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Gastrointestinal Health and Immunity of Milk Formula Supplemented with a Prebiotic Mixture of Short-Chain Galacto-oligosaccharides and Long-Chain Fructo-Oligosaccharides (9:1) in Healthy Infants and Toddlers: A Systematic Review with Meta-Analysis

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ABSTRACT

Prebiotics are substrates selectively utilized by microorganisms to confer health benefits to their hosts. Various prebiotics have been supplemented in standard milk formulas for infants who cannot be exclusively breastfed, aiming to provide benefits similar to those of breast milk. One of the most commonly used prebiotics is a mixture of 90% short-chain galacto-oligosaccharides and 10% long-chain fructo-oligosaccharides (scGOS/lcFOS [9:1]). Systematic review and meta-analysis were conducted to determine the effectiveness of scGOS:lcFOS (9:1) supplementation in standard milk formula for improving gastrointestinal health and immunity among healthy infants and toddlers, using parameters such as stool pH and intestinal colonization with beneficial bacteria. This systematic review was prepared in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines. Randomized clinical trials comparing scGOS/lcFOS (9:1)-supplemented formula versus placebo- or non-supplemented formula milk were eligible for inclusion. Related studies on gastrointestinal health and immunity among healthy infants up to five years old were searched from the earliest available date until February 29, 2024. Eighteen publications (number of participants=1,675) were selected for the systematic review, of which 11 were subsequently subjected to a meta-analysis. Results showed that the standard formula supplemented with scGOS/lcFOS (9:1) was well tolerated and conferred various gastrointestinal health and immunity to healthy infants and toddlers. These findings support the supplementation of standard milk formula with scGOS/lcFOS (9:1) for healthy infants and toddlers.

Keywords: Prebiotics; Short-chain galacto-oligosaccharides; Long-chain fructo-oligosaccharides; Gastrointestinal health; Immunity; Infants; Toddlers

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Conflict of Interest

The author has no financial conflicts of interest.

INTRODUCTION

The human gastrointestinal tract, as one of the largest interfaces within the body between host cells and environmental molecules (250–400 m^2), is vital for maintaining overall health. Various bacteria, archaea, and eukaryotes (collectively referred to as intestinal flora or gut microbiota) reside within the human gastrointestinal tract, where they have co-evolved with the host over time to form an intricate and mutually beneficial relationship [1,2]. It is estimated that the ratio of human to bacterial cells is approximately 1:1 [3]. The intestinal flora residing in the human gastrointestinal tract plays a crucial role in supporting its physiological roles. These microorganisms assist gastrointestinal epithelial cells in breaking down and absorbing nutrients, processing waste materials, producing crucial compounds (e.g., vitamins and short-chain fatty acids), and regulating innate and adaptive immunity of the gastrointestinal tract [1,4,5]. Disruption of this physiological interaction can lead to various health issues, including irritable bowel syndrome, inflammatory bowel disease, diabetes, and cancer. Therefore, maintaining a healthy intestinal flora by regulating the balance between commensal/beneficial and pathogenic bacteria is crucial [1,4-6].

Abundant and diverse intestinal flora serves as targets for improving health and treating diseases. The composition and metabolic signatures of intestinal flora can be modulated through dietary or non-dietary approaches [7,8]. One well-established dietary method involves the use of prebiotics. The current definition of a prebiotic is a substrate that is selectively utilized by host microorganisms to confer health benefits, which originally comprises oligosaccharides (naturally existing, such as human milk oligosaccharides and synthesized oligosaccharides). Notably, the scope of prebiotics has now been extended to include conjugated linoleic acid, polyunsaturated fatty acids, phenols, and phytochemicals [9].

Prebiotics are defined by three criteria: (i) the ability to resist host digestion, (ii) fermentation by intestinal flora, and (iii) selective stimulation of the growth and/or functionality of the intestinal flora associated with health [9]. Their ability to selectively foster the growth of beneficial microorganisms, such as those residing in the gastrointestinal tract, underpins their use in modulating the intestinal flora. Many prebiotics used in humans were initially recognized for their ability to stimulate the growth and function of *Lactobacillus* and *Bifidobacterium* species, while not promoting pathogens such as those in the class Clostridia or *Escherichia coli* [10-12]. In addition to *Lactobacillus* and *Bifidobacterium* species, prebiotics are also known to stimulate other bacteria in the human gastrointestinal tract, such as *Faecalibacterium prausnitzii* and *Anaerostipes* species [13,14].

To date, the two frequently studied prebiotics are fructans and galacto-oligosaccharides (GOS). Fructans include inulin and fructo-oligosaccharides (FOS), which consist of fructose chains arranged in a linear form with beta (2,1) glycosidic linkage [15]. GOS can be generated by extending lactose, lactulose, or raffinose family through enzymatic glycosylation with galactose [16]. The individual provision of FOS or GOS have been demonstrated to promote growth of *Lactobacillus* and *Bifidobacterium* species [9,15-17]. Using certain prebiotics to enrich the colonization and functionality of beneficial intestinal flora can improve gastrointestinal health and immunity, particularly in infants and toddlers.

When breastfeeding is not feasible, milk formula is commonly administered to infants. However, as bovine milk used in standard milk formulas lacks oligosaccharides, its provision does not support proper intestinal colonization by beneficial microbes (e.g., *Bifidobacterium*

and *Lactobacillus* species). Therefore, various types of prebiotics have been added to current standard formulas for infants [18]. However, it is important to note that specific prebiotics could generate different kind of health benefits. Thus, the findings from one prebiotic cannot be extrapolated to other prebiotics. As human milk oligosaccharides in breast milk serve as the gold standard for selecting prebiotics to be added to infant milk formula, a specific mixture of prebiotics was created by combining 90% short-chain GOS (derived from lactose with a degree of polymerization between 3 and 8) and 10% long-chain FOS (extracted inulin from chicory roots with a degree of polymerization >23) to resemble the molecular size distribution of human milk oligosaccharides [19,20].

Hence, it is of interest to conduct clinical trials on gastrointestinal health and immunity using a mixture of 90% short-chain GOS and 10% long-chain FOS (scGOS:lcFOS [9:1]). Therefore, a systematic review and meta-analysis was performed to determine the effectiveness of scGOS:lcFOS (9:1) supplementation in standard milk formula for improving gastrointestinal health and immunity among healthy infants and toddlers by measuring parameters, such as stool pH, intestinal colonization with beneficial bacteria, and fecal secretory immunoglobulin A.

MATERIALS AND METHODS

This systematic review and meta-analysis were constructed based on the Cochrane Guidelines for Systematic Reviews of Interventions and written according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 [21,22]. The review protocol is registered in PROSPERO (CRD42024528419).

Search strategy

Publications reporting the effects of scGOS/lcFOS (9:1) on gastrointestinal health and immunity in healthy infants and toddlers up to five years old were searched from the earliest available date until February 29, 2024, across five independent databases: PubMed, Science Direct, EBSCO Medline, JSTOR, and Cochrane Central Register of Controlled Trials. The search was conducted using the following terms: scGOS/lcFOS 9:1 OR FOSGOS 1:9 OR GOSFOS ratio OR prebiotics scGOS/lcFOS 9:1 OR short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides AND RCT OR randomized controlled trial OR randomized clinical trial. Reference lists from the identified publications and relevant organizations were also searched (**Fig. 1**). Only publications in English were included.

