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Short Communication

Effect of oligofructose supplementation on gut microflora and well-being in young children attending a day care centre☆

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Abstract

The effect of daily administration of oligofructose (OF) on 7–19 months old healthy children intestinal microflora, intestinal tolerance and well-being was assessed in a double blind placebo controlled study. The study comprised 8 days of observation, 21 days of supplementation, and 15 days of post-supplementation. Exclusion criteria included antibiotic use and intake of other prebiotic and probiotic at any time following enrolment. Faecal flora was analysed by culture methods, and health information was recorded daily. Bifidobacteria, tended to slightly increase with OF supplementation, but not with placebo ($p=0.095$). Simultaneously, a decrease in potential pathogens, significant for clostridia ($p=0.05$) but not for staphylococci ($p=0.09$) was observed in the OF group. These modifications did not persist during the post-supplementation period. OF supplementation were accompanied by less flatulence, diarrhoea, vomiting ($p<0.001$), and fever ($p<0.05$) events.

Keywords: Oligofructose; Intestinal microbiota; Tolerance; Children; Placebo-controlled trial

1. Introduction

The intestinal microbiota, harbouring more than 400 species, performs important metabolic and immunological functions and acts as a biological barrier against pathogens. Its composition has been found to vary during life. Initially sterile, the gastrointestinal tract (GIT) of newborn is rapidly colonized by bacteria from both the vaginal and faecal maternal floras and the surrounding environment through a complex process. During the two first years of life, upon introduction of solid food, a stable population of microflora becomes established, in which anaerobes predominate. During this period, a child is vulnerable to gastro-intestinal and other disorders, especially in day-care centres where infectious diseases are very frequent.

Dietary modulation of the gut microbiota by probiotics and prebiotics is an important feature in nutritional sciences. Belonging to current functional foods, prebiotics are defined as non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon and are non-digestible oligosaccharides (Aggett et al., 2003; Gibson and Roberfroid, 1995; Roberfroid, 1999). Among oligosaccharides, inulin and oligofructose (OF) have been shown to inhibit growth of pathogens, and to display clear bifidogenic properties. This has been demonstrated *in vitro* and *in vivo*, in animals and in healthy adults (for review see Kolida et al., 2002). Several studies have reported effects of infant formula supplemented with a mixture of oligosaccharides (combination of galacto-oligosaccharides and inulin) (for a review see Boehm et al., 2004; Haarman and Knol, 2005), but very few studies concerned the effects of the solely supplementation of OF in infants (Euler et al., 2005) and in young

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children during and just after the weaning period (Duggan et al., 2003; Moore et al., 2003; Saavedra and Tschernia, 2002). Moreover, in the latter, the impact of solely OF supplementation on intestinal microflora was not studied.

Thus, we aimed to investigate, through a randomised placebo-controlled and double-blind trial, the effects of a daily administration of oligofructose on young children intestinal microflora. Besides, we evaluated well-being and intestinal tolerance of oligofructose.

2. Methods

2.1. Subjects

This study was a double-blind placebo-controlled and was approved by the human subjects review committee (CCPPRB) of Saint-Germain-en-Laye (France) and written parental consent. Healthy full term children, with a normal birth weight, aged between 6 and 24 months and attending day-nurseries in Paris (France) were included. Clinical parameters such as age, type of feeding during the first weeks of life, allergic diseases and anthropometric values were based on the health record, the paediatrician's inclusion examination, and the parent's recall. Exclusion criteria were as follows: breast-feeding within the previous month before the beginning of the study, antibiotic treatments and diarrhoea within the previous 8 days, chronic gastrointestinal diseases, malabsorption, iron supplementation, intake of anti-secretory, anti-acid or anti-reflux medication, treatment with molecules active on gastrointestinal motility and laxatives, antibiotic treatments and intake of other prebiotics and/or probiotics (see below). Any other medication along the study was recorded in diaries.

2.2. Diet and experimental design

It was a 3 period-study including 8 days of observation without supplementation, followed by 21 days of supplementation by either 2 g/day of an oligofructose (OF), i.e. Beneo® P95 supplied by Orafti (Tienen, Belgium) (OF group), or 2 g/day of placebo, i.e. maltodextrin (control group) in one single daily administration. Both OF and maltodextrin were in powder form and packaged identically except for product code number. Subjects were randomly assigned to 1 of the 2 groups in a double-blind manner. Then, a 15 day-period of run-out observation followed the supplementation period (post-supplementation period). OF or placebo were added to food (cereals...) or drinks and taken by the young children at the day-nursery or at home. Intake of other specific prebiotics, probiotics (e.g. *Lactobacillus* and/or *Bifidobacterium*), and fermented milks were not allowed during the whole study. A list of foods to avoid was provided to each parent. Usual diet and beverage intakes were not modified during the trial period.

