Processed Infant Cereals as Vehicles of Functional Components

Magnus Domellöf, Christina West

Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden

Abstract

Cereals are the most common complementary foods all over the world and there is now a novel possibility to add functional components to target health problems that are not caused by a simple nutritional deficiency. So far there have been very few published trials on the addition of functional components to infant cereals. A single trial has suggested that infant cereals containing a combination of probiotics, prebiotics and zinc are an effective adjunct to oral rehydration solution in the treatment of acute gastroenteritis. Up to now there has been no evidence that infant cereals supplemented with probiotics or prebiotics have a preventive effect on diarrhea but a recent study has suggested that a milk fat globule membrane (MFGM) protein fraction added to an infant cereal reduces the risk of diarrhea in a developing country. There are some promising results suggesting that infant cereals supplemented with probiotics or prebiotics may prevent atopic eczema. The addition of prebiotic oligosaccharides to infant cereals may lead to softer stools, likely to benefit those infants who are suffering from constipation. More studies are needed to verify these results and to assess the effects of other functional components – especially probiotics, prebiotics, nucleotides, novel protein fractions and recombinant human milk proteins – added to infant cereals.

Introduction

Exclusive breastfeeding ensures optimal nutrition for healthy infants during the first 6 months of life. Thereafter, the feeding of nutrient-dense complementary foods, along with continued breastfeeding, is critical to ensure optimal health, growth and development of infants and young children. The complementary feeding period (6–24 months of age) is therefore an especially important target for nutritional interventions.
Cereals are the most common complementary foods all over the world. Both traditional and industrially produced infant cereals are based on common grains such as rice, maize, wheat, oat or sorghum and are often combined with milk or legumes such as soy.

Traditional home-made or locally produced cereals in developing countries are often poorly adapted to the nutritional needs of infants, leading to malnutrition [1]. The fortification of infant cereals with micronutrients (iron, zinc, vitamin A, etc.) has been shown to be an effective tool to prevent basic nutritional deficiencies [2, 3]. However, there is now a novel possibility to add functional components in order to target important public health problems that are not caused by a simple nutritional deficiency. Examples of such health problems are infectious diarrhea in developing countries and allergies in industrialized countries.

Functional components of foods are usually defined as those having additional health effects beyond basic nutrition. In a European consensus document, a food product or component was regarded as functional if it was demonstrated to beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved health and/or reduction of risk of disease [4].

Functional components in infant food products include long chain polyunsaturated fatty acids (LCPUFAs), probiotics, prebiotics, nucleotides, protein fractions, amino acids, structured triglycerides, polyamines, recombinant proteins, hormones and growth factors. Several of these functional components are presently added to commercially available infant food products even though their health benefits are not clearly proven [5].

Most of the functional components added to infant foods are originally bioactive components of breast milk since the feeding of human milk has been shown to promote many health benefits, e.g. more favorable development of the brain, gut and immune system, less risk of infectious diseases, diabetes or cancer [6–8]. It is thus logical that most of these functional components have been tested primarily as supplements to infant formula and very few trials have so far been performed of functional components added to processed infant cereals (table 1).

**Probiotics and Prebiotics**

During the postnatal period, breastfed but not formula-fed infants establish an intestinal flora rich in lactic acid bacteria, e.g. bifidobacteria and lactobacilli [9]. Possible mechanisms for the protective effect of breast milk against infections include growth inhibition of pathogenic microorganisms through the production of lactic, acetic and other organic acids, with a consequent decrease of intraluminal pH that inhibits the growth of some bacterial pathogens. In contrast, formula feeding tends to favor a flora associated with
a near-neutral pH of the feces [10]. Furthermore, bifidobacteria and lactobacilli compete with potentially pathogenic bacteria for nutrients and epithelial adhesion sites. The gut microbiota also modulates the recovery of substrates through fermentation of nondigestible carbohydrates and nitrogen salvage, and affects mucosal growth and the absorption of water and nutrients [11]. Accumulating evidence also indicates that the gut flora modulates mucosal physiology, barrier function and systemic immunologic and inflammatory responses [12]. The realization that the intestinal flora modifies the function of the gut immune system has led to the concept of probiotic and prebiotic therapy as possible means to reduce the risk of infections and allergies [13]. Probiotics are live organisms which when administered in adequate amounts confer a health benefit on the host [14]. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of a limited number of bacteria in the colon that have the potential to improve the host’s health [15]. Commercially available prebiotics are galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS) and inulin-type fructans [16].

