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PREBIOTIC EFFECT OF DAILY FRUCTOOLIGOSACCHARIDE INTAKE ON WEIGHT GAIN AND REDUCTION OF ACUTE DIARRHEA AMONG CHILDREN IN A BANGLADESH URBAN SLUM: A Randomized Double-masked Placebo-controlled Study

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Abstract: Fructooligosaccharide (FOS) is a typical prebiotic agent. A randomized, double-masked, placebo-controlled study was performed to evaluate the prebiotic effect of daily intake of an isotonic solution containing FOS on body weight gain and the reduction of diarrhea in children in an urban slum in Bangladesh over six consecutive months. We enrolled a total of 150 children, aged 25-59 months. Sixty-four children in the FOS group received 50 mL of isotonic solution with 2 g of FOS added, and 69 children in the placebo group were given an identical solution with 1 g of glucose added, once a day. The measurement of body weight was carried out every other day; height and arm circumference were measured once a month; and the children's mothers were interviewed to obtain data about diarrhea, the consistency and constitution of stool, other symptoms, and antibiotic treatment. As a result, the body weight gain during the six-month period was 0.86±0.55 kg in the FOS group and 0.89±0.48 kg in the placebo group, while the increase in height and arm circumference were not significantly different between the two groups. The number of diarrhea episodes during the six-month period was not significantly different. A significant reduction in the duration of diarrhea days and of duration per episode was observed in the FOS group (p =0.039 and p=0.008, respectively). In conclusion, daily intake of FOS was associated neither with the children's growth nor with the number of diarrhea episodes, but a significant reduction in the duration of diarrhea days was observed. Further studies are needed to confirm the effects of FOS by changing the doses and eliminating the influence of antibiotics.

Keywords: prebiotic effect, fructooligosaccharide (FOS), weight gain, diarrhea, RCT

INTRODUCTION

Diarrhea is a severe health problem that leads to mortality among children in developing countries. Clinical studies have clarified that some nondigestible carbohydrates with a molecular weight of more than 20,000 can alleviate diarrhea [1-8]. Fructooligosaccharide (FOS) is a mixture of 1-kestose (GF2), 28%; nystose (GF3), 60%; and β-fructofranosyl nystose (GF4), 12%; [9, 10]. It is completely safe [7, 9], has a sweet taste, and is easy to dissolve due to its small molecular weight (approximately 680). Prebiotics have already been identified as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or more bacteria in the colon [11, 12].

FOS is a typical nondigestible oligosaccharide and prebiotic agent. It is not hydrolyzed by any intestinal enzymes, is well fermented in the large intestine in human studies [13, 14], and improves the intestinal microflora in such a way that it becomes difficult for pathogenic microbes to proliferate in the human gastrointestinal tract [15-20]. The fermentation brings probiotic effects *in vitro* [21, 22], *in vivo* [21, 23], and also in humans [21, 24]. Using [U-^{14}C] FOS Oku, et al. have already shown that more than 99% of FOS is metabolized through fermentation by intestinal microbes [10, 25, 26]. The final products of fermentation are gases and short chain fatty acids such as acetate, propionate, and *n*-butyrate [10, 25, 27, 28]. Through the process of fermentation, harmful products such as ammonia, phenol, skatole, and indole are reduced due to the reduction of the en-

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zyme activity needed to synthesize these products [29, 30].

Furthermore, studies using animals and humans to determine the interaction of FOS supplementation with gastrointestinal function [31, 32], growth [29], immune response [33, 34], diarrhea in pigs [35], and traveler's diarrhea [36] have been assessed. However, the effect of FOS intake on the reduction of diarrhea and growth in children in the community has not been clarified [37]. Our hypothesis is that the daily intake of FOS-supplemented commercially developed isotonic solution improves the intestinal microflora and reduces diarrhea, thereby inducing an improvement in body weight gain in children. To assess the prebiotic effect on body weight gain and on the reduction of diarrhea, we performed a randomized, double-masked, placebo-controlled study for six consecutive months with children in an urban slum community of Bangladesh.

