Full Paper

Bifidobacteria Microflora
Vol. 13 (2), 51–63, 1994

Effective Dose of Lactosucrose on Fecal Flora and Fecal Metabolites of Humans

Hiroyoshi HARA,[1] Shou-Tou LI,[1] Masakuni SASAKI,[1]
Tsukasa MARUYAMA,[1] Atsushi TERADA,[1] Yukiko OGATA,[2]
Koki FUJITA,[2] Hiroshi ISHIGAMI,[2] Kozo HARA,[2]
Isao FUJIMORI[3] and Tomotari MITSUOKA[1]

[1]*Department of Food Hygiene, Nippon Veterinary and Animal Science University, 1–7–1 Kyonan-cho, Musashino, Tokyo 180,*
[2]*Ensuiko Sugar Refining Co., Ltd, 1–4–21 Yaesu, Chuo-ku, Tokyo 103, and*
[3]*Taiyo Pet Food Co., Ltd., Tokyo Kaijo Bldg. 2–3–3 Shiba, Minato-ku, Tokyo 105*

(Received for publication, June 2, 1994)

Abstract Lactosucrose (4^G-β-D-galactosylsucrose) was fermented *in vitro* by bifidobacteria and lactobacilli, and to a limited degree by the *Bacteroides fragilis* group, clostridia, eubacteria, and enterobacteriaceae. The effects of dietary lactosucrose on the fecal flora and fecal metabolites were studied in eight healthy volunteers (20–23 years of age) who ingested 3 g of lactosucrose/day for 7 days followed by 6 g of lactosucrose/day for 7 consecutive days. During lactosucrose intake, the counts of bifidobacteria were increased significantly ($p<0.001$), whereas the counts of clostridia, including *Clostridium perfringens*, and bacteroidaceae were decreased significantly ($p<0.05$) compared with the values before the intake. The total bacterial counts were decreased significantly ($p<0.05$) on day 14 during the intake. The frequency of occurrence of lecithinase-negative clostridia was decreased significantly ($p<0.05$) when compared with the values before and after the intake. No detectable changes occurred in the counts of other organisms throughout the experimental periods. Fecal concentrations of ammonia, sulfide, phenol, ethylphenol, skatol and indole were decreased significantly ($p<0.05$) during lactosucrose intake. Acetic acid and lactic acid were increased significantly ($p<0.05$) during the intake. Fecal enzyme activity of β-glucuronidase was decreased significantly ($p<0.05$) on day 14 of the intake. Serum very low density lipoprotein (VLDL) was increased significantly ($p<0.01$) on day 14 during the intake. Mean fecal pH values decreased from 6.3 to 5.9, and mean water content increased 3.6% during the intake. Fecal weight was increased slightly during the intake. The results obtained showed that the effective dose of lactosucrose for all healthy adults is 3 g/day.

Key words: enzyme activity; intestinal flora; lactosucrose; organic acids; putrefactive products; serum lipids

The intestinal flora is composed of 100 trillion bacteria including 100 species, and contains a variety of enzymes that perform extremely varied types of metabolism in the intestine. They not only contribute to normal physiological functions, but also participate significantly in the causation of various disease states by biotransforming a variety of ingested or endogenous compounds to beneficial or harmful derivatives. Thus, this transformation may influence drug efficacy, toxicity, carcinogenesis and aging (*17*).

Recently, it has been reported that lactosucrose [4^G-β-D-galactosylsucrose or *O*-β-D-galacto-pyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1←2)-β-D-fructofuranoside] selectively enhanced the growth of bifidobacteria in the human intestine (*21*, *34*) and inhibited intestinal clostridia of dogs and cats, resulting in the reduction of putrefactive products in the intestine (*30*, *31*).

In the present study, we examined the effects of lactosucrose on the fecal flora, weight, water content, pH, metabolic activities, and the serum lipid levels in eight healthy volunteers.

