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Prebiotic oligosaccharides change the concentrations of short-chain fatty acids and the microbial population of mouse bowel*

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Abstract: The purpose of this study was to clarify effects of selected oligosaccharides on concentrations of cecal short-chain fatty acids (SCFAs), total large bowel wet weight and wall weight, and cecal microbiota levels in mice. Mice were respectively given gavage of selected fructooligosaccharides (FOS), galactooligosaccharides (GOS), mannanoligosaccharides (MOS), and chitooligosaccharides (COS) [1000 mg/(kg body weight·d)]. Control group was given physiological saline solution. After 14 d treatment, SCFAs and lactate in mice cecum were significantly increased (P<0.05) by intake of oligosaccharides, especially FOS and GOS. Thus, providing these oligosaccharides as ingredients in nutritional formulas may benefit the gastrointestinal tract.

Key words: Oligosaccharides, Short-chain fatty acids (SCFAs), Bifidobacteria, Lactobacilli, Mouse
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INTRODUCTION

Prebiotics are food ingredients that selectively stimulate the growth and activities of specific bacteria in the gastrointestinal tract, usually bifidobacteria and lactobacilli (Roberfroid, 2000). And it is known that both the strains possess inhibitory activity towards the growth of pathogens, the ability to attach the intestinal epithelial cells, and other beneficial effects on the host (Greene and Klaenhammer, 1994; Fan *et al*., 2006). Furthermore, compared with other anaerobes in the gastrointestinal tract, lactobacilli and bifidobacteria have enzymes with lower activities, such as β-glucosidase, β-glucuronidase, urease, azoreductase, and nitrate reductase, which are involved in the formation of mutagens and carcinogens (Pool-Zobel *et al*., 1996).

The major fermentation products of prebiotic metabolism in large bowel are short-chain fatty acids (SCFAs), which had different effects on colon morphology and function such as supply of energy to the intestinal mucosa, lowering of the pH, and stimulation of sodium and water absorption (Scheppach, 1994). As one important SCFA, butyrate is associated with many beneficial biological functions in the colon. One of the important effects of butyrate on the DNA methylation is probably ascribed to modified gene expression, and the action mechanism is yet unclear, especially in the context of colon cancer (Pool-Zobel *et al*., 1996). However, butyrate may directly enhance cell proliferation of normal cells, but suppress cell proliferation of transformed cells. Furthermore, in the presence of butyrate, apoptoses may be enhanced in transformed cells but inhibited in normal cells (Hass *et al*., 1997).

Some indigestible oligosaccharides, such as galactooligosaccharides (GOS), fructooligosaccharides (FOS), mannanoligosaccharides (MOS), and chitooligosaccharides (COS), have been demonstrated to possess prebiotic activity in human (Campbell *et al*.,

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1997; Francis Suh and Matthew, 2000; Zentek *et al*., 2002; Wu *et al*., 2005; Shoaf *et al*., 2006). FOS has been shown to be indigestible by human enzymes in the small intestine, but is extensively fermented in the large bowel (Mitsuoka *et al*., 1987) to SCFA, which can be absorbed and metabolized by the host. Another prebiotic effect is also observed that bifidobacteria and lactobacilli, which play a beneficial role in improving gastrointestinal health, are increased by ingestion of FOS and GOS (McBain and Macfarlane, 2001; Smiricky-Tjardes *et al*., 2003).

To further investigate the role of selected oligosaccharides on concentrations of cecal SCFAs, total large bowel wet weight and wall weight, and concentrations of intestinal microbiota, and to discuss the mechanisms of oligosaccharides' action to the host, we have tested the effects on the large bowel by gavage of selected oligosaccharides in mice model.

MATERIALS AND METHODS

Oligosaccharides

Four commercial oligosaccharides were investigated for their effects on the intestine physiology of mice. The FOS (Meiji, Tokyo, Japan) contains 95% oligofructose with a degree of polymerization (DP) 2~7 and 5% of glucose, fructose and sucrose. The COS (Weikang, Shanghai, China) contains 90% deacetylated oligosaccharides with a DP 3~6. The MOS (East Angel, Hubei, China) with a DP 3~7 is at a purity of 95%, and the GOS (Deshipu, Xi'an, China) with a DP 3~8 was at a purity of 90%.

