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Effect of Xylooligosaccharide on the Growth of Bifidobacteria

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Abstract Xylooligosaccharide was found to have a favorable effect on human intestinal flora. Xylooligosaccharide was utilized by bifidobacteria, but it was not utilized by *Escherichia coli* and *Clostridium* spp. *in vitro*. *In vivo*, xylooligosaccharide (5 g/day) promoted the growth of bifidobacteria, lowered fecal pH and helped to maintain the fecal water content within normal range. These results showed that xylooligosaccharide can selectively promote the growth of bifidobacteria and help to establish favorable environmental conditions in the intestines.

Key words: xylooligosaccharide; bifidobacteria

An intestinal bacterial flora is closely related to human health conditions, and a predominance of *Bifidobacterium* is particularly considered to be beneficial (*2, 3, 5, 8, 9, 12*).

Extensive studies have shown that various *Bifidobacterium*-containing preparations and oligosaccharides used as energy sources by bifidobacteria are effective for stimulating the propagation of intestinal bifidobacteria (*13, 16*), but there are problems with presently available products regarding their development in the intestine (*14*), stability (Fuwa et al, 1982; presented at 1st Annual Meeting of Inst. of Neosugar, Tokyo), and selective utilization by intestinal bacteria (*6, 11, 17*). The purpose of this study was to solve these problems by using xylooligosaccharides. Here we describe the utilization of xylooligosaccharide by intestinal bacteria *in vitro*. And during oral administration of xylooligosaccharide at 5 g per day for 3 consecutive weeks, the effect on human intestinal flora, water content in feces and pH variation, which are considered to be related to the increase in bifidobacteria, are described.

MATERIALS AND METHODS

Preparation and composition of the test sugar (XO). The xylooligosaccharide used was obtained from birch wood xylan hydrolyzed by *Trichoderma*-derived enzyme xylanase (Sumyzyme TX, Shin Nihon Chemical Co., Ltd.). It was decolored with active charcoal powder, desalted and concentrated. The methods for preparation

Table 1. Availability of xylooligosaccharide by intestinal bacteria

Bacteria		XO	X1	X2	X3	G1
Bifidobacterium adolescentis	CIFL N0037	⫲	+	⫲	⫲	⫲
bifidum	aE318 (RIKEN)	±	±	−	−	⫲
infantis	CIFL N0050	⫲	nt	nt	nt	⫲
longum	CIFL N0044	⫫	⫲	⫫	±	⫲
breve	IV-14 (RIKEN)	±	±	−	−	⫲
Lactobacillus acidophilus	I-68 (RIKEN)	±	±	±	−	⫲
casei	I-139 (RIKEN)	±	±	−	−	⫲
fermentum	ATCC 14931	−	−	−	−	⫫
gasseri	ATCC 33323	±	±	±	±	⫲
salivarius	I-108 (RIKEN)	±	±	−	−	⫲
Streptococcus pyogenes	CIFL A0017	−	−	−	−	±
Eubacterium aerofaciens	S-12 (RIKEN)	−	−	−	−	⫲
lentum	CIFL N0059	−	−	−	−	−
limosum	V-60 (RIKEN)	−	±	−	−	+
nitritogenes	CIFL N0085	−	−	−	−	⫫
Propionibacterium granulosum	CIFL N0083	−	−	−	−	⫫
acnes	GAI #5468	−	−	−	−	⫫
Bacteroides fragilis	GAI #5562	±	+	−	−	+
vulgatus	V-114 (RIKEN)	+	⫫	±	±	⫫
bivius	GAI #5518	−	−	−	−	±
intermedius	46 (Nihon U.)	−	−	−	−	+
ovatus	ATCC 8483	−	±	−	−	±
thetaiotaomicron	GAI #5628	−	−	−	−	−
Clostridium perfringens	GAI #5526	−	−	−	−	+
paraputrificum	V-96 (RIKEN)	−	−	−	−	⫲
difficile	V-6 (RIKEN)	−	−	−	−	+
butyricum	GAI #7503	±	+	±	−	⫫
cadaveris	XI-10 (RIKEN)	−	−	−	−	⫫
Fusobacterium mortiferum	GAI #5442	−	−	−	−	±
necrophorum	GAI #5634	−	−	−	−	±
russii	GAI #0317	−	−	−	−	±
varium	GAI #5566	−	−	−	−	±
Escherichia coli	M-1 (RIKEN)	−	±	−	−	+
Staphylococcus aurens	CIFL A0012	−	−	−	−	⫫
epidermidis	CIFL A0018	−	−	−	−	⫲
haemolyticus	CK6-2 (Tokyo)	−	−	−	−	⫫
Peptostreptococcus productus	X-45 (RIKEN)	±	±	±	±	+
magnus	GAI #5528	−	−	−	−	−
Veillonella parvula	GAI #5602	−	−	−	−	−
Klebsiella pneumoniae	CIFL A0003	±	±	±	±	+
Enterococcus faecalis	ST-201 (RIKEN)	−	−	−	−	⫲
Mitsuokella multiacidus	VI-70 (RIKEN)	+	⫲	−	−	⫲
Enterobacter aerogenes	CK121-1 (Tokyo)	+	+	+	+	±
cloacae	CIFL A0001	±	±	±	±	±
Megamonas hypermegas	CIFL N0060	+	⫫	±	−	⫫
Morganella morganii	ME138-1 (Tokyo)	−	−	−	−	⫫

