The effect of a prebiotic supplemented formula on growth and stool microbiology of term infants

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Abstract

Background: The intestinal flora of breast-fed infants is generally dominated by bifidobacteria which have beneficial properties. Their presence is due to various compounds of breast milk including prebiotic substances.

Aim: This prospective, double blind, study compared the growth, acceptability and the proportion of bifidobacteria and clostridia in the stool flora of bottle-fed infants randomized to receive a formula with a specific mixture of 0.4 g/100 ml prebiotic galacto- and long-chain fructooligosaccharides or the same formula without added prebiotics.

Methods: Within 0–14 days after birth at term, healthy bottle-fed infants were enrolled to receive either a prebiotic formula or a standard formula. At recruitment anthropometric measurements were done. These were repeated at the age of 6 and 12 weeks. Stool samples were taken at inclusion and at the age of 6 weeks. The number of bifidobacteria and clostridia was determined by fluorescent in situ hybridization.

Results: There was good tolerance of the prebiotic formula. Somatic growth was similar in the two groups. Stool frequency was significantly higher in the prebiotic group (P=0.031). Infants in the prebiotic group had also softer stools as compared to the control group (P=0.026). Baseline values of microorganisms at study entry were similar. The percentage of faecal clostridia at the completion of the study was significantly lower in the prebiotic group (P=0.042), while the proportion of faecal bifidobacteria was higher in the prebiotic group as compared to the control group. However this difference did not reach statistical significance (P=0.262). The percentage of E. coli was lower in the prebiotic group but again this did not reach statistical significance (P=0.312).

Conclusion: An infant formula containing prebiotic oligosaccharides is well tolerated, leads to normal somatic growth and suppresses the numbers of clostridia in the faeces with a trend for higher percentage of stool bifidobacteria and lower percentage of E. coli.

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1. Introduction

Human milk contains approximately 1% oligosaccharides. In contrast infant formulae based on cow’s milk contain negligible
amounts of oligosaccharides [1–3]. The oligosaccharides in human milk comprise a large number (>130) of different compounds made up of units of glucose, galactose, N-acetylgalcosamine, fucose and sialic acid [4,5]. The biologic functions of these oligosaccharides are not fully understood [1]. However there is evidence that they play an important role in generating a colonic microflora that comprises predominantly bifidobacteria [6–8]. Also, the oligosaccharides in human milk contribute towards its anti-infective properties by preventing the adherence of pathogenic microorganisms to the intestinal mucosa [9,10]. The development of a bifidobacteria predominant flora is considered to be important for the health of infants since it inhibits both gram positive and gram negative pathogenic bacteria, it has a stimulatory effect on the immune system and it promotes the production of B vitamins [11,12]. Since analogues of the very complex human milk oligosaccharides are not commercially available, a mixture of long-chain fructo-oligosaccharides and trans galacto-oligosaccharides (GOS/lcFOS) has been developed which has a similar molecular weight profile to human milk oligosaccharides. This mixture has recently been shown to have a prebiotic effect in term and preterm infant formulas [13,14]. The purpose of this study was to investigate the growth, acceptability, and stool microbiology of infants fed a starting infant formula containing added prebiotic oligosaccharides.

2. Experimental design and methods

The study was based on a double blind, randomized parallel group design. The subjects comprised healthy term infants with birth weight between the 10th and 90th centiles, whose mothers had ceased breast feeding by the age of 14 days. Exclusion criteria included: infants with major congenital abnormalities or chromosomal disorders or with disease requiring systemic antibiotic treatment, infants from multiple births and also adopted or fostered infants. Written informed consent was obtained from the parents and the study had the approval of the hospital ethics committee.

2.1. Sample size

The primary outcome measure of this study was weight gain. The AAP Committee on the assessment of infant formulas has recommended that growth studies conducted for the purpose of assessing infant formulae should have the capability to detect differences in weight gain during the first 3 months of life of 3 g/day i.e. one-half of a standard deviation [15]. At 5% significance and 80% power, this required a group size of 64 infants. After allowing for dropouts (20%) the number of infants required to be recruited per group was 80 i.e. a total of 160 formula fed infants. Thus 160 formula fed infants were enrolled into the study. Infants were randomly assigned to receive either a standard term formula with 0.4% added GOS/lcFOS (PBF group) or the same formula without added prebiotics (STF group). Both formulas were provided by Numico Research, the Netherlands.

