Effects of infant formula containing a mixture of galacto- and fructooligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first 4 months of life

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Adding prebiotics or probiotics to infant formula to improve the intestinal flora of formula-fed infants is considered to be a major innovation. Several companies have brought relevant formulations onto the market. However, comparative data on the effects of pre- and probiotics on the intestinal microflora of infants are not available. The present study aimed to compare the effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the composition and metabolic activity of the intestinal microflora. Before birth, infants were randomised and double blindly allocated to one of three formulas. The prebiotic (GOS/FOS) group (n 19) received regular infant formula supplemented with a mixture of galacto-oligosaccharides and fructo-oligosaccharides (6 g/l). The probiotic (Bb-12) group (n 19) received the same formula supplemented with 6·0 × 1010 viable cells of B. animalis per litre. The standard group (n 19) received non-supplemented regular formula. A group of sixty-three breast-fed infants was included as a reference group. Faecal samples were taken at postnatal day 5 and 10, and week 4, 8, 12 and 16. Compared with the groups fed Bb-12 and standard formula, the GOS/FOS formula group showed higher faecal acetate ratio (69·7 % (SEM 2·7), 69·9 % (SEM 3·9) and 82·2 % (SEM 5·3); P<0·05) at 16 weeks. Differences in percentage of bifidobacteria between the GOS/FOS (59·2 % (SEM 7·7)), Bb-12 (52·7 % (SEM 8·0)) and the standard (51·8 % (SEM 6·4)) groups were not statistically significant at 16 weeks. Feeding infants GOS/FOS formula resulted in a similar effect on metabolic activity of the flora as in breast-fed infants. In the Bb-12 group, composition and metabolic activity of the flora were more similar to those of the standard group.


In breast-fed infants the intestinal microflora is dominated by bifidobacteria. In general, formula-fed infants have a more diverse flora (Bullen & Tearle, 1976; Benno et al. 1984; Balmer et al. 1989; Chierici et al. 1997; Hartmans et al. 2000). Fermentation by intestinal microflora results in the production of SCFA, which have different functions such as an energy source for colonocytes, regulating cell growth, lowering intestinal pH and inhibiting the growth of pathogens (Wang & Gibson, 1993). Branched SCFA, products of protein breakdown by intestinal bacteria, are potentially harmful. In breast-fed infants, the microflora produces high amounts of acetate and lactate which in combination with a lower pH restricts the growth of potential pathogens like Escherichia coli and Clostridium perfringens (Eklund, 1983; Wang & Gibson, 1993). In formula-fed infants, relatively high amounts of propionate and butyrate are found.

Complex neutral oligosaccharides have been identified as the most likely prebiotic factor in human milk that stimulates the growth of bifidobacteria in the infant gut (Brand et al. 1998; Engfer et al. 2000). Prebiotics are defined as ‘non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health’ (Gibson & Roberfroid, 1995). Like human milk oligosaccharides, prebiotics in infant nutrition stimulate the growth of bacteria that are already present in the large intestine. Several investigators have reported on approaching the prebiotic effect of human milk oligosaccharides by using a mixture of 90 % galacto-oligosaccharides (GOS) and 10 % fructo-oligosaccharides (FOS) in regular infant formula (Boehm et al. 2002; Moro et al. 2002). It was found that feeding infants GOS/FOS formula significantly increased the number of bifidobacteria.

Besides prebiotics, another approach to improve the intestinal microflora is to add probiotics to infant formula (Langhendries et al. 1995). Probiotics were originally defined as ‘live microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance’ (Fuller, 1989). The possible role of specific probiotic bacteria in recovery from atopic disease and treatment of rotavirus diarrhoea in children

Abbreviations: Bb-12, Bifidobacterium animalis strain Bb-12; DAPI, 4′,6-diamidino-2-phenylindole; FISH, fluorescence in situ hybridisation; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides.

