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# Dosage-Related Bifidogenic Effects of Galacto- and Fructooligosaccharides in Formula-Fed Term Infants

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**ABSTRACT**

**Background:** Human milk oligosaccharides have been shown to stimulate selectively the growth of *Bifidobacteria* and *Lactobacilli* in the intestine. In this study, the bifidogenic effect of an experimental prebiotic oligosaccharide mixture consisting of low-molecular-weight galactooligosaccharides and high-molecular-weight fructooligosaccharides was analyzed in 90 term infants.

**Methods:** Two test formulas were supplemented with either 0.4 g/dL or with 0.8 g/dL oligosaccharides. In the control formula, maltodextrin was used as placebo. At study day 1 and study day 28, the fecal species, colony forming units (cfu) and pH were measured and stool characteristics, growth, and side effects were recorded.

**Results:** At study day 1, the median number of *Bifidobacteria* did not differ among the groups (0.4 g/dL group, mean [interquartile range] 8.5 [1.9] cfu/g; 0.8 g/dL group, 7.7 [6.1] cfu/g; and the placebo group, 8.8 [6.1] cfu/g) (figures in square brackets are interquartile range). At the end of the 28-day feeding period, the number of *Bifidobacteria* was significantly increased for both groups receiving supplemented formulas (the 0.4 g/dL group, 9.3 [4.9] cfu/g; the 0.8 g/dL group, 9.7 [0.8] cfu/g) versus the placebo group (7.2 [4.9] cfu/g, $P < 0.001$). This effect was dose dependent (0.4 g/dL versus 0.8 g/dL, $P < 0.01$). The number of *Lactobacilli* also increased significantly in both groups fed the supplemented formulas (versus placebo, $P < 0.001$), but there was no statistically significant difference between the group fed formula with 0.4 g/dL oligosaccharides and the group fed formula with 0.8 g/dL oligosaccharides. The dosage of supplement significantly influenced the change in fecal pH ($P < 0.05$) (placebo, pH 5.5–6.1; 0.4 g/dL formula, pH 5.48–5.44; 0.8 g/dL formula, pH 5.54–5.19). Slight changes in the stool frequency resulted in a significant difference between the placebo group and the group fed the 0.8 g/dL formula at day 28 ($P < 0.01$). Supplementation had a significant dose-dependent influence on stool consistency (0.8 g/dL versus placebo, $P < 0.0001$; 0.8 g/dL versus 0.4 g/dL, $P < 0.01$). Supplementation had no influence on the incidence of side effects (crying, regurgitation, vomiting) or growth.

**Conclusions:** These data indicate that supplementation of a term infant's formula with a mixture of galacto- and fructooligosaccharides has a dose-dependent stimulating effect on the growth of *Bifidobacteria* and *Lactobacilli* in the intestine and results in softer stool with increasing dosage of supplementation. ***JPGN 34:291–295, 2002.*** **Key Words:** Galactooligosaccharides—Fructooligosaccharides—*Bifidobacteria*—*Lactobacilli*—Term infants—Dosage. © 2002 Lippincott Williams & Wilkins, Inc.

In utero, the fetus is sterile until the rupture of the fetal membranes. During vaginal delivery, the infant will acquire the initial microflora from the mother. After this initial inoculation of bacteria, the intestinal flora is modulated by several extrinsic factors (1–3). The type of diet is one factor that determines the composition of the intestinal microflora of breast-fed infants, which differs from the microflora of bottle-fed infants (4). In breast-fed infants, the intestinal microflora is dominated by *Bifidobacteria* and *Lactobacilli*, and this microbial pattern produces beneficial effects on intestinal function and on development of the immune system (5,6). Although the mechanisms of these effects are very complex and not fully understood, dietary interventions to establish an intestinal microflora dominated by *Bifidobacteria* and *Lactobacilli* are recommended (7–9).

