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COMPARISON OF THE NUTRITIONAL EFFECTS OF FRUCTO-OLIGOSACCHARIDES OF DIFFERENT SUGAR CHAIN LENGTH IN RATS

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ABSTRACT

The influence of fructo-oligosaccharides of different sugar chain length on growth, nitrogen balance, and mineral balance was examined in rats fed diets containing either sucrose, 1-kestose, nystose, or a mixture of these at 50 or 100 g/kg of diet. Rats were fed the experimental diets for six weeks, and nitrogen balance and mineral (Ca and Mg) balance were examined after the second and fourth week. Food intake and body weight gain did not differ significantly among the groups. Each fructo-oligosaccharide fed was found to increase Ca and Mg absorption dose-dependently during the first balance study period and the degrees of increase in Ca and Mg absorption were similar with each fructo-oligosaccharide fed regardless of the level present in the experimental diet. Also, each of the fructo-oligosaccharides was found to increase fecal excretion of nitrogen, but there was no clear dose-dependence and the extent of increase in fecal excretion of nitrogen was similar for each fructo-oligosaccharide fed. In conclusion, our findings demonstrate that ingestion of fructo-oligosaccharides leads to increases in Ca and Mg absorption and an increase in fecal excretion of nitrogen, however, differences in sugar chain length do not influence their nutritional effects considerably in rats.
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KEY WORDS: Fructo-oligosaccharides, Calcium, Magnesium, Absorption, Fecal nitrogen

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INTRODUCTION

Recently, the importance of indigestible carbohydrates in the diet has been reported by many
investigators, since these dietary components have several desirable effects for human health (1-4).
Indigestible carbohydrates, such as dietary fiber, indigestible oligosaccharides or resistant starch,
reach the large intestine intact and are fermented by bacteria in the intestinal lumen, resulting in
the production of organic acids such as acetate, propionate and butyrate (5-7). These acids may be
partially responsible for several of the physiological effects of indigestible carbohydrates (3,4).

The physiological effects of fructo-oligosaccharides (FOS), which are indigestible
carbohydrates, especially those of mixtures of fructo-oligosaccharide (FO) of different sugar
length, such as 1-kestose (GF_2), nystose (GF_3) and fructofuranosylnystose (GF_4), have been well
examined (5,8,9). The nutritional effects of FOS are known to include stimulation of mineral
absorption (10-13) and promotion of fecal nitrogen excretion (13,14). In almost all previous
studies, the authors used FOS consisting of a   mixture of GF_2, GF_3 and GF_4. A few reports have
shown that there are differences in fermentability and availability to intestinal bacteria among
these FOS in vitro (15). Some authors have pointed out that the stimulatory effect of indigestible
carbohydrates on mineral absorption is related to their influence on the osmolality of the intestinal
contents (16-18). After ingestion of GF_2, GF_3 or GF_4, the osmolality of the intestinal contents may
vary, even when the same amount of each FO is ingested. Thus, it seems that the physiological
and nutritional effects of FO of different chain length may differ. However, to our knowledge, the
effects of individual types of FO in vivo have not been investigated previously.

The nutritional effects of FOS on mineral absorption and fecal nitrogen excretion can be
estimated   quantitatively, and these effects are known to display clear dose dependency (14).
Therefore, we thought that if there are some differences in nutritional effect, comparing individual
types of FO, it should be possible to detect such differences. In the present study, we examined
whether there is a difference among FOS with respect to their nutritional effects by observing the
growth, nitrogen balance and mineral (Ca and Mg) balance in rats fed FOS-containing diets.

MATERIALS AND METHODS

Five-week-old male Sprague-Dawley rats (Clea Japan, Tokyo, Japan) were housed in
individual stainless-steel metabolic cages with wire-mesh bottoms in a temperature- and
humidity-controlled room (25°C and 55% relative humidity) with a 12-h light-dark cycle. Seven
experimental diets were used in this study. The composition of these diets is shown in Table 1.
Rats in the control group received a diet that contained sucrose at 100 g/kg of diet and rats in the
other 6 subgroups received a diet that contained sucrose at 50 g/kg of diet and one of the fructo-
oligosaccharides (GF_2: nystose, GF_3: 1-kestose or FOS: a mixture of these) at 50 or 100g/kg of

diet. There were 7 rats in each group. All rats were allowed free access to water and experimental diet for four weeks. Food intake and body weight were measured every second day. On the final day of the experiment, the rats were anesthetized with diethyl ether, killed by drawing whole blood.

