Title of paper

Promotive effects of nondigestible disaccharides on rat mineral absorption depend on kind of the saccharide.

Running head

Disaccharides and mineral absorption

Authors

Patchana Asvarujanon, PhD, Satoshi Ishizuka, PhD and Hiroshi Hara, PhD

Institution

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan

Corresponding Author

Hiroshi Hara

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University

Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan

Tel: +81-11-706-3352 Fax: +81-706-2504

E-mail: hara@chem.agr.hokudai.ac.jp

ABSTRACT

OBJECTIVE: We examined the effects of feeding nondigestible disaccharides, difructose anhydride III (DFAIII), maltitol, melibiose and cellobiose on calcium, magnesium and iron absorption in comparison to fructooligosaccharide (FOS) in normal and ovariectomized rats.

METHODS: In experiment 1, six groups of male Sprague-Dawley rats were fed either a control diet (100 g cellulose/kg diet) or test diets containing 30 g of FOS or the four nondigestible disaccharides in place of the cellulose in the control diet for 4 wk. In experiment 2, two groups of female Sprague-Dawley rats (sham or ovariectomized) were divided into four subgroups and fed either the control or test diet containing FOS, DFAIII and melibiose for 5 wk. Feces and cecal contents were collected to evaluate mineral absorption and cecal fermentation.

RESULTS: In experiment 1, calcium absorption in the all disaccharides groups except the cellobiose group, magnesium absorption in the all test diet groups, and iron absorption in the FOS, DFAIII and melibiose groups were higher than those in the control group, respectively. In ovariectomized rats (experiment 2), calcium absorption in the DFAIII and melibiose groups, magnesium absorption in the all test diet groups, and iron absorption in the DFAIII group alone were higher than those in the control group, respectively. Cecal organic acids were positively and pH was negatively correlated with the absorption of these minerals, although the effects varied.

CONCLUSIONS: Nondigestible disaccharides increase calcium, magnesium, and iron absorption in normal and ovariectomized rats; however, the effects depend on the disaccharide tested, a fact that may partly be associated with the cecal fermentation of these disaccharides.

KEY WORDS: Nondigestible disaccharides, calcium, magnesium, iron, intestinal absorption, ovariectomized, rats

Introduction

Mineral deficiencies remain an important nutritional issue, with calcium intake in many Asian countries below the recommended daily allowance. Such deficiency in calcium is known to lead to progressive bone loss.[1] Magnesium is the second most abundant intracellular cation in vertebrates, and its deficiency has also been implicated as a risk factor for osteoporosis.[2-3] Another mineral with a low intake is iron. Iron deficiency is one of the most common nutritional problems in the world[4] and is a major cause of anemia. The low bioavailability of dietary iron is an important factor causes of iron deficiency.

As has been demonstrated by in vivo and in vitro studies, ingestion of nondigestible saccharides, including various types of oligosaccharides,[5-6] sugar alcohol[7-8] and polysaccharides,[9-10] results in an increase in calcium absorption in rats. We previously showed that difructose anhydride III (DFAIII) stimulates calcium transport in the isolated mucosa of the rat small intestine.[11] The promotive effect of DFAIII in the small intestine has also been demonstrated with an in vivo study using ileorectostomized rats.[12] Maltitol is another nondigestible disaccharide reported to have promotive effects on calcium absorption in the small intestine. Previous studies suggest that these disaccharides in the intestinal lumen directly affect the epithelium of the small intestine and enhance calcium transport.[13]

Increases in fermentation in the large intestine with feeding of nondigestible saccharides is also thought to be involved in the promotion of calcium transport in the large intestine.[6,14] It is well known that fructooligosaccharide (FOS) increases calcium absorption dependent on cecal fermentation in rats.[15-16] Organic acids produced through microbial fermentation decrease the pH of the cecal contents, which solubilize calcium salts in the large intestine and increase calcium absorption.[17-19] It has been reported that short-chain fatty acids themselves promote calcium absorption directly[20-21] or indirectly through stimulation of mucosal cell proliferation in the large intestine.[22]

There is little information concerning the effect of nondigestible disaccharides on magnesium or iron absorption compared to that on calcium absorption. Ingestion of FOS has been shown to increase magnesium absorption in rats[23] and to prevent iron-deficiency anemia induced by gastric resection.[24] Feeding of DFAIII was reported to increase iron absorption and partially prevents iron-deficiency anemia induced by tannic acid.[25]

In the present study, we used FOS, which consists of 42% 1-ketose, 46% nystose and 9% fructofuranosylnystose,[26] as a standard oligosaccharide because FOS has been well characterized as a nondigestible, fermentable oligosaccharide. This study was conducted to compare the effects of nondigestible disaccharides on calcium, magnesium and iron absorption by using normal and ovariectomized (OVX) rats. The ovariectomized rat is a model of osteoporosis in post-menopausal woman. Disaccharides, DFAIII and Maltitol, have been reported to increase calcium absorption as mentioned above. The former promotes the absorption in the small and large intestines, and the latter in the small intestine. We included two other nondigestible disaccharides, melibiose and cellobiose, in this study.

Di-D-fructose-1, 2′:2, 3′-dianhydride (DFAIII) is a disaccharide consisting of two fructose residues with two glycoside bonds. Maltitol (α-D-glucopyranosyl-1, 4-sorbitol) is a disaccharide alcohol generated by the hydrogenation of maltose. Melibiose (6-*O*-∂-D-galactopyranosyl-D-glucose) is a disaccharide composed of galactose and glucose, and cellobiose (4-*O*-D-Glucopyranosyl-D-glucose) is composed of two glucose residues with a β-glycoside bond. There are no reports on the effects of the latter two disaccharides on mineral absorption in vivo.

Materials and Methods

This study was approved by the Hokkaido University Animal Committee, and animals were maintained in accordance with the guidelines of the Hokkaido University for the care and

use of laboratory animals.

Animal and Experimental protocol

Male (experiment 1) and female (experiment 2) Sprague-Dawley rats (6 wk old; Clea Japan, Tokyo, Japan) weighing about 150 g were housed in individual cages in a room with controlled temperature (22 ± 2℃), relative humidity (40% to 60%), and lighting (lights from 8:00 AM to 8:00 PM) throughout the study.

In experiment 1, the rats had free access to deionized water and a sucrose-based semipurified stock diet (shown in Table1) for an acclimation period of 7 days, and were then divided into six groups of eight rats using a randomized block design based on body weight. Rats of one group (control diet group) were fed the test diet prepared based on AIN-93G formulation[27] containing 100 g cellulose/kg diet (Table1), and those of other five groups were fed test diets containing 30 g of the five nondigestible saccharides in place of the cellulose in the control diet for 4 weeks. Saccharides tested were short-chain fructooligosaccharide (FOS; Meioligo-P, Meiji Seika, Tokyo, Japan), as a standard oligosaccharide, and four nondigestible disaccharides, di-D-fructose anhydride III (DFAIII, Nippon Beet Sugar MFG Co., Ltd., Obihiro, Japan), maltitol (MAL; Lesys, Towa Kasei, Natori, Japan), melibiose (MEL, 6-*O*-∂-D-galactopyranosyl-D-glucose, Nippon Beet sugar MFG., Co., Ltd, Obihiro, Japan) and cellobiose (CEL, JW cellobiose, Japan chemical and machinery Co., Ltd., Osaka, Japan). All rats were allowed free access to the test diets and deionized water throughout the test diet period. Body weight and food intake were measured daily.