Stools for bacteriological analysis were collected at 4 periods: (i) at the end of the observation period (D8), (ii) in the middle of the supplementation period (D18 ± 2 days), (iii) at the end of the supplementation period (D28 ± 2 days), (iv) at the end of the post-supplementation period (D42). They were collected in sterile containers, immediately placed in an anaerobic atmosphere, and transfer to the laboratory for bacterial analyses at 4 °C, within the two following hours.

2.3. Analysis of the faecal flora

Qualitative and quantitative analysis of the faecal flora was performed on the fresh stools as previously described (Butel et al., 1998). Briefly, dilutions of stools in a pre-reduced peptone liquid medium were spread on various media allowing the isolation and quantification of the main genera, i.e. staphylococci, enterococci, enterobacteria, lactobacilli for aerobic genera, and *Bacteroides*, *Clostridium* and *Bifidobacterium* for anaerobic genera. In particular, lactobacilli were isolated on Rogosa agar, and bifidobacteria on Wilkins Chalgren agar base containing D-glucose 10 g/l, kanamycin 7.5 mg/l, L-cysteine 0.5 g/l and Tween 80 (0.5% v/v). All media for anaerobic bacteria and Rogosa agar were incubated at 37 °C in an anaerobic chamber up to 5 days (duScientific, AES laboratories, France). Furthermore, enteropathogens were searched for, i.e. *Campylobacter* on Karmali agar, *Salmonella* on Hektoen agar, and *Clostridium difficile* on CCFA agar. Rotavirus and adenovirus were detected by ELISA tests (IDEIA adenovirus and IDEIA rotavirus, Dakocytomation, Trappes, France). Bacterial identifications were performed using routine laboratory methods and, for anaerobic bacteria, according to Wadsworth laboratory procedures (Jousimies-Somer et al., 2002). Bifidobacteria identification was performed according to the morphologic characteristics, the presence of F6PPK, and PCR amplification with 16S rDNA-gene targeted genus-specific primers adapted from Kok et al. (1996). Bacterial counts were expressed as the $\log_{10}$ CFU/g of faeces and the count threshold was 3 $\log_{10}$ CFU/g of faeces.

2.4. Tolerance and well-being

For each infant, parents or care-givers from the day-nursery daily evaluated the gastrointestinal symptoms and general health information. Recorded data were separated into three groups: (i) number and aspect of stools (i.e. hard, normally form, soft, watery), diarrhoea (*i.e.* at least three liquid stools per day), constipation; (ii) abdominal distension, abdominal pain, flatus, vomiting, regurgitation, decreased appetite, feeding refusal (anorexia and spitting), and fever; (iii) use of medication, occurrence of illnesses, growing teeth, or any unusual signs or symptoms perceived during the course of the study. For the assessment of (i) and (ii), parents or care-givers reported if the events occurred "less than usual", "as usual", "more than usual" or "much more than usual". Severe diarrhoea, abdominal pain and vomiting episodes led to the exclusion of the infant from the study. Slight gastrointestinal disorders, flatus, decreased appetite, and regurgitations were considered as signs of discomfort, not implicating to suspend the supplementation, except if the parents asked for it.

2.5. Statistical analysis

Data from children with major deviations and/or biological missing data before and during the whole supplementation period were excluded. Major protocol deviations were determined in blinded way. Modifications of the flora were investigated separately for each group using the Wilcoxon two-sample paired signed rank test at the middle of the supplementation period (between D8 and D18), at the end of the supplementation period (between D8 and D28) and at the end of the observation period (between D8 and D42). Differences between the two groups were tested with the Mann–Whitney rank sum test at the middle (D18) and the end of the supplementation period (D28) and at the end of the second observation period (D42). Simple linear regression was performed when appropriate. Fisher exact test was used to evaluate tolerance and occurrence of side effects between the two groups. All statistical computations were performed using version 5.0 of the StatView package (SAS Institute Inc.). Differences were considered as significant for $p \leq 0.05$.