Animals and treatments

Forty male Balb/c mice were housed for 6 d after receipt. Every 8 mice were housed in suspended stainless steel cage under a 12-h cycle of light and darkness. Mice were given free access to diet and water. The animal use protocol was reviewed and approved by the Laboratory Animal Center of Zhejiang Chinese Medical University.

Five groups of mice at approximately 6 weeks of age with mean body weight (BW) of 20 g were fed with commercial diet (SLACOM-M02-F, SLAC Laboratory Animal Co., Ltd., Shanghai, China). Control groups were given gavage of physiological saline solution [20 ml/(kg BW·d)]. FOS, MOS, COS, and GOS groups were rendered gavage of 50 mg/ml corresponding oligosaccharide solution [20 ml/(kg BW·d)], respectively. The duration of the study was 14 d. All experiments were in accordance with National Research Council guidelines for the care and use of laboratory animals.

Sample collection

In 14 d mice were given gavage of each oligosaccharide every day. At the end of experiment period, mice were killed by an intracardiac injection of sodium pentobarbital. A ventral midline incision was made and the cecum and colon excised. Immediately after removal, the cecum and colon with contents were weighed to determine total weight. Cecal contents were collected, pH measured, and a 0.4-g aliquot immediately processed for SCFA analysis. The remaining cecal contents were immediately placed into a sterile assay tube for bacterial enumeration. After removal of the appropriate samples, the tissues were cleaned with water, blotted to dry, and weighed to determine cecal and colonic total wall weight.

SCFA and lactate analysis

Lactate concentrations were determined by gas chromatography after methylation and chloroformic extraction from cecal contents (Holdeman *et al*., 1977). Malonic acid was used as internal standard. SCFAs were analyzed by gas chromatography according to Jouany (1982) on supernatant of thawed samples centrifuged at 8000×*g* for 10 min. 4-Methyl valeric acid was used as internal standard.

Bacterial enumeration

Samples for enumeration of selected genera of cecal bacteria were serially diluted 10-fold with anoxic one-fourth strength peptone-water immediately after collection; 100 μl of the appropriate dilutions were inoculated onto duplicate plates using selective media for the enumeration of different bacteria. Bacteria were counted on Wilkins-Chalgren agar (Oxoid; total anaerobes), nutrient agar (Oxoid; total aerobes), Beerens agar (Oxoid; bifidobacteria), Rogosa agar (Oxoid; lactobacilli), Kanamycin Esculin Azide agar (KAA, Oxoid; *Enterococcus*), and Eosin Methylene blue (EMB, Oxoid; *Enterobacteriaceae*). Plates were incubated aerobically or in an

anaerobic chamber (H_2:CO_2:N_2=5:10:85, v/v/v) for 24 or 72 h as appropriate. After incubation, single colonies were counted, and the results were expressed as the log values of the colony-forming unit (CFU) per gram of wet weight of cecal content.

Statistical analysis

Tests for the statistical significance of differences were compared by one way analysis of variance (ANOVA). All statistics were performed using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Total large bowel weight, wall weight, and pH

Total large bowel weight, wall weight, and pH are presented in Table 1. Total weights of the colon and cecum were higher (P<0.05) in mice administrated with the four oligosaccharides compared with the control group. In addition, mice in the COS group had a lower (P<0.05) total cecal weight compared with the other three oligosaccharides groups. Cecal wall weight was higher (P<0.05) as a result of oligosaccharides consumption expect the COS group. Colonic wall weight was unaffected by oral administration with oligosaccharides. As shown in Table 1, cecal pH values were lower (P<0.05) after the gavage of the four oligosaccharides in mice compared with the control group. In contrast, compared with the control group, fecal pH values were lower (P<0.05) in the FOS and the GOS groups.

SCFA and lactate

All selected oligosaccharides significantly increased the cecal concentrations of total SCFAs compared with the control group (Table 2). In addition, the FOS and the GOS groups had higher (P<0.05) total SCFA concentrations than the MOS and the COS groups. Acetate, propionate, and butyrate in the cecum were increased with administration of oligosaccharides, while the concentration of valerate was not affected. Similarly, all selected oligosaccharides significantly increased (P<0.05) the cecal concentration of lactate. Among the oligosaccharides, FOS group had the highest lactate concentration, although it was not obvious, compared with the MOS and the GOS groups statistically.

