Short-Chain Fructo-Oligosaccharide Administration Dose-Dependently Increases Fecal Bifidobacteria in Healthy Humans[1]

Yoram Bouhnik,*[2] Kouroche Vahedi,* Lotfi Achour,* Alain Attar,* Jérôme Salfati,*† Philippe Pochart,* Philippe Marteau,* Bernard Flourié,* Francis Bornet† and Jean-Claude Rambaud*

*INSERM U 290, Fonctions intestinales, métabolisme et nutrition, Hôpital Saint-Lazare, 75010 Paris, France and †Eridania Beghin-Say, Vilvoorde Research and Development Center, Nutrition and Health Service, B-1800 Vilvoorde, Belgique

ABSTRACT Short-chain fructo-oligosaccharides (SC-FOS) are a mixture of oligosaccharides consisting of glucose linked to fructose units (Gf_n; $n = \leq 4$), which are not digested in the human small intestine but are fermented in the colon where they specifically promote the growth of bifidobacteria. In healthy volunteers, we assessed the tolerance and the threshold dose of SC-FOS that significantly increased fecal bifidobacteria counts and the possibility of a dose-response relationship. Randomly divided into five groups and eating their usual diets, healthy volunteers (40: 18 males, 22 females) ingested in two oral doses for 7 d a powder mixture containing (g SC-FOS/d): 0, G_0; 2.5, $G_{2.5}$; 5, G_5; 10, G_{10}; 20, G_{20}. Stools were collected before (d1) and at the end (d8) of sugar consumption, and tolerance was evaluated using a daily chart. Total anaerobe counts were not affected by SC-FOS ingestion. Bifidobacteria counts at d8 were greater in groups G_{10} and G_{20} than in G_0 and $G_{2.5}$ ($P < 0.05$). Fecal pH did not differ among groups. A significant correlation between the dose of SC-FOS ingested and the fecal bifidobacteria counts was observed at d8 ($r = 0.53$; $P < 0.01$). Excess flatus was significantly more frequent in subjects consuming G_{20} than in those consuming G_0, $G_{2.5}$ or G_5 ($P < 0.05$), and more intense in G_{20} than in G_0 and G_5 groups ($P < 0.05$). In conclusion, the optimal and well-tolerated dose of SC-FOS that significantly increased fecal bifidobacteria in healthy volunteers consuming their usual diet is 10 g/d. J. Nutr. 129: 113–116, 1999.

KEY WORDS: • *bifidobacteria* • *colonic flora* • *fermentation* • *prebiotics* • *short-chain fructooligosaccharides* • *humans*

Short-chain fructo-oligosaccharides (SC-FOS)[3] are a mixture of oligosaccharides consisting of glucose linked to fructose units (Gf_n; $n \leq 4$) the links between fructose units are β-(1,2) (Hirayama et al. 1989). SC-FOS are produced commercially from sucrose using an enzymatic process (Clevenger et al. 1988). Ingested SC-FOS are poorly digested in the human small intestine (Molis et al. 1996) but are fermented in the colon by the resident microflora (Hosoya et al. 1988, McKellar and Modker 1989, Molis et al. 1996). Unlike other indigestible sugars such as lactose or lactulose, which are hydrolyzed by a wide variety of gut bacteria, SC-FOS are fermented in vitro only by a limited range of microorganisms that include most species of bifidobacteria (Gibson and Wang 1994, Hidaka et al. 1986, Mitsuoka et al. 1987, Wang and Gibson 1993). Indeed, bifidobacteria have relatively high activity of β-fructosidase that is selective for β-(1,2) glycosidic bonds present in SC-FOS (De Vries and Stouthamer 1967). After FOS hydrolysis, fructose serves as an efficient growth substrate for the bifidobacteria pathway of hexose fermentation, which is almost exclusively carried out by bifidobacteria (Scardovi 1965).

In light of the recent interest in "prebiotics" which selectively encourage the growth of species that are perceived as beneficial (Gibson and Roberfroid 1995), in humans dietary addition of SC-FOS at a dose of 12.5 g/d increased fecal bifidobacteria counts (Bouhnik et al. 1996). Dietary interventions that promote bifidobacteria may be beneficial because bifidobacteria, a saccharolytic genus, could contribute to the protection that breastfeeding provides against gut infections (Bullen and Willis 1971), and could be of interest in prevention of colon carcinogenesis (Bouhnik et al. 1996, Buddington et al. 1996). Other FOS, with longer chains obtained by partial hydrolysis of inulin, also increased fecal counts of bifidobacteria at a dose of 15 g/d (Gibson and Roberfroid 1995). More recently, a significant increase in bifidobacteria counts was obtained using FOS at a dose of 4 g/d (Buddington et al. 1996). The dose-response relationship between SC-FOS and the microflora has not been adequately established.

