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Fructooligosaccharides Alter Profiles of Fecal Short-Chain Fatty Acids and Bile Acids in Rats

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Abstract We investigated the effects of fructooligosaccharides and chicory inulin on the profiles of cecal and fecal short-chain fatty acids (SCFAs) and fecal bile acids in rats. Thirty-six Sprague Dawley male rats weighing about 190 g were randomly divided among four treatments; control diet, control diet + 6%(w/w) fructooligosaccharide (FOS), control diet + 6% chicory inulin oligosaccharide(CIOS), and control diet + 6% chicory inulin(CI). The rats were pair-fed and experimental diets were maintained for 5 weeks. Cecal and fecal pH was significantly decreased in rats that were fed fructooligosaccharides and chicory inulin. Cecal propionate was significantly elevated in rats fed CIOS diets, and butyrate was lower in rats fed FOS and CI than control values. Cecal lactate was significantly higher in the FOS group than in the control group. The fecal excretions of acetate and total SCFA were 200-300% higher in rats that were fed fructooligosaccharides and chicory inulin than in the control group. Lactate excretion was highest in rats that were fed FOS, followed by those fed CIOS and CI. The cholic acid and total bile acid concentrations in feces were significantly lower in the rats that were fed fructooligosaccharides and chicory inulin. The deoxycholic acid concentrations in wet feces were significantly lower in the groups of rats that ate CIOS (0.186 mM), FOS (0.274 mM), and CI (0.362 mM) than in the control group (0.595 mM). Among the fructans, short-chain fructooligosaccharide was more effective at decreasing colonic pH and lactate production, but medium-chain chicory inulin oligosaccharide was more effective at increasing fecal butyrate and lowering the fecal secondary bile acid concentration.

Keywords: fructooligosaccharide, inulin, short-chain fatty acids, lactate, secondary bile acids

Introduction

Oligosaccharides and chicory inulin are some of the most popular functional food components in world. They are referred as "prebiotics", which are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria such as bifidobacteria in the colon (1, 2).

The addition of inulin or oligofructose to the diet has been shown to significantly suppress aberrant crypt foci formation in the colon (3). This inhibition of colon cancer by oligosaccharide and inulin could be due to the alteration of the composition of microflora with a subsequent decrease in intestinal pH and pathogenic bacteria. Primary bile acids, cholic acid, and chenodeoxycholic acid are converted to secondary bile acids, deoxycholic acid, and lithocholic acid by colonic bacteria when the colonic pH is high. This is because the activity of bacterial 7-dehydroxylase is inhibited under acidic conditions (4). Bile acids (5), especially secondary bile acids, have been assumed to act at the promotion stages of the adenoma-carcinoma sequence in the colon (6, 7).

Inulin and oligofructose may be regarded as non-viscous, water-soluble dietary fibers of low-molecular weight because none of the molecules of fructose or glucose originating from these fiber sources appear in the portal blood. Instead, short-chain fatty acids (SCFAs) appear in the portal blood (1). Different oligosaccharide profiles and dietary fiber sources affect the absolute amounts of each SCFA as well as relative ratios of SCFA produced in the colon (8). This can affect pH in colon and feces. It has been shown *in vitro* as well as in living rats that acidic pH is very effective at lowering the concentration of soluble bile acids (9). Fecal bile acid excretion and bile acid concentration were shown to be affected by different kinds of soluble fibers in hamsters (10). Bile acid profiles were altered depending on the dietary fiber sources in rats (11).

An altered microbiota in the large bowel, depending on the substrates, can change relative and absolute SCFA concentrations, which affect colonic pH and bile acid profiles. It has been assumed that different functional attributes of inulin and oligofructose are due to differences in their chain lengths. Fructans consist of fructose polymers with a glucose molecule at the end, and their chain length varies from 2 units up to several hundred (12, 13). Therefore, this study was designed to determine whether two kinds of fructooligosaccharides and chicory inulin, which have different degrees of polymerization, have different effects on SCFA and lactic acid production and on dehydroxylation of primary bile acids in the large bowel.