Randomization was carried out using a block design and stratification. Doctors involved in the study were unaware of the type of milk given to each infant. The oligosaccharide mixture consisted of 90% GOS derived from lactose and 10% lcFOS (high molecular weight fraction of inulin extracted from chicory roots). This 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. At recruitment the infants' weight, length and head circumference were measured. These anthropometric measurements were repeated by the same person at the age of 6 and 12 weeks. Stool samples were collected from 64 infants (32 per group) randomly selected from each group immediately after enrolment and at 6 weeks of age. Each subject provided 4 stool samples: one on each of 2 consecutive days immediately after enrolment and one on each of 2 consecutive days at 6 weeks of age. Stool samples were collected as freshly as possible and immediately frozen at −20 °C. The stools were analyzed at Numico Research, the Netherlands for total bacterial count by staining the bacterial cells with 4,6-diamino-2-phenylindole (DAPI) and for the total number of bifidobacteria using fluorescent in situ hybridization.
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(FISH), using probe Bif 164 as described [16]. At least 25 microscopic fields per sample were automatically examined using an qOlympus AX70 epifluorescence microscope and appropriate image software.

At 6 weeks and 10 weeks post partum mothers completed a diary and questionnaire in which the intake of formula and stool characteristics (frequency and consistency, on a 5 point scale) were recorded for 5 days. Also a 24 hour recall was taken at the 13th week interview regarding formula intake, satisfaction and occurrence of possetting.

2.2. Statistical analysis

The results of the two formula groups were evaluated using t-tests for parametric data and by the Mann–Whitney test for non-parametric data. Binary and categorical data were assessed by chi-squared analysis.

3. Results

Almost equal numbers of infants in both groups dropped out during the study. Clinically relevant adverse events related to the study formula were not reported. In 9 cases (5 in the prebiotic group and 4 in the control group) infants dropped out of the study because the mother or the private paediatrician changed to another formula. In 7 cases (4 in the PBF and 3 in the STF) infants dropped out of the study because of minor gastrointestinal complaints. For 4 cases reasons for dropout are unknown. Thus 140 infants completed the study (70 in each group). At inclusion basic characteristics of the two groups were similar (Table 1). There was no difference between formulas with regards to infant satisfaction, tolerance and occurrence of possetting. Somatopic growth during the study for the PBF and the STF subjects was virtually the same (Table 2). With respect to the number of stools passed, stool frequency was significantly higher in the PBF group. There was also a significant influence of the supplementation on stool consistency. Infants of the PBF having softer stools as compared to controls. The median proportion of faecal bifidobacteria as a percentage of the total microorganisms was initially 5.36 in the PBF group and 8.04 in the STF group (Table 3). At 6 weeks of age the proportion of bifidobacteria in the PBF group had increased to 39.7% whereas little change was observed in the STF group. The percentage of clostridia in the stools was initially 0% for both groups but by 6 weeks it was significantly lower in the PBF group as compared to the STF group (0% vs. 3.29%) (Table 3). Stool pH was lower in the PBF group at 6 weeks but this did not reach statistical significance.

4. Discussion

Prebiotics are nondigestible food compounds 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 thereby improving host health [17]. Although undigested nitrogen and lipid-containing compounds may also have prebiotic effects, the most commonly studied and used compounds of infant feeds with proposed prebiotic nature are nondigestible carbohydrates [18–20]. The prebiotic oligosaccharide mixture used in the study was preferentially designed to stimulate bifidobacteria. The data demonstrated that supplementation of a standard term milk formula with this mixture for 6 weeks can stimulate bifidobacteria, indicating a bifidogenic effect of the oligosaccharide supplemented formula. Bifidobacteria are desirable not only because they protect infants from pathogenic intestinal microorganisms during a phase of insufficient immune response but also because their presence in the gut microflora of infants with a high risk for atopic disease reduces the risk for atopic disease [21–23]. The increase in the number of faecal bifidobacteria was accompanied by a decrease in the proportion of clostridia and also by a decrease in stool pH. Increased stool frequency and a significant change towards softer stools were also noted in the prebiotic supplemented group. Increased frequency and softer stool consistency is an effect of the supplementation which is of practical importance because hard stools and obstipation are common problems in newborn infants. The increased proportion of bifidobacteria in the stools of the PBF group is in line with the results of previous studies that investigated a GOS-lcFOS prebiotic mixture [13,14,24–26].