This study was approved by the Animal Committee of Meiji Seika Bioscience Laboratories, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Meiji Seika Bioscience Laboratories.

Ten and 24 days after the start of feeding the experimental diets, mineral balance and nitrogen balance were determined for a 5-day period, and all feces and urine were collected during this 5-day period in each case. The ratios of apparent absorption of calcium (Ca) and magnesium (Mg) were calculated from the following formulae:

$$\text{Apparent absorption} = (\text{intake} - \text{fecal excretion})/(\text{intake}) \times 100 \ (\%)$$

The concentrations of Ca and Mg in the diets, feces and urine were determined by means of a sequential plasma spectrometer (ICPS-5000; Shimadzu, Kyoto, Japan) as described previously (11). Samples of the diets and feces were first dried and then micropulverized. The micropulverized samples (approx. 100 mg each) were ashed at 600°C for 24 h. The ashed samples, dissolved in 4 ml of 2 N HCl, were diluted appropriately with distilled water for atomization. Urine was diluted appropriately with distilled water and subjected to atomization directly.

Ten and 24 days after the start of feeding the experimental diet, all feces and urine were collected, for a period of 5 days in each instance, for measurement of fecal and urinary nitrogen. Fecal and urinary nitrogen were determined after Kjeldahl digestion in an automatic analyzer (KIEL-AUTO DTP-3, Mitamura Riken Kogyo Inc., Tokyo, Japan).

Fructo-oligosaccharides (FOS; consisting of a mixture of 42% 1-kestose, 46% nystose and 9% 1F- β - fructo-furanosylnystose), nystose and 1-kestose (content above 95% in each instance) were obtained from Meiji Seika Kaisha, Ltd. (Tokyo, Japan). The chemical structures of these compounds are shown in Fig. 1.

FOS was manufactured from sucrose using fructosyltransferase (19). FO is not hydrolyzed in the rat by digestive enzymes, such as disaccharidase in the intestinal mucosa and β -amylase in pancreatic homogenates (5). Other dietary components were purchased from Oriental Yeast Co. (Tokyo, Japan). All other reagents were of analytical grade and were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

Data are expressed as mean values with standard deviation. Data were analyzed by one-way ANOVA, and significant differences among groups were determined by Tukey's test (SPSS Ver.6.0, SPSS Inc., Chicago, USA). Differences were considered significant at $p < 0.05$.

TABLE 1

Composition of the Diets and Designation

Group	Control	FOS 5% (10%)	GF$_2$ 5% (10%)	GF$_3$ 5% (10%)
Ingredient (g/kg diet)				
Casein	250	250	250	250
α -potato starch	95	95	95	95
Corn oil	60	60	60	60
Vitamin mix. [1]	10	10	10	10
Mineral mix. [1]	35	35	35	35
Cellulose	50	50	50	50
Sucrose	500	450	450	400
Fructo-oligosaccharides [2]	0	50 (100)	0	0
GF$_2$ [3]	0	0	50 (100)	0
GF$_3$ [4]	0	0	0	50 (100)
Chemical analysis (mmol/kg diet)				
Calcium	113	115	112	115
Magnesium	15.2	15.1	14.7	14.8
Phosphorus	88.1	91.7	90.4	89.1

[1] Prepared according to AIN-93G formulation.