Materials and Methods

Animals and diets Male Sprague- Dawley rats (Daehan Biolink Co., Chungbuk, Korea) weighing about 100 g were acclimated to the facility for 1 week while being fed a commercial pellet diet (Samyang Feed Co., Gangwon, Korea). The rats were kept individually in wire-mesh cages in a room maintained at 20±2°C and 50±5% relative

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humidity. When the rats weighed approximately 190 g, they were randomly divided into four groups of nine animals each, and then fed different experimental diets (Table 1) for 5 weeks.

The experimental diets were AIN76-based diets (14) that were supplemented with 6% oligosaccharide or chicory inulin, at 1% cholesterol, and 0.25% sodium cholate. We used a control diet and diets supplemented with fructooligosaccharide (FOS), chicory inulin oligosaccharide (CIOS), and chicory inulin (CI). The fructooligosaccharide (CJ Co., Seoul, Korea), which had 3-4 degrees of polymerization (DP), consisted of approximately 26% GF_2, 19.5% GF_3, and 9.5% GF_4+GF_5 on a dry substance basis. Chicory inulin (Sigma Chemical Co., USA) had about 35 DP. Chicory inulin oligosaccharide, which has mainly 5-7 DP, was produced from chicory inulin by endoinulinase from *Xanthomonas oryzae* No. 5 (15). The rats were fed *adlibitum* for 1 week and the FOS group consumed the least amount of food. Subsequently, other groups were pair-fed to the same food intake levels as the FOS group. The rats were weighed every other day; feces were collected during the last 4 days and were frozen for storage at -70°C. These experiments were performed according to the guidelines of animal experimentation appproved by Daegu University.

Sample collection The rats were anesthetized by administration of inhaled diethyl ether. The cecum was then excised and the contents were removed and weighed. Portions of the cecal contents and feces were collected into Eppendorf tubes containing a metaphosphoric acid solution (250 g/L) and were frozen at -70°C. Remaining portions were stored at -70°C for the analysis of lactate and bile acids.

Table 1. Composition of experimental diets (g/kg diet)

Ingredient	Control	FOS	CIOS	CI
Casein	200	200	200	200
Corn starch	404.5	404.5	404.5	404.5
Sucrose	168	58.3	108	108
Lard	40	40	40	40
Soybean oil	75	75	75	75
AIN-76 mineral mix*	40	40	40	40
AIN-76 vitamin mix*	10	10	10	10
Cholesterol	10	10	10	10
Sodium cholate	2.5	2.5	2.5	2.5
Cellulose	50	50	50	50
Fructooligosaccharide syrup	0	60(146.3)[1)]	0	0
Chicory inulin oligosaccharide	0	0	60[2)]	0
Chicory inulin	0	0	0	60[3)]

FOS, fructooligosaccharide; CIOS, chicory inulin oligosaccharide; CI, chicory inulin.
*AIN-76 mineral mix and AIN-76 vitamin mix from Teklad Co. (USA).
[1)]Composition of fructooligosaccharide syrup: 41% oligosaccharide (1-kestose + nystose + 1^F-fructofuranosyl nystose), 25% water, and 34% glucose and sucrose. The amount of FOS syrup added is in parenthesis.
[2)]DP5, 68%; DP > 5, 21%; inulin, 7%.
[3)]Approximately 35 DP.

Determination of short-chain fatty acids and lactate The cecal contents or feces were diluted four times with distilled water, and the pH was measured using a pH electrode (ORION model 420A, Thermo Electron Co., MA, USA) that was completely immersed in the sample solution. Approximately 0.4 g of cecal contents or feces in 0.8 mL of metaphosphoric acid solution (250 g/L) were mixed thoroughly with 3 mL of distilled water and centrifuged at 8000×g for 20 min at 4°C. The supernatant was filtered through a 0.45 μm filter (Millipore Co., MA, USA). One microliter of the filtrate was injected into a BP21 capillary column (25 m × 0.32 mm × 0.25 mm film thickness). Chromatographic analysis was performed using a gas chromatograph (Varian Star 3600X, Varian Inc., CA, USA) equipped with a flame ionization detector and a split injector. The injector temperature was 270°C and the detector temperature was 280°C. The carrier gas was N_2 and was set at a flow rate of 10 mL/min. The split flow ratio was 40 mL/min.