**Treatment of Acute Gastroenteritis**

Diarrheal diseases are a leading cause of mortality in infants and children worldwide, and continue to be a significant cause of morbidity in industrialized countries [17]. In recent years, the major advance in the treatment of acute gastroenteritis in children was the introduction of oral rehydration solution (ORS) in the early stages of illness [18]. In addition, rice-based ORS

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Component(s)</th>
<th>Main result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore et al. [40]</td>
<td>2003</td>
<td>FOS</td>
<td>Softer, more frequent stools</td>
</tr>
<tr>
<td>Duggan et al. [27]</td>
<td>2003</td>
<td>FOS</td>
<td>No prevention of diarrhea</td>
</tr>
<tr>
<td>Shamir et al. [22]</td>
<td>2005</td>
<td>*S. thermophilus, B. lactis, L. acidophilus, FOS, zinc</td>
<td>Shorter duration of acute gastroenteritis (treatment)</td>
</tr>
<tr>
<td>Moro et al. [32]</td>
<td>2006</td>
<td>GOS, FOS</td>
<td>Prevention of atopic eczema</td>
</tr>
<tr>
<td>Zavaleta et al. [55]</td>
<td>2006</td>
<td>MFGM protein fraction</td>
<td>Prevention of diarrhea</td>
</tr>
<tr>
<td>West et al. (unpubl. study, 2007)</td>
<td>2006</td>
<td>LF19</td>
<td>Prevention of atopic eczema; no prevention of diarrhea</td>
</tr>
</tbody>
</table>
is superior to ORS alone in reducing the frequency and stool volume in acute gastroenteritis [19]. However, nutritional interventions during the diarrheal illness are usually not helpful in reducing the duration of diarrhea, and current recommendations suggest that the normal diet is continued during mild diarrhea [20].

Studies have clearly shown that probiotics is a useful adjunct to rehydration therapy in treating acute, infectious diarrhea in adults and children and this has also been supported by a Cochrane meta-analysis showing that the duration of diarrhea is shortened by about 1 day and that patients receiving probiotics had fewer stools on treatment day 2–3 [21].

There is one published randomized controlled trial (RCT) on the effects of probiotics and prebiotics in infant cereals in the treatment of acute gastroenteritis: Shamir et al. [22] randomized 65 infants at 6–12 months of age, suffering from acute gastroenteritis and mild to moderate dehydration, to receive either a lactose-free soy protein-based rice cereal or the same cereal with added probiotics, prebiotics and zinc. The study was carried out in an ambulatory setting. The infants were first prescribed an ORS solution according to previously established guidelines. Thereafter, parents from both groups were instructed to feed 600 ml of cereals with or without supplements: *Streptococcus thermophilus*, *Bifidobacterium lactis* and *Lactobacillus acidophilus* (2 × 10^9 colony forming units each), FOS (0.3 g) and zinc (10 mg). The mean duration of diarrhea was 15 h shorter in the supplemented group compared to the control group (1.34 ± 0.71 vs. 1.97 ± 1.24 days, p = 0.017). There was no difference in time to resolution of fever or vomiting. On day 3, there was only one infant with watery stools in the supplemented group compared to 10 infants in the control group (p = 0.02). Unfortunately, due to the combined supplementation, it is not possible to know if this effect was due to the added probiotics, prebiotics or zinc. Zinc supplementation by itself has been shown in several trials to substantially reduce the duration and severity of symptoms in acute gastroenteritis [23].

**Prevention of Diarrhea**

Several RCTs have suggested that the addition of probiotics to infant formula may reduce the severity of diarrhea episodes. Recently, Weizman et al. [24] showed that a formula with *B. lactis* and a formula with *Lactobacillus reuteri* resulted in fewer and shorter episodes of diarrhea compared to standard formula. Thibault et al. [25] showed in a large trial (n = 971) that formula fermented with *Bifidobacterium breve* and *S. thermophilus* resulted in fewer cases of dehydration and fewer medical consultations but no difference in the incidence or duration of diarrhea episodes.