Outcomes

The primary outcomes were stool consistency, stool frequency, stool pH, intestinal colonization by beneficial and pathogenic bacteria, concentrations of fecal short-chain fatty acids and lactate, concentration of fecal secretory immunoglobulin A, and risk of gastrointestinal infection. The secondary outcomes were safety and physical growth (increments in weight, height, and head circumference); various immune parameters in the blood, including antibodies, cytokines, C-reactive protein, and leukocyte subsets; and risk of respiratory infection.

Eligibility criteria

Randomized clinical trials comparing formula milk supplemented with scGOS/lcFOS (9:1) with placebo or non-supplemented formula milk were eligible for inclusion. Only trials

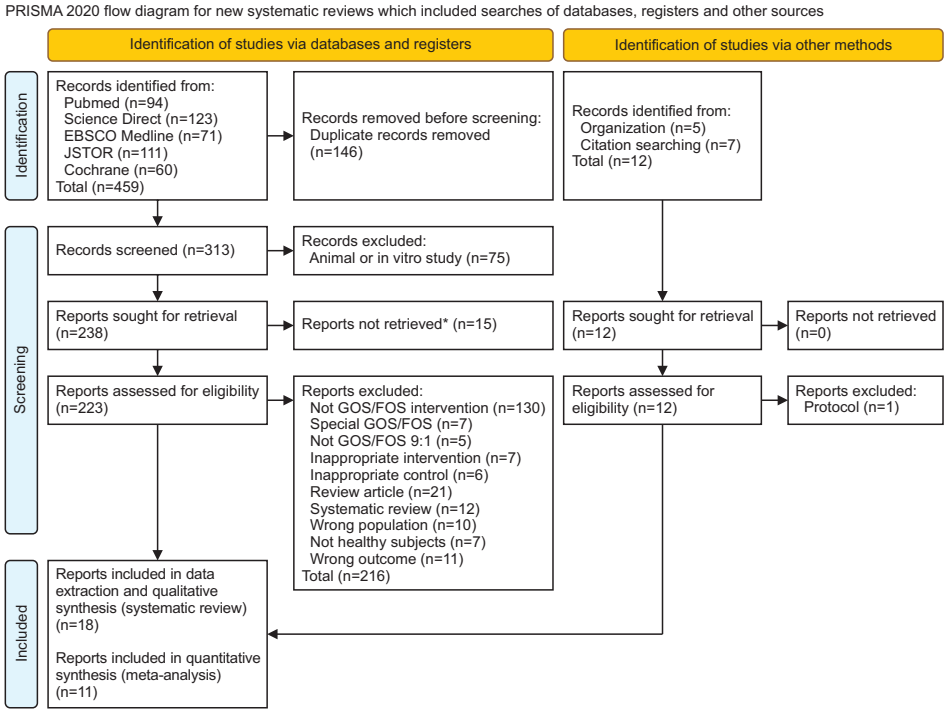

Fig. 1. Flow of information through various phases of the systematic review and meta-analysis. The flowchart follows the framework outlined in the Preferred Reporting Items for Systematic Review and Meta-Analyses 2020 statement [22]. A total of 18 studies, derived from 12 studies, were selected for the systematic review. Eleven studies were selected for the meta-analysis. *Only abstract or preceding report (n=13) or not in English (n=2). GOS: short-chain galacto-oligosaccharides, FOS: long-chain fructo-oligosaccha.

involving full-term healthy infants and/or toddlers up to five years old were included. Trials with at least one of the following outcomes were included: gastrointestinal health, gastrointestinal infection, or immunity.

Study selection and data extraction

Electronic searches were uploaded to the Rayyan Qatar Computing Research Institute [23]. The retrieved information included the location of the study, number of participants, demographic and baseline characteristics of the participants, details of the intervention and control conditions, study methodology, recruitment and completion rates, outcomes, and time points of measurement, as well as information regarding potential biases or confounders. Study selection and data extraction were performed independently by two investigators (V.S. and J.J.). Only recent duplicate registrations were included. Titles and abstracts were initially screened. Subsequently, each study was assessed to determine if it met the inclusion criteria. The reviewers resolved these discrepancies through discussions.

Quality assessment

Two investigators (V.S. and J.J.) independently assessed the risk of bias in the selected studies according to the Cochrane risk-of-bias tool for randomized trials (RoB 2), including bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in selection of the reported result [24]. The RoB 2 assessments were entered into an Excel spreadsheet (Microsoft Excel RoB2 Macro) [24]. These assessments were used to judge the overall risk of bias (low risk, concerns, or high risk). The reviewers resolved these discrepancies through

discussions. The overall certainty of the body of evidence was rated by using the Grading of Recommendations Assessment, Development and Evaluation approach [21,25]. The quality of evidence was rated low if there was a serious concern regarding the overall risk of bias, consistency of effect, imprecision, indirectness, and/or publication bias.

Meta-analysis

A meta-analysis was performed using the Review Manager (RevMan version 5.4. The Cochrane Collaboration, 2020). Heterogeneity was investigated using I2 parameters. If publications were considered heterogeneous (I2 ≥50%), a random-effects model was applied. Otherwise, a fixed effects model was used. A forest plot was generated if a minimum of three publications assessed the same parameters and reported them using the same method. The main results of interest were combined using the inverse-variance method, in which the magnitudes of the effects were measured as standardized mean differences with 95% confidence intervals (CIs). If the mean value was not reported, it was calculated using the provided median value. To evaluate publication bias, a funnel plot was generated by plotting the effect size of each study across the associated standard errors. A funnel plot was used to identify any signs of asymmetry that could indicate the presence of publication bias. Notably, only funnel plots for quantified parameters with I^2 <50% (i.e., homogenous) are displayed.

RESULTS

Search results

A total of 459 records were identified through the five selected database searches. After excluding 146 duplicate studies, 313 articles were screened. Of these, 75 records were excluded as they were animal or in vitro studies, 13 records were excluded for being only abstracts or proceedings reports, and 2 records were excluded because they were not published in English. Full-text articles for the remaining 223 records were retrieved, of which 216 were subsequently excluded, as shown in **Fig. 1**, resulting in seven relevant records. In addition, 11 relevant records were identified through citation searches and organizational information. Eighteen full-text publications were eligible for inclusion in the systematic review. Eleven publications were included in this meta-analysis.

Characteristics of publications and participants

The main characteristics of the included publications are summarized in **Table 1**. A total of 18 publications were derived from 12 distinct studies, of which two were from a trial by Bakker-Zierikzee et al. [26,27], three were from a trial led by Knol [28-30], three were from a trial by Moro et al. [31-33], and two were from a trial led by Vandenplas [34,35]. A total of 1,675 participants were included in this review. Most studies focused on neonates [26,27,31-41], while four studies included infants [28-30,42]. In addition, one study examined the benefits of growing-up milk supplemented with scGOS/lcFOS (9:1) for toddlers (i.e., aged 11–29 months at the beginning of the study) [43].

