BMC Pediatrics

RESEARCH

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Growth and gastrointestinal tolerance of healthy formula-fed infants: a multicentre, prospective observational study

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Abstract

Background Infant formula with human milk oligosaccharides (HMOs) and increased β-palmitate mimics breast milk nutritional composition and clinical benefits. We aimed to assess formula-fed infant growth, gastrointestinal tolerance, infections, and parental satisfaction with a partly fermented infant formula with an improved lipid profile (enriched with β-palmitate and docosahexaenoic/arachidonic acid) and short and long-chain oligosaccharides (scGOS/lcFOS [9:1]) and HMOs.

Methods A prospective descriptive observational study in healthy infants with formula feeding or breastfeeding (reference population) was conducted in six Spanish primary care centres following routine clinical practice. In the first, second and fourth month of life visits sociodemographic, clinical, and anthropometric variables (weight, length, head circumference), stool consistency (Brussels Infant and Toddler Stool Scale [BITSS]), gastrointestinal symptoms, infections incidence and associated healthcare resource utilisation, and caregivers' satisfaction with formula were collected. A descriptive statistical analysis was performed (STATA-v.14). Growth was estimated as the mean (standard deviation) increase in the anthropometric variables and z-scores.

Results A total of 61 formula-fed and 65 breastfed infants were included in the study (50.8% male). The average increase in weight, length and head circumference in the formula feeding and in the breastfeeding groups from the first to the fourth month of life was 2,566 (496) g, 9.7 (1.7) cm and 4.4 (1.0) cm, and 2,571 (702) g, 9.8 (1.8) cm and 4.4 (1.1) cm, respectively. The weight z-score was −0.1 (0.7) for formula-fed and 0.1 (1.1) for breastfed infants. In all visits, more than 88% of infants had loose/watery stools and most infants suffered gastrointestinal symptoms with low/medium frequency. In the fourth month of life visit, 16 (26.2%) formula-fed and 16 (24.6%) breastfed infants had infections, mainly respiratory, with 16% of formula-fed and 12% of breastfed infants requiring treatment. Most formula-feeding caregivers had a good/very good opinion of formula (85.2%). 75.4% infants drank the whole feeding bottle.

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Conclusions The growth, gastrointestinal tolerance, and incidence of infections of healthy formula-fed infants during the first four months of life were appropriate and in line with WHO standards. Formula feeding caregivers were satisfied with this partly fermented infant formula with an improved lipid profile and oligosaccharides.

Keywords Formula feeding, Breastfeeding, Growth, Gastrointestinal tolerance, β-palmitate

Background

The World Health Organization (WHO) recommends exclusive breastfeeding during the first six months of an infant's life as it is the optimal source of nutrients for newborn infants [1]. Breastfeeding positively correlates with less infectious and immune-mediated diseases, improved cognitive development and lower risk of being overweight and having type 2 diabetes later in life [2–4]. Moreover, nursing women have a reduced risk of type 2 diabetes and breast and ovarian cancer [3].

The WHO reports that 44% of infants aged 0–6 months are exclusively breastfed worldwide [5]. However, significant differences exist in breastfeeding rates across countries. For instance, in eleven European countries, the rate of breastfeeding (exclusive or mixed feeding) at six months ranged from 38% in Italy to 71% in Norway, with exclusive breastfeeding rates ranging from 10 to 39% across countries [6]. In Spain, 46% and 28% of infants received any breastfeeding or were exclusively breastfed, respectively [6]. Perceived insufficient milk, misinterpreted unsettled infant behaviours and the need to rest are some of the reported reasons for mixed or formula feeding [7, 8].

Although formulas cannot emulate the living, dynamic nature of breast milk and the mother-infant interaction during breastfeeding [7], formula composition is required to resemble breastfeeding in nutritional and clinical benefit. Breast milk's nutritional composition is unique with lactose as the most abundant nutrient, followed, in this order, by fat, human milk oligosaccharides (HMOs) and proteins [9]. In this context, infant formula is designed to mimic the nutritional composition of breast milk and is intended as an effective substitute when breastfeeding is not possible [10].

As the second largest macronutrient in breastmilk, fat provides 50% of the total energy content [9]. Palmitate represents almost 25% of fatty acids in breast milk [10]. Most of it is esterified in the sn-2 (β) position, which allows its absorption [11]. However, esterification in the sn-1 or sn-3, which are the most in infant formula, results in high levels of free palmitic acid in the infant's intestine. This binds to calcium, forming insoluble calcium soaps [11]. Calcium soaps are excreted with faeces and are associated with reduced calcium and fatty acid absorption and with hard and infrequent stools [11, 12]. Infants fed with formulas with higher β-palmitate levels show reduced excretion of palmitic acid-derived calcium soaps and increased fatty acids absorption. The consequences of this are better bone mineralisation for the growing skeleton, improved stool consistency and fatty acid metabolism and intestinal microbiome development [12, 13]. Docosahexaenoic (DHA) and arachidonic acid (ARA) are also two essential fatty acids present in breast milk important for infant growth, immune system, vision, cognitive development, and motor systems [10].

More than 150 oligosaccharide structures have been identified in breast milk [9]. Of these, 90% and 10% correspond to short and long chain oligosaccharides, respectively [14]. HMOs are complex, non-digestible and non-nutritional oligosaccharides in human breast milk but are almost absent in cow's milk [2, 15]. After lactose and lipids, HMOs are the third most abundant solids of breast milk [16]. HMOs composition vary between women and during lactation, and their concentration depends on the Lewis blood group and secretor status of the mother [17]. However, 2′-fucosyllactose and lacto-N-neotetraose are two of the most predominant HMOs [18]. Clinical trials and observational studies have shown HMOs beneficial effects for infant growth, immune protection and balanced microbiome development and that formula supplementation with HMOs is safe and well tolerated [2, 18].

A novel partly fermented (postbiotic-providing) infant formula was developed with an improved lipid profile, with increased levels of β-palmitate from milk fat and DHA/ARA. This formula also contains more than 100 different oligosaccharide structures, with HMOs and short chain galacto-oligosaccharides and long chain fructo-oligosaccharides (scGOS/lcFOS), in the 9:1 ratio in which they are present in breastmilk. The scGOS/lcFOS (9:1) prebiotic mixture, mimics the quantity but mainly the diversity and function of the oligosaccharides of breast milk [19–23]. More than 90 publications from more than 40 clinical trials have shown important clinical benefits of this infant formula, with fewer allergic symptoms and infections, while also promoting gut microbiome development and improved stool frequency and consistency [24, 25]. However, real-world studies may help to understand whether these benefits are also achieved in routine clinical practice.

With this study we aimed to determine the growth, gastrointestinal tolerance, infections, associated healthcare resource utilisation and parental satisfaction of healthy infants fed from their first to their fourth month of life with a partly fermented (postbiotic-providing) infant formula with an improved lipid profile (high β-palmitate

levels from milk fat and DHA/ARA) and oligosaccharides (scGOS/lcFOS [9:1] and HMOs).