[2] Meioligo-P® (concentration of oligosaccharides was greater than 95% of total mixture.)
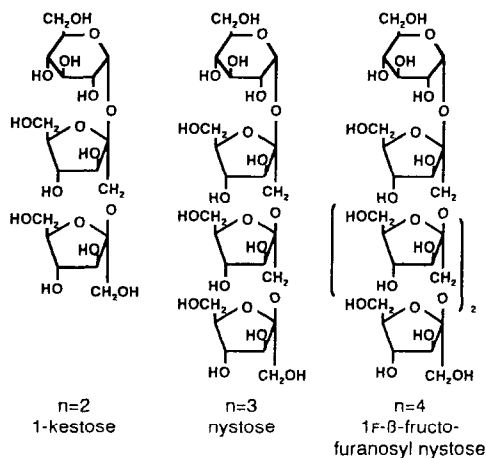
[3] 1-Kestose, [4] Nystose

FIG. 1. Chemical structure of the fructo-oligosaccharides.

RESULTS

Food intake and body weight gain (Table 2)   Body weight gain, total food intake and food efficiency did not differ significantly among the groups.

 Ca balance (Table 3)   During each of the balance study periods (10th -14th and 24th-28th day), a significant decrease in fecal Ca excretion and a significant increase in urinary excretion of Ca were observed in rats fed the 10% FO-containing diets.   These changes elicited by FO-feeding were dose-dependent in each instance and similar in degree regardless of the type of FO in the diet During the first period (10th - 14th day) of the balance study, the absorption values and the ratio of apparent absorption of Ca in rats fed the 10% FO-containing diets were higher than those in rats fed the control diet.

Mg balance (Table 4)   During each of the balance study periods (10th - 14th and 24th-28th day), a significant decrease in fecal Mg excretion was observed in rats fed the 10% FO-containing diets. During in the first balance study period (10th-14th day) , a significant increase in urinary excretion of Mg was observed in rats fed the 10% FO-containing diets. These changes elicited by FO-feeding were dose-dependent in each instance and similar in degree regardless of the type of FO in the diet. During each of the balance study periods (10th - 14th and 24th-28th day), the absorption values and ratio of apparent absorption of Mg in rats fed the 10% FO-containing diets were higher than those in rats fed the control diet. During the second balance study period (24th - 28th day), the ratio of apparent absorption of Mg in rats fed the 5% FO-containing diets was higher than that in rats fed the control diet.

TABLE 2
Body Weight and Food Intake

Group[1]	Body weight (g)			Total food intake (g)	Food efficiency[2]
	initial	final	gain		
Control	128 ± 3 [NS]	333 ± 15 [NS]	205 ± 13 [NS]	517 ± 25 [NS]	0.397 ± 0.019 [NS]
FOS 5%	128 ± 4	334 ± 10	207 ± 10	514 ± 17	0.402 ± 0.017
FOS 10%	127 ± 3	340 ± 12	213 ± 12	501 ± 27	0.425 ± 0.012
GF_2 5%	126 ± 3	336 ± 11	211 ± 14	511 ± 18	0.407 ± 0.015
GF_2 10%	127 ± 4	340 ± 21	213 ± 21	492 ± 36	0.433 ± 0.014
GF_3 5%	126 ± 3	341 ± 22	215 ± 23	515 ± 29	0.418 ± 0.033
GF_3 10%	126 ± 4	336 ± 12	210 ± 14	496 ± 24	0.424 ± 0.024

Values are mean ± SD (n=7).
NS = No significant difference
[1] See Table 1.
[2] Food efficiency = Body weight gain / Food intake