There are no published trials on the effect of infant cereals supplemented with probiotics on the prevention of diarrhea. However, preliminary results are available from an RCT by our group [West et al., unpubl. study] in which 179 mostly breastfed infants were assigned to cereals (rice and wheat porridge
with milk proteins) with or without *Lactobacillus paracasei* strain F19 (LF19). The recommended intake was at least one serving of cereals daily from 4 to 13 months of age. In the LF19 group, one serving contained $1 \times 10^8$ colony forming units. Compliance was good in both groups: mean intake was 0.85 ($\pm 0.45$) servings per day. There was no difference in the number of days with fever, respiratory illness or diarrhea between the groups. Infants in the LF19 group had fewer days with antibiotic prescriptions compared to placebo (1.6 vs. 2.2 days, $p = 0.044$).

The lack of effect on diarrhea morbidity cannot be explained by a low incidence or duration of gastroenteritis in the study of West et al. since the average number of days with diarrhea was 0.3 days/month compared to 0.2 days/month in the control group in the study of Weizman et al. The negative results of West et al. with regard to diarrhea support those of Thibault et al. (see above). However, in the study of Thibault et al., a more liberal definition of diarrhea was used resulting in a higher number of days with diarrhea (1.0 days/month). An alternative explanation for the lack of effect on diarrhea morbidity in the study of West et al. is that the preventive effect of probiotics on diarrhea is less pronounced in breastfed infants, as suggested by Oberhelman et al. [26] who found a decrease in the incidence of diarrhea in Peruvian 18- to 29-month-olds receiving a *Lactobacillus rhamnosus* GG supplement, an effect which was largely restricted to those toddlers who were not breastfed.

There is one published trial of prebiotic supplemented infant cereals assessing effects on diarrhea: Duggan et al. [27] randomized 282 breastfed Peruvian infants between the ages of 6 and 12 months to receive infant cereals (either rice- or oat-based at the choice of the family) with or without FOS supplementation (0.55/15 g serving) during 6 months. An identical study was performed in 349 infants with zinc added to both infant cereals. In both studies, FOS supplementation of formula was not associated with any difference in diarrhea prevalence or use of health care resources.

**Prevention of Atopic Eczema**

In a randomized study by Kalliomäki et al. [28, 29] in families at risk of allergy, capsules of *L. rhamnosus* GG given perinatally to mothers and infants resulted in a reduction in the prevalence of atopic eczema at 24 months of age (from 46 to 23%) without any effect on IgE concentrations at 24 months or skin prick tests at 4 years of age. Some studies have also suggested that probiotics may have a beneficial treatment effect on atopic eczema in children [30, 31].

There is only one published RCT on infant formula or cereals supplemented with probiotics or prebiotics, assessing the effect on atopic eczema: Moro et al. [32] randomized 259 term infants with a family history of atopy to infant formula with or without a mixture of 90% GOS and 10% FOS at a concentration of 8 g/l. A reduction was observed in the incidence of atopic
eczema up to 6 months of age from 23% in the control group to 10% in the prebiotic group (p = 0.014). IgE or other measures of allergy were not presented in that study.

Preliminary data from the study by West et al. suggest that the incidence of atopic eczema up to 13 months of age was 50% lower in the LF19-supplemented group compared to controls (11 vs. 22%, fig. 1), suggesting that probiotics in infant cereals have a preventive effect against this disease [West, unpubl. study]. However, there was no difference in IgE concentrations.

The study by West et al. confirms the results of two previously published trials showing a preventive effect of prebiotics/probiotics on atopic eczema. Both the studies of West et al. and Kalliomäki et al. suggest that the preventive effect of prebiotics/probiotics on atopic eczema is mediated by an IgE-independent mechanism. Since atopic eczema has been associated with disruption of the intestinal mucosal barrier, one possible mechanism of probiotics is a reduction in intestinal permeability and thereby a reduction of antigen transfer across the intestinal mucosa [33].

**Effect on Stool Consistency**

Seven RCTs of prebiotic supplements to infant formulas have been published [32, 34–39]. All except one was performed in term infants. Five of the studies used a mixture of 90% GOS and 10% FOS at a concentration of 4–10 g/l. Two of the studies used FOS at a concentration of 1.5–3 g/l. Most of these studies showed that added oligosaccharides resulted in increased stool frequency and a softer stool consistency.

