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REVIEW ARTICLE

Nutrition

Technical review by the ESPGHAN Special Interest Group on Gut Microbiota and Modifications on the health outcomes of infant formula supplemented with synbiotic

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Abstract

This technical review—one of five developed by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Special Interest Group on Gut Microbiota and Modifications (SIG-GMM)—supports the preparation of a position paper on the use of biotic- and synbiotic-supplemented infant formulas. This paper also presents the statements made by the SIG-GMM after performing a technical review to evaluate the clinical effects of synbiotic-supplemented infant formulas in healthy full-term infants (0–12 months), as emerged from studies published before 2024. The review focused on the following clinical outcomes (if available): anthropometric measurements, safety, tolerability, stool frequency and consistency, infantile colic or crying, gastrointestinal symptoms, infections and antibiotic use, and allergic disorders. Following the review, all members of the SIG anonymously voted on each statement, scoring them between 0 and 9. A statement was accepted when ≥75% of the members scored >6. The technical review identified 16

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randomized controlled trials that evaluated the clinical effects of synbiotic-supplemented infant formula in healthy full-term infants. The studies varied in terms of synbiotic composition, study design, intervention duration, and outcomes. Formulas supplemented with synbiotics studied so far were well tolerated and showed no significant difference compared to the non-supplemented formulas in growth parameters, gastrointestinal symptoms, stool characteristics, or safety. This technical review serves as the background for formulating recommendations on the use of synbiotic-supplemented infant formula in healthy infants studied so far.

KEYWORDS

anthropometry, biotics, breastfeeding, formula feeding, microbiome, nutrition

1 | INTRODUCTION

In 2020, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined synbiotics as a mixture of live microorganisms and substrate(s) selectively utilized by host microorganisms that confer a health benefit on the host.[1] ISAPP also distinguished a synergistic synbiotic, in which the prebiotic substrate is selectively utilized by the co-administered microorganism(s), from a complementary synbiotic, where the prebiotic component targets autochthonous microorganisms (the resident microbiota).[1] Infant formulas containing synbiotics have been used for more than 15 years. In 2011, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published a systematic review and comment by the Committee on Nutrition on the supplementation of infant formula with probiotics and/or prebiotics.[2] Only three randomized controlled trials (RCTs) evaluating four types of synbiotics were identified at that time.[3–5] Since then, several studies investigated diverse synbiotics in infant formulas.[6] Additionally, two systematic reviews were recently published.[7,8] Given these developments, the ESPGHAN Special Interest Group on Gut Microbiota and Modifications (SIG-GMM) has undertaken this updated technical review. Our aim is to evaluate the recent evidence on the use of synbiotic-supplemented formulas for infant nutrition, possibly warranting an update of the relevant societal clinical practice position paper.

What is Known

- Synbiotics are a mixture of live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host.
- Synbiotic-supplemented infant formulas are available for over 15 years, but their clinical benefit is unclear.

What is New

- Sixteen randomized controlled studies show that synbiotic-supplemented formulas are well-tolerated and do not raise growth or safety concerns.
- A synbiotic-supplemented formula softened stools compared to the control group, but there is no obvious evidence of benefit on infant crying or gastrointestinal symptoms.
- Some trials reported reducedrate of infections (gastrointestinal or respiratory) in the synbiotic group, although this result was inconsistent.

2 | METHODS

The ESPGHAN SIG-GMM published five technical reports to evaluate the safety and efficacy of infant formula supplemented with probiotics, prebiotics, synbiotics, postbiotics, and human identical milk oligosaccharide with the following priority research questions: (i) “Are there, and if so, which clinically relevant benefits have been demonstrated by the supplementation with any biotic to infant formula?” and (ii) “Should biotics be added to infant formula? If yes, which specific biotic and for which indications?” The present technical review aims to support the development of a position paper on the use of synbiotic-supplemented infant formula.

A literature review was conducted up to December 31, 2023, across Cochrane, DARE, CENTRAL, PubMed, and EMBASE databases, along with searches on ClinicalTrials.gov. The search terms included “synbiotic” OR “symbiotic” AND “formula” OR “infant formula” OR “infant nutrition.” Some studies preferred to use the term “probiotic and prebiotic combination” instead of “synbiotic,” so the articles our study group found while preparing other “probiotic” and “prebiotic” technical reviews were also looked at. Only English-language papers were considered. Peer-reviewed RCTs, meta-analyses, systematic reviews, and

previous ESPGHAN recommendations were used in the analyses. Reference lists obtained from the identified studies and key review articles, including previously published meta-analyses, were also evaluated.

We only included RCTs that evaluated healthy term-born infants under 1 year old who were receiving infant formula. We included studies that compared synbiotic-supplemented infant formula with non-supplemented infant formula or human milk in healthy infants. Synbiotics administered as a supplement, not being part of the formula composition when manufactured, were excluded. We also excluded formulas that contained partially or extensively hydrolyzed proteins. We excluded studies that dealt with preterm infants, infants with any condition, such as cow milk allergy, or any disease. We performed an initial screening of the titles, abstracts, and keywords of each identified record, and then retrieved the full text of potentially relevant publications. At least two reviewers independently assessed the eligibility of each potentially relevant trial using the inclusion criteria. Figure 1 displays a flowchart detailing the study selection process in accordance with PRISMA guidelines.

