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Effects of Fructooligosaccharides on the Absorption of Magnesium and Calcium by Cecectomized Rats

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Summary: We reported previously that feeding of fructooligosaccharides (FO) increased the apparent absorption of calcium (Ca), magnesium (Mg) and phosphorus (P) in rats. We suggested that there was an important correlation between this phenomenon and fermentation of FO in the large intestine. However, the precise mechanism remained to be characterized. Therefore, we performed a mineral-balance study to identify the segment of lumen in which FO affects mineral absorption, using cecectomized rats. Sham-operated rats and cecectomized rats were fed a control diet (without FO) or an FO-diet (containing 50 g of FO per kg of feed) for 28 days. Feeding of the FO-diet decreased the luminal pH in the cecum and colon in the sham-operated rats. In the cecectomized rats, feeding of the FO-diet also decreased the luminal pH in the colon. Thus, FO was fermented in the colon of the cecectomized rats. However, the acid composition of feces was altered by cecectomy. Feeding of the FO-diet increased the absorption of Ca and Mg in the sham-operated rats. In the cecectomized rats, the FO-diet increased the absorption of Mg but did not increase the absorption of Ca. These results suggest the mechanisms for the absorption of Ca and Mg when rats are fed an FO are different.

Introduction

The main site of absorption of minerals such as calcium and magnesium in the lumen was initially believed to be the small intestine [1]. However, recent reports indicate that Ca and Mg are absorbed from the large intestine, namely, the cecum and the colon [2, 3]. A competitive correlation between the absorption of Ca and Mg in the small intestine has been reported [4, 5], but independent mechanisms for the absorption of Ca and Mg [6; 7], have also been proposed. The absorption of both Ca and Mg can be accelerated by feeding rats a diet that includes poorly digestible or undigestible and fermentable carbohydrates, such as lactose [8], oligosaccharides [9] or resistant starch [10]. We reported previously that a diet that contained fructooligosaccharides (FO), which stimulate the growth of bifidobacteria, increased the absorption of Ca, Mg and phosphorus (P) in rats [11, 12]. Undigestible carbohydrates were fermented by the luminal bacteria in the large intestine, namely, the cecum and the colon. Many reports have suggested an important correlation between increases in absorption of minerals and fermentation of such undigestible carbohydrates in the large intestine [13, 14, 15, 16, 17, 18]. Several hypotheses about the mechanisms of these effects have been proposed: (i) lowering of the luminal pH raises the concentration of ionized minerals and accelerates the passive diffusion of minerals via paracellular pathways [10]; (ii) ab-

Abbreviations used: FO, fructooligosaccharides

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Materials and Meth

Diet and animals: Five-wee
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Surgical procedure for cece
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sorption of short chain fatty acids is accompanied by absorption of minerals [3, 16, 18]; and (iii) hypertrophy of the colon wall as result of a supply of undigestible carbohydrates is related to an enhanced capacity for absorption of minerals. We have now investigated the role of the large intestine in the absorption of Ca and Mg, using cecectomized rats in a mineral-balance study.

Materials and Methods

Diet and animals: Five-week-old male Sprague-Dawley rats (Clea Japan, Tokyo) were housed in individual stainless-steel metabolic cages in a temperature- and humidity-controlled room (25°C and 55% relative humidity). Seven rats were used in each experimental subgroup. The composition of each diet is shown in Table I. The calculated concentrations of Ca and Mg added agreed well with analyzed values (Tab. I). Half of the sham-operated and cecectomized rats received a diet that contained sucrose at 100 g/kg of diet. The other rats received a diet that contained sucrose at 50 g/kg and fructooligosaccharides (FO) at 50 g/kg of diet. All rats were fed these experimental diets for 28 days. On the final day of the experiment, the rats were anesthetized with diethyl ether. Blood was drawn by abdominal aortic puncture.