Table 1 Colonic and cecal total and wall weights and pH values in mice by gavage of different oligosaccharides for 14 d

Group	Total weight (g)		Wall weight (g)		Cecal pH	Fecal pH
	Cecum	Colon	Cecum	Colon		
Control	0.60±0.07^a	0.43±0.11^a	0.15±0.03^a	0.23±0.03	6.99±0.13^a	7.01±0.11^a
FOS	1.19±0.10^b	0.71±0.16^b	0.27±0.04^b	0.23±0.04	6.20±0.09^b	6.75±0.07^b
COS	0.90±0.07^c	0.56±0.08^c	0.18±0.04^a	0.24±0.06	6.52±0.08^b	6.90±0.10^a
MOS	1.08±0.06^b	0.64±0.10^b	0.23±0.05^b	0.22±0.04	6.47±0.10^b	6.82±0.08^a
GOS	1.01±0.08^b	0.69±0.09^b	0.26±0.06^b	0.24±0.07	6.34±0.09^b	6.73±0.07^b

FOS: fructooligosaccharides; COS: chitooligosaccharides; MOS: mannanoligosaccharides; GOS: galactooligosaccharides. Values in a column not sharing the same superscript differ significantly, P<0.05. Results are shown as mean±SD, n=8

Table 2 Cecal concentrations of short-chain fatty acids (SCFAs) and lactate in mice by gavage of different oligosaccharides for 14 d

Groups	SCFA contentions (μmol/g)					Lactate (μmol/g)
	Total SCFA	Acetate	Propionate	Butyrate	Valerate	
Control	41.01±5.42^a	30.09±2.09^a	7.43±0.16^a	2.59±0.31^a	0.90±0.25	2.50±0.13^a
FOS	63.58±4.70^b	47.59±2.40^b	9.04±0.19^b	6.23±0.39^b	0.72±0.27	7.84±0.35^b
COS	49.59±3.67^c	37.13±3.11^c	7.94±0.10^c	3.64±0.40^c	0.88±0.31	5.20±0.21^c
MOS	57.54±4.50^d	42.30±3.09^c	8.29±0.13^b	6.40±0.35^b	0.55±0.49	7.30±0.20^b
GOS	61.54±3.82^b	45.97±1.53^b	8.82±0.21^b	6.02±0.52^b	0.73±0.33	7.18±0.36^b

FOS: fructooligosaccharides; COS: chitooligosaccharides; MOS: mannanoligosaccharides; GOS: galactooligosaccharides. Values in a column not sharing the same superscript differ significantly, P<0.05. Results are shown as mean±SD, n=8

Bacterial concentrations

After 14 d of gavage, the concentrations of bifidobacteria and lactobacilli in the cecum were higher (P<0.05) in each oligosaccharide group than in the control group. Specially, FOS had the most obvious stimulating effect to bifidobacteria and lactobacilli (Table 3). In addition, the concentrations of *Enterococcus* and *Enterobacteriaceae* were significantly reduced (P<0.05) by oral administration of each oligosaccharide. However, the total aerobes in the cecum were not significantly affected (P>0.05) after the oligosaccharide treatment. The concentrations of total anaerobes were higher (P<0.05) in the FOS and the GOS groups than in the control group.

Table 3 Microbiota concentrations in the cecum of mice by gavage of different oligosaccharides for 14 d

Group	Bacteria counts (log CFU/g wet stool)					
	Enterococcus	*Enterobacteriaceae*	Bifidobacteria	Lactobacilli	Total aerobes	Total anaerobes
Control	5.3±0.18^{a}	7.4±0.24^{a}	5.8±0.20^{a}	7.6±0.13^{a}	5.8±0.24	7.9±0.12^{a}
FOS	3.5±0.11^{b}	6.1±0.12^{b}	7.3±0.16^{b}	9.3±0.21^{b}	5.1±0.17	8.5±0.13^{b}
COS	4.8±0.20^{c}	6.6±0.09^{b}	5.9±0.08^{c}	7.9±0.10^{c}	5.6±0.19	8.0±0.12^{a}
MOS	4.0±0.11^{b}	5.6±0.13^{c}	6.6±0.10^{d}	9.1±0.13^{b}	5.3±0.14	8.2±0.08^{a}
GOS	3.6±0.15^{b}	6.6±0.21^{b}	6.9±0.21^{bd}	9.0 ±0.25^{b}	5.2±0.16	8.4±0.09^{b}

