Dietary prebiotic oligosaccharides are detectable in the faeces of formula-fed infants

GUIDO E. MORO1, BERND STAHL2, SILVIA FANARO4, JÜRGEN JELINEK2, GÜNTHER BOEHM1,2 & GIOVANNI V. COPPA3

1Centre for Infant Nutrition, Macedonio Melloni Maternity Hospital, Milan, Italy, 2Numico Research Germany, Friedrichsdorf, Germany, 3Department of Paediatrics, University of Ancona Institute of Paediatrics, Ancona, Italy, and 4Department of Clinical and Experimental Medicine, Division of Neonatology, University of Ferrara, Ferrara, Italy

Abstract
Human milk oligosaccharides are not digested during intestinal passage and can be detected in stools. In this study it was investigated whether a prebiotic mixture of low-molecular-weight galacto-oligosaccharides (GOS) and high-molecular-weight fructo-oligosaccharides (FOS) can be detected in stool samples of formula-fed infants. The test formula was supplemented with 0.8 g/dl oligosaccharides (GOS + FOS). In the control formula, maltodextrins were used as placebo. Fecal flora was assessed at the beginning (day 1) and at the end of a 28-d feeding period (day 2). At day 2 the content of galacto- and fructo-oligosaccharides in the stool samples were measured. On study day 1, the number of bifidobacteria was not different among the groups (supplemented group: 7.7 (6.2) CFU/g; placebo group: 8.0 (6.0) CFU/g). At the end of the 28-d feeding period, the number of bifidobacteria was significantly higher in the group fed the supplemented formula when compared to placebo (supplemented group: 9.8 (0.7) CFU/g stool; placebo group: 7.1 (4.7) CFU/g stool; p < 0.001). In all infants fed the supplemented formula, GOS and FOS could be identified in the stool samples. That was not the case in infants fed the non-supplemented formula.

Conclusion: The present data confirm the bifidogenicity of oligosaccharides and indicate that dietary galacto-oligosaccharides and long chain fructo-oligosaccharides remain during the whole passage in the lumen of the gastrointestinal tract, similarly to human milk oligosaccharides.

Key Words: Galacto-oligosaccharides, long chain fructo-oligosaccharides, digestibility, infants

Introduction
In breastfed infants the intestinal microflora is dominated by bifidobacteria and lactobacilli [1], and this microbial pattern beneficially affects the intestinal function and the development of the immune system [2,3]. Although the mechanisms of these effects are very complex and not fully understood, dietary interventions to establish an intestinal microflora rich in bifidobacteria and lactobacilli are recommended [4,5].

The effect of human milk on the intestinal flora is caused by its content of selective agents which can stimulate the growth of bifidobacteria and lactobacilli. Oligosaccharides, which are a major component of human milk [6], have been identified as a “bifidogenic” factor of human milk [6–8]. The composition of neutral human milk oligosaccharides is very complex [6]. Due to their complexity, it is not possible to reproduce in infant formulas oligosaccharides identical to those of human milk. To mimic the prebiotic effect of human milk oligosaccharides, a mixture of 90% galacto-oligosaccharides (GOS) (derived from lactose [9]) and 10% fructo-oligosaccharides (FOS) (high-molecular-weight fraction of inulin extracted from chicory roots [10]) has been used. The mixture was designed to have a molecular size distribution similar to that of neutral human milk oligosaccharides [11].

Human milk oligosaccharides can be detected in the faeces of breastfed infants [12]. More recently, it has been shown that human milk oligosaccharides are resistant to enzymatic digestion in the upper gastrointestinal tract [13]. Non-digestibility and selective fermentation in the colon by potentially beneficial bacteria are prerequisites for the prebiotic effect of these dietary ingredients [4].
Thus, the aim of the present study was to investigate whether the mixture of galacto- and fructo-oligosaccharides can be detected in the faeces of term infants fed an infant formula supplemented with the prebiotic mixture of GOS and FOS.

**Patients and methods**

The study protocol was approved by the two ethical committees of the hospitals, and informed parental consent was obtained for each infant prior to enrolment in the study.

Thirty-two term infants, appropriate for gestational age, were randomly selected from a cohort of the population of a study designed to investigate the dose dependence of the bifidogenic effect of the prebiotic mixture [14]. In all infants, enteral nutrition was started with breast milk according to the practice of the hospital. Only when the mother was unable or decided not to breastfeed was the infant randomly assigned to one of three formula groups. The composition of the two formulas was identical, except for the supplemented oligosaccharides. The active formula was supplemented with 0.8 g/dl of the oligosaccharides mixture, and the control formula was supplemented with maltodextrines as placebo (Table I).

The most relevant clinical data of the formula-fed infants under study are summarized in Table II.

For microbiological analysis, stool samples were collected at the beginning when formula feeding started (study day 1) and 28 d after (study day 2). The stool samples of study day 2 were analysed for the presence of components of the oligosaccharides mixture.

A quantity of 0.2 g of fresh faecal sample was homogenized in a cryo-protective glycerol transport medium (glycerol 10 ml, oxoid 0.1 g, H$_2$O ad 100 ml) and immediately frozen at $-80^\circ$C. The samples were transported on dry ice. For the identification of bifidobacteria and lactobacilli, selective media were used (bifidobacteria: DIC medium (Bonaparte 1997); lactobacilli: Rogosa) as described previously [15].