Lactate was determined by colorimetric method. Lactate was converted into acetaldehyde by the addition of concentrated sulfuric acid. The acetaldehyde was detected by its color reaction with p-hydroxydiphenyl in the presence of copper ions and was measured by absorbance at 560 nm (16).

Fecal bile acid analysis Fecal bile acids (lithocholic, deoxycholic, chenodeoxycholic, and cholic acid) were extracted according to the method of Child *et al.* (17). Their butyl ester derivatives were prepared, and the final dried extract was dissolved in trichloromethane. Nordeoxycholic acid (Research Plus Inc., NJ, USA) was used as an internal standard (18). One microliter was injected into a gas chromatograph (Varian Star 3600X) equipped with a DB-5 capillary column (30 m × 0.32 mm × 1 μm film thickness) and a flame ionization detector. The injector and detector temperature were set at 300°C. The oven temperature was programmed to increase from 50°C to 200°C at a rate of 30°C/min. The temperature was then increased in increments of 4°C/min from 200°C to 285°C, which was maintained for 70 min. N_2 (14.5 mL/min) was the carrier gas.

Statistical analysis The results were expressed as means ± standard error of the mean (SEM), and the SPSS release 11.0 software package was used for the statistical analyses. Differences among groups that were significant at $p<0.05$ using one-way ANOVA were tested by Duncan's multiple range test at $p<0.05$. Correlations between variables were shown by Pearson correlation coefficients.

Results and Discussion

Body weight gain, food efficiency, and fecal conditions The food intake among the groups was identical because the rats were pair-fed. The body weight gain of the rats that were fed FOS or CI diets was smaller than that of the groups on control and CIOS diets (Table 2). The feed efficiency ratio of the CIOS group was higher than that of the CI group. As shown in Table 3, the wet weights of the daily fecal output of the rats fed fructooligosaccharides and chicory inulin were significantly higher

Table 2. Body weights and feed efficiency ratios in rats fed diets containing fructooligosaccharides

	Initial body weight (g)	Body weight gain (g/day)	Feed efficiency ratio (g/100 g)
Control	190.6±6.5	7.09±0.182^{a}	28.3±0.85^{ab}
FOS	188.7±7.0	6.39±0.155^{b}	26.4±0.90^{ab}
CIOS	188.3±7.9	7.36±0.193^{a}	29.3±0.97^{a}
CI	190.6±6.4	6.48±0.239^{b}	26.0±1.14^{b}

Values are means±SEM. Values in the same column with different superscript letters are significantly different. $p<0.05$.
Rats in the control, CIOS, and CI groups were pair-fed with a food intake equal to that of FOS group rats.

Table 3. Output and moisture content of feces in rats fed diets containing fructooligosaccharides

	Wet weight (g/day)	Dry weight (g/day)	Moisture (%)
Control	4.59±0.266^{b}	3.07±0.084^{b}	32.1±2.30^{c}
FOS	6.70±0.880^{a}	3.37±0.291^{b}	46.5±3.66^{a}
CIOS	7.67±0.365^{a}	4.26±0.076^{a}	43.6±2.39^{ab}
CI	6.49±0.380^{a}	3.98±0.193^{a}	38.0±2.42^{bc}

Values are means±SEM. Values in the same column with different superscript letters are significantly different. $p<0.05$.

than that of the control group. Dry fecal weights were also higher in rats fed CIOS and CI. The moisture content of the test rat feces was in the order of FOS > CIOS > CI > control and was 45% higher in feces from rats fed FOS than in feces from the control group. None of the groups showed symptoms of diarrhea.