MATERIALS AND METHODS

Preparation and composition of lactosucrose. Lactosucrose (LS) was prepared from lactose and sucrose by β-fructofuranosidase from *Arthrobacter* sp. K-1(*5*). The LS preparation was composed of 95.1% lactosucrose, 2.8% sucrose, 0.4% lactose, and 1.7% other oligosaccharides.

Fermentation of LS in vitro. A total of 103 strains of human intestinal bacteria including 22 strains of six species of bifidobacteria were used in this test. The saccharides tested were LS (Ensuiko Sugar Refining Co., Ltd., Tokyo), glucose (Wako Pure Chemical Co., Ltd., Tokyo), raffinose (Wako), lactulose (Sigma), fructo-oligosaccharides (FOS, Meiji Seika Co., Ltd., Tokyo), and soybean oligosaccharides refined (SOR, Calpis Food Industry Co., Ltd., Tokyo).

One milliliter of peptone yeast extract Fildes solution (PYF) broth with 0.5% of the saccharides were inoculated with 0.03 ml (10^8 CFU/ml) of the test organisms using an automatic multipoint inoculator 120 (Lifetec Co., Ltd., Saitama). The organisms were pre-cultured in PYF broth with 1% glucose, centrifuged at 3,000 rpm at 4°C for 20 min, and suspended at 0.5 ml with a diluent solution composed of 0.1% sodium thioglycolate, 0.1% L-cysteine HCl and 0.85% NaCl. The inoculated media were incubated anaerobically at 37°C for 96 hr by the steel wool method. After incubation, the pH of each medium was measured using an automatic multipoint pH meter 5051 (Lifetec Co., Ltd.), and scored in the following manner: −, pH >6.0; (+), pH 5.5–5.9; +, pH 5.0–5.4; ++, pH <4.9.

Subjects and diet. The subjects were eight healthy male volunteer students (Department of Food Hygiene, Nippon Veterinary and Animal Science University, Tokyo) who ranged in age from 20 to 23 years. They consumed the normal free-choice diet for 2 weeks before (CNT-1) and after (CNT-2) the LS period, as shown in Table 1. They were given the normal diet plus a cup of coffee with 3 g/day (LS-1) and 6 g/day (LS-2) of LS for the first and second weeks, respectively. None

Table 1. Experimental schedule

Diet ingested Experimental period	Control diet ← CNT 1 → Before intake	Lactosucrose ← LS-1 → During intake	 ← LS-2 → During intake	Control diet ← CNT 2 → After intake
Weeks	← 2 →	← 1 →	← 1 →	← 2 →
Lactosucrose (g/day)		3	6	
Sampling day	0	7	14	14
Item for analysis;				
Moisture content (%)	*	*	*	*
pH	*	*	*	*
Fecal weight (g)	*	*	*	*
Fecal flora	*	*	*	*
Metabolic products	*	*	*	*
Enzyme activity	*	*	*	*
Serum lipids	*	*	*	*

*: Items examined.

Table 2. Media and cultural methods for comprehensive investigation of intestinal flora

Medium	Organisms enumerated	Incubation method and dilution	Incubation at 37°C
Non-selective media			
Medium 10 (modified)	Fastidious anaerobes	Plate-in-bottle method 10^{-7}, 10^{-8}	4 days
EG agar	Anaerobes	Steel wool method replaced air with CO_2	3 days
BL agar	Anaerobes	10^{-6}, 10^{-7}, 10^{-8}	
Trypticase soy blood agar	Aerobes	Air 10^{-5}, 10^{-6}, 10^{-7}	1–2 days
Selective media		10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}	
BS agar	Bifidobacteria	Steel wool method replaced air with CO_2	3 days
ES agar	Eubacteria		
NBGT agar	Bacteroidaceae		
Neomycin Nagler agar	Clostridia		
VS agar	Veillonellae and Peptostreptococci		
LBS agar (modified)	Lactobacilli	Replaced air with CO_2	2 days
DHL agar	Enterobacteriaceae	Air	1 day
TATAC agar	Streptococci		2 days
PEES agar	Staphylococci		
NAC agar	Pseudomonas		
Potato dextrose agar	Yeasts and molds		
Media after heat treatment		10^{-1}, 10^{-3}, 10^{-5}	
BL agar and CW agar	Clostridia	Steel wool method replaced air with CO_2	2 days

of the subjects were given antibiotic treatment or other therapy or foods with abundant viable cultures for 1 month prior to and during the experiments.

