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.

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. 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. 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, the Quality of Reporting of Meta-analyses statement, and the Centre for Reviews and Dissemination group 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. 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 I² 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.

**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...
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 2005” in the tables). Similarly, 2 others were different publications from the same RCT24,25 and were considered as a single trial and referred to as “Moro et al”29,30 in this review (and as “Moro et al 2002” in the tables). A total of 11 trials were finally included in the review (Figure 1). Thirteen RCTs33-45 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 al32 provided the needed data. The first author of Bakker-Zierikzee et al21,22 and Bakker-Zierikzee et al23 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 and Ben et al43), GOS-FOS and acidic oligosaccharide in 1 trial (Fanaro et al28), FOS in 1 trial (Bettler and Euler25), a combination of polydextrose, GOS, and lactulose in 1 trial (Ziegler et al32), 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 al21,22 Bakker-Zierikzee et al23 and Ben et al24 and Decsi et al27). 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 al21,22 Bakker-Zierikzee et al23 and Costalos et al26 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 trials20,24,27,29,31 demonstrated significantly higher levels of bifidobacteria after supplementation with prebiotics. Two trials (Bakker-Zierikzee et al21,22 and Costalos et al26) 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 al23 did not find any significant differences between the 2 groups.

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 al28 Moro et al29,30 and Moro et al31) also evaluated the effect on lactobacilli colony counts. Fanaro et al28 and Moro et al29,30 demonstrated higher levels of lactobacilli in the stools after supplementation with prebiotics, whereas Moro et al31 found no difference in lactobacilli counts between the 2 groups.

#### Stool Colonization With Pathogenic Bacteria

Alliet et al20 Ben et al24 Costalos et al26 Decsi et al27 Fanaro et al28 and Moro et al29,30 reported this outcome.
Effects of prebiotic supplementation on enteric pathogens such as *E. coli*, *Klebsiella* species, *Clostridia*, enterococci, etc, were studied. Costalos et al26 showed a trend toward reduction in pathogenic bacteria in the prebiotic-supplemented groups. The data provided by Alliet et al20 and Decsi et al27 suggested a reduction in pathogenic bacteria in the prebiotic group. However Ben et al,24 Fanaro et al,28 and Moro et al29,30 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 al29,30) evaluated the effect of prebiotic supplementation on stool pH. All except Costalos et al26 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 (I²=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...
Table 3. Characteristics and Results of Trials Included in the Analysis

