Prevention of coprophagy modifies magnesium absorption in rats fed with fructo-oligosaccharides

BY ATSUTANE OHTA, SEIGO BABA, MASAKO OHTSUKI, ADUSA TAGUCHI AND TAKASHI ADACHI

Department of Nutritional Science Centre, Bioscience Laboratories, Meiji Seika Kaisha Ltd, Saitama 350-02, Japan

AND HIROSHI HARA

Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

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We developed a new type of anal cup for prevention of coprophagy and determined whether the absorption of Ca and Mg and the stimulatory effects of feeding fructo-oligosaccharides (FO) on the absorption of Ca and Mg were altered by prevention of coprophagy in rats. Rats were fed on a FO-free diet or a diet containing 50 g FO/kg for 2 weeks with or without prevention of coprophagy. FO-feeding increased the apparent absorptive ratio of Ca and Mg in rats with or without prevention of coprophagy. However, in the FO-fed groups the absorptive ratio of Mg in rats with prevention of coprophagy was higher than in rats without prevention of coprophagy. The Ca content of the femur was higher in rats fed on the FO-diet than in rats fed on the FO-free diet both with and without coprophagy. In conclusion, FO-feeding increased the absorption of Ca and Mg in rats both with and without coprophagy. Moreover, prevention of coprophagy enhanced the absorption of Mg in rats fed with FO. Coprophagy has to be considered when the effects of luminal fermentation or mineral absorption are examined in rats.

Coprophagy: Fructo-oligosaccharides: Calcium: Magnesium

Coprophagy occurs in many rodent species (Giovannetti, 1982). In the laboratory rat, large amounts of excreted faeces are re-ingested. Coprophagy has a positive effect on the supply of vitamins (Schulze & Haenel, 1969) and the digestibility of minerals such as Fe (Neale, 1982) or Ca (Cree *et al.* 1986). Therefore, in rat experiments, coprophagy may lead to errors in the evaluation of nutrient absorption and digestibility when the results of these studies are extrapolated to humans. Therefore, it is important to examine the consequences of coprophagy on intestinal absorption of nutrients.

We reported previously that a diet containing fructo-oligosaccharides (FO), increased the apparent absorption of Ca and Mg in rats. FO, which are not digestible by human enzymes, are fermented by luminal bacteria and stimulate the growth of bifidobacteria (Hidaka *et al.* 1991). The same effects have been observed with other indigestible carbohydrates, such as resistant starch (Schulz *et al.* 1993), lactulose (Heijnen *et al.* 1993) and inulin (Levrat *et al.* 1991). In all the previous studies the effects of indigestible carbohydrates were observed in rats that were allowed to practise coprophagy. However, there have been no observations of whether the stimulatory effects of such indigestible carbohydrates on mineral absorption occur in rats when coprophagy is prevented.

Many investigators have suggested that an increase in mineral absorption is related to the bacterial fermentation of these indigestible carbohydrates in the hindgut (Demigné *et al.* 1989; Levrat *et al.* 1991; Ohta *et al.* 1993, 1994*b*; Schulz *et al.* 1993). On the other hand, Jackson & Topping (1993) reported that the prevention of coprophagy altered the fermentation of indigestible carbohydrates in the hindgut, based on variation in the composition of short-chain fatty acids (SCFA) in the caecal contents in rats. Furthermore, the chemical forms of mineral salts in faeces which are re-ingested differ from those in the experimental diet. It has been suggested that the chemical forms of mineral salts in experimental diets change into other chemical forms such as chemical complexes or organic acid salts during luminal passage (Brink *et al.* 1992; Heijnen *et al.* 1993). Thus, it is thought that the prevention of coprophagy may affect mineral absorption and the stimulatory effect of indigestible carbohydrates on mineral absorption.

The purpose of the present experiments was to ascertain whether prevention of coprophagy modifies the absorption of Ca and Mg, the stimulatory effects of FO on the absorption of Ca and Mg, and the luminal fermentability of FO in the rat.

In previous studies, several methods for preventing coprophagy have been proposed (Neale, 1982; Cree *et al.* 1986; Zhang *et al.* 1992), but when we tried them in preliminary tests those methods required frequent attention and did not prevent coprophagy completely. Therefore, for use in this study we developed a new type of device for preventing coprophagy, namely a wire-mesh anal cup.