Profiles of short-chain fatty acids and lactate The profiles of short-chain fatty acids and lactate and the pH in the cecum and feces are shown in Table 4. Cecal pH, from lowest to highest, was in the order of FOS < CIOS and CI < control. Fecal pH was in the order of FOS < CI < CIOS < control. The pH was lower in the cecum than in the feces. Cecal acetate and total SCFA levels were not significantly different among groups. Cecal propionate was significantly elevated in the group fed CIOS, whereas butyrate was lower in the groups fed FOS and CI than in the control group. Cecal lactate was significantly higher in the FOS group than in the other groups. The fecal excretion of acetate was 200-300% higher in the rats that were fed fructooligosaccharides and chicory inulin than in the controls. The fecal propionate content was increased 139% in the rats that were fed chicory inulin, and the butyrate content was significantly greater in the rats fed chicory inulin oligosaccharide than those fed the control diet. Total SCFA excretion was increased about 200% over control values in the rats that were fed fructooligo-saccharides and chicory inulin. Lactate excretion was highest in the rats that were fed fructooligosaccharide (12.7 times that of the control group) followed by those fed chicory inulin oligosaccharide and chicory inulin. The microbial fermentation of oligosaccharides and dietary fiber is known to be involved in several physiological mechanisms that promote colonic health. One mechanism is the production of SCFAs. Different dietary fiber sources affect the relative concentration ratios of the different SCFAs in the colon (19), as observed in the work of Zoran *et al.* (20). They found that acetate, propionate, and butyrate were produced within the colonic lumen in molar ratios of 65:10:20 in rats consuming wheat bran diets, whereas the ratio was altered to 45:15:35 in rats consuming oat bran diets. Soy oliogosaccharide, inulin, and hydrolyzed inulin produced significantly higher propionate levels. Soy oligosaccharide and inulin compared with glucose produced significantly more butyrate after fermentation using human fresh fecal inocula for 24 hr (8).

The differences in SCFA and lactate profiles could have resulted from changes in gut microbiota. Campbell *et al.* (21) reported that rats fed oligosaccharide diets showed higher numbers of cecal bifidobacteria and total anaerobes and lower numbers of total aerobes, than in those given a control diet. Acetate production appeared to originate mainly from fermentation by glycolytic colonic bacteria and bifidobacteria (22). Bifidobacteria primarily produce acetate and lactate during fermentation (21). In the present study, diets supplemented with fructooligosaccharide or

Table 4. Short chain fatty acids and lactate in cecal contents and feces of rats fed diets containing fructooligosaccharides

Group	pH	Acetate	Propionate	n-Butyrate	Total SCFA	Lactate
Cecum (μmol/cecum)						
Control	6.25±0.045^{a}	97.37±14.66	62.58±8.350^{b}	9.154±3.189^{a}	171.70±24.83	2.61±0.243^{b}
FOS	5.19±0.096^{c}	144.71±26.09	93.15±19.69^{ab}	2.731±0.741^{b}	263.60±44.72	23.01±5.24^{a}
CIOS	5.42±0.137^{bc}	141.71±24.56	118.78±18.57^{a}	4.962±1.592^{ab}	276.54±42.92	11.09±2.74^{b}
CI	5.54±0.131^{b}	112.43±18.36	89.60±21.30^{ab}	1.960±0.506^{b}	213.28±39.78	9.29±1.84^{b}
Feces (μmol/day)						
Control	6.98±0.020^{a}	49.99±6.39^{b}	34.85±3.798^{b}	1.97±0.450^{b}	91.11±12.22^{b}	4.29±0.891^{b}
FOS	6.20±0.057^{d}	176.05±15.55^{a}	43.76±8.66^{b}	5.25±1.76^{b}	279.46±25.39^{a}	54.39±36.25^{a}
CIOS	6.71±0.043^{b}	197.61±26.03^{a}	51.99±6.45^{ab}	13.79±2.31^{a}	299.66±35.48^{a}	36.25±12.47^{a}
CI	6.49±0.517^{c}	149.41±98.51^{a}	83.21±18.99^{a}	5.85±1.09^{b}	270.51±49.68^{a}	32.03±6.30^{a}

Values are means±SEM. Values in the same column with different superscript letters are significantly different. $p<0.05$.

