Supplementation of prebiotics in infant formula

Ana Močić Pavić
Iva Hojsak
Referral Center for Pediatric Gastroenterology and Nutrition, Children’s Hospital Zagreb, Zagreb, Croatia

Background: In recent years prebiotics have been added to infant formula to make it resemble breast milk more closely and to promote growth and development of beneficial intestinal microbiota. This review aims to present new data on the possible positive effects of prebiotics in infant formula on intestinal microbiota (bifidogenic and lactogenic effect) and on clinical outcomes including growth, infections, and allergies. With that aim, a literature search of the Cochrane Central Register of Controlled Trials (CENTRAL), EMBASE, Scopus, PubMed/Medline, Web of Science, and Science Direct in the last 10 years (December 2003 to December 2013) was performed.

Results: Altogether 24 relevant studies were identified. It was found that during intervention, prebiotics can elicit a bifidogenic and lactogenic effect. As far as clinical outcomes were concerned, 14 studies investigated the effect of infant formula supplemented with prebiotics on growth and found that there was no difference when compared with non-supplemented infant formula. All available data are insufficient to support prebiotic supplementation in order to reduce risk of allergies and infections.

Conclusion: There is currently no strong evidence to recommend routine supplementation of infant formulas with prebiotics. Further well-designed clinical studies with long-term follow-up are needed.

Keywords: prebiotics, infant formula, growth, allergy, infections, supplementation

Introduction
Prebiotics are defined as non-digestible food ingredients that affect the host by selectively targeting growth and/or the activity of one or more bacteria in the colon that can improve health.1 Breast-fed infants have an intestinal microbiota dominated by Bifidobacterium and Lactobacillus and this is quite different from the intestinal microbiota of those fed with a standard infant formula.2,3 Human milk contains substantial quantities of prebiotics, oligosaccharides which undigested reach the colon and selectively serve as an energy source for desired bacteria, dominantly bifidobacteria.4

Intestinal microbiota have been considered an important physiological factor for different functions of the gut; most importantly, development of the immune system.5 In recent years, attempts have been made to make intestinal microbiota in formula-fed infants similar to those found in breast-fed infants, mostly by adding pro- and prebiotics. Human milk oligosaccharides are structurally very complex, have a huge diversity and currently are not available for commercial use.4 However, several prebiotics have been developed that have a positive effect on the colonization, growth, survival and function of commensal bacteria. Most commonly used prebiotics in infant formulas...
are a mixture of long-chain galacto-oligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS), both neutral oligosaccharides with proven prebiotic effect.\(^6\)

This review aimed to present available data on the role of prebiotics in infant formula on growth, infection rate and allergies, and on their influence on intestinal microbiota through the bifidogenic and lactogenic effect.

With that aim we performed a literature search which included The Cochrane CENTRAL, EMBASE, Scopus, PubMed/Medline, Web of Science, and Science Direct over the last 10 years (the period from December 2003 to December 2013). All relevant randomized controlled trials (RCT) were included. No other reports—including case series, retrospective trials, crossover trials and uncontrolled trials—were taken into consideration. We included only the studies which were performed on healthy term infants.

**Results**

**Trial characteristics**

The most commonly studied prebiotic was a 9:1 mixture of GOS and FOS, following with GOS, acidic oligosaccharides (AOS), combination of GOS, FOS and AOS, polydextrose (PDX) and GOS, PDX/GOS and lactulose (LOS), oligofructose and inulin. The prebiotic concentration ranged from 0.12 to 0.8 g/100 mL. Sample size of included studies varied from 20 to 1,130 infants and duration of intervention from 15 days to 6 months.