The effect of human milk on the intestinal flora is caused by its content of selective agents that can stimulate the growth of *Bifidobacteria* and *Lactobacilli*. Oligosaccharides, which are a major component of human milk (10), have been identified as a "bifidogenic" factor of human milk (11,12). Recently, human milk oligosaccharides were shown resistant to enzymatic digestion in the upper gastrointestinal tract (13). Nondigestibility and selective fermentation by potentially beneficial bacteria in the colon are prerequisites for a prebiotic effect of dietary ingredients (7–9).

The composition of neutral human milk oligosaccharides is very complex (10–15), and the relation between

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these different structures and their function is not well understood. Galactooligosaccharides and fructooligosaccharides have been used to stimulate *Bifidobacteria*, and several human studies have demonstrated the prebiotic effect of these compounds (16–19).

In a previous study performed in preterm infants, we tested the prebiotic capacity of an oligosaccharide mixture consisting of 90% galactooligosaccharides (derived from lactose) (20) and 10% fructooligosaccharides (high-molecular-weight fraction of inulin extracted from chicory roots) (16). The mixture was combined to mimic the molecular size distribution of human milk oligosaccharides and to benefit from a possible synergistic effect of both compounds to stimulate the growth of *Bifidobacteria* (21).

The mixture was used in a concentration of 1 g/dL, similar to the oligosaccharide content of human milk (10). The data of this study of preterm infants demonstrate that this mixture cannot stimulate intestinal *Bifidobacteria* in formula-fed infants. The number of *Bifidobacteria* found in the infants fed a formula supplemented with this oligosaccharide mixture was in the upper range of the values found in infants fed human milk (21), possibly indicating that a lower dosage also may be effective.

Several environmental differences exist between preterm infants who are treated in an intensive care unit and term infants treated under normal home conditions that influence the development of intestinal flora (22,23). Consequently, conclusions from the study of preterm infants cannot be extrapolated to healthy term infants.

Therefore, the aim of the current study was to investigate whether the mixture of galacto- and fructooligosaccharides also has a bifidogenic effect in term infants and whether this effect is dose dependent.

## PATIENTS AND METHODS

Ninety term infants, appropriate for gestational age, admitted to the Macedonio Melloni Maternity Hospital (n = 75) and to the Mangiagalli Hospital (n = 15), University of Milan, Italy, were eligible for the study. The ethics committees of the two hospitals approved the study, and parents gave informed consent before enrollment in the study.

Enteral nutrition was started with breast-feeding for all infants, according to the practice of the two hospitals. When the mother was not able to or decided not to breast-feed, the infant was randomly assigned to one of three formula groups. The composition of the three formulas was, apart from the supplemented oligosaccharides, identical. Two formulas were supplemented with the oligosaccharide mixture at different concentrations: 0.8 g/dL and 0.4 g/dL. The control formula was supplemented with maltodextrins as placebo. Table 1 shows the composition of the three formulas. Table 2 gives the most relevant clinical data of the formula-fed infants.

The infants were evaluated first when formula feeding started (study day 1) and then evaluated 28 days later (study day 2). At each study day, stools were collected, fecal flora and pH of the stool were determined, and stool characteristics and any side effects were recorded. Breast-feeding of more than 14 days or receiving antibiotic treatment were exclusion criteria.

**TABLE 1.** *Composition of the two studied formulas per deciliter*

| Formula | Control | 0.4 g/dL | 0.8 g/dL |
|---|---|---|---|
| Fat (g) | 3.6 | 3.6 | 3.6 |
| Carbohydrates (g) | 7.2 | 7.4 | 8.2 |
| Lactose (g) | 7.2 | 7.2 | 7.2 |
| Oligosaccharide mixture (g) | 0 | 0.4 | 0.8 |
| Maltodextrines (g) | 0.8 | 0.4 | 0 |
| Protein (g) | 1.5 | 1.5 | 1.5 |
| Whey/casein ratio | 60/40 | 60/40 | 60/40 |
| Minerals (g) | 0.4 | 0.4 | 0.4 |
| Osmolarity (mOsmol/L) | 285–295 | 285–295 | 285–295 |
| Energy content (kJ) | 291 | 291 | 291 |