In experiment 2, the rats had free access to deionized water and a sucrose-based semipurified stock diet (Table1) one day before operation. The rats were divided into two groups; one group of rats underwent bilateral ovariectomy (OVX) and the other group underwent bilateral laparotomy (sham). Both operations were performed under the same

anesthetic procedure (Nembutal: sodium pentobarbital 40 mg/kg of body weight; Abbott Laboratories, North Chicago, IL, USA). All animals had free access to deionized water and the stock diet for a 6-day recovery period. After postoperative recovery, sham and OVX rats were randomly assigned to four subgroups of 8-9 rats. Rats of the four subgroup were fed CON, FOS, DFA III, or MEL diets, respectively. Rats were fed the assigned test diets for 5 weeks. All rats were allowed free access to the test diets and deionized water throughout the test period. Body weight and food intake were measured daily.

Feces were collected during day 10-14 (in experiment 1) and the last 5 days of the test period (in both experiments) to evaluate absorption of calcium, magnesium and iron. On the last day of the experiment, all rats were killed under pentobarbital anesthesia (Nembutal: sodium pentobarbital 50 mg/kg of body weight; Abbott Laboratories). In experiment 1, the cecum was removed with their contents, weighed, frozen immediately with liquid nitrogen, and stored at –40°C until subsequent analyses. The cecal contents were collected by cutting open the frozen cecal wall and cecal wall was washed with saline and weighed. The content weight was evaluated as the difference in weight between the cecum with and without contents.

Analyses

Collected feces were freeze-dried and milled to a fine powder, and the powdered feces (approximately 1.5 g) were dry-ashed at a temperature elevated linearly to 550℃ for 6 hr, and then at 550℃ for 18 hr with an electric furnace (EYELA, TMF-3200, Tokyo Rikakikai, Tokyo, Japan). The ashed samples were treated with 5.49 mol/l HCl at 200℃ for 30 minutes and were dissolved in 0.82 mol/l HCl. Calcium, magnesium, and iron concentrations in the ashed sample solutions were measured by polarizing zeeman-effect atomic absorption spectrometry (Z-5310, Hitachi, Tokyo, Japan) after suitable dilution.

The cecal contents were diluted with 4 volumes of deionized water and homogenized

with a Teflon homogenizer. The pH of the homogenate was measured with a semiconducting electrode (ISFET pH sensor 0015-15C, HORIBA, Ltd., Kyoto, Japan) as the pH of the cecal contents. Lipids and proteins in the homogenate of the cecal contents were removed through treatment with chloroform, and the concentrations of individual organic acids (succinic, lactic, acetic, propionic, butyric and valeric) were measured by ion-exclusive chromatography using a high-pressure liquid chromatography (HPLC) system (Organic Acid Analysis System, Shimadzu Corporation, Kyoto, Japan) constructed with a solvent delivery system (SLC-10 AVP; Shimadzu), double ion-exchange column (Shim-pack SCR-102h, 8x300 mm; Shimadzu) and an electroconductivity detector (CDD-6A; Shimadzu), as previously described.[28]

Calculations and Statistical Analyses

Calcium, magnesium and iron absorption in this study was expressed as "net absorption", which was calculated by subtract the mineral excretion in feces from mineral intake using the equation 1), and was calculated the apparent absorption rate as "absorption rate" using the equation 2).

1) Net Ca, Mg, or Fe absorption (mmol/5 days or μmol/5 days)

= total Ca, Mg or Fe intake – Ca, Mg or Fe excretion in feces.

2) Ca, Mg, or Fe absorption rate (%)

$$= 100 \times \frac{\text{(total Ca, Mg or Fe intake – Ca, Mg or Fe excretion in feces)}}{\text{total Ca, Mg or Fe intake.}}$$

Each value represents the mean ± standard error. The effects of Time and Diet (in experiment 1) and Operation and Diet (in experiment 2) were analyzed by two-way analysis of variance (ANOVA). Duncan's multiple-range test was used to determine whether mean values were significantly different between groups ($P < 0.05$).[29] The correlation coefficients for the pH of cecal contents, total organic acid contents, total SCFA contents against mineral

absorption were calculated by the least squares method.[30]

Results

Experiment 1

There were no significant differences in body weight gain between the five oligosaccharide groups and that in the control group (Table 2); however, gain in the CEL group was significantly higher than in the MAL group. Food intake in the control group was higher than those in rats in each of the test diet groups, but there were no differences among the five oligosaccharide groups. Fecal dry weight excreted for 5 days was influenced by Time and Diet ($P < 0.001$). The output of the control group was higher than those of other groups in both the first (day 10-14) and second (day 24-28) balance periods, but there were no differences among the five oligosaccharide groups. Fecal output of all groups except for the control group on day 24-28 tended to be lower compared to those on day 10-14 ($P < 0.001$ for Time and Time x Diet).

There were no differences in calcium and magnesium intake among the diet groups on day 10-14, but the calcium intake of the control group was higher than those of the other diet groups on day 24-28 (data not shown). Net calcium and magnesium absorption was influenced by Time and Diet (Table 3). Magnesium balance was higher in the second period than in the first period, but calcium balance had the interaction between Time and Diet. Net calcium absorption in the DFAIII group was higher than that in all other groups except for the MEL group during day 24-28. Net magnesium absorption in the FOS group was higher than that in the control group in both periods.

There were no differences in iron intake among groups in the first period, and that in the control group during the second period was higher than those in the other groups except for the DFAIII group (data not shown). Net iron absorption was influenced by Time and Diet, and there was interaction between Time and Diet. Net iron absorption in the first period was higher in the DFAIII group compared to those of the MAL and CEL groups (Table 3). In the second period,

iron absorption in the DFAIII and MEL groups was higher than those in the control, MAL and CEL groups.

Changes in calcium and magnesium absorption rates were very similar to those in net calcium and magnesium balance (Figure 1-2). Calcium absorption rate of the DFAIII group in the second period was higher than those of the other groups except for the MAL and MEL groups. Magnesium absorption rates of the all oligosaccharide groups were higher than the control group in both balance periods. Changes in iron absorption rate were also similar to those in net balance (Figure 3). The rate in the DFAIII group alone was higher than that in the control group in the first period; however, those of the FOS, DFAIII and MEL groups were higher than that in the control group in the second period.

The wet weight of the cecal wall and cecal contents (g/100 g body weight) was higher in the FOS, DFAIII and MEL groups than in the control group (Table 4). The highest values for the cecal wall and contents were in the DFAIII and FOS groups, respectively. The pH of the cecal content was higher in the control group than in the other test diet groups. The pH value in the FOS group was the lowest among the groups.

Rats fed the DFAIII diet had the highest pools of succinic, acetic and propionic acids and total SCFAs in the cecal contents (Table 5). The lactic acid pools in the FOS and MEL groups were much higher than those in the other groups. The butyrate pool was highest in the MAL group, followed by that in the DFAIII group. The total organic acid pools of all the oligosaccharide groups were higher than that in the control group, with the highest value observed in the FOS group. The total SCFA pool was the highest in the DFAIII group, followed by that in the MAL group, and values in both groups were higher than that in the control group.

Experiment 2

Initial body weight was different between sham and OVX rats ($P = 0.008$, Table 6). Body weight gains and food intakes were higher in OVX rats than in sham rats ($P < 0.001$ for

Operation). Food intake in the DFAIII group was lower than that in the control group in both sham and OVX rats. Dry weight of feces excreted over the last 5 days was higher in the OVX rats than in the sham rats; however, there were no differences among diet groups. The uterine weight was much lower in OVX rats than in sham rats.

Ovariectomy influenced calcium, magnesium and iron intakes in the results of two way ANOVA (all $P < 0.001$, data not shown). Net calcium, magnesium and iron absorption was influenced by Operation (OVX) and Diet except for Diet for magnesium (Table 7). Net calcium absorption in OVX rats was higher in the DFA III group than in the control group. Net magnesium absorption was higher in three oligosaccharide groups (FOS, DFAIII, MEL) than in the control group in OVX rats, and absorption in the FOS and DFAIII groups, but not the control group were higher in OVX rats than in the sham rats, respectively. Iron absorption in OVX rats fed DFAIII was higher than those in all other groups.

