

## Short-term supplementation with dietary fructooligosaccharide and dietary mannitol elevated the absorption of calcium and magnesium in adult rats

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**ABSTRACT:** The effects of dietary fructooligosaccharide (FOS) and dietary mannitol on the absorption of Ca and Mg in a short term feeding trial were studied. Adult Wistar rats were divided into three groups and fed diets containing 0, 8% FOS or 8% mannitol for seven days. Daily intake and feces were monitored for three days to determine the apparent absorption of Ca and Mg. At the last day of the feeding trial, blood sample was collected from cecal vein to assess Ca and Mg levels. The cecum and colon were removed to analyze the parameters. The results showed that both dietary FOS and dietary mannitol significantly increased the apparent absorptions of Ca and Mg. Both dietary FOS and dietary mannitol significantly increased Ca concentration in cecal vein plasma, but did not affect Mg concentration. They significantly decreased Ca concentration and significantly increased soluble Ca concentration in cecal content dry matter (DM). The Mg concentration in colonic content DM was significantly decreased by feeding dietary FOS and dietary mannitol. FOS fermentation in cecum led to low cecal pH and increases in cecal organic acids concentration. Mannitol was fermented in cecum to induce low cecal pH and cecal wall extension. In conclusion, short-term supplementation with dietary FOS and dietary mannitol improved the apparent absorption of Ca and Mg. FOS and mannitol were fermented in cecum to elevate Ca absorption from cecum and to elevate Mg absorption in colon in rats.

**Keywords:** mannitol; FOS; Ca; Mg; absorption; cecum; rat

### INTRODUCTION

Due to the growing rate of population suffering from metabolic diseases like obesity and diabetes, indigestible short chain carbohydrates including monosaccharides, disaccharides, oligosaccharides, and sugar alcohols as low-caloric food substances have generated researchers' interest because they are resistant in the small intestine and do not increase blood glucose level and insulin secretion after ingestion. Indigestible short chain carbohydrates are fermented in hindgut and positively influence the absorption of nutrients, such as mineral elements, in the intestinal tract.

Fructooligosaccharide is a kind of low-caloric indigestible oligosaccharide, with 0.3–0.6 times the sweetness of sucrose. Fructooligosaccharide is

contained in fruits, vegetables, some grains, and cereals (Campbell et al. 1997). Previous studies have found that FOS improved mineral absorption in both animal and human intestine (van den Heuvel et al. 1999; Tahiri et al. 2003). The bacteria in the hindgut can ferment FOS, which results in increased organic acids production and a drop of luminal pH. Luminal minerals are more soluble under the acidification. Therefore, more luminal minerals are available to be absorbed from intestinal epithelium into the bloodstream.

Mannitol is a sugar alcohol with the formula  $C_6H_8(OH)_6$ . Compared with sucrose, mannitol has 40% caloric value and half sweet approximately in equal mass. Mannitol is distributed naturally in mushrooms, algae, trees, and many kinds of plants (Wisselink et al. 2002). Mannitol is partially absorbed

from the small intestine, but does not get converted into energy source (Saunders and Wiggins 1981). In the large intestine, mannitol is fermented by local bacteria (Dwivedi 1991). The major end products of fermentation are organic acid and intestinal gas (Bar 1990). Therefore, foods and beverages sweetened with mannitol in place of sucrose are helpful in controlling caloric intake and body weight.

The present study was designed to investigate the effects of dietary FOS and mannitol on the absorption of Ca and Mg and the mechanism in adult rats in one-week supplement. In this study, we try to compare Ca and Mg absorption after the ingestion of FOS and mannitol with digestible sugar (sucrose) ingestion, and also to observe the different effects of the contributions of FOS and mannitol fermentation in the large intestine on mineral absorption.