MATERIALS AND METHODS

Animals and diets

Five-week-old male Sprague-Dawley rats (Clea Japan, Tokyo, Japan) were housed in individual stainless-steel metabolism cages with wire-mesh bottoms in a temperature- and humidity-controlled room (25° and 55% relative humidity) with a 12 h light–dark cycle. Two experimental diets were used in this study. The compositions of these diets are shown in Table 1.

Rats in two subgroups received a diet that contained sucrose at 100 g/kg diet (control diet) and rats in the other two subgroups received a diet that contained sucrose at 50 g/kg diet and FO at 50 g/kg diet (FO diet). There were four experimental subgroups: control diet with the sham prevention of coprophagy (C+), FO diet with the sham prevention of coprophagy (F+), control diet with the prevention of coprophagy (C−), FO diet with the prevention of coprophagy (F−). There were seven to eleven rats in each group. All rats were allowed free access to water and experimental diets for 15 d. On the final day of the experiment the rats were anaesthetized with diethyl ether, blood was drawn by abdominal aortic puncture and the caecum and left femur were removed.

Prevention of coprophagy

In previous studies in rats, plastic collars or tail cups have been used for preventing coprophagy. The plastic collar damages the rat's neck. Sometimes faeces adhere to the bottom wire-mesh of the metabolism cage, and the plastic collar cannot prevent the re-ingestion of these faeces. The plastic tail cup which is fastened to the tail damages the rat's tail and is also gnawed by the rat. Moreover, this type of tail cup frequently moves out of place when the rat struggles hard to get rid of it (Neale, 1982; Zhang *et al.* 1992). Therefore rats given this type of plastic tail cup need frequent care. In a preliminary test we used previous methods for the prevention of coprophagy, but we could not confirm that coprophagy was completely prevented. In fact, we observed traces of faeces in the stomach contents of a few rats at killing. Thus, we developed a wire-mesh anal cup by modification

Table 1. *Composition of experimental diets*

Diet...	C	F
Ingredients (g/kg)		
Casein	250	250
Maize starch	495	495
Maize oil	60	60
Vitamin mixture*	10	10
Salt mixture*	35	35
Cellulose†	50	50
Sucrose	100	50
Fructo-oligosaccharides‡	—	50
Chemical analysis (mmol/kg)		
Calcium	118·8	117·3
Magnesium	18·7	18·5

* Prepared according to AIN-76 formulation (American Institute of Nutrition, 1977).
† Oriental Yeast Co., Tokyo, Japan.
‡ Meioligo-P® (Meiji Seika Kaisha, Ltd, Tokyo, Japan; concentrations of oligosaccharides were greater than 950 g/kg total mix).

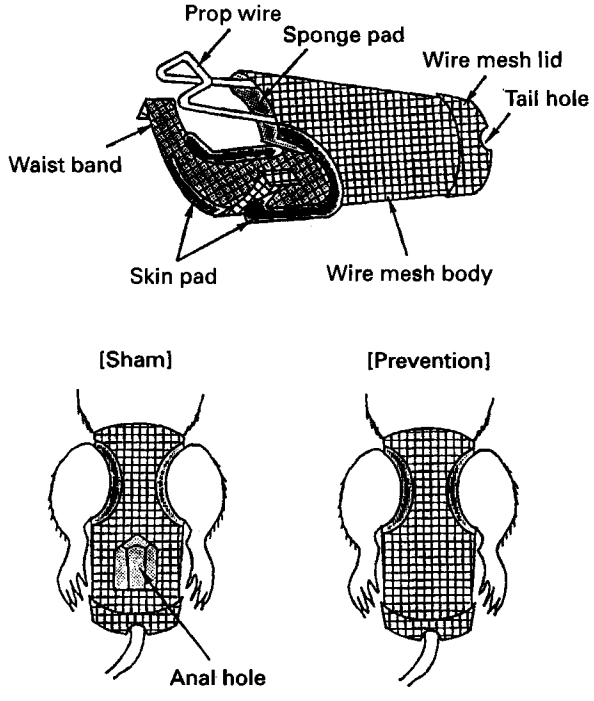


Fig. 1. The structure of the device for preventing coprophagy (a wire-mesh anal cup).

of the method using the plastic tail cup (Wang & Peters, 1963; Neale, 1982) and used it in the present study. The structure of this device is shown in Fig. 1.