For the microbiologic analysis, 0.2 g of a fresh fecal sample was homogenized in a cryoprotective glycerol transport medium (glycerol 10 mL, oxoid 0.1 g, $H_2O$ up to 100 mL volume) and immediately frozen at −80°C. The samples were transported on dry ice. To identify *Bifidobacteria* and *Lactobacilli,* selective media were used (*Bifidobacteria*, disseminated intravascular coagulation medium [Bonaparte 1997]; *Lactobacilli*, Rogosa), as described previously (24). The fecal samples also were analyzed for *Bacteroides*, *Clostridium* species, *Escherichia coli*, *Enterobacter*, *Citrobacter*, *Proteus*, *Klebsiella*, and *Candida*. The numbers are given as colony forming units (cfu)/g stool.

The pH was measured in the fresh stool sample using a multicolor indicator paper (accuracy ± 0.2, Spezialindikatorpapier Merck Eurolab GmbH, Darmstadt, Germany).

Stool characteristics were recorded with respect to consistency (score 1–5: 1 = watery; 2 = soft; 3 = seedy; 4 = formed; 5 = hard) and frequency. Stool consistency and color were evaluated using the appearance of the fresh sample. The consistency of each stool sample collected in the 2 study days was recorded and the mean of the scores obtained for each day was used to characterize the stool consistency of that day.

The incidence of crying (score 1–3: 1 = practically not crying; 2 = crying in connection with feeding; 3 = crying independently from the meals), regurgitation (score 1–3: 1 = 0 regurgitation; 2 = 1–2 regurgitations; 3 = > 2 regurgitations per day), and vomiting (score 1–3: 1 = 0 vomiting; 2 = 1 episode of vomiting; 3 = > 1 episode of vomiting per day) were recorded on the basis of the mother's interview.

For all infants, growth parameters were measured at each study day. Body weight was measured using a scale with an accuracy of ± 5 g. The crown–heel length was measured using a special board for newborn infants that has an accuracy of ± 1 mm.

### Statistics

Anthropometric data are given as mean ± standard deviation (SD). Respective homogeneity of groups was tested by one-way analysis of variance.

To account for data not normally distributed, the data for microflora and stool frequency and consistency were described as the median and interquartile ranges (25–75th percentile). Therefore, the influence of the feeding regimens on these pa-

**TABLE 2.** *Clinical data of the infants enrolled in the study*

| Supplementation group | Placebo | 0.4 g/dL Oligosaccharides | 0.8 g/dL Oligosaccharides |
|---|---|---|---|
| N (M/F) | 33 (17/18) | 30 (17/13) | 27 (12/15) |
| Gestational age (wk) | 39.6 ± 1.9 | 39.1 ± 2.1 | 39.8 ± 1.7 |
| Weight at birth (g) | 3,243 ± 427 | 3,228 ± 452 | 3,287 ± 390 |
| Length at birth (cm) | 49.7 ± 2.2 | 50.1 ± 1.9 | 50.3 ± 1.4 |
| Age at study entry (days) | 6.3 ± 2.1 | 6.8 ± 1.9 | 7.2 ± 2.2 |
| Feeding volume ($mg \cdot kg^{-1} \cdot d^{-1}$) | 162 ± 58 | 163 ± 64 | 173 ± 55 |
| Weight gain during study period (g/d) | 36.8 ± 8.3 | 35.1 ± 6.7 | 35.9 ± 6.5 |
| Length gain during study period (cm/wk) | 0.87 ± 0.16 | 0.88 ± 0.23 | 0.87 ± 0.17 |

rameters was investigated using nonparametric tests. The Kruskal-Wallis test was used for overall group effect. In case of significance, the Mann-Whitney test was performed for single group comparisons.