This work was performed in accordance with the Helsinki Declaration as updated in Tokyo, 1975.

Table 3. Utilization of six oligosaccharides by various intestinal bacteria

Organism	No. of strains	control	Glucose	Lacto-sucrose	Lactulose	FOS	SOR	Raffinose
Bifidobacterium								
B. longum	6	—	++	++	++	++	++	++
B. adolescentis	7	—	++	++	++	++	++	++
B. breve	3	—	++	++	++	++	++	++
B. bifidum	3	—	++	+	++	—	—	—
B. infantis	2	—	++	+	++	+	+	+
B. pseudocatenulatum	1	—	++	++	++	++	++	+
Lactobacillus								
L. acidophilus	3	—	++	+	+	++	+	(+)
L. salivarius	2	—	++	++	++	++	++	++
L. casei	2	—	+	++	++	—	—	—
Bacteroides								
B. vulgatus	12	—	++	+	+	+	(+)	(+)
B. distasonis	5	—	++	+	+	+	+	+
B. fragilis	3	—	++	+	+	++	+	++
B. thetaiotamicron	2	—	++	+	+	+	+	+
B. uniformis	1	—	++	++	+	++	+	++
B. melaninogenicus	1	—	+	—	—	—	—	—
Rikenella								
R. microfusus	1	—	++	+	+	+	(+)	(+)
Clostridium								
C. perfringens	6	—	++	(+)	+	(+)	(+)	(+)
C. bifermentans	2	—	+	+	(+)	(+)	—	—
C. paraputrificum	3	—	++	(+)	+	—	—	—
C. ramosum	2	—	++	+	+	+	(+)	(+)
C. butyricum	1	—	++	+	++	+	+	+
Clostridium spp.	4	—	++	+	+	(+)	(+)	—
Eubacterium								
E. aerofaciens	8	—	++	(+)	+	+	+	—
E. limosum	2	—	++	—	—	—	—	—
E. lentum	1	—	—	—	—	—	—	—
Eubacterium spp.	1	—	++	+	+	—	—	—
Peptostreptococcus								
P. parvulus	1	—	++	—	—	—	—	—
Escherichia								
E. coli	3	—	+	(+)	(+)	—	—	—
Salmonella								
S. enteritidis	1	—	++	(+)	—	—	—	(+)
S. typhimurium	1	—	++	(+)	(+)	—	—	(+)
Proteus								
P. mirabilis	1	—	++	(+)	(+)	(+)	(+)	(+)
P. vulgaris	1	—	++	+	+	(+)	(+)	—
Aeromonas								
A. hydrophyla	2	—	++	—	—	(+)	—	—
A. sobria	1	—	+	—	—	(+)	—	—
Enterococcus								
E. faecalis	2	—	++	+	+	(+)	(+)	(+)
Staphylococcus								
S. aureus	4	—	++	+	+	(+)	(+)	(+)

Bacillus								
B. cereus	1	—	++	+	+	+	+	+
B. mycoides	1	—	++	+	+	+	+	+

FOS, fructo-oligosaccharides; SOR, soybean oligosaccharides refined.
Judgment of bacterial growth: —; pH≧6.0, (+); 5.5≦pH<5.9, +; 5.0≦pH<5.4, ++; pH<4.9

Sampling of feces and blood. Freshly voided fecal samples were collected from each subject on immediately before lactosucrose intake, on days 7 and 14 during the intake, and on day 14 after the intake. The samples were weighed to determine fecal output and were stored immediately at 4°C. Fecal flora, water content and enzyme activities were analyzed within 3 hours after the collection of samples. The remainder of the samples were frozen at −80°C for later analysis of bacterial metabolites.

Blood samples were obtained 60 min before and after breakfast on the same day of collection of fecal specimens.