Methods

Study design and setting

A descriptive, multicentre, prospective observational study in healthy infants was conducted between May 2021 and October 2022 in six Spanish primary health care centres in Valencia (Serrería I, Serrería II, Malvarrosa and Trafalgar), Sevilla (Amante Laffon) and Madrid (El Restón). Infants were recruited by their paediatricians during the routine visit at first month (± 5 days) of life. Infants were followed up during routine clinical practice visits in their second (± 5 days) and fourth (± 5 days) month of life (Fig. 1).

Population

Eligible subjects were one month old (± 5 days), healthy infants born between 37 and 41 weeks of gestation, whose parents/legal guardians (hereafter, caregivers) signed the informed consent. The choice of starting exclusive breastfeeding or exclusive formula feeding was freely and voluntarily made by the caregivers prior to their inclusion in the study. Breastfed infants received breastmilk from the moment they were born. Formula feeding infants started feeding with a partly fermented (postbiotic-providing, derived from the Lactofidus™ fermentation process) infant formula with an improved lipid profile (high β-palmitate levels from milk fat and DHA/ARA) and oligosaccharides (scGOS/lcFOS [9:1] and HMOs) (Almirón Profutura® 1; Nutricia, Danone Specialised Nutrition, Spain). Infants who changed their type of feeding during the study period (three months), were excluded from the final analysis. Exclusion criteria included known intolerance/allergy to lactose or cow's milk; conditions requiring types of infant feedings other than those specified in the protocol; relevant diseases or disorders that contraindicated their inclusion or permanence in the study and,

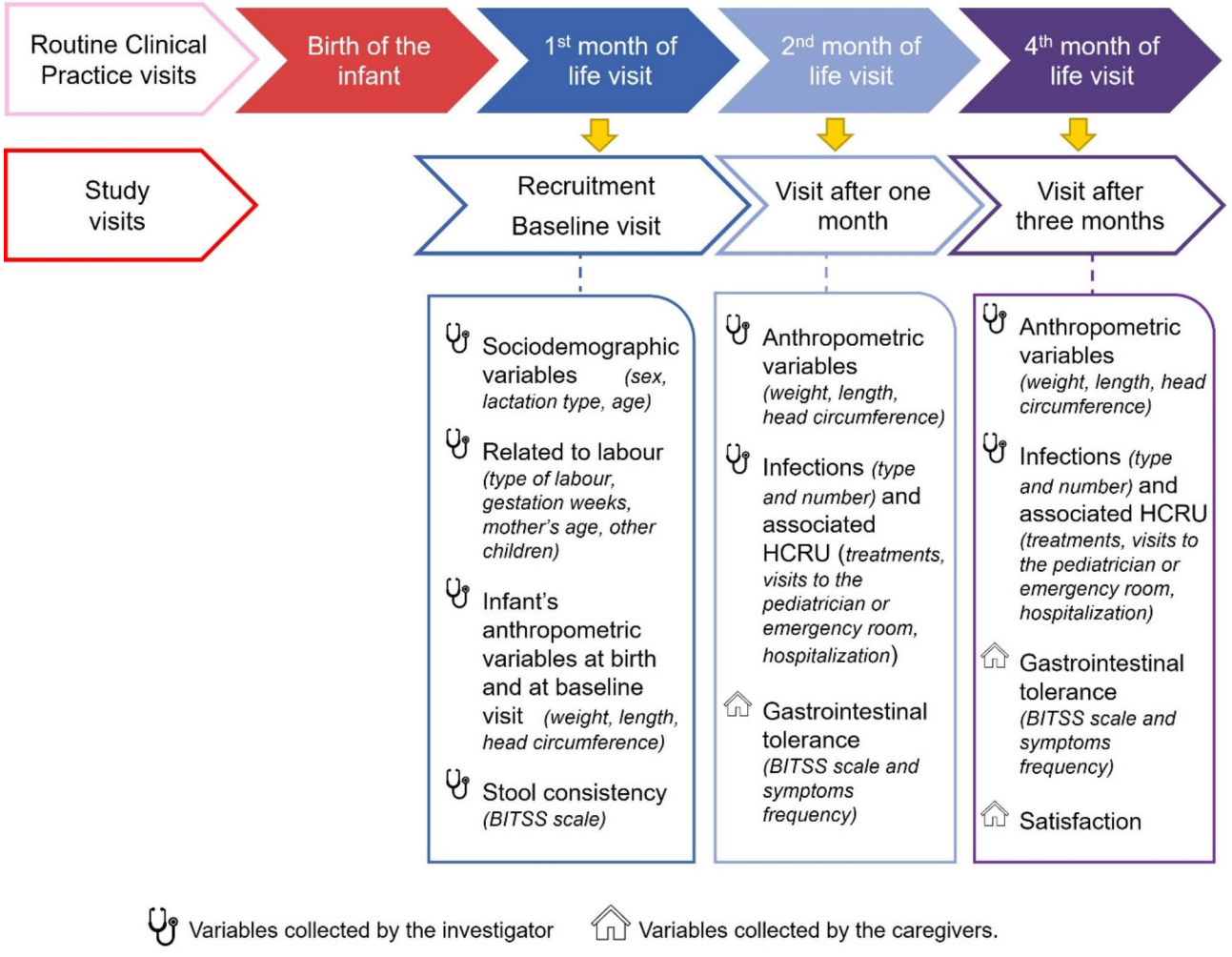

Fig. 1 Study design and study variables. HCRU: Healthcare Resource Utilisation; BITSS: Brussels Infant and Toddler Stool Scale

according to the investigators' criteria, caregivers' inability to follow the instructions or procedures of the study.

A population of exclusively breastfed infants was included as a reference group. Both breastfeeding and the infant formula, provided free of charge to subjects, were given ad libitum.

With regard to sample size, the number of infants under one year of age in Spain, which was 359,045 in January 2020, and the percentage of formula-fed infants in Spain were taken into account [26, 27]. Sample size was calculated using a 95% confidence interval, a standard deviation (SD) of 5.1 based on WHO data [28], and a precision of 5% to estimate an increase of 25.8 g/day [28]. It was necessary to enrol a minimum of 60 infants in each group.

Study objectives

The primary objective of the study was to determine the growth, defined as the average weight gain at two and four months of age from the first month of age, of healthy formula-fed infants. The secondary objectives were to evaluate infant anthropometric measurements (length and head circumference increase; length-for-age, weight-for-age, and head circumference-for-age z-scores), to determine the gastrointestinal tolerance, and to describe the type, severity, duration, and associated healthcare resource utilisation (HCRU) of infections. Additionally, satisfaction with the formula from the perspective of caregivers was assessed.

The aim of the study was not to compare the benefits of formula feeding with breastfeeding, because the benefits of breastfeeding exceed those of other feeding options. The objective of the study was to take the breastfeeding group as a reference to analyse whether growth is adequate when formula feeding is chosen for personal or health reasons.