SUBJECTS AND METHODS

Study Population

The trial took place in Mirpur, an urban slum community with 800 households in the city of Dhaka, Bangladesh between December 2004 and June 2005. Mirpur is densely populated and has poor sanitary and hygienic conditions. Diarrhea is known as a serious health problem among children in the slums of Dhaka. The International Center for Diarrhea Diseases Research (ICDDR, B) has established a demographic surveillance system in this area, and a sampling frame was available. Children aged 25-59 months and their mothers were recruited randomly based on the demographic database prepared by ICDDR, B. Children who were being breast-fed, those who suffered from chronic diarrhea or malnutrition (weight-for-height z-score <-2), and/or those who were receiving antibiotic therapy were excluded.

A total of 158 children were enrolled, but 8 out of 158 did not participate. Therefore, 150 children were randomly assigned to each of two groups, the prebiotic (FOS) or the placebo (glucose) group.

The trial was approved by the Ethical Committee of Siebold University of Nagasaki Prefecture, and by the Ethical Review Committee of ICDDR, B. Informed consent was obtained from all parents. The children were provided medical care in the ICDDR, B-run clinic for any serious illnesses during the study period. Oral rehydration therapy was performed for most of the diarrhea cases observed.

Study Outcome

The primary study outcome was body weight gain and a reduction in the number of diarrhea episodes. The secondary outcome was a decrease in the cumulative duration of diarrhea days, the duration of diarrhea days per diarrhea episode, and the number of defecations per day during a diarrhea episode. According to the World Health Organization [38], diarrhea is defined as the passage of three or more loose or watery stools in a 24-h period. An episode of diarrhea was defined as a period beginning with a day when the subject experiences more than three loose stools and ending with the last diarrhea day followed by at least two consecutive days without diarrhea. Severe diarrhea was defined as either persistent or invasive diarrhea. Persistent diarrhea was defined as having a duration of more than 14 days, while invasive diarrhea was defined as diarrhea with macroscopic blood.

Study Protocol

The intervention involved the daily intake for six consecutive months of commercially produced isotonic solution (Pocari Sweat, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) with the addition of fructooligosaccharide (FOS, Meiji Seika Kaisha Ltd., Tokyo, Japan), the chemical structure of which is 1^{F}-(β-fructofranosyl)$_{n-1}$-sucrose, where n varies from 2 to 4 (e.g., 2, 1-kestose (GF2), 28%; 3, nystose (GF3), 60%; and 4, 1^{F}-β-fructofranosyl nystose (GF4), 12%). The placebo group received an identical bottle of placebo solution with glucose (Nihon Shokuhin Kako CO., Ltd., Tokyo, Japan). FOS and glucose were of an analytical grade. The commercially produced isotonic solution masked the slight differences in both sweetness and taste between the two solutions. In order to reduce the osmotic pressure of the solution, 1.85 g of "Pocari Sweat" powder was dissolved in 50 mL of water, and 2 g of FOS or 1 g of glucose was added to achieve equal energy. The total osmotic pressure was 0.234 Osmol/kg for the FOS solution and 0.278 Osmol/kg for the glucose solution, measured by the cryoscopy freezing-point method using the Osmometer OM802 (Asahi Life Science Co., Ltd., Tokyo, Japan). The main nutrients and the concentration of electrolytes in these solutions are shown in Table 1. Forty mL of each test solution was freshly prepared every morning, and then 50 mL was transferred to a bottle for each child.

Prior to the start of the trial, randomization was carried out using the master randomization code, and two sets of code envelopes were made by an independent statistician, who was not involved in the study in any way. No collaborator or field research assistant knew the group to which any of the children belonged, or the content of any particular solution bottle.