Various concentrations of scGOS/lcFOS (9:1) (hereafter referred to as GOS/FOS) were assessed in the included studies. Four studies evaluated a GOS/FOS concentration of 0.4 g/dL [31,32,41,42], five studies assessed 0.6 g/dL [26,27,34,35,39], and the majority evaluated 0.8 g/dL [28-33,36-38,40]. In addition, a study on growing-up milk assessed 1.2 g/dL of GOS/FOS in toddlers [43]. Thirteen publications compared GOS/FOS supplementation with a non-supplemented standard formula [26-30,34,35,37-39,41-43], whereas five compared GOS/

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Table 1. Characteristics and results of the included studies

Study	Group and duration	Measured outcomes	Results
Bakker-Zierikzee et al., 2005 [26]	Intervention 1: scGOS/lcFOS, 0.6 g/dL (n=19) Intervention 2: 6.0×10^9 cfu *Bifidobacterium animalis* Bb-12/100 mL formula (n=19) Control: non-supplemented standard formula (n=19) Breastfed reference group (n=63) Duration of intervention=16 wk	Intestinal bifidobacteria and stool pH at day 5 and 10 and 4, 8, 12, and 16 wk; fecal short-chain fatty acids and lactate at day 5nd 10 and 4, 8, 12, and 16 wk.	Compared with the groups fed Bb-12 and standard formula, the GOS/FOS formula group showed higher fecal acetate ratio (69.7%, 69.9%, and 82.2%, respectively; p<0.05) and lactate concentration (11.3, 3.1, and 34.7 mmoL/kg feces, respectively) and lower pH (6.6, 7.1, and 5.6, respectively; p<0.05) at 16 wk. Differences in percentage of bifidobacteria between the GOS/FOS (59.2%), Bb-12 (52.7%), and standard (51.8%) groups were not statistically significant at 16 wk. Feeding infants with the GOS/FOS formula resulted in a similar effect on metabolic activity of the flora as in breast-fed infants. In the Bb-12 group, composition and metabolic activity of the flora were more similar to those in the standard group.
Bakker-Zierikzee et al., 2006 [27]	Intervention 1: scGOS/lcFOS, 0.6 g/dL (n=19) Intervention 2: 6.0×10^9 cfu *Bifidobacterium animalis* Bb-12/100 mL formula (n=19) Control: non-supplemented standard formula (n=19) Breastfed reference group (n=63) Duration of intervention=16 wk	Fecal sIgA at postnatal day 5 and 10, and 4, 8, 12, 16, 20, 24, 28, and 32 wk.	During the intervention, prebiotic formula-fed infants showed a trend towards higher fecal sIgA levels compared with the standard formula-fed infants, reaching statistical significance at 16 wk of age. In contrast, probiotic formula-fed infants showed a highly variable fecal sIgA concentration with no statistically significant differences compared with the standard formula group. Formula-fed infants may benefit from infant formulas containing a prebiotic mixture of GOS and FOS, as a clear trend toward increase fecal sIgA secretion was observed. Adding viable *B. animalis* strain Bb-12 to infant formula did not show any significant trend in this regard.
Béghin et al., 2021 [39]	Intervention 1: fermented infant formula+scGOS/lcFOS (n=70) Intervention 2: scGOS/lcFOS, 0.6 g/dL (n=70) Intervention 3: fermented infant formula (n=70) Control: non-supplemented standard formula (n=70) Breastfed reference group (n=70) Duration of intervention=6 mo	Growth outcomes (weight-height-head circumference) at birth, baseline, and 2, 4, and 6 mo; stool consistency at 0, 2, 4, and 6 mo; intestinal flora at baseline and 2 and 4 mo; fecal pH, short-chain fatty acids, and D- & L-lactate at baseline and 2 and 4 mo; fecal sIgA at baseline and 2, 4, and 6 mo; adverse events were recorded.	All tested formulas were associated with normal growth and were well tolerated. At four months of age, the median sIgA concentration in the fermented infant formula+scGOS/lcFOS group was significantly higher than the control group (p=0.03) and more similar to the concentrations observed in the breastfed-reference group. Bifidobacteria increased over time in all the groups. The fermented infant formula+scGOS/lcFOS combination resulted in a microbiota composition and metabolic activity that was closer to that of the breastfed infants' microbiome.
Bisceglia et al., 2009 [40]	Intervention: scGOS/lcFOS, 0.8 g/dL (n=39) Control: standard formula with maltodextrin (n=37) Duration of intervention=28 d	Growth outcomes (weight-height-head circumference), adverse event and stool frequency at day 28; transcutaneous bilirubin at postnatal 2, 24, 48, and 72 h, and day 5, 7, 10, and 28.	Neonates receiving prebiotics showed significantly higher number of stools upon dietary intervention compared to those on placebo (p<0.001; day 28, 3.4±0.0.9 vs. 1.7±0.9, respectively). Neonates whose formula was supplemented with prebiotics had lower transcutaneous bilirubin levels, which were statistically significant from 72 h of life (5.46±1.6 vs. 7.07±2.49; p<0.05) and throughout the duration of the dietary intervention (day 28, 2.41±0.4 vs. 2.85 0.5; p<0.05).
Bruzesse et al., 2009 [42]	Intervention: scGOS/lcFOS, 0.4 g/dL (n=96) Control: non-supplemented standard formula (n=105) Duration of intervention=12 mo	The incidence of intestinal and respiratory tract infections and growth outcomes (weight and height) were monitored for 12 mo.	The incidence of gastroenteritis was lower in the supplemented group than in the control group (0.12±0.04 vs. 0.29±0.05 episodes/child/12 mo; p=0.015). The number of children with ›3 episodes tended to be lower in the prebiotic group (17/60 vs. 29/65; p=0.06). The number of children with multiple antibiotic courses/year was lower in children receiving prebiotics (24/60 vs. 43/65; p=0.004). A transient increase in body weight was observed in children on prebiotics compared to controls during the first 6 mo of follow-up. Prebiotic administration reduce intestinal and, possibly, respiratory infections in healthy infants during the first year of age.
Chatchatee et al., 2014 [43]	Intervention: lcPUFA+scGOS/lcFOS, 1.2 g/dL (n=348) Control: non-supplemented standard growing-up milk formula (n=349) Cow-milk reference group (n=37) Duration of intervention=52 wk	The primary outcome was the number of episodes of URTI or GIIs based on a combination of participant's reported illness symptoms; secondary outcomes were total number and duration of episodes of URTI and/or GII; parents' absence from work because of the child's illness; number, duration, and season of participants' absence from day care center; number and type of all infections diagnosed by a physician or investigator; and growth outcomes (weight and height) at 1 and 52 wk.	Children in the active group had a decreased risk of developing at least 1infection compared with the control group (299/388 [77.1%] vs. 313/379 [82.6%], respectively; relative risk 0.93, 95% CI 0.87–1.00). A trend toward a reduction (p=0.07) in the total number of infections was observed in the active group, which was significant when confirmed by one of the investigators (268/388 [69.1%] vs. 293/379 [77.3%], respectively; relative risk 0.89, 95% CI 0.82–0.97; p=0.004). More infectious episodes were observed in the cow's milk group, when compared with both the GUM groups (34/37 [91.9%] vs. 612/767 [79.8%], respectively; relative risk 1.15, 95% CI 1.04–1.28).