The present study was designed to determine the digestive tolerance and the threshold dose of SC-FOS that leads to a

[1] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[2] To whom correspondence should be addressed at INSERM U 290, Hôpital Saint-Lazare, 107 bis rue du Faubourg Saint-Denis, 75475 Paris cedex 10, France.

[3] Abbreviations used: cfu, colony-forming unit; Gf_n, glucose linked to fructose units; GF_2, 44% 1-kestose; GF_3, 46% nystose; GF_4, 10% $1^F\beta$-fructofuranosyl nystose; SC-FOS, Short-chain-fructooligosaccharides.

Manuscript received 28 February 1998. Initial review completed 11 June 1998. Revision accepted 15 September 1998.

significant increase in fecal bifidobacteria and the possibility of a dose-response relationship in healthy volunteers consuming their usual diet.

SUBJECTS AND METHODS

Subjects. Healthy volunteers (40: 18 males and 22 females), ages 18–47 y (mean 29.6), participated in the study. Not one had any history of gastrointestinal disease, except for appendectomy. No antibiotics or laxatives had been taken during the 2 mo before the study. No other medication was allowed during the investigation period. Subjects gave written informed consent to the protocol, which was approved by the local Ethics Committee (CCPPRB Paris Saint-Louis, Hôpital Saint Louis, Paris, France).

Experimental design. The volunteers were randomly assigned to five groups (eight subjects per group). For 7 d they ingested in two oral doses at the end of the two main meals a powdered mixture of 20 g of the following composition: G_0 group (SC-FOS 0 g and saccharose 20 g) used as placebo; $G_{2.5}$ group (SC-FOS 2.5 g and saccharose 17.5 g); G_5 group (SC-FOS 5 g and saccharose 15 g); G_{10} group (SC-FOS 10 g and saccharose 10 g); and G_{20} group (SC-FOS 20 g and saccharose 0 g). Throughout the study, subjects consumed their usual diet with the exception of products containing high levels of FOS such as onion, asparagus, wheat, rye, triticale and Jerusalem artichoke. They avoided fermented dairy products containing viable bifidobacteria which increases fecal bifidobacteria within a few days of consumption thus avoiding bias in favor of bifidobacteria that would mask the feeding effects of the SC-FOS treatment (Bouhnik et al. 1992). We used SC-FOS from Actilight® (Béghin Meiji Industries, Neuilly sur Seine, France) which consisted of 44% 1-kestose (GF_2), 46% nystose (GF_3) and 10% 1^F-β-fructofuranosyl nystose (GF_4).

Tolerance was evaluated using a daily chart where the symptoms (excess flatus, borborygmi, bloating, abdominal pain) were graded from 0 (no symptoms) to 3 (severe symptoms). Frequency and consistency of stools were also noted, and diarrhea was defined as one or more watery stools, or more than three stools per day.

Stool collection. Stool samples were collected on the day before sugar consumption and on d8 of sugar consumption in plastic containers under anaerobic conditions (AnaerocultA; Merck, Darmstadt, Germany), immediately stored at 4°C and analyzed within 4 h. We previously determined that no variations existed in bacterial counts and pH when the same stools were analyzed fresh vs. 4 h after freezing (Bouhnik et al. 1992).

Bacterial enumeration and pH. Fecal samples were homogenized with a high-speed blender (Ultraturax; Labortechnik, Staufer, Germany) and serially diluted 10-fold in solution for anaerobes (saline, glucose and cysteine). Of each dilution 100 μL was inoculated in appropriate medium. Wilkins-Chalgren agar (Difco Laboratories Inc., Detroit, MI) was used to isolate Wilkins-Chalgren counts, considered to be representative of anaerobic bacteria. Although such conditions for recovering stools can kill extremely strict oxygen-intolerant organisms present in the colon, conditions for samples analysis were consistent throughout all studies. Beerens' medium (Beerens 1990) was used to isolate bifidobacteria; anaerobes bacteria and bifidobacteria were cultured for 7 and 5 d, respectively, at 37°C in usual anaerobic conditions (Gas Pak system; BBL, Cockeysville, MD) with Anaerocult A. An aliquot of stool was diluted fivefold in distilled water, homogenized and the pH measured with a pH meter (Bioblock, Illkirch, France).