Surgical procedure for cecectomy: Rats were deprived of diet for 24 h before surgery, although they had access to water. They were anesthetized by intraperitoneal injection of Nembutal (sodium pentobarbital, 35 mg/kg body wt; Abbot Laboratories, North Chicago, IL). The cecum was surgically removed by the method of Lambert [19]. Sham surgeries were performed on the rats of N and NFO groups. The abdominal cavity was exposed for about 20 min, the same length of time as required for the cecectomy procedure. After surgery, rats were cared for as described by Nishimura *et al* [20]. Rats were not allowed any diet for the first 24 h after surgery.

Mineral-balance studies: Four, 10, 17 and 24 days after the start of the experiment, rats were subjected to a mineral-balance study for 5 days. All feces and urine were collected for 5-day periods in each case. The apparent absorption of minerals and the retention of minerals were calculated from the following formulae: Apparent absorption = (intake-fecal excretion) / (intake) × 100 (%); Retention = (intake-fecal excretion-urinary excretion) / (intake) × 100 (%)

Quantitation of Ca, Mg and P: The amounts of Ca, Mg and P in diets, feces and urine were determined with a sequential plasma spectrometer (ICPS-5000; Shimazu, Tokyo). Diet and feces were first dried and then micropulverized after weight measurement. Micropulverized samples (100 mg) were reduced to ash at 600°C for 24 h in the presence of 1 ml of nitric acid. The ash samples, dissolved in 4 ml of 2 N HCl, were diluted appropriately with distilled water for atomization.

Table I: Composition of experimental diet (g/kg) and designations of the four group of rats

	N*1	NFO*2	O*3	OFO*4
Casein	250	250	250	250
Corn starch	495	495	495	495
Corn oil	60	60	60	60
Vitamin mix.[a]	10	10	10	10
Salt mix.[a]	35	35	35	35
Cellulose	50	50	50	50
Sucrose	100	50	100	50
Frucutooligo™	—	50	—	50

a) Prepared according to AIN-76 prescription (Ca, 5.2 g; Mg, 0.5 g; P, 4.0 g/kg diet)
b) Meioligo-P®
(The concentration of oligosaccharides was above 95%, w/w)
*1 Rats with sham operation
*2 Rats with sham operation fed the FO diet
*3 Rats with cecectomy
*4 Rats with cecectomy fed the FO diet

Urine was diluted appropriately with 0.1 N HCl and subjected directly to atomization.

Quantitation of lactate and short chain fatty acids in the feces: Fecal short chain fatty acids were quantitated by gas-liquid chromatography (series II-5890; Hewlett-Packard) after extraction of feces with diethyl ether. Fecal lactate was quantitated by the method of Noll [21].

Chemicals: Fructooligosaccharides (FO) are a mixture of oligosaccharides consisting of approximately 42% 1-ketose, 46% nystose and 9% 1^{F}-β-fructofuranosyl nystose. FO was manufactured from sucrose by fructosyltransferase [22]. FO are not hydrolyzed in the rat by digestive enzymes, such as disaccharidase of intestinal mucosa and α-amylase of pancreatic homogenates [23]. FO were obtained from Meiji Seika Kaisha, Ltd. (Tokyo). Other dietary components were purchased from Oriental Yeast Co. (Tokyo). All other reagents were of analytical grade and were purchased from Wako Pure Chem. Ind., Ltd. (Osaka).

Statistics: Tukey's test was used to compare group means. Significance was recognized at $p < 0.05$.

Results

Body weight and food intake (Tab. II): Initial body weight and final body weight were similar for all the groups. Total food intake was also similar in all groups.