3. Results and discussion

Our study focused on children 6 to 24 months old attending day-care centres, a target population known for a high risk of common infectious diseases. During our study, common infections (pharyngitis, otitis, bronchitis...) and antibiotics uses were so frequent that only 20 children out of 35 included went to a complete end of the study (57%), all exclusions being due to antibiotic prescriptions. After the beginning of the supplementation, all children from the OF group completed the study versus 5 out of 15 controls. Thus, children who provided at least 3 stool samples and no antibiotic treatment during the 6 weeks of the study, consisted of 10 in the OF group and 10 in the control one. The characteristics of the children, were similar between the two groups: median age ± S.D. was 14.2 ± 3.5 for the OF group and 12.6 ± 3.4 for the control group. Eight children in the OF group and 7 in the control group were breastfed at birth and breast feeding lasted less than 5 months except for one, belonging to the OF group, who was breast-fed during 10 months. Only 3 children in the OF group and 2 in the control group missed one or two intake(s) during the three weeks of supplementation.

3.1. Faecal flora composition

The number of colonized children and the mean level of colonization for each bacterial group are summarized in Table 1. Modifications in bacterial levels between the beginning (D8) and the end (D28) of the supplementation are compared in Fig. 1.

In terms of aerobic flora, few children were colonized with *Staphylococcus* sp., whereas all, except one, were colonized at high level with enterococci and enterobacteria. Concerning the anaerobic flora, all children were colonized with high levels of *Bacteroides*, *Clostridium* and *Bifidobacterium* and rarely with *Eubacterium*, the latter being detected only at dominant levels because of lack of a selective medium. Lactobacilli were seldom identified. This corresponds with the observation that lactobacilli form unstable transient populations in the infants' intestinal flora (Stark and Lee, 1982). Neither bacterial enteropathogen nor rotavirus or adenovirus was isolated.

The intestinal microflora changed slightly all over the study, but only in the OF-supplemented group.

In the OF group, bifidobacterial levels increased at the end of the supplementation period, tending to differ from the control group ($p = 0.095$), even though initial levels were similar. The mean level in bifidobacteria rose in the OF group from 9.1 (D8) to 9.5 (D28) $\log_{10}$ CFU/g of faeces, and slightly decreased in the control group from 9.2 to 9.0 $\log_{10}$ CFU/g of faeces. Similar results were obtained in full term infant, after a 5 week feeding, with an OF supplemented formula (1.5 and 3 g/L) by Euler et al. (2005) who reported minimal effect on fecal flora. However bifidobacterial counts in OF supplemented

Table 1
Level of colonization and number of colonized infants in the OF and control groups

	OF group				Control group			
Day of sampling	D8	D18	D28	D42	D8	D18	D28	D42
Nb of infants	10	10	10	9	12	12	10	10
Aerobes								
Staphylococci	4.3 ± 0.6 (3) [a]	4.3 ± 0.5 (3)	4.6 [b] (1) [c]	4.4–4.9 [b] (2)	4.4 ± 0.7 (4)	4.7 ± 1.5 (6)	5.8 ± 1.5 (5)	4.5 ± 0.7 (3)
Enterococci	6.8 ± 0.8 (10)	6.7 ± 1.0 (10)	6.6 ± 1.5 (10)	6.7 ± 1.1 (9)	6.7 ± 1.4 (12)	6.9 ± 1.1 (11)	6.4 ± 1.7 (9)	6.1 ± 1.5 (10)
Enterobacteria	7.9 ± 1.0 (9)	6.5 ± 1.7 (10)	7.2 ± 1.6 (10)	6.8 ± 0.8 (8) [d]	7.7 ± 1.4 (11)	7.2 ± 1.2 (12)	7.4 ± 1.2 (10)	7.7 ± 1.3 (9)
Lactobacillus	7.6 [b] (1)	ND [e]	8.3 [b] (1)	ND	ND	ND	ND	ND
Anaerobes								
Bacteroides	8.4 ± 1.6 (10)	8.4 ± 1.2 (9)	8.6 ± 1.0 (9)	8.0 ± 1.5 (8)	8.3 ± 1.7 (12)	8.2 ± 0.6 (11)	8.4 ± 1.4 (10)	8.4 ± 0.9 (10)
Clostridium	7.6 ± 1.8 (10)	6.7 ± 1.4 (10) [d]	7.2 ± 1.8 (10) [c]	7.3 ± 1.3 (9)	7.0 ± 1.8 (12)	7.4 ± 1.9 (12)	7.2 ± 1.5 (10)	7.5 ± 1.5 (10)
Bifidobacterium	9.1 ± 0.9 (10)	9.2 ± 0.8 (10)	9.5 ± 0.8 (10) [c]	8.7 ± 0.7 (9)	9.2 ± 0.7 (12)	9.1 ± 0.8 (12)	9.0 ± 0.7 (10)	9.0 ± 0.6 (10)
Yeast	4.1 [b] (1)	4.1 [b] (1)	3.1–4.1 [b] (2)	3.3–5.2 [b] (2)	3.4–4.1 [b] (2)	3.8 ± 0.5 (3)	4.2 ± 0.6 (4)	3.7 ± 0.7 (4)

[a] Bacterial counts in colonized infants, expressed as mean ± SD $\log_{10}$ CFU/g, number of colonized infants in brackets.
[b] Individual values.
[c] Different from the control group at the same time with $p \leq 0.1$.
[d] Significantly different from the control group at the same time ($p \leq 0.05$).
[e] ND = not detected, the threshold count was 3 $\log_{10}$ CFU/g.