TABLE 3. Ca balance

Group [*]	Control	FOS 5%	FOS 10%	GF$_2$ 5%	GF$_2$ 10%	GF$_3$ 5%	GF$_3$ 10%
10 - 14 days							
Intake (mmol/day)	2.6 ± 0.2 [NS]	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.5 ± 0.2	2.6 ± 0.2	2.7 ± 0.2
Fecal excretion (mmol/day)	0.7 ± 0.2 [a]	0.5 ± 0.1 [ab]	0.2 ± 0.1 [c]	0.5 ± 0.2 [abc]	0.2 ± 0.1 [c]	0.6 ± 0.3 [ab]	0.3 ± 0.2 [cb]
Urinary excretion (mmol/day)	13.3 ± 2.0 [d]	51.1 ± 25.2 [cd]	98.8 ± 31.8 [ab]	67.7 ± 21.1 [bc]	117.4 ± 37.3 [a]	48.9 ± 19.5 [cd]	128.6 ± 43.8 [a]
Absorption (mmol/day)	1.9 ± 0.1 [c]	2.1 ± 0.1 [bc]	2.3 ± 0.1 [ab]	2.1 ± 0.1 [bc]	2.2 ± 0.2 [ab]	2.1 ± 0.2 [bc]	2.4 ± 0.3 [a]
Absorption (%)	73.1 ± 4.4 [a]	79.4 ± 4.8 [ab]	90.6 ± 2.8 [c]	81.1 ± 5.8 [ab]	90.6 ± 3.6 [c]	78.9 ± 8.0 [a]	88.3 ± 7.3 [bc]
24 - 28 days							
Intake (mmol/day)	2.9 ± 0.3 [NS]	2.8 ± 0.1	2.9 ± 0.2	3.0 ± 0.2	2.8 ± 0.2	3.0 ± 0.2	2.8 ± 0.2
Fecal excretion (mmol/day)	1.0 ± 0.2 [a]	0.9 ± 0.1 [ab]	0.7 ± 0.2 [bc]	1.0 ± 0.2 [a]	0.6 ± 0.2 [c]	0.9 ± 0.2 [abc]	0.7 ± 0.2 [abc]
Urinary excretion (mmol/day)	15.4 ± 3.5 [c]	33.6 ± 19.1 [c]	112.5 ± 4.9 [ab]	51.6 ± 12.7 [c]	113.4 ± 34.8 [ab]	67.4 ± 31.5 [bc]	151.8 ± 58.1 [a]
Absorption (mmol/day)	2.0 ± 0.2 [ab]	1.9 ± 0.2 [b]	2.2 ± 0.1 [a]	2.1 ± 0.1 [ab]	2.2 ± 0.2 [a]	2.1 ± 0.2 [a]	2.1 ± 0.3 [a]
Absorption (%)	66.9 ± 3.0 [a]	66.8 ± 4.8 [a]	76.2 ± 4.4 [c]	68.4 ± 3.4 [ab]	78.1 ± 5.0 [bc]	71.2 ± 4.5 [abc]	74.3 ± 7.3 [bc]

Values are mean ± SD (n=7).
NS = No significant difference
Values not sharing a common superscript letter are significantly different ($p<0.05$).
*See TABLE 1.

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TABLE 4. Mg balance

Group*	Control	FOS 5%	FOS 10%	GF₂ 5%	GF₂ 10%	GF₃ 5%	GF₃ 10%
10 - 14 days							
Intake (μmol/day)	327.8 ± 23.9 [NS]	325.6 ± 14.1	315.1 ± 13.2	313.0 ± 11.6	308.3 ± 25.1	323.0 ± 29.5	329.4 ± 27.4
Fecal excretion (μmol/day)	66.1 ± 25.5 [a]	49.8 ± 14.0 [abc]	29.3 ± 5.9 [bc]	44.3 ± 14.7 [abc]	28.3 ± 7.5 [c]	54.5 ± 21.0 [ab]	35.4 ± 9.9 [bc]
Urinary excretion (μmol/day)	134.3 ± 16.7 [b]	166.5 ± 20.7 [a]	168.0 ± 15.2 [a]	151.1 ± 21.0 [ab]	162.7 ± 18.6 [ab]	155.8 ± 17.1 [ab]	170.5 ± 19.7 [a]
Absorption (μmol/day)	261.7 ± 24.1 [NS]	275.8 ± 16.4	285.7 ± 14.7	268.7 ± 8.8	279.9 ± 26.0	268.5 ± 17.8	294.1 ± 24.3
Absorption (%)	80.0 ± 7.0 [a]	84.7 ± 4.1 [abc]	90.7 ± 1.9 [cd]	86.0 ± 4.3 [abc]	90.7 ± 2.6 [cd]	83.4 ± 5.4 [ab]	89.3 ± 2.7 [bc]
24 - 28 days							
Intake (μmol/day)	366.2 ± 32.2 [NS]	357.4 ± 17.7	359.8 ± 27.2	379.9 ± 27.5	356.7 ± 26.0	372.0 ± 27.2	357.2 ± 19.8
Fecal excretion (μmol/day)	110.3 ± 24.7 [a]	75.7 ± 17.8 [bc]	51.0 ± 13.5 [cd]	83.0 ± 20.0 [ab]	47.9 ± 10.4 [d]	67.0 ± 10.1 [bcd]	53.6 ± 14.9 [c]
Urinary excretion (μmol/day)	157.7 ± 14.9 [ab]	189.2 ± 27.2 [bc]	217.3 ± 24.2 [c]	209.5 ± 38.1 [c]	221.1 ± 19.4 [c]	142.4 ± 15.8 [c]	161.8 ± 39.8 [ab]
Absorption (μmol/day)	255.9 ± 23.5 [a]	281.8 ± 25.4 [ab]	308.8 ± 19.8 [b]	296.9 ± 26.5 [ab]	308.8 ± 18.0 [b]	305.0 ± 24.0 [b]	303.6 ± 21.6 [b]
Absorption (%)	70.0 ± 5.2 [a]	78.8 ± 5.1 [b]	85.9 ± 3.1 [c]	78.2 ± 5.1 [b]	86.7 ± 2.2 [c]	82.0 ± 2.4 [bc]	85.0 ± 4.0 [c]