Food and FO intake for each period and fecal dry weight (Tab. III): Food intake was similar for all the groups in each experimental period. Intakes of FO by rats fed the FO-containing diet

Table II: Body weight and food intake of the rats fed experimental diets for 28 days

Group*	Initial weight (g)	Final weight (g)	Food intake (g)
N	122 ± 3 ns	356 ± 14 ns	572 ± 32 ns
NFO	121 ± 5	374 ± 16	596 ± 31
O	121 ± 5	355 ± 13	572 ± 30
OFO	121 ± 3	357 ± 19	596 ± 35

Mean ± S.D. (n = 7). ns = No significant difference between any values.
* See legend to Table I

(NFO and OFO) was also similar in each period. However, feces dry weight of rats in OFO group were about 30% heavier than those of rats in other groups in each period.

Luminal pH (Tab. IV): The pH of ileal contents did not differ significantly among the various groups. The pH of cecal contents was lower in the NFO group than in the N group. The pH of colon contents in the NFO and OFO groups was lower than in the N and O groups.

Fecal levels of lactate and short chain fatty acids (Tab. V): FO significantly increased the fecal concentration of acetate in both the sham-operated and cecectomized rats. Concentrations of L-lactate and butyrate were higher in the OFO rats than in the other rats.

Mg balance (Tab. VI): FO significantly decreased the fecal excretion of Mg in both the sham-operated and cecectomized rats in each period. Moreover, FO significantly increased the apparent Mg absorption ratio in both the sham-operated and the cecectomized rats in each period. Cecectomy reduced the apparent Mg absorption ratio in both the FO-fed and the FO-free rats.

Ca balance (Table VII): The fecal excretion of Ca by NFO rats was lower than by rats fed other diets. Moreover FO significantly increased the apparent Ca absorption ratio in the sham-operated rats during all but the final 5-day, but not in the cecectomized rats.

Discussion

The cecum of the rat is the main site of fermentation of undigestive carbohydrates. In the cecectomized rats, the FO diet lowered the luminal pH in the colon (Tab. II). Thus, FO was fermented in the colon of the cecectomized rats. In the sham-operated rats, fecal dry weights were not increased by the FO diet. However, in the cecectomized rats, fecal dry weights were increased by the FO diet. The increase was equal

Table III: Food intake, FO intake and fecal weight

Days	Group*	Food intake (g/day)	FO intake (g/day)	Fecal dry weight (g/day)
4–8 days	N	19.1 ± 1.5 ns		1.26 ± 0.05 ab
	NFO	18.6 ± 1.6	0.93 ± 0.08	1.17 ± 0.12 a
	O	19.4 ± 1.0		1.34 ± 0.12 b
	OFO	20.0 ± 0.9	1.00 ± 0.04	2.00 ± 0.12 c
10–14 days	N	20.4 ± 1.4 ns		1.45 ± 0.10 a
	NFO	21.2 ± 1.4	1.06 ± 0.07	1.51 ± 0.16 a
	O	20.2 ± 1.1		1.53 ± 0.10 a
	OFO	21.7 ± 1.4	1.09 ± 0.07	2.06 ± 0.18 b
17–21 days	N	21.1 ± 2.2 ns		1.50 ± 0.11 a
	NFO	23.1 ± 1.2	1.15 ± 0.06	1.66 ± 0.14 a
	O	21.5 ± 2.0		1.63 ± 0.13 a
	OFO	21.9 ± 1.2	1.09 ± 0.06	2.17 ± 0.22 b
24–28 days	N	21.3 ± 1.7 ns		1.45 ± 0.10 a
	NFO	22.5 ± 1.1	1.12 ± 0.05	1.51 ± 0.16 a
	O	21.9 ± 2.1		1.53 ± 0.10 a
	OFO	22.8 ± 1.8	1.14 ± 0.09	2.05 ± 0.18 b

Mean ± S.D. (n = 7). ns = No significant difference between any values.
Values with different superscripts are significantly different ($p < 0.05$).
* See legend to Table I