For comparison of the frequency of positive cultures for *Bacteroides*, *Clostridium* species, *E. coli*, *Enterobacter*, *Citrobacter*, *Proteus*, *Klebsiella*, and *Candida* the $\chi^2$ test was performed.

All tests were performed on an α-level of 5%. *P* values greater than 0.05 were considered significant. StatView 5.0 software (SAS Institute Inc., Cary, NC, U.S.A.) was used.

## RESULTS

At the first study day, the numbers of fecal *Bifidobacteria* did not differ among the groups, median (interquartile range): placebo, 8.8 (6.1) cfu/g; formula supplemented with 0.4 g/dL oligosaccharides, 8.5 (1.9) cfu/g; formula supplemented with 0.8 g/dL oligosaccharides, 7.7 (6.1) cfu/g). During the study period, the number of fecal *Bifidobacteria* increased in both groups that received the supplemented formulas but remained nearly constant in the placebo group. Therefore, at the end of the 28-day feeding period, the number of *Bifidobacteria* in the stools was significantly higher in both groups fed the supplemented formulas than in the stools of the placebo group, median (interquartile range): placebo, 7.2 (4.9) cfu/g; formula supplemented with 0.4 g/dL oligosaccharides, 9.3 (1.6) cfu/g; formula supplemented with 0.8 g/dL, 9.7 (0.8) cfu/g), but there was also a statistically significant difference between the group fed the 0.4 g/dL formula and the group fed the 0.8 g/dL formula ($P < 0.01$) (Fig. 1).

At the beginning of the study period, the number of *Lactobacilli* in the stools did not differ among the groups: placebo, 3.4 (0.2); 0.4 g/dL formula, 3.3 (0.2); 0.8 g/dL formula, 3.4 (0.2). During the study period, the number increased in both groups that received supplemented formulas, and at study day 2, the number was significantly higher ($P < 0.01$) in both groups fed the supplemented formulas than in the placebo group: median (interquartile range): placebo, 3.4 (1.8) cfu/g; 0.4 g/dL formula, 5.9 (1.5) cfu/g; 0.8 g/dL formula, 5.6 (2.1) cfu/g). There was no statistically significant difference

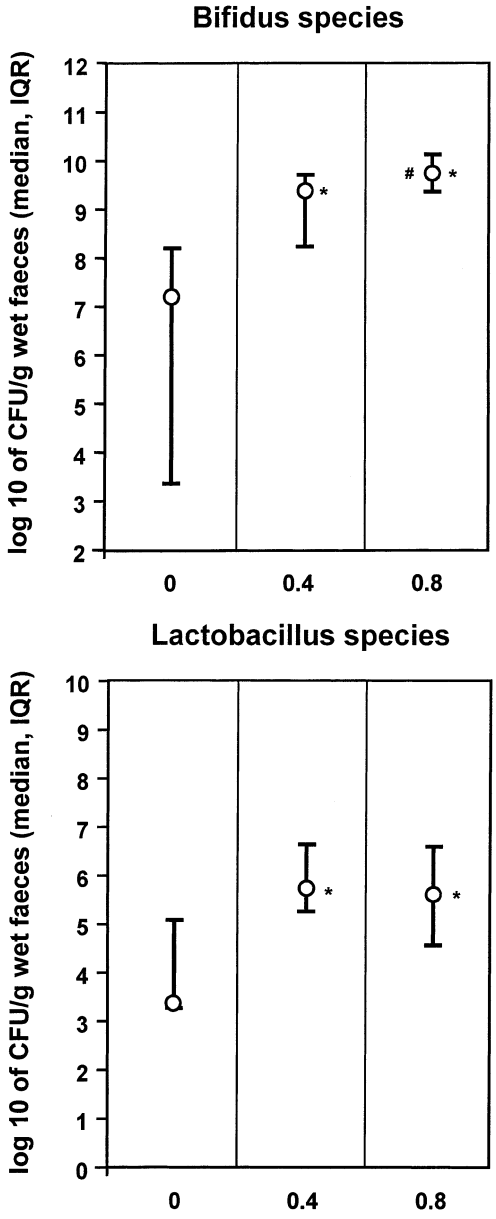


**FIG. 1.** Influence on the counts of *Bifidobacteria* and *Lactobacilli* of various oligosaccharide supplementations to infant formulas after a 28-day feeding period. IQR = interquartile range.