Data extraction included study characteristics, synbiotic composition, patient characteristics, intervention details, and follow-up duration. The review focused on the following clinical outcomes (if available): anthropometric measurements, safety, tolerability, stool frequency and consistency, infantile colic or crying, gastrointestinal symptoms, infections and antibiotic use, and allergic disorders. Microbiota composition was not a priority focus of this technical report. The Cochrane Collaboration tool was used to assess the risk of bias, which was evaluated by three authors for each included study.[9] The ESPGHAN SIG-GMM reports evidence and statements related to each specific synbiotic. The modified Delphi process was used to establish a consensus on the statements. all members of the SIG anonymously voted on each statement, scoring them between 0 and 9. A statement was accepted when ≥75% of the members scored >6.

3 | RESULTS

Sixteen RCTs enrolled healthy infants (an inclusion criterion) and reported clinical outcomes (Table 1). Additionally, we identified one narrative review,[6] one recent systematic review with network meta-analysis on infant formulas supplemented with probiotics or synbiotics,[7] and another systematic review and meta-analysis on infant formulas supplemented with biotics and respiratory infections.[8] The most commonly prebiotic component is represented by long-chain (lc) fructooligosaccharides (FOS) and short-chain (sc) galactooligosaccharides (GOS) added to infant formulas up to 0.8 g/100 mL. The risk of bias is reported in Table 2.

The studies are herein presented and summarized according to their specific probiotic and prebiotic components.

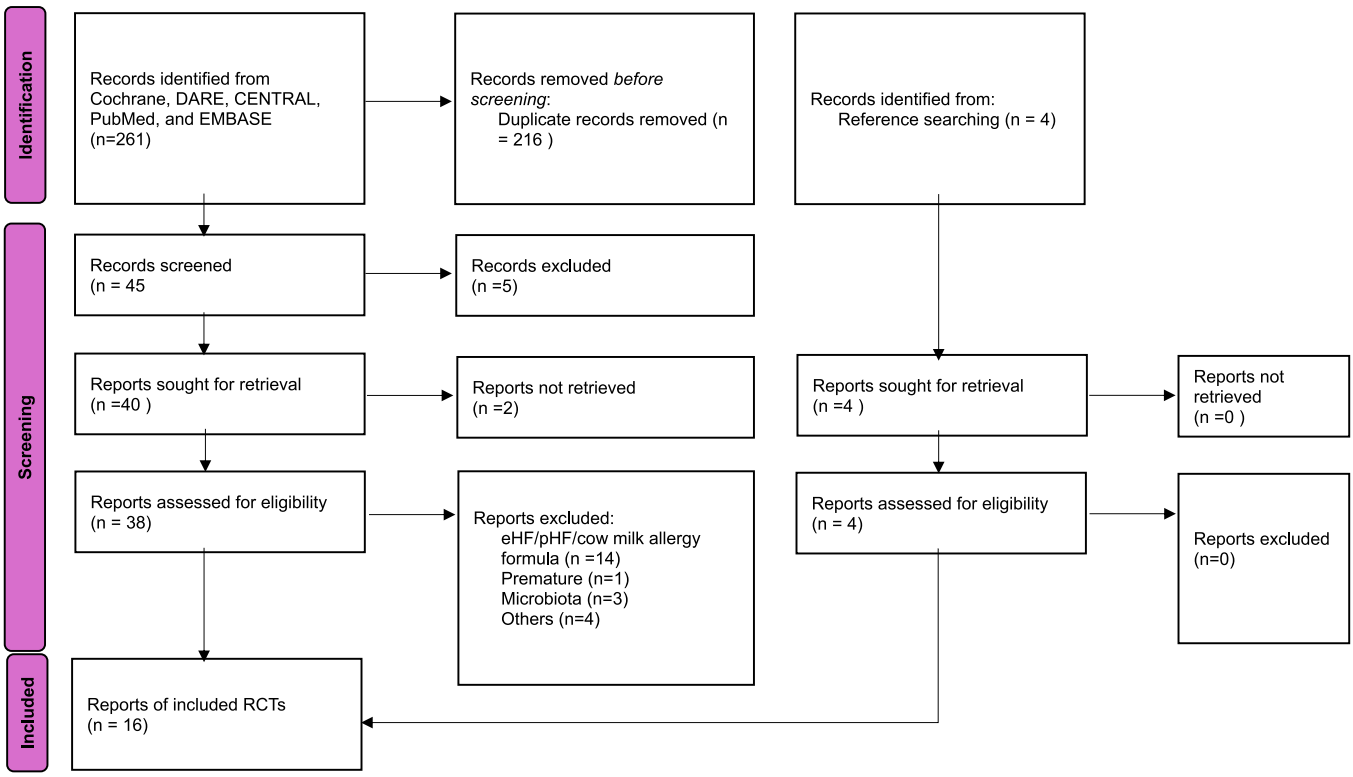

FIGURE 1 Flow chart of identification of studies. eHF, extensively hydrolyzed formula; pHF, partially hydrolyzed formula.

TABLE 1 The main characteristics of the trials were included in the technical report.