Table IV: Luminal pH on the final day of rats fed experimental diets

Group*1	ileum *2	cecum *3	colon *4
N	6.99 ± 0.62 ns	6.47 ± 0.27 a	6.89 ± 0.56 a
NFO	6.67 ± 0.41	5.36 ± 0.14 b	5.70 ± 0.41 b
O	6.97 ± 0.55		6.56 ± 0.34 a
OFO	6.47 ± 0.64		5.43 ± 0.22 b

Mean ± S.D. (n = 7)
Values with different superscripts are significantly different ($p < 0.05$).
*1 See legend to Table I
*2 5 cm proximal to the cecum
*3 1 cm distal to the ileal-cecal junction
*4 5 cm distal to the ileal-cecal junction

to about half the weight of FO ingested (Tab. III). Thus, it appears that some of the ingested FO was fermented in the cecectomized rats fed an FO diet.

Ca and Mg are absorbed from both the small intestine and the large intestine, which includes the cecum. Using the average of all data of apparent absorption ratios for the four periods (see Tab. VI and VII), we attempted to characterize the absorption of Mg (Fig. 1) and Ca (Fig. 2).

According to our results, large amounts of Mg were absorbed via the cecum in the rats fed an FO-free diet (10.9%: the difference in apparent Mg absorption between the rats of N group and the rats of O groups, Fig. 1). The ratio of apparent Mg absorption that was due to FO in the sham operated rats (NF: 23.9%; the difference in apparent Mg absorption between the rats of N group and the rats of NFO group, Fig. 1) to apparent Mg absorption that was due to FO in the cecectomized rats (OF: 17.6%; the difference in apparent Mg absorption between the rats of O group and the rats of OFO group, Fig. 1) was about 70%. Therefore, we concluded that, in the case of absorption of Mg, the stimulatory effect of FO occurred mainly in the colon or the colon compensated for the cecum. And there was almost no absorption of Ca via the cecum in the rats fed FO-free diet that did not contain other fermentable carbohydrates (3.0%: the difference in apparent Ca absorption between the rats of N group and the rats of O group, Fig. 2). The ratio of apparent Ca absorption that was due to FO in the sham operated rats (NF: 11.4%; the difference in apparent Ca absorption between the rats of N group and the rats of NFO group, Fig. 2) to apparent Ca absorption that was due to FO in the cecectomized rats (OF: 0.8%; the difference in apparent Ca absorption between the rats of O group and the rats of OFO group, Fig. 2) was about 7%. Therefore, we concluded that the stimulatory effect of FO on the absorption of Ca occurred mainly in the cecum.

There have been studies on the effects of lactose on mineral absorption [24, 25, 26, 27]. It has been reported that feeding of lactose increases absorption of Ca and Mg [8]. However, the mechanism of this effect of lactose has not been clarified. Rémécy *et al* reported that inulin [9], lactulose and pectin [14] all increase the flux of Ca from the cecum. These carbohydrates have similar characteristics, being poorly digestible or undigestible and are fermentable by luminal bacteria.

There are several hypotheses about the mechanism of the stimulatory effect of these carbohydrates on the absorption of minerals. It has been proposed that a decrease in luminal pH increases absorption of minerals. Several reports indicate that Ca inhibits the absorption of Mg [28, 29]. Brink *et al* investigated whether decreases in absorption of Mg were caused by production of Ca-Mg-P complexes in the lumen [5]. Passive transport via the paracellular pathway of Ca and Mg has been discussed [2, 7]. Feeding of poorly digestible or undigestible and fermentable carbohydrates has been reported to in-

