

## Original Article

# Supplementation of infant formula with native inulin has a prebiotic effect in formula-fed babies

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**Objectives:** In this study we investigated the effects of native inulin in formula-fed babies. The influence of inulin on the microbial composition, pH, consistency and amount of faeces, and on frequency of defecation was assessed. **Methods:** In this study a daily dosage of 0.25 g/kg/d was used: 3 weeks of inulin consumption were followed by 3 weeks without or vice versa. The study group consisted of 14 babies with an average age of 12.6 weeks ( $\pm$  6.4 weeks) and the average intake of inulin was 1.5 ( $\pm$  0.3) g/d. **Results:** The consumption of inulin increased the content of *Bifidobacterium* and *Lactobacillus* in the faeces of formula-fed babies, without affecting the number of *Bacteroides* or the total anaerobic count. With inulin there was a trend for stools to become softer and for the amount of faeces to increase significantly. Frequency of defecation was not affected by the consumption of inulin. No adverse effects were reported during the periods of inulin consumption. **Conclusions:** We conclude that, with native inulin, a prebiotic effect can be observed in formula-fed babies. Inulin may therefore be a useful ingredient in infant formulae.

**Key Words:** inulin, babies, colonic microbiota, infant formula, prebiotic

## Introduction

Inulins are  $\beta$ - (2,1)-fructans that occur as a reserve carbohydrate in many plants.<sup>1</sup> As such we consume these components in moderate amounts with our daily diet. They are found in vegetables such as wheat, onions and leeks and in fruits such as bananas. Inulins are also available as a food ingredient and are used in a wide variety of food products. Many applications of inulins are based on the prebiotic features of these fructans: they are able to stimulate growth of bifidobacteria in the human colon, thereby possibly providing health benefits to the host.<sup>2</sup> This property may also be useful in baby food formulae, which serve to replace mother's milk.

Next to lactose (68 g/l), fat (37 g/l) and protein (10-12 g/l) human milk also contains 10 – 20 g/l of oligosaccharides. These oligosaccharides have a complex molecular structure and consist of a variety of sugars. Galactose, glucose, fucose, N-acetylglucosamine and sialic acid are the constituents of these oligosaccharides.<sup>3</sup> This rich diversity is unique to human milk. They are not digested in the babies' gastrointestinal tract<sup>4</sup> and a variety of functions are attributed to them, including prebiotic activity and anti-infective action.<sup>5</sup> Other factors such as the presence of lactoferrin and lysozyme and the low buffer capacity also play a role in the prebiotic properties of human breast milk.<sup>6</sup>

Distinctive differences in intestinal microbiota between breast fed babies and bottle fed ones have been reported<sup>7,8,9</sup>. *Bifidobacterium* and *Lactobacillus* are the main species found in breast-fed babies, while formula-fed babies have an adult-type flora with a predominance of *Streptococcus*, *Enterobacteriaceae* and *Bacteroides*. One of the purported

beneficial properties of *Bifidobacterium* and *Lactobacillus* is activation of the immune system<sup>10,11</sup> and it has been suggested that the fact that bottle fed babies' death rate and infection of contagious diseases is significantly higher than that of breast-fed babies<sup>12</sup> is related to this difference. Obviously these differences in microbial composition are caused by differences in the composition of human breast milk and formula milk.

In bovine milk the oligosaccharides described above are absent, meaning that baby formulae based on cow's milk lack these prebiotic properties. In order to improve the functionality of baby formulae attempts are made to improve the nutritional features by including prebiotic oligosaccharides<sup>e.g. 13,14,15</sup> or probiotic bacteria.<sup>e.g. 16</sup>

For the prebiotics applied so far much attention has been paid to oligosaccharides derived from sucrose and inulin (fructo-oligosaccharides<sup>17</sup>) or lactose (galacto-oligosaccharides), or mixtures thereof.<sup>13</sup> Native inulin (a natural polydisperse mixture of fructan polymers derived from chicory roots) has not been tested in baby formulae thus far. In this study we investigated the effects of inulin on the microbial composition of the faecal microbiota and stools of formula-fed babies.