Publication	Design	Biotics	Study period	Study population	Primary outcome	Duration
Cooper et al.[10] 2016, South Africa	DB, RCT	*B. lactis* CNCM-I-3446 (1×10^7 CFU/g) + BMOS generated from whey permeate (8 g/L; GOS, 3′-SL, 6′-SL)	October 2008 and October 2013	Healthy, full-term, newborn infants were recruited from HIV-positive mothers Synbiotic formula ($n = 207$) Control ($n = 214$)	Fecal bifidobacteria count at 10 days, and daily weight gain (g/day) between 10 days and 4 months.	12 months
Simeoni et al.[11] 2016, France, Poland	DB, RCT multicenter	*B. lactis* CNCM-I-3446 (1×10^7 CFU/g) + BMOS generated from whey permeate (8 g/L; GOS, 3′-SL, 6′-SL)	–	Synbiotic formula ($n = 32$) Control ($n = 30$) Breastfed group ($n = 29$)	The impact of a synbiotic formula on gut microbiota composition in healthy infants.	3 months
Radke et al.[12] 2017, Germany, France, the Netherlands	DB, RCT multicenter	*B. lactis* CNCM-I-3446 (1×10^7 CFU/g) + BMOS generated from whey permeate (8 g/L; GOS, 3′-SL, 6′-SL)	October 2008 to December 2013	Synbiotic formula ($n = 206$) Control ($n = 207$) Breastfed group ($n = 63$)	The mean incidence of diarrhea and all infections with fever during the first 6 months and 1 year of life.	12 months
Castanet et al.[13] 2020, France, Greece, Austria	DB, RCT multicenter	*B. lactis* CNCM-I-3446 ($3.7 \pm 2.1 \times 10^4$ CFU/g) + BMOS generated from whey permeate (6 g/L; GOS, 3′-SL, 6′-SL) Native bovine lactoferrin	September 2009 to December 2010	Probiotic formula ($n = 44$) Synbiotic formula ($n = 43$) Control formula ($n = 40$) Breastfed ($n = 75$)	The fecal calprotectin mean levels (µg/g of feces) at 2 and 4 weeks of age.	8 weeks
Bocquet et al.[14] 2013, France	DB, RCT multicenter	*B. lactis* CNCM-I-3446 (1×10^7 CFU/g) + GOS-FOS (9:1; 4 g/L)	February 2008 and November 2009	Probiotic formula ($n = 267$) Synbiotic formula ($n = 261$)	The mean number of infectious episodes during the first year of life.	12 months
Puccio et al.[4] 2007, Italy	DB, RCT	*B. longum* BL999 (BAA-999, 2×10^7 CFU) GOS and a FOS (9:1; 4 g/L)	–	Synbiotic formula ($n = 42$) Control formula ($n = 55$)	Weight gain during the first 4 months of the life of infants.	112 days
Chua et al.[15] 2017, Thailand	Exploratory, DB RCT	*B. breve* M-16V (7.5×10^8 CFU/100 mL) scGOS/lCFOS (9:1, 8 g/L)	June 2011 and April 2013	153 subjects delivered by C-section synbiotic ($n = 52$), prebiotic ($n = 51$), control formula ($n = 50$). Non-randomized reference vaginally delivered infant ($n = 30$)	The determination of total fecal bifidobacteria.	24 weeks
Phavichtir et al.[16] 2020, Thailand	Exploratory, DB RCT	Syn4: *B. breve* M-16V (1×10^4 CFU/100 mL) + scGOS/lCFOS (9:1, 8 g/L) Syn6: *B. breve* M-16V (1×10^6 CFU/100 mL) + scGOS/lCFOS (9:1, 8 g/L)	May 2013 and September 2015	Syn4 formula ($n = 81$) Syn6 formula ($n = 82$) Control formula ($n = 84$)	To evaluate the bifidogenic effect of an infant formula containing synbiotics with two doses of *B. breve* M-16V (either 1×10^4 cfu/mL or 1×10^6 cfu/mL), in combination with scGOS/lcFOS (9:1) in healthy infants aged 6–19 weeks.	8 weeks

TABLE 1 (Continued)