Table V: The pH and concentrations of various acids in feces

Group*	pH	Acids concentration (mM)				
		Acetate	Propionate	Butyrate	D-Lactate	L-Lactate
N	7.1 ± 0.2 a	17.4 ± 7.9 a	5.1 ± 8.7 ns	0.5 ± 0.7 a	11.0 ± 4.8 ns	9.2 ± 2.9 a
NFO	6.8 ± 0.5 b	33.3 ± 11.3 b	7.0 ± 3.6	1.2 ± 1.2 ab	8.2 ± 5.5	7.7 ± 4.7 a
O	7.6 ± 0.5 a	29.5 ± 9.9 ab	5.9 ± 3.2	1.5 ± 1.1 ab	3.8 ± 9.5	4.5 ± 5.0 a
OFO	6.0 ± 0.5 c	43.7 ± 11.7 b	2.9 ± 5.9	4.3 ± 4.2 b	14.5 ± 10.1	56.6 ± 32.6 b

Mean ± S.D. (n = 7). ns = No significant difference between any values.
Values with different superscripts are significantly different ($p < 0.05$).
* See legend to Table I

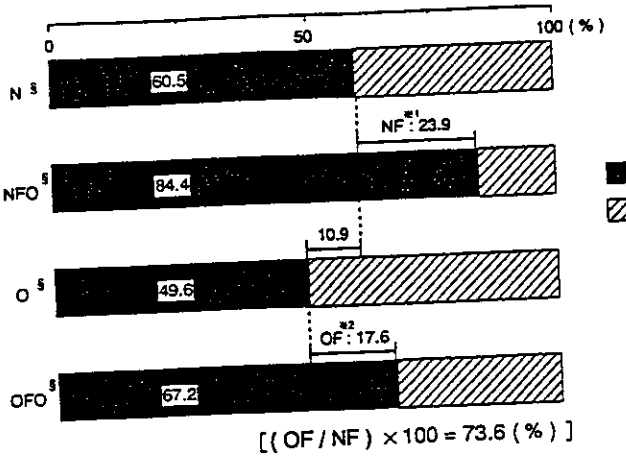

Figure I: Characterization of the apparent absorption of Mg. Values are the average of all datas of apparent absorption ratio for the four periods.
§ names of experimental subgroup, see Table I.
※1 NF: increace was due to FO in the sham operated Rats.
※2 OF: Increace was due FO in the Cecectomized Rats.

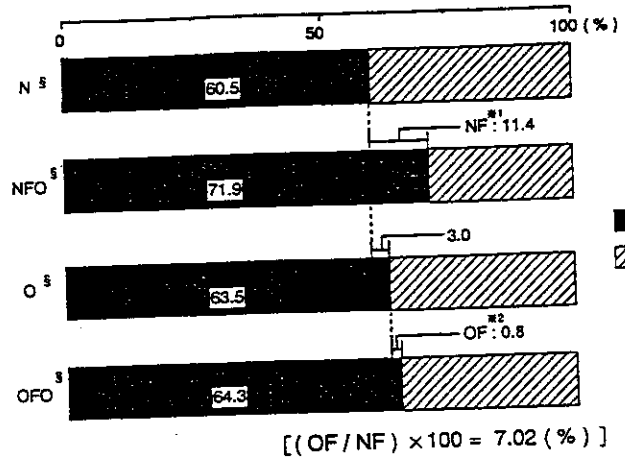

Figure II: Characterization of the apparent absorption of Ca. Values are the average of all datas of apparent absorption ratio for the four periods.
§ names of experimental subgroup, see Table I.
※1 NF: increace was due to FO in the sham operated Rats.
※2 OF: Increace was due FO in the Cecectomized Rats.

crease levels of ionized Ca and Mg in the lumen [10]. In our present study, feeding an FO diet lowered the luminal pH in both the sham-operated and the cecectomized rats.

A second hypothesis is that short chain fatty acids, produced by fermentation of carbohydrates, accelerate the absorption of Ca and Mg from both the cecum and the colon directly. Lutz *et al* reported that short chain fatty acids accelerated the absorption of Mg from both the cecum and the colon and that acetate and butyrate accelerated absorption of Ca from the distal colon [3, 17]. Wasserman *et al* described production of a highly absorbable Ca-lactate complex [25, 27]. Ca-acetate complexes pass more readily across cell membranes than ionized Ca [18]. We found that the acid composition of feces was altered and that the fecal concentration of L-lactate was raised by cecectomy. The change in acid composition may be related to why feeding an FO diet did not increase the absorption of Ca in the cecectomized rats.