Using our method of a wire-mesh anal cup for preventing coprophagy, frequent care was not necessary as it was for other methods (Neale, 1982; Jackson & Topping, 1993) because our wire-mesh anal cup rarely came off and was not gnawed or broken by rats. Moreover,

moist stools did not adhere to the wire-mesh anal cup, thus collection of stools was easy. Rats in the C− and F− groups were prevented access to the stool by the attachment of the wire-mesh anal cup to the waist of the rats with a rubber band. Rats in the C+ and F+ groups were allowed access to the stool by the attachment of the coprophagy-permitting wire-mesh anal cup (sham) with a pentagonal hole (22 mm × 22 mm) around the anus of the rat. Before the present experiment we observed, using a 24 h video tape recorder for monitoring (Video camera CM 32665-B03, Camera control unit TK-U895, TimeLap Video Cassette Recorder BR-9000, Victor Co. Ltd, Tokyo, Japan), that rats with the sham anal cups were able to re-ingest their faeces. Coprophagy occurred about 3–4 times per rat per day. This frequency of coprophagy was similar to previous data (Ebino, 1993), and this suggests that the coprophagy-permitting wire-mesh anal cup does not inhibit coprophagy remarkably. These wire-mesh anal cups were attached during the experimental period.

Mineral balance studies

At 4 and 10 d after feeding the experimental diets, rats were subjected to a mineral-balance study for 5 d. All faeces and urine were collected for a 5 d period in each case. The apparent absorptive and retention ratios for Ca and Mg were calculated from the following formulas:

$$\text{apparent absorption} = (\text{intake} - \text{faecal excretion})/(\text{intake}) \times 100\,(\%),$$

$$\text{retention} = (\text{intake} - \text{faecal excretion} - \text{urinary excretion})/(\text{intake}) \times 100\,(\%).$$

The Ca and Mg concentrations in the diets, faeces, urine and femur were determined with a sequential plasma spectrometer (ICPS-5000; Shimadzu, Kyoto, Japan) as described previously (Ohta *et al.* 1994*b*). Diets and faeces were first dried and then micropulverized. Micropulverized samples (approximately 100 mg) were ashed at 600° for 24 h. The ashed samples, dissolved in 4 ml 2 M-HCl, were diluted appropriately with distilled water for atomization. Urine was diluted appropriately with distilled water and subjected to atomization directly.

Quantitation of short-chain fatty acids in faeces

From 7 to 10 d after feeding the experimental diet, all faeces were collected for quantitation of short-chain fatty acids (SCFA). Faecal SCFA were quantitated by GLC (series II-5890; Hewlett-Packard, Pennsylvania, USA) after extraction of SCFA with diethyl ether from faeces (Whitehead *et al.* 1976).

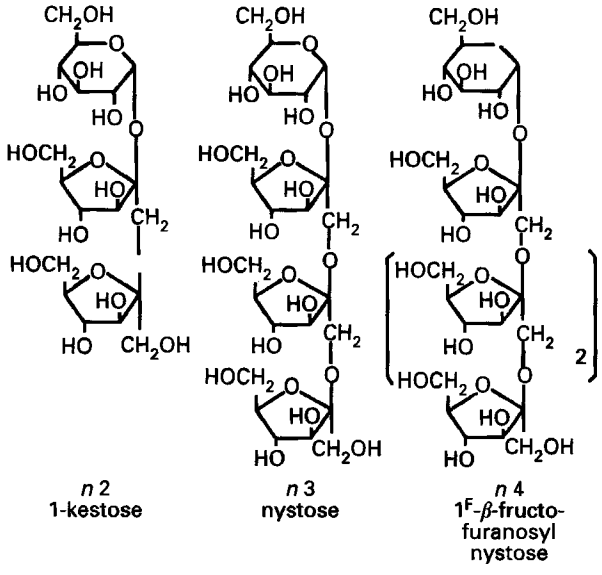
Chemicals

The FO mixture (Meioligo-P®, Meiji Seika Kaisha, Ltd., Tokyo, Japan) consisted of 42% 1-kestose, 46% nystose and 9% 1F-β-fructofuranosyl nystose. The chemical structure of the FO mixture is shown in Fig. 2.