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was elucidated in several studies (Isolauri et al. 2000; Kalliomaki et al. 2001). However, to date, only one study has focused on the effects of probiotics on the intestinal microflora of infants (Langhendries et al. 1995).

Recognising the possible health benefits of a gut flora dominated by bifidobacteria, the question is whether pre- or probiotics should be used to reach the best possible effect. In the present study, we investigated the effects of adding either prebiotic oligosaccharides or probiotic bacteria, to the same standard infant formula, on the composition and metabolic activity of the intestinal microflora in infants. To make an optimal comparison of the prebiotic and probiotic formulas, the study was performed in one population under comparable environmental conditions. We hypothesised that infants fed either prebiotics or probiotics will develop an intestinal microflora dominated by bifidobacteria. Bifidobacteria produce acetate and lactate, which have a lowering effect on intestinal pH. Therefore, similar to breast-fed infants, we expected to find a lower pH, higher ratios of acetate and higher amounts of lactate in the faeces of infants fed prebiotic and probiotic formulas, compared with infants fed standard formula.

Subjects and methods
Subjects

Sixty-three pregnant women who had decided to breast-feed, and fifty-seven who had chosen not to, were recruited during their last trimester of pregnancy. Infants with normal birth weight and no congenital abnormality, congenital disease or gastrointestinal disease were enrolled within 3 d after delivery. The study was approved by the ethical committee of the Medical Centre St. Radboud, Nijmegen, The Netherlands. Written informed consent was obtained from the parents before enrolment in the study.

Feeding groups

Infants of mothers who had decided not to breast-feed were randomly and double blindly allocated to one of three formula groups (GOS/FOS, Bb-12 or standard). Randomisation included a block size of 3 and was carried out by a person not involved in the study. The formula tins containing the different products were coded using a number the infants received at inclusion. The standard formula group (n 19) received a regular, non-supplemented infant formula (Nutrilon I; Nutricia, Zoetermeer, The Netherlands). The main compositional data of the standard formula at the standard dilution of 131 g/l are given in Table 1. The prebiotic formula group (GOS/FOS; n 19) received the same standard infant formula supplemented (6 g/l) with a mixture of trans-galacto-oligosaccharides (Vivinal GOS; Borculo Domon Ingredients, Zwolle, The Netherlands) and fructo-oligosaccharides (Raftilin HPFOS; Orafti Active Food Ingredients, Tienen, Belgium). The mixture comprised 90 % GOS and 10 % FOS in order to closely resemble the spectrum of molecular masses of the neutral oligosaccharide fraction in human milk (Stahl et al. 1994). The probiotic formula group (Bb-12; n 19) received the standard infant formula supplemented with 6·0 × 10^{10} viable cells of Bifidobacterium animalis per litre (Christian Hansen Ltd, Hørsholm, Denmark). B. animalis strain Bb-12 (sometimes referred to as B. lactis) is a thoroughly investigated probiotic and has been found to survive passage through the gastrointestinal tract of adults and infants (Langhendries et al. 1995; Fukushima et al. 1998; Alander et al. 2001). Several studies have demonstrated that during a period of daily ingestion of viable Bb-12 cells, the number of faecal bifidobacteria increases significantly (Link-Amster et al. 1994; Schiffrin et al. 1995; Alander et al. 2001).

The shelf life of the probiotic formula was tested during storage. After 12 months of storage, 1·0 × 10^{10} (SEM 0·5 × 10^{10}) colony-forming units of B. animalis were recovered. The study formulas were fed ad libitum during the study period. Mothers were instructed to heat the water to a maximal temperature of 45 °C before adding the milk powder. This was to avoid hot spots in the liquid milk during microwaving, possibly leading to killing of the bacteria. Mothers who had decided to breast-feed were stimulated to continue breast-feeding during the course of the study and were supported by a lactation consultant when needed. At termination of breast-feeding their infants received one of the three formulas. Compliance was assessed by counting the number of unused formula tins during each visit and comparing the amount of formula consumed with the recorded food intake.