Bacteriological analysis. The fecal flora was analyzed using the method and media of Mitsuoka et al. (*18*) as shown in Table 2. Two grams of feces were suspended in 18 ml of anaerobic diluent and mixed thoroughly, then a decimal dilution series (10^{-1}–10^{-8}) was prepared. From appropriate dilutions, 0.05-ml aliquots were inoculated to 4 non-selective and 11 selective media. In addition, dilutions (10^{-1}–10^{-5}) of the fecal specimens were heated at 80°C for 10 min to select for clostridial spores and a portion of 0.1 ml of each dilution was inoculated onto BL agar and CW agar (Nissui Seiyaku, Co., Ltd., Tokyo). After incubation, each plate was examined for bacterial colonies. The identification of 16 bacterial groups, yeasts and molds was performed with Gram-reaction, colonial and cellular morphologies, spore formation, aerobic growth, and selected biochemical characteristics. The bacterial counts are expressed as the $\log_{10}$ of the number of bacteria per gram wet feces.

Analysis of fecal metabolites. Measurement of fecal ammonia was carried out using a potentiometer IOL-30 (DKK Co., Ltd., Tokyo) with ammonia gas sensing electrode 1716 using the method of Terada et al. (*32*). For sulfide analysis, 1 g of feces was dissolved in 44 ml of distilled water, to which was added 5 ml of S-DIMA (40 g NaOH, 10 g L-ascorbic acid, 9.3 g EDTA 2Na, 500 ml glycerine, and 485.7 ml distilled water), and then measured using a sulfide electrode 7100 (DKK Co., Ltd.). The concentrations of fecal organic acids were determined by high-performance liquid chromatography organic acid analysis system (HPLCOA, Shimazu Seisakusho Co., Ltd., Tokyo). One gram of fecal samples was homogenized with 5 ml of distilled water and centrifuged at 3,000 rpm at 4°C for 20 min. A 10-ml portion of the suspension was analyzed on HPLCOA with a two-column serial connection of SCR-102H (8 mm I.D.×300 mm L.) added a guard column SCR-102H (6 mm I.D.×50 mm L.). Operating parameters were as follows: column oven temperature, 40°C; flow rate of mobile phase (5 mM *p*-toluenesulfonic acid aqueous solution), 0.8 ml/min.; flow rate of buffer (20 mM Bis-Tris aqueous

solution containing 5 mM *p*-toluenesulfonic acid and 100 mM EDTA), 0.8 ml/min. Fecal contents of indole, skatol and phenols were analyzed by gas chromatography using the method of Yoshihara (*35*). Fecal enzyme activities were determined using the method of Rowland et al. (*24*).

Assay of serum lipids. Serum phospholipids (*27*), triglycerides (*16*), free fatty acids (*26*), and cholesterol (*13*) were measured enzymatically, and lipoprotein was measured by use of a turbidimetric method (*25*) and agarose gel electrophoresis assay (*23*).

Measurement of fecal properties. Fecal water contents were measured by using approximately 1 g samples weighed before and after drying in a vacuum oven 625 (Ikemoto Rika Kogyo Co., Ltd., Tokyo) at 105°C. Fecal pH values were determined with a flat glass electrode (DKK Co., Ltd., Tokyo). The weight of fecal output for 24 hr was measured for 3 consecutive days, including the days of sampling for the bacterial analyses.

Statistical analysis of data. Student's *t*-test and the method of Fisher's direct probability calculation were used for statistical analysis of the fecal flora. Student's *t*-test was used for analysis of pH value, moisture content, weight, enzyme activities and metabolites of feces and serum lipids.

RESULTS

Utilization of LS In Vitro

The results of fermentation test *in vitro* of six saccharides including LS are shown in Table 3. LS was fermented by all the bifidobacteria tested, lactobacilli including *L. acidophilus*, *L. salivarius* and *L. casei* as effectively as glucose, and other bacteria, such as *Bacteroides fragilis* group, clostridia, *Staphylococcus aureus*, *Enterococcus faecalis*, bacilli, *Salmonella*, and *Escherichia coli* to a lesser degree than glucose. FOS, SOR and raffinose showed similar fermentation patterns, except that they were not fermented by *B. bifidum*, *L. casei*, *C. paraputrificum*, *E. coli*, and *Salmonella* (except for raffinose).