FO were manufactured from sucrose using fructosyltransferase (*EC* 3.2.1.26 obtained from *Aspergillus niger* ATCC 20611; Hidaka *et al.* 1988). FO are not hydrolysed in the rat by digestive enzymes, such as the disaccharidase of the intestinal mucosa or the α-amylase (*EC* 3.2.1.1) of pancreatic homogenates (Oku *et al.* 1984). Other dietary components were purchased from Oriental Yeast Co. (Tokyo, Japan). All other reagents were of analytical grade and were purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan).

Statistical methods

Data are expressed as mean values with their standard errors. Data were analysed by two-way (diet and prevention of coprophagy) ANOVA, and significant differences between groups were determined by Tukey's test (SPSS Ver.6.0, SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P < 0{\cdot}05$.



$1^F(1\text{-}\beta\text{-fructofuranosyl})_{n-1}$-sucrose

Fig. 2. Chemical structures of the fructo-oligosaccharides.

Table 2. *Body-weight gain (g/rat per d) and feed intake (g/rat per d) in rats fed on a control diet (C) or a fructo-oligosaccharides-containing diet (F) with (+) or without (−) coprophagy allowed**

(Mean values with their standard errors for five to ten rats in each group)

Diet...	C				F				Statistical significance of effects of:		
Coprophagy...	+		−		+		−		Diet (D)	Coprophagy (C)	D × C
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Body-wt gain	2·0	0·3	2·2	0·3	1·4	0·3	2·0	0·2	NS	NS	NS
Feed intake	14·4	0·5	14·1	0·3	13·5	0·2	13·6	0·3	NS	NS	NS

* For details of diets and procedures, see Table 1 and pp. 776–778.

RESULTS

Prevention of coprophagy

During the first balance study period, one rat in the C+ group, two in the F+ group, two in the C− group and two in the F− group took off their anal cups. During the second balance study period, one rat in the F− group took off his anal cup. All data from these rats were excluded from the data analyses.

Feed consumption and body-weight gain

Total feed consumption was similar in all groups (Table 2). Body-weight gain did not differ significantly among the groups (Table 2).

Table 3. *Apparent absorption and retention values for calcium and magnesium in rats fed on a control diet (C) or a fructo-oligosaccharides-containing diet (F) with (+) or without (−) coprophagy allowed**

(Mean values with their standard errors for five to ten rats in each group)

Diet...	C				F				Statistical significance of effects of:		
Coprophagy...	+		−		+		−		Diet (D)	Coprophagy (C)	D × C
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Calcium											
Days 3–7											
Intake (mmol/d)	1·68	0·05	1·66	0·03	1·59	0·03	1·59	0·03	NS	NS	NS
Absorption (%)	45·4^{b}	1·3	44·0^{b}	1·6	60·9^{a}	2·9	66·8^{a}	1·8	$P < 0·001$	NS	NS
Retention (%)	44·4^{b}	1·3	42·8^{b}	1·5	59·3^{a}	3·0	64·2^{a}	1·9	$P < 0·001$	NS	NS
Days 10–14											
Intake (mmol/d)	1·89	0·07	1·86	0·04	1·76	0·04	1·81	0·05	NS	NS	NS
Absorption (%)	47·7^{b}	2·7	47·7^{b}	2·1	52·9^{a}	4·2	59·6^{a}	2·8	$P = 0·008$	NS	NS
Retention (%)	46·9^{ab}	2·9	46·9^{b}	2·1	51·4^{ab}	4·3	57·7^{a}	2·9	$P = 0·019$	NS	NS
Magnesium											
Days 3–7											
Intake (mmol/d)	0·264	0·008	0·261	0·006	0·252	0·005	0·252	0·005	NS	NS	NS
Absorption (%)	58·8^{c}	2·3	55·9^{c}	1·9	76·3^{b}	2·3	87·9^{a}	0·5	$P < 0·001$	$P = 0·021$	$P < 0·001$
Retention (%)	38·6^{b}	3·2	32·7^{b}	1·3	50·5^{a}	4·0	47·5^{a}	3·3	$P < 0·001$	NS	NS
Days 10–14											
Intake (mmol/d)	0·298	0·010	0·293	0·007	0·278	0·007	0·285	0·008	NS	NS	NS
Absorption (%)	52·0^{c}	3·7	52·0^{c}	2·2	68·1^{b}	3·6	83·6^{a}	1·4	$P < 0·001$	$P = 0·006$	$P = 0·006$
Retention (%)	34·8^{b}	3·0	29·7^{b}	1·5	44·3^{a}	2·7	50·1^{a}	2·2	$P < 0·001$	NS	$P = 0·030$

$^{a, b, c}$ Mean values with unlike superscript letters were significantly different (Tukey): $P < 0·05$.

