REVIEW ARTICLE

Prebiotic Supplementation in Full-term Neonates

A Systematic Review of Randomized Controlled Trials

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Objective: To systematically review randomized controlled trials evaluating the efficacy and safety of prebiotic supplementation in full-term neonates.

Data Sources: Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE, and CINAHL databases and proceedings of relevant conferences.

Study Selection: Eleven of 24 identified trials (n=1459) were eligible for inclusion.

Intervention: Trials comparing formula milk supplemented with or without prebiotics, commenced at or before age 28 days and continued for 2 weeks or longer.

Main Outcome Measures: Stool colony counts (bifidobacteria, lactobacilli, and pathogens), pH, consistency, frequency, anthropometry, and symptoms of intolerance.

Results: Six trials reported significant increases and 2 reported a trend toward increases in bifidobacteria counts after supplementation. Meta-analysis estimated significant reduction in stool pH in infants who received prebiotic supplementation (weighted mean difference, –0.65; 95% confidence interval, –0.76 to –0.54; 6 trials). Infants who receive a supplement had slightly better weight gain than did controls (weighted mean difference, 1.07 g; 95% confidence interval, 0.14-1.99; 4 trials) with softer and frequent stools similar to breastfed infants. All but 1 trial reported that prebiotic supplementation was well tolerated. In that trial, diarrhea (18% vs 4%; $P=.008$), irritability (16% vs 4%; $P=.03$), and eczema (18% vs 7%; $P=.046$) were reported more frequently by parents of infants who received prebiotic supplements.

Conclusions: Prebiotic-supplemented formula is well tolerated by full-term infants. It increases stool colony counts of bifidobacteria and lactobacilli and results in stools similar to those of breastfed neonates without affecting weight gain. Larger trials with long-term follow-up are needed to determine whether these short-term benefits are sustained.

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BACTERIAL COLONIZATION OF the sterile neonatal gut starts immediately after birth and consists predominantly of bifidobacteria and lactobacilli. These pioneer bacteria modulate gene expression in host epithelial cells, create a favorable permanent habitat for themselves, and prevent growth of harmful bacteria. Early colonization is thus a critical determinant of the permanent gut flora that may beneficially affect the individual's health throughout life by preventing conditions such as colon cancer, inflammatory bowel disease, allergic diseases, diabetes, and obesity.[1,2]

Human milk contains various "oligosaccharide prebiotics" that promote the beneficial gut flora, making breastfeeding very important especially in the first month of life.[3-6] However, breastfeeding may not be possible for various reasons. Formula feeding at such a critical stage of development may result in failure to develop normal gut flora and colonization with potential pathogens such as staphylococci and *Escherichia coli*.[7-9] Supplementation of formula milk with prebiotic oligosaccharides such as galactose oligosaccharide (GOS) and fructose oligosaccharide (FOS) is therefore being explored to overcome this problem.[10,11]

Prebiotic oligosaccharides are short-chain carbohydrates with a degree of polymerization between 2 and 60 and are nondigestible by human or animal digestive systems. The defining property of prebiotics is their ability to selectively stimulate the growth of bifidobacteria and lactobacilli in the large intestine.[12] The prebiotic oligosaccharides in turn are fermented by the gut flora, resulting in the release of hydrogen and carbon dioxide gas and short-chain fatty acids such as butyrate. The short-chain fatty acids reduce the pH of the stools, resulting in more acidic

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stools, which in turn leads to a mild laxative effect with softening and increased frequency of stools. This could be beneficial in preventing the constipation that is frequently observed in formula-fed infants. In addition, the acidic pH prevents growth of pathogens, promotes further growth of healthy organisms, and promotes integrity of colonic epithelial cells. The immediate adverse effects of prebiotics are abdominal pain, regurgitation, and flatulence, which are related to excessive gas production in the gut. These adverse effects can result in failure to adhere to treatment and hence limit the short-term as well as long-term potential benefits of prebiotics.

A narrative review by Fanaro et al[13] reported that prebiotic mixture specifically stimulates the growth of bifidobacteria and lactobacilli and reduces the growth of pathogenic bacteria. They also concluded that prebiotic supplementation results in changes in stool pH and short-chain fatty acid levels that are similar to those of breastfed infants. However, these conclusions were based on the results of 6 trials (of which only 3 were randomized controlled trials [RCTs]) in a neonatal population. A Cochrane review studied the effect of prebiotic supplementation for the prevention of allergic disease and food hypersensitivity in infants.[14] Only 2 of the 7 studies included in the review reported on allergic disease outcome. Meta-analysis of these studies found no significant difference in eczema, but significant heterogeneity was detected. There was insufficient evidence to determine the role of prebiotic supplementation of infant formula for the prevention of allergic disease and food hypersensitivity. This review did not evaluate the effect of prebiotic supplementation on intestinal bacterial flora, which is a prerequisite for the potential benefits of prebiotics.

Considering the significance of gut colonization in the early neonatal period and the recently published RCTs in this population, we undertook this systematic review to determine the effectiveness of prebiotic supplementation on gut colonization with normal and pathogenic bacteria, the physical characteristics of stool, and growth as measured by anthropometry in full-term neonates.

METHODS

We followed guidelines from the Cochrane neonatal review group,[15] the Quality of Reporting of Meta-analyses statement,[16] and the Centre for Reviews and Dissemination group[17] for undertaking and reporting this systematic review and meta-analysis. To be included in this review, the trials had to meet the following criteria.

Only randomized and quasi-randomized trials were included. Case series, retrospective trials, crossover trials, and uncontrolled trials were not eligible.

Trials involving full-term neonates were eligible for inclusion. Trials were excluded if the postnatal age at randomization was greater than 28 days. Trials on preterm neonates (<37 weeks at birth) were excluded because their physiology and nutritional requirements are different from those of full-term neonates.