Calcium absorption rate was also influenced by Operation and Diet ($P < 0.001$). The absorption rate was higher in rats fed DFAIII and MEL compared to that in rats fed the control and FOS diets in both sham and OVX groups (Figure 4). Magnesium absorption rates in the OVX rats were higher in the three oligosaccharide groups compared to that in control group (Figure 5). In the control diet groups, the absorption rate in sham rats was higher than that in OVX rats. There were no significant differences among diet groups in sham rats. The iron absorption rate in the DFAIII group was higher than that in the control group in sham rats, and higher than those in the other three groups in OVX rats (Figure 6).

Discussion

In the present study, the effects of the ingestion of four disaccharides, DFAIII, MAL, MEL and CEL on calcium, magnesium and iron absorption were compared to those of FOS and control (only cellulose) diets. All disaccharides were effective in increasing the absorption of at least one mineral; however, the effects varied markedly among these nondigestible saccharides.

Calcium absorption was increased most effectively by DFAIII and MEL, followed by MAL, but these three disaccharides had similar effects on magnesium absorption. Iron absorption was increased more effectively by DFAIII and MEL than by the other two disaccharides. In female OVX rats, iron absorption was enhanced by DFAIII ingestion alone. These results suggest that the mechanisms involved in the promotion of mineral absorption, especially those of calcium and iron are different between these disaccharides. The reason for limited effects of MEL and FOS on iron absorption in OVX rats compared to that in normal male and sham female rats, is not yet known.

We examined both net absorption (net balance) and absorption rate (%) as food intakes were differed among the diet groups, between male and female and between sham and OVX rats. In male rats (Exp. 1), net calcium and iron absorption were clearly increased by the ingestion of DFAIII and MEL (Table 3), even though intakes of these minerals were reduced in all the oligosaccharide groups. This is reflected in the high values for these absorption rates in the DFAIII and MEL groups in the second balance period (Figs. 1 and 3, right panels). This result shows that these two disaccharides have good potential for enhancing calcium and iron absorption. Deficiencies in these two minerals are apparent in many countries. DFAIII and MEL may be beneficial foodstuffs for improving these mineral deficiencies. This finding again reveals that the effects of nondigestible disaccharides on mineral absorption depend on the type of disaccharide.

The mechanisms for the promotion of mineral absorption by nondigestible saccharides are proposed both in the small and large intestines, including the cecum. Increasing cecal fermentation is the established mechanism involved in the promotion of calcium and magnesium absorption in rats.[6,14,31] The proposed mechanisms are (1) the expansion of the lumen surface due to enlargement of the cecum, and (2) the increase in soluble mineral concentrations in the large intestinal contents as a result of decreasing pH.[17-19] Some studies have suggested that SCFAs

produced from these saccharides are responsible for the enhancement of calcium absorption in the large intestine.[20-21,32] The results in Table 2 show that the nondigestible disaccharides used in this study were fermentable because fecal dry weight was lower in the groups fed the four disaccharides compared to the control group fed cellulose. Increases in fermentation were supported by the larger pools of organic acids; however, these pools varied markedly among the groups fed the four disaccharides. The highest level of organic acid including SCFA was in the FOS group, which was followed by the MEL group. The organic acid pools of rats fed MAL and CEL were less than a half of the levels in the FOS and MEL groups, showing that these disaccharides are relatively less fermentable. The absence of or only minor effects of MAL and CEL on calcium and iron absorption may be associated with the lower fermentability of these disaccharides.

We next evaluated correlations between several cecal parameters and mineral absorption (Table 8). Absorption of the three minerals in the second period was negatively correlated to the cecal pH and was positively correlated to total organic acid pool and cecal weight (wall and contents), which suggests that the cecal fermentation is involved in the promotion of mineral absorption for those tested. In the second period, calcium, but not magnesium absorption, was correlated to the acetic and propionic acid pools. These SCFAs have been shown to promote calcium absorption as described above.[20-21,32] Magnesium absorption was correlated to the lactic acid pool and pH and not to SCFA pool. Lactic acid is a stronger acid than SCFA. This result demonstrates that lower pH, not the existence of organic acids, is important for magnesium absorption in the large intestine. In contrast to the second period, no correlations between these cecal parameters and calcium absorption were found in the first period. Ingestion of DFAIII did, however, increase calcium absorption in the first period (Fig. 1 left panel). Factors other than cecal fermentation may contribute to the enhancement of calcium absorption by DFAIII. The promotive effects in the small intestine may be involved in the DFAIII-induced increase in

calcium absorption. Mitamura et al.[12] have reported that DFAIII enhanced calcium absorption in the small intestine, and Mineo et al.[33] have shown that DFAIII directly affects the intestinal epithelial cells and activates passage through tight junctions to increase calcium absorption in the small intestine.

Iron absorption was the most strongly stimulated among that of the three minerals by ingestion of nondigestible disaccharides, especially DFAIII. The absorption rate of iron was strongly correlated to SCFA pools in the first period, but not in the second period, in which, the absorption was strongly correlated to the cecal enlargement (weight of the cecal wall and contents). There is no direct evidence that iron is absorbed from the large intestine; however, Sakai et al.[25] have shown that the cecal fermentation of FOS is involved in the prevention of anemia induced by gastric resection. However, the mechanisms for iron absorption from the large intestine still need to be clarified.

We used OVX rats in which it has been reported that net calcium balance is impaired by increases in the intestinal calcium secretion and by calcium malabsorption with decreasing levels of estradiol.[34] In experiment 2, the uterine weight was much lower in OVX rats than in sham rats, indicating the success of the surgical procedure in all OVX rats. We observed that OVX impaired calcium and magnesium, but not iron absorption (Figure 4-6). Reduced net calcium absorption by OVX was restored by feeding DFA III or MEL, which agrees with previous results obtained by using OVX rats.[12] The present study also showed that magnesium absorption impaired by OVX was restored by feeding of DFAIII or MEL as well as FOS. These results suggest that feeding these disaccharides can benefit impaired calcium and magnesium absorption in postmenopausal women.[35-37]

In conclusion, the ingestion of nondigestible disaccharides increases calcium, magnesium and iron absorption in normal and OVX rats; however, the beneficial effects of disaccharide ingestion on mineral absorption differ according to disaccharide type.

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FIG. 1 Calcium absorption rate in rats fed control or test diets containing 30 g nondigestible oligosaccharide/kg diet at 2 wk (day 10-14) and 4 wk (day 24-28) after the start of feeding the test diets in male rats (experiment 1). Each value represents the mean ± standard error (n = 8). *P* values estimated by two-way analysis of variance (Time, Diet and Time x Diet were <0.001, < 0.001, 0.039, respectively). Values not sharing a letter are significantly different, $P < 0.05$. FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol, MEL: Melibiose, CEL: Cellobiose

FIG. 2 Magnesium absorption rate in rats fed control or test diets containing 30 g nondigestible oligosaccharide/kg diet at 2 wk (day 10-14) and 4 wk (day 24-28) after the start of feeding the test diets in male rats (experiment 1). Each value represents the mean ± standard error (n = 8). *P* values estimated by two-way analysis of variance (Time, Diet and Time x Diet were < 0.001. < 0.001, 0.013, respectively). Values not sharing a letter are significantly different, $P < 0.05$. FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol, MEL: Melibiose, CEL: Cellobiose

FIG. 3 Iron absorption rate in rats fed control or test diets containing 30 g nondigestible oligosaccharide/kg diet at 2 wk (day 10-14) and 4 wk (day 24-28) after the start of feeding the test diets in male rats (experiement 1). Each value represents the mean ± standard error (n = 8). *P* values estimated by two-way analysis of variance (Time, Diet and Time x Diet were < 0.001, < 0.001, 0.005, respectively). Values not sharing a letter are significantly different, $P < 0.05$. FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol, MEL: Melibiose, CEL: Cellobiose