## MATERIAL AND METHODS

**Animals and diets.** Twenty-one adult male Wistar rats were housed in individual wire-mesh stainless steel cages in an air-conditioned room maintained at  $23 \pm 1^\circ\text{C}$  with 50–60% relative humidity. The light was set up as a constant cycle of 12 h light (8:00–20:00)/12 h darkness (20:00–8:00). Rats were acclimatizing to laboratory conditions for one week preceding the feeding trial. The rats ( $403.2 \pm 48.1$  g body weight) were randomly divided into three treatment groups, each group being fed one of the experimental diets (control diet, FOS diet, mannitol diet) for seven days. There were seven rats in each group. Diets and water were available *ad libitum* during the entire experimental period.

The experimental diets were as follows: the control diet (C) consisted of standard laboratory chow (AIN-93G) (Reeves et al. 1993). The FOS diet and the mannitol diet containing 8% FOS and 8% mannitol respectively were created by replacement of equal amounts of sucrose in the control diet with FOS and mannitol. The composition of the experimental diets is shown in Table 1.

During the experiment, the amount of food supplied, diet residues, and diet waste were measured daily. Feces were collected for 24 h daily for three days in days 4–6 of the feeding trial. On day 7 the rats were fasting for 8 h, then were re-fed by the control diet for 3 h again. After that, the feed was removed from the cages and three solutions were delivered directly to the stomach using a gavage strategy. The procedure used a syringe attached to a curved steel gavage needle that was inserted down the esophagus to the

Table 1. Composition of experimental diets

Ingredients	Control	FOS	Mannitol
$\alpha$ -Corn starch (g/kg)	562	562	562
Casein (g/kg)	200	200	200
Sucrose (g/kg)	100	20	20
Corn oil (g/kg)	70	70	70
Cellulose powder (g/kg)	20	20	20
Vitamin mix (g/kg)	10	10	10
Mineral mix (g/kg)	35	35	35
L-Cystine (g/kg)	3	3	3
D-Mannitol (g/kg)	0	0	80
Fructooligosaccharide (g/kg)	0	80	0
Gross energy (kcal/g)	4.94 (calculation)		

control = control diet (AIN-93G) (Reeves et al. 1993), FOS = experimental diet containing 8% fructooligosaccharide, mannitol = experimental diet containing 8% mannitol

entrance of the stomach. Sucrose, FOS, and mannitol were diluted in distilled water. Sucrose solution, FOS solution, and mannitol solution were given to the rats in the control diet group, the FOS diet group, and the mannitol diet group, respectively. The delivered quantity was a half of the average daily intake of sucrose or FOS or mannitol for each rat. Three hours after gavage, the rats were anaesthetized with diethyl ether for blood collection from cecal vein, and finally sacrificed by exsanguinations from the celiac artery.

**Sample collection and analysis.** Blood samples were collected from cecal vein in syringes treated with heparin to determine the mineral level. Plasma was obtained by centrifugation at 3000 rpm for 15 min at  $20^\circ\text{C}$ . The cecum and colon of each rat were collected with the digesta, and stored at  $-30^\circ\text{C}$ . The pH value of the contents of cecum and colon was measured with a pH meter (TWIN Horiba Ltd., Kyoto, Japan). The concentrations of organic acids in the cecal content were measured with high-performance liquid chromatography (HPLC) (column: 2 Shim-pack SCR-102H, detector: Shimadzu CDD-10A; Shimadzu Corp., Kyoto, Japan). A part of the digesta of cecum and colon was oven-dried for 24 h, comminuted and ashed in a  $550^\circ\text{C}$  muffle furnace to determine Ca and Mg levels. The rest of cecal digesta and colonic digesta was mixed with deionized water respectively and centrifuged at  $14\,000\text{ g}$  at  $4^\circ\text{C}$  for 10 min. The supernatant was diluted to measure soluble Ca and Mg. Ca and Mg levels in diets, feces, cecal digesta, colonic digesta, and blood were determined by an

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atomic absorption spectrophotometer AA-7000 (Shimadzu Corp.). Feed efficiency and apparent Ca and Mg absorption were calculated as follows:

feed efficiency = weight gain/feed intake × 100%

apparent mineral absorption =  
(intake – fecal excretion)/intake × 100%

**Ethics.** Animals were cared for and sacrificed in accordance with the guidelines for animal experiments approved by the institutional Ethics Committee of the Okayama University (experimental protocol No. 51735).