Four trained field research assistants and four health attendants were recruited from the study area. A field research assistant and a health attendant formed a team for house visits and confirmed the screening criteria. Four

Table 1. Nutrients and concentration of electrolytes in FOS and placebo solutions

	FOS solution	Placebo solution
Nutrients (per 100 mL)		
Energy (kcal)	16.0	16.0
Carbohydrate (g)	3.4	3.4
Protein (g)	0.0	0.0
Fat (g)	0.0	0.0
Added FOS (g)	4.0	0.0
Added glucose (g)	0.0	2.0
Electrolytes (mmol/L)		
Na^+	21.0	21.0
K^+	5.0	5.0
Ca^{2+}	0.5	0.5
Mg^{2+}	0.25	0.25
Cl^-	16.5	16.5
$Citrate^{3-}$	3.3	3.3
$Lactate^-$	1.0	1.0

FOS, fructooligosaccharide.
Data were obtained from Otsuka Pharmaceutical Co., Ltd.

teams distributed the test solution daily and directly administered it to each child. They interviewed the mother of each child every day, and measured each child's body weight on alternative days. The interview questions were the number of defecations, consistency and constitution of stool (hard, loose, or watery; visible blood and/or mucus in the feces), abdominal or other symptoms, and treatment and administration of antibiotics. The research assistants conducted their activities according to a manual using checklists. If the child was not at home, the mother was asked to keep one bottle and to give it to the child within a day. Body weight was measured using a digital scale with 0.1-kg precision (UNI-scale). Height and arm circumference were measured using a wooden perpendicular scale and a TALC tape measure, respectively in the field clinical office once a month. Measurements were carried out at least two times.

Data Management and Statistical Analysis

The study releaved a 0.06 kg mean difference with a 0.12 kg standard deviation in body weight gain, based on the assumptions from previous studies in Bangladesh [39, 40], using a two-sided alpha of 0.05 and a power of 80%. The requirement of more than 63 children per group was established, and assuming a 16.7% (20/120) drop-out rate, the calculated number of children required for the study was 75 in each group.

Data were collected by the field research assistants according to forms that were reviewed by the supervising staff in ICDDR, B. Data were entered and checked logically and in terms of range. Thereafter, cleaning and analysis were performed using SPSS ver.11 for Windows, Japan (SPSS Inc., Japan). For comparison of the categorical data, chi-square or Fisher's exact test was used. Continuous data were compared with Student's *t*'test for normally distributed data and the Welch or Mann-Whitney *U* test for non-parametric data. Pearson's correlation was used to evaluate the relation of the number of diarrhea episodes or cumulative days of diarrhea with the body weight gain. The data were expressed as means and standard deviations with the significance level considered to be less than 0.05.

RESULTS

Characteristics of Children at Baseline

The flow chart of randomization procedure is shown in Figure 1. The characteristics of the participants initially allocated to each group and those who completed the study are shown in Table 2. The characteristics were not significantly different for the initial groups. Age in the FOS group (n=75) was 46.4±9.8 months, and that in the placebo group (n=75) was 46.5±9.2 months. The number of children who completed the study was 64 in the FOS group and 69 in the placebo group. The percentage of males was significantly higher in the FOS group than in the placebo group (p=0.037).

Dose Levels of FOS Administered

According to the field research assistants, all the children liked to drink the solutions, and none refused the administration. The average dose level of the administration of FOS was 0.147±0.235 g per kg of body weight at the start of the study period and 0.138±0.222 g per kg of body weight at the end of the study.

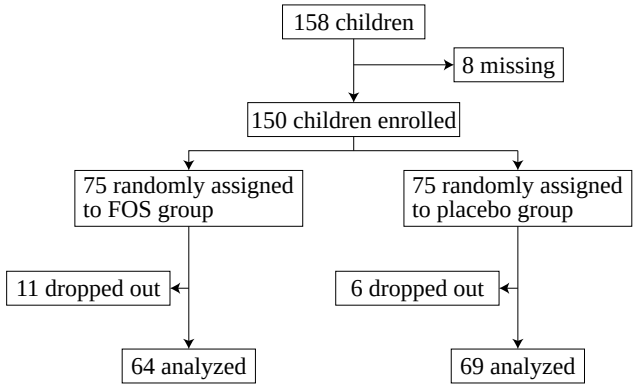

Figure 1. Flow chart of randomization procedure and attrition of study participants