There is one published trial of prebiotic supplemented infant cereals assessing effects on stool quality: Moore et al. [40] randomized 56 infants to receive either a rice-based cereal with milk protein supplemented with FOS or the equivalent amount of maltodextrin (placebo) from 4 to 12 months of age.
Infants receiving FOS had significantly more stools per day (1.99 ± 0.62 vs. 1.58 ± 0.66, p = 0.02). For infants receiving FOS, stool consistency was less likely to be described as ‘hard’ and more likely to be described as ‘soft’ or ‘loose’.

Other Effects of Probiotics and Prebiotics
Formulas and cereals supplemented with probiotics and prebiotics are generally well accepted and result in the same growth as standard formula. An increase in fecal bifidobacteria has been shown in several studies of oligosaccharide supplements to formula [34, 35, 37, 38], supporting their prebiotic effect. Except for effects on diarrhea and stool quality (see above), most studies show no effect on other gastrointestinal symptoms, e.g. regurgitation, vomiting, colics, etc.

Immunological effects of probiotics and prebiotics added to infant formulas or cereals (e.g. effects on immunoglobulin responses to childhood immunizations) have been suggested in some [West, unpubl. study; 41] but not all trials [27].

Other and Novel Functional Components
LCPUFAs are fundamental for central nervous system growth and development. Several studies have shown that LCPUFA supplementation of infant formula may lead to short-term effects on psychomotor development and visual acuity, but recent Cochrane meta-analyses of trials in preterm and term infants have concluded that there is still no evidence for long-term benefits [42, 43]. Since LCPUFA supplementation theoretically would have a better effect the earlier it is given during the first year of life [44], it has not primarily been considered for supplementation to infant cereals and there are no published studies of LCPUFA-supplemented infant cereals.

Compared to cow’s milk, human milk has higher concentrations of nucleotides. Several RCTs of infant formulas supplemented with nucleotides have suggested beneficial effects on infant immune response but so far there is no evidence of a dose response or a consistency in the specificity of the response [45–49]. So far there have been no published trials of infant cereals supplemented with nucleotides.

Human recombinant lactoferrin and lysozyme have recently been expressed in rice [50, 51] and may have a protective effect against enteral infections [52], but as of yet, there are no clinical studies on these recombinant proteins added to infant formula, infant cereals or cereal-based ORS solutions.

However, novel protein components can also be found in bovine milk. A bovine milk protein fraction containing enriched milk fat globule membranes (MFGM) has recently become available. This fraction contains 120 proteins and other components, including several with possible antiviral and antibacterial activity (e.g. butyrophilin, MUC1, lactadherin, lactoferrin, sphingomyelin, sphingosine, sphingosine-1-phosphate).
gangliosides and sialic acid) as well as micronutrient binding proteins (e.g. lactoferrin, folic acid binding protein) [53]. Lönnertal et al. [54] and Zavaleta et al. [55] recently presented results from an RCT, in which 6- to 11-month-old infants (n = 550) in periurban Lima, Peru, were fed a micronutrient-fortified, cereal- and milk-based meal (40 g/day) with (a) MFGM or (b) skim milk proteins twice daily for 6 months. The incidence of diarrhea was lower in the MFGM group (5.8 episodes/child/year vs. 6.3 in the control group; p < 0.05). S-Cu and vitamin B12 were significantly higher in the MFGM group than in the skim milk group but there was no difference in anemia prevalence, hemoglobin, serum ferritin, serum Zn, serum folate or growth between groups.

**Effect Modifiers**

It is not always possible to extrapolate the results from trials of bioactive components given as a separate supplement (e.g. drops, capsules) to the same bioactive component added to infant cereals. As an example, we have shown that iron given as a separate supplement has different effects on hemoglobin and serum ferritin compared to the same dose of iron given as iron-fortified foods [56]. Similarly, it is not recommended to extrapolate the results from trials of a functional component in infant formula to the same functional component added to infant cereals. Infants receiving infant cereals are commonly also breastfed, in contrast to formula-fed infants who are usually not breastfed, and the functional components may have different effects depending on whether the infant is breastfed or not [26]. It is also important to consider which liquid is used to reconstitute infant cereals. In the Peruvian study by Duggan et al. [27], the infant cereal powder was mixed with milk, breast milk or water. It is likely that these liquids will influence the effect of the functional components differently. Furthermore, it is important not to generalize effects of probiotics and prebiotics since different strains of bacteria in different doses as well as different combinations of oligosaccharides in different doses may have different clinical effects [10, 57]. Even though most tested functional components are well tolerated, safety aspects and long-term outcomes should be included in all clinical trials.

