British Journal of Nutrition (2005), **93**, Suppl. 1, S57–S60
DOI: 10.1079/BJN20041354

Application of prebiotics in infant foods

Gigi Veereman-Wauters*

Queen Paola Children's Hospital, ZNA, Lindendreef 1, 2020, Antwerp, Belgium

The rationale for supplementing an infant formula with prebiotics is to obtain a bifidogenic effect and the implied advantages of a 'breast-fed-like' flora. So far, the bifidogenic effect of oligofructose and inulin has been demonstrated in animals and in adults, of oligofructose in infants and toddlers and of a long-chain inulin (10 %) and galactooligosaccharide (90 %) mixture in term and preterm infants. The addition of prebiotics to infant formula softens stools but other putative effects remain to be demonstrated. Studies published post marketing show that infants fed a long-chain inulin/galactooligosaccharide mixture (0·8 g/dl) in formula grow normally and have no side-effects. The addition of the same mixture at a concentration of 0·8 g/dl to infant formula was therefore recognized as safe by the European Commission in 2001 but follow-up studies were recommended. It is thought that a bifidogenic effect is beneficial for the infant host. The rising incidence in allergy during the first year of life may justify the attempts to modulate the infant's flora. Comfort issues should not be confused with morbidity and are likely to be multifactorial. The functional effects of prebiotics on infant health need further study in controlled intervention trials.

Prebiotics: Infant food: Oligofructose: Galactooligosaccharides: Inulin: Bifidogenic

Why breast is best

Breast-feeding has been the ideal mode of feeding for the newborn infant. In addition to favouring an intimate bond between mother and child, breast-feeding protects against atopy (Gdalevich *et al.* 2001) and infections (Pettigrew *et al.* 2003). Human breast milk is a superior nutrient not only on philosophical grounds but also because its varying composition is particularly well adapted to the baby's needs. Mother's milk is a functional food 'par excellence': it contains semi-essential nutrients, free amino acids, enzymes, hormones, growth factors, polyamines, nucleotides and oligosaccharides. The later are present in concentrations of 10–12 g/l in human milk and are thought to favour the growth of bifidobacteria that characterize the breast-fed baby's intestinal flora.

It is well known that the type of feeding influences the installation of the intestinal flora after birth. During birth, oral inoculation with maternal intestinal and vaginal flora first causes colonization of the neonate's sterile gastrointestinal tract. Exclusively breast-fed infants harbor predominantly bifidobacteria, and low numbers of *Escherichia coli* and bacteroides. Formula-fed infants harbor equal number of these various commensal bacteria (Harmsen *et al.* 2000).

Bifidobacteria are fermentative and lower stool pH. A Bifidus-dominated flora is considered protective for the host as it may activate the immune system and inhibit invading pathogens (Gibson & Roberfroid, 1995). Some evidence suggests that atopic infants harbor less bifidobacteria (Bjorksten *et al.* 2001). The immature intestinal barrier facilitates antigen transfer causing some degree of mucosal inflammation. Inflammation leads to increased permeability and may impair the equilibrium of the intestinal flora. These factors could be important in the development of food allergy (Fig. 1). Thus, the current working hypothesis is that the intestinal microflora plays a role early on in the development of allergy and that a Bifidus-dominated flora may be protective.

Competing with the breast factor

Although the majority of mothers start to breast-feed the first day, the percentage of exclusively breast-fed babies at 4–6 months varies widely. Some numbers indicating the percentage of exclusive breast-feeding until weaning to solid foods are: 2–15 % in African countries, 20–55 % in Latin America, 55–61 % in Sweden, 14–21 % in Great Britain. In an attempt to maintain a Bifidus-dominated flora upon their introduction, formulas and toddlers' food products have recently been enriched with prebiotics (Vandenplas, 2002). Beneficial commensal bacteria are called probiotics. Prebiotics are non-digestible substances that are substrates to and favour the growth of potentially health-promoting bacteria, in this case bifidobacteria (Roberfroid, 2001).

Breast milk oligosaccharides (present in 1–1·2 %) show an impressive structural diversity. More than 130 different structures have been identified. Some of the most prominent are mono- and difucosyllactose, lacto-*N*-tetraose, mono- and difucosylated derivates, fucosylated lacto-*N*-hexaoses and lacto-*N*-octaoses. Commercial prebiotics are galacto-oligosaccharides and inulin-type fructans.

