

The capacity of short-chain fructo-oligosaccharides to stimulate fecal Bifidobacteria: a randomized controlled dose-response relationship study in healthy humans

Yoram Bouhnik (1), Laurent Raskine (2), Guy Simoneau (3), Damien Paineau (4), Francis Bornet (4).

(1) Hépatogastroentérologie et Assistance Nutritive, Hôpital Lariboisière, Paris, France

(2) Bactériologie et Virologie, Hôpital Lariboisière, Paris, France

(3) Unité de Recherche Thérapeutique, Hôpital Lariboisière, Paris, France

(4) Nutri-Health, Rueil-Malmaison, France

Corresponding author:

Prof. Yoram Bouhnik, MD, PhD

Hôpital Lariboisière, 2, rue Ambroise Paré, 75475 PARIS Cedex 10, FRANCE

E-mail : yoram.bouhnik@lrb.ap-hop-paris.fr

Tel: 00 33 1 49 95 25 75; Fax: 00 33 149 95 25 77

Short title: Dose-response relation of scFOS and bifidobacteria

ABSTRACT

Background: Short-chain fructo-oligosaccharides (scFOS) are well-known for their bifidogenicity. In a large study comprising 200 healthy volunteers, we determined the bifidogenic properties of 7 non-digestible carbohydrates administered at a dose of 10 g/d in human diet, and performed dose-response relationships of the bifidogenic substrates at doses ranging from 2.5 to 10 g/d, and compared them to a placebo. The aim of this presentation is to give more details about dose-response effects of short-chain fructo-oligosaccharides (scFOS).

Methods: Forty healthy volunteers (18 males, 22 females) eating their usual diets were randomly divided into 5 groups of 8 subjects and received scFOS at a dose of 2.5, 5.0, 7.5 and 10 g/d or a placebo for 7 d. Stools were collected before (d8) and at the end (d15) of sugar consumption, and tolerance was evaluated with a daily chart.

Results ($m \pm SEM$): Bifidobacteria counts were higher in scFOS than in placebo groups for all doses tested [2.5 g/d ($P=0.02$); 5 g/d ($P=0.03$); 7.5 g/d ($P=0.01$); 10 g/d ($P=0.003$)]. A significant correlation between the dose of ingested scFOS and the fecal bifidobacteria counts was observed at d15 ($r^2 = 0.307$, $P < 0.001$). Total anaerobes increased at the dose of 10 g/d. No significant differences were found for *Bacteroides*, *Lactobacillus*, enterobacteria and pH in any group. Digestive symptom frequency was not different between scFOS at all doses tested and placebo. Bloating was significantly more intense during scFOS ingestion at doses 2.5 and 5 g/d, but not at doses of 7.5 and 10 g/d. Excess flatus, borborygmi and abdominal pain were not different than placebo at all doses tested.

Conclusions: This study showed that scFOS is bifidogenic and well tolerated at doses ranging from 2.5 to 10 g/d, with a dose-response relationship in healthy volunteers.

BACKGROUND

Short chain fructo-oligosaccharides (scFOS) are a mixture of oligosaccharides consisting of glucose linked to fructose units; links between fructose units are β -(1,2)[1]. They are produced commercially from sucrose using an enzymatic process. Ingested scFOS are poorly digested in the human small intestine but are fermented in the colon by the resident microflora [2].

In light of the recent interest in "prebiotics" which have been defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacterial species in the colon"[3], it has been shown in humans that dietary addition of short-chain fructo-oligosaccharides (scFOS) at doses from 4 to 12.5 g/d led to an increase in fecal counts of bifidobacteria [4,5]. Such bifidobacteria-promoting dietary interventions can be perceived as beneficial, as bifidobacteria are a saccharolytic genus, and could contribute to the protection that breast feeding provides against gut infections [6], and play a role in the prevention of colon carcinogenesis [4,5].