Data analysis. Fecal concentrations of total anaerobes and bifidobacteria were expressed as log colony-forming unit (cfu)/g wet weight. The results were expressed as means ± SEM. Repeated measures analysis of variance was used to compare: (a) the bacterial population levels and pH at d1 in each group with treatment as factor and (b) the bacterial concentrations and pH between d1 and d8 in each group with time and treatment as factors. Following a significant F test ($P < 0.05$), the Newman-Keuls test was used to identify different means (Linton and Gallo 1975). The correlation between the dose of SC-FOS ingested and bifidobacteria counts measured on d8 was tested by the Spearman correlation. Symptoms experienced with SC-FOS were compared to those with placebo using the Wilcoxon signed rank test.

RESULTS

Fecal bacterial counts and pH. Total anaerobe counts did not increase due to SC-FOS ingestion in any group (**Table 1**). Bifidobacteria counts did not increase in groups G_0 and $G_{2.5}$ but they were significantly greater in groups G_5, G_{10} and G_{20} at d8 than in d1 ($P < 0.05$) (Table 1). In these groups, the greater bifidobacteria count was evident in absolute concentration and in the percentage of bifidobacteria among total anaerobes (**Fig. 1**). Bifidobacteria counts increased from d1 to d8 in six of eight subjects (75%) in G_5, and in eight of eight subjects (100%) in G_{10} and G_{20}. Fecal pH did not change between d1 and d8 in any group (Table 1).

Dose-effect of SC-FOS on bifidobacteria concentrations. Bifidobacteria counts did not differ significantly among groups at d1 (Table 1). At d8, they were significantly higher in groups G_{10} and G_{20} than in groups G_0 and $G_{2.5}$; counts in group G_5 did not differ from other groups. A significant correlation between the dose of ingested SC-FOS and the fecal bifidobacteria counts was observed at d8 ($r = 0.53$; $P < 0.01$).

Digestive tolerance. Digestive symptoms were reported by 37.5% (15/40) subjects; 27.5% (12/40) of subjects complained of excess flatus, 20% (8/40) of bloating, 15% (6/40) of borbo-

TABLE 1

Fecal anaerobes and bifidobacteria counts and fecal pH in healthy volunteers consuming various doses of short-chain fructooligosaccharides (SC-FOS) for 7 d[1]

	Total anaerobe counts			Bifidobacteria counts			pH		
SC-FOS dose	d1	d8	P^2	d1	d8	P	d1	d8	P
g/d	*log cfu/g stool*			*log cfu/g stool*					
0	9.94 ± 0.25	9.93 ± 0.23	0.91	8.63 ± 1.23	8.27 ± 1.08[b]	0.72	6.20 ± 0.67	7.01 ± 0.78	0.09
2.5	9.81 ± 0.28	9.87 ± 0.25	0.86	8.05 ± 1.10	8.18 ± 1.09[b]	0.86	6.74 ± 0.33	6.44 ± 0.54	0.12
5	9.88 ± 0.50	10.19 ± 0.22	0.12	8.10 ± 0.83	9.05 ± 0.44[ab]	0.04	6.59 ± 0.60	6.54 ± 0.54	0.71
10	9.80 ± 0.40	10.15 ± 0.31	0.09	8.00 ± 1.25	9.52 ± 0.32[a]	0.02	6.83 ± 0.38	6.83 ± 0.42	0.95
20	9.98 ± 0.14	10.29 ± 0.15	0.05	8.18 ± 0.93	9.53 ± 0.60[a]	0.01	6.67 ± 0.35	6.36 ± 0.56	0.15

[1] Data are means ± SEM, $n = 8$.
[2] P-value for the difference between d1 and d8.
Means in a column not sharing a superscript are significantly different, $P < 0.05$.
cfu, colony-forming unit.