**Conclusion**

Even though several randomized trials have been published on the addition of functional components to infant formulas, there are very few published trials on the addition of functional components to infant cereals (table 1).

Probiotics have been shown to be an effective adjunct to ORS therapy in the treatment of acute gastroenteritis and a single trial has suggested that this is also true for infant cereals containing a combination of probiotics, pre-
Functional Components in Infant Cereals

Biotics and zinc. As of yet there is no evidence that infant cereals supplemented with probiotics or prebiotics have a preventive effect on diarrhea but a recent study has suggested that an MFGM protein fraction added to an infant cereal reduces the risk of diarrhea in a developing country. There are some promising results suggesting that infant cereals supplemented with probiotics or prebiotics may reduce the incidence of atopic eczema. It is also possible that the addition of prebiotic oligosaccharides to infant cereals increases stool frequency and leads to a softer stool consistency, likely to benefit those infants who are suffering from constipation.

Since the number of studies is very small, all of the above results need to be verified in further studies. The promising initial results showing clinical effects of infant cereals with added functional components on treatment and prevention of acute gastroenteritis and prevention of atopic eczema are especially interesting. More studies are needed to assess the effects of functional components – especially probiotics, prebiotics, nucleotides, novel protein fractions and recombinant human milk proteins – added to infant cereals and cereal-based ORS solutions.

References


Discussion

Dr. Pazvakavambwa: Would the etiology of the diarrhea, viral diarrhea versus non-viral, make a difference? When these probiotics are added to the cereal, is it after cooking, is the cereal warm, heated, or at what stage is it added?

Dr. Domellof: In a recent meta-analysis, there was a significant effect on viral gastroenteritis [1]. Regarding the second question, most of these cereals are manufactured with probiotics already added. Since they are living organisms, the product should not be heated to more than serving temperature in the home.
Dr. Simell: Why have none of the studies characterized the infectious agent causing the diarrhea? The other question I have regards the zonulin. Many of you are familiar with the recent data from Sapone et al. [2] looking at the function of zonulin as a gut permeability regulator, where gluten especially seems to be a very important contributor to the permeability of the gut. Have you or others looked at the possible negative effects of the cereals on the effect of probiotics? As suggested in your studies, there may be some negative effects as well. Has the zonulin concentration changed in any of these cases?

Dr. Domellöf: We did not observe any negative interaction between the cereals themselves and the effects of the probiotics, even though the trial was not designed to study that. Regarding intestinal permeability, this has been suggested to be a mechanism for the effect of probiotics on atopic eczema [3]. We have not specifically looked at zonulin. With regard to pathogenic agents, we did not investigate them in this study. However, in Sweden, bacterial diarrhea is very rare and the most common cause of viral diarrhea is rotavirus.