**Statistical analysis.** Data was shown as means ± SD. Significant difference between the data was analyzed by Tukey-Kramer's test using the statistical software StatView, Version 5.0 by SAS Institute Inc. Significance of difference was justified to be at  $P < 0.05$ .

## RESULTS

Body weight, feed intake, daily weight gain, feed efficiency, and digestibility of dry matter and crude ash of the fecal collection period (days 4–6 of the feeding trial) are shown in Table 2. Initial body weight and final body weight were similar among the experimental groups. Feed intake in the mannitol diet group was significantly higher than that in the control diet group and the FOS diet group. Daily weight gain, feed efficiency, and dry matter digestibility were not statistically different among the experimental groups. Crude ash digestibility in the rats in the FOS diet group and the mannitol

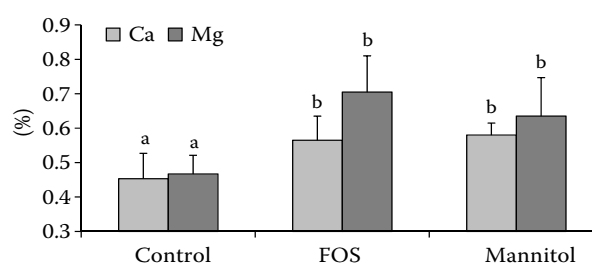


Figure 1. Effects of dietary FOS and mannitol on apparent absorptions of Ca and Mg absorption on days 4–6 of the feeding trial in rats fed the experimental diets

control = control diet (AIN-93G) (Reeves et al. 1993), FOS = experimental diet containing 8% fructooligosaccharide, mannitol = experimental diet containing 8% mannitol data are mean ± SD (six rats were in the control diet group and seven rats were in the FOS diet group and the mannitol diet group respectively). Significance of difference between the data was analyzed by Tukey-Kramer test using add-in statistical software for MS Excel (SSRI Co., Tokyo, Japan). Differences were considered significant at  $P < 0.05$

diet group was significantly higher than that in the control diet group.

The apparent absorption of Ca and Mg in days 4–6 of the feeding trial are shown in Figure 1. The apparent absorption of Ca and Mg was significantly increased by FOS feeding and mannitol feeding, compared with the control diet. But between the FOS diet group and the mannitol diet group, the apparent absorption of Ca and Mg did not significantly differ.

The concentration of Ca and Mg in cecal vein plasma is shown in Figure 2. The Ca concentrations in cecal vein plasma in the FOS diet group

Table 2. Body weight, feed intake, daily weight gain, feed efficiency, dry matter digestibility, and crude ash digestibility in rats

	Control ( $n = 6$ )	FOS ( $n = 7$ )	Mannitol ( $n = 7$ )
Initial body weight (g)	404.1 ± 57.2	401.8 ± 43.6	403.6 ± 43.6
Final body weight (g)	420.4 ± 55.0	419.4 ± 34.7	407.3 ± 41.1
Daily weight gain (g/day)	2.3 ± 1.8	2.5 ± 2.2	0.5 ± 2.8
Feed intake (g/day)	17.2 ± 0.3 <sup>a</sup>	17.4 ± 0.3 <sup>a</sup>	18.1 ± 0.7 <sup>b</sup>
Feed efficiency (%)	4.5 ± 3.3	4.8 ± 4.1	1.1 ± 5.1
Dry matter digestibility (%)	94.7 ± 0.7	94.4 ± 0.7	94.6 ± 1.2
Crude ash digestibility (%)	44.5 ± 5.8 <sup>a</sup>	53.8 ± 5.6 <sup>b</sup>	57.4 ± 7.5 <sup>b</sup>

control = control diet (AIN-93G) (Reeves et al. 1993), FOS = experimental diet containing 8% fructooligosaccharide, mannitol = experimental diet containing 8% mannitol

data are mean ± SD, significance of difference between the data was analyzed by Tukey-Kramer test using add-in statistical software for MS Excel (SSRI Co., Tokyo, Japan), differences were considered significant at  $P < 0.05$