In a recent study comprising 200 healthy volunteers, we determined the bifidogenic properties of 7 non-digestible carbohydrates administered at a dose of 10 g/d in human diet : Four non-digestible carbohydrates were found to be statistically different from placebo, *i.e.* bifidogenic at 10 g/d: scFOS, soybean-oligosaccharides, galacto-oligosaccharides and type III resistant starch; we therefore performed a dose-response relationships of these 4 substrates at doses ranging from 2.5 to 10 g/d, and compared them to a placebo [7]. The effects of the 7-d treatment were found significantly different among the 4 non-digestible carbohydrates ($P=0.009$). A trend was found for doses tested ($P= 0.06$) suggesting that the relation between doses and bifidobacteria counts could be different among the 4 non-digestible

carbohydrates, and that among them, a dose-response effect could be present. As we previously demonstrated a dose-response relation between scFOS and bifidobacteria at higher doses [8], we found of interest to investigate a possible dose-response relation between scFOS and bifidobacteria at lower doses.

SUBJECTS AND METHODS

Subjects

Forty healthy volunteers [18 males, 22 females, age 29 ± 1.3 (mean \pm SEM)] were included. Exclusion criteria were: history of gastrointestinal disease except for appendectomy; antibiotics or laxatives taken during the two months before the study; other medication during the investigation period. Subjects gave written informed consent to the protocol, which was performed in accordance with local legislation, the ICH guidelines and the principles laid down in the current revision of the Declaration of Helsinki, and was approved by an Ethical Committee (« Comité Consultatif pour la Protection des Personnes se prêtant à la Recherche Biomédicale » of Paris-Saint Louis, France).

The scFOS tested was Actilight™ [Beghin Meiji, Paris, France ;which consist of 44% 1-kestose (GF2), 46% nystose (GF3) and 10% 1F- β -fructofuranosyl nystose (GF4),the placebo was 50% sucrose - 50% fully digestible waxy maize derived maltodextrins (DE6.5) (Cerestar, Vilvoorde, Belgium).

Experimental Design

To evaluate possible dose-response effects on the intestinal microflora, 40 volunteers were randomized into 5 groups of 8 subjects and ingested scFOS at a daily dose of 2.5, 5.0, 7.5 or 10 g/d, from d8 to d14, in two oral doses after lunch and dinner or the placebo. All subjects consumed their usual daily diet from the pre-inclusion day (d0) to the end of the study (d15). They were instructed to exclude from their diet fermented dairy products containing viable bifidobacteria and limit consumption of food products containing high level of non-digestible oligosaccharides such as onion, asparagus, wheat, rye, triticale and Jerusalem artichoke.

Digestive symptoms

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

Stool collection

Stools were recovered twice, on the d8-morning before the start of the scFOS consumption, and on the d15-morning, i.e., after 7d of scFOS consumption (from d8 to d14). Samples were collected in plastic containers rendered anaerobic (Anaerocult A; Merck, Darmstadt, Germany), immediately stored at 4 °C, transferred to the laboratory and analyzed. The procedure from stool emission to bacteriological analysis lasted less than 1 hour.

Bacterial counts and pH

Fecal samples (1 g) were introduced in the first pre-weighed tube of the dilution series and thoroughly mixed, then further tenfold dilutions were made up to 10^{-9} in a reduced diluant (cysteinated $\frac{1}{4}$ strength Ringer diluant). 0.1 ml of each dilution was spread on plates with different selective media to outnumber several bacterial genera: total anaerobic counts (Wilkins-Chalgren agar), *Bifidobacterium* (Beerens' medium), *Lactobacillus* (MRS agar), *Bacteroides* (BBE agar) and *Enterobacteria* (McConkey agar). The tests were duplicated for the first two media. Plates of the first three media were incubated anaerobically for 5 to 7 d, MRS agar for 48 hours under atmosphere enriched in CO₂ and McConkey agar aerobically for 48 hours. Colony counts were obtained and expressed as a log of the colony-forming units (CFU) per

gram of fresh feces. Extemporarily, the fresh stool pH was measured by pH meter (Bioblock, Illkirch, France).

Data analysis

Descriptive statistics used mean and standard error of mean ($m \pm \text{sem}$).

Efficacy analysis: The comparisons between each dose of scFOS versus placebo were performed on variations of bacteria counts (differences after-before treatment for bifidobacteria and other bacteria) using the unpaired t-test. Therefore, a dose-response relationship was sought using the linear regression model.