Dr. Guandalini: Thank you, a very interesting presentation and also very promising in terms of future studies. I want also to go along the same line of questions by adding some comments and also try to answer the questions that were raised. You are right, the effect of probiotics is evident mainly if not exclusively on diarrhea of viral origin, specifically rotavirus. There are a few studies in children with acute diarrhea, including our own [4] which remains the largest published in terms of the number of children studied, that looked at etiology with a rather comprehensive approach. We found something which other studies have since confirmed, including subsequent meta-analyses: that probiotics, or more precisely lactobacilli such as the Lactobacillus GG we utilized, are only active in the context of viral, not bacterial, diarrheas. Of course, it is conceivable that other probiotics may have different mechanisms of action and thus different efficacy. We really know close to nothing about the pharmacodynamics, pharmacokinetics of these products. I think we are just beginning to understand how these microorganisms work. Furthermore, I would like to add that probiotics have been found to be effective for instance in preventing antibiotic-associated diarrhea, obviously a very common problem. And then there is the issue of nosocomial diarrhea which is also of utmost importance. Already in 1994 in Baltimore Saavedra et al. [5] documented the efficacy of Bifidobacterium bifidum and the Streptococcus thermophilus in preventing the spread of rotaviral diarrhea in the wards. Subsequently there were two more studies, one by Szajewska et al. [6] which also showed the protective efficacy of Lactobacillus GG in a hospital ward, particularly in terms of preventing the spread of rotavirus-associated severe diarrhea. A subsequent study from Italy, however in conflict with these observations, failed to show a protective effect by Lactobacillus GG in hospitalized children [7]. So in essence and as Dr. Morelli delineated yesterday, I really think probiotics represent an exciting area, definitely worth pursuing. Chances are within 10 years, in the next Nestlé workshops, we will be discussing much more about their efficacy. Finally, on the question of permeability, it is clear to me that probiotics do have an effect in regulating intestinal permeability, as shown by several in vitro and in vivo studies. There was a pilot study in a few Crohn’s patients [8], where the double-sugar technique was used to investigate the effect of Lactobacillus GG on the permeability of the intestine, and it was very clear in all patients that there was a striking improvement, albeit transient, as it regressed in approximately 4 months in spite of ongoing intake.

Dr. Haschke: Three short comments. The first refers to the study by Moran [9], where he found a reduction in atopic manifestations by feeding infants a hydrolyzed formula, as we saw without prebiotics. In the group without prebiotics he found that 25% of the infants had atopic eczema. This is far beyond any of the other studies
which show that with hydrolyzed formulas the prevalence of atopic eczema is 10–11%. This is exactly what he achieved in this group. So it is not clear, especially as this was a hydrolyzed formula, whether this was an effect of the prebiotics or of the particular protein composition together with the prebiotics. It is very difficult to extrapolate to non-hydrolyzed formulas and to cereals. The second comment is related to the Isolauri study on the supplementation of lactating mothers and their infants with Lactobacillus GG during the perinatal period and thereafter. You showed the results until 2 years. Two weeks ago Dr. Isolauri presented the results until 7 years; the clinical results, and the preventive effect of Lactobacillus GG is still evident until 7 years of age; these are clinical data for atopic eczema. The third comment refers to whether probiotics or certain strains of probiotics are effective in bacterial or viral diarrhea, which is not clear. It depends very probably on which strain we are using. There is a study which was recently conducted at the ICDDR in Dacca where the Lactobacillus ST11 strain was tested; this strain had a clear effect on bacterial diarrhea but not on viral diarrhea. So we still have to learn a lot about the efficacy of individual strains in different situations before we can really make a general recommendation. I think it is very important to look at the cause of diarrhea.

Dr. Ribeiro: I have a different concept about this and the real results that have been presented in the literature to date. We have done three trials now with Lactobacillus GG and when we compared the data published so far on so-called diarrhea with our cases, e.g. 5 ml/kg/h of stool output, we could not see a difference over time in our area. At one time rotavirus was around 40% of the etiology and now more recently it is around 70–75%. There was no difference in terms of diarrhea duration and also in stool output. But what is promising is when you look at the prevention studies in daycare centers we see fewer longer cases of diarrhea; so we could observe a decrease in the duration in less persistent cases. I don't know what your idea is when faced with severe diarrhea regardless of the etiology as seen in our setting. A point that I am very concerned about is that we keep talking about probiotics as something that could be extrapolated everywhere in the world, and we have to be concerned about the microbiota we are dealing with; I don't think we can compare Swedish and north European microbiota. What is the environment, what is the colonization of the intestine in another part of the world compared to here in Brazil where we have a completely different intestinal colonization?

Dr. Domellöf: I agree with you that it is very important to conduct studies on prevention of diarrhea in low income regions.