<sup>a,b</sup>means within rows with different superscripts differ ( $P < 0.05$ )

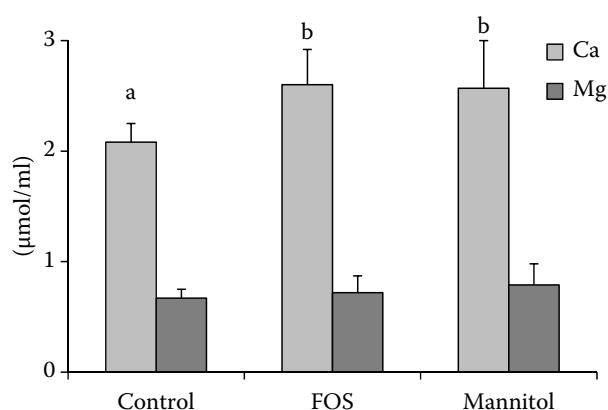


Figure 2. Effects of dietary FOS and mannitol on the concentrations of Ca and Mg in cecal vein plasma in rats control = control diet (AIN-93G) (Reeves et al. 1993), FOS = experimental diet containing 8% fructooligosaccharide, mannitol = experimental diet containing 8% mannitol data are mean  $\pm$  SD (six rats were in the control diet group and seven rats were in the FOS diet group and the mannitol diet group, respectively). Significance of difference between the data was analyzed by Tukey-Kramer test using add-in statistical software for MS Excel (SSRI Co., Tokyo, Japan). Differences were considered significant at  $P < 0.05$

and in the mannitol diet group were significantly higher than those in the control diet group, but no significant difference between the two indigestible

diet groups was observed. The Mg concentrations in cecal vein plasma in rats did not significantly differ among the three experimental groups.

The parameters of cecum and colon in rats are shown in Table 3. Compared with the control diet, cecal pH was significantly decreased by the diets with FOS and mannitol. The total weight and the content weight of cecum in the FOS group and the mannitol group were significantly higher than in the control group. Cecal tissue weights and cecal moisture in rats in the mannitol group were significantly higher than in the FOS group and the control group. Colonic pH in rats in the FOS group and the mannitol group was significantly higher than that in the control group. The total weight, the tissue weight, and the content weights of colon were not significantly different between the three experimental groups. Colonic moisture in the mannitol group was significantly higher than in the FOS group and the control group.

Organic acids concentration in cecal contents in rats is shown in Table 4. Compared with the control diet group, succinic acid concentration was significantly increased by mannitol feeding. But the concentration of succinic acid in rats in the FOS group was not different neither from that in the mannitol diet group nor in the control diet group. The concentrations of lactic acids and butyric acids