Tolerance analysis: A global analysis of the observed frequencies was performed on the 5 groups during the treatment period and for each symptom using a Chi-2 test.

Symptom intensity was noted every day as follows: 0: no symptom; 1: mild symptoms; 2: moderate symptoms; 3: severe symptoms, resulting in a daily score.

All daily scores were added for each symptom 1) before the treatment period from d1 to d8 (a.m.) and 2) during the treatment period from d8 (p.m.) to d15; in order to test the differences (doses versus placebo) on the variation of the total score (differences During-Before treatment). The same procedure was used (ANOVA, Fisher's test).

RESULTS

Fecal bacterial counts and pH

Bifidobacteria counts increased in scFOS groups at doses of 2.5, 5, 7.5 and 10 g/d. Total anaerobes increased at the dose of 10 g/d (Table 1). No significant differences were found for *Bacteroides*, *Lactobacillus*, enterobacteria and pH in any group (table 2).

Dose-effect of scFOS on bifidobacteria concentrations

Bifidobacteria counts did not differ significantly among groups at d1. A significant correlation between the dose of ingested scFOS and the fecal bifidobacteria counts was observed on the d15 – d8 difference ($r^2 = 0.307$, $P < 0.001$) (figure 1)

Digestive tolerance

Digestive symptom frequency, as assessed by cumulative daily scores, was not different between scFOS at all doses tested and placebo (Table 3). Bloating was significantly more intense during scFOS ingestion at doses 2.5 and 5 g/d, but not at doses of 7.5 and 10 g/d (Table 4). Excess flatus, borborygmi and abdominal pains were no different from placebo at all doses tested (Table 4). No diarrhea was reported by any subject.

DISCUSSION

This placebo controlled dose-response study shows that 7-d ingestion of scFOS at a dose from 2.5 to 10 g/d, which was well tolerated, led to a significant increase in fecal bifidobacteria in healthy volunteers. This is the first study which demonstrates the bifidogenic effect of sc-FOS from such low-dose (2.5g/d). Moreover, it must be stressed that the increase in bifidobacteria counts was correlated with the dose of ingested scFOS. We previously reported such effect in healthy volunteers using another dose-response relationship study. However, the optimal and well tolerated dose of scFOS which led to a significant increase in fecal bifidobacteria under usual diet was 10 g/d. These differences are probably due to the relatively small size of each sample, which decreased the power of the study. The increase in total anaerobes observed at the dose of 10 g/d is probably due to the high stimulation of colonic microflora induced by prebiotics [8,9]. Fecal bifidobacteria and anaerobes levels observed in this study were similar to those found in previous studies [3,5,10] in healthy volunteers using scFOS.

ScFOS has been studied extensively and its bifidogenic effect was demonstrated in well-controlled human trials [3-5,8]. However, Roberfroid et al. concluded from a compilation work that in a large population, there seems to be no dose-response effect of these non-digestible carbohydrates on bifidobacteria for daily intake doses between 4 and 40 g/d [11]. Here, we found a linear dose-response relationship from 2.5 to 10 g/d, suggesting a dose-effect relationship. Such a relationship has been previously found using scFOS, but the range of doses was larger, from 5 to 20 g/d [8], and in another trial using a mixture of galacto- and fructooligosaccharides as supplementation of a term infant's formula [12]. It was not found with others

substrates, such as galacto-oligosaccharides alone, resistant starch and soybean oligosaccharides [7].

We did not find any significant reduction in any other genus. There is little published data in human available in the literature for comparison. Contradictory results have been reported in this field, as Gibson et al. [3] showed a significant reduction in *Bacteroides* using 15 g/d oligofructose, although Rao [9] found an increase in *Bacteroides* using oligofructose at dose of 5g/d. Moreover using oligofructose at 8 g/d, 2 authors did not find any effect on *Bacteroides* [13,14]. The reasons for these discrepancies are unclear.