Publication	Design	Biotics	Study period	Study population	Primary outcome	Duration
Gil-Campos et al.[17] 2012, Spain	DB, RCT	*L. fermentum* CECT5716 (1×10^7 CFU/g) with/without GOS (0.3 g/100 mL)	May 2009 and September 2010	Synbiotic formula ($n = 61$) Prebiotic formula ($n = 60$)	Average weight gain between baseline and 4 months of age.	6 months
Maldonado et al.[18] 2012, Spain	DB, RCT	*L. fermentum* CECT5716 (2×10^8 CFU/day) with/without GOS (0.3 g/100 mL)	May 2008 and July 2009	Synbiotic formula ($n = 97$) Prebiotic formula ($n = 91$)	The incidence of infections, including gastrointestinal, respiratory, otitis, urinary, and other less common infections.	6 months
Szajewska et al.[19] 2017, Poland	DB, RCT multicenter	*Lactobacillus paracasei* ssp. *paracasei* strain F19 (*Lactobacillus* F19); 1×10^9 CFU per L of ready-to-use formula with or without GOS/FOS	January 2011 and March 2016	Synbiotic formula ($n = 90$) Prebiotic formula ($n = 92$)	Growth (body weight, length, and head circumference) during the first year of life.	12 months
Chouraqui et al.[3] 2008, France	DB, RCT	(EF1) *Bifidobacterium longum* BL999 (BL999), *Lactobacillus rhamnosus* LP (LPR), GOS + scFOS (9:1, 4 g/L) (EF2) *Bifidobacterium longum* BL999, *Lactobacillus paracasei* ST11, GOS + scFOS (9:1, 4 g/L)	October 2004 and 2005	EF1 synbiotic formula ($n = 54$) EF2 synbiotic formula ($n = 60$) Probiotic formula ($n = 60$) Control formula ($n = 53$)	Weight gain in infants fed the study formulas from 14 to 112 days of age.	12 months
Nieto-Ruiz et al., 2019[20] Sepulveda-Valbuena et al., 2021[21] Herrmann et al., 2021,[22] Spain	DB, RCT	*B. longum* subsp. *infantis* CECT 7210 *Lacticaseibacillus rhamnosus* LCS-742 a FOS and inulin (ratio 1:1)	–	Synbiotic formula ($n = 85$) Control formula ($n = 85$) Breastfed ($n = 50$)	Neurocognitive development at 2nd, 3rd, 4th, 6th, 12th, 18th, 30th months, 4 and 6 years, and functional magnetic resonance imaging at 6 years.	6 years
Rozé et al.[23] 2012, France	DB, RCT multicenter	*Lacticaseibacillus rhamnosus* LCS-742 *Bifidobacterium longum* subsp *infantis* M63; 96% GOS, 4% scFOS	–	Synbiotic formula ($n = 48$) Control formula ($n = 49$)	Weight at 6 months of age.	6 months
Vlieger et al.[5] 2009, The Netherlands	RCT	*L. paracasei* ssp. *paracasei*/g (*L. casei* CRL-431) + *B. animalis* ssp. *lactis* (Bb12) GOS 2.4 g/L	November 2004 and January 2007	Synbiotic formula ($n = 67$) Control formula ($n = 59$)	Differences in growth parameters at 3 months of age.	6 months
Cohen et al.[24] 2013, France	DB, RCT	*Streptococcus thermophilus* NCC 2496, *Streptococcus salivarius* DSM 13084, *Lacticaseibacillus rhamnosus* LPR CGMCC 1.3724, Raftilose (FOS)	November 2007 and April 2009	Synbiotic formula ($n = 112$) Control formula ($n = 112$)	Incidence of acute otitis media in each group in the 12 months.	12 months

Abbreviations: 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose; B., *Bifidobacterium*; BMOS, bovine milk-derived oligosaccharides; BW, birth weight; CFU, colony forming unit; DB, double-blind; FOS, fructooligosaccharides; GOS, galactooligosaccharides; HIV, human immunodeficiency virus; L, *Lactobacillus*; Lc, *Lacticaseibacillus*; lcFOS, long-chain fructooligosaccharides; Lig, *Ligilactobacillus*; Lim, *Limisolactobacillus*; RCT, randomized controlled trial; scGOS, short-chain galactooligosaccharides.

TABLE 2 Risk of bias of included studies.

Publication	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Cooper et al.[10]	Unclear	High risk	Low risk	Unclear	Low risk	Unclear	Unclear
Simeoni et al.[11]	Unclear	Unclear	Low risk	Low risk	Low risk	Low risk	Low risk
Radke et al[12]	Low risk	Unclear	Low risk	Low risk	Low risk	Unclear	Unclear
Castanet et al.[13]	Low risk	Low risk	Low risk	Low risk	Unclear	Unclear	Unclear
Bocquet et al.[14]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Puccio et al.[4]	Unclear	High risk	Unclear	Unclear	Unclear	Unclear	Unclear
Chua et al.[15]	Low risk	Unclear	Unclear	Unclear	Low risk	Low risk	Low risk
Phavichtir et al.[16]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Gil-Campos et al.[17]	Low risk	Low risk	Unclear	Low risk	Low risk	Unclear	Low risk
Maldonado et al.[18]	Unclear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Szajewska et al.[19]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Chouraqui et al.[3]	Unclear	Unclear	Low risk	Low risk	Unclear	Unclear	Unclear
Nieto-Ruiz et al.,[20] Sepulveda-Valbuena et al.,[21] Herrmann et al.[22]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Rozé et al.[23]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Vlieger et al.[5]	Low risk	Unclear	High risk	High risk	Low risk	Low risk	Unclear
Cohen et al.[24]	Low risk	Low risk	Low risk	Low risk	Unclear	Unclear	Unclear

3.1 | *Bifidobacterium animalis* ssp. lactis (CNCM I-3446) and bovine milk-derived oligosaccharides (BMOS)

Four double-blind RCTs have been published evaluating *Bifidobacterium* (B.) *lactis* CNCM I-3446 in addition to prebiotics.[10–13] In four studies, the prebiotic component was a mixture of BMOS generated from whey permeate (containing GOS and other milk oligosaccharides, such as 3′- and 6′-sialyllactose [3′- and 6′-SL])[10–13] In three studies, the probiotic and prebiotic content used in the synbiotic formulation was the same (*B. lactis* CNCM I-3446 1×10^7 CFU and BMOS as 8 g/L).[10–12] Another RCT used the same synbiotic at different concentrations of *B. lactis* CNCM I-3446 ($3.7 + 2.1 \times 10^4$ CFU/g powder formula) and BMOS (6 g/L in reconstituted formula) with additional native bovine lactoferrin (1 g/L).[13] In all studies, mean weight, length, body mass index, and head circumference-for-age *z*-scores were not significantly different between the synbiotic and control formula groups.[10–13] However, in one study, only in boys born by vaginal delivery, those consuming the synbiotic formula had a significantly greater percentage of bone mineral content at 4 months ($p = 0.005$), and a significantly greater adjusted mean lean mass (grams) at 12 months ($p = 0.002$) compared to those consuming the control formula.[10] These studies found a lower consistency of stools in the synbiotic group compared to the control infants.[10–13] No study showed a significant benefit in, spitting up,[10,11,13] or colic.[10–13] Radke et al.[12] showed no difference in flatulence between the synbiotic and control groups during the 12-month study period, except at 3 months: the proportion of infants who had never flatulence was higher in the synbiotic-supplemented formula group.