Trials comparing formula milk supplemented with prebiotics vs placebo or unsupplemented formula milk were eligible for inclusion. The prebiotics could be GOS, FOS, or both. The supplementation should have commenced within 28 days of life and continued for at least 2 weeks. Trials comparing a combination of prebiotics and probiotics vs controls were excluded. Trials in which the intervention formula had different composition than the control formula (apart from prebiotics) were excluded.

Trials with at least 1 of the following outcome measures were included: stool characteristics such as pH, consistency, and frequency; stool colony count of bifidobacteria and lactobacilli; stool colonization with enteric pathogenic bacteria such as *E coli*; weight gain during the first 12 months of life; and symptoms of intolerance such as excessive vomiting, diarrhea, regurgitation, and excessive irritability.

IDENTIFICATION AND ASSESSMENT OF TRIALS

The Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane library, issue 2, 2008), PubMed (1966 to May 2008), EMBASE (1980 to May 2008), and CINAHL databases, as well as proceedings of the pediatric academic society meetings (published in *Pediatric Research* from 1980) and pediatric gastroenterology conferences (from 1980 onward) were searched. PubMed was searched by means of the following Medical Subject Headings words: *oligosaccharides* AND *infant formula* AND *infant* OR *infant, newborn*. The search was repeated using the text word *prebiotic* instead of *oligosaccharides*. Finally, the search was repeated with the text word *inulin*. Related articles of the included trials were searched on PubMed fortnightly until May 2008 to identify any additional trials.

In addition, the reference lists of identified trials and key review articles were searched. No language restrictions were applied. Two of us (S.R. and R.S.) searched the literature independently and assessed the eligibility of trials for inclusion in the review. Any differences were resolved by discussion with the third reviewer (S.P.).

The methodologic quality of the included trials in terms of internal validity was assessed by the 2 reviewers (S.R. and R.S.), using the Jadad scoring system.[18] In the event of disagreement, consensus was reached by discussion with the third reviewer (S.P.).

The 2 reviewers (S.R. and R.S.) independently extracted the data. Inconsistencies were resolved by discussion among all 3 reviewers. All authors of studies were contacted to provide additional information and clarification regarding the data and methods of their trials.

STATISTICAL ANALYSIS

Meta-analysis was done with Review Manager 4.3 software (http://www.cc-ims.net/RevMan). Weighted mean difference and 95% confidence interval were calculated. Heterogeneity was estimated by the I^2 statistic. A fixed-effects model was used. The results were also cross-checked by using the random-effects model. Funnel plots were used to identify the possibility of publication bias.[19]

RESULTS

TRIAL SELECTION

Searching PubMed by using the search term *oligosaccharides* returned a total of 45 relevant articles. Replacing it with the text word *prebiotics* returned a total of 37 relevant articles. Replacing the word with *inulin* returned 3 articles. After removing the overlapping articles, a total of 55 potentially relevant articles were identified. Careful scrutiny of these 55 publications and additional articles obtained by searching related articles on PubMed

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and other databases produced a total of 13 articles that were eligible for inclusion.[20-32]

Of these 13 articles, 2 were different publications from the same trial.[21,22] They were considered as a single trial and referred to as "Bakker-Zierikzee et al[21,22] (and as "Bakker-Zierikzee et al[21,22] 2005A" in the tables). Similarly, 2 others were different publications from the same RCT[29,30] and were considered as a single trial and referred to as "Moro et al[29,30]" in this review (and as "Moro et al,[29,30] 2002" in the tables). A total of 11 trials were finally included in the review (**Figure 1**). Thirteen RCTs[33-45] were excluded for reasons given in **Table 1**.

SUMMARY OF FINDINGS

Methodologic Quality

The reviewers agreed on all of the methodologic assessments. Authors were contacted for clarifications and/or additional data given the inadequate reporting in individual trials included in the review. Authors of Alliet et al,[20] Costalos et al,[26] Decsi et al,[27] and Zeigler et al[32] provided the needed data. The first author of Bakker-Zierikzee et al[21,22] and Bakker-Zierikzee et al[23] advised us to contact a coauthor, who did not respond to our 3 requests. There was no response from the remaining authors. The details of the quality of individual trials are presented in **Table 2**.

Trial Characteristics

Eleven RCTs (n=1459) were included in the review. Nine were considered to be of good quality, with Jadad scores of 3 or more. On the basis of the information from the publications, the Jadad scores were assessed to be less than 3 in 2 RCTs.[23,24] The supplementation was with GOS in 2 trials (Bakker-Zierikzee et al[23] and Ben et al[24]), GOS-FOS and acidic oligosaccharide in 1 trial (Fanaro et al[28]), FOS in 1 trial (Bettler and Euler[25]), a combination of polydextrose, GOS, and lactulose in 1 trial (Ziegler et al[32]), and GOS-FOS in the remaining 6 trials. The sample size in individual trials ranged from 34 to 297. The concentration of prebiotics ranged from 0.15 to 0.8 g/dL. Four trials had a group of breastfed infants as a reference group (Bakker-Zierikzee et al,[21,22] Bakker-Zierikzee et al,[23] and Ben et al,[24] and Decsi et al[27]). The duration of supplementation varied from 2 weeks to 6 months. Outcomes assessed varied in individual trials and included stool characteristics; stool bifidobacteria, lactobacilli, and pathogenic bacterial colony counts/pH/fatty-acid profile/IgA/short-chain fatty acid levels; symptoms of intolerance (regurgitation, diarrhea, and excessive crying); anthropometry; allergy; plasma lipid profile; and calcium absorption at different times after supplementation during the trial period. The trial characteristics are shown in **Table 3**.