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## Methods & Materials

### Subjects and experimental design

The subjects were 14 infants (male: 10, female: 4) living in the orphanage of the Civic Children's Hospital (Seoul, Korea) aged on average 12.4 weeks (see Table 1). All were bottle fed before the weaning period and in good health.

The subjects were randomly assigned to either Group 1 (7 subjects, 5 male, 2 female), which started with the inulin treatment, or Group 2 (7 subjects, 5 male, 2 female), which started with the control treatment (formula without native inulin). For the inulin treatment the subjects were fed milk formula containing inulin (0.25 g/kg/d) three times a day for three weeks; the control treatment consisted of the same formula without the inulin supplement. Appropriate amounts of inulin were mixed with the powder formula. After 3 weeks the treatment was switched; there was no washout period between the treatments. Faecal samples were collected as described below after 3 weeks of treatment and after 6 weeks of the reverse treatment.

Native inulin (Frutafit® IQ) was supplied by Sensus (Roosendaal, Netherlands). The composition of the powdered formula is given in Table 2.

The study protocol was approved by the Medical Ethical Committee of the hospital. The hospital received a financial compensation for contributing to this study.

### Analysis

#### Faeces collection

Faeces was collected from the subject's diaper just after a bowel movement, mixed with sterilized phosphate buffer (10 %, w/w, 0.2 M sodium phosphate, pH 7.0), frozen and stored until further analysis.

#### Analysis of microbial composition of faeces

The total numbers of anaerobic bacteria, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* were determined by plate counting. A sample of frozen faeces (0.5 g) was homogenized in 4.5ml sterilized 0.2 M sodium phosphate buffer, pH 7.0. This homogenate was further diluted as appropriate for inoculating the various media. BL agar (Difco C220-17, USA) was used for total anaerobic bacteria, BS agar medium (*Bifidus* selective agar medium) added to BL agar with antibiotic for *Bifidobacteria*, M-LBS agar medium (*Lactobacillus* selective agar medium), and NBGT agar medium for *Bacteroides*. The composition of the various media is found in detail elsewhere<sup>18</sup>. Each diluted solution was spread on the selective agar medium and cultivated anaerobically at 37 °C for 72 hours.

#### Examination of bowel parameters

The amount of faeces and frequency of defecation were recorded at a daily basis by the hospital nurses. The amount of the excreta was determined by weighing the diapers and subtracting the weight of an empty diaper. Consistency of the faeces was scored on a scale from 1-4, in which 1 was watery, 2 was muddy, 3 was clay-like and 4 was hard pellets. The records of the hospital also tracked data on the babies' health situation during the trial.

**Table 1.** Characteristics of the subjects

Parameter	Average (range)	SD
Age (wks)	12.6 (5-24)	6.4
Initial weight (g)	5006 (3750-6960)	1169
Final weight (g)	5926 (4630-7640)	1019
Weight gain (g)	9 21 (170-1920)	604
Growth (g/wk)	153 (28-320)	101
Inulin intake (g/d)	1.5 (1.0-1.75)	0.3

The average data and standard deviation were calculated for all 14 subjects. Final weight and weight gain were measured after 6 weeks.

**Table 2.** Composition of baby food formula.

Component	Per 100 g
Fat (g)	27.0
Linoleic acid (g)	3.5
$\gamma$ -linolenic acid (mg)	14.0
DHA (mg)	70.0
Arachidonic acid (mg)	22.0
Protein (g)	12.2
Carbohydrate (g)	56.0
Oligosaccharides (g)†	1.5
Minerals (g)	1.52
Vitamin mix (mg)‡	94.4
Others components (mg)§	805.0
Energy content (kcal)	516.0

† A mixture of raffinose, galactooligosaccharide and fructooligosaccharide was used (each 500 mg per 100 g); ‡ Vitamin A (1700 IU), vitamin B1 (0.3 mg), vitamin B2 (0.6 mg), vitamin B6 (0.3 mg), vitamin B12 (2 µg), vitamin C (50 mg), vitamin D3 (380 IU), vitamin E (6 IU), nicotinic amide (5 mg), folic acid (100 µg), biotin (20 µg), pantothenic acid (3 mg), inositol (35 mg) and  $\beta$ -carotene (60 µg); § L-arginine (480 mg), L-cysteine (200 mg), taurine (30 mg), lactoferrin (80 mg), nucleotide (15 mg)

#### Statistical analysis

Mean and standard deviation were calculated for all variables. A t-test was used to determine statistical significance (two-sided;  $p < 0.05$ ) between the treatments. The frequency and consistency of bowel movements were analyzed by the chi-square test.