A portion of bacterial culture fluid was inoculated in PYF broth with 0.5% various sugar. After incubation at 37°C, for 4 days, pH was determined for criteria of bacterial growth.

Judgement of bacterial growth: ΔpH=(test pH)−(control pH).

−, ΔpH<1.0; ±, 1.0≦ΔpH<1.5; +, 1.5≦ΔpH<2.0; ⫫, 2.0≦ΔpH<2.5; ⫲, 2.5≦ΔpH; nt, not tested.

XO, xylooligosaccharide mixture mainly composed of xylobiose; X1, xylose; X2, xylobiose; X3, xylotriose; G1, glucose.

and purification of xylooligosaccharide are described in the Japanese Patent Publication (No. 62-155095). This saccharide (XO) was composed of 22% xylose, 58% xylobiose, 13% xylotriose and 7% other saccharides. It was further purified by fractionation with column chromatography, and crystalline xylose, xylobiose and xylotriose were obtained and used in *in vitro* experiments.

In vitro *carbohydrate utilization by intestinal bacteria.* The strains used in this test were *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Peptostreptococcus*, *Lactobacillus*, *Enterococcus*, *Escherichia coli* and other strains (Table 1), and were obtained from the culture collection of the Institute of Anaerobic Bacteriology (School of Medicine of Gifu University), the Calpis Intestinal Flora Laboratory, RIKEN (the Institute of Physical and Chemical Research), Tokyo Sogo Rinsyo Kensa Center and School of Dentistry of Nihon University.

Culture medium used: glucose, xylooligosaccharide, xylose, xylobiose or xylotriose was added to the Peptone-Yeast-Fildes medium (*4*), with each made up to a 0.5% final concentration and sterilized in an autoclave at 120°C for 20 min. The bacterial strains for the study were incubated on BL agar plate, then inoculated twice on modified GAM broth and incubated under anaerobic conditions at 37°C for 24 hr. A 0.03-ml portion of this culture fluid was inoculated in 1.5 ml of the PYF medium and the pH was determined after anaerobic incubation at 37°C for 4 days (96 hr). The anaerobic jar had an atmosphere of nitrogen gas containing 10% CO_2 and 3% H_2. The degree of utilization was determined by the decrease in pH (see Table 1 footnote). Control experiment was performed with sugar-free medium.

Oral administration of xylooligosaccharide. Nine healthy men, aged 50 to 60 years, were given xylooligosaccharide at 5 g daily for 3 weeks, during which their daily food intake was not limited except for the omission of live microorganism preparations, fermented beans, drugs and other bifidus factors. Feces were analyzed twice before the initial administration, at the 1st, 2nd and 3rd week after initial administration, and the 6th week after the final administration. The analytical items were intestinal flora, pH, water content and volatile fatty acids (VFA), according to the procedures of Mitsuoka et al (*9*).

Data processing. Values are mean$\pm$SD. A Student t test was used to evaluate the significance of the changes observed. Moreover, chi-square test was used regarding fecal water content. We could determine whether xylooligosaccharide maintains the fecal water content within normal range, taking the sample numbers during administration and both before and after administration, and grouping them according to whether they are within or beyond normal range. The results were classified in a 2×2 table and we examined them using chi-square test.