OUTCOMES OF INTEREST

Stool Colonization With Bifidobacteria and/or Lactobacilli

Nine of the 11 trials evaluated the effect of prebiotic supplementation on the colony counts of bifidobacteria in the stools (Table 3 and **Table 4**). The stools were analyzed at various time intervals (1 week to 6 months) after the supplementation was commenced. Bakker-Zierikzee et al,[21,22] Bakker-Zierikzee et al,[23] and Costalos et al[26] reported the colony counts of bifidobacteria as a percentage of the total bacterial counts. All other trials presented the data as actual colony counts per gram of stool. Six trials[20,24,27-29,31] demonstrated significantly higher levels of bifidobacteria after supplementation with prebiotics. Two trials (Bakker-Zierikzee et al[21,22] and Costalos et al[26]) reported that, although not statistically significant, the prebiotic-supplemented group had a higher percentage of bifidobacteria in the total bacterial count at all ages during the study period. Bakker-Zierikzee et al[23] did not find any significant differences between the 2 groups.

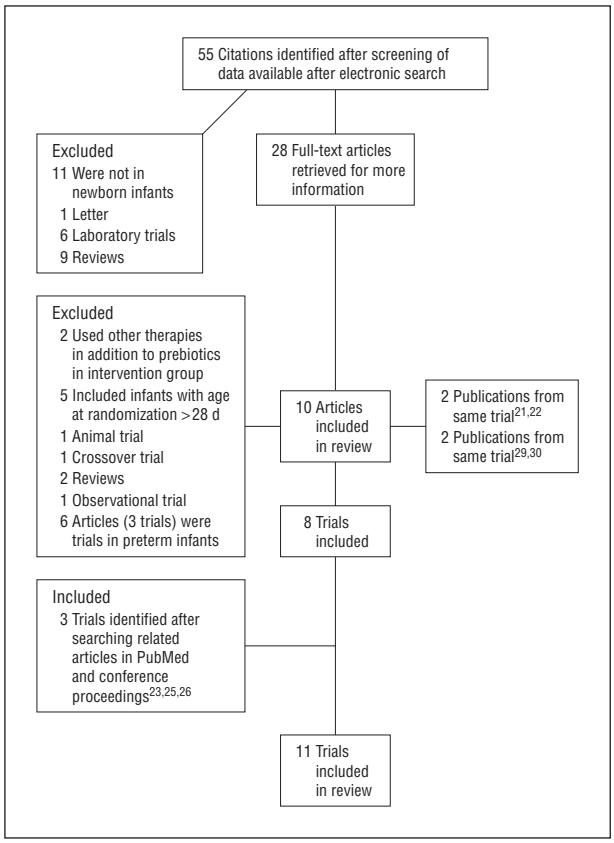

Figure 1. Process of trial selection.

Meta-analysis was not possible because of significant heterogeneity in the methods for measuring and reporting colony counts and the timing of estimation. Even after gathering additional information from the trial authors, few data were available in a format that could be combined.

Three trials (Fanaro et al,[28] Moro et al,[29,30] and Moro et al[31]) also evaluated the effect on lactobacilli colony counts. Fanaro et al[28] and Moro et al[29,30] demonstrated higher levels of lactobacilli in the stools after supplementation with prebiotics, whereas Moro et al[31] found no difference in lactobacilli counts between the 2 groups.

Stool Colonization With Pathogenic Bacteria

Alliet et al,[20] Ben et al,[24] Costalos et al,[26] Decsi et al,[27] Fanaro et al,[28] and Moro et al[29,30] reported this outcome

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Table 1. Randomized Trials Excluded From the Review

Trial	Group	Reason for Exclusion
Puccio et al,[33] 2007	Full-term infants	Intervention group received combination of probiotics and prebiotics
Haarman and Knol,[34] 2005	Full-term infants	Age of trial infants was 28 to 90 d
Euler et al,[35] 2005	Full-term infants	Crossover trial
Savino et al,[36] 2006	Infants with colic	Intervention group received combination prebiotics and partially hydrolyzed whey protein, and high palmitic acid content; control formula also had prebiotic content (0.4 g/dL of GOS-FOS)
Knol et al,[37] 2005	Infants	Age at randomization >28 d
Brunser et al,[38] 2006	Infants approximately 4 mo old	Age at randomization >28 d
Kukkonen et al,[39] 2007	Infants at risk of allergy	Intervention group received combination of probiotics and prebiotics
Schmelzle et al,[40] 2003	Healthy newborn infants <2 wk	In addition to prebiotics, intervention formula contained partially hydrolyzed whey protein, modified vegetable oil with high β-palmitic acid content, and starch; control group received standard newborn formula without any of these supplements
Scholtens et al,[41] 2006	Infants on weaning diet	Age of infants 4-6 mo
Fuentes et al,[42] 2005	Full-term infants 37-42 wk[a]	In addition to prebiotics, intervention formula contained partially hydrolyzed whey protein, modified vegetable oil with high β-palmitic acid content, and starch; control group received standard newborn formula without any of these supplements
Boehm et al,[43] 2002	Preterm infants ≤32 wk[a]	Preterm infants
Kapiki et al,[44] 2007	Preterm infants ≤36 wk[a]	Preterm infants
Mihatsch et al,[45] 2006	Preterm infants <1500 g and 24-31 wk[a]	Preterm infants

Abbreviation: GOS-FOS, galactose oligosaccharide and fructose oligosaccharide.
[a] Indicates gestational age.