Dr. Brunser: I agree with Dr. Haschke in the sense that we don’t have a clear understanding of the way in which prebiotics and probiotics work. Many years ago we did a study in which we provided nucleotides to children and studied the incidence of diarrhea. We discovered that the nucleotides indeed prevented the first episodes of diarrhea, and we were happy because it was known that nucleotides improve T-cell immunity and to this we attributed the effect [10]. However, about 4 years ago an article appeared showing that nucleotides increase the growth of bifidobacteria in the gut; thus when studying the effects of a probiotic or a prebiotic one has to be very careful because they may act simultaneously through different mechanisms in the chain of events that prevent diarrhea. The other point is that one of the effects of prebiotics is that they enhance the recovery of the normal flora. We recently published an article in Pediatric Research [11] demonstrating that after children had been treated with amoxicillin for a week, the administration of a mixture of inulin and fructo-oligosaccharides resulted in the recovery of normal bifidobacteria counts. It took about 3 or 4 weeks for the controls to obtain normal bifidobacteria counts [11]. One minor point, in a study of gastric permeability in young adults, medical students who acutely drank alcohol or smoked were fed yogurt with Lactobacillus johnsonii (La1). Measurement of gastric
mucosal permeability demonstrated that La1 and also smoking, surprisingly, decreased the urinary excretion of sucrose [12]. The effect of smoking is probably due to nicotine that induces vasoconstriction in the mucosa and this decreases excretion. But it was felt that La1 induced improvement in permeability [13]. So the problem is that more than one mechanism may operate simultaneously many times, and one has to look for effects in unexpected areas.

**Dr. Solomons:** Looking at your final slide on cereal, it is impressive that the treatment is a major constraint to the preservation of certain organisms in their living state; but it also supports the notion that perhaps some of the chemical compounds are sensitive. So the challenge to the cereal industry, in their manufacture of complementary feeding, is to make products that never get heated and in which the water is safe. So I would like to see the industry go in that direction – an innovative new idea, in my way of thinking. As a second point would be maternal education to give the mothers confidence to prepare foods without the terminal heating, which would destroy what we hope to be ‘bioactive’ at the moment of consumption by the infant.

**Dr. Haschke:** Just to reply to Dr. Solomons’ comment. The solution exists; it is the single packaging of cereals, because cereals are given to infants beyond 6 months of age so they are not as susceptible to certain bacteria as very small infants during the first few weeks of life. The problem of contamination is almost always due to inadequate storage of the product or use of contaminated water, so heating of water and cooling it down before mixing it with the cereal and single bag sachets is possible, but this unfortunately increases the cost. This is a solution which is not the first choice for developing countries.

**Dr. Giovannini:** What is your personal opinion: is it better to add probiotics to the food by sachet or should it be in the food? There are two points: (1) how long is the efficacy because storage and transport are sometimes problematic, and (2) stability, in the case of probiotics in food cold is needed.

**Dr. Domellöf:** I think both of these options have to be explored and the recommendation might be different in different settings and different cultures. Probably the addition of probiotics as a component of the complementary food would improve compliance because, as we heard yesterday, taking a separate supplement is more demanding for the parents, but on the other hand you have the stability issues that were mentioned.

**Dr. Cardoso:** The bifidogenic effect of breast milk or formula with probiotics is easy to understand. But infants more than 6 months old who receive other types of foods in different countries are eating a lot of potentially functional ingredients at meals that can have a bifidogenic effect. How can you separate this when you give a fructo-oligosaccharide or galacto-oligosaccharide to these infants with cereals? You noted some differences in terms of intestinal transit, soft feces or recuperation of tissue, etc. How can you separate this?

**Dr. Domellöf:** The softening of stools is not a bifidogenic effect, it is an effect of the oligosaccharides themselves. There are several studies that have documented an increase in bifidobacteria after giving infant formula with probiotics [14]. Stool cultures have not usually been performed in the published trials on probiotics in infant cereals, but it would be interesting to verify the bifidogenic effect also in infants with a mixed diet.

**Dr. Jongpiputvanich:** You mentioned that there is no consistent beneficial effect of some functional components such as LCPUFA nucleotides even in full-term babies. Why do we have to add these nutrients to supplementary feeding for older babies? It seems to me that if one nutrient is added, the price will increase. It looks like a cosmetic change, not a beneficial effect.

**Dr. Domellöf:** Most of these components have been tested primarily in infant formula but several studies now suggest that they actually may have positive health
effects during the weaning period. On the other hand, I agree with you that it is important to consider cost-effectiveness and that possible health benefits must be verified in properly powered clinical studies before bringing these products to the market.

References