Table 2. Characteristics of children at baseline: enrolled and analyzed

	Initially allocated		Completed study	
	FOS (n=75)	Placebo (n=75)	FOS (n=64)	Placebo (n=69)
Age (mo)	46.5 (9.2)	46.4 (9.8)	46.5 (9.1)	46.3 (9.4)
Males (%)	52.0	38.6	53.1[a]	34.8
Body weight (kg)	12.8 (2.0)	12.6 (2.0)	12.9 (2.0)	12.6 (2.1)
Height (cm)	94.9 (8.6)	93.8 (7.8)	95.0 (8.3)	93.8 (8.1)
MUAC (cm)	15.0 (1.0)	15.1 (1.1)	15.0 (1.1)	15.1 (1.2)

FOS, fructooligosaccharide; MUAC, arm circumference. Values were expressed as mean and (standard deviation: S.D.). The number of enrolled children was 150; a group of 75 children were allocated to the FOS-group, and the other 75 were to the placebo group. Sixty-four children of the FOS-group and 69 children of the placebo group completed the study and their data were used for analysis. a: significantly different from the placebo group, at p=0.037.

Growth and the Number of Children Experiencing Diarrhea

Data on body weight gain after six months' daily intake of the two kinds of test solutions are shown in Table 3. The average body weight gain in the FOS group was 0.86±0.55 kg, while that in the placebo group was 0.89±0.48 kg. There were no significant differences in the increase in body weight, height and arm circumference between either the FOS and placebo groups or the males and females in each group.

The number of children who experienced one or more episodes of diarrhea was 39 out of 64 children in the FOS group and 44 out of 69 children in the placebo group. The difference between the groups was not significant. The number of children experiencing persistent diarrhea was only one in the FOS group and two in the placebo group.

The Number and Duration of Diarrhea Episodes

Table 4 shows the average number of diarrhea episodes, the cumulative diarrhea days over the six-month period, the duration of diarrhea per episode, and the number of defecations per day of diarrhea. The average number of diarrhea episodes was 1.3±1.6 in the FOS group and 2.0±2.8 in the placebo group. There were apparently fewer episodes of diarrhea in the FOS group than in the placebo group, but the difference was not significant.

The cumulative number of diarrhea days in the FOS group was 3.3±4.9 days, significantly fewer than that in

Table 3. Growth during the six-month period and the number of children who experienced diarrhea

	FOS (n=64)	Placebo (n=69)
Body weight gain (kg)	0.86 (0.55)	0.89 (0.48)
Height gain (cm)	2.76 (0.71)	2.73 (0.68)
MUAC gain (cm)	0.24 (0.39)	0.27 (0.41)
Children reporting diarrhea (number)[*1]	39	44
Children reporting persistent diarrhea (number)[*2]	1	2

FOS, fructooligosaccharide; MUAC, arm circumference. Values were expressed as mean and (S.D.). [*1]: the number of children who experienced one or more episodes of diarrhea; [*2]: the number of children who experienced persistent diarrhea. No difference was observed between the two groups.

Table 4. The comparison of diarrhea episodes between FOS and placebo groups

	FOS (n=63)	Placebo (n=67)	p-values
Number of diarrhea episodes (number)	1.3 (1.6)	2.0 (2.8)	0.098
Cumulative diarrhea days	3.3 (4.9)	6.3 (10.8)[a]	0.039
Duration of diarrhea days per episode[*1]	2.5 (1.8)	3.2 (2.4)[b]	0.008
Number of defecations per day of diarrhea[*1]	2.5 (1.7)	2.2 (1.4)	0.096
Visible blood in feces (number)	1	1	1.000
Mucus in feces (number)	9	14	0.365

Values were expressed as mean and (S.D.). The total numbers (64 in FOS group, and 69 in placebo group) excluding persistent diarrhea of FOS and placebo groups were 63 and 67, respectively. [*1]: n=84 in FOS group, and n=147 in FOS group, respectively. a, b: significantly different from the placebo group, respectively.