The third hypothesis is that hypertrophy of the colon wall is related to an enhanced capacity for absorption of minerals. The colon wall of rats fed an FO-containing diet exhibited hypertrophy. However, we obtained no information relevant to this hypothesis in the present study.

Karbach suggested that there were different mechanisms for absorption of Ca and Mg in the colon [2]. Our results support such a possibility and, clearly, the effects of FO on the absorption of Ca and Mg in the large intestine were different.

Table VI: Magnesium balances

Days	Group[*]	Intake (mg/day)	Fecal excretion (mg/day)	Urinary excretion (mg/day)	Absorption (mg/day)[2]	Absorption ratio (%)[3]	Retention (mg/day)[4]	Retention ratio (%)[5]
4–8 days	N	795 ± 0.62	297 ± [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table VI. Magnesium balances

Days	Group*1	Intake (mg/day)	Fecal excretion (mg/day)	Urinary excretion (mg/day)	Absorption (mg/day)*2	Absorption ratio (%)*3	Retention (mg/day)*4	Retention ratio (%)*5
4–8 days	N	7.85 ± 0.63 ab	2.96 ± 0.34 b	2.83 ± 0.28 b	4.89 ± 0.55 b	62.2 ± 3.8 b	2.05 ± 0.68 ab	25.7 ± 6.4 ab
	NFO	7.42 ± 0.63 a	0.91 ± 0.29 c	4.17 ± 0.46 c	6.51 ± 0.71 c	87.7 ± 4.3 c	2.35 ± 0.37 ab	31.6 ± 4.4 ab
	O	7.76 ± 0.39 ab	3.86 ± 0.71 a	1.98 ± 0.37 a	3.90 ± 0.73 a	50.3 ± 9.1 a	1.92 ± 0.41 a	24.7 ± 5.1 a
	OFO	8.19 ± 0.36 b	2.45 ± 0.24 b	3.06 ± 0.39 b	5.74 ± 0.42 bc	70.0 ± 3.2 b	2.67 ± 0.30 b	32.6 ± 3.3 b
10–14 days	N	8.34 ± 0.57 ab	3.16 ± 0.49 b	3.03 ± 0.56 b	5.19 ± 0.55 b	62.2 ± 5.1 b	2.16 ± 0.52 ns	25.8 ± 3.8 ns