between the group fed the 0.4 g/dL formula and the group fed the 0.8 g/dL formula (Fig. 1).

Oligosaccharide supplementation had not significant effect on the number of infants with positive culture for *Bacteroides*, *Clostridium* species, *E. coli*, *Enterobacter*, *Citrobacter*, *Proteus*, *Klebsiella*, and *Candida*. The fecal pH increased in the placebo group from 5.50 ± 0.63 at the beginning of the study to 6.1 ± 0.66 at the end of the study. In the group fed the 0.4 g/dL formula, there was no significant change in pH (first measurement, 5.48 ± 0.48; second measurement, 5.44 ± 0.53). In the group fed the 0.8 g/dL formula, the pH decreased from 5.64 ± 0.58 at the first measurement to 5.19 ± 0.40 at the end of the feeding period. The influence of the diet on the change in fecal pH was significant ($P < 0.05$).

Stool frequency increased only in the group fed the 0.8 g/dL formula (Fig. 2). The range of the stool frequency in this group was between 1 to 5, and no infant had diarrhea during the study period.

Supplementation significantly influenced the stool consistency scores (Fig. 2). In the placebo group, the score increased, that is, the stools became harder. There was no significant change in the group fed the 0.4 g/dL formula. In the group fed the 0.8 g/dL formula, the consistency changed to softer stools, close to the scores found in the reference group (Fig. 2).

The different diets did not influence the incidence of crying, regurgitation, or vomiting (data not shown). Weight gain and length increment were similar among the groups (Table 2).