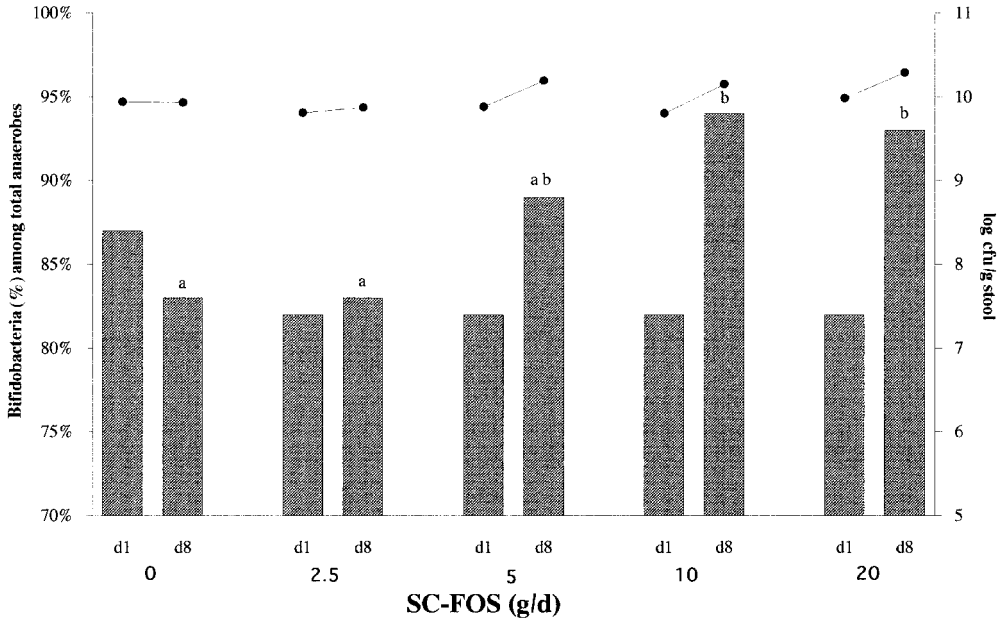

FIGURE 1 Dose-related effect of the 7-d ingestion of short-chain fructo-oligosaccharides (SC-FOS) on fecal bifidobacteria and total anaerobes in five groups of healthy volunteers. Circles represent mean densities of total anaerobes, and bars indicate percentages of bifidobacteria among total anaerobes for d1 and d8 in each group. Values are means, $n = 8$. Means at d8 not sharing a superscript are significantly different, $P < 0.05$. cfu, colony-forming unit.

rygmi and 20% (8/40) of abdominal pain (**Table 2**). Excess flatus was significantly more frequent in subjects who consumed G_{20} than in those who consumed G_0, $G_{2.5}$ and G_5 ($P < 0.05$); group G_{10} did not differ from other groups. No significant differences existed among groups in other symptoms. None of the subjects reported diarrhea or nausea. Excess flatus, as assessed by cumulative daily scores, was significantly more intense in G_{20} than in G_0 and G_5 groups (**Table 3,** $P < 0.05$). For the other symptoms, no significant differences existed among groups.

DISCUSSION

This study shows that 7-d ingestion of SC-FOS at a dose of 10 g/d, which was well-tolerated, significantly increased fecal bifidobacteria counts in healthy volunteers. Moreover, the increase in bifidobacteria counts was correlated with the dose of ingested SC-FOS. The study was performed under usual dietary conditions, without previous diet restriction, which could have altered colonic bacterial balance and, whatever initial bifidobacteria counts they had, an increase in bifidobacteria counts was noted in 75% of our subjects at the dose of 5 g/d, and in 100% of them at doses of 10 and 20 g/d. Anaerobe counts did not change between d1 and d8 except in the G_{20} group; bifidobacteria increased in absolute terms and as a percentage of anaerobes in G_5, G_{10} and G_{20} groups. Fecal bifidobacteria and anaerobe basal and stimulated population levels observed in this study were similar to those found in previous studies (Bouhnik et al. 1996, 1997; Gibson et al. 1995) in healthy volunteers and in elderly persons (Kleessen et al. 1997) using SC-FOS or other oligosaccharides. Therefore, the dietary treatment increases bifidobacteria levels to a level above typical baseline. A slight acidification of fecal contents during SC-FOS ingestion has been observed in animals (Hidaka et al. 1986) and humans (Mitsuoka et al. 1987). In the present study, as in our previous study (Bouhnik et al. 1996), fecal pH did not change during the ingestion of SC-FOS. However, fecal pH is not necessarily an accurate indicator of fermentation and acidity in the more proximal colon (Florent et al. 1985).