### Stool colonization with bifidobacteria (bifidogenic effect)

Twelve of the 24 included studies evaluated the effect of prebiotic supplementation on the bifidobacteria in the stools (Table 1). The majority of published studies\(^7\)–\(^9\) demonstrated significantly higher levels of bifidobacteria after supplementation, while two trials\(^15\),\(^16\) reported a higher count of bifidobacteria; however, this was not statistically significant. Salvini et al\(^17\) in their small explorative study (sample size 20) reported a long-lasting bifidogenic effect, which continued even 6 months after intervention was stopped. One study looked at the difference in the stool colonies of bifidobacteria in infants fed on prebiotic-supplemented formula and breast-fed infants and found that formula supplemented

### Table 1 Prebiotic-supplemented infant formula and bifidogenic effect

<table>
<thead>
<tr>
<th>Author</th>
<th>N/age</th>
<th>Duration of intervention</th>
<th>Setting</th>
<th>Prebiotic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakker-Zierikzee et al 2005</td>
<td>34/ at birth</td>
<td>4 months</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.6 g/100 mL</td>
<td>Trend toward higher counts in prebiotic group</td>
</tr>
<tr>
<td>Costalos et al 2008</td>
<td>160/14 days</td>
<td>15 days</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.4 g/100 mL</td>
<td>Trend toward higher counts in prebiotic group</td>
</tr>
<tr>
<td>Decsi et al 2005</td>
<td>69/12 weeks</td>
<td>12 weeks</td>
<td>Healthy term infants, enrolled 14 days</td>
<td>GOS/FOS 0.4 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Fanaro et al 2005</td>
<td>31/6 weeks</td>
<td></td>
<td>Healthy term infants</td>
<td>1. GOS/FOS 0.6 g + AOS 0.2 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td></td>
<td>159/12 weeks</td>
<td></td>
<td>Healthy infants</td>
<td>2. AOS 0.2 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Fanaro et al 2009</td>
<td>90/4 weeks</td>
<td></td>
<td>Healthy term infants</td>
<td>GOS 0.5 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Moro et al 2006</td>
<td>206/6 months</td>
<td></td>
<td>Term infants at high risk for atopy</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Ben et al 2004</td>
<td>147/6 months</td>
<td></td>
<td>Healthy term infants</td>
<td>GOS 0.24 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Ben et al 2008</td>
<td>164/3 months</td>
<td></td>
<td>Term infants; formula feeding within 4 weeks after birth</td>
<td>GOS 0.24 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Salvini et al 2011</td>
<td>20/6 months</td>
<td></td>
<td>Healthy term infants of HCV-positive mothers</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>Long-lasting bifidogenic effect</td>
</tr>
<tr>
<td>Scalabrin et al 2012</td>
<td>230/60 days</td>
<td></td>
<td>Healthy term infants</td>
<td>GOS + PDX 0.4 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Veerman-Wauters et al 2011</td>
<td>110/28 days</td>
<td></td>
<td>Healthy term infants</td>
<td>SYNI 0.4 g/100 mL</td>
<td>SYNI 0.8 and GOS/FOS – comparable to breast-fed infants</td>
</tr>
</tbody>
</table>

**Abbreviations:** GOS, short-chain galacto-oligosaccharides; FOS, long-chain fructo-oligosaccharides; HCV, hepatitis C virus; PDX, polydextrose; AOS, acidic oligosaccharides; SYNI, Synergy1 (consists of 50:50 oligofructose and long-chain inulin).
with prebiotics has a bifidogenic effect comparable to that of breast milk.\textsuperscript{18}

### Stool colonization with lactobacilli (lactogenic effect)

As for the lactogenic effect, we identified only four studies that evaluated the prebiotic effect on the counts of lactobacilli colonies (Table 2). All studies presented the data as actual colony counts per gram of stool. Three studies\textsuperscript{6,9,18} reported higher levels of lactobacilli in the stool after prebiotic supplementation; in contrast, Moro et al\textsuperscript{12} found no difference in colony counts. None of those studies included breast-fed infants.

#### Growth

Overall 14 studies measured growth as an outcome (Table 3). Growth was validated by body weight, length, and head circumference. In six of the studies,\textsuperscript{7,10,19,20} in addition to the prebiotic and control groups, a group of breast-fed infants was included. All identified trials reported no difference in growth among the groups.