Table 2. Jadad Score for Assessment of Trial Quality

Trial	Randomization	Method to Create Randomization Clear and Appropriate	Double-blind	Methods of Blinding Appropriate	Description of Withdrawal or Dropout	Total Score
Alliet et al,[20] 2007	Yes	Yes	Yes	Yes	Yes	5
Bakker-Zierikzee et al,[21,22] 2005A	Yes	Not clear	Yes	Yes	Yes	4
Bakker-Zierikzee et al,[23] 2005B	Yes	Not clear	Yes	Not clear	Not clear	2
Ben et al,[24] 2004	Yes	Not clear	Not clear	Not clear	Not clear	1
Bettler and Euler,[25] 2006	Yes	Yes	Yes	Not clear	Yes	4
Costalos et al,[26] 2008	Yes	Yes	Yes	Yes	Yes	5
Decsi et al,[27] 2005	Yes	Yes	Yes	Yes	Yes	5
Fanaro et al,[28] 2005	Yes	Not clear	Yes	Yes	Yes	4
Moro et al,[29,30] 2002	Yes	Not clear	Yes	Yes	Not clear	3
Moro et al,[31] 2006	Yes	Yes	Yes	Yes	Yes	5
Ziegler et al,[32] 2007	Yes	Not clear	Yes	Not clear	Yes	3

(**Table 5**). Effects of prebiotic supplementation on enteric pathogens such as *E coli*, *Klebsiella* species, clostridia, enterococci, etc, were studied. Costalos et al[26] showed a trend toward reduction in pathogenic bacteria in the prebiotic-supplemented groups. The data provided by Alliet et al[20] and Decsi et al[27] suggested a reduction in pathogenic bacteria in the prebiotic group. However Ben et al,[24] Fanaro et al,[28] and Moro et al[29,30] did not find significant differences between prebiotic and control groups.

Stool pH

Eight trials (Alliet et al,[20] Bakker-Zierikzee et al,[21,22] Bakker-Zierikzee et al,[23] Ben et al,[24] Costalos et al,[26] Decsi et al,[27] Fanaro et al,[28] and Moro et al[29,30]) evaluated the effect of prebiotic supplementation on stool pH. All except Costalos et al[26] reported that prebiotic supplementation resulted in a significantly lower stool pH compared with controls. Pooling of the available data from 6 trials estimated a statistically significant reduction in stool pH in the prebiotic-supplemented group (weighted mean difference, –0.65; 95% confidence interval, –0.76 to –0.54) (**Figure 2**). However, significant statistical heterogeneity was noted between the trials for this outcome (I^2=81%; $P<.001$).

Stool Consistency

Costalos et al,[26] Fanaro et al,[28] Ziegler et al,[32] Moro et al,[29,30] and Moro et al,[31] assessed the stool consistency after

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Table 3. Characteristics and Results of Trials Included in the Analysis