Study variables and measurements

Sociodemographic and clinical characteristics

Baseline infant characteristics, recorded in the first month of life visit, included sex, age and lactation type. Labour-related variables were type of labour, weeks of gestation, mother's age and number and order of sibling in family unit.

Growth: anthropometric measurements and z-scores

At birth and at each visit, the infants' weight, length, and head circumference were measured by the paediatrician using standardised procedures.

Gastrointestinal tolerance: stool characteristics and gastrointestinal symptoms

Stool consistency and gastrointestinal tolerance from the previous week were recorded during the second and fourth month of life visits. To assess stool consistency, caregivers used the *Brussels Infant and Toddler Stool Scale* (BITSS) and classified their infants' stools as hard, formed, loose or watery based on their resemblance to one of the seven photographs included in the scale [29]. Clinicians registered the value from the first month of life visit. To evaluate gastrointestinal tolerance, caregivers recorded the frequency of their infants' feeding and after-feeding discomfort, feeding and after-feeding regurgitation, feeding and after-feeding vomiting, flatulence, bloating and after-feeding satisfaction.

Infections and associated HCRU

The occurrence of infections was collected from the first to the second and from the second to the fourth month of life. The number, type (respiratory, gastrointestinal, skin, otitis, others [conjunctivitis, fever]), and duration of the infections were registered. Treatment type and duration, number of visits to the paediatrician (emergency or booked) and to the accident and emergency department (A&E), and hospitalisations associated with the infections were recorded to estimate the HCRU.

Caregivers' satisfaction with formula feeding

During the second and fourth month of life visits, caregivers completed an ad-hoc formula satisfaction questionnaire (only formula-fed group). Questions and answering options included: 'What is your general opinion about formula feeding?' Very good/Good/Good enough/Bad/Very bad; and 'In the last week, how much of the product did your infant take?' Less than half/More than half but not all/Whole feeding bottle.

Statistical analysis

Absolute and relative frequencies were calculated to describe qualitative variables, whereas centrality and dispersion measures (mean, standard deviation, quartiles, minimum and maximum) were calculated for quantitative variables. STATA v.14 was used for data analysis.

Sociodemographic and clinical characteristics were compared for baseline differences between the formula feeding and breastfeeding groups using t-test for normal homoscedastic distributions, Mann-Whitney U test for distributions not meeting those requirements and the Pearson Chi^2 test for categorical variables. The same tests were used to evaluate the differences between both groups during follow-up. Only the differences that were statistically significant are indicated. P-value< 0.05 was regarded as statistically significant.

Regarding the infants' growth, differences from the first month of life in mean weight, length and head circumference were calculated to estimate their growth in both groups. Weight-for-age, length-for-age and head

circumference-for-age z-scores were calculated using WHO Child Growth Standards [30].

Percentages of infants with low (never or rarely), medium (sometimes) and high frequency (always or often) of gastrointestinal symptoms were calculated for each symptom for both groups.

Compliance with ethics guidelines

The study was conducted in accordance with the Declaration of Helsinki, followed Good Clinical Practices according to the International Conference on Harmonisation and was evaluated and approved by the reference Ethics Committee for Research on Medicinal Products of the Hospital Clínico Universitario of Valencia, Spain (approval reference number: 211/20). All parents or legal guardians of the subjects gave their written informed consent.

Results

Sociodemographic and clinical characteristics

A total of 159 infants were invited to participate. Of them, 142 were included, 66 and 76 in the formula feeding and breastfeeding groups, respectively (Fig. 2). From these, 61 and 65 infants completed the study, respectively. The most common reasons for drop-out were parental decision ($n = 2$) and transition to mixed feeding ($n = 6$) for the formula feeding and breastfeeding groups, respectively.

The mean (SD) age of the infants at enrolment was 1.0 (0.1) months and 50.8% of them were male. The mean weight, length and head circumference at birth was 3,280 (382) g, 49.8 (1.9) cm and 34.3 (1.0) cm, respectively. The mean weeks of gestation were 39.3 (1.1), and mother's mean age was 33.7 (4.9) years. Most infants were the first or the second in the family unit. Formula feeding and breastfeeding infants showed similar baseline characteristics with no statistically significant differences. However, a higher proportion of formula-fed infants were born by C-section ($p = 0.021$) (Table 1).

Growth

From the first to the fourth month of life, formula-fed infants showed a mean (SD) increase of 2,566 (496)g in weight (Fig. 3a), 9.7 (1.7) cm in length (Fig. 3b) and 4.4 (1.0) cm in head circumference (Fig. 3c). These results were consistent with those of the breastfeeding reference group ($p = 0.895$ for growth, $p = 0.696$ for weight and $p = 0.808$ for length). In addition, in the fourth month of life, formula-fed infants' mean z-scores for weight and length (Fig. 3d), were consistent with breastfeeding. Only statistically significant differences were found between groups in head circumference z-score ($p = 0.010$). All results were in line with WHO standards [30].

Gastrointestinal tolerance

88.5%, 91.8% and 88.5% of formula-fed infants had loose or watery stools in their first, second and fourth month of life, respectively (Fig. 4). In the breastfeeding group, 96.9% in the first and second month of life, and 89.2% in