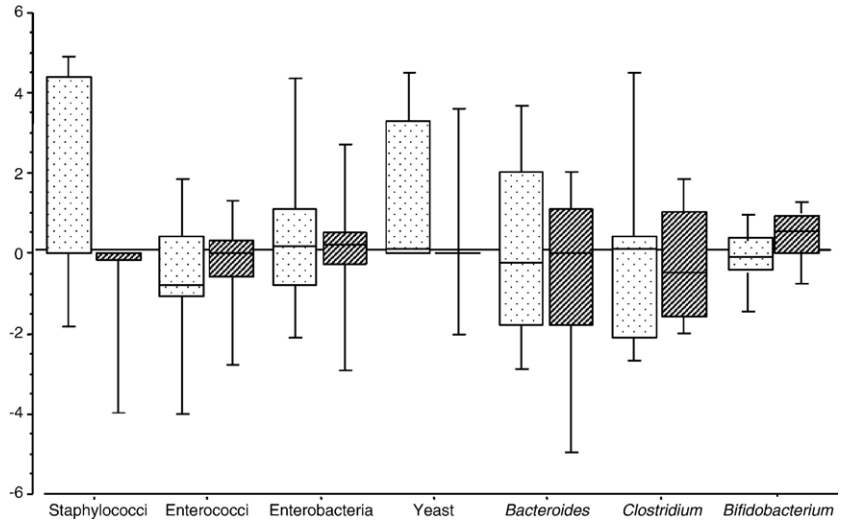

Fig. 1. Difference in bacterial level between D28 and D8 (log_{10}CFU/g), control group and OF group. The boxplot shows the median (central horizontal line), the 25th centile (lower box border), and the 75th centile (upper box border). The lower and upper horizontal lines refer to the 10th and the 90th centile, respectively.

infants were as high as in breast-fed ones (mean level 9.1 CFU/g of faeces). Initial levels of bifidobacteria were similarly high in both groups at the beginning of the study (ca 9 log_{10} CFU/g). Such, high bifidobacterial levels are quite usual for children of this age group (Stark and Lee, 1982). These high initial levels may explain the relatively small additional increase in bifidobacterial levels with OF supplementation. In healthy human adults, who have lower levels of bifidobacteria, OF supplementation (5 g, 8 g or 15 g/day) increased bifidobacterial levels from 8.8 to 9.5 log_{10}CFU/g of faeces (Gibson et al., 1995). In our study, OF supplementation at a dose of 2 g/day led to the same high bifidobacterial level, *i.e.* 9.5 log_{10} CFU/g of faeces. Moreover, several studies in adults (Bouhnik et al., 1999; Garleb et al., 1996; Tuohy et al., 2001) and in experimental models (Catala et al., 1999; Howard et al., 1995) have shown that the bifidogenic effect of OF is dependent on the initial level of bifidobacteria: the greatest increase in bifidobacterial levels correlates with a low initial population level. In the present study, both groups exhibited a trend towards a higher increase in bifidobacterial level when initial levels are lower (correlation coefficients: $r=-0.6$ with OF, $r=-0.7$ for controls). Indeed, wide individual variations probably explain no statistical significance (Fig. 2). However, the number of children with an increase in bifidobacterial levels at the end of the supplementation period was significantly higher in the OF group than in the control group. In fact, in the OF group, the bifidobacterial level increased in 7/10 children, above 0.5 log_{10} CFU/g in 5/10, was stable in 1 and decreased in 2 (Fig. 2). In controls, the bifidobacterial level increased in only 3 children ($p=0.05$ vs. the OF group), always remaining under 0.5 log_{10} CFU/g, and decreased in the other 7. Modifications in bifidobacterial level did not persist after the run-out observation period without supplementation (D42). A similar return close to the initial bifidobacterial level was observed in animal models (Campbell et al., 1997; Le Blay et al., 1999) and in human (Rao, 2001) in the same 2 weeks delay. It is logical to hypothesize that the population of bifidobacteria increases in the presence of substrate, *e.g.* OF, which is moreover not metabolised by all other bacterial groups (Langlands et al., 2004), and then decreases when this energy source is no longer available.