Questionnaires

Demographic, clinical and anthropometric data of the mother were collected prior to delivery. Information on delivery was obtained from the mother at day 5 after delivery. Information on the infants’ food intake, formula tolerance, stool characteristics, health and anthropometrics was obtained from questionnaires at postnatal day 5, 10 and 28 and once every 4 weeks thereafter until the end of the study.

Faecal samples

Parents were asked to take faeces samples from their infants, at postnatal day 5, 10, 28 and once every 4 weeks thereafter. The samples were taken from the diaper as soon as possible after defecation, collected in faeces containers (Greiner Laboratory, Alphen a/d Rijn, The Netherlands) and stored immediately at −20 °C by the parents. During the study period, the investigators visited the participants regularly, to collect faeces samples and

Table 1. Composition of the study formulas per litre

<table>
<thead>
<tr>
<th></th>
<th>Standard formula</th>
<th>GOS/FOS formula</th>
<th>Bb-12 formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>2800</td>
<td>2800</td>
<td>2800</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Casein:whey</td>
<td>40:60</td>
<td>40:60</td>
<td>40:60</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Total carbohydrates (g)</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>GOS (g)</td>
<td>–</td>
<td>5-4</td>
<td>–</td>
</tr>
<tr>
<td>FOS (g)</td>
<td>–</td>
<td>0-6</td>
<td>–</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>75</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>–</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Bb-12 (CFU)</td>
<td>–</td>
<td>–</td>
<td>6·0 × 10^{10}</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>540</td>
<td>540</td>
<td>540</td>
</tr>
<tr>
<td>P (mg)</td>
<td>270</td>
<td>270</td>
<td>270</td>
</tr>
<tr>
<td>Mg (mg)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Na (mg)</td>
<td>190</td>
<td>190</td>
<td>190</td>
</tr>
<tr>
<td>K (mg)</td>
<td>680</td>
<td>680</td>
<td>680</td>
</tr>
<tr>
<td>Cl⁻ (mg)</td>
<td>430</td>
<td>430</td>
<td>430</td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Zn (mg)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

GOS, galacto-oligosaccharides; FOS, fructo-oligosaccharides; Bb-12, Bifidobacterium animalis strain Bb-12; CFU, colony-forming units.
questionnaires. Infant formula was supplied on request. Faeces samples were transported to the laboratory in a portable freezer (minimal temperature −15 °C; MRFD-015; Veba Meditemp, Uden, The Netherlands).

Preparation of faecal samples

For the determination of SCFA, 1 g of faeces was thawed in iced water, diluted 10 × in MilliQ and homogenised for 10 min using a stomacher. The homogenised faeces were stored at −20 °C.

Fluorescence in situ hybridisation (FISH) analysis and lactic acid measurements, the samples were thawed in iced water, diluted 10 × (v/v) in PBS, pH 7.4, and homogenised for 10 min using a stomacher. The homogenised faeces were stored at −20 °C.