* For details of diets and procedures, see Table 1 and pp. 776–778.

Table 4. *Femur weight and mineral contents in rats fed on a control diet (C) or a fructo-oligosaccharides-containing diet (F) with (+) or without (−) coprophagy allowed**

(Mean values with their standard errors for five to ten rats in each group)

Diet...	C				F				Statistical significance of effects of:		
Coprophagy...	+		−		+		−		Diet (D)	Coprophagy (C)	D × C
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Dry wt (mg)	186	6	183	5	196	7	194	5	NS	NS	NS
Ash wt (mg)	109	3	110	3	118	4	116	4	$P = 0.043$	NS	NS
Ca (mmol/bone)	0·889[b]	0·051	0·833[b]	0·040	1·065[a]	0·056	1·011[a]	0·040	$P = 0.001$	NS	NS
Mg (μmol/bone)	40·9[b]	1·9	47·7[a]	1·5	47·2[ab]	2·1	42·1[ab]	1·5	NS	NS	$P = 0.002$

[a, b] Mean values with unlike superscript letters were significantly different (Tukey): $P < 0.05$.

* For details of diets and procedures, see Table 1 and pp. 776–778.

Table 5. *pH of caecal contents and faecal excretion (μmol/d) of individual short-chain fatty acids of rats fed on a control diet (C) or a fructo-oligosaccharides-containing diet (F) with (+) or without (−) coprophagy allowed**

(Mean values with their standard errors for five to ten rats in each group)

Diet...	C				F				Statistical significance of effects of:		
Coprophagy...	+		−		+		−		Diet (D)	Coprophagy (C)	D × C
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Caecum											
pH	7·27[a]	0·19	7·49[a]	0·15	5·88[b]	0·21	6·00[b]	0·15	$P < 0.001$	NS	NS
Faeces											
Acetate	91·0[c]	16·1	70·4[c]	7·1	442·6[a]	79·6	266·8[b]	49·5	$P < 0.001$	$P = 0.032$	NS
Propionate	15·6[b]	5·3	15·6[b]	1·7	140·4[a]	36·0	31·8[b]	9·7	$P < 0.001$	$P < 0.001$	$P < 0.001$
Butyrate	3·7[b]	1·1	8·1[b]	0·9	69·0[a]	28·3	29·2[ab]	9·3	NS	$P < 0.001$	NS

[a, b, c] Mean values with unlike superscript letters were significantly different (Tukey): $P < 0.05$.

* For details of diets and procedures, see Table 1 and pp. 776–778.

Calcium balance (*Table 3*)

During the first period (4th–9th day) of the balance study the apparent absorptive ratio of Ca and the Ca-retention ratio in rats fed on FO-containing diets were higher than in rats fed on the control diet. In rats fed on the control diet these values were not altered by preventing coprophagy. However, in the FO groups the apparent absorptive ratio of Ca and the Ca-retention ratio in rats with prevention of coprophagy tended to be higher than in rats without coprophagy (not significant, $q = 2{\cdot}959$).

During the second period (10th–15th day) of the balance study all results were similar to those during the first period except that the Ca-retention ratio in the FO group with coprophagy was not higher than in both control groups.

Magnesium balance (*Table 3*)

During both the first and second periods of the balance study the apparent absorptive ratio of Mg and the Mg-retention ratio in rats fed on FO-containing diets were higher than in rats fed on the control diet. In the rats fed on the control diet these values were not altered by preventing coprophagy. In contrast, in rats fed on the FO-containing diet the apparent absorptive ratio of Mg in rats without coprophagy was significantly higher than in rats with coprophagy.

Calcium and magnesium contents of femur (*Table 4*)

In rats both with and without coprophagy the Ca content of the femur in rats fed on the FO-diet was significantly higher than in rats fed on the control diet. In rats fed on the control diet the Mg content of the femur in rats without coprophagy was higher than that in rats with coprophagy.