Fecal Flora Analysis

The effects of LS intake on the composition of fecal flora are shown in Table 4. The levels of bifidobacteria were significantly ($p<0.001$) increased during LS intake, while the levels of lecithinase-positive clostridia including *Clostridium perfringens* and bacteroidaceae were significantly ($p<0.05$) decreased during the intake, expect for the levels of *C. perfringens* in one volunteer. The percentage of bifidobacteria to total bacteria were improved from 10.5% before the intake to 38.9% and 38.1% on days 7 and 14 in the LS period, respectively. The levels of total bacteria were significantly ($p<0.05$) decreased on day 14 in the LS period. In frequency of occurrence, lecithinase-negative clostridia during LS intake were decreased significantly ($p<0.05$) when compared with before and after the intake. No detectable changes occurred in the levels of other organisms throughout the experimental period.

Table 4. Effect of lactosucrose intake on fecal flora of 8 human volunteers

Organism	Before intake day 0	During intake		After intake day 14
		day 7	day 14	
Total bacteria	11.01±0.11[a]	10.94±0.11	10.87±0.07*	10.93±0.18
Bifidobacteria	10.03±0.15 (100)[b]	10.53±0.11*** (100)	10.45±0.11*** (100)	9.92±0.17 (100)
Bacteroidaceae	10.80±0.12 (100)	10.63±0.10* (100)	10.50±0.11** (100)	10.72±0.19 (100)
Eubacteria	9.95±0.38 (100)	9.57±0.39 (100)	9.58±0.36 (100)	9.64±0.42 (100)
Peptococcaceae	9.55±0.26 (100)	9.14±0.69 (100)	9.19±0.58 (100)	9.69±0.47 (100)
Megasphaerae	9.08±0.67 (88)	8.94±0.52 (63)	8.60±0.67 (88)	9.46±0.41 (88)
Curved rods	9.03±0.49 (50)	8.80 (25)	8.60 (25)	9.05±0.48 (50)
Veillonellae	6.80±1.21 (50)	6.65 (25)	7.43 (25)	6.79±0.16 (38)
Clostridia				
Lecithinase-positive	5.74±1.38 (88)	3.78±0.92* (88)	3.62±0.89* (88)	4.40±1.39 (100)
Lecithinase-negative	9.33±0.35 (100)	8.90±0.52 (50)*	8.50±0.59 (38)*	9.26±0.50 (100)
Lactobacilli	7.68±1.78 (100)	7.28±1.16 (100)	7.77±1.08 (100)	7.34±1.55 (100)
Enterobacteriaceae	8.81±1.22 (100)	8.51±0.55 (100)	8.11±1.17 (100)	8.79±0.46 (100)
Pseudomonas	3.41±0.85 (63)	3.94±0.53 (38)	4.04±0.75 (38)	3.01±0.32 (88)
Streptococci	8.43±0.75 (100)	8.53±1.11 (100)	8.56±0.52 (100)	8.61±0.87 (100)
Staphylococci	4.70±1.36 (75)	3.62 (25)	3.10 (25)	3.44±1.40 (38)
Bacilli	4.28±1.66 (63)	2.97±0.94 (38)	3.19 (25)	2.54 (25)
Yeasts	3.24±0.52 (63)	3.50±0.55 (63)	3.60±0.96 (63)	3.09±0.55 (75)
Molds	1.60 (13)	(0)	2.30 (13)	(0)

[a] Data expressed as mean log number per gram feces±S.D.

[b] Figures in parentheses are frequency of occurrence (%).

Significant difference from the counts of day 0 (before the intake):

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Fecal Metabolic Products

A significant decrease ($p<0.05$) in the amounts of fecal ammonia, sulfide, indole, phenol, and skatol were observed during LS intake, compared with those before the intake (Table 5).