Effects of prebiotics in infant food

Preterm infants

An infant food company has recently launched formulas for preterm and term infants containing a combination of long-chain inulin (10 %) and galactooligosaccharides (90 %). Terminology

* **Corresponding author:** Dr G. Veereman-Wauters, fax +32 16 402348, email gveereman@skynet.be

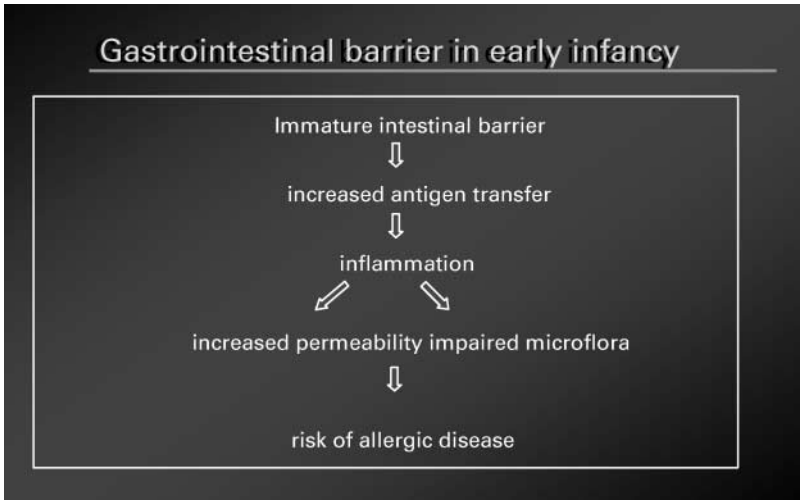

Fig. 1. Gastrointestinal barrier in early infancy.

may be confusing; indeed, the long-chain inulin component was named fructooligosaccharide (FOS) to easily combine with the term galactooligosaccharide (GOS; for an extensive discussion on nomenclature of inulin-type fructans see the paper by Roberfroid in this supplement).

In preterm infants of about 31weeks' gestational age and about 1 week old, a double-blind, randomized controlled study was performed comparing standard formula with formula containing 1 g of the prebiotic mixture/dl. A group fed fortified human milk was studied in parallel. During the 28 d study period, the number of faecal bifidobacteria and lactobacilli increased in the prebiotic formula group to levels seen in the breast-fed group. The difference in composition of the faecal flora between the standard formula and the prebiotic formula group was highly significant. Moreover, stool consistency and stool frequency were similar in the breast-fed and the supplemented group (Boehm *et al.* 2002). The prebiotic mixture might also have improved Ca absorption as indicated by a similar urinary Ca/P ratio in prebiotic-fed and breast-fed babies (Marini *et al.* 2003).

Term infants

Several studies have been performed with the same prebiotic mixture in term infants.

In a double-blind, randomized study, ninety term infants were fed either a standard formula or a formula supplemented with 0·4 or 0·8 g/dl of a preparation containing high-molecular-weight inulin (10 %) and galactooligosaccharides (90 %). Data were compared with a parallel breast-fed group. At the end of the 28 d study period, the number of faecal bifidobacteria was significantly increased in a dose-dependent manner in the supplemented infants. Supplemented infants also significantly increased their number of faecal lactobacilli but this was not dose-dependent. Stool pH decreased, stool consistency and stool frequency increased in a dose-dependent manner. Growth, crying, regurgitation or vomiting was similar in all groups (Moro *et al.* 2002, 2003).

Introduction of the supplemented formula (0·8 g/dl) after 4 weeks of standard formula also induced the growth of bifidobacteria. Moreover, prebiotic-fed and breast-fed babies had the same subgroups of induced bifidobacteria (Knol *et al.* 2002).

The prebiotic mixture (0·8 g/dl) described earlier was incorporated in a commercial formula also containing a partially hydrolysed protein and a high β-palmitic acid level (Omneo®; Nutricia, Numico and Conformil®; Milupa, Numico). A preliminary study documented the bifidogenic effect of this formula (Knol *et al.* 2001). In a double-blind randomized study, 150 infants under 2 weeks of age received this novel or a standard formula until the age of 12 weeks. The infants were growing normally and tolerated both formulas well. Stools of infants receiving the new formula contained more faecal bifidobacteria and were softer. Serum biochemistry and amino acid profiles were similar after 6 weeks (Schmelzle *et al.* 2003). Comparative follow-up of infants fed the new commercial formula with a lower concentration of the prebiotic mixture (0·4 g/dl), standard formula or human milk showed that growth and body composition were comparable after 2 months of intervention, but the number of faecal bifidobacteria was higher in the test group than in the infants receiving standard formula (Rigo *et al.* 2001).