We observed a linear relationship between the increase in fecal bifidobacteria counts and the amount of SC-FOS ingested, suggesting that the concentrations of bifidobacteria in the human colon could be regulated by oral doses of FOS. A dose-effect relationship previously was found using another

TABLE 2

Subjects reporting digestive intolerance symptoms in healthy volunteers consuming various doses of short-chain fructo-oligosaccharides (SC-FOS)[1]

SC-FOS dose	Excess flatus	Bloating	Borborygmi	Abdominal pain	Subjects reporting symptoms
g/d	*n (%)*				
0 (placebo)	1 (12.5[b])	0	0	1 (12.5)	2 (25)
2.5	1 (12.5[b])	1 (12.5)	1 (12.5)	1 (12.5)	2 (25)
5	1 (12.5[b])	1 (12.5)	0	0	1 (12.5)
10	3 (37.5[ab])	2 (25)	2 (25)	4 (50)	4 (50)
20	6 (75[a])	4 (50)	3 (37.5)	2 (25)	6 (75)

[1] Data are number (%) of subjects in each group, $n = 8$.
[2] Values in a column not sharing a superscript are significantly different, $P < 0.05$.

TABLE 3

Intensity of digestive intolerance symptoms in healthy volunteers consuming various doses of short-chain fructo-oligosaccharides (SC-FOS)[1,2]

SC-FOS dose	Excess flatus	Bloating	Borborygmi	Abdominal pain
g/d				
0 (placebo)	0.25 ± 0.71[b]	0	0	0.63 ± 1.77
2.5	1.25 ± 3.54[ab]	0.25 ± 0.71	0.88 ± 2.47	0.75 ± 2.12
5	0.38 ± 1.06[b]	0.38 ± 1.06	0	0
10	1.63 ± 3.46[ab]	1.88 ± 3.94	1.25 ± 2.55	1.38 ± 2.72
20	3.75 ± 3.96[a]	2.25 ± 3.11	1.38 ± 2.07	1.13 ± 2.47

[1] Data are means ± SEM of symptoms reported during the 7-d study, $n = 8$. Means not sharing a superscript are significantly different, $P < 0.05$.
[2] Symptoms intensity was noted as follow: 0: no symptom; 1–7: mild symptoms; 8–14: moderate symptoms; 15–21: severe symptoms.

bifidogenic substrate, galacto-oligosaccharides, but the minimal dose that significantly increased fecal bifidobacteria counts was 10 g/d, and the maximal well-tolerated dose was 15 g/d (Ito et al. 1990). In our study using SC-FOS, the range between efficient and maximal tolerated doses was greater. Notably, the degree of change in the fecal flora necessary to cause benefit is unknown and a statistically significant result does not ensure physiological consequences.

In a threshold study evaluating symptomatic response to varying levels of SC-FOS ingested regularly by 14 healthy volunteers, excessive flatus and borborygmi were recorded by about 10% of volunteers at 10 g/d of SC-FOS and excessive flatus, borborygmi and bloating by about 20–30% of volunteers at 20 g/d (Briet et al. 1995). In another study in which 10 volunteers ingested 15 g/d FOS for 12 d, gaseous symptoms such as abdominal cramps, excess flatus and bloating were significantly more severe in subjects ingesting the FOS than in sucrose-fed control subjects ($P < 0.05$) (Stone-Dorshow and Levitt 1987). However, with the exception of flatulence, these symptoms, if present, were usually mild and did not increase (or decrease) during the course of the 12-d period. In our previous study of 10 healthy volunteers who ingested 12.5 g/d of SC-FOS for 12 d, only bloating was found to be significantly more frequent during SC-FOS ingestion period than during the placebo ingestion ($P < 0.05$), but was very mild and present only in 5/10 volunteers (Bouhnik et al. 1997). From all these results, apparently the most common symptoms noted during SC-FOS administration are excess flatus and/or bloating, but only a minority of subjects experiences them and they are usually very mild.

In conclusion, this study permitted us to determine 10 g/d to be the optimal and well-tolerated dose of SC-FOS which leads to a significant increase in colonic bifidobacteria in healthy volunteers consuming their usual diet.

LITERATURE CITED

Beerens, H. (1990) An elective and selective medium for *Bifidobacterium* spp. Lett. Appl. Microbiol. 11: 155–157.

Bouhnik, Y., Flourié, B., D'Agay-Abensour, L., Pochart, P., Gramet, G., Durand, M. & Rambaud, J. C. (1997) Administration of transgalacto-oligosaccharides increases faecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. J. Nutr. 127: 444–448.

Bouhnik, Y., Pochart, P., Marteau, P., Arlet, G., Goderel, I. & Rambaud, J. C. (1992) Fecal recovery in humans of viable Bifidobacterium sp. ingested in fermented milk. Gastroenterology 102: 875–878.