RESULTS

Carbohydrate Utilization Assay by Intestinal Bacteria

Table 1 shows the extent of utilization of xylooligosaccharide (XO) and its saccharide components, xylose (X1), xylobiose (X2) and xylotriose (X3), as well

Table 2. Effect of XO on intestinal flora

Organisms	Before administration (n=18)	During administration (n=27)	After administration (n=9)
Enterobacteriaceae	7.42±1.32 (100)[a]	7.14±1.39 (100)	6.98±1.34 (100)
Streptococcus	7.68±1.13 (100)	7.09±1.12 (100)	8.11±1.29 (100)
Staphylococcus	2.98±0.57 (56)	2.76±0.27 (26)	2.70±0.57 (22)
Yeasts	4.05±1.03 (72)	4.14±1.30 (74)	4.19±1.48 (78)
Pseudomonas	2.72±0.57 (33)	2.65±0.49 (7)	4.34 (10)
Corynebacterium	3.20±0.90 (17)	2.88±0.52 (19)	2.75±0.92 (22)
Bacillus	7.08 (6)	6.57±1.97 (17)	4.05±1.34 (22)
Lactobacillus	7.04±1.17 (100)	6.91±1.21 (100)	7.25±1.60 (89)
Bifidobacterium	9.71±0.44 (100)	10.11±0.39 (100)**[b]	10.12±0.48 (100)
Eubacterium	10.02±0.42 (100)	10.02±0.45 (100)	9.98±0.55 (100)
Bacteroidaceae	10.27±0.51 (100)	10.14±0.61 (100)	10.58±0.29 (100)
B. fragilis group	9.82±0.54 (100)	9.56±0.83 (100)	10.06±0.45 (100)
Megamonas hypermegas	8.73±0.45 (56)	8.81±0.50 (56)	9.05±0.71 (67)
Peptostreptococcus	9.21±0.87 (94)	9.20±0.82 (93)	9.60±1.17 (100)
Clostridium perfringens	4.52±1.77 (33)	4.58±0.92 (44)	3.87±0.96 (67)
Clostridium—other	9.38±0.50 (100)	9.44±0.64 (100)	9.81±0.43 (100)
Veillonella	6.87±1.70 (89)	6.19±1.74 (89)	6.96±1.65 (89)
Megasphaera	5.35±3.18 (11)	8.55±0.26 (22)**	7.25±2.33 (22)
Total	10.69±0.36 (100)	10.71±0.43 (100)	10.96±0.33 (100)

[a] Means±S.D. of log no. of bacteria/g wet feces (%: frequency of occurrence).
[b] Significant difference from the counts before administration: $P<0.01$**

as that of glucose. Intestinal bacteria seem to have more difficulty in utilizing saccharides of higher molecular weight, such as xylose, xylobiose, and xylotriose. However, among the *Bifidobacterium* species, *B. adolescentis* showed strong utilization of disaccharide xylobiose and trisaccharide xylotriose as energy sources. Moreover, a xylooligosaccharide mixture mainly composed of xylobiose was well utilized by *B. adolescentis*, *B. infantis* and *B. longum*. As for other bacteria, all *Lactobacillus* species utilized glucose, while xylooligosaccharide was only slightly utilized, except for by *L. fermentum*. Xylooligosaccharide was used slightly by some of *Bacteroides* species, but the extent of utilization was lower than that of glucose. *Staphylococcus* and *Escherichia coli* did not utilize xylooligosaccharide as an energy source, but utilized glucose well. Most *Clostridium* species utilized glucose, but not xylooligosaccharide.

Effect on Intestinal Bacterial Flora

Table 2 shows the changes in intestinal microflora of the nine subjects who were administered xylooligosaccharide. There was no change in the total bacterial counts throughout the experimental period. The counts of *Bifidobacterium* increased significantly during administration of xylooligosaccharide (t-test, significant at 1%), while those of *Bacteroides* and *Clostridium* did not increase during administration. In individual data (Fig. 1), an increase in the counts of *Bifidobacterium* was recorded in 7 of 9 subjects. The remaining two subjects had very high