FISH analysis was performed as described previously (Langendijk et al. 1995; Jansen et al. 1999; Harmsen et al. 2000) with some slight modifications. Paraformaldehyde-fixed samples were applied to gelatin-coated glass slides (polytetrafluoroethylene-coated eight-well (1 cm2/well) object slides; CBN lab suppliers, Drachten, The Netherlands) and air-dried. The dried samples were dehydrated in 96% ethanol for 10 min. Hybridisation buffer (20 mm-Tris–HCl, 0·9 m-NaCl, 0·1% SDS; pH 7·2) with Cy3-labelled *Bifidobacterium*-specific probe Bif164mod (5′-CAT CCG GYA TTA CCA CCC; 10 ng/μl), was preheated and added to the dried samples. Bif164mod is modified version of probe S-G-Bif-0164-a-A-18 (Langendijk et al. 1995), which detects the presence of bifidobacteria including the *B. animalis* species (Satokari et al. 2001). The slides were incubated overnight in a dark moist chamber at 50 °C. After hybridisation the slides were washed for 30 min in 50 ml of preheated washing buffer (20 mm-Tris–HCl, 0·9 m-NaCl; pH 7·2) and briefly rinsed in MilliQ. For staining all bacteria, the samples were incubated with 0·25 ng 4,6-diamidino-2-phenylindole (DAPI)/μl in PBS for 5 min at room temperature. After DAPI staining the slides were briefly rinsed in MilliQ, dried, mounted with VectaShield (Vector Laboratories, Burlingame, CA, USA) and covered with a coverslip. The slides were automatically analysed using an Olympus AX70 epifluorescence microscope with automated image analysis software (Analysis 3.2; Soft Imaging Systems GmbH, Münster, Germany). The percentage of bifidobacteria per sample was determined by analysing twenty-five randomly chosen microscopic positions. At each position the percentage of bifidobacteria was determined by counting all cells with a DAPI filter set (SP100; Chroma Technology Corp., Brattleboro, VT, USA) and counting all bifidobacteria using a Cy3 filter set (41 007; Chroma Technology Corp.).

Short-chain fatty acid analysis

The SCFA acetic, propionic, n-butyric, isobutyric and n-valeric acids were quantitatively determined using a Varian 3800 gas chromatograph (Varian, Inc., Walnut Creek, CA, USA) equipped with a flame ionisation detector. The sample (0·5 μl) was injected at 80 °C into the column (Stabilwax, 15 m × 0·53 mm, film thickness 1·00 μm; Restek Co., Bellafonte, PA, USA) using He as carrier gas (20·7 kPa). New columns were conditioned overnight at 200 °C. After injection of the sample, the oven was heated to 160 °C at a rate of 16 °C/min, followed by heating to 220 °C at 20 °C/min and finally maintained at a temperature of 220 °C for 1·5 min. The temperature of the injector and the detector was 200 °C. After every ten samples the column was cleared by injection of 0·5 μl formic acid (1%, by vol.) to avoid memory effects of the column, followed by injection of 0·5 μl standard SCFA mix (1·77 mM-acetic acid, 1·15 mM-propionic acid, 0·72 mM-n-butyric acid, 0·72 mM-isobutyric acid, 0·62 mM-n-valeric acid; Sigma-Aldrich) to monitor the occurrence of memory effects. SCFA concentrations were determined using 2-ethylbutyric acid as an internal standard. Faecal SCFA concentrations are dependent on the consistency of stools.

**Lactate**

Homogenised faeces were thawed on ice and centrifuged for 5 min at 14 000 rpm, then 100 μl of the supernatant was heated for 10 min at 100 °C to inactivate all enzymes. Lactate was determined enzymatically using an L-lactic acid detection kit with D- and L-lactate dehydrogenase (Boehringer Mannheim, Mannheim, Germany).

**pH**

After storage at −20 °C, faecal samples were thawed and the pH was measured directly in the faeces at room temperature using a Handy-lab pH meter (Schott Glas, Mainz, Germany) equipped with an Inlab 423 pH electrode (Mettler Toledo, Columbus, OH, USA).

**Data analysis**

Prior to the study, power calculations showed that to detect a difference in the percentage of bifidobacteria between the intervention formula groups (GOS/FOS and Bb-12) and the standard formula group of 30% with SD of 25%, thirteen infants should be included per group. Because of an expected drop-out of 30% in the formula groups, more infants than calculated were included in the study. The SPSS statistical package (version 11.0; SPSS Inc., Chicago, IL, USA) was used for analysis of the results. All values were checked for normality by visual inspection of the normal probability plots. Differences in percentage of bifidobacteria, pH, relative amounts of SCFA and lactate between the groups were tested for significance using ANOVA. In the case of a significant difference (*P*<0.05), groups were compared by using the Bonferroni post hoc test.