## Results

### Growth

Table 1 shows the overall characteristics of the babies. No differences in growth were observed between the periods of inulin-treatment and the periods without inulin: average weight increase with inulin was 509 ( $\pm$  372) g in 3 weeks, whereas without inulin this increase was 411 ( $\pm$  394) g in 3 weeks ( $p=0.5051$ ). Also there were no adverse effects (like diarrhoea) observed during the control and inulin treatments.

### Microbial composition of faeces

The microbial composition of the faeces with and without inulin treatment is shown in Table 3. The content of total anaerobic bacteria and *Bacteroides* did not differ between the two treatments. A trend was found for the total number anaerobic bacteria increasing with inulin treatment ( $p=0.0618$ ) and for *Bacteroides* no significance was reached ( $p=0.5224$ ). A significant increase in *Bifido bacterium* sp. was found when the numbers of control treatment and inulin-treatment were compared ( $p=0.0163$ ).

**Table 3.** Microbial composition of faeces

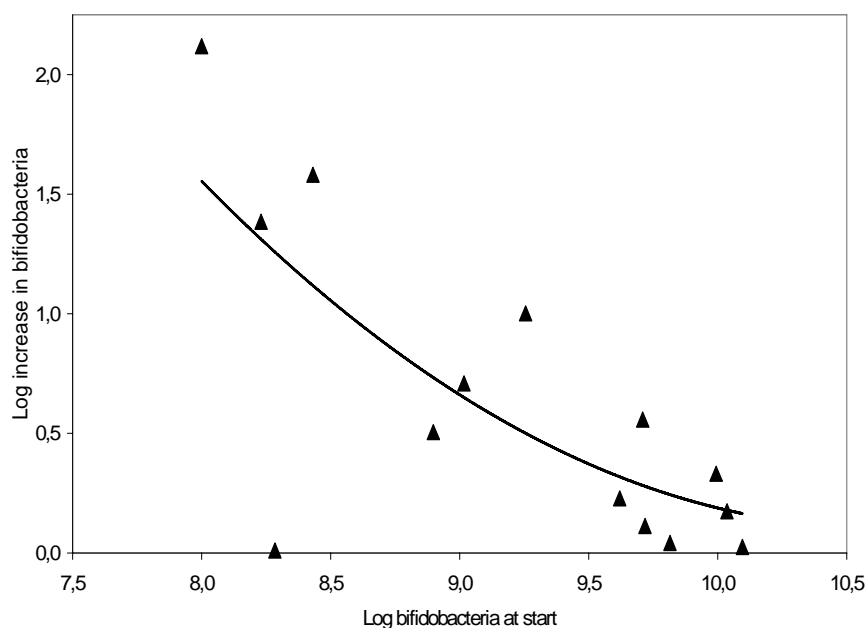
Bacteria	Inulin treatment	Control treatment
Total anaerobes	10.58 (0.224)	10.27 (0.344)
<i>Bacteroides</i>	9.51 (0.389)	9.40 (0.344)
<i>Bifidobacterium</i>	9.85 (0.523) *	9.22 (0.741)
<i>Lactobacillus</i>	9.09 (0.377) *	8.61 (0.741)

All data are expressed in log colony forming units per g of faecal matter as the average of 14 subjects with the standard deviation in parentheses. Data with \* in the same row are significantly different ( $p < 0.05$ ).