### Table 2 Prebiotic-supplemented infant formula and lactogenic effect

<table>
<thead>
<tr>
<th>Author</th>
<th>N / age</th>
<th>Duration of intervention</th>
<th>Setting</th>
<th>Prebiotic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanaro et al 2005\textsuperscript{11}</td>
<td>31/at birth</td>
<td>6 weeks</td>
<td>Healthy term infants</td>
<td>1. GOS/FOS 0.6 g + AOS 0.2 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Moro et al 2006\textsuperscript{12}</td>
<td>206/at birth</td>
<td>6 months</td>
<td>Term infants at high risk for atopy</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Ben et al 2008\textsuperscript{16}</td>
<td>164/at birth</td>
<td>3 months</td>
<td>Term infants; formula feeding within 4 weeks after birth</td>
<td>GOS 0.24 g/100 mL</td>
<td>Increased in prebiotic group</td>
</tr>
<tr>
<td>Salvini et al 2011\textsuperscript{17}</td>
<td>20/at birth</td>
<td>6 months</td>
<td>Healthy term infants of HCV-positive mothers</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>Long-lasting lactogenic effect</td>
</tr>
</tbody>
</table>

### Abbreviations:

GOS, short-chain galacto-oligosaccharides; FOS, long-chain fructo-oligosaccharides; AOS, acidic oligosaccharides; NS, not significant; HCV, hepatitis C virus.

### Table 3 Prebiotic-supplemented infant formula and growth

<table>
<thead>
<tr>
<th>Author</th>
<th>N / age</th>
<th>Duration of intervention</th>
<th>Setting</th>
<th>Prebiotic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moro et al 2003\textsuperscript{12}</td>
<td>90/at birth</td>
<td>4 weeks</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.4 and 0.8 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Moro et al 2006\textsuperscript{13}</td>
<td>206/at birth</td>
<td>6 months</td>
<td>Term infants at high risk for atopy</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Ben et al 2004\textsuperscript{16}</td>
<td>147/at birth</td>
<td>6 months</td>
<td>Healthy term infants</td>
<td>GOS 0.24 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Ben et al 2008\textsuperscript{16}</td>
<td>164/at birth</td>
<td>3 months</td>
<td>Term infants; formula feeding within 4 weeks after birth</td>
<td>GOS 0.24 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Costalos et al 2008\textsuperscript{16}</td>
<td>160/\textless 14 days</td>
<td>15 days</td>
<td>Healthy term infants, enrolled \textless 14 days</td>
<td>GOS/FOS 0.4 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Fanaro et al 2005\textsuperscript{11}</td>
<td>31/at birth</td>
<td>6 weeks</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.4 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Ziegler et al 2007\textsuperscript{16}</td>
<td>226/\textless 14 days</td>
<td>120 days</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.4 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Bettler et al 2006\textsuperscript{19}</td>
<td>297/at birth</td>
<td>12 weeks</td>
<td>Healthy term infants</td>
<td>FOS 0.3 and 0.15 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Decsi et al 2005\textsuperscript{16}</td>
<td>69/at birth</td>
<td>12 weeks</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.4 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Alliet et al 2007\textsuperscript{16}</td>
<td>225/at birth</td>
<td>6 months</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.6 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Piemontese et al 2011\textsuperscript{10}</td>
<td>830/\textless 8 weeks</td>
<td>1 year</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.68 g/100 mL + LOS 0.8 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Brunser et al 2006\textsuperscript{17}</td>
<td>91/3.5 months old</td>
<td>13 weeks</td>
<td>Healthy term infants</td>
<td>FOS 0.2 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Ribeiro et al 2012\textsuperscript{20}</td>
<td>133/9–48 months</td>
<td>108 days</td>
<td>Healthy term infants</td>
<td>PDX/GOS 0.5 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Ashley et al 2012\textsuperscript{11}</td>
<td>419/12–16 days</td>
<td>120 days</td>
<td>Healthy term infants</td>
<td>1. PDX/GOS 0.4 g/100 mL</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Abbreviations:

GOS, short-chain galacto-oligosaccharides; FOS, long-chain fructo-oligosaccharides; PDX, polydextrose; LOS, lactulose; NS, not significant; AOS, acidic oligosaccharides.
### Allergy

Results are summarized in the Table 4. Two studies\(^{12,21}\) found a positive effect on the reduction of the risk for atopic dermatitis; however the children included in one study were infants with high risk of atopy\(^{12}\) and in the other study, only healthy full-term infants were included.\(^{21}\) Arslanoglu et al\(^{22}\) showed a protective effect of prebiotic supplementation on allergies in a follow-up period of 2 years; however, that effect was not seen after 5 years of follow-up.\(^{23}\) Moreover, both studies had a high dropout rate which should be taken into consideration when interpreting the results.