Because it is not possible to assign breast- and bottle-feeding double blindly and to ensure adequate randomisation, no statistical analyses were performed to compare the breast-feeding group with any of the formula feeding groups. When an infant changed from breast- to formula feeding, it was considered a drop-out and only the samples taken during the period of complete breast-feeding were included in the study. Samples taken after the switch from breast- to formula feeding were not included in the study.

**Results**

In total, 120 infants were included in the study between January 2000 and May 2003. Fifty-seven infants started on
formula feeding directly after birth and were divided equally
among the formula groups. Of the sixty-three infants who were
fed breast milk directly after birth, twenty-four switched to for-

mula feeding before the age of 16 weeks and five infants dropped
d out. The characteristics of the study subjects are shown in Table
2. In the formula groups, thirteen infants dropped out of the study
within the first 16 weeks after birth: four in the standard group,
five in the GOS/FOS group and four in the Bb-12 group. Reasons for
dropping out included colic, suspicion of cows’ milk allergy,
constipation and practical problems.

Faecal bifidobacteria

The percentage of bifidobacteria in the faeces of infants at the age
of 5 d, 10 d, and 4, 8, 12 and 16 weeks is shown in Fig. 1 for the different
feeding groups. Although not statistically significant, the GOS/FOS

-Feeding group tends to have a higher percentage of bifidobacteria in the total
bacterial count at all ages compared with the standard and Bb-12
 formula groups. Percentages of bifidobacteria in the formula
groups are comparable to that found in the breast-fed group.

pH

The pH values measured in the faeces of the formula-fed infants are
shown in Fig. 2. The lowest pH was found in infants fed on breast
milk. Faecal pH of infants fed the GOS/FOS formula was lower
than in the standard (P<0.05 at all ages except 5 d) and the Bb-12
 formula groups (P<0.05 from week 8 on). At age 10 d, the faecal
pH of infants fed the Bb-12 formula was significantly lower than
that in the standard formula group (P=0.001).

Short-chain fatty acids

The total amount of SCFA in the faeces is shown in Table 3. The percentages of the different SCFA from the total amount of SCFA
are shown in Table 4. The table includes data from all available
faeces samples that were large enough (0.5 ml) to perform the
SCFA analysis. There were no statistically significant differences
in total SCFA concentration found between the formula groups.

However, already after 10 d, differences in SCFA profiles could
be seen between infants fed on GOS/FOS formula and infants
fed on standard or Bb-12 formula. Infants fed the GOS/FOS for-

mula had higher percentages of acetate and lower percentages of
propionate, butyrate and iC4-5 SCFA (isobutyrate, isovalerate and
valerate) compared with infants fed the standard or the Bb-12 for-

mula. There were no differences in the relative amounts of SCFA
in the faeces of infants fed Bb-12 formula compared with infants
fed the standard formula.

Lactate

The concentrations of lactate (mmol/kg faeces) in all groups are
shown in Table 3. Already from 5 d of age, the GOS/FOS formula
group (NS) and the group fed on breast milk had higher amounts
of faecal lactate than did the standard and Bb-12 formula groups.
No differences were found between the Bb-12 group and the stan-

dard formula group.

Discussion

With the aim of stimulating the typical intestinal microbial ecology
of breast-fed infants in formula-fed infants, the present study com-
pared the effects of adding either prebiotics or probiotics to a stan-

dard infant formula. We found that infant formula containing a
mixture of GOS and long-chain FOS induced a metabolic activity
of the intestinal microflora similar to that in breast-fed infants
(high acetate, lactate and low pH). We also observed that infant for-

mula containing viable bifidobacteria induced a metabolic activity
comparable to that in infants fed standard infant formula (SCFA pat-

tern typical for mixed-type flora, neutral pH).