**Table 4.** Stool parameters

Parameter	Inulin treatment	Control treatment
pH	6.31 (0.34)	6.51 (0.49)
Consistency	1.8 (2.7)	2.4 (4.2)
Amount (g)	167 (80) **	75 (30)
Frequency	1.6 (2.4)	1.2 (1.6)

All data are given as the average of 14 measurements with the standard deviation in parentheses. Faecal pH and amount (in g) were measured as described, consistency was scored on a scale from 1-4 (watery to hard). Data with \*\* in the same row are significantly different ( $p < 0.01$ ).



**Figure 1.** Increase in *Bifidobacterium* sp. in formula-fed babies as a function of the original number of *Bifidobacterium* sp. The faecal content of *Bifidobacterium* sp. was determined without and with inulin supplementation of the formula, and the logarithmic increase was calculated.

When the individual results were plotted against the original value of bifidobacteria, it was observed that, when the starting value was lower, a higher increase was found (Fig 1). It is also noteworthy that two subjects showed no response during inulin treatment (Fig 1).

For *Lactobacillus* sp. a significant increase during inulin-treatment was found ( $p = 0.020$ ).

### Effects on stools

Despite the large variations in the various parameters of bowel habit (as shown by the large standard deviations; see Table 4) some differences and trends could be discerned. The pH of the faeces decreased slightly during inulin treatment (from  $6.51 \pm 0.49$  to  $6.31 \pm 0.34$ ), but this decrease did not reach statistical significance ( $p = 0.2206$ ).

Defecation frequency was increased slightly compared to control, from  $1.2 (\pm 1.6)$  times per day to  $1.6 (\pm 2.4)$  times per day during inulin treatment ( $p = 0.1900$ ), whereas faecal consistency became softer following 3 weeks of inulin-treatment. A trend towards a decreased consistency was found: it changed from an average  $2.4 (\pm 4.2)$  to  $1.8 (\pm 2.7; p = 0.0584)$ . We found an increase in the amount of

faeces: during inulin treatment the amount was  $167 (\pm 80)$  g versus  $75 (\pm 30)$  g during the control ( $p = 0.0088$ ). The magnitude of the changes in stool parameters was not correlated with the magnitude of the changes in microbial composition of the faeces; for instance, changes in faecal consistency as described above were not correlated with the change in content of bifidobacteria or lactobacilli (data not shown).

### Discussion

This study shows that supplementation with native inulin of a standard infant formula containing oligosaccharides has a prebiotic effect in formula-fed Korean babies: an increase of *Bifidobacterium* sp. and *Lactobacillus* sp. was found. At the same time there was no increase in *Bacteroides* sp. or in total anaerobic count. Plate counting techniques were used to assess changes in microbial composition of the faeces. Despite the potential shortcomings of these techniques the data clearly show prebiotic changes in this composition. The numbers of *Bifidobacterium*, *Lactobacillus* and *Bacteroides* which are found with other techniques (e.g. FISH or DGGE <sup>e.g. 19</sup>) do not deviate from

what we find in this study. In the study by Favier et al.<sup>20</sup> these genera were identified by molecular methods as the major constituents of an infant's faecal microbiota. Moreover, Boehm et al.<sup>21</sup> using plate counting, report similar numbers in microbial composition of the faecal flora of formula-fed babies.

The changes in microbial composition were accompanied by changes in faecal consistency and amount. Only the latter reached statistical significance, but with the trend observed for consistency the data are indications that inulin leads to enhanced fermentation in the colon. In this respect our data are not essentially different from the data found in adults.<sup>22,23,24</sup>

The dosage at which these effects were observed in babies seems lower than published for effects in adults. When compared on a body weight basis however, the difference is much less or even absent. For native inulin prebiotic effects in adults have been reported in the range from 0.2 g/kg/d<sup>22</sup> to 0.5 g/kg/d.<sup>23</sup> The dosage applied in our study (0.25 g/kg/d) is well within this range.