A decrease in colonic pH might reduce the risk of developing colonic cancer, since an inverse correlation between stool pH and colon cancer risk was observed [15,16]. A slight acidification of fecal contents during scFOS ingestion was observed in animals [17] and in humans [13]. In our previous studies, fecal pH did not change during the ingestion of scFOS [5,8]. However, as the fecal pH is the net sum of the degree of short chain fatty acid absorption and bicarbonate secretion during passage through the colon, fecal pH does not reflect the pH in the colon under physiological conditions [18,19].

Symptoms relating to gas production in the gut are widely reported in human prebiotic feeding studies, but remain very mild at recommended intakes [20,21]. When compared to placebo, we did not find any significant digestive intolerance symptoms except minor bloating in scFOS. Neither was a dose-response relationship

for digestive symptoms observed. In a threshold study evaluating symptomatic response to varying levels of scFOS ingested regularly by 14 healthy volunteers, excessive flatus and borborygmi were recorded by about 10% of volunteers at 10 g/d of scFOS and excessive flatus, borborygmi and bloating by about 20-30% of volunteers at 20 g/d [22]. In another study in which 10 volunteers ingested 15g/d FOS for 12 d, gaseous symptoms such as abdominal cramps, excess flatus and bloating were all significantly more severe in subjects ingesting the FOS than in sucrose control subjects ($P < 0.05$) [23]. 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 comprising 10 healthy volunteers who ingested 12.5 g/d of scFOS for 12 d, only bloating was found significantly more frequent during the scFOS ingestion period than during placebo ingestion ($P < 0.05$), but was very mild and present in 5/10 volunteers only [5]. From all these results, it appears that the most common symptoms noted during scFOS administration are excess flatus and /or bloating, but only a minority of subjects experiences them and they are usually very mild.

CONCLUSION

This study confirmed that scFOS is bifidogenic and well tolerated in healthy volunteers. For the first time, a bifidogenic effect appeared at 2.5 g/d of scFOS, and a dose-response relationship was demonstrated from 2.5 to 10 g/d..

COMPETING INTERESTS

Yoram Bouhnik, Laurent Raskine, Guy Simoneau: no affiliations

Damien Paineau, Francis Bornet: Nutri-Health SA, Rueil-Malmaison, France

AUTHORS' CONTRIBUTIONS

Yoram Bouhnik: study design, data collection, data analysis, writing of the manuscript

Laurent Raskine: data collection

Guy Simoneau: data collection, data analysis

Damien Paineau: writing of the manuscript

Francis Bornet: study design, writing of the manuscript

ACKNOWLEDGEMENTS

This study was supported by a grant from the Health & Nutrition Group, Eridania-Beghin Say, B-1800, Vilvoorde, Belgium.

REFERENCES

1. M. Hirayama, N. Sumi, H. Hidaka (1989). Purification and properties of fructooligosaccharides producing beta-fructofuranosidase from *Aspergillus niger* ATCC 20611. *Agric Biol Chem*; (53): 667-673.
2. C. Molis, B. Flourie, F. Ouarne, M. F. Gailing, S. Lartigue, A. Guibert, F. Bornet, J. P. Galmiche (1996). Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. *Am J Clin Nutr*; 3 (64): 324-8.
3. G. R. Gibson, M. B. Roberfroid (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr*; 6 (125): 1401-12.
4. R. K. Buddington, C. H. Williams, S. C. Chen, S. A. Witherly (1996). Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. *Am J Clin Nutr*; 5 (63): 709-16.
5. Y. Bouhnik, B. Flourie, M. Riottot, N. Bisetti, M. F. Gailing, A. Guibert, F. Bornet, J. C. Rambaud (1996). Effects of fructo-oligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. *Nutr Cancer*; 1 (26): 21-9.
6. C. L. Bullen, A. T. Willis (1971). Resistance of the breast-fed infant to gastroenteritis. *Br Med J*; 770 (3): 338-43.
7. Y. Bouhnik, L. Raskine, G. Simoneau, E. Vicaut, C. Neut, B. Flourié, F. Brouns, F. Bornet (2004). The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *Am J Clin Nutr*; 80 (6): 1658-64.
8. Y. Bouhnik, K. Vahedi, L. Achour, A. Attar, J. Salfati, P. Pochart, P. Marteau, B. Flourie, F. Bornet, J. C. Rambaud (1999). Short-chain fructo-oligosaccharide