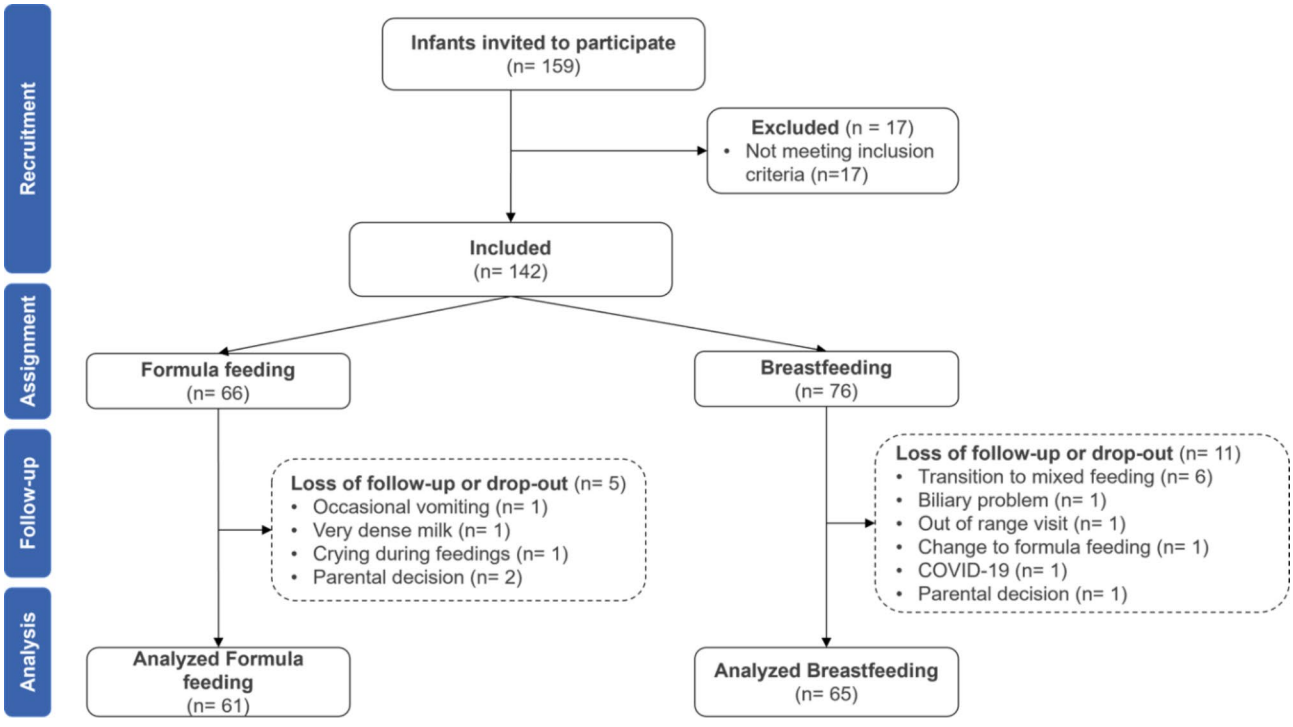

Fig. 2 Flow chart of patient recruitment

Table 1 Sociodemographic and clinical characteristics

		Total population	Formula feeding	Breastfeeding	*p*-value
Sex, n (%)	Male	64 (50.8%)	33 (53.2%)	31 (47.7%)	0.472*
	Female	62 (49.2%)	28 (46.8%)	34 (52.3%)	
Type of labour, n (%)	C-section	21 (16.7%)	15 (24.6%)	6 (9.2%)	**0.021***
	Vaginal labour	105 (83.3%)	46 (75.4%)	59 (90.8%)	
Type of feeding, n (%)	Breastfeeding	65 (51.6%)	-	-	-
	Formula feeding	61 (48.4%)			
Weeks of gestation, mean (SD)		39.3 (1.1)	39.1 (1.1)	39.4 (1.1)	0.092†
Mother's age (years), mean (SD)		33.7 (4.9)	33.1 (6.0)	34.3 (3.5)	0.400†
Infants' age at baseline visit (months), mean (SD)		1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.890†
Number of children in family unit, n (%)	1	58 (46.0%)	28 (45.9%)	30 (45.2%)	0.805†
	2	54 (42.9%)	25 (41.0%)	29 (44.6%)	
	3	9 (7.1%)	5 (8.2%)	4 (6.2%)	
	4	3 (2.4%)	2 (3.3%)	1 (1.5%)	
	5	1 (0.8%)	1 (1.6%)	0 (0.0%)	
	9	1 (0.8%)	0 (0.0%)	1 (1.5%)	
Order of sibling in family unit	1st	56 (44.4%)	28 (45.9%)	28 (43.1%)	0.970†
	2nd	56 (44.4%)	25 (41.0%)	31 (47.7%)	
	3rd	9 (7.1%)	5 (8.2%)	4 (6.2%)	
	4th	3 (2.4%)	2 (3.3%)	1 (1.5%)	
	5th	1 (0.8%)	1 (1.6%)	0 (0.0%)	
	8th	1 (0.8%)	0 (0.0%)	1 (1.5%)	
Anthropometric measurements at birth, mean (SD)	Weight (g)	3,280 (382)	3,274 (398)	3,286 (370)	0.851^
	Length (cm)	49.8 (1.9)	49.9 (1.7)	49.7 (2.1)	0.608^
	H. circumference (cm)	34.3 (1.0)	34.3 (0.9)	34.3 (1.2)	0.980^

p-values in bold indicate statistically significant differences; * Pearson Chi^2 test; † Mann-Whitney U test; ^ t-test. H. circumference: Head circumference; SD: standard deviation

the fourth month, had loose or watery stools. No hard stools were detected in any of the infants at any timepoint. Differences were not statistically significant at any timepoint ($p = 0.067$, $p = 0.210$ and $p = 0.900$ in the first, second and fourth month of life, respectively).

Formula feeding and breastfeeding resulted in adequate gastrointestinal tolerance with no statistically significant differences between groups. In general, most caregivers reported low or medium frequency for most symptoms (Additional File 1). However, 32.8% of formula-fed and 32.3% of breastfed infants suffered from flatulence with high frequency in their second month of life. 15.4% of breastfed infants showed feeding and after-feeding regurgitation with high frequency. In the fourth month of life, high-frequency symptoms were reported in less than 15% infants for each category.

After-feeding satisfaction was good in both groups. More than 75% of infants seemed always or often satisfied after feeding in their second and fourth month of life from the caregiver's perspective (Additional File 1).

Infections and associated HCRU

In the formula feeding group, a total of 12 infants (19.7%) suffered 12 infections episodes from their first to their second month of life, and 16 infants (26.2%) had 21 infections episodes from their second to their fourth month of life (Table 2). The number of infants with infections in the reference breastfeeding group followed the same pattern and showed no statistically significant differences with the formula feeding group ($p = 0.690$ and $p = 0.835$ in the first to second and in the second to fourth month of life periods, respectively). In the whole period, 19 (31.1%) formula-fed, and 23 (35.4%) breastfed infants had infections ($p = 0.614$), of whom 11 and 5 infants had more than one infection, respectively. Infants were mainly treated in primary care setting with a booked appointment. In both groups, respiratory infections were the most common infection: 81.0% of formula-fed and 70.6% of breastfed infants infections from the second to the fourth month of life. Infections had a mean duration of four to seven days.

Nine (14.8%) and ten (16.4%) formula-fed infants in each period, and eight breastfed infants in both periods (12.3%), required treatment. Differences were not statistically significant ($p = 0.688$ and $p = 0.512$, respectively). Antipyretics were the most common prescribed treatment. In the formula feeding group, the mean (SD) duration of treatment with antipyretics was 2.7 (1.5) days in the first period (first to second month) and 3.0 (1.4) days in the second period (second to fourth month). In the breastfeeding group, the mean duration was 1.5 (0.7) days in both periods.