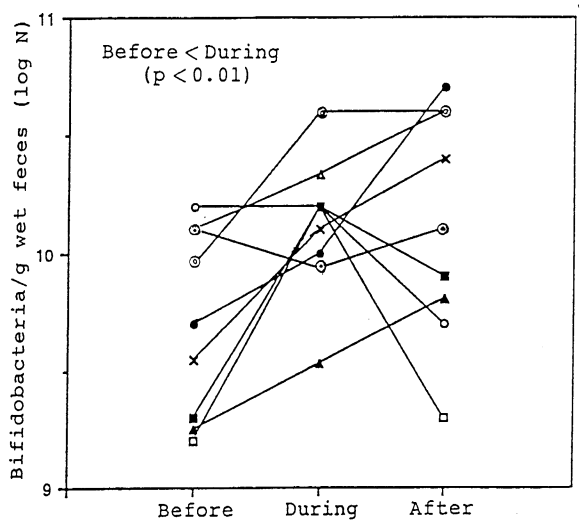

Fig. 1. Effect of XO on counts of *Bifidobacterium* in intestine. ×, ○, ●, □, ■, △, ▲, ◎, ◉: each value of nine volunteers. Before[a], before XO administration; During[b], during XO administration; After[c], 6th week after the final XO administration; XO[d], xylooligosaccharide mixture mainly composed of xylobiose.

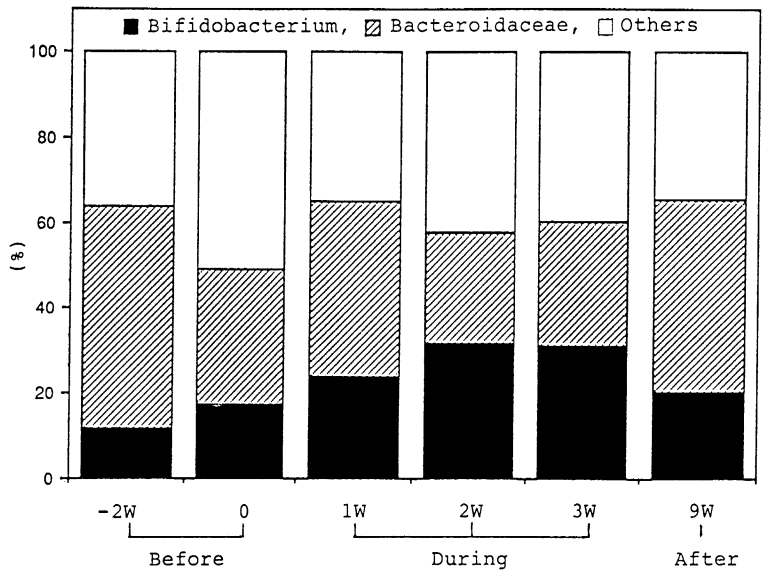

Fig. 2. Changes of intestinal flora of volunteers by the administration of XO. Column shows the percentage of each bacterial group to total counts in the feces. [a,b,c,d]: see the footnote to Fig. 1.

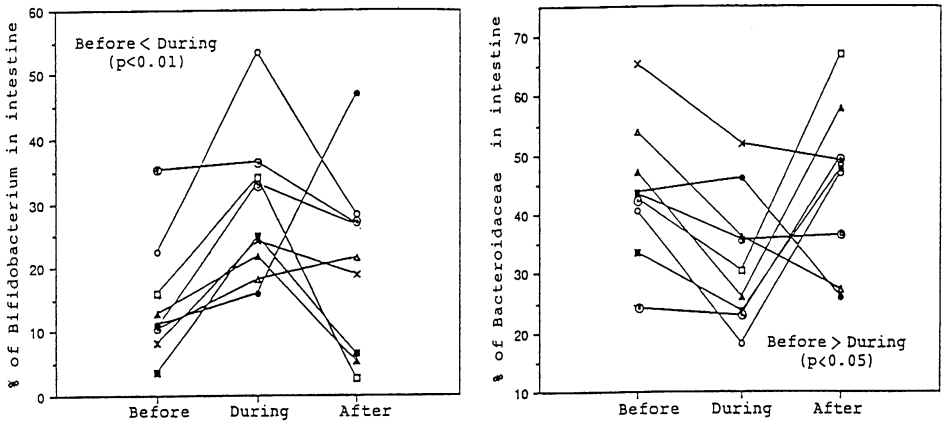

Fig. 3. Effect of XO on ratio of *Bifidobacterium* and Bacteroidaceae in intestine.[a,b,c,d]: see the footnote to Fig. 1. ×, ○, ●, □, ■, △, ▲, ◎, ◉: symbols are the same as in Fig. 1.