The pH of caecal contents and faecal excretion of short-chain fatty acids (*Table 5*)

The pH of the caecal contents was lower in the FO groups than in the control groups. In rats with coprophagy the faecal excretion rates of acetate, propionate and butyrate were higher in the FO group than in the control group. However, in rats without coprophagy, only the faecal excretion of acetate was higher in the FO group than in the control group. Moreover, in rats fed on the FO-containing diet, faecal excretions of acetate, propionate and butyrate were higher in the rats with coprophagy than in the rats without coprophagy.

DISCUSSION

A few rats took off their anal cups, but generally we did not need to attend to the anal cup throughout the experimental period due to the use of the new wire-mesh-type anal cup. An unexpected advantage of using this anal cup was that diets and urine did not contaminate the faeces. No change in the size of this device was necessary during the experimental period, because a sponge-pad between the rat and the device permitted growth of the rat.

In the present study the prevention of coprophagy did not alter the absorption of Ca and Mg in rats which were allowed free access to the FO-free purified diet. Tadayyon & Lutwak (1968) reported that the prevention of coprophagy decreased the apparent absorptive ratios of both Ca and Mg by about 10%. In their study the consumption of diet was restricted and rats were fed on diets containing several levels of Ca and P. Previously, we reported that increased Ca or P in the diet decreased the absorption of Mg markedly. The same result was reported by Brink *et al.* (1992). In our opinion, statistically analysing the absorptive ratios of Ca or Mg in rats under several different dietary conditions to evaluate the effect of coprophagy is not appropriate.

Cree *et al.* (1986) also reported that the apparent absorption of Ca decreased when coprophagy was prevented with an anticoprophagy cage in rats fed on a non-purified diet. However, Zhang *et al.* (1992) reported that the prevention of coprophagy with a collar or an anal cup did not alter the bioavailability of Fe from a diet containing $FeSO_4$, but did alter the bioavailability of Fe from a high-crude-fibre diet in anaemic rats. We speculated that the effect of coprophagy on mineral absorption is more remarkable in rats fed on a non-purified diet, such as a high-dietary-fibre diet, than in rats fed on a purified diet.

FO-feeding increased the apparent absorption of Ca and Mg in rats both with and without coprophagy in the present study. Moreover, prevention of coprophagy increased the absorption of Ca and especially Mg in rats fed on the FO-containing diet. The reason for the higher absorptive ratio of Mg when coprophagy is prevented is not known. A decrease in the luminal pH by the luminal fermentation of indigestible carbohydrates increases the soluble fraction of minerals and increases the absorption of these minerals (Demigné *et al.* 1989; Rémésy *et al.* 1993; Schulz *et al.* 1993). We also observed decreases in the caecal pH as a result of FO-feeding in the present study. However, the pH of the caecal contents in the FO group without coprophagy was the same as that in the FO group with coprophagy. These results suggest that other mechanisms are involved in the enhancement of mineral absorption. We suggest two mechanisms for the increases in Ca and Mg absorption due to the prevention of coprophagy in rats fed on the FO-containing diet in the present study. First, the prevention of coprophagy may alter the intestinal microflora in rats, as has been reported previously (Gustafsson & Fitzgerald, 1960; Ebino, 1993), and this may affect the absorption of minerals. The change of composition of faecal SCFA in this study supports this hypothesis. The second hypothesis is that the increased Ca intake caused by re-ingestion of faeces may decrease the absorption of Mg. The apparent absorptive ratio of Mg was higher than that of Ca in rats fed on the FO-containing diet with the prevention of coprophagy. Thus, the Ca:Mg ratio in faeces was higher than the Ca:Mg ratio in the diet. Increased Ca intake decreased the absorption of Mg, as previously reported (Brink *et al.* 1992; Ohta *et al.* 1994*a*).

In the present study the Ca content of the femur was also increased by FO-feeding in rats both with and without coprophagy. We previously reported that an increase of Ca absorption raised the Ca content of the femur in normal weaning rats (Ohta *et al.* 1993). In contrast, Tadayyon & Lutwak (1968) reported that increased absorption of Ca did not raise the Ca content of the femur. Disagreement between these results may also be caused by differences in dietary conditions.

In conclusion, FO-feeding increased the absorption of Ca and Mg and increased the Ca content of the femur in rats with prevention of coprophagy. Moreover, prevention of coprophagy particularly enhanced the stimulatory effect of FO on the absorption of Mg. Coprophagy has to be considered when the effects of luminal fermentation on mineral absorption are examined in rats.

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