Caregivers' satisfaction with formula feeding

More than 80% of formula feeding caregivers reported having a good or very good opinion about formula feeding in both study visits (Table 3). Moreover, 67.2% and

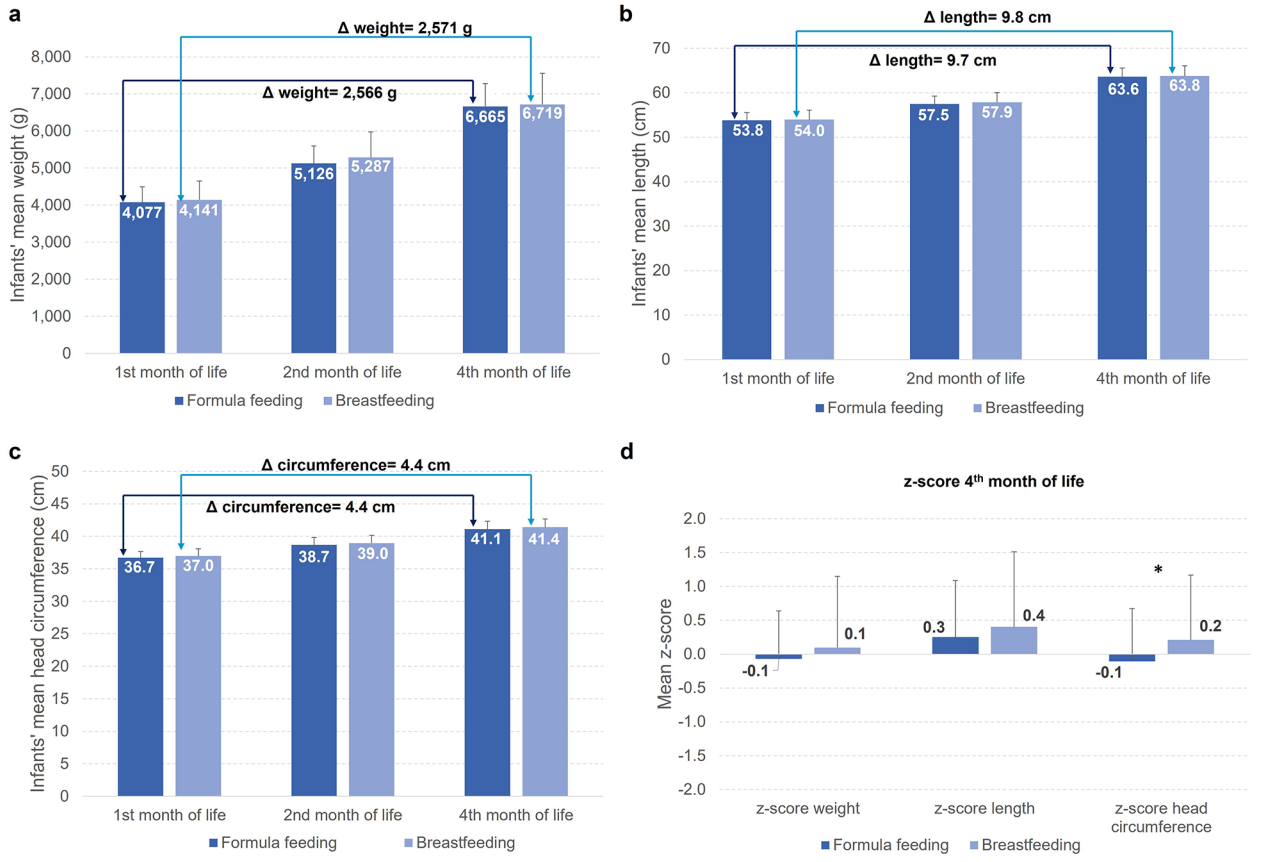

Fig. 3 Formula feeding and breastfeeding infants' growth. Formula feeding and breastfeeding infants mean weight (**a**), length (**b**) and head circumference (**c**) at the different visits. (**d**) Formula feeding and breastfeeding infants mean z-scores at the fourth month of life visit. Error bars represent standard deviation. *Statistically significant differences $p = 0.010$ (Mann-Whitney U test)

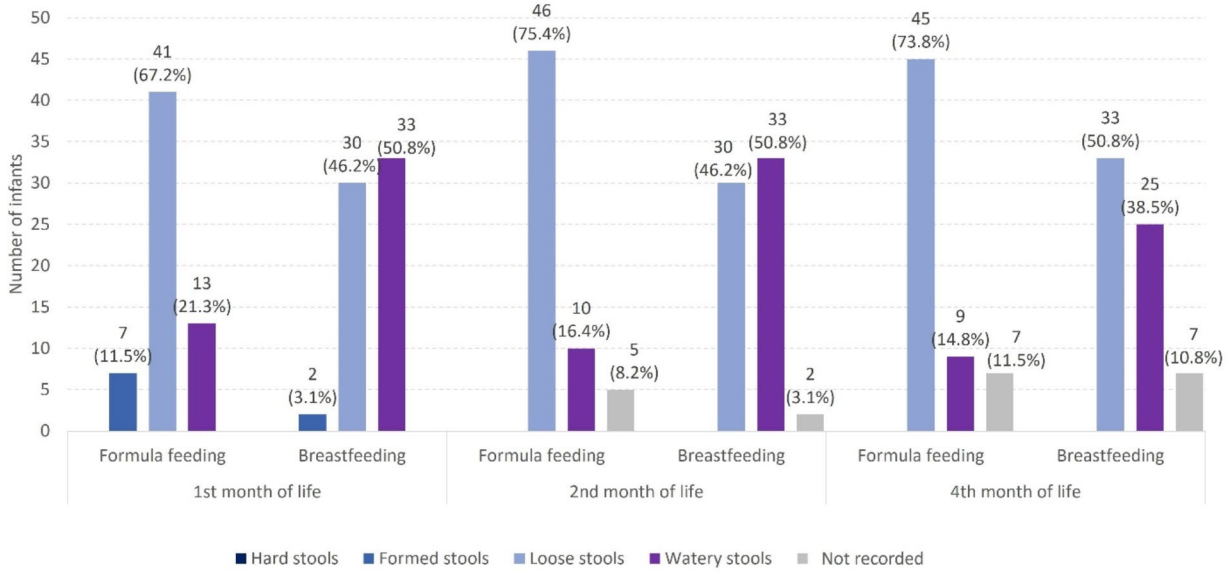

Fig. 4 BITSS scale results of both groups at one, two and four months of life

Table 2 Incidence and type of infections in formula-fed and breastfed infants

Infections	From 1st to 2nd month of life				From 2nd to 4th month of life			
	Formula feeding	*N*	Breastfeeding	*N*	Formula feeding	*N*	Breastfeeding	*N*
Infants with infections, n (%)	**12 (19.7%)**	**61**	**11 (16.9%)**	**65**	**16[†](26.2%)**	**61**	**16[‡](24.6%)**	**65**
Total infections per group, n	**12**	**-**	**11**	**-**	**21**	**-**	**17**	**-**
Respiratory, n (%)	10 (83.3%)	12	7 (63.6%)	11	17 (81.0%)^	21	12 (70.6%)	17
Gastrointestinal, n (%)	1 (8.3%)		1 (9.1%)		1 (4.8%)		3 (17.6%)	
Skin, n (%)	0 (0.0%)		0 (0.0%)		0 (0.0%)		1 (5.9%)	
Otitis, n (%)	0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)	
Others, n (%)	1 (8.3%)		3 (27.3%)		3 (14.3%)		1 (5.9%)	
Conjunctivitis	1 (100%)	1	2 (66.7%)	3	3 (100.0%)	3	1 (100%)	1
Fever	0 (0.0%)		1 (33.3%)		0 (0.0%)		0 (0.0%)	