Table 5. Concentration and daily excretion of fecal bile acids in rats fed diets containing fructooligosaccharides

Group	Cholic acid	Chenodeoxycholic acid	Deoxycholic acid	Lithocholic acid	Sum of Bile Acids
			(μmol/g wet feces)		
Control	30.99±2.30[a]	0.420±0.118	0.595±0.139[a]	0.437±0.207	32.72±2.19[a]
FOS	25.03±1.94[b]	0.460±0.137	0.274±0.036[b]	0.219±0.044	26.12±1.74[b]
CIOS	21.52±1.74[b]	0.187±0.052	0.186±0.044[b]	0.191±0.079	22.16±1.74[b]
CI	20.28±2.22[b]	0.285±0.067	0.362±0.052[b]	0.115±0.038	21.17±2.32[b]
			(μmol/day)		
Control	132.69±13.27	1.72±0.508	2.41±0.449	1.64±0.663	139.53±12.88
FOS	138.30±16.32	2.88±0.971	1.53±0.267	1.10±0.197	144.55±17.18
CIOS	145.43±13.76	1.21±0.323	1.26±0.547	1.26±0.494	149.64±13.68
CI	118.85±15.57	1.72±0.437	2.16±0.368	0.695±0.264	124.21±15.95

Values are means±SEM. Values in the same column with different superscript letters are significantly different. $p<0.05$.

Table 6. Correlations of lactate or individual SCFA concentrations with pH of feces and cecal contents

SCFA	Cecal pH	Fecal pH
Lactate	-0.590**	-0.519**
Acetate	-0.078	-0.536**
Propionate	-0.039	-0.135
Butyrate	0.342*	-0.088
Total SCFA	0.062	-0.499**

**$p<0.01$ *$p<0.05$.

chicory inulin oligosaccharide produced amounts of lactate in the cecum and in the feces that were higher than those in the control diet. High concentrations of lactate can be an etiological factor for chronic fermentative diarrhea (23, 24). However, fructooligosaccharide or chicory inulin levels as high as 6% of total diet did not cause diarrhea in this study.

We found that fecal excretion of propionate was significantly increased in rats fed chicory inulin. This result was in agreement with the findings by Kim *et al.* (25), who observed that rats fed diets of 5% chicory extract or 5% inulin showed greater cecal propionate concentrations.

The rapid and extensive absorption of SCFAs from the large bowel is enchanced at lower pHs, and lactate is absorbed from the colon more slowly than acetate (26). Thus, the ratio of lactate to total SCFA could be observed to be higher in the feces than in the cecum (Table 4).

Currently, we do not know mechanisms for the differences in SCFA profiles among the different kinds of fructooligosaccharides. One possibility is that the differences are attributable to different degrees of polymerization. Inulin is a fructan with long chain unit (approximately 35 DP), chicory inulin oligosaccharide (5-7 DP) is an oligofructose, and fructooligosaccharide (3-4 DP) is a short-chain fructooligosaccharide. Owing to its longer chain length, inulin is less soluble than oligofructose (12). Long-chain fructans (42% with a DP>21) were associated with longer transit time when compared to short-chain fructans (a median DP of 3). Abdominal symptoms after fructan intake increased with increasing dose and decreasing chain length (13). Ingestion of fructooligosaccharides led to increases in Ca and Mg absorption in growing rats, but no difference was found between GF_2 and GF_3 (27). Rats fed short-chain fructooligosaccharides showed more rapid recovery from post-gastrectomy anemia and more efficient absorption of iron than the rats fed inulin (28). Kruger *et al.* (29) reported that inulin (DP>23) showed a more significant effect than fructooligosaccharide on calcium bioavailability in growing rats (DP 2-8).

Bile acid profiles As shown in Table 5, the fecal concentrations of cholic acid, deoxycholic acid, and total bile acids were significantly decreased in the rats that were fed fructooligosaccharides and chicory inulin. The daily excreted amounts of primary bile acids, secondary bile acids, and total bile acids were not significantly different among groups. Nonetheless, there was a tendency toward decreased excretion of deoxycholic acid in rats that were fed fructooligosaccharide and decreased excretion of lithocholic acid in the rats that were fed chicory inulin.