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Table 1. (Continued) Characteristics and results of the included studies

Study	Group and duration	Measured outcomes	Results
Costalos et al., 2008 [41]	Intervention: scGOS/lcFOS, 0.4 g/dL (n=70) Control: non-supplemented standard formula (n=70) Duration of intervention=6 wk	Growth outcomes (weight-height-head circumference) at 6 and 12 wk. Stool frequency, consistency, and pH at baseline and 6 weeks (only from 64 participants, 32 per group). The number of bifido-bacteria, clostridia, and E. coli were measured at baseline and 6 weeks.	Somatic growth was similar between the groups. Stool frequency was significantly higher in the prebiotic group (p=0.031). Infants in the prebiotic group also had softer stools compared with the control group (p=0.026). Baseline values of microorganisms at study entry were similar. The percentage of fecal clostridia at the completion of the study was significantly lower in the prebiotic group (p=0.042), whereas the proportion of fecal bifidobacteria was higher in the prebiotic group compared with the control group. However, this difference did not reach statistical significance (p=0.262). The percentage of *E. coli* was lower in the prebiotic group, but this difference also did not reach statistical significance (p=0.312).
Knol et al., 2005 [28]	Intervention: scGOS/lcFOS, 0.8 g/dL (n=15) Control: non-supplemented standard formula (n=19) Breastfed reference group (n=19) Duration of intervention=6 wk	At study onset and after 4 and 6 wk, fecal samples were examined for the number of bifidobacteria, pH, short chain fatty acids, and lactate.	After 6 wk, the mean proportion of bifidobacteria was significantly higher in the prebiotic group (59.6% vs. 49.5% in the control group; p<0.05). Compared with the control group, infants in the prebiotic group had a lower stool mean pH, increased proportion of acetate, and decreased proportion of propionate. The mean pH in the intervention and control groups were 5.7 and 6.3, respectively (p<0.001).
Haarman et al., 2005 [29]	Intervention: scGOS/lcFOS, 0.8 g/dL (n=15) Control: non-supplemented standard formula (n=19) Breastfed reference group (n=19) Duration of intervention=6 wk	*Bifidobacterium* species at the beginning and end of the study	The results showed a significant increase in the total amount of fecal bifidobacteria (54.8±9.8% to 73.4±4.0%) in infants who received the prebiotic formula, with a diversity of *Bifidobacterium* species similar to breast-fed infants. The intestinal microbiota of infants who received a standard formula resembled a more adult-like distribution of bifidobacteria and contained relatively more *B. catenulatum* and B. adolescentis (2.71±1.92% and 8.11±4.12%, respectively, vs. 0.15±0.11% and 1.38±0.98%, respectively, for the prebiotic group). In conclusion, the specific prebiotic infant formula used induced fecal microbiota that closely resembled the microbiota of breastfed infants, inducing at the level of the different *Bifidobacterium* species
Haarman et al., 2006 [30]	Intervention: scGOS/lcFOS, 0.8 g/dL (n=15) Control: non-supplemented standard formula (n=19) Breastfed reference group (n=19) Duration of intervention=6 wk	*Lactobacillus* species at the beginning and end of the study.	During the 6-wk intervention period, a significant increase was observed in the total percentage of fecal lactobacilli in the breastfed (0.8±0.3% vs. 4.1±1.5%) and prebiotic (0.8±0.3% vs. 4.4±1.4%) groups. The *Lactobacillus* species distribution in the prebiotic group was comparable to breastfed infants, with relatively high levels of *L. acidophilus*, *L. paracasei*, and *L. casei*. On the other hand, the standard formula-fed infants had more *L. delbrueckii* and less *L. paracasei* compared with breast-fed and prebioticfed infants. An infant milk formula containing a specific mixture of prebiotics is able to induce a microbiota that closely resembles the microbiota of breastfed infants.
Moro et al., 2002 [31]	Intervention 1: scGOS/lcFOS, 0.4 g/dL (n=30) Intervention 2: scGOS/lcFOS, 0.8 g/dL (n=27) Control: standard formula with maltodextrin (n=33) Breastfed reference group (n=15) Duration of intervention=4 wk	Fecal microbial species, colony forming units, and pH were measured at day 1 and 28; stool frequency and consistency, growth outcome (weight and height), and adverse event were recorded at day 28.	At study day 1, the median number of bifidobacteria did not differ among the groups (0.4 g/dL group=8.5 cfu/g; 0.8 g/dL group=7.7 cfu/g; and placebo group=8.8 cfu/g). At the end of the 28-day feeding period, the number of bifidobacteria significantly increased in both the groups receiving supplemented formulas (0.4 g/dL group=9.3 cfu/g; 0.8 g/dL group=9.7 cfu/g) vs. the placebo group (7.2 cfu/g; p<0.001). This effect was dose dependent (p<0.01). The number of lactobacilli also increased significantly in both the supplemented formula-fed vs. placebo-fed groups (p<0.001). However, no statistically significant difference was observed between the groups fed formula with 0.4 g/dL and 0.8 g/dL oligosaccharides. The dosage of supplement significantly influenced the change in fecal pH (p<0.05) (placebo: pH 5.5–6.1; 0.4 g/dL formula: pH 5.48–5.44; 0.8 g/dL formula: pH 5.54–5.19). Slight changes in stool frequency resulted in a significant difference between the placebo and 0.8 g/dL formula-fed groups at day 28 (p<0.01). Supplementation had a significant dose-dependent influence on stool consistency (0.8 g/dL vs. placebo, p<0.0001; 0.8 g/dL vs. 0.4 g/dL, p<0.01). Supplementation had no influence on the incidence of side effects (crying, regurgitation, vomiting) or growth.
Moro et al., 2003 [32]	Intervention 1: scGOS/lcFOS, 0.4 g/dL (n=30) Intervention 2: scGOS/lcFOS, 0.8 g/dL (n=27) Control: standard formula with maltodextrin (n=33) Breastfed reference group (n=15) Duration of intervention=4 wk	Bifidobacteria and lactobacilli cfu in infants receiving scGOS/lcFOS as compared with breastfed infants.	A supplementation of scGOS/lcFOS had a stimulating effect on the growth of bifidobacteria and lactobacilli in the intestine. This resulted in stool characteristics similar to those found in human-milk-fed infants. A dosage of 0.4 g/dL of scGOS/lcFOS results in significant effects; however, this effect can be enhanced to a level observed in breastfed infants by increasing the dosage to 0.8 g/dL.

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Table 1. (Continued) Characteristics and results of the included studies