Simultaneously with the trend to increase in bifidobacterial populations, we observed a decrease in staphylococci and clostridia levels, significant for the latter. Staphylococcal colonization tended to be lower (number of colonized children and colonization level) in the OF group compared to controls ($p=0.09$), the differences being kept during the whole OF supplementation period, but not following the run out observation period. Clostridial level decreased during OF supplementation as compared to initial levels ($p=0.06$), with a significant difference from controls at D18 ($p=0.05$).

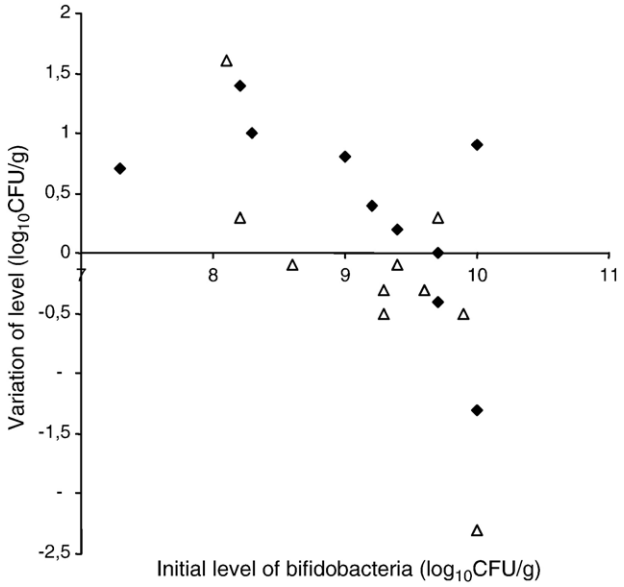

Fig. 2. Variation in *Bifidobacterium* level after supplementation (at D28) with regard to the initial level (at D8), △ control group; ♦ OF group.

Bifidobacteria are thought to lower the intestinal pH through their fermentation end-products, *i.e.* acetic and lactic acids, leading to the inhibition of the growth of potentially pathogenic bacteria. OF was previously demonstrated to lower *Clostridium* sp. and *E. coli in vitro* (Gibson and Wang, 1994; Wang and Gibson, 1993) and *in vivo* in humans (Gibson et al., 1995), as well as to restore the ecological balance in animal models (Campbell et al., 1997). Likewise, OF was shown to decrease both the occurrence and the severity of cecal lesions in an animal model of neonatal necrotising enterocolitis through the decrease in pathogenic clostridial populations linked to an increase in bifidobacterial levels or through a direct inhibiting effect (Butel et al., 2002).

3.2. Tolerance and well-being

The whole study comprised 6730 observations over 840 days (42 days/child). No intolerance phenomenon induced any exclusion from the protocol. There were no adverse events. Treatments for minor events were similar in the two groups and consisted of anti-pyretics, fluidifiants, cough syrups, and/or homeopathy drugs. A few studies already demonstrated tolerance of OF supplementation in same aged children at doses up to 3 g/days (Moore et al., 2003; Saavedra and Tschernia, 2002) and clinical benefits with an impact on febrile illness, diarrhoea and upper respiratory illness (Saavedra and Tschernia, 2002), but none of these studies deal with intestinal microflora. In our study, the intake of OF at a daily dose of 2 g was well tolerated by the children: no significant side effects were observed during the whole study. Feeding refusal, abdominal pain and frequency and consistency of the stools were similar between the two groups ($p>0.05$). All subjects exhibited normal growth during the study. Moreover, our results confirm that OF exerts beneficial effects to health and well-being. Indeed, the number of infectious diseases requiring antibiotic treatment, the number of episodes of flatulence, diarrhoea, vomiting and fever were significantly lower in the OF group as compared to the control group ($p<0.001$, $p<0.05$ for fever) as well as the number of children who start to vomit ($p<0.05$). The decrease in flatulence observed with OF supplementation might be linked to the decrease in clostridia, which are gas-producing bacteria.

To conclude, our study is the first one to evaluate the effects of OF supplementation on modulation of microflora with tolerance and well-being in young children attending day-care centres. The results of this study indicate good tolerance and clinical benefits of OF supplementation. Despite the slight modulation of intestinal microflora, a link between clinical benefits and flora's modification cannot be excluded. Further studies on a larger number of children are needed.

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