Values are mean ± SD (n=7).
NS = No significant difference
Values not sharing a common superscript letter are significantly different (p<0.05).
*See TABLE 1.

N balance (Table 5)   With each type of FO examined, FO-feeding increased fecal nitrogen excretion during each of the balance study periods. The degree of increase in fecal nitrogen in rats fed FO was similar for each type of FO tested. During the first balance study period, the increase was dose dependent, but during the second balance study period, the dose dependence of the increase was not clearly evident. The nitrogen retention ratios did not differ among the groups.

DISCUSSION

To our knowledge, there has been no previous   investigation comparing the nutritional effects of individual types of FO of different chain length in rats in vivo. However, the indigestibility and fermentability of FOS have been well examined (5,7).

FOS are known to escape digestion in the small intestine and reach the large intestine intact, where they are fermented by bacteria in the lumen. Bacterial fermentation of carbohydrates in the large intestine leads to production of organic acids and gas, bacterial propagation, and production of heat which results in a loss of energy for the host estimated to be equal to 50% of the energy content of the carbohydrate (20). Recently, Molis et al. estimated that the energy content of FOS is about one-half that of sucrose (21). In the present study, food intake, body weight gain and feed efficiency did not differ among groups. Assuming that the energy content of FOS is one-half that of sucrose, the total energy intake of rats fed 10% FOS-containing diet is calculated to be about 95% of that of rats fed the control diet. Thus, the difference in total energy intake is small. We did not detect any difference in body weight gain or feed efficiency as a reflection of the difference in energy intake. At least, it seems that there is no large difference in energy efficiency among FOS of different chain length.

In this study, feeding FOS-containing diets to rats led to increases in Ca and Mg absorption. Especially, during the first balance study period, these effects were linearly dose-dependent. Several authors have speculated that the stimulatory effects of indigestible carbohydrates on mineral absorption are due to   their osmotic effects   (16-18). The molecular weight of GF_2 is 504 and that of GF_3 is 666. Therefore, the osmotic effect of GF_2 is greater than that of GF_3, when the same amount of GF_2 or GF_3 is fed. However, the apparent levels of absorption of Ca or Mg, comparing rats fed GF_2 or GF_3 in the present study, were very similar. Delzenne et al. have reported that the stimulatory effect on Ca and Mg absorption comparing inulin and its hydrolysate were similar (15). These results suggest that the osmotic effect of indigestible carbohydrates is not the main reason for their stimulatory effect on mineral absorption.