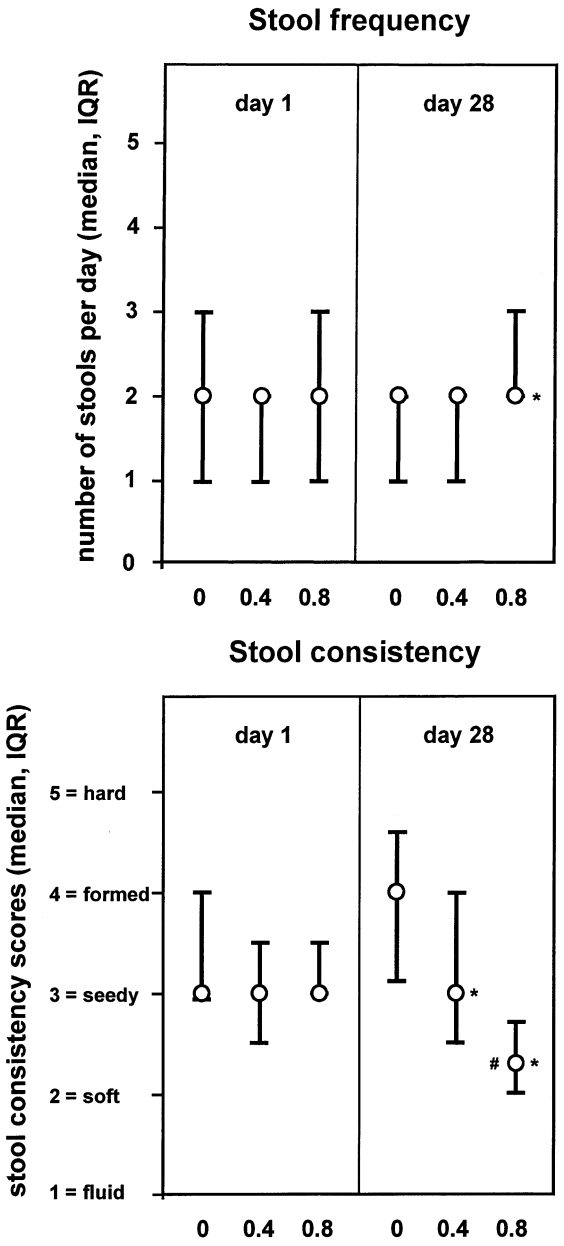


**FIG. 2.** Influence on stool frequency and consistency of various oligosaccharide supplementations to infant formulas after a 28-day feeding period. IQR = interquartile range.

## DISCUSSION

Infant formula supplementation with a mixture of galactooligosaccharides and fructooligosaccharides with high molecular weight leads to increased numbers of the fecal *Bifidobacteria* and *Lactobacilli*. A change in consistency to softer stools and, less pronounced, to a higher stool frequency accompanies this increase. Supplementation also significantly influences the pH of the stools.

The effect of supplementation on the number of *Bifidobacteria,* stool consistency, and stool pH was dose dependent, indicating that oligosaccharides reach the colon and interact quantitatively with the intestinal flora.

The effect of the supplementation on stool characteristics is of practical importance because it may decrease the adverse effects associated with the higher incidence of hard stools or constipation in infants fed standard infant formula as compared with breast-fed infants (24,25). Increased formula osmolarity also could influence stool consistency. In the current study, 0.8 g carbohydrates were added to all study formulas, increasing the osmolarity within a small range (< 5 mOsmol/L). Because the effect of this supplementation on stool consistency could not be seen in the placebo group, which received formula supplemented with maltodextrins, the stool characteristics probably were influenced mainly by changes in the intestinal flora.

Regarding the optimal dosage of the galacto- and fructooligosaccharide mixture, our data demonstrate that a concentration of 0.4 g/dL is bifidogenic and influences stool characteristics and fecal pH as well. However, these supplementation effects were more pronounced in the group fed the 0.8 g/dL formula. Furthermore, the bifidogenic effect is much more homogenous in the group fed the 0.8g/dL formula, indicated by nearly simi-

lar counts of *Bifidobacteria* in all infants after 28 days. This supports the results of a study in adults using different dosages of short-chain fructooligosaccharide (26). In that adult study, a dose-dependent increase of fecal *Bifidobacteria* was also observed.

In our study, galactooligosaccharides derived from lactose were the dominating oligosaccharides in the formula supplement. In human milk, galactose is a major component of human milk oligosaccharides, even if with a different structure. Furthermore, the galactooligosaccharides used in this study have been widely used in infant feeding (17), because they are present in all lactose-reduced or lactose-free products in which lactose has been enzymatically digested (21). To date, no side effects have been reported. More recently, Guesry et al. (27) studied fructooligosaccharides as the only supplement in a formula for term infants at an intake of up to 3 g/day, which is approximately 10 times higher than in our study. They could not demonstrate a bifidogenic effect, nor did they observe side effects. In our study, supplementation also did not influence the incidence of regurgitation, vomiting, or crying, which underlines the safety of the oligosaccharides mixture.

Using the current data, we cannot evaluate to what extent the galactooligosaccharides or the fructooligosaccharides are responsible for the observed effects. However, from the data in the literature (17), a synergistic effect of both ingredients can be assumed. The intensity of the bifidogenic effect of the mixture may indicate that such a synergistic effect took place.

In summary, supplementation of a formula for term infants with a mixture of galacto- and fructooligosaccharides stimulates the growth of *Bifidobacteria* and *Lactobacilli* in the intestine and results in softer stools in a dose-dependent manner. A dosage of 0.4 g/dL results in significant effects, but the effects can be enhanced homogeneously to a level observed in breast-fed infants by increasing the dosage to 0.8 g/dL.

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