With regards to the effect of a prebiotic formula on the numbers of pathogenic microorganisms, results of previous studies are conflicting with three studies showing no effect of oligosaccharide supplementation on the numbers of potentially pathogenic bacteria, [24,25,27] while three others reported reduced numbers of clinically relevant pathogens in the faecal flora [13,28,29]. With regards to faecal clostridia one previous study reported reduced proportions following the introduction of prebiotics [13] while one study reported no effect of prebiotic supplementation on the population of clostridia [24]. A reduction in the population of clostridia is beneficial not only because of their potential to cause intestinal infection but also because of their connection with atopic disease [30].

Stool pH was reduced with the GOS/lcFOS formula when compared to a formula without prebiotic supplementation, and was similar to the faecal pH found in breast-fed infants [14]. The similarity in stool pH pattern indicates that the studied prebiotic mixture stimulates not only the bifidobacteria but also the whole intestinal flora in the same direction.

| Table 3 Median (range) proportion of faecal bifidobacteria, clostridia and E. coli as a percentage of the total microorganisms |
|----------------|----------------|----------------|----------------|
| Microorganism | PBF            | STF            | P*             |
| Bifidobacteria|                |                |                |
| 0 weeks       | 5.36 (0.00–96.70) | 8.04 (0.00–94.96) | 0.782          |
| 6 weeks       | 39.69 (0.00–143.30) | 14.87 (0.00–101.00) | 0.262          |
| Clostridia    |                |                |                |
| 0 weeks       | 0 (0.00–67.04)  | 0 (0.00–29.29)  | 0.45           |
| 6 weeks       | 5.63 (0.00–88.51) | 0 (0.00–71.70)  | 0.595          |
| E. coli       |                |                |                |
| 0 weeks       | 4.06 (0.00–69.32) | 4.06 (0.00–59.31) | 0.312          |

* Mann–Whitney test.
as breast-fed infants [18]. Regarding the optimal dosage of the GOS-lcFOS mixture our data demonstrate that a concentration of 0.4 g/dl is bifidogenic. Previous studies have demonstrated that these effects of supplementation are more pronounced when a formula containing 0.8 g/dl of prebiotics is used [25]. Another study investigated the influence of the duration of supplementation on the numbers of bifidobacteria and it came to the conclusion that the numbers of bifidobacteria increase significantly with time [14]. In our study GOS represented the dominating oligosaccharides in the formula supplement. Also in human milk galactose represents a major component of the human milk oligosaccharides. On the basis of the present data we cannot answer the question to which extent the GOS or the FOs are responsible for the observed effects. However from the data in the literature a synergistic effect of both ingredients can be assumed. With regards to stool characteristics similar to our results previous studies found that oligosaccharides softened stool consistency and increased stool frequency without causing diarrhea [25,26]. This effect was dose dependent.

Regarding somatic growth, the present study did not demonstrate any significant effect of prebiotics on growth rate. Three previous studies reported on growth characteristics. Two of them examined growth from birth to the age of 1 month in preterm [14] and term [25] infants and found no significant group differences. In the third study [27] weight gain was higher in the study formula but only in girls and only during the first 6 weeks of the 12-week study period. Head circumference was greater after 12 weeks but again only in girls. Furthermore the sum of skinfold measurements was higher but only in boys. With regards to formula acceptability the mothers’ responses in the questionnaires indicated that the study formula was well accepted by their infants and that there was a low incidence of feeding-related problems. No adverse effects were reported during the study.

In conclusion this study demonstrated that a prebiotic supplemented infant formula was well accepted and tolerated by infants aged 0 months to 3 months and resulted in anthropometric growth data that are similar to that of infants fed by a regular starters formula. Infants fed this formula produced softer stools with a microflora having a significantly lower proportion of clostridia and a trend for higher proportion of bifidobacteria and lower proportion of E. coli as compared with infants given a standard infant formula. This latter difference may confer health advantages by providing some protection against gastrointestinal infections and allergy. These potential benefits should form the basis for further studies.

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References

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