Correlations of lactate or individual SCFA concentrations with pH of cecal contents and feces are shown in Table 6. The cecal pH was negatively correlated with lactate ($r=-0.590$, $p<0.01$) in cecum and positively correlated with butyrate concentration ($r=0.342$, $p<0.05$). The fecal pH was negatively correlated with lactate ($r=-0.519$, $p<0.01$), acetate ($r=-0.536$, $p<0.01$), and total SCFA ($r=-0.499$, $p<0.01$) concentrations. It has been shown that galactomannooligosaccharides with DP 5 were more effective than ones with DP 7 on the growth of *Bifidobacterium* spp (30). This study might indicate that bifidogenetic effects of fructans were associated with different degrees of polymerization. In the present study, short-chain fructooligosaccharide produced lactate and lowered the pH in large intestines of rats to a greater degree than did medium-chain or long-chain fructans.

The conversion of primary bile acids to carcinogenic secondary bile acids by colonic bacteria is inhibited under acidic conditions because the activity of bacterial 7-dehydroxylase is inhibited below pH 6.5 (4). In fact it was shown *in vitro* as well as in rats that an acidic pH was very effective at lowering the concentration of soluble bile acids

(9).

It has been shown that the ratio of anaerobic bacteria to aerobic bacteria is greater in the feces than in the cecum. 7-dehydroxylation is carried out only by anaerobic bacteria, and that the luminal pH is higher in the distal colon than in the proximal colon (31). From this standpoint, the fecal bile acid profile, rather than the cecal bile acid profile, reflects the conversion of primary bile acids into secondary bile acids in the large bowel. In this study, the fecal concentrations of cholic acid, deoxycholic acid, and total bile acid were decreased in rats fed fructooligosaccharide, chicory inulin oligosaccharide, and chicory inulin. However, the excretion of individual bile acid and total bile acid in the feces did not differ among the groups. As rats were fed in pairs on diets supplemented with sodium cholate at the level of 0.25%, all of the groups consumed the same amount of cholic acid. Cholic acid became the predominant bile acid in cecum and feces. This is the reason that the primary bile acid concentration was much higher than the secondary bile acid concentration (Table 5). The decreased concentration of bile acid was associated, in part, with a larger fecal output (Table 3). Nonetheless, significantly decreased concentrations of cholic acid and deoxycholic acid and a tendency toward lowered fecal excretion of secondary bile acids may be regarded as positive effects of the consumption of fructooligosaccharides and chicory inulin.

Oligosaccharides and fructans may act differently than dietary fiber on bile acid metabolism. Dietary fibers themselves also seem to act differently on bile acid metabolism. Some dietary fibers, such as psyllium, seem to act similarly to cholestyramine, which is a known bile acid sequestrant with cholesterol-lowering potential (32). Wheat bran may act differently from soluble fibers on bile acid metabolism. The consumption of brown bread rather than white bread significantly decreased the concentration of cholic acid in the feces (33). The consumption of whole grain bread corresponding to 11 g of supplemental fiber per day decreased fecal excretion of secondary bile acids in healthy men and women (34). Wheat bran was more protective against the development of colon cancer than oat bran (20). However, oat bran produced a more acidic luminal pH than wheat bran and more butyrate, which may protect against colon cancer development (35, 36). One of the mechanisms involved in the protective effect of wheat bran could be lowered concentrations of primary and secondary bile acids (33, 34) in the large bowel.

In conclusion, the present study showed that ingestion of fructooligosaccharides and chicory inulin increased the cecal and fecal concentrations of lactate and SCFAs. They also decreased the fecal concentrations of bile acids, including secondary bile acids. Short-chain fructooligosaccharide was more effective at decreasing colonic pH and lactate production, but chicory inulin oligosaccharide was more effective at increasing fecal butyrate and lowering fecal secondary bile acid concentration.

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