FIG. 4 Calcium absorption rate in sham-operated and ovariectomized female rats fed control or test diets containing 30 g nondigestible oligosaccharide/kg diet for 5 wk after the start of feeding the test diets (experiment 2). Each value represents the mean ± standard error (n = 8 in the sham rats; n = 9 in the ovariectomized group). *P* values estimated by two-way analysis of variance (Operation, Diet and Operation x Diet were < 0.001, < 0.001, 0.296, respectively). Values not sharing a letter are significantly different, $P < 0.05$. FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MEL: Melibiose

FIG. 5 Magnesium absorption rate in sham-operated and ovariectomized female rats fed control or test diets containing 30 g nondigestible oligosaccharide/kg diet for 5 wk after the start of feeding the test diets (experiment 2). Each value represents the mean ± standard error (n = 8 in the sham rats; n = 9 in the ovariectomized group). *P* values estimated by two-way analysis of variance (Operation, Diet and Operation x Diet were 0.156, < 0.001, 0.017, respectively). Values not sharing a letter are significantly different, $P < 0.05$. FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MEL: Melibiose

FIG. 6 Iron absorption rate in sham-operated and ovariectomized female rats fed control or test diets containing 30 g nondigestible oligosaccharide/kg diet for 5 wk after the start of feeding the test diets (experiment 2). Each value represents the mean ± standard error (n = 8 in the sham rats; n = 9 in the ovariectomized group). *P* values estimated by two-way analysis of variance (Operation, Diet and Operation x Diet were 0.573, 0.002, 0.566, respectively). Values not sharing a letter are significantly different, $P < 0.05$. FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MEL: Melibiose

TABLE 1

COMPOSITION OF STOCKS[1] AND THE TEST DIETS[2]

ELEMENT	g/kg diet
Casein[3]	250
Corn oil	50
Mineral mixture[4]	35
Vitamin mixture[4]	10
Choline bitartate	2.5
Cellulose	100
Sucrose	To make 1 kg

[1]Crystallized cellulose (100 g/kg diet; Avicel PH102, Asahi Chemical Industry Co., Ltd., Tokyo, Japan) was added to the stock diet.

[2]Crystallized cellulose (30 g/kg diet) was replaced with fructooligosaccharide(FOS), di-D-fructose anhydride III (DFA III), maltitol (MAL), melibiose (MEL) and cellobiose (CEL).

[3]Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).

[4]Mineral and vitamin mixtures were prepared according to the AIN-93G formulation.

TABLE 2

BODY WEIGHT GAIN, FOOD INTAKE, AND FECAL OUTPUT OF RATS FED THE CONTROL OR TEST DIETS FOR 4 WK[1,2] (EXPERIMENT 1)

Diet	n	Initial body weight	Body weight gain	Food intake	Fecal output	
					day 10-14	day 24-28
		g	g	g/d	dry g/5 days	
Control	8	214 + 2.96 [a]	167 ± 10.3 [ab]	25.1 ± 0.57 [a]	15.4 ± 0.76 [b]	18.5 ± 0.64 [a]
FOS	8	214 + 3.98 [a]	156 ± 8.56 [ab]	22.5 ± 0.60 [b]	13.3 ± 0.61 [c]	10.5 ± 0.53 [dg]
DFA III	8	215 + 5.63 [a]	162 ± 12.1 [ab]	22.0 ± 0.44 [b]	12.1 ± 0.60 [cd]	10.4 ± 0.62 [defg]
MAL	8	217 + 3.06 [a]	149 ± 6.85 [b]	22.2 ± 0.44 [b]	12.1 ± 0.64 [cde]	10.7 ± 0.48 [g]
MEL	8	217 + 2.23 [a]	162 ± 7.83 [ab]	22.1 ± 0.49 [b]	12.0 ± 0.36 [cdef]	9.3 ± 0.35 [defg]
CEL	8	217 + 4.25 [a]	179 ± 6.38 [a]	23.4 ± 0.47 [b]	12.7 ± 0.59 [c]	10.6 ± 0.55 [dfg]
ANOVA (*P* value)						
Time		-	-	-	< 0.001	
Diet		0.978	0.266	< 0.001	< 0.001	
Time x Diet		-	-	-	< 0.001	

[1] Each value represents mean ± standard error of the mean.

[2] Values in a column not sharing a superscript differ significantly, $P < 0.05$.

FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol, MEL: Melibiose, CEL: Cellobiose

TABLE 3

NET CALCIUM, NET MAGNESIUM, AND NET IRON ABSORPTION IN RATS FED THE CONTROL OR TEST DIETS FOR 4 WK [1,2] (EXPERIMENT 1)

Diet	Net Ca absorption		Net Mg absorption		Net Fe absorption	
	Day 10-14	Day 24-28	Day 10-14	Day 24-28	Day 10-14	Day 24-28
	mmol/5 days		mmol/5 days		µmol/5 days	
Control	9.74 ± 0.27 [abc]	8.10 ± 0.60 [d]	1.46 ± 0.07 [e]	1.74 ± 0.09 [bcd]	27.8 ± 1.92 [abc]	18.8 ± 3.26 [cd]
FOS	10.6 ± 0.38 [ab]	8.83 ± 0.54 [cd]	1.75 ± 0.09 [bc]	1.99 ± 0.08 [a]	33.1 ± 2.49 [ab]	24.6 ± 2.63 [bcd]
DFA III	10.3 ± 0.30 [abc]	11.1 ± 0.53 [a]	1.62 ± 0.04 [cde]	1.93 ± 0.10 [ab]	37.5 ± 1.36 [a]	31.7 ± 1.92 [ab]
MAL	9.76 ± 0.48 [abc]	9.12 ± 0.38 [bcd]	1.51 ± 0.04 [de]	1.79 ± 0.06 [abc]	25.7 ± 3.32 [bc]	16.8 ± 2.51 [d]
MEL	10.2 ± 0.13 [abc]	10.2 ± 0.48 [abc]	1.63 ± 0.04 [cde]	1.88 ± 0.09 [ab]	29.6 ± 2.10 [ab]	28.7 ± 4.51 [b]
CEL	10.4 ± 0.43 [ab]	8.10 ± 0.49 [d]	1.63 ± 0.06 [cde]	1.70 ± 0.08 [bcde]	26.5 ± 1.35 [bc]	6.19 ± 3.21 [e]
ANOVA (*P* value)						
Time	< 0.001		< 0.001		< 0.001	
Diet	0.004		0.008		<0.001	
Time x Diet	0.007		0.618		0.024	

[1] Each value represents mean ± standard error of the mean.

[2] Values in a column not sharing a superscript differ significantly, $P < 0.05$.

FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol, MEL: Melibiose, CEL: Cellobiose

TABLE 4

WEIGHT OF THE CECAL WALL, CECAL CONTENTS, AND THE pH OF THE CECAL CONTENTS OF RATS FED CONTROL OR TEST DIETS FOR 4 WK [1,2] (EXPERIMENT 1)

Diet	Cecal wall	Cecal contents	pH
	wet g/100 g body weight	wet g/100 g body weight	
Control	0.14 ± 0.01 [d]	0.53 ± 0.06 [c]	7.22 ± 0.12 [a]
FOS	0.23 ± 0.02 [b]	2.05 ± 0.17 [a]	5.76 ± 0.06 [e]
DFA III	0.27 ± 0.02 [a]	1.65 ± 0.18 [b]	5.95 ± 0.04 [de]
MAL	0.19 ± 0.01 [c]	1.01 ± 0.06 [c]	6.40 ± 0.08 [bc]
MEL	0.21 ± 0.01 [bc]	1.71 ± 0.11 [b]	6.24 ± 0.10 [cd]
CEL	0.15 ± 0.01 [d]	0.78 ± 0.09 [cd]	6.61 ± 0.20 [b]
ANOVA *P* value	< 0.001	< 0.001	< 0.001

[1] Each value represents mean ± standard error of the mean.