<table>
<thead>
<tr>
<th>Trial</th>
<th>Intervention</th>
<th>Outcomes Assessed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alliet et al,20 2007</td>
<td>Intervention: GOS/lf-FOS, 0.6 g/dL (n = 86) Control: unsupplemented formula (n = 90) Duration of supplementation: 6 mo</td>
<td>Serum cholesterol and triglyceride levels at ages 8 and 26 wk; stool pH, stool colony counts of bifidobacteria, pathogenic Enterobacteriaceae and clostridia at ages 8 and 26 wk; anthropometry at ages 8, 12, and 16 wk</td>
<td>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 28 wk in prebiotic group</td>
</tr>
<tr>
<td>Bakker-Zierikzee et al,25 2005A</td>
<td>Prebiotic group: GOS, 0.6 g/dL; Breastfed reference group (n = 63) Duration of supplementation: 16 wk</td>
<td>Intestinal flora, fecal short-chain fatty acids, and stool pH on days 5 and 10 at ages 4, 8, 12, and 16 wk; fecal IgA on days 5 and 10 and every 4 wk until age 32 wk</td>
<td>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</td>
</tr>
<tr>
<td>Bakker-Zierikzee et al,25 2005B</td>
<td>Prebiotic group: GOS, 0.6 g/dL; Breastfed reference group (n = 63) Duration of supplementation: 16 wk</td>
<td>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, 5, 12, and 16</td>
<td>No differences between groups for all outcomes</td>
</tr>
<tr>
<td>Ben et al,26 2004</td>
<td>Prebiotic group: GOS, 0.24 g/dL (n = 69) Breastfed reference group (n = 26) Duration of supplementation: 6 mo</td>
<td>Stool colony counts of bifidobacteria and pathogenic bacteria; stool pH; stool SCFA; symptoms and signs of intolerance at ages 3 and 6 mo</td>
<td>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</td>
</tr>
<tr>
<td>Bettler and Euler,25 2006</td>
<td>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</td>
<td>Weight, length, and head circumference at ages 4, 8, and 12 wk; adverse effects; serum chemistry panel</td>
<td>No difference in physical growth between groups; all formulas well tolerated; FOS 0.3-g/dL group had less constipation than other groups</td>
</tr>
<tr>
<td>Costalos et al,26 2008</td>
<td>Prebiotic group: GOS-lf-FOS, 0.4 g/dL (n = 80) Control: unsupplemented standard formula (n = 80) Duration of supplementation: 15 d</td>
<td>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</td>
<td>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)</td>
</tr>
<tr>
<td>Decsi et al,27 2005</td>
<td>Prebiotic group: GOS, 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</td>
<td>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</td>
<td>Stool colony counts of bifidobacteria at age 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</td>
</tr>
<tr>
<td>Fanaro et al,28 2005</td>
<td>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</td>
<td>Fecal flora, stool characteristics, stool pH, SCFA after 6 wk of supplementation; increase in weight (g/d) and length (cm/wk) during trial period</td>
<td>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</td>
</tr>
<tr>
<td>Moro et al,29 2002</td>
<td>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</td>
<td>Fecal flora, stool pH, stool characteristics on day 28; symptoms and signs of intolerance; anthropometry during trial period</td>
<td>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</td>
</tr>
<tr>
<td>Moro et al,29 2006</td>
<td>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</td>
<td>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</td>
<td>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 lactobacillus colony counts between groups; less regurgitation and crying in prebiotic group</td>
</tr>
<tr>
<td>Ziegler et al,29 2007</td>
<td>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</td>
<td>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</td>
<td>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</td>
</tr>
</tbody>
</table>

Abbreviations: AOS, acidic oligosaccharide; FOS, fructose oligosaccharide; GOS, galactose oligosaccharide; PDX, polydextrose; lf-FOS, long-chain FOS; LOS, lactulose; SCFA, short-chain fatty acids.
prebiotic supplementation. All reported that the stools were softer in the prebiotic-supplemented group.