Table 3. Parameters of cecum and colon in rats

	Control ( $n = 6$ )	FOS ( $n = 7$ )	Mannitol ( $n = 7$ )
<b>Cecum</b>			
pH	$7.6 \pm 0.2^a$	$6.4 \pm 0.4^b$	$6.9 \pm 0.2^c$
Whole weight (g)	$4.50 \pm 0.68^a$	$7.70 \pm 1.26^b$	$6.68 \pm 1.61^b$
Tissue weight (g)	$0.70 \pm 0.06^a$	$0.75 \pm 0.11^a$	$0.95 \pm 0.16^b$
Content weight (g)	$3.8 \pm 0.6^a$	$6.9 \pm 1.2^b$	$5.3 \pm 1.7^b$
Moisture (%)	$80.2 \pm 2.6^a$	$82.5 \pm 3.2^a$	$87.8 \pm 2.9^b$
Ash (% DM)	$28.8 \pm 0.7^a$	$24.1 \pm 1.8^b$	$21.4 \pm 0.7^b$
<b>Colon</b>			
pH	$7.7 \pm 0.1^a$	$6.8 \pm 0.4^b$	$6.8 \pm 0.2^b$
Whole weight (g)	$2.67 \pm 0.59$	$2.94 \pm 1.25$	$3.73 \pm 0.78$
Tissue weight (g)	$1.23 \pm 0.06$	$1.18 \pm 0.20$	$1.35 \pm 0.18$
Content weight (g)	$1.44 \pm 0.60$	$1.76 \pm 1.07$	$2.38 \pm 0.83$
Moisture (%)	$60.3 \pm 5.9^a$	$68.0 \pm 9.1^a$	$79.3 \pm 8.2^b$
Ash (% DM)	$29.6 \pm 1.7^a$	$25.4 \pm 1.3^b$	$22.8 \pm 2.7^b$

control = control diet (AIN-93G) (Reeves et al. 1993), FOS = experimental diet containing 8% fructooligosaccharide, mannitol = experimental diet containing 8% mannitol, DM = dry matter

data are mean  $\pm$  SD, significance of difference between the data was analyzed by Tukey-Kramer test using add-in statistical software for MS Excel (SSRI Co., Tokyo, Japan), differences were considered significant at  $P < 0.05$

<sup>a-c</sup>means within rows with different superscripts differ ( $P < 0.05$ )

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Table 4. Concentration of organic acids ( $\mu\text{mol/g}$  content) in cecal contents in rats

	Control ( $n = 6$ )	FOS ( $n = 7$ )	Mannitol ( $n = 7$ )
Succinic acid	$2.5 \pm 2.7^a$	$18.0 \pm 12.9^{ab}$	$26.1 \pm 15.6^b$
Lactic acid	$2.4 \pm 0.4^a$	$15.2 \pm 2.4^b$	$10.2 \pm 5.7^b$
Formic acid	$0.35 \pm 0.19$	$0.98 \pm 1.58$	$1.52 \pm 1.41$
Acetic acid	$27.2 \pm 8.4^a$	$60.5 \pm 10.6^b$	$12.6 \pm 6.4^c$
Propionic acid	$10.1 \pm 3.4^a$	$14.2 \pm 1.8^b$	$5.3 \pm 2.3^c$
Isobutyric acid	$3.1 \pm 3.2$	$7.1 \pm 3.6$	$9.2 \pm 5.2$
Butyric acid	$3.1 \pm 0.9^a$	$7.0 \pm 1.8^b$	$5.9 \pm 1.1^b$
Isovaleric acid	$0.71 \pm 0.43$	$0.36 \pm 0.02$	$1.33 \pm 1.39$
Valeric acid	$0.41 \pm 0.03$	–	$0.68 \pm 0.0$
Total acids	$62.0 \pm 27.4^a$	$138.2 \pm 36.6^b$	$69.5 \pm 35.5^a$

control = control diet (AIN-93G) (Reeves et al. 1993), FOS = experimental diet containing 8% fructooligosaccharide, mannitol = experimental diet containing 8% mannitol

data are mean  $\pm$  SD, significance of difference between the data was analyzed by Tukey-Kramer test using add-in statistical software for MS Excel (SSRI Co., Tokyo, Japan), differences were considered significant at  $P < 0.05$

<sup>a-c</sup>means within rows with different superscripts differ ( $P < 0.05$ )

in rats in the FOS diet group and the mannitol diet group were significantly higher than those in rats in the control diet group. The concentrations of acetic acid and propionic acid were significantly increased by FOS feeding, but were significantly decreased by mannitol feeding, compared with the control diet.

The total acids concentration in cecal contents in the FOS diet group was significantly higher than in the control diet group and the mannitol diet group. Between the control diet group and the mannitol diet group, the total acids concentrations in cecal contents were similar.