[2] Values in a column not sharing a superscript differ significantly, $P < 0.05$.

FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol

MEL: Melibiose, CEL: Cellobiose

TABLE 5

CONCENTRATIONS OF SCFA AND OTHER ORGANIC ACIDS IN THE CECAL CONTENTS OF RATS FED CONTROL OR TEST DIETS FOR 4 WK [1,2] (EXPERIMENT 1)

	Succinic acid	Lactic acid	Acetic acid	Propionic acid	n-butyric acid	Total SCFA [3]	Total organic acid [4]
	μmol / cecum contents						
Control	30.0 ± 7.60 [b]	7.58 ± 0.83 [c]	53.8 ± 8.51 [b]	7.26 ± 1.34 [b]	6.77 ± 1.43 [c]	70.9 ± 11.3 [c]	108 ± 19.0 [e]
FOS	11.1 ± 4.27 [b]	469 ± 68.8 [a]	54.8 ± 14.3 [b]	17.0 ± 2.52 [b]	1.26 ± 0.66 [c]	73.0 ± 16.5 [c]	554 ± 71.3 [a]
DFA III	67.7 ± 32.7 [a]	21.8 ± 2.12 [c]	153 ± 16.4 [a]	48.0 ± 12.4 [a]	25.2 ± 4.41 [b]	228 ± 29.3 [a]	317 ± 33.7 [c]
MAL	36.6 ± 9.25 [ab]	14.3 ± 2.11 [c]	63.6 ± 6.89 [b]	16.8 ± 2.16 [b]	53.9 ± 8.82 [a]	138 ± 13.8 [b]	189 ± 17.4 [de]
MEL	2.07 ± 1.05 [b]	344 ± 26.6 [b]	48.2 ± 6.87 [b]	11.2 ± 2.46 [b]	4.37 ± 1.27 [c]	64.7 ± 7.85 [c]	411 ± 33.3 [b]
CEL	23.6 ± 9.38 [b]	84.6 ± 35.4 [c]	58.3 ± 7.88 [b]	11.6 ± 3.48 [b]	21.7 ± 4.28 [b]	93.9 ± 14.6 [bc]	202 ± 35.0 [d]
ANOVA							
P value	0.085	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1] Each value represents mean ± standard error of the mean.

[2] Values in a column not sharing a superscript differ significantly, $P < 0.05$.

[3] Total SCFA: sum of acetic acid, propionic acid, n-butyric acid, iso-butyric acid, iso-valeric acid, and n-valeric acid.

[4] Total organic acid includes SCFAs, succinic and lactic acids.

FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol, MEL: Melibiose, CEL: Cellobiose

TABLE 6

INITIAL BODY WEIGHT, BODY WEIGHT GAIN, FOOD INTAKE, FECAL OUTPUT AND UTERINE WEIGHT OF RATS FED THE CONTROL OR TEST DIETS FOR 5 WK[1,2] (EXPERIMENT 2)

	n	Initial body weight	Body weight gain	Food intake	Fecal output	Uterine weight
		g	g	g/d	dry g/5 days	g/100 g body weight
Sham						
Control	8	157 ± 3.28	98.8 ± 4.86 [c]	18.9 ± 0.29 [d]	5.10 ± 0.15 [bc]	0.23 ± 0.03 [ab]
FOS	8	156 ± 4.02	93.0 ± 7.22 [c]	17.9 ± 0.21 [de]	5.15 ± 0.37 [bc]	0.20 ± 0.02 [b]
DFA III	8	158 ± 2.38	87.9 ± 4.16 [c]	17.2 ± 0.16 [e]	4.85 ± 0.33 [c]	0.23 ± 0.02 [ab]
MEL	8	156 ± 2.90	94.8 ± 7.15 [c]	18.3 ± 0.21 [d]	4.60 ± 0.33 [c]	0.25 ± 0.02 [a]
Ovariectomized						
Control	9	162 ± 2.00	167 ± 6.77 [ab]	22.0 ± 0.39 [ab]	5.79 ± 0.39 [ab]	0.02 ± 0.01 [c]
FOS	9	162 ± 2.86	175 ± 7.28 [a]	22.7 ± 0.46 [a]	6.13 ± 0.29 [a]	0.02 ± 0.01 [c]
DFA III	9	162 ± 1.45	154 ± 4.00 [b]	20.7 ± 0.33 [c]	5.71 ± 0.26 [ab]	0.02 ± 0.01 [c]
MEL	9	162 ± 2.89	158 ± 5.60 [b]	21.5 ± 0.45 [bc]	5.39 ± 0.25 [abc]	0.02 ± 0.01 [c]
ANOVA (*P* value)						
Operation		0.008	< 0.001	< 0.001	0.005	< 0.001
Diet		0.979	0.108	< 0.001	0.207	0.359
Operation x Diet		0.966	0.328	0.046	0.371	0.328

[1] Each value represents mean ± standard error of the mean.

[2] Values in a column not sharing a superscript differ significantly, $P < 0.05$.

FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol, MEL: Melibiose, CEL: Cellobiose

TABLE 7

NET CALCIUM, NET MAGNESIUM, AND NET IRON ABSORPTION IN RATS FED THE CONTROL OR TEST DIETS FOR 5 WK [1,2] (EXPERIMENT 2)

Diet	Net Ca absorption	Net Mg absorption	Net Fe absorption
	mmol/5 days	mmol/5 days	µmol/5 days
Sham			
Control	7.27 ± 0.28 [bc]	1.20 ± 0.04 [c]	7.62 ± 1.62 [b]
FOS	6.02 ± 0.20 [d]	1.11 ± 0.04 [c]	9.72 ± 2.48 [b]
DFA III	7.05 ± 0.22 [c]	1.22 ± 0.03 [c]	15.5 ± 2.52 [b]
MEL	7.80 ± 0.17 [abc]	1.27 ± 0.05 [bc]	14.0 ± 1.92 [b]
Ovariectomized			
Control	7.30 ± 0.36 [bc]	1.20 ± 0.09 [c]	11.3 ± 0.94 [b]
FOS	7.11 ± 0.30 [c]	1.46 ± 0.09 [a]	13.0 ± 1.47 [b]
DFA III	8.15 ± 0.27 [a]	1.49 ± 0.06 [a]	21.5 ± 2.78 [a]
MEL	7.88 ± 0.19 [ab]	1.42 ± 0.03 [ab]	13.9 ± 1.65 [b]
ANOVA (*P* value)			
Operation	0.003	< 0.001	0.041
Diet	< 0.001	0.073	< 0.001
Operation x Diet	0.062	0.043	0.481

[1] Each value represents mean ± standard error of the mean.

[2] Values in a column not sharing a superscript differ significantly, $P < 0.05$.

FOS: Fructooligosaccharide, DFA: Difructose anhydride III, MAL: Maltitol, MEL: Melibiose, CEL: Cellobiose

TABLE 8

CORRELATIONS BETWEEN CALCIUM, MAGNESIUM, IRON ABSORPTION RATE AND OTHER VARIABLES IN RATS FED CONTROL OR TEST DIETS FOR 4 WK1,2 (EXPERIMENT 1)

	pH	Total organic acid	Total SCFA	Cecum wall weight	Cecum content	Lactic	Acetic	Propionic
Day 10-14								
% Ca	**-0.132**	**0.233**	**0.073**	**0.287**	**0.293**	**0.148**	**0.114**	**0.076**
% Mg	**-0.621*** **	**0.540*** **	**0.201**	**0.642*** **	**0.600*** **	**0.410** **	**0.229**	**0.289**
% Fe	**-0.372***	**0.326***	**0.525*** **	**0.578*** **	**0.555*** **	**0.061**	**0.587*** **	**0.505** **
Day 24-28								
% Ca	**-0.464** **	**0.559*** **	**0.393***	**0.418** **	**0.501*** **	**0.337***	**0.429** **	**0.434** **
% Mg	**-0.689*** **	**0.680*** **	**0.156**	**0.496*** **	**0.726*** **	**0.579*** **	**0.161**	**0.254**
% Fe	**-0.469** **	**0.434** **	**0.229**	**0.491*** **	**0.566*** **	**0.334***	**0.252**	**0.326***

1Significant correlations (*, P < 0.05, **, P < 0.01, ***, P < 0.001)

2 Total SCFA: sum of acetic acid, propionic acid, n-butyric acid, iso-butyric acid, iso-valeric acid, and n-valeric acid.