Study	Group and duration	Measured outcomes	Results
Moro et al., 2005 [33]	Intervention: scGOS/lcFOS, 0.8 g/dL (n=16) Control: standard formula with maltodextrin (n=16) Duration of intervention=4 wk	Growth outcomes (weight and height) at day 28; fecal bifidobacteria at day 1 and 28; presence of scGOS and lcFOS in stool samples of formula-fed infants at day 28.	On study day 1, the number of bifidobacteria was not different among the groups (supplemented group=7.7 cfu/g; placebo group=8.0 cfu/g). At the end of the 28-d feeding period, the number of bifidobacteria was significantly higher in the group fed the scGOS/lcFOS-supplemented formula compared with the control group (supplemented group=9.8 cfu/g stool; placebo group=7.1 cfu/g stool; $p<0.001$). In all infants fed the supplemented formula, GOS and FOS could be identified in the stool samples. That was not the case in infants fed the non-supplemented formula.
Scholtens et al., 2008 [34]	*[number of participants at week 26]* Intervention: scGOS/lcFOS, 0.6 g/dL (n=75) Control: non-supplemented standard formula (n=81) Breastfed reference group (n=31) Duration of intervention=26 wk	Fecal sIgA at 26 wk; intestinal microbiota and fecal pH at 8 and 26 wk; adverse events were recorded.	An infant milk formula with 6 g/L scGOS/lcFOS resulted in a higher concentration of fecal sIgA, suggesting a positive effect on mucosal immunity. In addition, the percentages of bifidobacteria were higher in the scGOS/lcFOS group (60.4%) than in the control group (52.6%, $p=0.04$). The percentages of Clostridium species were 0.0% and 3.27%, respectively ($p=0.006$).
Raes et al., 2010 [35]	*[number of participants at week 26]* Intervention: scGOS/lcFOS, 0.6 g/dL (n=75) Control: non-supplemented standard formula (n=81) Breastfed reference group (n=31) Duration of intervention=26 wk	Sera IgG-IgM-IgA-IgE titers at 8 and 26 wk; leukocyte subsets at 8 and 26 wk; sera C-reactive protein and cytokines at 8 and 26 wk.	No significant differences were observed between both the formula groups in the studied immune parameters at 8 and 26 wk. Supplementation of infant formula with a mixture of prebiotic oligosaccharides did not alter the basal level of the measured parameters of the developing immune system in healthy infants with a balanced immune system during the first 6 mo of life in comparison to feeding a standard infant formula or exclusive breast-feeding.
Salvini et al., 2011 [36]	Intervention: scGOS/lcFOS, 0.8 g/dL (n=10) Control: standard formula with maltodextrin (n=10) Duration of intervention=6 mo	Growth outcomes (weight-lengthhead circumference) at 3, 6, and 12 mo; fecal counts of bifidobacteria and lactobacilli at 3, 6, and 12 mo; fecal pH at 3, 6, and 12 mo; sera IgE titer at 3 and 12 mo; sera specific IgG anti HBs-antigen titer at 12 mo; leukocyte subsets at 12 mo.	All infants had a normal weight gain, length growth, and head circumference increment, and no differences were observed between the groups for all measurements. Prebiotic supplementation resulted in more fecal bifidobacteria ($p<0.0001$) and lactobacilli ($p=0.0044$) compared with the control group. The supplementation influenced fecal pH ($p=0.0005$), with significantly higher pH values in the control group. Except for T lymphocyte titers at 12 mo of age, which were lower in the control group ($p=0.017$), the groups did not differ at each evaluation in the measured leukocyte populations. The diet did not influence the IgE levels in the serum ($p=0.27$). At 12 mo of age, the anti-hepatitis B virus surface antibody titers did not differ between the feeding groups. Both formulas were well tolerated, with no adverse effects recorded.
Teoh et al., 2022 [37]	*[PP population fully formula-fed]* Intervention 1: scGOS/lcFOS, 0.8 g/dL (n=29) Intervention 2: phospholipid-coated lipid droplets+scGOS/lcFOS (n=35) Control: non-supplemented standard formula (n=28) Breastfed reference group (n=66) Duration of intervention=12 mo	Growth outcomes (weight, height, and head circumference) at baseline and 4, 8, 13, and 17 wk; stool frequency and consistency at 1, 2, 3, and 4 mo; adverse events were recorded.	Equivalence of daily weight gain was demonstrated between intervention 1 and 2 groups after additional correction for ethnicity and birthweight (difference in estimated means of 0.1 g/d, 90% CI [−2.30, 2.47]; equivalence margin±3 g/d). No clinically relevant group differences were observed in secondary growth outcomes, tolerance outcomes or number, and severity or relatedness of adverse events. This study corroborates that an infant formula with large, milk phospholipid-coated lipid droplets supports adequate growth and is well tolerated and safe for use in healthy infants.
Veereman-Wauters et al., 2011 [38]	Intervention 1: SYN1 (50 oligofructose:50 OS) 0.4 g/dL (n=15) Intervention 2: SYN1 (50 oligofructose:50 FOS) 0.8 g/dL (n=13) Intervention 3: scGOS/lcFOS, 0.8 g/dL (n=11) Control: non-supplemented standard formula (n=15) Breastfed reference group (n=23) Duration of intervention=4 wk	Weight gain at 2 and 4 wk; length gain at 4 wk; stool frequency and consistency at baseline and 2 and 4 wk; total bacteria, bifidobacteria, Bacteroides, clostridia, and lactic acid bacteria at day 3, 14, and 28; bifidobacteria cfu at day 3, 14, and 28; tolerance and adverse events were recorded.	During the first month of life, weight, length, intake, and crying increased significantly across all the groups. Regurgitation and vomiting scores remained low and similar. Stool frequency decreased significantly and similarly in all the formula groups but was lower than that in the breast-fed group. All the prebiotic groups maintained soft stools, only slightly harder than those of the breast-fed infants. The standard group had significantly harder stools at 2 and 4 weeks compared with 1 ($p<0.001$ and $p=0.0279$). The total number of fecal bacteria increased in all the prebiotic groups (9.82, 9.73, and 9.91 to 10.34, 10.38, and 10.37 log10 cells/g feces, respectively; $p=0.2298$) and more closely resembled the breast-fed pattern. Numbers of lactic acid bacteria, Bacteroides, and clostridia were comparable. In the SYN1 0.8 g/dL and scGOS/lcFOS groups, *Bifidobacterium* counts were significantly higher at day 14 and 28 compared with day 3, and were comparable with the breast-fed group. Tolerance and growth were normal.

scGOS: short-chain galacto-oligosaccharides, lcFOS: long-chain fructo-oligosaccharides, SYN1: orafti synergy1 (50 oligofructose:50 fructo-oligosaccharides), sIgA: secretory IgA, URTI: upper respiratory tract infection, GII: gastrointestinal infection, GUM: growing-up milk, CI: confidence interval.

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FOS supplementation with a maltodextrin-supplemented standard formula [31-33,36,40]. Notably, several studies have also assessed other types of interventions, in addition to GOS/FOS, including other prebiotics [38], probiotics [26,27], bioactive compounds produced via fermentation [39], and large milk phospholipid-coated lipid droplets [37]. However, this systematic review and meta-analysis focused only on analyzing the gastrointestinal health and immunity of GOS/FOS supplementation, as compared to non-supplemented or maltodextrin-supplemented standard milk formulas (as the control group).

Quality assessment

The risk of bias assessment for all included publications was performed using Rob 2 tool, with the results depicted in **Fig. 2**. Rob 2 tool derives the risk of bias based on algorithms that are informed by answers to specific signaling questions. The structure provided by these signaling questions aims to make the assessment easier and more efficient to use, as well as to improve agreement between assessors [24]. Among the 18 selected publications, 13 had a low risk of bias, two had some concerns, and three had a high risk of bias. Two publications from one study were assessed as having some concerns due to bias in the selection of the reported results [29,30]. Both publications served as exploratory studies in testing quantitative polymerase chain reaction to detect various bacterial species; therefore, this method was probably not listed in a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis. In addition, three publications from another study were assessed as having a high risk of bias in the measurement of the outcome [31-33] because no information was found regarding the awareness of outcome assessors about the intervention received by study participants, or influence on the assessment outcome due to knowledge of the received intervention. This issue arose because the Rob 2 tool was recently developed for assessing risk of bias in randomized trials, and many old studies, such as those [31-33], may not meet the current high standard of performing randomized clinical trials.