Table 5. Levels of Ca and Mg in the contents of cecum and colon in rats

	CON ( $n = 6$ )	FOS ( $n = 7$ )	MAN ( $n = 7$ )
<b>Cecum</b>			
Total Ca ( $\mu\text{mol/g DM}$ )	$2052.9 \pm 125.5^a$	$1557.8 \pm 119.4^b$	$1332.8 \pm 133.3^c$
Soluble Ca ( $\mu\text{mol/g DM}$ )	$134.9 \pm 24.1^a$	$446.4 \pm 98.0^b$	$359.2 \pm 83.1^b$
Soluble Ca/total Ca (%)	$6.57 \pm 1.13^a$	$29.19 \pm 8.90^b$	$27.58 \pm 8.59^b$
Total Mg ( $\mu\text{mol/g DM}$ )	$171.6 \pm 48.2^{ab}$	$112.0 \pm 22.7^a$	$190.7 \pm 54.8^b$
Soluble Mg ( $\mu\text{mol/g DM}$ )	$13.8 \pm 5.7$	$14.2 \pm 7.2$	$18.2 \pm 8.3$
Soluble Mg/total Mg (%)	$8.1 \pm 2.2$	$12.7 \pm 5.7$	$9.5 \pm 4.8$
<b>Colon</b>			
Total Ca ( $\mu\text{mol/g DM}$ )	$3040.6 \pm 1241.6$	$2959.6 \pm 1604.9$	$3367.2 \pm 1182.3$
Soluble Ca ( $\mu\text{mol/g DM}$ )	$122.3 \pm 34.5$	$174.3 \pm 140.3$	$216.0 \pm 89.6$
Soluble Ca/total Ca	$5.83 \pm 1.93$	$10.73 \pm 10.47$	$15.73 \pm 7.02$
Total Mg ( $\mu\text{mol/g DM}$ )	$256.9 \pm 31.2^a$	$161.0 \pm 97.4^b$	$126.6 \pm 37.5^b$
Soluble Mg ( $\mu\text{mol/g DM}$ )	$16.2 \pm 2.9^a$	$29.7 \pm 10.2^b$	$17.8 \pm 6.2^{ab}$
Soluble Mg/total Mg (%)	$6.3 \pm 1.2^a$	$18.4 \pm 8.8^b$	$14.1 \pm 3.9^b$

control = control diet (AIN-93G) (Reeves et al. 1993), FOS = experimental diet containing 8% fructooligosaccharide, mannitol = experimental diet containing 8% mannitol, DM = dry matter

data are mean  $\pm$  SD, significance of difference between the data was analyzed by Tukey-Kramer test using add-in statistical software for MS Excel (SSRI Co., Tokyo, Japan), differences were considered significant at  $P < 0.05$

<sup>a-c</sup>means within rows with different superscripts differ ( $P < 0.05$ )



The levels of Ca and Mg in cecal contents and colonic contents dry matter are shown in Table 5. In the dry matter of cecal contents, the concentrations of Ca in the FOS diet group and the mannitol diet group were significantly lower than in the control diet group. The concentration of Ca in cecal contents in the mannitol diet group was significantly higher than in the FOS diet group. The concentration and the proportion of soluble Ca in total Ca in cecum contents in the FOS diet group and the mannitol diet group were significantly higher than in the control diet group. The concentration of Mg in cecal content in the FOS group and the mannitol group was not significantly different from that in the control group, but cecal Mg concentration in the mannitol diet group was significantly lower than that in the FOS diet group. In the dry matter of colonic contents, the Ca concentration, soluble Ca concentration, and the proportion of soluble Ca in total Ca were not significantly different between the experimental groups. Colonic Mg concentrations in the FOS diet group and the mannitol diet group were similar and significantly lower than in the control diet group. Colonic soluble Mg concentration in the FOS group was significantly higher than in the control diet group. The proportion of soluble Mg in total Mg in rats was significantly increased by FOS feeding and mannitol feeding when compared with the rats in the control diet group.