Trial	Intervention	Outcomes Assessed	Results
Alliet et al,[20] 2007	Intervention: GOS-lcFOS, 0.6 g/dL (n = 86) Control: unsupplemented formula (n = 90) Duration of supplementation: 6 mo	Serum cholesterol and triglyceride levels at ages 8 and 26 wk; stool pH, stool colony counts of bifidobacteria, pathogenic *Escherichia coli* and clostridia at ages 8 and 26 wk; anthropometry at ages 8, 12, and 16 wk	Serum cholesterol and triglyceride levels not different between groups; stool pH lower in prebiotic group at ages 8 and 26 wk; significantly higher stool colony counts of bifidobacteria at age 26 wk, lower counts of *E coli* at age 8 wk and lower counts of clostridia at age 26 wk in prebiotic group
Bakker-Zierikzee et al,[21,22] 2005A	Prebiotic: GOS-FOS, 0.6 g/dL (n = 19) Control: unsupplemented formula (n = 19) Breastfed reference group (n = 63) Duration of supplementation: 16 wk	Intestinal flora, fecal short-chain fatty acids, and stool pH on days 5 and 10 and at ages 4, 8, 12, and 16 wk; fecal IgA on days 5 and 10 and every 4 wk until age 32 wk	Trend toward higher stool colony counts of bifidobacteria in prebiotic group vs standard formula group; stool pH lower in prebiotic group; fecal IgA levels higher in prebiotic group at age 16 wk
Bakker-Zierikzee et al,[23] 2005B	Prebiotic group: GOS, 0.6 g/dL (n = 17) Control: unsupplemented formula (n = 17) Breastfed reference group (n = not known) Duration of supplementation: 16 wk	Bifidobacteria as percentage of total No. of bacteria in stools; short-chain fatty acids, lactates, pH of stools on days 5 and 10 and weeks 4, 8, 12, and 16	No differences between groups for all outcomes
Ben et al,[24] 2004	Prebiotic group: GOS, 0.24 g/dL (n = 69) Control: unsupplemented formula (n = 52) Breastfed reference group (n = 26) Duration of supplementation: 6 mo	Stool colony counts of bifidobacteria and pathogenic bacteria; stool pH; stool SCFA; anthropometry; symptoms of intolerance at ages 3 and 6 mo	Stool colony counts of bifidobacteria higher and pathogenic *E coli* lower in the prebiotic group; stool pH lower in prebiotic group; no difference in anthropometry or symptoms of intolerance between groups
Bettler and Euler,[25] 2006	Prebiotic group: FOS, 0.3 g/dL (n = 101); FOS, 0.15 g/dL (n = 98) Control: unsupplemented formula (n = 98) Duration of supplementation: 12 wk	Weight, length, and head circumference at ages 4, 8, and 12 wk; adverse effects; serum chemistry panel	No difference in physical growth between groups; all formulas well tolerated; FOS 0.3-g/dL group had less constipation than other groups
Costalos et al,[26] 2008	Prebiotic group: GOS-lcFOS, 0.4 g/dL (n = 80) Control: unsupplemented standard formula (n = 80) Duration of supplementation: 15 d	Anthropometry at ages 6 and 12 wk; stool for bifidobacteria, clostridia, and *E coli* at age 6 wk; stool characteristics at ages 6 and 10 wk	Growth during trial period same in both groups; no difference in symptoms of intolerance; stools softer and more frequent in prebiotic group; stool pH not different between groups; trend toward higher stool bifidobacteria as percentage of total bacterial count in prebiotic group; percentage of fecal clostridia at completion of trial significantly lower in prebiotic group ($P = .04$)
Decsi et al,[27] 2005	Prebiotic: GOS-FOS, 0.4 g/dL (n = 21) Control: formula supplemented with maltodextrin, 0.8 g/dL (n = 24) Breastfed reference group (n = 52) Duration of supplementation: 12 wk	Intestinal flora on days 14 and 28 of supplementation, weekly stool pH; symptoms and signs of intolerance; allergic disease in first 12 mo of life	Stool colony counts of bifidobacteria at 14 and 28 d of supplementation higher in prebiotic group; stool colony counts of pathogenic *E coli* lower in prebiotic group; stool pH lower in prebiotic group; no difference in symptoms of intolerance such as excessive irritability, vomiting, regurgitation, or atopy
Fanaro et al,[28] 2005	Prebiotic group 1: GOS-FOS, 0.6 g/dL and AOS, 0.2 g/dL (n = 15) Prebiotic group 2: AOS, 0.2 g/dL (n = 16) Control group: maltodextrin as placebo (n = 15) Duration of supplementation: 6 wk	Fecal flora, stool characteristics, stool pH, SCFA after 6 wk of supplementation; increase in weight (g/d) and length (cm/wk) during trial period	Stool pH lower in prebiotic group 1; infants fed combination of acidic and neutral oligosaccharides had higher colony counts of lactobacilli and bifidobacteria at 6 wk of supplementation; no difference in colony counts of pathogenic bacteria between groups; stools softer in both prebiotic groups; no difference in length and weight gain between groups during trial period; no difference in incidence of crying, regurgitation, or vomiting between groups
Moro et al,[29,30] 2002	Prebiotic group 1: GOS-FOS, 0.4 g/dL (n = 30) Prebiotic group 2: GOS-FOS, 0.8 g/dL (n = 27) Control group: maltodextrin as placebo (n = 33) Duration of supplementation: 4 wk	Fecal flora, stool pH, stool characteristics on day 28; symptoms and signs of intolerance; anthropometry during trial period	Stool colony counts of bifidobacteria and lactobacilli higher in prebiotic groups; stool pH on day 28 lower in prebiotic group 2; no difference in colony counts of pathogenic bacteria; stools softer and more frequent in prebiotic group 2; no difference in anthropometry between groups; no difference in symptoms of intolerance
Moro et al,[31] 2006	Intervention: hydrolyzed milk supplemented with GOS-FOS, 0.8 g/dL (n = 129) Placebo: hydrolyzed milk supplemented with maltodextrin, 0.8 g/dL (n = 130) Duration of supplementation: 6 mo	Atopic dermatitis at ages 3 and 6 mo; stool frequency and consistency, stool lactobacilli and bifidobacteria at ages 3 and 6 mo; vomiting, regurgitation, and crying	Less atopic dermatitis in prebiotic group; stools softer and more frequent in prebiotic group; higher colony counts of bifidobacteria in prebiotic group; no difference in lactobacilli colony counts between groups; less regurgitation and crying in prebiotic group
Ziegler et al,[32] 2007	Prebiotic group 1: PDX-GOS-LOS, 0.4 g/dL (n = 74) Prebiotic group 2: PDX-GOS-LOS, 0.8 g/dL (n = 76) Group 3: standard formula (n = 76) Duration of supplementation: 120 d	Anthropometry at ages 14, 30, 60, 90, and 120 d; tolerance at ages 14, 30, 60, 90, and 120 d; stool consistency at ages 30, 60, 90, and 120 d	No difference in weight, length, and head circumference at all time points; higher risk of diarrhea and eczema in prebiotic group 1; higher risk of excessive irritability in prebiotic group 2

Abbreviations: AOS, acidic oligosaccharide; FOS, fructose oligosaccharide; GOS, galactose oligosaccharide; PDX, polydextrose; lcFOS, long-chain FOS; LOS, lactulose; SCFA, short-chain fatty acids.

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Table 4. Stool Colonization With Bifidobacteria After Supplementation

Trial	Measure	Age	Prebiotic	Control	Authors' Conclusion
Alliet et al,[20] 2007	Colony counts (cells/g of stool)	8 wk; 26 wk	1.06 E + 10 (8.36 E + 9)[a]; 1.63 E+10 (7.68 E+10)[a]	8.05 E+9 (9.14 E + 9)[a]; 9.89 E+9 (6.34 E+9)[a]	Higher counts in prebiotic group
Bakker-Zierikzee et al,[21,22] 2005A	Bifidobacteria as % of total bacterial count	16 wk	59.2 (SEM, 7.7)	51.8 (SEM, 6.4)	Trend toward higher counts in prebiotic group
Bakker-Zierikzee et al,[23] 2005B	Bifidobacteria as % of total bacterial count	4, 8, 12, and 16 wk	Not given	Not given	No difference
Ben et al,[24] 2004	Colony counts (log CFU/g of stool)	3 mo	9.0 (1.8)[a]	7.2 (1.2)[a]	Increased in prebiotic group
		6 mo	7.9 (1.3)[a]	6.0 (0.9)[a]	Increased in prebiotic group
Costalos et al,[26] 2008	Bifidobacteria as % of total bacterial count	6 wk	39.69 (0.00-143.3)[b]	14.87 (0.00-101.00)[b]	Trend toward higher counts in prebiotic group
Decsi et al,[27] 2005	Bifidobacteria colony counts (log CFU/g of stool)	2 wk	11.25 (1.83)[a]	8.07 (0.49)[a]	Increased in prebiotic group
		4 wk	11.82 (2.59)[a]	7.61 (0.87)[a]	Increased in prebiotic group
Fanaro et al,[28] 2005	Colony counts (log CFU/g of stool)	6 wk	9.61 (0.7)[a]	8.75 (0.5)[a]	Increased in prebiotic group
Moro et al,[29,30] 2002	Colony counts (log CFU/g of stool)	4 wk	9.7 (0.8)[c]	7.2 (4.9)[c]	Increased in prebiotic groups
Moro et al,[31] 2006	Colony counts (log CFU/g of stool)	3 mo	9.56 (0.9)[c]	8.3 (1.1)[c]	Increased in prebiotic groups
		6 mo	10.28 (0.7)[c]	8.65 (1.2)[c]	Increased in prebiotic group