In contrast to the bifidogenic effect of the long-chain inulin (10 %) and galactooligosaccharide (90 %) mixture, short-chain oligofructose alone had no effect at doses of 200, 400 and 600 mg per bottle for 2 weeks (Guesry *et al.* 2000).

Thus, in preterm and term infants, a mixture of long-chain inulin (10 %) and galactooligosaccharides (90 %) is bifidogenic. The effect is greater at a concentration of 0·8 than at 0·4 g/dl. The pattern of composition of the population of bifidobacteria is comparable to the one found in breast-fed infants. Growth and body composition of supplemented infants is unaltered. The supplementation of prebiotics to infant formula appears safe. A summary of the currently published data is found in Fig. 2. Early evidence led to the publication of a Statement by the Scientific Committee on Food of the European Commission on 13 December 2001 in which addition of the prebiotic mixture at a concentration of 0·8 g/dl to infant formula is considered safe (http://europe.eu.int/comm-food). It was recommended, however, that additional data on growth, body composition, nutrient availability and water balance be obtained.

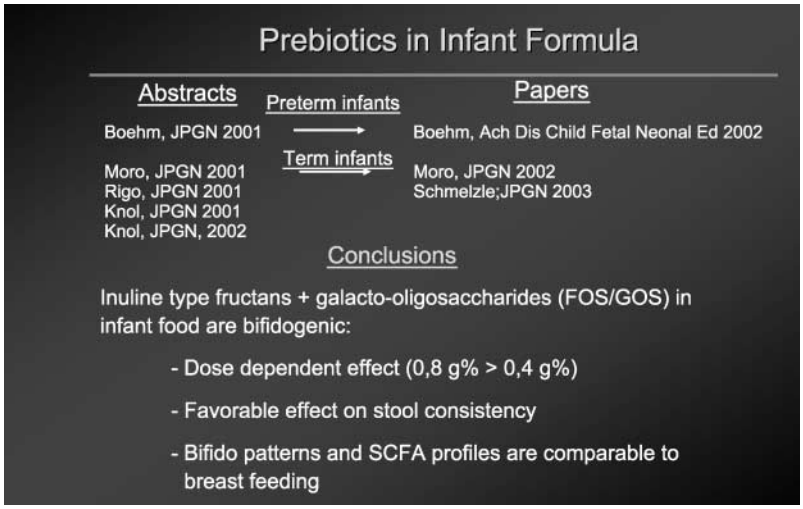

Fig. 2. Prebiotics in infant formula, summary.

Toddlers

Prebiotics have been added to weaning foods in an attempt to conserve or restore a Bifidus-dominant flora in toddlers. Indeed, a higher count of bifidobacteria may prevent rotaviral enteritis (Saavedra *et al.* 1994) and protect against allergy (Bjorksten *et al.* 2001).

The addition of oligofructose to cereals, leading to an average daily consumption of 1·1 g in healthy children aged between 4 and 24 months, significantly decreased the number of infectious episodes as indicated by fever and medical visits. Control subjects had more sick days and a higher intake of antibiotics. Growth was similar in the supplemented and non-supplemented group. Episodes of emesis, regurgitation and perceived discomfort were lower in the supplemented children but there were no differences in flatulence or constipation (Saavedra *et al.* 1999; Tschernia *et al.* 1999; Saavedra & Tschernia, 2002).

The putative influence of prebiotics on the immune response was examined in a double-blind study in which fifty infants aged 7–9 months received a mixture of oligofructose (70 %) and inulin (30 %) at a concentration of 1 g per 25 g of dry weight cereal during 4 weeks prior to measles vaccination. This led to an average daily intake of 0·2 g prebiotic/kg body weight. The rise in post vaccination anti-measles IgG was significantly higher in the supplemented infants. No differences were noted in gastrointestinal tolerance (Firmansyah *et al.* 2001; Haschke *et al.* 2001).

Tolerance to oligofructose in cereals was assessed in healthy children aged 4–12 months in a double-blind randomized study. With a daily consumption of 0·74 g/d on average and 3 g/d maximum, tolerance to the prebiotic was excellent. Supplemented children had softer stools. There were no differences in episodes of crying, emesis or colic. Stool pH was similar in both groups (Moore *et al.* 2003).