No study showed a different rate of infection between the study groups. Overall, and regardless of delivery mode, the mean number of infections, adverse events and severe AEs was equivalent in the control and the synbiotic groups.[10,12,13]

3.2 | *B. animalis* ssp. lactis (CNCM I-3446) and prebiotics (scGOS/lcFOS)

Boquet et al.[14] tested the *B. lactis* CNCM I-3446 (10^7 CFU/g) associated with a mixture of GOS/FOS (9:1; 0.4 g/100 mL) in 261 neonates compared to 267 neonates fed the probiotic formula, with the same amount

of probiotic without prebiotic. Growth parameters were not significantly different between the synbiotic and probiotic formula groups. Tolerance, as measured by daily stool frequency and consistency and overall acceptance of the formula, was not different between groups throughout the study. During the first year of life, the number and site of infections and the mean frequency of antibiotic use were not different between the groups.[14]

3.3 | *Bifidobacterium breve* M-16V and prebiotics (scGOS/lcFOS)

Six RCTs assessed the effect of *B. breve* M-16V as supplemented in infant formula with the addition of a prebiotic component.[15,16,25–28] However, four publications were excluded because the tested formula was based on extensive[25,26] or partially hydrolyzed proteins[27] and another study because it reported only additional data from a previous RCT on microbiota composition.[28] One included RCT recruited 153 newborns delivered by cesarean section randomized to receive either the synbiotic formula ($n = 52$) (infant formula plus 0.8 g/100 mL scGOS/lCFOS and *B. breve* M-16V, 7.5×10^8 CFU/100 mL), prebiotic formula ($n = 51$) (infant formula plus 0.8 g/100 mL scGOS/lCFOS) or the control formula ($n = 50$). Thirty subjects (vaginally delivered) were included in the not-randomized reference group until 16 weeks of age (intervention period).[15] The primary outcome was the determination of total fecal bifidobacteria, but it also included growth and safety outcomes.[15] In another trial, two groups of infants were fed the synbiotic formula consisting of 0.8 g/100 mL scGOS/lcFOS and *B. breve* M-16V at a dose of either 1×10^4 CFU/mL (Syn4) ($n = 81$) or 1×10^6 CFU/mL (Syn6) ($n = 82$) for 6 weeks and compared for intestinal microbiota changes to a control group fed a standard formula ($n = 84$) and to breastfed infants ($n = 43$).[16] Data on growth, stool frequency consistency, and safety were also provided.[16] Growth parameters did not differ between the groups in the two abovementioned studies.[15,16] Fecal frequency did not differ between the study groups, while fecal consistency was significantly softer in the synbiotic group during the intervention period in one study.[16] The percentage of patients experiencing any adverse event was similar in the synbiotic and the control groups in the two included studies.[15,16]

3.4 | *Bifidobacterium longum* BL999 (BAA-999, BB536) and GOS/FOS

Puccio et al.[4] published in 2007 the first RCT evaluating the effect of an infant formula containing a probiotic (*B. longum* BL999 at a dose of 2×10^7 CFU) and prebiotics (90% GOS and 10% FOS, 4 g/L) compared to infant formula, from 14 to 112 days of life. Growth parameters as well as the incidence of adverse effects did not differ between the groups. Compared to infants fed a control formula ($n = 55$), infants in the synbiotic group ($n = 42$) had a significantly increased stool frequency (2.2 ± 0.7 vs. 1.8 ± 0.9 occurrences/day, $p = 0.018$), had less constipation ($p = 0.03$) and less respiratory tract infections (relative risk [RR]: 0.6; 95% confidence interval [CI]: 0.37–1.03). Stool consistency, crying, colic, regurgitation, and vomiting showed no statistically significant differences between the two groups.[4]