Abbreviation: CFU, colony-forming unit.
[a] Mean or mean (SD).
[b] Median (range).
[c] Median (interquartile range).

Table 5. Effect of Prebiotic Supplementation on Stool Colonization With Potentially Pathogenic Bacteria

Trial	Measure	Age	Prebiotic	Control	Authors' Conclusion
Alliet et al,[20] 2007	Colony counts (cells/g of stool), *Escherichia coli* FISH analysis	8 wk; 26 wk	5.80 E+8 (5.78 E+8)[a]; 3.99 E+8 (4.79 E+8)[a]	1.03 E+9 (7.25 E+8)[a]; 6.80 E+8 (1.07 E+9)[a]	Lower counts of pathogenic *E coli* at age 8 wk and clostridia at age 26 wk in prebiotic group
	Colony counts (cells/g of stool), *Clostridium* spp FISH analysis	8 wk; 26 wk	1.30 E+8 (2.52 E+8)[a]; 2.77 E+8 (4.91 E+8)[a]	5.59 E+8 (1.15E+9)[a]; 7.58 E+8 (7.83 E+8)[a]	NA
Ben et al,[24] 2004	Colony counts (CFU/g of stool), *E coli*	3 mo; 6 mo	Actual values not given	Actual values not given	No difference
Costalos et al,[26] 2008	*E coli* as % of total bacterial count	6 wk	1.95 (0.00-69.32)[b]	0 (0.00-59.31)[b]	Trend toward lower counts in prebiotic group
Decsi et al,[27] 2005	Colony counts (log CFU/g of stool), *E coli*	2 wk; 4 wk	9.60 (1.39)[a]; 10.57 (1.60)[a]	10.08 (1.96)[a]; 9.68 (1.42)[a]	NA
Fanaro et al,[28] 2005	Colony counts of *E coli*, *Clostridium* spp, *Proteus* spp, *Klebsiella* spp, *Candida* spp, *Bacteroides* spp, *Enterobacter* spp, and *Citrobacter* spp	6 wk	Actual values not given	Actual values not given	No difference
Moro et al,[29,30] 2002	Colony counts of *Bacteroides* spp, *Clostridium* spp, *E coli*, *Enterobacter* spp, *Citrobacter* spp, *Proteus* spp, *Klebsiella* spp, and *Candida* spp	4 wk	Actual values not given	Actual values not given	No difference

Abbreviations: CFU, colony-forming unit; FISH, fluorescence in situ hybridization; NA, not available; spp, species.
[a] Mean (SD).
[b] Median (range).

prebiotic supplementation. All reported that the stools were softer in the prebiotic-supplemented group.

Stool Frequency

Costalos et al,[20] Moro et al,[29,30] and Moro et al[31] reported on stool frequency. All reported a higher frequency of stools in prebiotic-supplemented infants. The higher frequency of stools was considered to be similar to the frequency in breastfed infants and hence was reported by the investigators as a beneficial outcome rather than as diarrhea.

Physical Growth During the First Year of Life

Nine trials (Alliet et al,[20] Ben et al,[24] Bettler and Euler,[25] Costalos et al,[26] Decsi et al,[27] Fanaro et al,[28] Moro et al,[29,30] Moro et al,[31] and Ziegler et al[32]) evaluated the effect of prebiotic supplementation on physical growth at various ages in the first year of life. All reported no difference in physical growth between the 2 groups. However, pooled meta-analysis of the data from 4 trials showed that infants in the prebiotic group had slightly better weight gain during the trial period than

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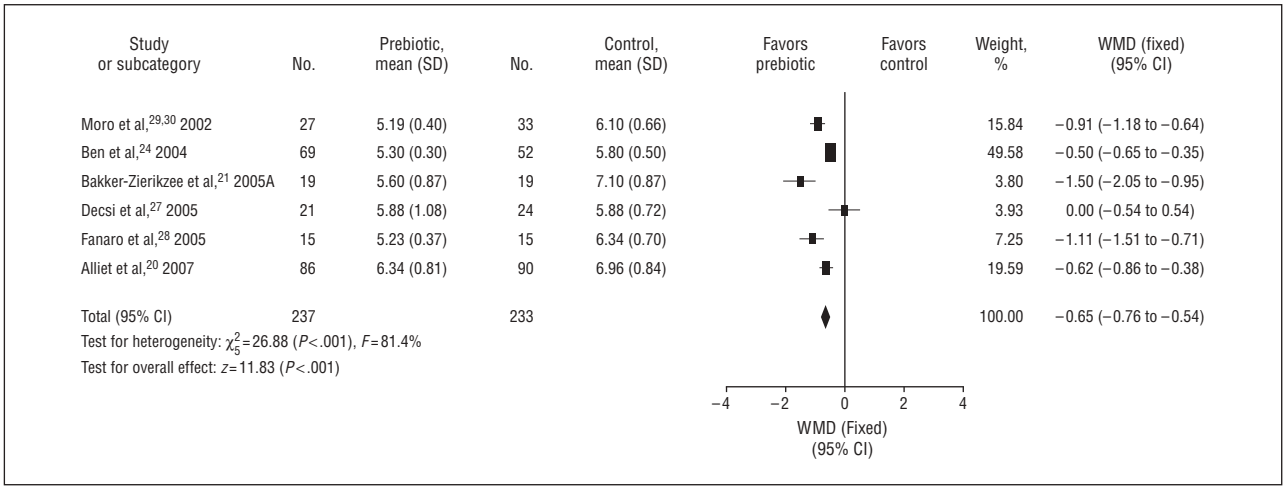