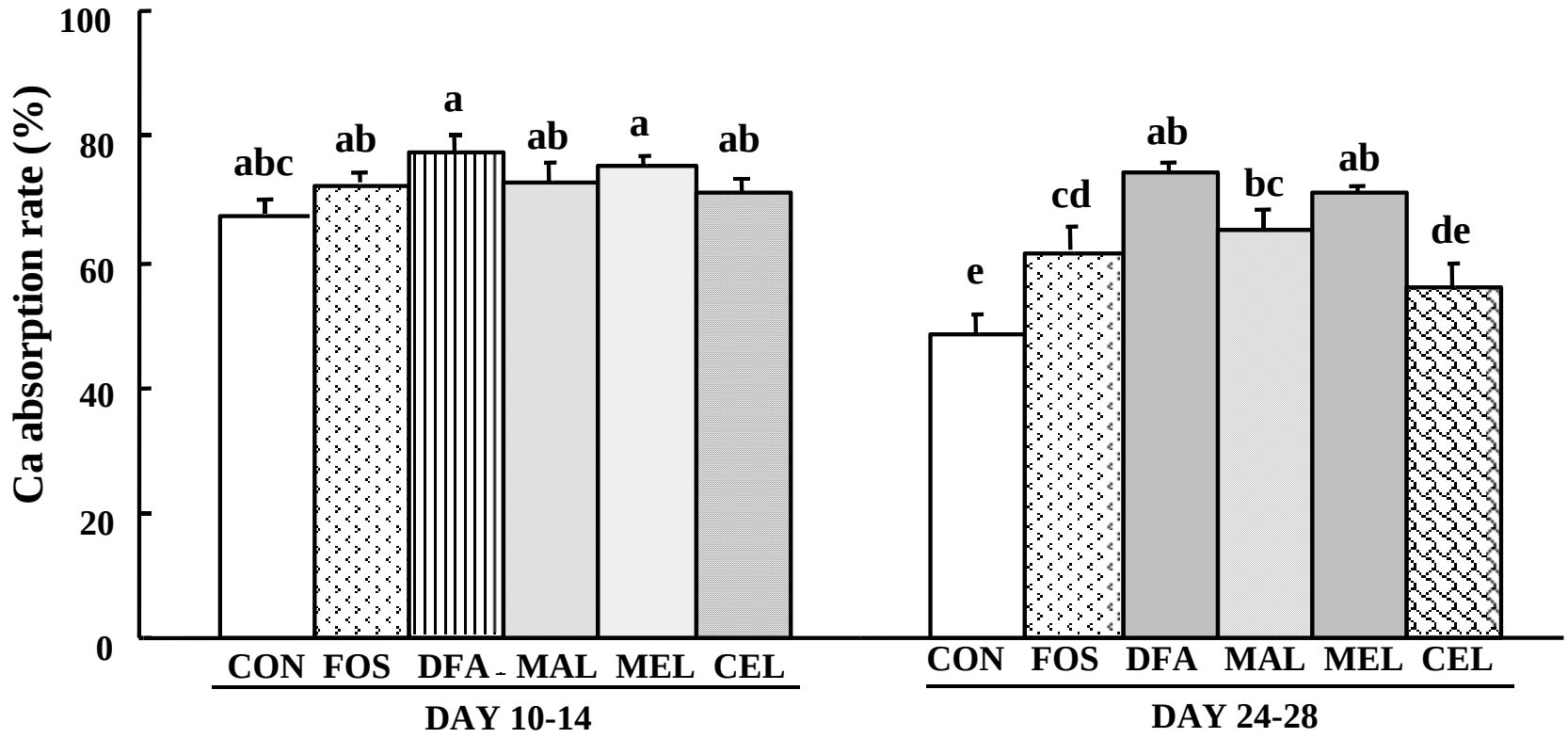
Ca absorption rate (%)
100
80
60
40
20
0
abc
ab
a
ab
a
ab
e
cd
ab
bc
ab
de
CON FOS DFA MAL MEL CEL
DAY 10-14
CON FOS DFA MAL MEL CEL
DAY 24-28

FIG. 1

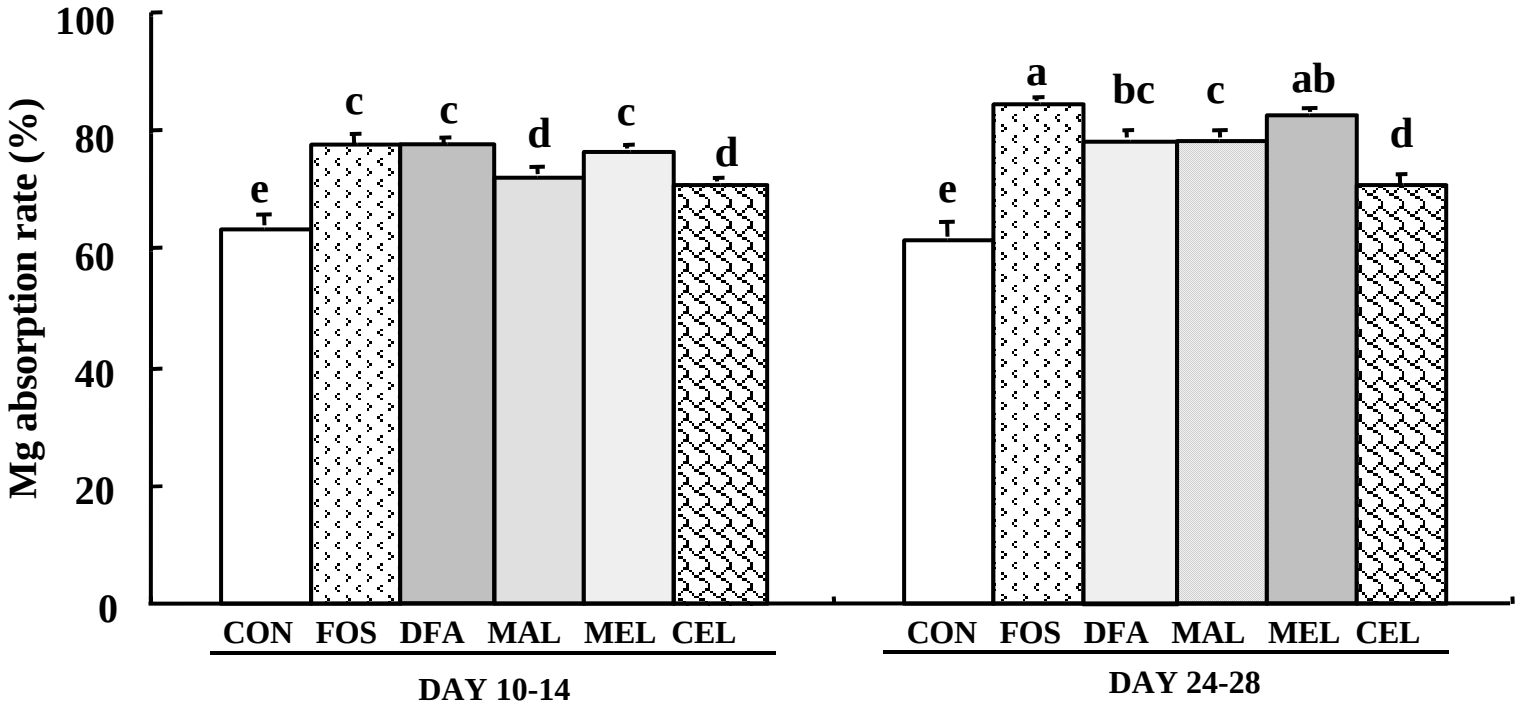
Mg absorption rate (%)
100
80
60
40
20
0
e
c
c
d
c
d
a
bc
c
ab
d
CON FOS DFA MAL MEL CEL
CON FOS DFA MAL MEL CEL
DAY 10-14
DAY 24-28