3.5 | *Limisolactobacillus fermentum* CECT5716 and GOS

We identified four papers (based on three RCTs) testing a synbiotic formula containing *Lactobacillus* (L.) *fermentum* (currently named *Limosilactobacillus fermentum*) CECT5716 and prebiotic GOS (0.3 g/100 mL) in healthy infants.[17,18,29,30] However, one was excluded because it was a follow-up study at 3 years of life[29] and another because tested protein hydrolyzed formulas.[30] In the study by Gil-Campos et al.,[17] 61 infants were in the synbiotic group and 60 infants were fed a control formula for the first 6 months of life. The effect of a follow-on formula with GOS (0.4 g/100 mL) plus *L. fermentum* CECT5716 (2×10^8 CFU/day) was compared to the follow-on formula with only GOS in 6 months old infants (synbiotic group $n = 97$, control prebiotic group $n = 91$) followed for 6 months.[18] No significant differences in growth parameters were observed among groups at any study points.[17,18] Stool frequency and consistency, flatulence, regurgitation, sleeping hours and behavior were similar in both groups during the 6 months study.[17] The odds ratio (OR) of having at least one gastrointestinal or respiratory infection was 0.36 (95% CI: 0.08–0.97) and 0.77 (95% CI: 0.36–1.66), respectively, but the difference was only significant ($p = 0.025$) for gastrointestinal infections. The incidence rate of gastrointestinal infections in infants of the control group was three times higher than in the synbiotic group ($p = 0.018$).[17] The number of infants that needed to be treated to reduce one event of diarrhea was 5. No difference was observed in febrile episodes or antibiotic treatments.[17] In the other included trial, the synbiotic formula significantly reduced gastrointestinal infections by 46% ($p = 0.032$), total respiratory infections by 26% ($p = 0.022$), and recurrent respiratory infections by 72%.[18] No significant differences were found for otitis, urinary tract, and other infections possibly because of the low number of events obtained in both study groups. Regarding antibiotic treatment and the number of fever episodes, no significant differences between study groups were

observed. Events of diarrhea associated with antibiotic treatments were detected in 17.5% of control infants versus 9.6% of infants in the synbiotic group (nonsignificant).[18] No adverse effects associated with synbiotic supplementation were detected during the studies.[17,18]

3.6 | *Lactobacillus paracasei* ssp. *paracasei* strain F19 plus FOS and GOS

One RCT evaluated an infant formula with FOS and GOS ($n = 91$) and *.paracasei* (current name *Lacticaseibacillus paracasei)* ssp. *paracasei* strain F19 (10^9 CFU) ($n = 97$) for the first 12 months of life.[19] There was no difference in the growth parameters and in the adverse effects between the two groups. During the study, a significant difference was reported in stool consistency (reported as more "loose" stools) in the synbiotic group compared with the control group.[19] There were no significant differences between groups in days with fever, vomiting, eczema, gastrointestinal or total respiratory infections, wheezing episodes, use of antibiotics, unscheduled doctor's visits, and hospitalization. A significant reduction in the number of episodes of lower respiratory tract infections was found in the synbiotic-supplemented formula group compared with the prebiotic-supplemented formula group at 12 months (RR: 0.34; 95% CI: 0.13–0.85, number needed to treat [NNT]: 10, 95% CI: 5–57).[19]

3.7 | Combination of mixture of probiotics and prebiotics

We identified 10 studies evaluating the combination of different strains of probiotics and prebiotics in infant formulas. A summary of the characteristics and main results of the studies are herein reported according to the specific probiotic strains used.

3.8 | *B. longum* BL999 plus *L. rhamnosus* LPR or *L. paracasei* ST11 and scGOS/lcFOS

In one RCT, healthy full-term infants were fed with a control formula or one of the following three experimental formulas (all three containing *B. longum* BL999) from ≤2 to 16 weeks of age: BL999 (1.29×10^8 CFU) and *L. rhamnosus* LPR (6.45×10^8 CFU); BL999 combined with *L. rhamnosus* LPR plus 4 g/L of 90% GOS/10% scFOS, *or B. longum* BL999 (2.58×10^8 CFU), *L. paracasei* ST11 (2.58×10^8 CFU), and 90% GOS to 10% scFOS (4 g/L). Infants were evaluated at 2, 4, 8, 12, 16, and 52 weeks of age.[3] Growth parameters were similar between infants fed the synbiotic formulas and control groups. There was no difference in digestive tolerance considered as the frequency of flatulence, colic, spitting up, and vomiting between the groups. Liquid stools occurred significantly more frequently in the *B. longum* BL999-*L. paracasei* ST11-GOS/SCFOS group than in both the control group and the *B. longum* BL999-*L. rhamnosus* LPR group (OR: 2.79; 95% CI: 1.48–5.29, $p = 0.008$). Stool frequency was significantly higher in infants in the *B. longum* BL999-*L. rhamnosus* LPR + GOS/scFOS group compared with the control group (2.1 days vs. 1.6 days, $p = 0.03$).[3] No difference in adverse effects between groups was reported.[3] Another RCT tested *B. longum* BL999 plus *L. rhamnosus* LPR and prebiotics (FOS and inulin), but it was excluded from the analysis because it enrolled healthy 12-month-old infants.[31]

3.9 | *Bifidobacterium infantis* IM1 and *Lacticaseibacillus rhamnosus* LCS-742 plus a mix of FOS and inulin

We identified eight publications (all about one study: COGNIS study[20–22,32–36]) evaluating the effect of *B. infantis* IM1 *(B. longum* subsp. *infantis* CECT 7210) and *Lacticaseibacillus rhamnosus* LCS-742 plus a mix of FOS and inulin (ratio 1:1) added to a formula containing long-chain polyunsaturated fatty acid (LCPUFA), milk fat globule membrane (MFGM) gangliosides, nucleotides, and sialic acid, enrolling 170 full-term infants aged 0–2 months (85 in the experimental formula group, 85 in the control formula) and 50 breastfed infants as reference group. The primary aim of the COGNIS study was the assessment of the neurodevelopment of children, while secondary outcomes included behavior and temperament, cortical visual evoked potentials, growth, infectious events, and the effect of fatty acids and fatty acid desaturase polymorphisms. According to our inclusion criteria, we evaluated three publications from the COGNIS study.[20–22] No differences in growth and neurodevelopment were found between formula-fed groups from enrollment up to 18 months.[20,21] Since the experimental formula was supplemented with additional components that may influence the neurocognitive effects that are beyond the scope of our paper, these results will not be further discussed herein and have been previously reported.[33] At 12 months of age, significantly fewer infections were reported in the synbiotic group ($p = 0.044$), particularly respiratory ($p = 0.031$) and gastrointestinal infections ($p = 0.030$), than in control groups.[22]