The concentrations of total organic acids, lactic acid, and acetic acid were significantly ($p<0.05$) increased during the intake (Table 6).

Table 5. Effect of lactosucrose intake on fecal ammonia and fecal putrefactive products of 8 human volunteers

Product	Before intake Day 0	During intake		After intake Day 14
		Day 7	Day 14	
Ammonia	244.8±139.9[a]	96.4±48.0*	87.3±46.0*	248.0±79.8
Sulfide	5.1± 1.6	3.3± 1.2*	3.1± 1.4*	4.1± 1.7
Phenol	31.1± 19.9	11.4± 3.2*	11.9± 6.5*	21.0± 9.1
p-Cresol	54.0± 30.3	37.7±32.2	35.4±35.6	48.5±31.6
Ethylphenol	9.5± 9.5	2.1± 2.2	1.8± 2.2	5.5± 5.5
Indole	53.8± 23.4	16.4±10.5**	13.6± 8.7**	31.8±18.1
Skatol	21.9± 20.7	9.3±12.9	5.9± 4.9	17.8±14.6

[a] Data expressed as mean±SD of μg/g wet feces.
Statistically significant at *=$p<0.05$, and **=$p<0.01$ levels when compared with the values before the intake.

Table 6. Effect of lactosucrose intake on fecal organic acids of 8 human volunteers

Product	Before intake Day 0	During intake		After intake Day 14
		Day 7	Day 14	
Total organic acids	26.48±3.39[a]	33.70±7.12*	35.12±7.23*	28.00±4.11
Pyruvic	2.51±1.30	2.73±2.11	2.81±1.83	2.63±1.41
Malic	1.12±0.42	2.05±1.24	2.22±1.22	1.42±1.30
Succinic	2.18±1.26	3.21±1.86	3.87±2.50	2.64±1.52
Lactic	2.62±0.87	4.08±2.44**	4.01±0.80*	2.81±1.13
Formic	1.02±0.68	1.33±0.52	1.30±0.51	1.20±0.80
Acetic	8.76±1.03	12.30±1.22*	11.51±1.19*	9.13±1.12
Propionic	3.25±0.47	4.20±1.70	4.17±1.21	3.50±0.86
iso-Butyric	0.20±0.07	0.15±0.07	0.42±0.58	0.17±0.07
Butyric	2.88±1.15	2.00±0.88	2.70±0.99	2.58±0.66
iso-Valeric	0.24±0.24	0.07±0.11	0.18±0.11	0.23±0.41
Valeric	1.70±1.02	1.58±1.33	1.93±1.59	1.69±0.98

[a] Data expressed as mean±SD of mg/g wet feces.
Statistically significant at *=$p<0.05$ and **=$p<0.01$ levels when compared with the values before the intake.

Fecal Bacterial Enzyme Activities

Fecal β-glucuronidase activity was significantly ($p<0.05$) decreased on day 14 during LS intake compared with before the intake (Table 7). Other enzyme activities showed also a tendency to decrease during LS intake.

Lipid Analysis

The effect of LS intake on serum lipids is shown in Table 8. No significant changes occurred in the levels of total lipid, total cholesterol, phospholipid, triglycerides, lipoprotein and HDL-cholesterol throughout the experimental period. Low-density lipoprotein (LDL) and VLDL were increased on day 14 in the LS period, in particularly, significantly ($p<0.05$) in fasting VLDL, but in five volunteers, decreased slightly from 440.0±79.8 (mean±SD) mg/dl in fasting (433.0±92.6

Table 7. Effect of lactosucrose intake on fecal enzyme activities of 8 human volunteers

Enzyme	Before intake Day 0	During intake		After intake Day 14
		Day 7	Day 14	
β-Glucuronidase	13.25± 5.31[a]	8.11± 6.54	5.96± 4.29*	21.57±15.68
Nitroreductase	2.64± 0.43	1.80± 0.89	1.95± 0.70	2.11± 0.49
Azoreductase	1.25± 0.79	0.79± 0.61	0.70± 0.23	1.02± 0.39
β-Glucosidase	43.48±30.52	38.96±33.62	16.89±11.22	38.98±33.45

[a] Data expressed as mean±SD of μmol/h/g wet feces.