The components of the oligosaccharide mixture were analysed using high-performance anion-exchange chromatography. A faecal sample (200 mg) from each infant was stored in 2.0 ml of culture medium (equal to 1:10, w/v dilution). The sample was further diluted with deionized water (1:1, v/v) and stored overnight at 4°C. The sample was centrifuged at 5000 rpm for 15 min. The supernatant was filtered through a 0.22-$\mu$m membrane (Millipore, Bedford, MA, USA). The sample was further diluted (1:200, v/v) with deionized water for chromatographic analyses.

High-performance anion-exchange chromatography (HPAEC) was performed on a HPAEC-system AI 450 equipped with a CarboPac PA-1 precolumn (4×25 mm) and a CarboPac PA-1 column (4×250 mm). A 25-$\mu$l fecal solution sample was injected, by means of an AMS autosampler. The detector was a pulsed amperometric detector PAD II (all: Dionex, Sunnyvale, CA, USA). Two different methods were used to detect low-molecular-weight oligosaccharides and high-molecular-weight oligosaccharides [16].

**Statistics**

Anthropometric data are given as means ± standard deviation (SD). The respective homogeneity of groups was tested by one-way analysis of variance (ANOVA). To account for data not normally distributed, the data on the microflora were described as medians and interquartile ranges (IQR, 25th–75th percentile). As a consequence, the influence of the feeding regimens on these parameters was investigated using non-parametric tests. For an overall group effect, the Kruskal-Wallis test was used. All tests were performed on an alpha-level of 5%. $P$-values < 0.05 were considered significant. The software StatView 5.0 (SAS Institute Inc.) was used.

**Results and discussion**

On study day 1, the number of bifidobacteria was not different among the groups (supplemented group: 7.7

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**Table I.** Composition of the two studied formulas per decilitre.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Placebo</th>
<th>Supplemented formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (g)</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>- Lactose (g)</td>
<td>7.2</td>
<td>7.2</td>
</tr>
<tr>
<td>- GOS/FOS mixture (g)</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>- Maltodextrines (g)</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Whey/casein ratio</td>
<td>60/40</td>
<td>60/40</td>
</tr>
<tr>
<td>Minerals (g)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Energy content (kcal)</td>
<td>71</td>
<td>71</td>
</tr>
</tbody>
</table>

**Table II.** Clinical data of the infants enrolled in the study.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Placebo</th>
<th>Supplemented formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$ (M/F)</td>
<td>16 (9/7)</td>
<td>16 (8/8)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.5 ± 1.8</td>
<td>39.6 ± 1.8</td>
</tr>
<tr>
<td>Weight at birth (g)</td>
<td>3,265 ± 403</td>
<td>3,249 ± 398</td>
</tr>
<tr>
<td>Length at birth (cm)</td>
<td>49.7 ± 2.2</td>
<td>50.1 ± 1.6</td>
</tr>
<tr>
<td>Age at study entry (d)</td>
<td>6.2 ± 2.1</td>
<td>6.9 ± 2.1</td>
</tr>
<tr>
<td>Feeding volume (ml/kg×d)</td>
<td>166 ± 59</td>
<td>171 ± 56</td>
</tr>
<tr>
<td>Weight gain during study period (g/d)</td>
<td>36.5 ± 7.9</td>
<td>36.1 ± 6.7</td>
</tr>
<tr>
<td>Length gain during study period(cm/week)</td>
<td>0.88 ± 0.16</td>
<td>0.87 ± 0.17</td>
</tr>
</tbody>
</table>
Digestibility of prebiotic oligosaccharides

for the beneficial bacteria of the human intestine. In addition to that, β-linked galactose is the key structural element of the GOS representing the majority of molecules in the GOS/FOS mixture, but also an important characteristic of all human milk oligosaccharides [6].

In conclusion, the data of the present study demonstrate that dietary GOS as well as long chain FOS are present during the whole gastrointestinal passage, and this underlines their capability to act as prebiotic ingredients for infant formulas.

References


Figure 1. HPAE chromatography according to [16] of diluted human faecal samples. (A) Chromatogram of a faecal sample from a GOS/FOS-fed infant. (B) Chromatogram of a faecal sample from a standard formula (i.e. non-supplemented)-fed infant. The peaks eluting between 25 min and 35 min represent individual structures of galacto-oligosaccharides (GOS). The peaks eluting between 45 min and 53 min represent individual structures of fructo-oligosaccharides (FOS).

(6.2) CFU/g; placebo group: 8.0 (6.0) CFU/g. At the end of the 28-d feeding period, the number of bifidobacteria was significantly higher in the group fed the supplemented formula when compared to the placebo group (supplemented group: 9.8 (0.7) CFU/g stool; placebo group: 7.1 (4.7) CFU/g stool; \( p < 0.001 \)).

In all infants fed the supplemented formula, GOS and FOS could be identified in the respective stool samples (Figure 1A). That was not the case in all infants fed the non-supplemented formula (Figure 1B).

The data demonstrate clearly that GOS as well as FOS are present during the whole passage in the lumen of the gastrointestinal tract. The same effect has been shown for human milk oligosaccharides using the same methodology as in the present study [12].

Resistance to digestion is an essential prerequisite for the prebiotic function of an ingredient. The bifidogenic effect of the GOS/FOS mixture has been demonstrated in several studies, which is in line with the present findings [14,15,17–19].

The core structure of human milk oligosaccharides consists of monosaccharides in β-glycosidic linkage. This is also the distinctive feature of the galacto-oligosaccharides and long-chain fructo-oligosaccharides [6]. The enzymes of the human gastrointestinal tract are not able to hydrolyse these glycosidic bonds; this holds especially true for the brush border glycohydrolases of the small intestine [20]. This is one of the prerequisites of a prebiotic compound to be available