On the other hand, when compared with the dosages used by others in studies with babies, the dosage in the present study seems low. For instance, in their study with preterm infants Boehm et al.<sup>15,21</sup> used about 1 g/kg/d of the oligosaccharide mixture (a mixture of short chain galacto-oligosaccharide and long chain inulin, 9:1, w/w). Similar dosages were applied in other studies.<sup>13,15</sup> Low dosages of fructooligosaccharides were without effect on the faecal microbial composition.<sup>17</sup> An explanation may be that the subjects in our study already were being bottle-fed for a considerable time on a formula containing oligosaccharides. The total daily dosage is therefore higher than from the addition of the native inulin alone. It may also be that the chain length distribution of native inulin was suitable for a prebiotic effect. Boehm et al.<sup>21</sup> have shown that fructooligosaccharides alone do not exhibit a bifidogenic response<sup>17</sup> whereas a mixture of these oligosaccharides with long chain inulin does have such an effect. They suggested that the mixture has a chain length distribution more or less equal to that of the oligosaccharides in mother's milk. In line with this we suggest that the chain length distribution present in native inulin with a degree of polymerization ranging from 3 to 60 also favours a bifidogenic effect.

The dosage is also low when compared to mother's milk, which contains 10-20 g/l of oligosaccharides. Not all of these carbohydrates show bifidogenic properties; probably only the low molecular weight saccharides are responsible for this effect.<sup>25</sup> This implies that the daily prebiotic dosage obtained from mother's milk is lower than expected from the concentration alone.

The type of dose response curve (a higher bifidogenic response with a lower starting value of *Bifidobacterium* sp.) has also been reported for adults.<sup>26,27</sup> In adults it is also not uncommon to find individuals who do not respond to inulin consumption in terms of changes in faecal microbial composition.<sup>28</sup> Taken together it follows from our data that in formula-fed babies native inulin behaves as a prebiotic in much the same way as in the adult population.

Next to the bifidogenic effect we also found a clear positive effect on the faecal *Lactobacillus* content. For

adults significant changes in this genus have not been reported<sup>28,29</sup>, but in infants Boehm et al.<sup>21</sup> and Moro et al.<sup>13</sup> showed increases in *Lactobacillus* sp. similar to the present study. It may well be that the longer fructan chains as present in native inulin are responsible for this stimulatory effect on *Lactobacillus*.<sup>30</sup>

Both the increase in *Bifidobacterium* and *Lactobacillus* could have health benefits. With regard to the difference in *Bifidobacterium* composition in allergic and healthy infants<sup>31</sup> it could be interesting to study the effects of native inulin on the allergic response of infants. There are already indications that inulins may affect the immune system in a positive way.<sup>30,32</sup> Inulins may stimulate mineral absorption in infants just as they do in adults.<sup>33</sup>

In summary we conclude that the addition of native inulin to baby formula elicits a prebiotic response in formula-fed babies. The significant increase in stool amount and the trend for softer stool consistency due to inulin consumption may offer benefits to infants that suffer from constipation or hard stools. Native inulin may therefore help improve the babies' health. Further research is required to show the health benefits.

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Original Article

## Supplementation of infant formula with native inulin has a prebiotic effect in formula-fed babies

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### 在婴儿配方奶粉中补充天然菊粉能有效为婴儿带来益生原作用

主旨：在本课题里，我们调查了天然菊粉在食用婴儿配方奶粉的婴儿中的作用。主要针对菊粉对微生物组分，pH，排粪量，粪便密度以及排粪次数进行评估。

方法：在本研究中，我们使用的剂量为 0.25 克/公斤/日：三星期连续摄入菊粉紧跟随三星期空白或者相反过来。以 14 名平均年龄为 12.6 星期(±6.4 星期)婴儿组成，平均菊粉摄取量为 1.5 克/日。

结果：菊粉的摄入在不影响粪便中细菌总数或厌氧菌总数的情况下，增加了粪便中双歧杆菌及乳酸菌的含量比例。随着菊粉的摄取，婴儿粪便的分量明显增加，硬度降低。排便次数不受菊粉摄取的影响，在期间，并没有发现摄取菊粉有任何不良反应。

结论：菊粉的摄入能为婴儿带来明显的益生原作用，菊粉能作为婴儿配方奶粉中有效的功能性配料。

關鍵字：菊粉、嬰兒、結腸菌落、嬰兒配方、益生菌。