Figure 2. Stool pH after at least 4 weeks of supplementation. CI indicates confidence interval; WMD, weighted mean difference. Because of rounding, weight percentages do not total 100.

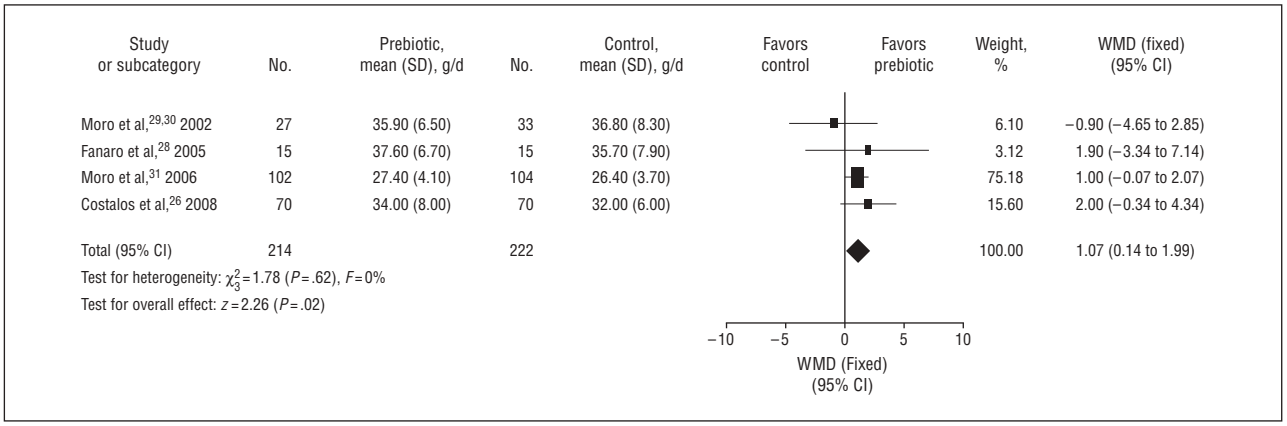

Figure 3. Weight gain during the trial period. CI indicates confidence interval; WMD, weighted mean difference.

did controls (weighted mean difference, 1.07 g; 95% confidence interval, 0.14-1.99 g) (**Figure 3**).

Tolerance

Eight trials (Ben et al,[24] Bettler and Euler,[25] Costalos et al,[26] Decsi et al,[27] Fanaro et al,[28] Moro et al,[29,30] Moro et al,[31] and Ziegler et al[32]) reported this outcome. All except Ziegler et al reported that prebiotic supplementation was well tolerated and that the incidence of symptoms such as excessive irritability, crying, regurgitation, and vomiting was not different between the 2 groups.

Ziegler et al[32] evaluated the effect of 2 different combinations of prebiotics at 2 different intake levels on the growth and tolerance in healthy formula-fed, full-term infants (N=226) up to 120 days of age. Infants were randomly assigned to receive a control formula (n=76), the control formula with 0.4 g/dL of a prebiotic blend (n=74), or the control formula with 0.8 g/dL of the prebiotic blend (n=76). There were no statistically significant differences in any group for growth measurements at any time during the study period. Significant differences in stool consistency were detected among the 3 formula groups at 30, 60, and 90 days of age ($P<.001$, $P=.03$, and $P=.004$, respectively), with the supplemented-formula groups having looser stools than the control group. The 0.8-g/dL group had significantly higher stool frequency than the control and 0.4-g/dL groups at 30 days of age ($P=.02$ and $P=.02$, respectively), but all of the groups were similar at 60, 90, and 120 days of age. They found a significant increase in 3 categories of adverse events: diarrhea (0.4 g/dL vs control, 18% vs 4%; $P=.008$), eczema (0.4 g/dL vs control, 18% vs 7%; $P=.046$), and irritability (0.8 g/dL vs control, 16% vs 4%; $P=.03$). The risk of eczema was higher (18% vs 4%; $P=.008$) in the 0.4-g/dL group than in the 0.8-g/dL group. The authors concluded that infants receiving the prebiotic mixture achieved normal growth and stool characteristics more similar to those of breastfed infants in comparison with controls. They advised considering the risk of possible intolerance against the benefits of prebiotics.

COMMENT

The results of our systematic review show that, in full-term neonates, prebiotic supplementation of formula milk results in higher stool colony counts of bifidobacteria. This effect was consistent across most of the trials irrespective of the heterogeneity among studies with regard to the dosage, duration of supplementation, and method

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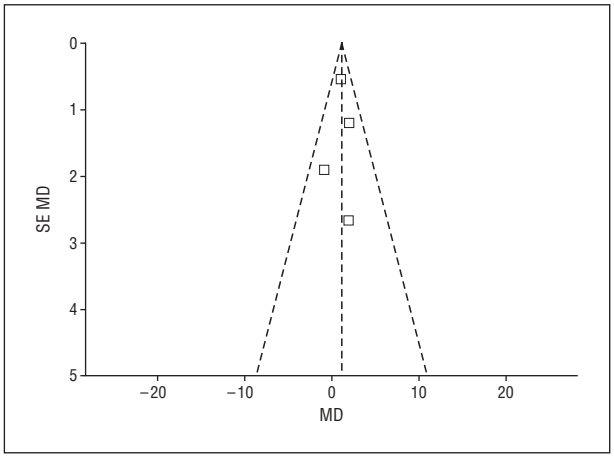

Figure 4. Funnel plot for weight gain. SE MD indicates standard error of mean difference.