FIG. 2

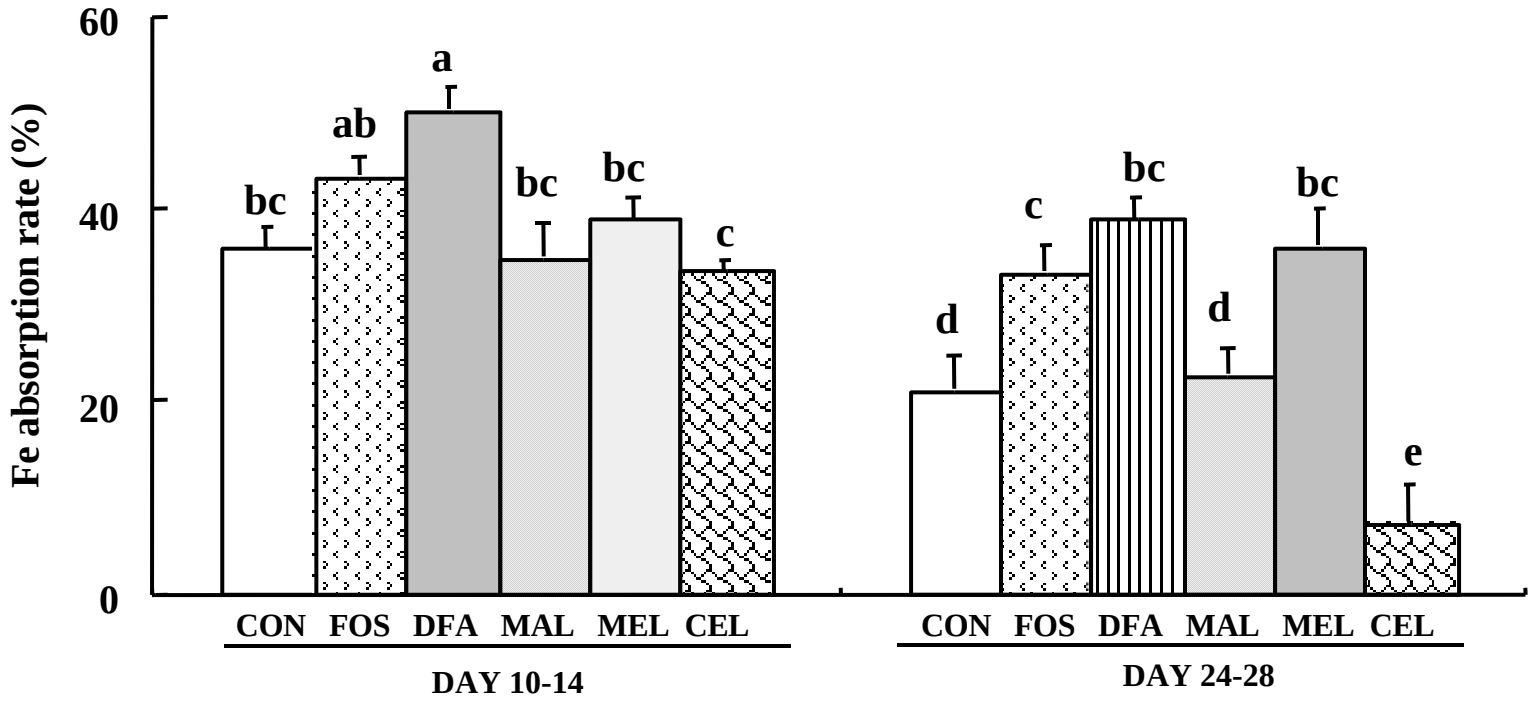
Fe absorption rate (%)
60
40
20
0
bc
ab
a
bc
bc
c
d
c
bc
d
bc
e
CON FOS DFA MAL MEL CEL
DAY 10-14
CON FOS DFA MAL MEL CEL
DAY 24-28

FIG. 3

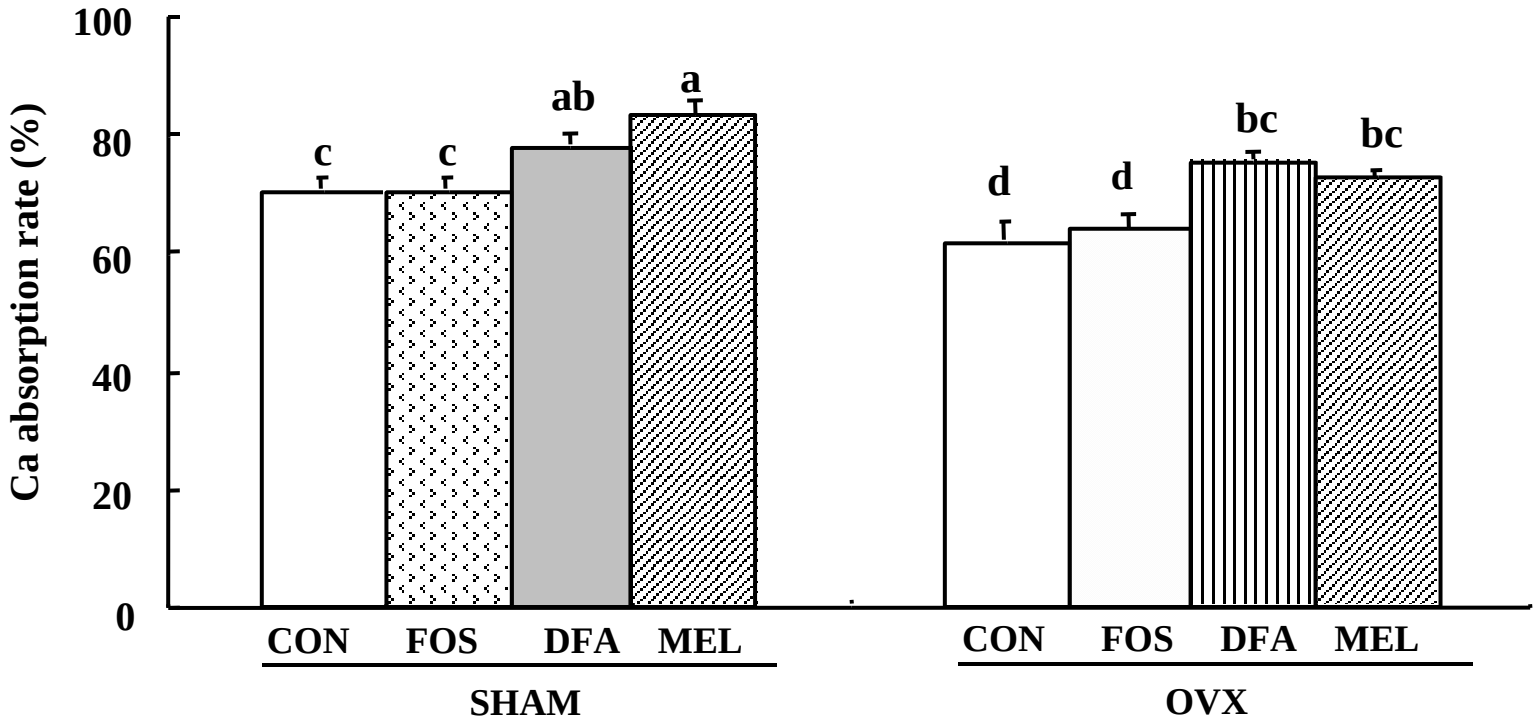
Ca absorption rate (%)
100
80
60
40
20
0
c
c
ab
a
d
d
bc
bc
CON
FOS
DFA
MEL
CON
FOS
DFA
MEL
SHAM
OVX