### Stool Frequency

Costalos et al., Moro et al., and Moro et al 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., Ben et al., Bettler and Euler, Costalos et al., Decsi et al., Fanaro et al., Moro et al., Moro et al., and Ziegler et al.) 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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**Table 4. Stool Colonization With Bifidobacteria After Supplementation**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Measure</th>
<th>Age</th>
<th>Prebiotic</th>
<th>Control</th>
<th>Authors’ Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alliet et al., 2007</td>
<td>Colony counts (cells/g of stool)</td>
<td>8 wk</td>
<td>1.06 E+10</td>
<td>8.05 E+9</td>
<td>Higher counts in prebiotic group</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 wk</td>
<td>(5.36 E+9)</td>
<td>E+9</td>
<td></td>
</tr>
<tr>
<td>Bakker-Zierikzee et al</td>
<td>Bifidobacteria as % of total bacterial count</td>
<td>16 wk</td>
<td>59.2 (SEM, 7.7)</td>
<td>51.8 (SEM, 6.4)</td>
<td>Trend toward higher counts in prebiotic group</td>
</tr>
<tr>
<td>Costalos et al, 2008</td>
<td>Colony counts (log CFU/g of stool)</td>
<td>6 wk</td>
<td>9.0 (1.5)</td>
<td>7.2 (1.2)</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Moro et al, 31</td>
<td>Colony counts (log CFU/g of stool)</td>
<td>6 wk</td>
<td>7.9 (1.3)</td>
<td>6.0 (0.9)</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Decsi et al, 2007</td>
<td>Colony counts (log CFU/g of stool)</td>
<td>2 wk</td>
<td>11.25 (1.83)</td>
<td>8.07 (0.49)</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Fanaro et al, 2005</td>
<td>Colony counts (log CFU/g of stool)</td>
<td>6 wk</td>
<td>11.82 (2.59)</td>
<td>7.61 (0.87)</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Moro et al, 2005</td>
<td>Colony counts (log CFU/g of stool)</td>
<td>4 wk</td>
<td>9.75 (0.5)</td>
<td>7.2 (4.9)</td>
<td>Increased in prebiotic groups</td>
</tr>
<tr>
<td>Moro et al, 2006</td>
<td>Colony counts (log CFU/g of stool)</td>
<td>6 mo</td>
<td>10.35 E+9</td>
<td>8.3 (1.1)</td>
<td>Increased in prebiotic groups</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Trial</th>
<th>Measure</th>
<th>Age</th>
<th>Prebiotic</th>
<th>Control</th>
<th>Authors’ Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alliet et al., 2007</td>
<td>Colony counts (cells/g of stool), E. coli FISH analysis</td>
<td>8 wk</td>
<td>5.80 E+8</td>
<td>1.03 E+9</td>
<td>Higher counts in prebiotic group</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 wk</td>
<td>(5.78 E+8)</td>
<td>(7.68 E+8)</td>
<td></td>
</tr>
<tr>
<td>Bakker-Zierikzee et al</td>
<td>Bifidobacteria as % of total bacterial count</td>
<td>8 wk</td>
<td>1.30 E+8</td>
<td>5.59 E+8</td>
<td>NA</td>
</tr>
<tr>
<td>Costalos et al, 2008</td>
<td>Colony counts (CFU/g of stool), E. coli</td>
<td>6 wk</td>
<td>9.61 (0.7)</td>
<td>8.75 (0.5)</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Decsi et al, 2005</td>
<td>Colony counts (log CFU/g of stool), E. coli</td>
<td>2 wk</td>
<td>9.60 (1.39)</td>
<td>10.08 (1.96)</td>
<td>NA</td>
</tr>
<tr>
<td>Fanaro et al, 2005</td>
<td>Colony counts (log CFU/g of stool), E. coli</td>
<td>6 wk</td>
<td>10.57 (1.60)</td>
<td>10.08 (1.96)</td>
<td>NA</td>
</tr>
<tr>
<td>Moro et al, 2002</td>
<td>Colony counts (log CFU/g of stool), E. coli</td>
<td>4 wk</td>
<td>Actual values not given</td>
<td>Actual values not given</td>
<td>No difference</td>
</tr>
</tbody>
</table>

**Abbreviations:** CFU, colony-forming unit; FISH, fluorescence in situ hybridization; NA, not available; spp, species.

- a Mean (SD).
- b Median (range).
- c Mean or mean (SD).

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Eight trials (Ben et al., 2004; Decsi et al., 2005; Fanaro et al., 2005; Moro et al., 2002; Alliet et al., 2007; Bakker-Zierikzee et al., 2005; Ziegler et al., 2006; Costalos et al., 2007) 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. 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.

**Tolerance**

Eight trials (Ben et al., 2004; Bettler and Euler, 2005; Costalos et al., 2004; Decsi et al., 2005; Fanaro et al., 2005; Moro et al., 2002; Moro et al., 2006; Ziegler et al., 2006) 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. 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...
of supplementation.

Lower counts of healthy gut flora before the commencement of formula milk 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. However, some studies have shown a dose-dependent stimulating effect on the growth of bifidobacteria and lactobacilli in the intestine. 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 and Costalos et al (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.

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. 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 for the primary outcomes of stool pH and weight gain do not suggest such a possibility (Figure 4 and Figure 5).

Ziegler et al reported an increased incidence of atopic eczema in the prebiotic-supplemented group. However, the large RCT by Moro et al 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. The Cochrane review that reported the meta-analysis of results of these 2 trials 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. 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 oligо-
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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REFERENCES


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The advantages of not going steady far outweigh the advantages of going steady in high school. Steady dating tends to stunt the development of personality.

—From the educational pamphlet “Teenage Maturity” by Daniel Lowry, 1965