To investigate whether infant formula containing pre- or pro-

biotics can induce an intestinal microflora comparable to that in
breast-fed infants, one should ideally compare the results of
both formula groups with those observed in the breast-fed group.
However, we did not statistically compare the breast-feed-

ing group with the formula feeding groups because it is not
possible to randomise and double blindy assign infants to
breast-feeding and also because of the obvious selection bias
due to social and educational differences between breast- and for-

mula feeding mothers (Ford & Labbok, 1990). By limiting stat-

istical analysis to the formula groups, we are still able to
compare the effect of the prebiotic and probiotic component on
gut flora.

Although all formulas were well accepted and tolerated, thir-

teen of the fifty-seven formula-fed infants dropped out before
the age of 16 weeks. The number of drop-outs was not signifi-
cantly different (P=0.334) between groups. The reasons given
for dropping out were similar between the feeding groups,
which therefore did not give rise to selection bias.

Despite different product compositions and the somewhat
lower dosage of GOS/FOS in the present study (0.6 g/100 ml),
the percentage of bifidobacteria found in the GOS/FOS group
(64 % at 8 weeks of age) is similar to that observed by other
investigators: 76 % after 6 weeks, 0.8 g/100 ml (Schmelzle et

al. 2003), 69 % after 6 weeks, 0.8 g/100 ml (Knof et al. 2003); and
65 % after 6 weeks, 0.8 g/100 ml (Knof et al. 2002). For unknown
reasons, the percentage of bifidobacteria in the standard formula
group in the present study is higher than that observed previously.
Differences in composition of the formula (other than the carbo-
hydrates) may play a role.

Results of the probiotic group are difficult to compare with
other studies because, to date, only one study has reported the

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### Table 2. Characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Standard formula (n 19)</th>
<th>GOS/FOS formula (n 19)</th>
<th>Bb-12 formula (n 19)</th>
<th>Breast milk (n 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>12</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>7</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td><strong>Place of birth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At home</td>
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<td>Hospital</td>
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<td>9</td>
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<td><strong>Mode of delivery</strong></td>
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<tr>
<td>Vaginal</td>
<td>14</td>
<td>16</td>
<td>18</td>
<td>59</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><strong>Birth weight (g)</strong></td>
<td>3601</td>
<td>3318</td>
<td>3481</td>
<td>3651</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>sd</strong></td>
<td>501</td>
<td>602</td>
<td>524</td>
<td>601</td>
</tr>
</tbody>
</table>

GOS, galacto-oligosaccharides; FOS, fructo-oligosaccharides; Bb-12, Bifidobacterium animalis strain Bb-12.
effect of a probiotic infant formula on the intestinal microflora but did not give any quantitative data on the stimulation of bifidobacteria (Langhendries et al. 1995).

In the present study, we found that feeding infants an infant formula supplemented with a mixture of galacto-oligosaccharides and fructo-oligosaccharides has a marked effect on the metabolic activity of the intestinal microflora. In general, the pH and relative amounts of SCFA in faeces of our breast-fed group and the standard formula group are similar to the findings of others (Ogawa et al. 1992; Siigur et al. 1993; Edwards et al. 1994). However, the relative amounts of SCFA, the lactate concentration and the pH in the faeces of the GOS/FOS group are comparable to values seen in breast-fed infants. High relative amounts of acetate and high concentrations of lactate, together with the low faecal pH, create conditions that are less favourable (colonisation resistance) for Enterobacteriaceae and other potential opportunistic pathogens that can be

Fig. 1. Percentage of bifidobacteria from total number of bacterial cells per gram of faeces (wet weight) in infants fed breast milk (---), formula supplemented with a mixture of galacto-oligosaccharides and fructo-oligosaccharides (6 g/l; —–), formula supplemented with Bifidobacterium animalis strain Bb-12 (6·0 × 10^10 viable cells/l; —●—) and standard formula (—–), between birth and 16 weeks of age (d, day; w, week). Values are means with their standard error shown by vertical bars. No statistically significant differences were found between groups (P>0.05).