	NFO	8.48 ± 0.57 ab	1.05 ± 0.18 a	4.58 ± 0.62 c	7.44 ± 0.55 d	87.6 ± 2.1 c	2.86 ± 1.00	33.3 ± 9.7
	O	8.07 ± 0.44 a	4.01 ± 0.37 c	1.96 ± 0.44 a	4.06 ± 0.66 a	50.1 ± 6.2 a	2.10 ± 0.30	26.0 ± 2.8
	OFO	8.90 ± 0.57 b	2.85 ± 0.48 b	3.75 ± 0.82 bc	6.04 ± 0.40 c	68.1 ± 4.2 b	2.30 ± 0.72	25.9 ± 8.3
17–21 days	N	8.66 ± 0.89 ns	3.35 ± 0.37 b	2.81 ± 0.39 a	5.31 ± 0.58 ab	61.3 ± 2.0 b	2.49 ± 0.32 ab	28.8 ± 1.9 ab
	NFO	9.24 ± 0.48	1.16 ± 0.13 a	5.21 ± 0.67 c	8.07 ± 0.52 c	87.4 ± 1.7 d	2.86 ± 0.72 a	30.9 ± 7.0 a
	O	8.58 ± 0.82	4.15 ± 0.42 c	2.11 ± 0.66 a	4.43 ± 1.04 a	51.2 ± 8.6 a	2.33 ± 0.73 ab	26.8 ± 7.3 ab
	OFO	8.97 ± 0.48	4.33 ± 0.45 c	4.33 ± 0.45 b	6.19 ± 0.37 b	69.1 ± 4.9 c	1.86 ± 0.31 b	20.8 ± 3.8 b
24–28 days	N	8.74 ± 0.72 ns	3.83 ± 0.42 bc	3.17 ± 0.48 bc	4.92 ± 0.53 bc	56.2 ± 3.6 bc	1.74 ± 0.43 ns	19.9 ± 4.7 ns
	NFO	8.98 ± 0.43	1.55 ± 0.26 a	6.03 ± 0.80 a	7.43 ± 0.37 a	82.8 ± 2.5 a	1.39 ± 0.59	15.6 ± 6.7
	O	8.74 ± 0.84	4.66 ± 0.73 c	2.30 ± 0.61 c	4.09 ± 0.83 c	46.6 ± 7.8 c	1.79 ± 0.60	20.3 ± 5.7
	OFO	9.32 ± 0.75	3.58 ± 0.84 b	4.27 ± 1.05 b	5.75 ± 1.09 b	61.5 ± 9.7 b	1.48 ± 0.59	15.8 ± 6.3
Average*6	N	8.40 ± 0.76 ns	3.32 ± 0.51 b	3.12 ± 0.76 ab	5.07 ± 0.55 ab	60.5 ± 4.4 a	1.95 ± 0.68 ns	23.3 ± 7.7 ns
	NFO	8.53 ± 0.87	1.17 ± 0.32 c	5.00 ± 0.94 c	7.36 ± 0.77 c	86.4 ± 3.4 b	2.37 ± 0.90	27.8 ± 9.9
	O	8.29 ± 0.74	4.17 ± 0.63 a	2.09 ± 0.52 a	4.12 ± 0.81 a	49.5 ± 7.7 c	2.04 ± 0.55	24.5 ± 5.8
	OFO	8.86 ± 0.67	2.91 ± 0.68 b	3.85 ± 0.86 bc	5.93 ± 0.61 b	67.2 ± 6.6 a	2.08 ± 0.67	23.8 ± 8.4