### Respiratory and gastrointestinal infections

Results of the trials investigating the role of prebiotics on infection prevention are summarized in Tables 5 and 6.

**Table 4 Prebiotic-supplemented infant formula and atopic dermatitis**

<table>
<thead>
<tr>
<th>Author</th>
<th>N/age</th>
<th>Duration of intervention</th>
<th>Setting</th>
<th>Prebiotic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moro et al 2006(^{12})</td>
<td>206 at birth</td>
<td>6 months</td>
<td>Term infants at high risk for atopy</td>
<td>Extensively hydrolyzed whey formula + GOS/FOS 0.8 g/100 mL</td>
<td>↓ AD</td>
</tr>
<tr>
<td>Ziegler et al 2007(^{20})</td>
<td>226/14 days</td>
<td>120 days</td>
<td>Healthy term infants</td>
<td>1. PDX + GOS 0.4 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Arslanoglu et al 2008(^{22})</td>
<td>259/6 months</td>
<td>6 months</td>
<td>Term infants at high risk for atopy</td>
<td>2. PDX + GOS + LOS 0.8 g/100 mL</td>
<td>2 years follow-up</td>
</tr>
<tr>
<td>Gruber et al 2010(^{21})</td>
<td>1,130/8 weeks</td>
<td>1 year</td>
<td>Healthy term infants</td>
<td>Extensively hydrolyzed whey formula + GOS/FOS 0.8 g/100 mL</td>
<td>↓ AD</td>
</tr>
</tbody>
</table>

**Table 5 Prebiotic-supplemented infant formula and respiratory tract infections**

<table>
<thead>
<tr>
<th>Author</th>
<th>N/age</th>
<th>Setting</th>
<th>Prebiotic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arslanoglu et al 2007(^{24})</td>
<td>259/6 months</td>
<td>Term infants with a parental history of atopy</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>No significant difference in the incidence of respiratory tract infections</td>
</tr>
<tr>
<td>Arslanoglu et al 2008(^{22})</td>
<td>259/6 months</td>
<td>Term infants with a parental history of atopy</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>No significant difference in the incidence of respiratory tract infections</td>
</tr>
<tr>
<td>Bruzzeze et al 2009(^{25})</td>
<td>342/5–120 days</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.4 g/100 mL</td>
<td>No significant difference in the incidence of respiratory tract infections</td>
</tr>
<tr>
<td>van Stuijvenberg et al 2011(^{26})</td>
<td>830/8 weeks</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.68 g + AOS 0.12 g/100 mL</td>
<td>No significant difference in the incidence of respiratory tract infections</td>
</tr>
<tr>
<td>Ribeiro et al 2012(^{20})</td>
<td>133/9–48 months</td>
<td>Healthy term infants</td>
<td>PDX/GOS 0.5 g/100 mL</td>
<td>No significant difference in the incidence of respiratory tract infections</td>
</tr>
</tbody>
</table>

**Abbreviations:** AD, atopic dermatitis; GOS, short-chain galacto-oligosaccharides; FOS, long-chain fructo-oligosaccharides; PDX, polydextrose; LOS, lactulose; AOS, acidic oligosaccharides; NS, not significant.

Arslanoglu et al\(^{24}\) reported fewer episodes of physician-diagnosed overall and upper respiratory tract infections and fewer antibiotic prescriptions. Bruzzeze et al\(^{25}\) reported a lower incidence of gastroenteritis in the supplemented group. On the other hand, the study by van Stuijvenberg et al\(^{26}\) found a non-significant difference in the number of fever episodes.