- administration dose-dependently increases fecal bifidobacteria in healthy humans. *J Nutr*, 1 (129): 113-6.
9. A. V. Rao (1999). Dose-response effects of inulin and oligofructose on intestinal bifidogenesis effects. *J Nutr*, 7 Suppl (129): 1442S-5S.
 10. Y. Bouhnik, B. Flourie, L. D'Agay-Abensour, P. Pochart, G. Gramet, M. Durand, J. C. Rambaud (1997). Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *J Nutr*, 3 (127): 444-8.
 11. M. B. Roberfroid, J. A. Van Loo, G. R. Gibson (1998). The bifidogenic nature of chicory inulin and its hydrolysis products. *J Nutr*, 1 (128): 11-9.
 12. G. Moro, I. Minoli, M. Mosca, S. Fanaro, J. Jelinek, B. Stahl, G. Boehm (2002). Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *J Pediatr Gastroenterol Nutr*, 3 (34): 291-5.
 13. T. Mitsuoka, H. Hidaka, T. Eida (1987). Effect of fructo-oligosaccharides on intestinal microflora. *Nahrung*; 5-6 (31): 427-36.
 14. NDO: Healthy Food for the Colon. Symposium LUW,, Wageningen University., 1997. *Prebiotic effect of the (fructosyl-1-fructose) Fm-type inulin hydrolysate in humans*. E. Menne, N. Guggenbuhl
 15. S. L. Samelson, R. L. Nelson, L. M. Nyhus (1985). Protective role of faecal pH in experimental colon carcinogenesis. *J R Soc Med*; 3 (78): 230-3.
 16. S. L. Malhotra (1982). Faecal urobilinogen levels and pH of stools in population groups with different incidence of cancer of the colon, and their possible role in its aetiology. *J R Soc Med*; 9 (75): 709-14.

17. H. Hidaka, T. Eida, T. Takizawa, T. Tokunaga, Y. Tashiro (1986). Effects of fructo-oligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora*; (5): 37-50.
18. D. F. Evans, G. Pye, R. Bramley, A. G. Clark, T. J. Dyson, J. D. Hardcastle (1988). Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut*; 8 (29): 1035-41.
19. C. Florent, B. Flourie, A. Leblond, M. Rautureau, J. J. Bernier, J. C. Rambaud (1985). Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an in vivo study). *J Clin Invest*; 2 (75): 608-13.
20. G. R. Gibson, E. R. Beatty, X. Wang, J. H. Cummings (1995). Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology*; 4 (108): 975-82.
21. J. J. Rumessen, S. Bode, O. Hamberg, E. Gudmand-Hoyer (1990). Fructans of Jerusalem artichokes: intestinal transport, absorption, fermentation, and influence on blood glucose, insulin, and C-peptide responses in healthy subjects. *Am J Clin Nutr*; 4 (52): 675-81.
22. F. Briet, L. Achour, B. Flourie, L. Beaugerie, P. Pellier, C. Franchisseur, F. Bornet, J. C. Rambaud (1995). Symptomatic response to varying levels of fructo-oligosaccharides consumed occasionally or regularly. *Eur J Clin Nutr*; 7 (49): 501-7.
23. T. Stone-Dorshow, M. D. Levitt (1987). Gaseous response to ingestion of a poorly absorbed fructo-oligosaccharide sweetener. *Am J Clin Nutr*; 1 (46): 61-5.