Mean ± S.D. (n = 7). ns = No significant difference among values. Values with different superscripts are significantly different ($p<0.05$).

*1 See legend to Table I

*2 Intake – fecal excretion

*3 (Absorption/intake) * 100

*4 Intake – fecal excretion – urinary excretion

*5 (Retention/intake) * 100

*6 Average is the average value of all datas for the four periods.

Table VII: Calcium balances

Days	Group*1	Intake (mg/day)	Fecal excretion (mg/day)	Urinary excretion (mg/day)	Absorption (mg/day)*2	Absorption ratio (%)*3	Retention (mg/day)*4	Retention ratio (%)*5
4–8 days	N	100 ± 8 ns	35.5 ± 5.1 b	1.69 ± 0.80 a	65.1 ± 11.0 a	64.4 ± 6.3 a	63.4 ± 11.2 a	62.7 ± 6.7 a
	NFO	98 ± 8	19.1 ± 4.2 a	3.40 ± 0.71 b	79.1 ± 7.5 b	80.6 ± 4.0 b	75.7 ± 7.8 b	77.1 ± 4.1 b
	O	102 ± 5	31.6 ± 4.9 b	1.81 ± 0.90 a	70.2 ± 5.6 ab	68.9 ± 4.5 a	68.4 ± 5.5 ab	67.2 ± 4.8 a
	OFO	106 ± 5	32.3 ± 1.6 b	3.47 ± 1.28 b	73.3 ± 5.2 ab	69.4 ± 2.2 a	69.9 ± 4.1 ab	66.1 ± 1.4 a
10–14 days	N	107 ± 7 ns	38.2 ± 6.2 b	0.97 ± 0.58 a	68.8 ± 6.5 a	64.3 ± 4.9 a	67.8 ± 6.4 a	63.4 ± 5.0 a
	NFO	112 ± 8	26.9 ± 5.6 a	3.22 ± 0.78 b	85.3 ± 5.0 b	76.1 ± 4.2 b	82.0 ± 5.4 b	73.2 ± 4.1 b
	O	106 ± 6	35.6 ± 3.1 b	0.69 ± 0.21 a	70.4 ± 5.6 a	66.4 ± 2.9 a	69.7 ± 5.4 a	65.8 ± 2.9 a
	OFO	115 ± 7	39.5 ± 6.2 b	2.38 ± 1.10 b	75.3 ± 4.4 a	65.7 ± 4.0 a	72.9 ± 3.8 a	63.6 ± 4.2 a
17–21 days	N	111 ± 11 ns	44.9 ± 4.5 a	0.75 ± 0.50 a	66.2 ± 7.8 a	59.6 ± 2.0 a	65.5 ± 7.8 a	58.9 ± 2.2 a
	NFO	122 ± 6	35.1 ± 4.9 b	3.09 ± 1.60 b	87.0 ± 7.1 b	71.2 ± 4.1 c	84.0 ± 6.7 b	68.7 ± 3.9 b
	O	113 ± 11	41.6 ± 3.2 ab	0.74 ± 0.33 a	71.0 ± 8.8 a	62.9 ± 2.5 ab	70.3 ± 8.6 a	62.2 ± 2.6 a
	OFO	116 ± 6	40.3 ± 5.1 ab	2.06 ± 1.09 ab	75.5 ± 4.0 a	65.3 ± 3.3 b	73.4 ± 3.8 a	63.5 ± 3.7 a
24–28 days	N	112 ± 9 ns	52.1 ± 4.8 ns	0.87 ± 0.57 a	60.1 ± 7.2 ns	53.5 ± 3.5 ns	59.2 ± 7.1 ns	52.7 ± 3.6 ns
	NFO	119 ± 6	47.8 ± 6.6	3.52 ± 1.55 b	71.0 ± 5.3	59.8 ± 4.5	67.5 ± 6.0	56.9 ± 5.1
	O	115 ± 11	51.4 ± 11.8	0.69 ± 0.12 a	63.4 ± 6.4	55.6 ± 6.7	62.7 ± 6.5	55.0 ± 6.7
	OFO	120 ± 10	51.6 ± 7.0	2.77 ± 1.43 b	68.7 ± 10.9	56.9 ± 6.3	66.0 ± 10.1	54.7 ± 6.1
Average*6	N	108 ± 10 ns	42.7 ± 8.1 ns	1.07 ± 0.70 a	65.1 ± 8.5 a	60.4 ± 6.2 a	64.0 ± 8.5 a	59.4 ± 6.2 ns
	NFO	113 ± 11	32.2 ± 12.0	3.31 ± 1.18 b	80.6 ± 8.8 b	71.9 ± 8.8 b	77.3 ± 9.0 b	69.0 ± 8.7
	O	109 ± 10	40.1 ± 9.9	0.98 ± 0.67 a	68.8 ± 7.1 a	63.5 ± 6.6 ab	67.8 ± 6.9 ab	62.5 ± 6.4
	OFO	114 ± 9	40.9 ± 8.7	2.67 ± 1.28 b	73.2 ± 6.9 ab	64.3 ± 6.1 ab	70.5 ± 6.5 ab	62.0 ± 5.9

Mean ± S.D. (n = 7). ns = No significant difference among values. Values with different superscripts are significantly different ($p<0.05$).

*1 See legend to Table I

*2 Intake – fecal excretion

*3 (Absorption/intake) *100

*4 Intake – fecal excretion – urinary excretion

*5 (Retention/intake) *100

*6 Average is the average value of all datas for the four periods.

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