### Discussion

Currently available data show that prebiotic supplementation of infant formula can yield bifidogenic and lactogenic effects similar to those found in breast-fed babies. That trend was reported by all studies using the prebiotic mixture GOS/FOS. On the other hand, acidic oligosaccharides failed to yield the same effect when used alone, but when used in combination with the GOS/FOS mixture, an effect was observed.\(^{11}\) As far as concentration is concerned, both effects have been
achieved with the concentration recommended by the Scientific Committee on Food of the European Commission (ie, lower than 8 g/100 mL of milk).27

However, although presented studies proved that prebiotics have a bifidogenic and lactogenic effect, their clinical relevance still remains unknown. Moreover, we are not aware whether or not that effect on intestinal microbiota early in life could be long-lasting or whether it could also have clinical relevance later in life. Currently available data assessed only short-term clinical effects including growth, allergy and infection rate. As far as growth was concerned, none of the studies showed a significant difference between the supplemented and non-supplemented group.6–12,16,19,20,28–31 However, several limitations should be considered: first of all, the role model for ideal infant growth is not the formula-fed but the breast-fed infant; moreover, none of those studies assessed long-term prebiotic effect on infant growth.

There is some evidence that a prebiotic supplementation may prevent eczema.12,21 However, the evidence is weak; moreover, studies which measured long-lasting effect had very high dropout during the follow-up.22,23 It is still unclear whether the use of prebiotics should be restricted only to infants at high risk of allergy or whether they should also be used in low-risk populations. For the evidence-based recommendation on the use of prebiotics in infant formula for allergy prevention, we need more well-performed randomized controlled trials with long-term follow-up.

The role of prebiotics in the prevention of respiratory and gastrointestinal infections is still controversial; the number of well-designed clinical trials is limited. Regarding the effect on upper respiratory tract infections, the data is not unequivocal; there are two studies both on the same cohort, one during the use of prebiotics and the other during the follow-up.22,24 Both studies found a decreased incidence of respiratory tract infections. However, the follow-up study had a very high drop-out rate and, furthermore, intention-to-treat analysis was not performed.22 In these two studies the significant difference in the number of gastrointestinal infections was not observed. Because there is no clear evidence, further recommendation of the role of prebiotics in the prevention of infections could not be given.

The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition published a Position Paper in 2011 on the role of supplementation of infant formula with pro- and prebiotics in different clinical outcomes.32 The overall recommendation of that systematic review was that there was not enough evidence to recommend routine supplementation of infant formula with pro- and prebiotics. However, it was also clearly stated that supplementation does not raise safety concerns regarding side effects and infants’ growth.

Table 6 Prebiotic-supplemented formula and gastrointestinal infection

<table>
<thead>
<tr>
<th>Author</th>
<th>N/age</th>
<th>Duration of intervention or follow-up</th>
<th>Setting</th>
<th>Prebiotic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arslanoglu et al 200724</td>
<td>259/&lt;6 months</td>
<td>6 months</td>
<td>Term infants with a parental history of atopy</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Arslanoglu et al 200822</td>
<td>259/&lt;6 months</td>
<td>6 months</td>
<td>Term infants with a parental history of atopy</td>
<td>GOS/FOS 0.8 g/100 mL</td>
<td>2 years follow-up (134 children finished follow-up): NS</td>
</tr>
<tr>
<td>Ribeiro et al 201220</td>
<td>133/9–48 months</td>
<td>108 days</td>
<td>Healthy term infants</td>
<td>PDX/GOS 0.5 g/100 mL</td>
<td>NS</td>
</tr>
<tr>
<td>Bruzzesse et al 200925</td>
<td>342/15–120 days</td>
<td>1 year</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.4 g/100 mL</td>
<td>Lower number of gastrointestinal infections NS</td>
</tr>
<tr>
<td>van Stuijvenberg el al 201126</td>
<td>830/&lt;8 weeks</td>
<td>1 year</td>
<td>Healthy term infants</td>
<td>GOS/FOS 0.68 g + AOS 0.12 g/100 mL</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: GOS, short-chain galacto-oligosaccharides; FOS, long-chain fructo-oligosaccharides; PDX, polydextrose; AOS, acidic oligosaccharides; NS, not significant.

References