^Included 2 infections of COVID-19; † 3 patients with ≥ 2 infections; ‡ 1 patient with ≥ 2 infections

Table 3 Satisfaction with formula feeding: General satisfaction and quantity taken

		2nd month of life	4th month of life
What is your general opinion of formula feeding? N (%)	Very good	**32 (52.5%)**	**34 (55.7%)**
	Good	**20 (32.8%)**	**18 (29.5%)**
	Good enough	1 (1.6%)	1 (1.6%)
	Bad	0 (0.0%)	0 (0.0%)
	Very bad	0 (0.0%)	0 (0.0%)
	Not recorded	8 (13.1%)	8 (13.1%)
Inthe last week, how much of the product did your infant take? N (%)	Less than half	1 (1.6%)	0 (0.0%)
	More than half but not all	**11 (18.0%)**	7 (11.5%)
	Whole feeding bottle	**41 (67.2%)**	**46 (75.4%)**
	Not recorded	8 (13.1%)	8 (13.1%)

In bold are the answers with a frequency ≥ 15% for each question

75.4% infants had taken the whole feeding bottle in the preceding week of the second and fourth month of life visits, respectively (Table 3).

Discussion

Human milk is the preferred nutritional choice for newborns. Breast milk reduces the impact of infections in infants, allergic manifestations and metabolic diseases, and promotes the development of balanced intestinal microbiota [31]. Formula feeding is a good alternative when breastfeeding is not possible [1, 10]; however, little evidence is available to further demonstrate its suitability and benefits in the real-world setting. This descriptive study, following routine clinical practice, shows the benefits of a partly fermented infant formula with an improved lipid profile (high β-palmitate levels from milk fat and DHA/ARA) and oligosaccharides (scGOS/lcFOS [9:1] and HMOs) for infant growth, gastrointestinal tolerance, incidence of infection and associated HCRU and parental satisfaction with a 3-month follow-up.

In this study, 61 formula-fed and 65 breastfed healthy infants were included and followed from their first to their fourth month of life visits. The higher proportion of formula-fed infants born by C-section is in line with previous results [32–38]. Type of labour influences the initiation and duration of breastfeeding [35]. Different studies have highlighted the stress, pain, fear of failure and the need of help after surgery associated with breastfeeding [32, 36–38].

The increase in weight, length and head circumference was comparable between formula feeding and breastfeeding groups. There were only differences between groups in terms of head circumference z-score. Nevertheless, both groups increased their head circumference by 4.4 cm. Only when calculating the head circumference z-score a statistically significant difference did emerge, which was considered not clinically relevant. Furthermore, both groups had age-appropriate growth in all parameters, in line with WHO standards in all cases [30]. This suggests that this partly fermented formula with an improved lipid profile and oligosaccharides promotes the development and growth of the infant in accordance with breastfeeding at least in the first four months of life. These results are comparable to other real-world studies evaluating the growth of infants fed with formulas supplemented with HMOs [39, 40], as HMOs supplementation has shown benefits for infant growth [2]. Therefore, these results show how the infant formula under study, with the addition of certain components to infant formula that resemble breastmilk composition, despite the differences, can provide nutritional value and promote infants' growth in an important phase of child development. This would be useful in situations where, either for personal reasons or for health reasons, breastfeeding is

not possible. In this context, clinicians need alternatives for the adequate growth of infants.

Infant formula is often associated with harder stools, constipation and gastrointestinal symptoms [41, 42]. However, in this study more than 88% of formula-fed infants had loose or watery stools during the study period and none had hard stools according to BITSS scale; this may be attributed to the increased β-palmitate levels from the milk fat in this infant formula. Higher β-palmitate levels correlate with reduced formation of calcium soaps and softer stools, more similar to breastfed infants [11, 12, 43]. Most caregivers of formula-fed and breastfed infants reported low or medium frequency for most gastrointestinal symptoms, suggesting that the formula was well-tolerated. Good gastrointestinal tolerance has also been shown with other formulas with increased β-palmitate in clinical trials and real-world studies [43, 44].

Higher incidence of atopic dermatitis and respiratory infections, such as bronchitis and bronchiolitis, have been associated with conventional formulas compared with breastfeeding or mixed feeding in a clinical trial [45] and real-world study [46]. In our study, the infection rate was comparable between groups, with 31.1% of formula-fed and 35.4% of breasted infants having infections, mainly respiratory, similar to other studies carried out with formulas supplemented with HMOs [24, 40]. HMOs, which are not present in conventional formulas, may account for this improvement due to their beneficial effect of promoting immune system development [2, 18]. Similarly to the incidence of infections, HCRU was consistent between groups.

The level of caregiver satisfaction with this infant formula was very high. Infants also seemed satisfied with formula feeding, as most of them had taken the whole feeding bottle in the preceding week of the fourth month of life visit.

The study had several limitations. Firstly, the amounts of formula ingested by the participants were not recorded, nor was the volume of breastmilk. The amount of formula or breastmilk to be ingested was established by the paediatrician according to routine clinical practice, based on the number of feedings in both groups, the frequency, what the caregivers reported and the weight and growth of the infants. As the development and growth of the infants were within normal limits and in line with WHO standards, it was assumed that in both cases the recommendations of the paediatrician were followed in accordance with standard practice and that infants' calorie intake was enough. Secondly, the study included a small sample of subjects from which it is difficult to draw inferences from the general Spanish population, and even though the aim was not to compare the study population with the reference group, a sufficient size was obtained to analyse baseline differences and to determine growth in our sample. Infants from different geographic regions in Spain were included. Although inferences cannot be made for the rest of the population, this study shows robust results on infants' growth, and they can be applied to other populations and geographic areas. Thirdly, this was a real-world study and therefore there was no randomisation of subjects to groups, which would imply a selection bias. However, in this case randomisation would not be appropriate, as the general recommendation is that breastfeeding should be continued until six months of age. The baseline characteristics of the infants were analysed, and no significant differences were observed between the groups, with the only exception of the type of delivery. In addition, the recruitment process by the paediatricians was completely free and voluntary, and families were informed that they could withdraw from the study at any point, to minimize the risk of coercion or undue influence. Moreover, gastrointestinal tolerance and stool consistency were assessed by caregivers in relation to previous weeks, which could lead to recall bias and reflect subjective results based on caregivers' perceptions. Since gastrointestinal signs and symptoms may be difficult for caregivers to interpret, we used a validated stool consistency scale and a short and simple symptom frequency questionnaire in our study.

Conclusions

This prospective descriptive observational study showed appropriate growth of healthy formula-fed infants during the first four months of life, consistent with WHO standards and in line with the growth of the breastfeeding reference group. Gastrointestinal tolerance and incidence of infections were also adequate and consistent in both groups. In addition, caregivers of formula-fed infants were satisfied with this partly fermented infant formula with an improved lipid profile (high β-palmitate levels from milk fat and DHA/ARA) and oligosaccharides (scGOS/lcFOS [9:1] and HMOs).