FIGURE

Figure 1: Correlation between the dose of ingested scFOS and the fecal bifidobacteria counts on the d15 – d8 difference

TABLES

Table 1: Faecal bifidobacteria and total anaerobe counts (m±SEM, log cfu/g) in 40 healthy volunteers assigned to a 7-d consumption of short-chain fructo-oligosaccharides (scFOS) at a dose from 2.5 to 10 g/d or a placebo

scFOS dose (g/d)	Total anaerobes			Bifidobacteria		
	d8*	d15*	P**	d8*	d15*	P**
0 (placebo)	12.59 ± 0.17	12.54 ± 0.12	-	10.06 ± 0.29	9.57 ± 0.21	-
2.5	12.32 ± 0.21	12.55 ± 0.14	NS	9.15 ± 0.59	9.39 ± 0.70	0.02
5.0	12.48 ± 0.18	12.66 ± 0.14	NS	10.21 ± 0.21	10.67 ± 0.22	0.03
7.5	12.45 ± 0.16	12.55 ± 0.13	NS	9.28 ± 0.49	9.85 ± 0.35	0.01
10	11.65 ± 0.37	12.68 ± 0.14	0.03	9.00 ± 0.81	10.18 ± 0.60	0.003

*days 1-8 was a run-in period during which no treatment occurred, but subjects excluded from their diet fermented dairy products containing viable bifidobacteria and limited consumption of food products containing high level of non-digestible oligosaccharides.

** Statistical analyses were performed using an unpaired t-test

Table 2: Bacterial counts and pH (m±SEM, log cfu/g) in 40 healthy volunteers assigned to a 7-d consumption of short-chain fructo-oligosaccharides (scFOS) at a dose from 2.5 to 10 g/d or a placebo

scFOS dose (g/d)	<i>Lactobacillus</i>			<i>Bacteroides</i>			<i>Enterobacteria</i>			pH		
	d8*	d15*	P**	d8*	d15*	P**	d8*	d15*	P**	d8*	d15*	P**
0 (placebo)	5.21 ± 0.36	5.18 ± 0.41	-	8.76 ± 0.45	8.53 ± 0.32	-	6.93 ± 0.29	7.07 ± 0.37	-	6.94 ± 0.13	6.91 ± 0.11	-
2.5	4.66 ± 0.48	5.14 ± 0.60	NS	8.93 ± 0.28	9.08 ± 0.17	NS	6.93 ± 0.26	7.28 ± 0.33	NS	6.68 ± 0.18	6.75 ± 0.14	NS
5.0	5.34 ± 0.66	5.99 ± 0.67	NS	8.87 ± 0.18	9.09 ± 0.28	NS	7.34 ± 0.33	7.81 ± 0.32	NS	6.48 ± 0.15	6.52 ± 0.11	NS
7.5	5.37 ± 0.40	5.97 ± 0.39	NS	8.74 ± 0.25	8.61 ± 0.29	NS	6.30 ± 0.29	6.78 ± 0.26	NS	6.66 ± 0.13	6.78 ± 0.13	NS
10	5.40 ± 0.41	5.73 ± 0.83	NS	8.51 ± 0.41	9.39 ± 0.26	NS	6.28 ± 0.40	6.58 ± 0.32	NS	7.05 ± 0.21	7.20 ± 0.25	NS

*days 1-8 was a run-in period during which no treatment occurred, but subjects excluded from their diet fermented dairy products containing viable bifidobacteria and limited consumption of food products containing high level of non-digestible oligosaccharides.

** Statistical analyses were performed using an unpaired t-test

Table 3: Observed frequencies for digestive symptoms (n and % of column totals), during the treatment period in 40 healthy volunteers assigned to a 7-d consumption of short-chain fructo-oligosaccharides (scFOS) at a dose from 2.5 to 10 g/d or a placebo

scFOS dose (g/d)	Excess flatus		Bloating		Borborygmi		Abdominal pain	
	n	%	n	%	n	%	n	%
0 (placebo)	6/8	75	5/8	62.5	4/8	50	3/8	37.5
2.5	7/8	87.5	6/8	75	5/8	62.5	4/8	50
5.0	6/8	75	4/8	50	5/8	62.5	3/8	37.5
7.5	7/8	87.5	4/8	50	3/8	37.5	5/8	62.5
10	7/8	87.5	6/8	75	4/8	50	5/8	62.5