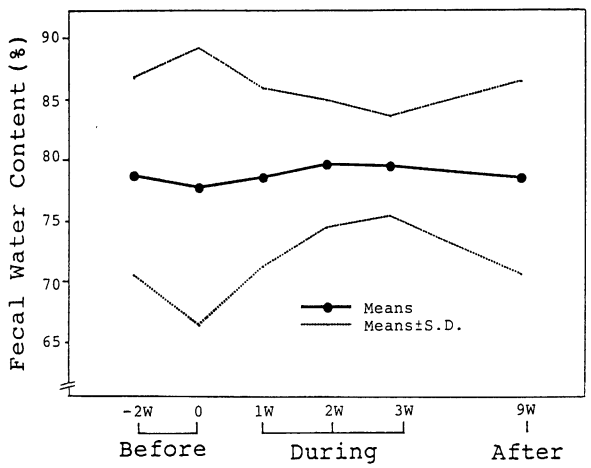

Fig. 4. Changes of fecal water content of volunteers by the administration of XO. [a,b,c,d]: see the footnote to Fig. 1.

counts of *Bifidobacterium* before administration.

Figure 2 shows the relative percentage of each bacterial group to total bacteiral counts in the feces. *Bifidobacterium* increased to 24–32%, compared with 12–17% before administration of xylooligosaccharide (*t*-test, significant at 1% level). The percent of bifidobacteria significantly decreased after discontinuation of administration and there was no significant difference between the levels before and after administration. On the other hand, *Bacteroides*, which had accounted for 52% at 2 weeks before administration, decreased to 26% at the 2nd week after administration had begun. In the individual data, all the subjects showed an increased

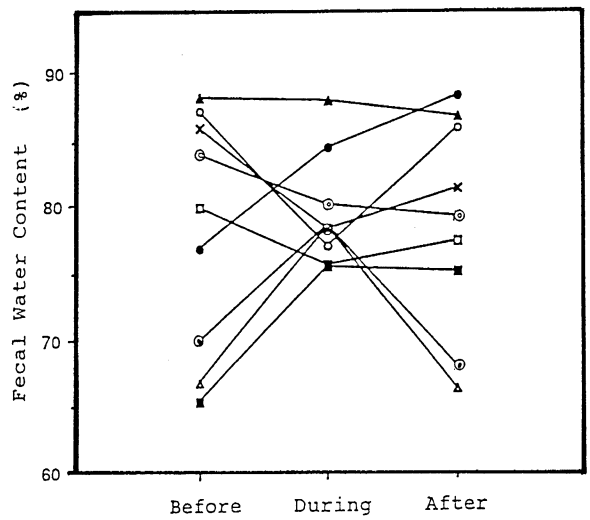

Fig. 5. Effect of XO on fecal water content. ^{a,b,c,d}: see the footnote to Fig. 1. ×, ○, ●, □, ■, △, ▲, ◎, ◉: symbols are the same as in Fig. 1.

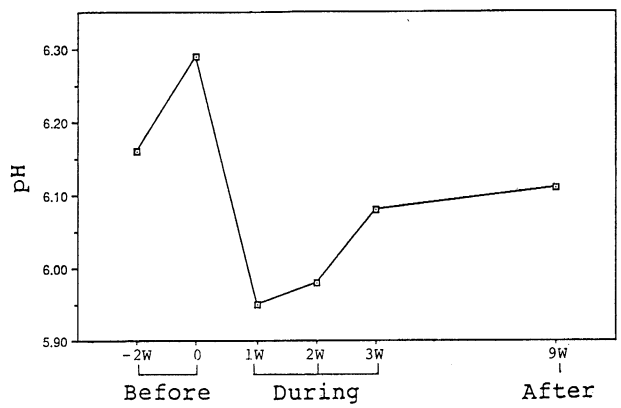

Fig. 6. Changes of fecal pH of volunteers by the administration of XO. ^{a,b,c,d}: see the footnote to Fig. 1.

relative percent of bifidobacteria during administration of xylooligosaccharide. The relative percent of *Bacteroides* dropped in 7 of the 9 subjects (Fig. 3).