3.10 | *L. rhamnosus* LCS- 742 and *B. longum* subsp infantis M63 and 96% GOS and 4% scFOS

In one RCT, 48 neonates were fed from birth with a synbiotic formula containing *L. rhamnosus* LCS-742 and *B. longum* subsp. *infantis* M63, 96% GOS and 4%

sc-FOS, enriched with bovine a-lactalbumin, using a native whey protein content with high a-lactalbumin concentration (34% of soluble proteins) for 6 months. The control group of 49 neonates was fed a standard formula.[23] There was no significant difference between the experimental and the control groups in growth parameters at 6 months. At 1 month, infants exhibited less crying or fussiness in the synbiotic group than in the control group ($p = 0.02$). The synbiotic formula showed a protective effect against the occurrence of mild atopic dermatitis at 6 months of age.[23]

3.11 | *L. paracasei* ssp. *paracasei* and *B. animalis* ssp. *lactis* plus GOS

One RCT randomized 126 newborns (7 days of age) to receive an infant formula containing the prebiotic GOS (0.24 g/100 mL) supplemented with *L. paracasei* ssp. *paracasei* (1×10^7 CFU) and *B. animalis* spp. *lactis* (1×10^7 CFU) ($n = 67$) or the same prebiotic formula with no probiotic supplementation ($n = 59$) for 3 months.[5] No significant differences were observed in growth parameters between the study groups at 3 and 6 months of life. During the first 3 months, the synbiotic group had a higher stool frequency (1.29 vs. 1.52 times/day, respectively; $p = 0.04$) and stool consistency score than the prebiotic group (2.57 vs. 2.36, respectively, $p = 0.05$). For both parameters, no difference between groups was noted later, during the observation period. There were no significant differences between groups in crying and sleeping hours. No difference was reported in the number of parent-diagnosed infections, antibiotic use, visits to the general practitioner, and adverse events.[5]

3.12 | *Streptococcus thermophilus* NCC 2496, *Streptococcus salivarius* DSM 13084, *L. rhamnosus* LPR CGMCC 1.3724, and Raftilose (FOS)

In one RCT, healthy infants aged 7–13 months delivered by C-section were randomized to receive either the synbiotic ($n = 112$) or the control follow-up formula ($n = 112$). The synbiotic formula contained *S. thermophilus* NCC 2496 (1×10^7 CFU), *S. salivarius* DSM 13084 (2.5×10^7 CFU), *L. rhamnosus* LPR CGMCC 1.3724 (1×10^7 CFU), and Raftilose (FOS). No difference in the rate of infections and adverse events was noted during the 12-month study period.[24]

4 | DISCUSSION

Data regarding synbiotics added to infant formula in presumed healthy infants are limited and heterogeneous by the population of infants recruited, outcomes, type and dosage of synbiotics, and duration of monitoring. The first RCT to address this issue was performed by Puccio et al.[4] One recent systematic review and network meta-analysis evaluating the health effects of infant formula supplemented with probiotics or synbiotics in infants and toddlers, included seven studies assessing synbiotics, but no separate evaluation of the results of these trials was performed.[7] Another systematic review on infant formula supplemented with prebiotics, probiotics or synbiotics focused on incidence of respiratory tract infections in infants and children.[8] However, only three studies using synbiotics (two enrolling infants) were included.[8] The aim of this technical review was to summarize the currently available data and assess the clinical effects, safety, and tolerability of the combined administration of probiotics and prebiotics in infant formula. Growth parameters and adverse events were not different between infants fed synbiotic formulas and control formulas (Table 3, Statement 1). In most studies, infants fed with a synbiotic formula showed softer stools compared to the ones in the control group, likely attributable to the prebiotic component (Table 3, Statement 2). Also, there are no studies on the effect of synbiotics on stool consistency in constipated infants. However, at present, there is no evidence that synbiotic formulas reduce infant crying or gastrointestinal symptoms. It is important to underline that this technical report did not consider studies that evaluated formulas with hydrolyzed proteins for their possible different digestive, motor, and immune effects (Table 3, Statement 3). Moreover, formulas with hydrolyzed proteins often present other components (i.e., reduced lactose or beta-palmitate) that can have a significant impact on gastrointestinal symptoms, consistency and acidity of stools, and different microbiota with possible clinical relevance. Likewise, trials enrolling preterm infants or subjects with comorbidities were also excluded from the analysis.