FIG. 4

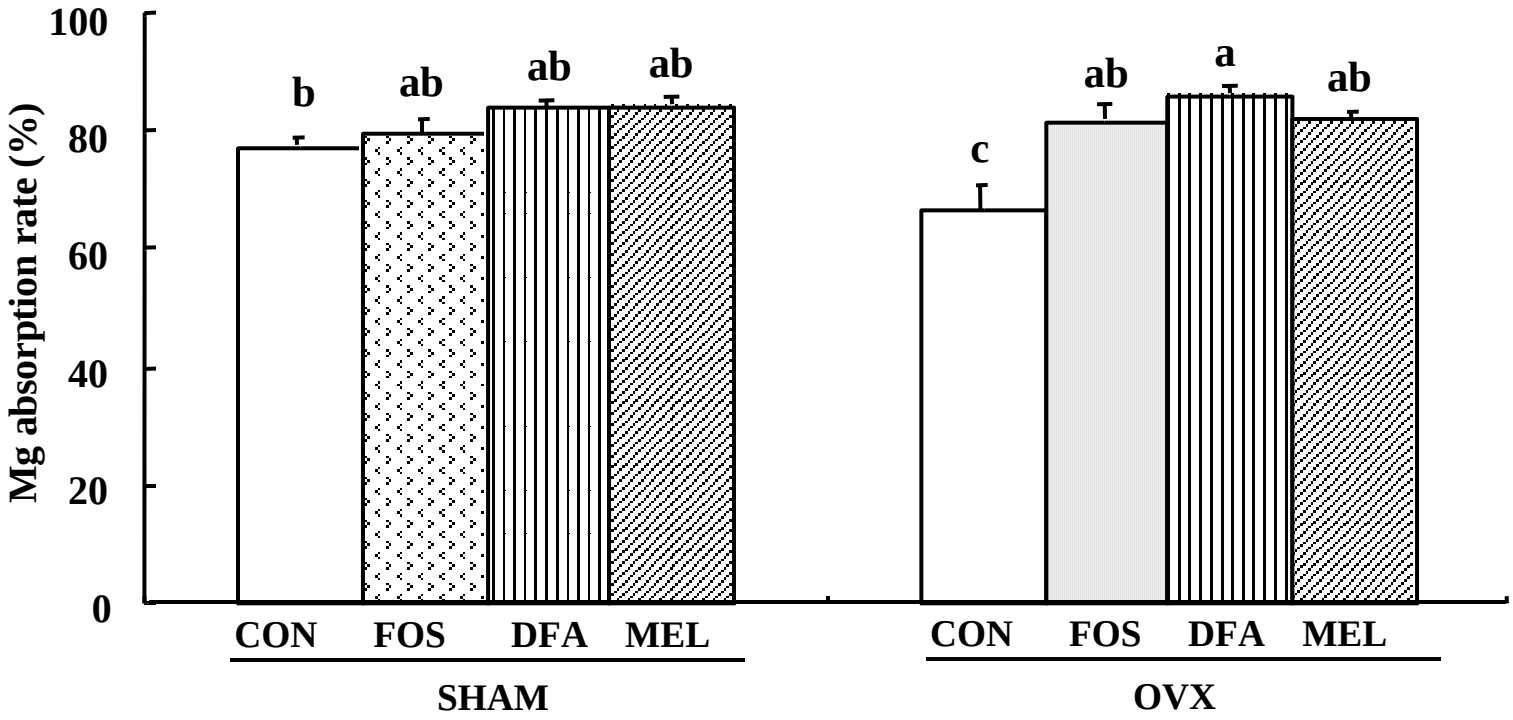
Mg absorption rate (%)
100
80
60
40
20
0
b
ab
ab
ab
c
ab
a
ab
CON
FOS
DFA
MEL
CON
FOS
DFA
MEL
SHAM
OVX

FIG. 5

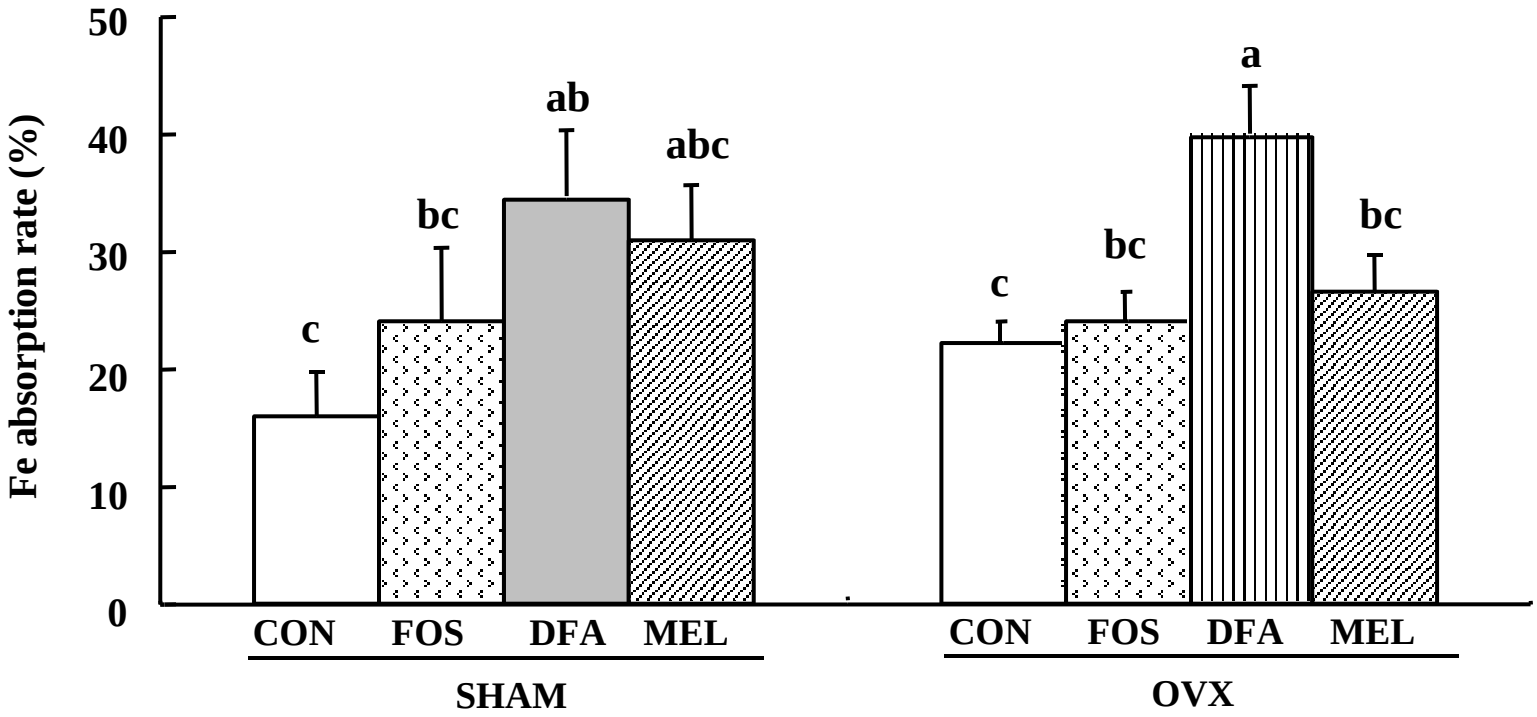
Fe absorption rate (%)
50
40
30
20
10
0
c
bc
ab
abc
c
bc
a
bc
CON
FOS
DFA
MEL
SHAM
CON
FOS
DFA
MEL
OVX

FIG. 6