019-9860-z

Lind, M. V., Larnkjær, A., Mølgaard, C., & Michaelsen, K. F. (2018). Breastfeeding, breast milk composition, and growth outcomes. *Nestle Nutrition Institute Workshop Ser, 89*, 63–77. https://doi.org/10.1159/000486493

Mahowald, M. A., Rey, F. E., Seedorf, H., Turnbaugh, P. J., Fulton, R. S., Wollam, A., … Gordon, J. I. (2009). Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. *Proceedings of the National Academy of Sciences, 106*(14), 5859–5864. https://doi.org/10.1073/pnas.0901529106

Marlett, J. A., & Cheung, T.-F. (1997). Database and quick methods of assessing typical dietary fiber intakes using data for 228 commonly consumed foods. *Journal of the American Dietetic Association, 97*(10), 1139–1151. https://doi.org/10.1016/S0002-8223(97)00275-7

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., … Ventura, M. (2017). The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews, 81*(4), 1–67. https://doi.org/10.1128/mmbr.00036-17

Morrison, D. J., & Preston, T. (2016). Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes, 7*(3), 189–200. https://doi.org/10.1080/19490976.2015.1134082

Neveu, V., Perez-Jimenez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., … Scalbert, A. (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database*, *2010*, bap024–bap033. https://doi.org/10.1093/database/bap024.

Norin, E., Midtvedt, T., & Björkstén, B. (2004). Development of faecal short-chain fatty acid pattern during the first year of life in Estonian and Swedish infants. *Microbial Ecology in Health and Disease, 16*(1), 8–12. https://doi.org/10.1080/08910600410026364

Ocké, M., de Boer, E., Brants, H., van der Laan, J., Niekerk, M., van Rossum, C., … Maly, M. (2012). PANCAKE – Pilot study for the Assessment of Nutrient intake and food Consumption Among Kids in Europe. *EFSA Supporting Publications, 9*(9), 1–120. https://doi.org/10.2903/sp.efsa.2012.EN-339

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: Metabolism of nutrients and other food components. *European Journal of Nutrition, 57*(1), 1–24. https://doi.org/10.1007/s00394-017-1445-8

Rubio-del-Campo, A., Alcántara, C., Collado, M. C., Rodríguez-Díaz, J., & Yebra, M. J. (2020). Human milk and mucosa-associated disaccharides impact on cultured infant fecal microbiota. *Scientific Reports, 10*(1), 1–12. https://doi.org/10.1038/s41598-020-68718-4

Salas-Salvadó, J., Rubio, M. A., Barbany, M., Moreno, B., & Grupo colaborativo de la, SEEDO. (2007). Consenso SEEDO 2007 para la evaluación del sobrepeso y la obesidad y el establecimiento de criterios de intervención terapéutica. *Med Clin (Barc), 128*(5), 184–196. https://doi.org/10.1016/S0025-7753(07)72531-9.

Salminen, S. (2004). Influence of mode of delivery on gut microbiota composition in seven years old children. *Gut, 53*(9), 1388–1389. https://doi.org/10.1136/gut.2004.041640

Saturio, S., Nogacka, A. M., Suárez, M., Fernández, N., Mantecón, L., Mancabelli, L., … Gueimonde, M. (2021). Early-life development of the bifidobacterial community in the infant gut. *International Journal of Molecular Sciences, 22*(7), 3382–3394. https://doi.org/10.3390/ijms22073382

Sevelsted, A., Stokholm, J., Bønnelykke, K., & Bisgaard, H. (2015). Cesarean section and chronic immune disorders. *Pediatrics, 135*(1), e92–e98. https://doi.org/10.1542/peds.2014-0596

Slimani, N., Fahey, M., Welch, A., Wirfält, E., Stripp, C., Bergström, E., … Riboli, E. (2002). Diversity of dietary patterns observed in the European Prospective Investigation into Cancer and Nutrition (EPIC) project. *Public Health Nutrition, 5*(6b), 1311–1328. https://doi.org/10.1079/PHN2002407

Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., … Petrosino, J. F. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature, 562*(7728), 583–588. https://doi.org/10.1038/s41586-018-0617-x

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal, 474*(11), 1823–1836. https://doi.org/10.1042/BCJ20160510

Tsukuda, N., Yahagi, K., Hara, T., Watanabe, Y., Matsumoto, H., Mori, H., … Matsuki, T. (2021). Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. *ISME Journal, 15*(9), 2574–2590. https://doi.org/10.1038/s41396-021-00937-7

Turroni, F., Peano, C., Pass, D. A., Foroni, E., Severgnini, M., Claesson, M. J., … Ventura, M. (2012). Diversity of bifidobacteria within the infant gut microbiota. *PloS ONE, 7*(5), 20–24. https://doi.org/10.1371/journal.pone.0036957

United States Department of Agriculture (USDA). (2004). Food Composition Databases. Retrieved January 12, 2022, from 2004 website: https://ndb.nal.usda.gov/ndb/.

van den Elsen, L. W. J., & Verhasselt, V. (2021). Human milk drives the intimate interplay between gut immunity and adipose tissue for healthy growth. *Frontiers in Immunology, 12*, 645415–645424. https://doi.org/10.3389/fimmu.2021.645415

van Esch, B. C. A. M., Porbahaie, M., Abbring, S., Garssen, J., Potaczek, D. P., Savelkoul, H. F. J., & van Neerven, R. J. J. (2020). The Impact of milk and its components on epigenetic programming of immune function in early life and beyond: Implications for allergy and asthma. *Frontiers in Immunology, 11*, 2141–2155. https://doi.org/10.3389/fimmu.2020.02141

World Health Organization (WHO). (2008). Interpretando los Indicadores de Crecimiento. In *Curso de Capacitación sobre la Evaluación del Crecimiento del Niño*. Retrieved from http://www.who.int/childgrowth/training/c_interpretando.pdf.

World Health Organization (WHO). (2011). Exclusive breastfeeding for six months best for babies everywhere. Retrieved from https://www.who.int/ mediacentre/news/statements/2011/breastfeeding_20110115/en/.

World Health Organization (WHO). (2019). *Child growth standards OMS Anthro [version 3.2.2]*.

Xu, J., Bjursell, M. K., Himrod, J., Deng, S., Carmichael, L. K., Chiang, H. C., … Gordon, J. I. (2003). A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. *Science, 299*(5615), 2074–2076. https://doi.org/10.1126/science.1080029

Yassour, M., Vatanen, T., Siljander, H., Hämäläinen, A. M., Härkönen, T., Ryhänen, S. J., … Xavier, R. J. (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Science Translational Medicine, 8*(343), 343rac81-343rac103. https://doi.org/10.1126/scitranslmed.aad0917.

Zhao, J., Zhang, X., Liu, H., Brown, M. A., & Qiao, S. (2018). Dietary protein and gut microbiota composition and function. *Current Protein & Peptide Science, 20*(2), 145–154. https://doi.org/10.2174/1389203719666180514145437