Each type of FO fed increased fecal nitrogen excretion. Indigestible carbohydrates serve as an energy source for intestinal bacteria and stimulate their growth (22,23). The nitrogen sources for bacterial protein synthesis are undigested dietary protein, digestive enzymes, mucosal cells and nitrogen compounds derived from blood such as amino acids, urea or ammonia. Two mechanisms may contribute to the increase in fecal nitrogen elicited by ingestion of indigestible

TABLE 5. Nitrogen balance

Group	Control	FOS 5%	FOS 10%	GF$_2$ 5%	GF$_2$ 10%	GF$_3$ 5%	GF$_3$ 10%
10 - 14 days							
Intake (mmol/day)	44.1 ± 3.2	[NS] 44.4 ± 1.9	44.3 ± 1.9	42.4 ± 1.6	41.3 ± 3.4	45.8 ± 4.2	45.5 ± 3.8
Fecal excretion (mmol/day)	1.55 ± 0.25 [a]	2.66 ± 0.36 [a]	3.10 ± 0.64 [b]	2.67 ± 0.41 [b]	2.93 ± 0.40 [bc]	2.83 ± 0.54 [b]	3.65 ± 0.59 [c]
Urinary excretion (mmol/day)	19.9 ± 2.4 [NS]	20.4 ± 2.5	17.4 ± 2.3	18.6 ± 1.4	16.8 ± 2.0	19.3 ± 4.1	17.9 ± 3.4
Net balance** (mmol/day)	22.6 ± 3.6 [NS]	21.4 ± 1.1	23.7 ± 1.6	21.2 ± 1.4	21.6 ± 2.0	23.7 ± 1.9	24.0 ± 2.6
Retention*** (%)	51.2 ± 6.1 [NS]	48.2 ± 3.4	53.7 ± 4.2	50.0 ± 2.5	52.3 ± 2.2	52.0 ± 6.0	52.8 ± 6.0
24 - 28 days							
Intake (mmol/day)	49.8 ± 4.4 [NS]	47.9 ± 2.4	47.5 ± 3.6	51.2 ± 3.7	48.8 ± 3.6	50.9 ± 3.7	46.9 ± 2.6
Fecal excretion (mmol/day)	1.97 ± 0.22 [NS]	3.59 ± 0.48	3.59 ± 0.85	3.92 ± 0.39	3.65 ± 0.67	3.69 ± 0.41	3.83 ± 0.70
Urinary excretion (mmol/day)	28.2 ± 3.3 [NS]	26.0 ± 2.6	24.3 ± 2.3	25.4 ± 3.9	24.6 ± 1.8	27.0 ± 3.3	23.9 ± 2.4
Net balance** (mmol/day)	19.6 ± 3.6 [NS]	18.3 ± 3.4	19.6 ± 2.3	19.0 ± 2.0	20.6 ± 2.0	20.2 ± 2.8	19.2 ± 1.9
Retention*** (%)	39.3 ± 6.1 [NS]	38.1 ± 5.9	41.3 ± 3.1	37.1 ± 4.4	42.2 ± 2.2	39.6 ± 4.8	41.0 ± 4.0

Values are mean ± SD (n=7).
NS = No significant difference
Values not sharing a common superscript letter are significantly different (p<0.05).
*See TABLE 1.
**Net balance = Intake - (Fecal excretion + Urinary excretion)
***Retention = Net balance x 100

carbohydrates. It has been shown that dietary fiber such as oat fiber leads to higher fecal nitrogen by enhancing fecal bulk and accelerating the digestive transit (24). Indigestible oligosaccharides are known to increase the transfer of blood urea nitrogen to the large intestine due to their osmotic effect. In the present study, a strong correlation between fecal dry weight and fecal nitrogen excretion was observed in both balance study periods (10-14 days: r=0.73769, 24-28 days: r=0.70806). It has been reported that the main constituent of fecal nitrogen is protein derived from bacterial sources (25). This observation suggests that the increase in fecal nitrogen may be dependent on the proliferation of intestinal bacteria, which is stimulated by FOS-feeding. There are reported to be some differences between GF_2 and GF_3 in availability to intestinal bacteria in vitro (15). With respect to the fecal nitrogen excretion, no remarkable difference was observed among the types of FOS tested. This indicates that there is no remarkable difference in their stimulatory effect on the proliferation of intestinal bacteria in vivo.

In conclusion, each of the types of fructo-oligosaccharide fed to rats was similarly effective to increase Ca and Mg absorption and fecal excretion of nitrogen. These findings suggest that the differences in sugar chain length among fructo-oligosaccharides do not influence their nutritional effects considerably in rats.

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