Changes in Fecal Water Content

The average fecal water content in the nine subjects, with the standard deviation of the mean, is shown in Fig. 4. It varied between 66 and 90% before administration and tended to congregate at the 80% level during administration. The mean values were nearly 79%, while the standard deviation of approximately 10%

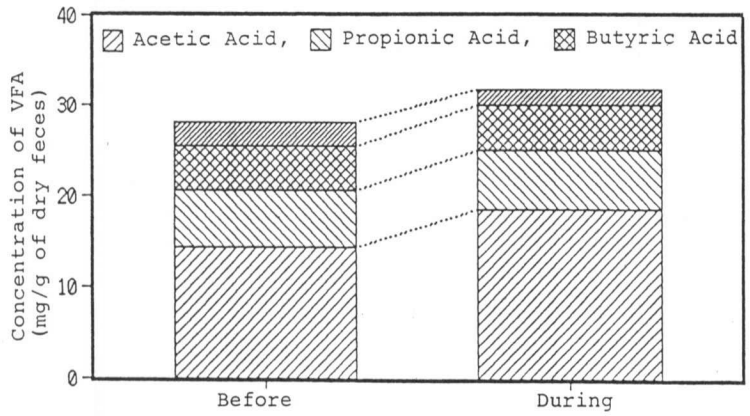

Fig. 7. Effect of XO on the fecal excretion of volatile fatty acid (VFA). ^{a,b,c,d}: see the footnote to Fig. 1.

before administration decreased with time: they were 7.3, 5.2 and 4.1% at 1, 2 and 3 weeks from the start of administration, respectively. The normal range of water content of feces is considered to be 70–80% (*7*). As for individual data (Fig. 5), only 3 subjects were within the normal range of fecal water content before administration and after discontinuation of administration, but 7 subjects were within the normal range during administration. The intake of xylooligosaccharide caused a significant increase in the number of subjects whose fecal water content was within normal range (chi-square: 2×2 table, significant at 5% level).

pH Changes of Feces

Figure 6 shows the mean fecal pH values of the nine subjects, which ranged from 6.16 to 6.29 before administration and from 5.95 to 6.08 during the period of xylooligosaccharide intake.

VFA Changes in Feces

Figure 7 shows the analytical results of volatile fatty acids (VFA) in the feces of five subjects who showed markedly lowered fecal pH. The total amount of organic acids in the feces was 28.32 ± 10.62 mg (mean$\pm$SD), of which acetic acid accounted for 50.40 ± 2.6%, propionic acid for 22.12 ± 3.97% and butyric acid for 16.25 ± 3.98%, coming to a total of nearly 90% of the organic acids in the feces. The amounts of organic acids rose to 31.75 ± 5.79 mg during administration of xylooligosaccharide, and the percentage of acetic acid remained at 57.54 ± 3.48%, indicating a significant increase (*t*-test, significant at 1% level).

DISCUSSION

The ability of intestinal bacteria to utilize xylooligosaccharide as an energy source was studied *in vitro* and *in vivo*. The *in vitro* results indicated that xylooligo-

saccharide is used by bifidobacteria, but not by *Clostridium* and *E. coli*. As this xylooligosaccharide is very stable at pH 7 or less (*1*), it is not degraded by the low-pH gastric fluid, nor by human and animal digestive enzymes (Okazaki et al, 1989; presented at 12th Carbohydrate Symposium, Osaka). Therefore, xylooligosaccharide can reach the lower digestive tract without being absorbed and can be utilized effectively as a growth-stimulating substance for bifidobacteria. In addition, xylobiose, a main component of xylooligosaccharide, is utilized by *Bifidobacterium*, especially by *B. adolescentis*, which is resident in most adults' intestines (*10*). So this xylooligosaccharide might be suitable as a bifidus factor in adults. In fact, in the *in vivo* experiment, when the subjects ingested xylooligosaccharide, bifidobacteria counts increased from the 10% pre-administration level to 32% at the 2nd week after the start of administration. As the relative percentage of bifidobacteria decreased after discontinuation of the administration, we concluded that the increase had been due to the effect of xylooligosaccharide. As administration of xylooligosaccharide selectively increased bifidobacteria and hardly caused any increase in other bacteria, xylooligosaccharide was found to be an effective bifidus factor.

Fecal pH decreased during administration of xylooligosaccharide. In this study, significant difference in the amount of fecal ammonia was not observed (data not shown), but increased fecal acetic acid was observed during administration. These results suggest that this lowered pH might be caused mainly by the increased amount of acetic acid. As a low pH stimulates the intestinal lining and promotes intestinal peristalsis, improvement of constipation might be expected (*15*, *18*). Also, evidence that ingested xylooligosaccharide tends to maintain fecal water content within normal level indicates that both hard and soft feces attain a suitable fecal firmness.

In summary, xylooligosaccharide increased the amount of intestinal bifidobacteria, lowered the fecal pH and maintained the fecal water content within normal range, which all contributed to the establishment of suitable environmental conditions for the intestine. These effects of xylooligosaccharide could be attained with a daily dose of 2 g.

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