Abbreviations

A&E	Accident and Emergency department
ARA	Arachidonic acid
BITSS	Brussels Infant and Toddler Stool Scale
DHA	Docosahexaenoic acid
HCRU	Healthcare resource utilisation
HMOs	Human milk oligosaccharides
lcFOS	Long chain fructo-oligosaccharides
scGOS	Short chain galacto-oligosaccharides
SD	Standard deviation
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-025-05446-6.

Additional File 1

Acknowledgements
The authors would like to thank all the infants and their families who participated in the study, and the collaborating investigators: Ignacio Sorribes Monrabal, Amelia Peris Vidal, Beatriz Tomás Aguirre, Gracia Castellanos Gómez, María Jesús Muñoz del Barrio, Ana Ramírez González, Ana Hervás Matamala, Ana Malo de Molina, Adriana Céspedes, Manuel Herreros, María José Gómez, Celestin Ebobo, Agata Radwanska, Maite Asensi Monzó, Susana Peñarroja Peirats, Ana Gómez Palonés, Idoia Hernández Monleón, Jose Luis Martin, Manolo Begara and Laura Núñez Martínez. Finally, the authors would like to acknowledge Ester de la Cruz, who received honoraria for medical writing.

Author contributions
LBB, EV, FJPS and CMC contributed to the conception and design of the study, to the analysis and interpretation of data and to the first draft and revision of the manuscript. LBB, AA, PS, UM, CCR, BA and CMC contributed to data collection. All authors revised and approved the final version of the manuscript and accept responsibility for the accuracy and integrity of the final manuscript as submitted.

Funding
The study was financed by Nutricia-Danone Specialized Nutrition.

Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was evaluated and approved by the reference Ethics Committee for Research on Medicinal Products of the Hospital Clínico Universitario of Valencia. All parents or legal guardians of the subjects gave their written informed consent.

Consent for publication
Not applicable.

Competing interests
LBB and CMC have received honoraria and support from infant nutrition industries for participation in presentations, courses, expert committees, and support with registration, travel and accommodation for attending conferences related to paediatrics. EVD, FJPS work for an independent research entity and have received fees for their contribution to project development and medical writing. The rest of the authors declare no conflicts of interest related to this study. The rest of the authors declare no conflicts of interest related to this study. The funding source of this study (Nutricia-Danone Specialized Nutrition) did not interfere with the conduct, the analysis nor the interpretation of the study findings.

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Received: 23 February 2024 / Accepted: 19 January 2025
Published online: 25 March 2025

References

1. World Health Organization (WHO). Breastfeeding. https://www.who.int/health-topics/breastfeeding#tab=tab_2. Accessed 16 October. 2023.
2. Vandenplas Y, Berger B, Carnielli VP, Ksiazyk J, Lagstrom H, Sanchez Luna M, et al. Human milk oligosaccharides: 2'-Fucosyllactose (2'-FL) and Lacto-N-Neotetraose (LNnT) in infant Formula. Nutrients. 2018;10(9):1161.
3. Victora CG, Bahl R, Barros AJ, Franca GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387(10017):475–90.
4. Pereyra-Elias R, Quigley MA, Carson C. To what extent does confounding explain the association between breastfeeding duration and cognitive development up to age 14? Findings from the UK Millennium Cohort Study. PLoS ONE. 2022;17(5):e0267326.
5. World Health Organization WHO. Infant and young child feeding. https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding. Accessed 16 October 2023.
6. Theurich MA, Davanzo R, Busck-Rasmussen M, Diaz-Gomez NM, Brennan C, Kylberg E, et al. Breastfeeding Rates and Programs in Europe: a Survey of 11 National Breastfeeding committees and representatives. J Pediatr Gastroenterol Nutr. 2019;68(3):400–7.
7. Perez-Escamilla R, Tomori C, Hernandez-Cordero S, Baker P, Barros AJD, Begin F, et al. Breastfeeding: crucially important, but increasingly challenged in a market-driven world. Lancet. 2023;401(10375):472–85.
8. Pierro J, Abulaimoun B, Roth P, Blau J. Factors Associated with Supplemental Formula Feeding of Breastfeeding infants during Postpartum Hospital stay. Breastfeed Med. 2016;11:196–202.
9. Kim SY, Yi DY. Components of human breast milk: from macronutrient to microbiome and microRNA. Clin Exp Pediatr. 2020;63(8):301–9.
10. Martin CR, Ling PR, Blackburn GL. Review of infant feeding: key features of breast milk and infant formula. Nutrients. 2016;8(5):279.
11. Rivero Urgell M, Santamaría Orleans A, Rodríguez-Palmero Seuma M. The importance of functional ingredients in pediatric milk formulas and cereals. Nutrición Hospitalaria. 2005;20(2):135–46.
12. Yao M, Lien EL, Capeding MR, Fitzgerald M, Ramanujam K, Yuhas R, et al. Effects of term infant formulas containing high sn-2 palmitate with and without oligofructose on stool composition, stool characteristics, and bifidogenicity. J Pediatr Gastroenterol Nutr. 2014;59(4):440–8.
13. Havlicekova Z, Jesenak M, Banovcin P, Kuchta M. Beta-palmitate - a natural component of human milk in supplemental milk formulas. Nutr J. 2016;15:28.
14. Fanaro S, Boehm G, Garssen J, Knol J, Mosca F, Stahl B, et al. Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. Acta Paediatr. 2005;94(0):22–6.
15. Hegar B, Wibowo Y, Basrowi RW, Ranuh RG, Sudarmo SM, Munasir Z, et al. The role of Two Human Milk Oligosaccharides, 2'-Fucosyllactose and Lacto-N-Neotetraose, in Infant Nutrition. Pediatr Gastroenterol Hepatol Nutr. 2019;22(4):330–40.
16. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012;22(9):1147–62.
17. Kunz C, Meyer C, Collado MC, Geiger L, Garcia-Mantrana I, Bertua-Rios B, et al. Influence of gestational age, Secretor, and Lewis Blood Group Status on the Oligosaccharide content of human milk. J Pediatr Gastroenterol Nutr. 2017;64(5):789–98.
18. Plaza-Diaz J, Fontana L, Gil A. Human milk oligosaccharides and Immune System Development. Nutrients. 2018;10(8):1038.
19. Urashima T, Hirabayashi J, Sato S, Kobata A. Human milk oligosaccharides as essential tools for Basic and Application studies on Galectins. Trends Glycoscience Glycotechnology. 2018;30(172):SE51–65.
20. Stahl B, Thurl S, Zeng JR, Karas M, Hillenkamp F, Steup M, et al. Oligosaccharides from human milk as revealed by Matrix-assisted laser Desorption/Ionization Mass Spectrometry. Analyt Biochem. 1994;223:218–26.
21. Coulier L, Timmermans J, Bas R, Van Den Dool R, Haaksman I, Klarenbeek B, et al. In-depth characterization of prebiotic galacto-oligosaccharides by a combination of analytical techniques. J Agric Food Chem. 2009;57(18):8488–95.
22. Finke B, Stahl B, Pritschet M, Facius D, Wolfgang J, Boehm G. Preparative continuous annular chromatography (P-CAC) enables the large-scale fractionation of Fructans. Agric Food Chem. 2002;50:4743–8.
23. Boehm G, Fanaro S, Jelinek J, Stahl B, Marini A. Prebiotic concept for infant nutrition. Acta Paediatr Suppl. 2003;91(441):64–7.
24. Vandenplas Y, de Halleux V, Arciszewska M, Lach P, Pokhylko V, Klymenko V et al. A Partly Fermented Infant Formula with Postbiotics Including 3'-GL, Specific Oligosaccharides, 2'-FL, and Milk Fat Supports Adequate Growth, Is Safe and Well-Tolerated in Healthy Term Infants: A Double-Blind, Randomised, Controlled, Multi-Country Trial. Nutrients. 2020;12(11).
25. Rodriguez-Herrera A, Mulder K, Bouritius H, Rubio R, Munoz A, Agosti M et al. Gastrointestinal Tolerance, Growth and Safety of a Partly Fermented Formula