Publication	Domain 1a	Domain 1b	Domain 2	Domain 3	Domain 4	Domain 5	Overall bias
Bakker-Zierikzee et al., 2005 [26]	Low	Low	Low	Low	Low	Low	Low
Bakker-Zierikzee et al., 2006 [27]	Low	Low	Low	Low	Low	Low	Low
Béghin et al., 2021 [36]	Low	Low	Low	Low	Low	Low	Low
Bisceglia et al., 2009 [37]	Low	Low	Low	Low	Low	Low	Low
Bruzesse et al., 2009 [42]	Low	Low	Low	Low	Low	Low	Low
Chatchatee et al., 2014 [43]	Low	Low	Low	Low	Low	Low	Low
Costalos et al., 2008 [38]	Low	Low	Low	Low	Low	Low	Low
Knol et al., 2005 [28]	Low	Low	Low	Low	Low	Low	Low
Haarman and Knol, 2005 [29]	Low	Low	Low	Low	Low	Some concerns	Some concerns
Haarman and Knol, 2006 [30]	Low	Low	Low	Low	Low	Some concerns	Some concerns
Moro et al., 2002 [31]	Some concerns	Low	Some concerns	Low	High	Some concerns	High
Moro et al., 2003 [32]	Some concerns	Low	Some concerns	Low	High	Some concerns	High
Moro et al., 2005 [33]	Some concerns	Low	Some concerns	Low	High	Some concerns	High
Scholtens et al., 2008 [34]	Low	Low	Low	Low	Low	Low	Low
Raes et al., 2010 [35]	Low	Low	Low	Low	Low	Low	Low
Salvini et al., 2011 [39]	Low	Low	Low	Low	Low	Low	Low
Teoh et al., 2022 [40]	Low	Low	Low	Low	Low	Low	Low
Veereman-Wauters et al., 2011 [41]	Low	Low	Low	Low	Low	Low	Low

Fig. 2. Risk of bias assessment in the selected studies. The Cochrane risk-of-bias assessment tool for randomized trials version 2 (Rob2) was employed for this evaluation [24]. The Microsoft Excel RoB2 Macro, encompassing six domains, was used to assess bias in the selected studies. Domain 1a assessed the randomization process, whereas Domain 1b investigated the timing of identification or recruitment of participants. Domain 2 assessed any deviations from intended interventions. Domain 3 investigated any missing outcome data. Domain 4 assessed measurement of the outcome. Domain 5 investigated selection of the reported result. Results of each domain were categorized as low risk (green), some concerns (yellow), or high risk (red). The overall bias for each publication was determined based on the results of all domains. A detailed explanation on "Some Concerns" and "High" is provided in the "Quality Assessment" subsection of the Results.

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It is important to note that a study with a high risk of bias does not imply that the study has a low quality, because the concept of quality is not well defined and could include study characteristics (e.g., performing a sample size calculation) that are not inherently related to bias in the study's results. The Rob 2 tool replaces the notion of assessing study quality with assessing risk of bias, which refers to systematic deviation from the effect of intervention that would be observed in a large randomized trial without any flaws [24]. Arguably, the Rob 2 tool might not be suitable to assess older study or publication for the following reasons [24,44,45]: (i) incomplete reporting of trial protocols and statistical methods in older publications; (ii) changes in methodology or experimental techniques due to technological advancements, which may render older techniques inadequate, leading to an overestimation of risk of bias; (iii) incomplete description of detail baseline characteristics, which is common in older publications and hinders the ability to determine whether any baseline discrepancies are due to deliberate bias; (iv) gap in information regarding intervention departures from original plans is usually observed in older publications, obstructing proper evaluation of bias arising from planned treatment deviation; (v) outdated statistical approaches for assessing baseline imbalances, hence older publications might not provide a complete picture of the prognostic elements necessary for a thorough evaluation of such modifications; (vi) incomplete reporting of outcomes evaluation and blinding, which is often observed in older publications and increases the risk of bias because blinding is critical for bias evaluation; and (vii) selective reporting in older publications, making it difficult to assess whether the published results were selectively chosen. Therefore, although the Rob 2 tool offers a robust framework for bias evaluation in randomized clinical trials, its application to older studies may raise the risk of bias. Thus, when assessing potential bias in older publications, it is important to proceed with caution and consider the context of the observed limitations.

Safety and physical growth

None of the 18 publications reported any serious adverse events following the administration of a standard formula supplemented with GOS/FOS. Among these, nine publications assessed the effect of GOS/FOS-supplemented standard milk formula on growth increment (i.e., weight, height, and head circumference) within the first year of life [31,33,36-42], whereas one study assessed growth increment among toddlers [43]. No difference in physical growth was observed between the intervention and control groups in most studies, except for one [42]. In that study, the mean body weight and height in the GOS/FOS supplementation group were significantly higher than those in the control group during follow-up. However, mean body weights were similar between the groups at 9 and 12 months of follow-up [42].

Meta-analyses were subsequently performed on weight and height gains. As shown in **Supplementary Fig. 1**, upon comparison of weight gain (g per day) among the four publications [31,36,40,41], no significant mean difference was observed between the GOS/FOS and control groups (p=0.35), although the GOS/FOS group had a higher mean weight gain (mean difference=0.11; 95% CI=−0.12 to 0.34). The heterogeneity measure (I^2) in this comparison was 0%, indicating that the compared studies were homogeneous; hence, a fixed-effects model of the meta-analysis was used. This was further confirmed by the funnel plot, as shown in **Supplementary Fig. 2**. A subsequent comparison of length gain (in cm/week) was conducted among the four publications shown in **Supplementary Fig. 3** [31,36,40,41]. No significant difference was observed between the GOS/FOS and control groups (p=0.22), although the GOS/FOS group had a slightly higher mean height gain (mean difference=0.03; 95% CI=−0.02 to 0.08). I^2 was 0%; hence, a fixed-effects model was used, supported by the funnel plot shown in **Supplementary Fig. 4**.

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Intestinal colonization

Ten studies evaluated the effects of GOS/FOS supplementation on intestinal colonization by bifidobacteria [26,28,29,31,32,34,36,38,39,41]. In addition, six studies evaluated the effect of GOS/FOS supplementation on intestinal colonization by lactobacilli [30-32,36,38,39]. All publications demonstrated higher levels of bifidobacteria or lactobacilli upon supplementation with GOS/FOS, with only two publications reporting non-significant differences (although increasing trends were observed in the GOS/FOS group) between the GOS/FOS and control groups [26,41]. A meta-analysis was subsequently performed on the percentage of bifidobacteria observed in the stools of infants from the GOS/FOS and control groups, as shown in **Fig. 3** [28,29,34,41]. The average proportion of bifidobacteria in the GOS/FOS group was indeed significantly higher than in the control group (p<0.00001; mean difference=15.58; 95% CI=−3.98 to 22.18). The I^2 value was 49%; hence, a fixed-effects meta-analysis model was used. This was supported by a funnel plot (**Supplementary Fig. 5**).

Four studies also evaluated the effect of GOS/FOS supplementation on intestinal colonization by pathogenic bacteria, including *Clostridium* species and *E. coli* [34,38,39,41]. A reduction in pathogenic bacteria upon GOS/FOS supplementation was observed in all publications, with two studies reporting significant differences between the GOS/FOS and control groups [34,39].

Stool frequency and consistency

Six publications assessed the effect of GOS/FOS supplementation on stool frequency and/or consistency [31,37-41]. Regarding stool frequency, three publications reported that GOS/FOS supplementation significantly correlated with higher stool frequency [39-41]. In addition, one publication demonstrated that 0.8 g/dL of GOS/FOS induced a higher stool frequency than both 0.4 g/dL GOS/FOS and standard formula [31]. With regard to stool consistency, four of five publications reported that children receiving the GOS/FOS (9:1)-supplemented standard formula had significantly softer stools than those receiving the standard formula alone [31,38,39,41].