FOS: fructooligosaccharides; COS: chitooligosaccharides; MOS: mannanoligosaccharides; GOS: galactooligosaccharides. Values in a column not sharing the same superscript differ significantly, P<0.05. Results are shown as mean±SD, n=8

DISCUSSION

The purpose of this test was to study the effects of prebiotic oligosaccharides on the concentrations of intestinal SCFAs and cecal microbiota in mice. In humans, pH, SCFA concentration, and bacterial count can be measured only in stools, which accounts imperfectly for fermentation occurring mainly in the proximal part of the large intestine (Florent *et al*., 1985). Accordingly, we chose to use mice, a model allowing accessibility to cecal contents, which was validated for fermentation studies of nondigestible oligosaccharides. The present study demonstrated that the intake of selected prebiotic oligosaccharides improved concentrations of cecal SCFAs, especially FOS and GOS. The total weight of the colon or cecum was also increased after administration of oligosaccharides. In addition, the concentrations of bifidobacteria and lactobacilli were increased by oligosaccharides, while the concentrations of *Enterococcus* and *Enterobacteriaceae* were reduced.

SCFA, particularly acetate, propionate, and butyrate, are the dominating end-products of bacteria fermentation in the large bowel. The above metabolites are formed mainly from polysaccharide, oligosaccharide, protein, peptide, and glycoprotein precursors (Macfarlane and Macfarlane, 2002). In this test, it was observed that intake of selected oligosaccharides, especially FOS and GOS, improved concentrations of total cecal SCFAs including butyrate. By producing a greater concentration of butyrate, the preferred energy source for colonocytes, a trophic effect may be resulted within the gastrointestinal tract. While these oligosaccharides differ in their chemical compositions, they probably are fermented similarly by the microbiota of mice. However, compared with other oligosaccharides, COS had the weakest effect on the concentrations of SCFAs. Lactate in the cecum was also increased by administration of all oligosaccharides. In previous report, it was considered that SCFA and lactate could affect the transport processes of colonic epithelial cells, energy metabolism, growth, and cellular differentiation (Cummings, 1995).

The elevation noted in cecal total weight and wall weight after the oligosaccharide gavage may result from SCFAs, which may increase crypt depth and cell density by providing energy source and normalizing cell proliferation. Other researchers have demonstrated higher cecal weight and cecal wall weight caused by FOS compared with a diet with no fiber (Younes *et al*., 1995). However, in the present test, the wall weight of the colon was not affected by the selected oligosaccharides. This discrepancy may result from the fact that mice are cecal fermentors. In addition, the pH of cecum was significantly lowered by ingestion of each oligosaccharide, while fecal pH value was only reduced by FOS and GOS. The change

of pH was probably caused by the great levels of total SCFA productions.

The increases of bifidobacteria and lactobacilli in mice cecum were observed by the intake of selected oligosaccharides. The concentrations of both *Enterococcus* and *Enterobacteriaceae* were reduced in all the oligosaccharide groups. In addition, the total anaerobes of the cecum were stimulated with all the oligosaccharide treatments. Bifidobacteria population may result in changes in the microbial ecology of the colon, which is detrimental to other anaerobic bacterial species (Gibson and Wang, 1994). It was demonstrated that the intakes of oligofructose and inulin led to significant increase in bifidobacteria and decrease in potential pathogens (Gibson *et al*., 1995). Bifidobacteria, together with some lactobacilli species, play an important role in the eco-physiology of the intestinal microbiota, such as resistance to infection and diarrhoea disease (Saavedra *et al*., 1994) and stimulation of immune system activity (Kirjavainen *et al*., 2002).

In conclusion, SCFAs and lactate of the cecum in mice were increased by the intake of selected prebiotic oligosaccharides, especially FOS and GOS. The cecal total weight and wall weight were also increased, which may be a result of SCFA change. In addition, these selected oligosaccharides stimulating the bifidobacteria and lactobacilli in the cecum may be useful in promoting gastrointestinal health. Thus, providing these oligosaccharides as ingredients in nutritional formulas could benefit the health of the gastrointestinal tract.

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