with Specific Prebiotics in Healthy Infants: A Double-Blind, Randomized, Controlled Trial. Nutrients. 2019;11(7).
26. Instituto Nacional de Estadística (INE). Encuesta Nacional de Salud 2017. MSCBS-INE 2017 2017. https://www.ine.es/ss/Satellite?L=es_ES&c=INESeccion_C&cid=1259926457058&p=1254735110672&pagename=ProductosYServicios/PYSLayout&param1=PYSDetalle&param3=1259924822888#:~:text=A%2520los%25206%2520meses%252C%2520un,natural%2520(39%252C0%2525).&text=Durante%2520el%2520tiempo%2520de%2520ocio%2520un%252508%252C4%2525%2520de%2520hombres,de%2520Salud%2520en%2520Espa%25C3%25B1a%25202020.]. Accessed 27 October 2023.
27. Instituto Nacional de Estadística (INE). Población residente por fecha, sexo y edad 2020. https://www.ine.es/jaxiT3/Datos.htm?t=56934. Accessed 27 October 2023.
28. World Health Organization (WHO). 4-month weight increments (g) GIRLS/BOYS. Birth to 24 months (z-scores). https://www.who.int/docs/default-source/child-growth/child-growth-standards/indicators/weight-velocity/boys-simplified-field-table--4-month-increments-birth-to-24-months-(z-scores).pdf?sfvrsn=38c7fce9_0.
29. Huysentruyt K, Koppen I, Benninga M, Cattaert T, Cheng J, De Geyter C, et al. The Brussels Infant and Toddler Stool Scale: a study on Interobserver Reliability. J Pediatr Gastroenterol Nutr. 2019;68(2):207–13.
30. World Health Organization (WHO). WHO child growth standards: growth velocity based on weight, length and head circumference: methods and development. 2009. https://www.who.int/publications/i/item/9789241547635.
31. Ayechu-Muruzabal V, van Stigt AH, Mank M, Willemsen LEM, Stahl B, Garssen J, et al. Diversity of Human Milk Oligosaccharides and effects on Early Life Immune Development. Front Pediatr. 2018;6:239.
32. Hobbs AJ, Mannion CA, McDonald SW, Brockway M, Tough SC. The impact of caesarean section on breastfeeding initiation, duration and difficulties in the first four months postpartum. BMC Pregnancy Childbirth. 2016;16:90.
33. Chen C, Yan Y, Gao X, Xiang S, He Q, Zeng G, et al. Influences of Cesarean Delivery on Breastfeeding practices and Duration: a prospective cohort study. J Hum Lact. 2018;34(3):526–34.
34. Erbaydar NP, Erbaydar T. Relationship between caesarean section and breastfeeding: evidence from the 2013 Turkey demographic and health survey. BMC Pregnancy Childbirth. 2020;20(1):55.
35. Wu Y, Wang Y, Huang J, Zhang Z, Wang J, Zhou L, et al. The association between caesarean delivery and the initiation and duration of breastfeeding: a prospective cohort study in China. Eur J Clin Nutr. 2018;72(12):1644–54.
36. Chaplin J, Kelly J, Kildea S. Maternal perceptions of breastfeeding difficulty after caesarean section with regional anaesthesia: a qualitative study. Women Birth. 2016;29(2):144–52.
37. Tully KP, Ball HL. Maternal accounts of their breast-feeding intent and early challenges after caesarean childbirth. Midwifery. 2014;30(6):712–9.
38. Jikijela TP, James S, Sonti BSI. Caesarean section deliveries: experiences of mothers of midwifery care at a public hospital in Nelson Mandela Bay. Curationis. 2018;41(1):e1–9.
39. Jochum F, Meyer-Krott M, Hubler T, Lorenz M, Bedikian R, Zakarian J, et al. Real-world evidence study on tolerance and growth in infants fed an infant formula with two human milk oligosaccharides vs mixed fed and exclusively breastfed infants. Mol Cell Pediatr. 2023;10(1):7.
40. Roman Riechmann E, Moreno Villares JM, Dominguez Ortega F, Carmona Martinez A, Pico Sirvent L, Santana Sandoval L, et al. Real-world study in infants fed with an infant formula with two human milk oligosaccharides. Nutr Hosp. 2020;37(4):698–706.
41. Forsyth B, McCarthy PL, Leventhal JM. Problems of early infancy, formula changes, and mothers' beliefs about their infants. J Pediatr. 1985;106(6):1012–7.
42. Quinlan PT, Lockton S, Irwin J, Lucas AL. The relationship between stool hardness and stool composition in breast- and formula-fed infants. J Pediatr Gastroenterol Nutr. 1995;20(1):81–90.
43. Nowacki J, Lee H-C, Lien R, Cheng S-W, Li S-T, Yao M, et al. Stool fatty acid soaps, stool consistency and gastrointestinal tolerance in term infants fed infant formulas containing high sn-2 palmitate with or without oligofructose: a double-blind, randomized clinical trial. Nutr J. 2014;13:105.
44. Mao M, Zhang L, Ge J, Yan J, Northington R, Yao M, et al. Infant feeding regimens and gastrointestinal tolerance: a Multicenter, prospective, observational cohort study in China. Glob Pediatr Health. 2018;5:2333794X17750271.
45. Plaza-Diaz J, Ruiz-Ojeda FJ, Morales J, de la Torre AIC, Garcia-Garcia A, de Prado CN et al. Effects of a Novel Infant Formula on Weight Gain, Body Composition, Safety and Tolerability to infants: the INNOVA 2020 study. Nutrients. 2022;15(1).
46. Gomez-Acebo I, Lechosa-Muniz C, Paz-Zulueta M, Sotos TD, Alonso-Molero J, Llorca J, et al. Feeding in the first six months of life is associated with the probability of having bronchiolitis: a cohort study in Spain. Int Breastfeed J. 2021;16(1):82.

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