As shown in **Fig. 4A**, a meta-analysis of stool frequency was performed in four studies [31,37,39,41]. The GOS/FOS group had a higher average stool frequency (number of stools per day) compared to the control group (mean difference=0.72; 95% CI=0.02 to 1.06). The GOS/FOS group exhibited a significantly higher stool frequency per day than the control group (p=0.04). Because the heterogeneity measure I^2 was 95%, a random-effects model of meta-analyses was used. Next, a meta-analysis of stool consistency was performed in three studies shown in **Fig. 4B** [31,37,41]. Of note, Teoh et al. [37] used a different scale to rate stool consistency (i.e., 1–4). Hence, the scoring was adjusted to a scale of 1–5 (i.e., the scale

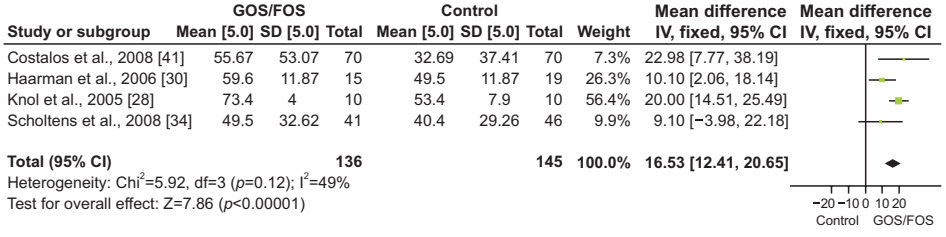

Fig. 3. Forest plot of clinical studies assessing the intestinal colonization by bifidobacteria in healthy infants fed with scGOS/lcFOS (9:1)-supplemented standard milk formula versus control. The individual study effect estimates (green boxes) and pooled effect estimate (diamond) are shown. The values are presented as mean difference with 95% CI, determined using a generic inverse-variance random effects model. Heterogeneity was quantified using the I^2 statistic.
GOS/FOS: scGOS/lcFOS (9:1)-supplemented standard milk formula, control: non-supplemented or maltodextrin-supplemented standard milk formula, CI: confidence interval, IV: inverse variance, SD: standard deviation.

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used in other studies) for this analysis. The calculation reconfirmed that the GOS/FOS group had significantly softer stools than the control group (p=0.0006; mean difference=−0.20; 95% CI=−0.31 to −0.08). The I^2 was 87%, suggesting that the compared publications were heterogeneous; hence, a random-effects meta-analysis model was required.

Stool pH

Seven studies investigated the effect of GOS/FOS (9:1) supplementation on lowering stool pH [26,28,31,34,36,39,41]. All studies observed lower stool pH upon GOS/FOS supplementation, with one publication demonstrating that 0.8 g/dL was more effective than 0.4 g/dL of GOS/FOS in lowering stool pH [31]. As shown in **Fig. 4C**, a subsequent meta-analysis was conducted on six studies [28,31,34,36,39,41]. The I^2 value was 87%; hence, a random-effects model of meta-analysis was used. The calculation confirmed that there was a significant difference in mean pH between the GOS/FOS and control groups (p<0.00001), with the GOS/FOS group exhibiting a lower mean pH (mean difference=−0.75; 95% CI=−1.01 to −0.49).

Fecal short-chain fatty acids and lactate

Three studies assessed the effect of GOS/FOS supplementation on increasing concentrations of fecal short-chain fatty acids and lactate [26,28,39]. All studies reported that the GOS/FOS-supplemented standard formula was associated with higher levels of fecal acetate, but with lower or similar levels of fecal propionate, butyrate, isobutyrate, isovalerate, and valerate

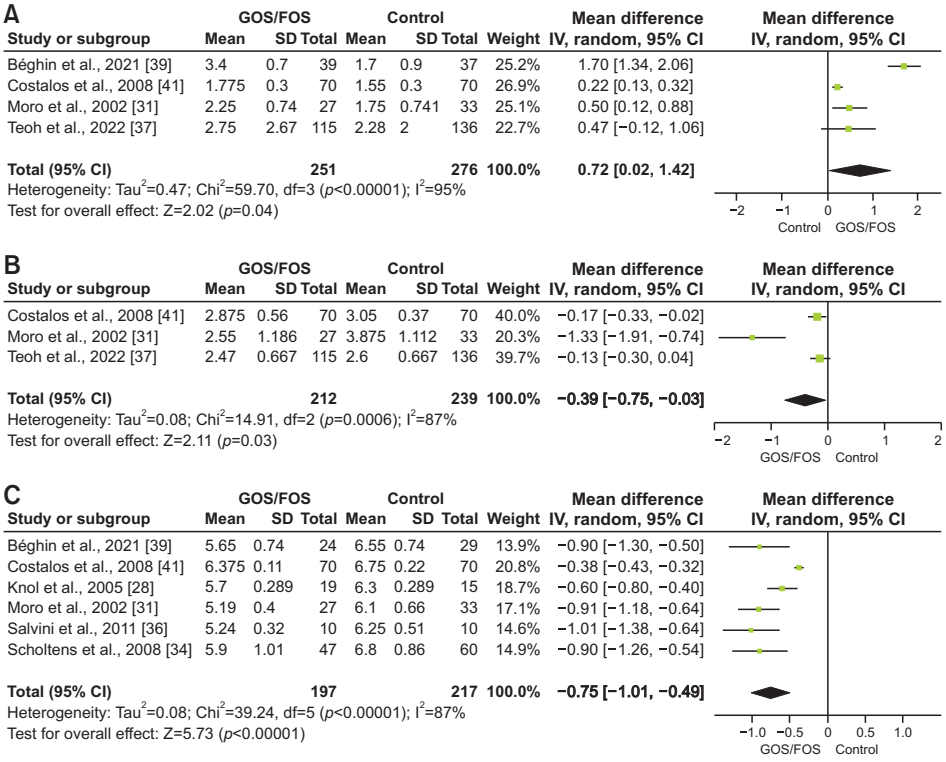

Fig. 4. Forest plot of clinical studies assessing various characteristics of stool in healthy infants fed with scGOS/lcFOS (9:1)-supplemented standard milk formula versus control. Characteristics of (A) stool frequency, (B) stool consistency, and (C) stool pH were evaluated. The individual study effect estimates (green boxes) and pooled effect estimate (diamond) are shown. The values are presented as mean difference with 95% CI determined using a generic inverse-variance random effects model. Heterogeneity was quantified using the I^2 statistic.
AGOS/FOS: scGOS/lcFOS (9:1)-supplemented standard milk formula, control: non-supplemented or maltodextrin-supplemented standard milk formula, CI: confidence interval, IV: inverse variance, SD: standard deviation.

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compared to those administered with the standard formula. GOS/FOS supplementation also correlated with higher levels of D- and L-lactate in the stools.

Immune markers and risks of infection

Three studies investigated the effect of GOS/FOS supplementation on increasing the concentrations of secreted immunoglobulin A in stools [27,34,39]. All studies reported that GOS/FOS-supplemented standard formulations increased the levels of fecal secreted immunoglobulin A (sIgA). However, this elevation was not observed in the concentration of serum immunoglobulin A [35], suggesting that supplementation with GOS/FOS appeared to directly improve mucosal immunity but not systemic immunity, as its administration would modulate the gut microbiome [46]. This hypothesis was partly supported by two studies that did not report any increase in various immune parameters in the blood upon GOS/FOS supplementation [35,36]. Interestingly, two studies investigated the protection effect of GOS/FOS supplementation against gastrointestinal infection and/or upper respiratory tract infection in children [42,43]. The improvement in mucosal immunity correlated with a reduction in intestinal and, presumably, respiratory infections among healthy infants during the first year of age [42]. In young children, the protection conferred by GOS/FOS supplementation was not as strong as that observed in infants. Nonetheless, a trend towards protection against gastrointestinal or respiratory infections was observed in a subset of the cohort [43].

A meta-analysis was performed on the concentrations of fecal sIgA among the three studies shown in **Fig. 5** [27,35,39]. The I^2 was 0%; hence, a fixed-effects model of the meta-analysis was used. A funnel plot of sIgA is shown in **Supplementary Fig. 6**. This calculation supported the notion that there was a significant difference in the concentration of fecal sIgA (mg/g feces) between the GOS/FOS and control groups (p=0.01), with the GOS/FOS group exhibiting a higher concentration of fecal sIgA (mean difference=0.06; 95% CI=0.01 to 0.11).