* Significant difference ($p<0.05$) from the values of day 0 (before the intake).

Table 8. Effect of lactosucrose intake on serum lipid of 8 human volunteers

Lipid	Before intake Day 0	During intake Day 14	After intake Day 14
Total lipids (mg/dl)	560.3±73.8[b]	560.9±82.3	558.0±109.9
	561.3±72.8	566.0±70.4	584.1±132.4
Total cholesterol (mg/dl)	177.3±24.2	173.0±24.4	174.7± 30.5
	173.8±26.1	167.4±22.9	171.1± 29.3
Triglycerides (mg/dl)	95.0±26.3	105.8±36.3	95.0± 40.7
	103.1±46.3	121.7±36.8	125.0± 59.5
Phospholipids (mg/dl)	198.7±18.1	194.9±22.9	200.3± 26.4
	196.9±20.0	192.6±20.1	201.7± 32.1
α-Lipoprotein (%)	34.8± 2.5	36.0± 3.0	37.5± 4.7
	33.9± 3.4	33.7± 3.3	33.7± 4.5
Preβ-Lipoprotein (%)	22.2± 3.3	22.8± 4.0	21.0± 4.0
	20.9± 4.1	23.2± 4.0	24.2± 3.7
β-Lipoprotein (%)	39.9± 3.3	39.8± 3.5	40.6± 3.4
	40.3± 5.8	38.0± 3.8	38.6± 2.7
HDL-cholesterol (mg/dl)	46.0± 8.6	44.1± 7.9	44.9± 8.3
	44.3± 8.5	43.6± 6.7	43.0± 8.8
LDL[a] (mg/dl)	423.9±73.3	450.1±77.1	439.4± 83.3
	412.1±86.8	429.1±75.5	423.9± 76.5
VLDL[a] (mg/dl)	126.7±47.7	150.3±59.6*	125.8± 56.9
	115.6±30.7	167.1±53.2	162.4± 78.0

[a] LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein.

[b] Data are expressed as mean±SD. The upper and lower lines are the values of before and after meal, respectively.

Statistically significant at *=$p<0.05$ level when compared with the values before the intake.

Table 9. Effect of lactosucrose intake on fecal weight, pH and water content of 8 human volunteers

Item	Before intake Day 0	During intake		After intake Day 7
		Day 7	Day 14	
Fecal weight (g)	95.6±16.2[a]	104.7±16.9	108.0±17.9	97.1±18.9
pH	6.3± 0.3	5.9± 0.3*	5.8± 0.4*	6.2± 0.2
Water content (%)	74.9± 4.2	78.4± 5.2	79.6± 2.5*	75.9± 2.7

[a] Data expressed as mean±SD.

* Significant difference ($p<0.05$) from the values of day 0 (before the intake).

mg/dl after meal) and 114.2±44.3 mg/dl (103.6±37.8 mg/dl) before LS intake to 439.2±94.6 mg/dl in fasting (430.4±83.8 mg/dl after meal) and 107.4±28.6 mg/dl (102.0±33.2 mg/dl) on day 14 of the intake, respectively.

Fecal Properties

Fecal water content was significantly ($p<0.05$) increased on day 14 in LS period, whereas fecal pH values were significantly ($p<0.05$) decreased during LS intake (Table 9). Fecal weight was increased at the rate of 9.5% and 13.0% on days 7 and 14 in the LS period, respectively, compared with before LS intake. The offensive odor of the feces was remarkably decreased during LS intake.

DISCUSSION

Although Fujita et al. (*6*) reported that LS was fermented by all species of *Bifidobacterium*, except for *B. bifidum in vitro*, our results showed that LS was fermented by all *Bifidobacterium* species including *B. bifidum*. The fermentation of LS by the intestinal bacteria was similar to that of lactulose (*9*).