## DISCUSSION

Indigestible monosaccharides, disaccharides, oligosaccharides, and sugar alcohols escape metabolism in the small intestine, but can be metabolized by bacterial flora in the large intestine. The fermentation of these short chain carbohydrates contributes to the absorption of bone-relevant minerals such as calcium and magnesium and improves bone mineral content (Weaver et al. 2011; Weisstaub et al. 2013). Ca retention was increased after day 11 of the supplementation of indigestible oligosaccharides, but this beneficial effect did not continue after day 25 in rats (Ohta et al. 1994). Dietary galactooligosaccharide initially increased intestinal calcium absorption in days 8–10 and in days 18–20 in ovariectomized rats, but no longer in days 28–30 (Chonan et al. 1995). In human studies, the improvement in intestinal calcium absorption induced by the supplementa-

tion of short chain FOS and lactulose lasted only 9 days (van den Heuvel et al. 1999). In the present study, the supplement of the experimental diets was continued for 7 days as short-term to observe the effects of FOS and mannitol on the intestinal absorption of Ca and Mg.

It has been reported that FOS has promoting effects on mineral absorption, especially on Ca and Mg absorption. In rats, Ohta et al. (1994) found that 5% dietary FOS increased the absorption of Ca and Mg in normal rats. When the rats were fed the low-Mg, high-Ca, and high-P diet, FOS significantly increased the Mg absorption. The related mechanism was that FOS was fermented by the bacteria in the large intestine to reduce luminal pH to stimulate mineral solubility to increase mineral absorption. We have previously demonstrated that 6% and 8% dietary mannitol improved the absorption of Ca and Mg in the large intestine, and increased the retention of Ca and Mg in tibia and femur after four weeks feeding in growing rats (Xiao et al. 2013). Mannitol performed similar fermentation as FOS. Mannitol was utilized by cecal bacteria to reduce cecal pH to increase the solubility of Ca and Mg. Mannitol feeding could lead to an enlarged cecal wall to provide larger surface area to facilitate the mineral absorption in the large intestine.

The results of this study support the above statement. The feeding of the experimental diets with 8% FOS and 8% mannitol enhanced the absorption of Ca and Mg in this study. The parameters in the cecum revealed that FOS and mannitol were fermented in the cecum. The cecal weights and cecal pH in the rats were reduced by both dietary FOS and dietary mannitol. Dietary FOS induced lower cecal pH than dietary mannitol did, and at the same time the cecal tissue weights were enlarged by mannitol feeding but not by FOS feeding. The different effects of FOS and mannitol on cecal wall tissue and pH may combine with the different pattern of organic acids produced from their fermentation in cecum. The quantity and distribution of organic acids depend on the structure and mass of indigestible substances that are fermented by certain bacteria species, leading to a corresponding organic acids pattern. The ratios of short chain fatty acids (SCFAs; 61 : 14 : 7 and 13 : 5 : 6 for acetic : propionic : butyric acids) in the rats fed dietary FOS and dietary mannitol (Table 4) suggest that FOS and mannitol were fermented in

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different pathways. The significant higher amount of total acids in cecum in this study suggested that FOS led to a greater production of organic acids than mannitol did. When the abdominal cavity was opened, more gas production in the rats fed dietary mannitol than in those fed dietary FOS was observed visually. Morishita (1994) reported that 5%-mannitol diet significantly decreased the population of *Viridans streptococci* and *Bifidobacteria* and significantly increased the population of *Fusiform* bacteria in cecum in rats. But FOS intake could increase the number of *Bifidobacterium* in healthy adults (Tokunaga et al. 1993). In another research, FOS intake induced a significant increase in the population of *Lactobacillus* spp. and *Bifidobacterium* spp., which came along with increased levels of butyric acid and acetic acid in human colon *in vitro* (Sivieri et al. 2014). These indigestible carbohydrates had different effects on bacteria population because when indigestible carbohydrates enter the large intestine, the population of one genus may increase at the expense of other bacterial species which use carbon source in similar pathways. SCFAs (especially butyric acids) accelerate epithelial cell proliferation, thereby increasing cecal tissue weight (Sakata 1986). Due to SCFAs the cecal epithelial mitotic activity was higher at pH 7.0 than at pH 5.0 in rats (Ichikawa and Sakata 1997). Therefore, the action of SCFAs on cecal epithelial proliferation may be more effective with cecal pH 6.9 in the rats fed dietary mannitol than with cecal pH 6.4 in the rats fed dietary FOS in this study.