DISCUSSION

A systematic review and meta-analysis of clinical trials were conducted to evaluate the gastrointestinal health and immunity of a standard formula supplemented with a mixture of short-chain GOS and long chain FOS in a ratio of 9:1, compared to non-supplemented or maltodextrin-supplemented standard formulas, among healthy infants and toddlers. Eighteen full-text publications were selected for the systematic review, 11 of which were further subjected to a meta-analysis. Notably, the majority of the selected studies were

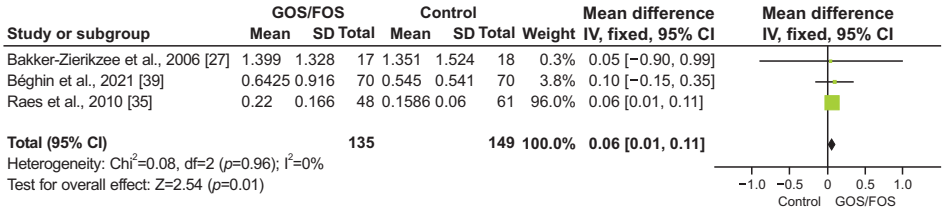

Fig. 5. Forest plot of clinical studies assessing the concentration of fecal secretory immunoglobulin A in healthy infants fed with scGOS/lcFOS (9:1)-supplemented standard milk formula versus control. The individual study effect estimates (green boxes) and pooled effect estimate (diamond) are shown. The values are presented as mean difference with 95% CI determined using a generic inverse-variance fixed effects model. Heterogeneity was quantified using the I^2 statistic.
GOS/FOS: scGOS/lcFOS (9:1)-supplemented standard milk formula, control: non-supplemented or maltodextrin-supplemented standard milk formula, CI: confidence interval, IV: inverse variance, SD: standard deviation.

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assessed to have a low risk of bias. Findings indicate that GOS/FOS supplementation in the standard formula was well tolerated by the study participants across the reviewed studies. Healthy infants or toddlers in the GOS/FOS group also had similar physical growth to those in the control group, reinforcing the notion that GOS/FOS supplementation in the standard formula is not detrimental to body weight, height, and head circumference [47].

Among the various gastrointestinal benefits evaluated in this systematic review, healthy infants in the GOS/FOS group consistently showed higher intestinal colonization of bifidobacteria, reinforcing the findings of previous systematic reviews on various prebiotics in infants [47,48]. Higher intestinal colonization by lactobacilli was also observed in this review, aligning with findings from a previously published review [47], but differing from another review [48]. The discrepancy with the latter publication may be attributed to the specific prebiotic choice analyzed in our review, scGOS/lcFOS (9:1). Additionally, higher levels of fecal acetate and lactate, which are fermentation products of bifidobacteria and lactobacilli, were consistently found in our review as well [48,49]. GOS/FOS supplementation was associated with a reduction in the number of fecal pathogenic bacteria. This reinforced the findings of another published review, which reported that GOS (alone or in combination with another prebiotic) decreased the levels of *Clostridium difficile* [48].

Other gastrointestinal benefits of GOS/FOS supplementation that were consistently observed in this review included higher stool frequency, softer stool, and lower stool pH. As these characteristics were also found among exclusively breastfed infants, these findings reinforce the notion that a mixture of scGOS/lcFOS (9:1) mimics the molecular size and distribution of human milk oligosaccharides in human milk. Furthermore, the scGOS/lcFOS (9:1)-supplemented standard formula exemplifies a standard formula designed to more closely resemble human milk [19,20,49].

This review also examined whether the GOS/FOS group demonstrated improved immune parameters and better protection against gastrointestinal and respiratory infection compared to the control group. In agreement with higher intestinal colonization by bifidobacteria and lactobacilli and increased acetate production, higher concentrations of fecal sIgA were observed among healthy infants in the GOS/FOS group. Dietary fiber, including prebiotics, promotes the intestinal sIgA response via the induction of short-chain fatty acids (particularly acetate), thus explaining the linear relationship between scGOS/lcFOS, bifidobacteria and lactobacilli, and sIgA [50-52]. This finding aligns with a published study, demonstrating that prebiotic supplementation increases sIgA concentration in colostrum or transitional milk [53]. Additionally, GOS/FOS supplementation was noted to confer protection against gastrointestinal and/or upper respiratory tract infections in healthy infants and toddlers. However, as only two studies in this review assessed this aspect, further research is required to evaluate the protective effects conferred by GOS/FOS against pathogens. Nonetheless, other reviews on prebiotics have reported a trend towards protection against infections among infants and children following prebiotic supplementation [54,55], suggesting that this would be an interesting parameter to be assessed in future studies.

While most publications in this review focused on healthy neonates or infants, only one publication focused on healthy toddlers aged 11–29 months [43]. As the study population in this publication was distinct from the others and the concentration of GOS/FOS exceeded 0.8 g/dL per serving [56], this publication was excluded from the meta-analysis. In the future, it

will be of interest to perform a separate meta-analysis of studies assessing the health benefits of scGOS/lcFOS (9:1) in toddlers or young children.

Several limitations were identified in the publications included in this systematic review. Since the focus was on scGOS/lcFOS (9:1), results from studies using different concentrations of GOS/FOS (0.4, 0.6, and 0.8 g/dL) were combined. Studies published over the past two decades that used different measurement methods and presented data in different measurement units were also analyzed. The assessed studies reported data at different time points, such as weight and length gain data were obtained at weeks 4, 10, and 12. This may have resulted in data variation, potentially skewing the meta-analysis and limiting the inclusion of additional studies. To address this, data collection was restricted to week 12 for meta-analysis to limit the duration of data collection and ensure that any impact on the measured parameters would be mainly from the intervention product (as the study participants were not yet weaned). In addition, one publication presented its data solely in graph form [26], thereby requiring estimation of actual values, which further complicated the meta-analysis. Contact with the corresponding author was not pursued, as the publication was dated (2005) and prior attempts by other researchers to reach the author were unsuccessful.

In conclusion, this systematic review shows that milk formula supplemented with scGOS/lcFOS (9:1) improves gastrointestinal health and immunity in healthy infants and toddlers. Infants in the GOS/FOS group consistently showed higher intestinal colonization by bifidobacteria, higher stool frequency, softer stools, lower stool pH, higher levels of fecal acetate, higher levels of fecal D- and L-lactate, and higher sIgA levels. Notably, serious adverse events were not observed upon the administration of a standard formula supplemented with GOS/FOS, indicating that this standard formula was also safe for consumption. This supports the supplementation of standard milk formula with certain prebiotics to improve the gastrointestinal health and immunity in healthy infants and toddlers.

SUPPLEMENTARY MATERIALS

Supplementary Fig. 1
Forest plot of clinical studies assessing weight gain in healthy infants fed with scGOS/lcFOS (9:1)-supplemented standard milk formula versus control.

Supplementary Fig. 2
Funnel plot for weight gain.

Supplementary Fig. 3
Forest plot of clinical studies assessing length gain in healthy infants fed with scGOS/lcFOS (9:1)-supplemented standard milk formula versus control.

Supplementary Fig. 4
Funnel plot for length gain.

Supplementary Fig. 5
Funnel plot for intestinal colonization of bifidobacteria.

Supplementary Fig. 6

Funnel plot for secretory immunoglobulin A.

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