In a similar double-blind randomized set-up as described for preterm and term infants, the long-chain inulin (10 %) and galactooligosaccharide (90 %) mixture was tested in fully bottle-fed infants who were weaned to solid food. The number of faecal bifidobacteria increased significantly in the supplemented infants receiving 4·5 g/d (Scholtens *et al.* 2003).

One study so far used prebiotics in diseased infants: breast-fed Peruvian infants with diarrhoea. The addition of oligofructose with or without Zn to cereals had no effect on the prevalence of diarrhoea (Duggan *et al.* 2003).

In summary, in healthy toddlers' up to 3 g oligofructose/d is well tolerated and 4·5 g of a mixture of long-chain inulin (10 %) and galactooligosaccharides (90 %) is bifidogenic. Prebiotics may stimulate the immune response in healthy infants but their effect in disease remains to be demonstrated. Toddlers taking prebiotics have softer stools.

In vitro *data*

Artificial models that simulate gastrointestinal transit have been used to study nutrient availability. These models consist of successive incubation chambers containing a particular food mixed with digestive juices. The solubilization of nutrients and minerals is assessed and suggests their availability for absorption (Bosscher *et al.* 2003*a*). Using that incubation techniques, it was demonstrated that inulin supplementation at a concentration of 0·4 g/dl to standard infant formula improves Ca availability (Bosscher *et al.* 2003*b*).

Future directions

One of the most challenging current research areas is the potential beneficial effect of prebiotics on the immune system of young infants (Miniello *et al.* 2003). Prebiotics in early nutrition may have very profound effects on the intestinal barrier, internal milieu and defence mechanisms. If they do, is their effect long-lasting? The supposed early 'window of opportunity' has not been well defined yet. Are there long-term health benefits related to an early treatment window?

Many questions remain regarding the spontaneous evolution of the intestinal flora, the influence of dietary factors, the impact of modulation or manipulation of the flora. The optimal composition and dosage of prebiotics should also be studied. Prebiotics may reduce the risk of disease but should they be used in case of illness?

Caution in the care of newborns demands evidence and not the extrapolation of isolated facts. Clinical studies are difficult

to perform in this age group but that does not imply that empirical actions are to be preferred. *In vitro* models may prove useful in selecting adequate and safe prebiotics. The functional effects of prebiotics on infant health also need to be further studied *in vivo* in controlled intervention trials.

References

Bjorksten B, Sepp E, Julge K, Voor T & Mikelsaar M (2001) Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* **108**, 516–520.

Boehm G, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B & Marini A (2002) Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. *Arch Dis Child Fetal Neonatal Ed* **86**, F178–F181.

Bosscher D, Van Caillie-Bertrand M & Deelstra H (2003*a*) Do thickening properties of locust bean gum affect the amount of calcium, iron and zinc available for absorption from infant formula? *In vitro* studies. *Int J Food Sci Nutr* **54**, 261–268.

Bosscher D, Van Caillie-Bertrand M, Van Cauwenbergh R & Deelstra H (2003*b*) Availabilities of calcium, iron, and zinc from dairy infant formulas is affected by soluble dietary fibers and modified starch fractions. *Nutrition* **19**, 641–645.

Duggan C, Penny M, Hibberd P, Gil A, Huapaya A, Cooper A, Coletta F, Emenhiser C & Kleinman RE (2003) Oligofructose-supplemented infant cereal. 2. Randomized, blinded, community-based trials in Peruvian infants. *Am J Clin Nutr* **77**, 937–942.

Firmansyah A, Pramita G, Carrie Fassler A, Haschke F & Link-Amster H (2001) Improved humoral immune response to measles vaccine in infants receiving infant cereal with fructo-oligosaccharides. *J Pediatr Gastroenterol Nutr* **31**, A521.

Gdalevich M, Mimouni D, David M & Mimouni M (2001) Breast-feeding and the onset of atopic dermatitis in childhood: a systematic review and meta-analysis of prospective studies. *J Am Acad Dermatol* **45**, 520–527.

Gibson GR & Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* **125**, 1401–1412.

Guesry P, Bodanski H, Tomsit E & Aeschlimann J (2000) Effect of 3 doses of fructo-oligosaccharides in infants. *J Pediatr Gastroenterol Nutr* **31**, Suppl. 2, S252.