Bouhnik, Y., Flourié, B., Riottot, M., Bisetti, N., Gailing, M. F., Guibert, A. (1996) Effects of fructo-oligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. Nutr. Cancer 26: 21–29.

Briet, F., Achour, L., Flourié, B., Beaugerie, L., Pellier, P., Franchisseur, C. (1995) Symptomatic response to varying levels of fructo-oligosaccharides consumed occasionally or regularly. Eur. J. Clin. Nutr. 49: 501–507.

Buddington, R. K., Williams, C. H., Chen, S. C. & Witherly, S. A. (1996) Dietary supplement of Neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. Am. J. Clin. Nutr. 63: 709–716.

Bullen, C. L., & Willis, A. T. (1971) Resistance of the breastfed infant to gastroenteritis. Brit. Med. J. 3: 338–343.

Clevenger, M. A., Turnbull, D., Inoue, H., Enomoto, M., Allen, J. A. (1988) Toxicological evaluation of Neosugar: genotoxicity, carcinogenicity and chronic toxicology. J. Am. Coll. Toxicol. 7: 643–662.

De Vries, W. & Stouthamer, A. H. (1967) Carbohydrate metabolism in Bifidobacterium bifidum var. Pennsylvanicus. Biochem. Biophys. Acta. 136: 415–425.

Florent, C., Flourié, B., Leblond, A., Rautureau, M., Bernier, J. J. & Rambaud, J. C. (1985) Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an in vivo study). J. Clin. Invest. 75: 608–613.

Gibson, G. R., Beatty, E. R., Wang, X. & Cummings, J. H. (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 108: 975–982.

Gibson, G. R. & Roberfroid, M. B. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125: 1401–1412.

Gibson, G. R. & Wang, X. (1994) Enrichment of bifidobacteria from human gut contents by oligo-fructose using continuous culture. FEMS Microbiol. Lett. 118: 121–127.

Hidaka, H., Eida, T., Takizawa, T., Tokunaga, T. & Tashiro, Y. (1986) Effects of fructo-oligosaccharides on intestinal flora and human health. Bifidobacteria Microflora 5: 37–50.

Hirayama, M., Sumi, N. & Hidaka, H. (1989) Purification and properties of fructo-oligosaccharides producing beta-fructofuranosidase from Aspergillus niger ATCC 20611. Agric. Biol. Chem. 53: 667–673.

Hosoya, N., Dhorranintra, B. & Hidaka, H. (1988) Utilization of [U^{14}-C] fructo-oligosaccharides in man as energy resources. J. Clin. Biochem. Nutr. 5: 67–74.

Ito, M., Deguchi, Y., Miyamoti, A., Matsumoto, K., Kikuchi, H., Matsumoto, K. (1990) Effects of administration of galacto-oligosaccharides on the human fecal microflora, stool weight and abdominal sensation. Microbial. Ecol. Health. Dis. 3: 285–292.

Kleessen, B., Sykura, B., Zunft, H. J. & Blaut, M. (1997) Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. Am. J. Clin. Nutr. 65: 1397–1402.

Linton, M. & Gallo, P. S. (1975) The Practical Statistician Simplified Handbook of Statistics. Wadsworth, Belmont, CA.

McKellar, R. C. & Modker, H. W. (1989) Metabolism of fructo-oligosaccharides by Bifidobacterium spp. Appl. Microbiol. Biotechnol. 31: 537–541.

Mitsuoka, T., Hidaka, H. & Eida, T. (1987) Effect of fructo-oligosaccharides on intestinal microflora. Die Nahrung 31: 427–436.

Molis, C., Flourié, B., Ouarné, F., Gailing, M. F., Lartigue, S., Guibert, A. (1996) Digestion, excretion, and energy value of fructo-oligosaccharides in healthy humans. Am. J. Clin. Nutr. 64: 324–328.

Scardovi, V. (1965) The fructose-6-phosphate shunt as a peculiar pattern of hexose degradation in the genus Bifidobacterium. Ann. Microbiol. Enzymol. 15: 19–24.

Stone-Dorshow, T. & Levitt, M. D. (1987) Gaseous response to ingestion of a poorly absorbed fructo-oligosaccharides sweetener. Am. J. Clin. Nutr. 46: 61–65.

Wang, X., & Gibson, G. R. (1993) Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J. Appl. Bacteriol. 75: 373–380.