Table 4: Intensity of digestive symptoms (scores) in 40 healthy volunteers assigned to a 7-d consumption of short-chain fructo-oligosaccharides (scFOS) at a dose from 2.5 to 10 g/d or a placebo

scFOS dose (g/d)	Excess flatus			Bloating			Borborygmi			Abdominal pain		
	d1-d8*	D8-d15*	P**	d1-d8*	D8-d15*	P**	d1-d8*	d8-d15*	P**	d1-d8*	d9-d15*	P**
0 (placebo)	4.12 ± 1.35	4.12 ± 1.45	-	3.87 ± 1.80	2.62 ± 0.92	-	3.25 ± 1.80	2.75 ± 1.47	-	1.50 ± 1.00	0.50 ± 0.26	-
2.5	1.75 ± 0.45	3.62 ± 1.28	NS	1.50 ± 0.42	3.12 ± 1.28	0.03	1.37 ± 0.49	3.00 ± 1.36	NS	1.12 ± 0.61	1.12 ± 0.61	NS
5.0	3.00 ± 1.08	5.25 ± 1.71	NS	1.75 ± 0.97	3.37 ± 1.76	0.03	1.75 ± 0.92	2.87 ± 1.42	NS	0.87 ± 0.51	0.87 ± 0.51	NS
7.5	3.50 ± 0.86	3.50 ± 0.68	NS	1.75 ± 1.03	1.50 ± 0.62	NS	1.75 ± 0.64	0.62 ± 0.32	NS	2.12 ± 0.87	2.12 ± 0.87	NS
10	3.37 ± 1.16	4.75 ± 1.03	NS	2.25 ± 1.06	2.62 ± 1.16	NS	1.50 ± 0.75	1.62 ± 0.86	NS	2.12 ± 0.81	2.12 ± 0.81	NS

*days 1-8 was a run-in period during which no treatment occurred, but subjects excluded from their diet fermented dairy products containing viable bifidobacteria and limited consumption of food products containing high level of non-digestible oligosaccharides.

** Statistical analyses were performed using Fisher's test

Symptom intensity was noted every day using a 4-grade scale 0: no symptom; 1: mild symptoms; 2: moderate symptoms; 3: severe symptoms. Cumulative daily scores were calculated for each period [D1, D8 a.m.], [D8 p.m., D15] and are reported in the present table as $m \pm \text{sem}$. Severity of symptoms and cumulative daily scores can be related via the following scale: 0: no symptom; 1-7: mild symptoms; 8 -14: moderate symptoms; 15-21: severe symptoms.

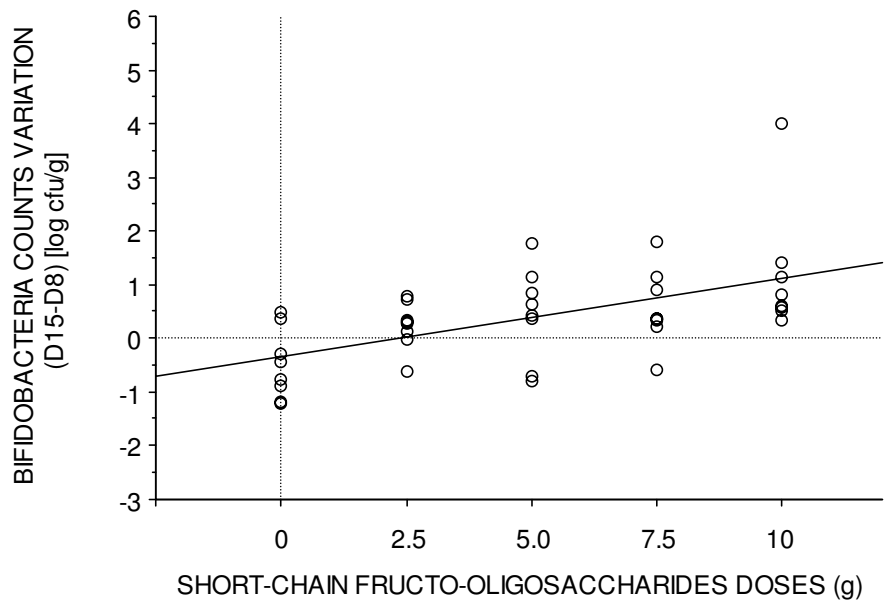


Figure 1