Harmsen H, Wildeboer-Veloo A, Raangs G, Wagendorp A, Klijn N, Bindels J & Welling G (2000) Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* **30**, 61–67.

Haschke F, Firmansyah A, Meng M, Steenhout P & Carrie A-L (2001) Functional food for infants and children. *Monatsschr Kinderheilkd* **149**, Suppl. 1, S66–S70.

Knol J, Poelwijk E & Van der Linde E (2001) Stimulation of endogenous Bifidobacteria in term infants by an infant formula containing prebiotics. *J Pediatr Gastroenterol Nutr* **32**, 399.

Knol J, Steenbakkers GMA, Van der Linde EGM, Gross S, Helm K, Klarczyk M, Schoepfer H & Kafka C (2002) Bifidobacterial species that are present in breast-fed infants are stimulated in formula fed infants by changing to a formula containing prebiotics. *J Pediatr Gastroenterol Nutr* **34**, 477.

Marini A, Negretti F, Boehm G, Li Destri M, Clerici-Bagozzi D, Mosca F & Agosti M (2003) Pro- and pre-biotics administration in preterm infants: colonization and influence on faecal flora. *Acta Paediatr Suppl* **441**, 80–81.

Miniello VL, Moro GE & Armenio L (2003) Prebiotics in infant milk formulas: new perspectives. *Acta Paediatr Suppl* **441**, 68–76.

Moore N, Chao C, Yang L-P, Storm H, Oliva-Hemker M & Saavedra JM (2003) Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. *Br J Nutr* **90**, 581–587.

Moro G, Minoli I, Mosca M, Fanaro S, Jelinek J, Stahl B & Boehm G (2002) Dosage-related bifidogenic effects of galacto- and fructo-oligosaccharides in formula fed term infants. *J Pediatr Gastroenterol Nutr* **34**, 291–295.

Moro G, Mosca F, Miniello V, Fanaro S, Jelinek J, Stahl B & Boehm G (2003) Effects of a new mixture of prebiotics on faecal flora and stools in term infants. *Acta Paediatr Suppl* **441**, 77–79.

Pettigrew M, Khodaee M, Gillespie B, Schwartz K, Bobo J & Foxman B (2003) Duration of breastfeeding, daycare and physician visits among infants 6 months and younger. *Ann Epidemiol* **13**, 431–435.

Rigo J, Pieltain C, Studzinski F, Knol J & Bindels J (2001) Clinical evaluation in term infants of a new formula based on pre-biotics, β-palmitate, and hydrolised proteins. *J Pediatr Gastroenterol Nutr* **32**, 402.

Roberfroid M (2001) Prebiotics: preferential substrates for specific germs? *Am J Clin Nutr* **73**, Suppl., 406S–409S.

Saavedra JM & Tschernia A (2002) Human studies with probiotics and prebiotics: clinical implications. *Br J Nutr* **87**, Suppl. 2, S241–S246.

Saavedra J, Bauman N, Oung I, Perman J & Yolken R (1994) Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhea and shedding of rotavirus. *Lancet* **344**, 1046–1049.

Saavedra JM, Tschernia A, Moore N, Abi-Hanna A, Coletta F, Emenhiser C & Yolken R (1999) Gastrointestinal function in infants consuming a weaning food supplemented with oligofructose a prebiotic. *J Pediatr Gastroenterol Nutr* **29**, A95.

Schmelzle H, Wirth S, Skopnik H, Radke M, Knol J, Bockler H, Bronstrup A, Wells J & Fusch C (2003) Randomized double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolyzed protein, a high beta-palmitic acid level, and nondigestible oligosaccharides. *J Pediatr Gastroenterol Nutr* **36**, 343–351.

Scholtens P, Alles M, Van der Linde E & Knol J (2003) Introduction of solid weaning foods with added prebiotic oligosaccharides affect the composition of the intestinal microflora. *J Pediatr Gastroenterol Nutr* **36**, A566.

Tschernia A, Moore N, Abi-Hanna A, Yolken R, Coletta F, Emenhiser C & Saavedra JM (1999) Effects of long-term consumption of a weaning food supplemented with oligofructose, a prebiotic, on general infant health status. *J Pediatr Gastroenterol Nutr* **29**, A58.

Vandenplas Y (2002) Oligosaccharides in infant formula. *Br J Nutr* **87**, Suppl. 2, S293–S296.