LS escapes digestion by intestinal enzymes. Thus, it is not absorbed in the small intestine, and passes directly into the colon where it is fermented by LS-fermenting bacteria such as bifidobacteria which produces short chain fatty acids. The significant increase observed in bifidobacteria during LS intake (3 g or 6 g/day) was similar to those during intake of various oligosaccharides [lactulose 3 g (*29*), xylooligosaccharides 5 g (*22*), FOS 8 g (*9*), 4′-galactosyllactose 8 g (*20*), galacto-oligosaccharides 10 g (*12*), transgalactosyl oligosaccharides (TOS) 10 g (*18*), soybean oligosaccharides extract (SOE) 10 g (*8*), palatinose 12 g (*14*), isomalto-oligosaccharides 13.5 g (*15*), raffinose 15 g (*2*), or lactulose 30 g (*11*) per day] for healthy adults.

The significant decrease observed in *C. perfringens*, lecithinase-negative clostridia and bacteroidaceae during LS intake was similar to those during intake of raffinose (*2*), TOS (*28*), and lactulose (*29*), and suggests that an increase in fermentative anaerobes inhibits the growth of proteolytic bacteria during lactulose administration as reported by Vince et al. (*33*).

A decrease in clostridia has not been observed during intake of other oligosaccharides in previous reports (*8, 9, 12, 14, 15, 20, 22*). The discrepancies between our data and hitherto reported results may be due to heat treatment to detect lecithinase-positive clostridia and the use of Medium 10 for lecithinase-negative clostridia in our study, because the detection rate of clostridia (lecithinase-positive and lecithinase-negative) was markedly increased when these methods were used (unpublished data).

The tendency for enterobacteriaceae to decrease by day 14 during LS intake was similar to that of lactulose intake (*29*) but not to that of TOS (3 g/day) (*28*). The significant decrease in bacteroidaceae during LS intake showed a negative correlation with the increase in the number of fecal bifidobacteria and contributed to the decrease in total bacteria.

The putrefactive products in the human intestine are mainly composed of

potentially toxic substances (*3*, *33*) such as ammonia, sulfide, indole and phenols produced from amino acids and urea. The significant decrease of fecal ammonia concentration despite an increase in fecal weights in this study due to LS fermentation may lead to inhibition of deaminase and urease activity by decreasing the pH and increasing short chain fatty acids (*7*) in the large intestine. The decreased fecal sulfide concentration during LS intake showed a positive correlation with the decrease in *C. perfringens* and bacteroidaceae which produce hydrogen sulfide (*19*).

The enzyme activities of β-glucuronidase, nitroreductase, azoreductase, and β-glucosidase have been shown to be associated with a wide variety of intestinal bacteria (*1*). The decrease in fecal β-glucuronidase activity during LS intake was the same as the results of polydextrose (*4*) and lactulose (*29*) intake, and may be be a result of the decreased activity in *C. perfringens* and bacteroidaceae.

It has been reported that intake of dietary fiber leads to a decrease in serum lipids, blood sugar, blood pressure, and body weight. Hidaka et al. (*9*, *10*) demonstrated that FOS intake reduced serum triglycerides and free fatty acids in patients with hyperlipidemia. They (*10*) also reported a tendency for serum total cholesterol and apoprotein A to decrease after FOS intake for 1 month in patients with hypercholesterolemia. The reason for the increase in serum VLDL on day 14 during LS intake was not clear. It may be that an increase in apolipoprotein during LS intake results from the absorption of LS metabolites that affect hepatic function and are synthesized into apolipoprotein in the liver.

Ogata et al. (*21*) reported that the minimum effective dose of LS was 1–2 g/day for an increase of bifidobacteria, a decrease in clostridia, ammonia and sulfide in the feces. The results in present study showed that 3 g LS/day is sufficient effective dose for an increase in short chain fatty acids, a decrease in indole and phenol, and a significant lowering of pH for all healthy adults. Furthermore, although the intake of 3 g LS/day was associated with decreased β-glucuronidase and increased water content in the healthy adults, the difference was not significant (unlike 6 g LS/day intake). From these results, it was suggested that the effective dose of LS for healthy adults is 3 g/day.

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