Table 5. Other diseases, symptoms and antibiotic treatment during the six-month period

	FOS (n=50)	Placebo (n=51)
Measles	1	2
Cough	44	44
Angular stomatitis	49	51
Ear discharge	50	51
Vomiting	15	18
Antibiotics treatment	50	51

No significant difference was detected between the two groups.

the placebo group (6.3±10.8 days), at p=0.039. The duration per diarrhea episode in the FOS group (2.5±1.8) was also significantly shorter than that in the placebo group (3.2 ±2.4), at p=0.008. However, the number of defecations per days of diarrhea was not significantly different between the FOS group (2.5±1.7) and the placebo group (2.1±1.4).

The number of children with macroscopic blood in feces was one in each group. Mucus in feces occurred in nine children in the FOS group and 14 in the placebo group. The difference was not statistically significant.

Other Symptoms and Antibiotic Treatment

The data on other symptoms were collected for 50 out of 64 children in the FOS group and for 51 out of 69 children in the placebo group, as shown in Table 5. Cough was observed in 44 out of 50 children in the FOS group and 44 out of 51 children in the placebo group. The difference was not significant. Most of the children experienced angular stomatitis and ear discharge. Chloramphenicol antibiotic treatment was administered to almost all of the children in the two groups.

Association of Diarrhea with Body Weight Gain

The number of episodes and duration of diarrhea had no significant correlation with body weight gain in either the FOS (r=-0.092, r=-0.64) or placebo group (r=-0.088, r= -0.44).

DISCUSSION

The prebiotic effect of daily FOS intake on growth and on the episodes and duration of diarrhea was studied in free-living children in an urban slum in Bangladesh. The difference in growth between the FOS and placebo groups was not significant, nor did the number of diarrhea episodes differ between the two groups. Many factors other than the prebiotic effect might directly and/or indirectly affect growth and diarrhea. It has been reported that it is difficult to detect the benefit of the prebiotic effect in healthy populations [36, 37].

Three factors may explain this. First, the dose of FOS, on average 0.147 g per kg of body weight, might be too small to reduce the number of diarrhea episodes. We selected the dose on the basis of the following two points: 1) dose transitory diarrhea can be completely avoided; and 2) can promote the intestinal microflora. In adults, approximately 15 g of daily single intake (or 0.3 g per kg of body weight) may induce hyperosmotic transitory diarrhea [26, 41, 42], and 1-2 g of daily single intake improved intestinal microbes [15]. Considering that 0.5-2.5 g of nondigestible oligosaccharides is supplemented to 100 g dry weight of some brands of artificial formula milk in Japan (0.05-0.3 g per 100 mL of formula solution), it may be possible to test higher doses of FOS to examine the prebiotic effects in the future. Further studies are needed to identify the optimal dose level of FOS for children.

Secondly, most children were reported to have symptoms of cough, angular stomatitis, and/or ear discharge and to be under treatment with chloramphenicol antibiotics, which interfere with the proliferation of not only harmful but also beneficial microbes [25, 27, 43, 44]. Chloramphenicol is a broad spectrum antibiotic, and it has been shown to reduce intestinal microbes in rats [25]. The prebiotic effect might be disturbed by the use of antibiotics.

Thirdly, this study was conducted from the cool and dry winter season to the hot and rainy season of Bangladesh. Since many of the children had been treated with antibiotics for respiratory infectious diseases in winter, the result may have differed if the study had started in the rainy season.

On the other hand, the average number of days of diarrhea during the six-month period and the number of days of diarrhea per episode were shorter in the FOS group than in the placebo group. Intake of FOS may reduce the duration of diarrhea in the children. But the shorter duration of diarrhea did not relate with weight gain, presumably because of the effects of antibiotics. Further studies are required to determine the prebiotic effect of FOS under conditions in which antibiotics effects have been eliminated. The optimal dose level and affordable administration methods of prebiotics for mitigating the disease burden of diarrhea should be also studied.

In conclusion, daily intake of FOS was associated neither with the children's growth nor with the number of diarrhea episodes, but a significant reduction in the duration of diarrhea days was observed. Further studies are needed to confirm the effects of FOS by changing the doses and eliminating the influence of antibiotics.

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