The level of Ca and Mg in cecal vein plasma showed that the feeding of FOS and mannitol increased Ca absorption in cecum, but not Mg absorption in this segment. The lower cecal pH resulted from high production of organic acid in cecum led by FOS feeding. Mannitol feeding did not change total organic acids production. The reduced cecal pH was induced by the production of lactic acids and succinic acids (Hoshi 1994). The reduction in luminal pH can raise the concentration of mineral cations (Raschka and Daniel 2005). Lower pH could increase the amount of soluble and ionized mineral to facilitate its absorption (Lupton et al. 1985). SCFAs from the fermentation of indigestible carbohydrates are the main factor for their positive effects on mineral absorption (Younes et al. 1996). SCFAs reportedly improved mineral absorption by several ways

such as increased epithelial absorptive capacity and increased intestinal blood flow and fluid, and regulated electrolyte exchanges between mineral and hydrogen (Topping and Clifton 2001). In this study, FOS feeding is more potent to reduce cecal pH than mannitol feeding. On the contrary, mannitol feeding markedly enlarged cecal wall tissue, leading to a greater exchange surface area to absorb Ca in cecum.

Cecum is the site where the highest rate of Ca absorption takes place in rats (Karbach and Feldmeier 1993). Especially when acidic fermentation took place in cecum, Ca absorption shifted toward the large intestine (Younes et al. 1996), which was supported by our results. The results of this study provided the information that apparent Mg absorption was increased by feeding FOS and mannitol. But the concentration of Mg in cecal vein plasma was increased neither by dietary FOS, nor dietary mannitol. In previous studies, the intestinal segment, where the absorption of Ca and Mg was increased by mannitol feeding, was determined by the non-absorbable marker CWC-Cr. The result declared the increment of the absorption of Ca and Mg took place in the large intestine (Xiao et al. 2013). Therefore, Mg tended to be absorbed more in the colon after the ingestion of mannitol. Ohta et al. (1995) reported FOS consumption could increase the absorption of Mg, and about a half of the increase occurred in the colon and rectum. The difference of Ca and Mg in the absorbed segment might be due to the distinct cellular and paracellular mechanisms of their transport in the intestine (Karbach and Feldmeier 1991). The colon was recognized as the major site of Mg absorption in both Mg-deficient and Mg-well-supplied rat (Chutkow 1966). Thus, FOS and mannitol contributed to Ca absorption in the cecum and to Mg absorption in the colon. In this study, the proportion of soluble mineral in luminal total mineral was increased by both FOS and mannitol feeding, due to the reduced intestinal pH. The more soluble minerals are more effective to increase their absorption by the intestinal epithelial cell. But the effect of luminal Ca presence on Mg absorption has been arguing (Schaafsma 1997). Some of the publications support high Ca intakes could reduce active Mg absorption via a competition for a common carrier, or via the paracellular pathway via Ca-induced change in the tight junction permeability to Mg (Behar 1975).

Therefore, after the intake of FOS and mannitol, the excess Ca might preclude Mg absorbed in cecum, and massive soluble Mg flowed into the colon and was absorbed.

## CONCLUSION

In conclusion, we have established that the absorption of Ca and Mg was improved by short-term feeding indigestible FOS and mannitol. Cecal fermentation of FOS and mannitol increased the solubility of Ca and Mg, facilitated Ca absorption in the cecum and contributed to Mg absorption in the colon.

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