Fig. 2. pH values of faeces in infants fed breast milk (---), formula supplemented with a mixture of galacto-oligosaccharides and fructo-oligosaccharides (6 g/l; —–), formula supplemented with Bifidobacterium animalis strain Bb-12 (6·0 × 10^10 viable cells/l; —●—) and standard formula (—–), between birth and 16 weeks of age (d, day; w, week). Values are means with their standard error shown by vertical bars. Mean values were significantly different from those of the standard formula group: *P<0.05. Mean values were significantly different from those of the Bb-12 formula group: †P<0.05.
Concentration of lactate (mmol/kg faeces) and total short-chain fatty acids (mmol/kg faeces) in the faeces of infants, between birth and 16 weeks of age, of the different study groups* (Mean values with their standard error and number of determinations)

<table>
<thead>
<tr>
<th></th>
<th>Standard formula</th>
<th>GOS/FOS formula</th>
<th>Bb-12 formula</th>
<th>Breast milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactate</td>
<td>SCFA</td>
<td>Lactate</td>
<td>SCFA</td>
</tr>
<tr>
<td>5 days</td>
<td>Mean</td>
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<td>54.7</td>
<td>14.0</td>
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*No statistically significant differences were found between groups (P > 0.05).

Table 3. Concentration of lactate (mmol/kg faeces) and total short-chain fatty acids (mmol/kg faeces) in the faeces of infants, between birth and 16 weeks of age, of the different study groups.

In the present study, adding viable B. animalis strain Bb-12 to a standard infant formula did not have a distinct effect on the number of bifidobacteria and metabolic activity of the intestinal microflora. Nevertheless, several studies have shown that specific probiotic bacteria including strain Bb-12 may have a role in the prevention and treatment of different diseases (Saavedra et al. 1994; Isolauri et al. 2000; Isolauri, 2001). According to the present study, adding viable B. animalis strain Bb-12 to a standard infant formula did not have a distinct effect on the number of bifidobacteria and metabolic activity of the intestinal microflora. Nevertheless, several studies have shown that specific probiotic bacteria including strain Bb-12 may have a role in the prevention and treatment of different diseases (Saavedra et al. 1994; Isolauri et al. 2000; Isolauri, 2001). According to the
original definition by Fuller (1989), probiotics should change microbial balance to have a health effect. Our results do not support such an effect on microflora of a widely used probiotic strain (*B. animalis*, Bb-12). It is possible that health effects of the probiotic strain Bb-12 already occur in the small intestine or do not require major changes in the intestinal microflora more distally.

In conclusion, feeding infants a formula containing the prebiotic GOS/FOS mixture resulted in high relative amounts of faecal acetate, high concentration of faecal lactate and a low faecal pH. In the infants who received the standard formula or the formula with added viable cells of *B. animalis* strain Bb-12, a similar effect on metabolic activity of the intestinal flora in the prebiotic group only. The observed shift from a more proteolytic (putrefaction) to a more saccharolytic colon physiology could be considered a health benefit for the infant.

### Acknowledgements

We would like to thank the parents and other caregivers for their participation in this study; Esmeralda van der Linde, Rob Slump and Jochem Steenbakkers for analysis of the faecal samples; and Bertha Stallinga and Martine van de Brink for data collection. Klaartje de Jong is acknowledged for her efforts in the initial phase of designing the study.

### References


### Table 4. Relative amount of short-chain fatty acids (percentage of total short-chain fatty acids) in the faeces of infants, between birth and 16 weeks of age, of the different study groups

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Total SCFA, sum of acetate, propionate, butyrate, isobutyrate, valerate and isovalerate; iC4-5 SCFA, sum of isobutyrate, isovalerate and valerate; GOS, galacto-oligosaccharides; FOS, fructo-oligosaccharides; Bb-12, *Bifidobacterium animalis* strain Bb-12.

Mean values were significantly different from those of the Bb-12 formula group: †P < 0.05.

Mean values were significantly different from those of the Bb-12 formula group: *P < 0.05.

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