A few studies evaluating different synbiotic-supplemented formulas reported reduced rate of infections (gastrointestinal or respiratory infections) in the synbiotic group.[4,17–19,22] However, this result was inconsistent among studies and age groups (Table 3, Statement 4). In one study, the synbiotic (*B. longum* BL999 [BAA-999, BB536]and GOS/FOS) group reported less respiratory tract infections (RR: 0.6; 95% CI: 0.37–1.03).[4] *L. fermentum* CECT5716 and GOS supplemented infant formula showed three times less gastrointestinal infections than the control formula with no difference in episodes of fever in one study[17] and a reduction by 46% of gastrointestinal infections, by 26% of total respiratory infections and by 72% of recurrent respiratory infections in another trial.[18] *Lactobacillus paracasei* ssp. *paracasei* strain F19 plus FOS and GOS also significantly reduced the number of episodes of lower respiratory tract infections compared with the

TABLE 3 Statements.

Statements		Median/mean	Votes
1	In presumed healthy infants, formulas with added synbiotics studied so far have shown no differences in anthropometric parameters compared to non-supplemented infant formulas.	9/8.9	8 (2×)/9 (18×)
2	Supplementation of infant formula with synbiotics studied so far suggests softer stool consistency and more frequent defecation in presumed healthy infants compared to the non-supplemented formula control group. However, the clinical relevance of this remains uncertain and inconsistent among studies.	9/8.6	7 (2×); 8 (3×); 9 (15×)
3	The synbiotics studied so far did not show any difference compared to the control group(s) in regurgitation, crying, fussiness, or colic in presumed healthy infants.	9/8.8	6; 8; 9 (18×)
4	There is insufficient data regarding a decreased prevalence of infections or reduced antibiotic use in infants receiving synbiotic-supplemented formula studies so far.	9/8.7	7 (2×); 8; 9 (17×)
5	All studies confirmed the safety and tolerance of synbiotic-supplemented formulas. However, no consistent clinical benefits were demonstrated in healthy infants who received synbiotic-supplemented formulas.	9/8.9	8 (2×); 9 (18×)

prebiotic-supplemented formula group at 12 months (RR: 0.34, 95% CI: 0.13–0.85, NNT: 10, 95% CI: 5–57).[19] At 1 year, Infants fed with *B. infantis* IM1 (*B. longum* subsp. *infantis* CECT 7210) and *L. rhamnosus* LCS-742 plus a mix of FOS and inulin added to a formula containing LCPUFA, MFGM gangliosides, nucleotides, and sialic acid reported significant less respiratory and gastrointestinal infections than the control groups.[22]

5 | CONCLUSION

The ESPGHAN SIG-GMM considers the available data suggesting that the tested synbiotic-supplemented infant formulas are safe but calls for caution in over interpretation of the clinical results (Table 3, Statement 5). In the future, the efficacy and safety of each synbiotic product should be further established. This technical report serves as the background for formulating recommendations on the use of synbiotic-supplemented infant formula in healthy infants studied so far.

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CONFLICT OF INTEREST STATEMENT

Silvia Salvatore has participated as a clinical investigator, advisory board member, consultant, and/or speaker for Aurora Biofarma, Danone, D.M.G., Erbozeta, Humanas, Nestle Health Science, and Noos. Ener Cagri Dinleyici has participated as a clinical investigator, advisory board member, consultant, and speaker for BioGaia, Biocodex, Nestle Health Science, Nestle Nutrition Institute, and Nutricia. Hania Szajewska serves as a board member of the International Scientific Association for Probiotics and Prebiotics (ISAPP), a role that is unpaid and voluntary. She has participated as a clinical investigator, advisory board member, consultant, and speaker for several companies, including Arla, BioGaia, Biocodex, Danone, Dicofarm, Nestlé, NNI, Nutricia, Mead Johnson, and Novalac. Roberto Berni Canani has participated as investigator and speaker for Biostime, Ch. Hansen, Dr. Schar, Mead Johnson, Nestlé, Novalac, and Sanofi. Pedro Gutierrez-Castrellón has been participating as clinical investigator, and/or advisory board member, and/or consultant, and/or speaker for Abbott Nutrition, Biogaia, Nestle Nutrition Institute, and AB-Biotics. Iva Hojsak received a fee for consultation or lecture from Abbott, BioGaia, Biocodex, Nestle, Ewopharma, and Sandoz. Flavia Indrio has participated as a clinical investigator, advisory board member, consultant, and speaker for several companies, including, BioGaia, Danone, Nestlé, NNI, and Abbot. Walter Mihatsch participated as a clinical investigator and/or advisory board member, and/or consultant and/or speaker for Alzchem, Danone, Nestle Nutrition Institute, and Neobiomics. Pedro Gutierrez-Castrellón has been participating as clinical investigator, and/or advisory board member, and/or consultant, and/or speaker for Abbott Nutrition, Biogaia, Nestle Nutrition Institute, and AB-Biotics. Rok Orel participated as a

clinical investigator, advisory board member, consultant, and speaker for several companies, including Medis, Ewopharma, Nutricia, Novalac, and Nestlé. Johannes B. van Goudoever participated as clinical investigator for the National Institute for Public Health and the Environment (RIVM) and Danone Research. He is director and founder of the National Human Milk Bank and member of the National Health Council. Yvan Vandenplas participated as a clinical investigator, and/or advisory board member, consultant, and/or speaker for Abbott Nutrition, Alba Health, Arla, Biogaia, Danone, ELSE Nutrition, Friesland Campina, Nestle Health Science, Nestle Nutrition Institute, Nutricia, Pileje, and United Pharmaceuticals (Novalac).

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