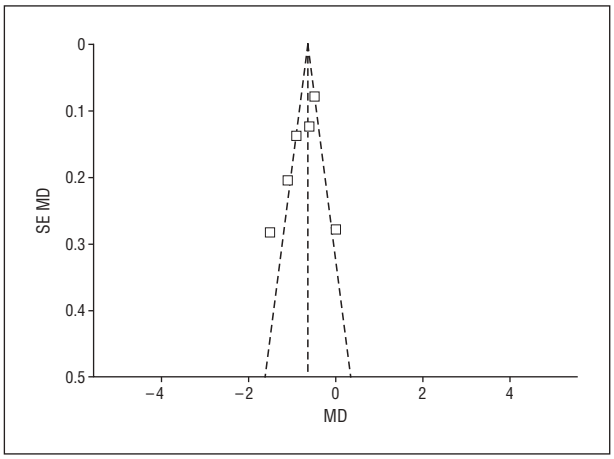

Figure 5. Funnel plot for pH. SE MD indicates standard error of mean difference.

of estimation and reporting of the results. In addition, stools in the supplemented group had higher lactobacilli counts, lower pathogenic bacteria counts, and more acidic pH and were softer and more frequent, similar to those of breastfed neonates.

Most of the trials showed a statistically significant increase in stool colony counts of bifidobacteria after prebiotic supplementation. Even studies that did not show statistically significant differences reported a trend toward higher stool colony counts of bifidobacteria in the prebiotic group. None of the studies showed a decrease in stool colony counts of bifidobacteria after prebiotic supplementation. The response to exogenous prebiotics is reported to depend on the baseline mass of healthy gut flora before the start of the supplement rather than the dose of prebiotics.[11] However, some studies have shown a dose-dependent stimulating effect on the growth of bifidobacteria and lactobacilli in the intestine.[29] In the absence of specific data, we can only speculate that the lack of significant benefits in some of the outcomes in the studies by Bakker-Zierikzee et al[21-23] and Costalos et al[26] (Table 3) may be related to lower counts of healthy gut flora before the commencement of supplementation.

The rationale for doses of 0.15 to 0.8 g/dL in various trials appears to be an attempt to achieve a maximum bifidogenic effect with minimal intolerance in the form of flatulence, abdominal distention, colic, etc. The European Scientific Committee on Food recommendation indicates that prebiotics can be added up to a maximum of 0.8 g per 100 mL of formula milk.[13,46,47]

In addition to the bifidogenic effect, we assessed the physical growth of these infants because of the theoretical risk of lower weight gain after prebiotic supplementation. Animal and human trials have suggested that prebiotics may reduce hunger and food consumption, possibly mediated via gut hormones, and may be a modality for prevention and treatment of obesity.[48-51] Although such effects may be beneficial in adolescents and adults, reduced weight gain can be detrimental during the immediate postnatal period. It is reassuring that all trials (n=9) that reported this outcome did not find such a detrimental effect of prebiotic supplementation. In fact, the meta-analysis of results from 4 trials showed that the prebiotic-supplemented group had slightly greater weight gain than did controls.

Excessive carbon dioxide and hydrogen gas released after fermentation of prebiotics in the colon has been shown to increase adverse effects such as flatulence, regurgitation, and vomiting. The neonates in these studies tolerated the prebiotic supplementation very well, without any increase in vomiting, irritability, or diarrhea.

When interpreting these short-term positive results, it is important to consider the possibility of publication bias wherein trials with negative results are not published. However, the funnel plots[19] for the primary outcomes of stool pH and weight gain do not suggest such a possibility (**Figure 4** and **Figure 5**).

Ziegler et al[32] reported an increased incidence of atopic eczema in the prebiotic-supplemented group. However, the large RCT by Moro et al[31] reported beneficial effects of prebiotic supplementation in reducing the incidence of atopic dermatitis and wheezing when followed up at 6 months as well as at 2 years of age.[31,52] The Cochrane review[14] that reported the meta-analysis of results of these 2 trials[31,32] did not find a statistically significant difference in the incidence of eczema in the prebiotic group. The mechanism of action of prebiotics in the prevention of allergic diseases is thought to be mediated via promoting the growth of healthy bacteria in the gut early in infancy, leading to "host-microbe cross-talk" and immunomodulation.[53] Current evidence is thus inadequate to derive any firm conclusions regarding the use of prebiotics for prevention of atopic diseases.

In summary, our results show that prebiotic supplementation of formula milk in full-term neonates is well tolerated and results in various short-term beneficial effects, including increased stool colony counts of bifidobacteria and lactobacilli, decreased counts of pathogenic enteric bacteria, more acidic stools, and softer and frequent stools, without adversely affecting weight gain. Larger population-based trials with continued long-term follow-up into adulthood are needed to find out whether these short-term benefits relate to improved general health and reduced morbidities. Until then, routine supplementation of formula milk with prebiotic oligo-

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saccharides cannot be recommended. Although further research continues, the issue of cost cannot be neglected, given that prebiotic-supplemented formula available in supermarkets costs approximately 15% to 20% more than regular formula.

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