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Fructooligosaccharides enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats

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Phytic acid (PA) and fructooligosaccharides (FOS) such as inulin are two food components that are able to modify mineral absorption negatively or positively. The influence of PA and FOS on the cecal and apparent mineral absorption as well as on the mineral status (plasma, hepatic, and bone) were investigated in four groups of rats fed one of the experimental diets: a fiber-free (FF) diet, a FF diet containing 7 g/kg PA (FF + PA), a diet containing 100 g/kg inulin (FOS), or a FOS diet containing 7 g/kg PA (FOS + PA). The cecal enlargement together with the acidification of cecal pH in rats adapted to FOS diets led to an improved Ca and Mg cecal absorption. Mineral apparent absorption was significantly enhanced by FOS ingestion (Ca, +20%; Mg, +50%; Fe, +23%; Cu, +45%), whereas PA decreased this factor only for trace elements (Fe, −48%; Zn, −62%; Cu, −31%). These inhibitory effects of a FF + PA diet have repercussions on blood (Mg, −15%; Fe, −12%; transferrin saturation −31%), liver (Mg, −18%; Fe, −42%; Zn, −25%), and bone (Zn, −25%) variables. However, the introduction of FOS into a PA diet counteracted these observed deleterious effects by stimulating bacterial hydrolysis of PA (+60% in rats adapted to FOS + PA compared to those fed the FF + PA diet) and by improving cecal absorption of minerals. (J. Nutr. Biochem. 11:500–508, 2000)

Keywords: absorption; calcium; fermentation; large intestine; magnesium; trace elements

Introduction

Phytic acid (PA) or *myo*-inositol hexaphosphate is present in seeds of all kinds and is concentrated in the aleurone and germ at levels of 3–6%.[1] Because this molecule is charged with six phosphate groups, phytic acid has been shown to bind mineral cations such as Ca, Zn, or Fe in the gastrointestinal tract, making dietary minerals unavailable for absorption and endogenously secreted minerals unavailable for reabsorption into the body.[2] Thus, PA is considered to be the major factor causing impaired absorption of several essential minerals. To improve mineral absorption, PA levels may be decreased by phytase, an enzyme that catalyzes the stepwise hydrolysis of phytate to phosphate and inositol via penta- to monophosphates. In the rat cecum, phytate hydrolysis due to microbial activity has been reported.[3] Our previous work has shown that the presence of fermentable carbohydrates such as resistant starch in diets can stimulate bacterial proliferation in the cecum, together with enhancement of PA breakdown and facilitation of divalent cations absorption.[4,5]

It was thus of interest to study the consequences of the presence of another soluble fiber in human foods on mineral absorption and status, and its interaction with PA. Inulin

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Table 1 Composition of diets

Diet	FF	FOS	FF + PS	FOS + PS
	(g/kg)			
Casein*	150	150	150	150
Corn oil*	50	50	50	50
Wheat starch*	755	655	745	645
Fructooligosaccharides†	0	100	0	100
Phytic acid‡	0	0	10	10
Mineral mix§	35	35	35	35
Vitamin mix§	10	10	10	10

*Casein, corn oil, and wheat starch were purchased from Louis François, St. Maur, France.
†Fructooligosaccharides: chicory inulin degree of polymerization (DP) ranges between 3 and 50, with average DP of 9; supplied by COSUCRA, Pecq-Warcoing, Belgium.
‡Dodecasodium salt from corn (Sigma Chemical Co., St. Louis, MO USA) contains 67% of phytic acid.
§All diets contained 5.2 g/kg Ca, 500 mg/kg Mg, 50 mg/kg Fe, 48 mg/kg Zn, and 6 mg/kg Cu. Mineral content of all diets was analyzed before the beginning of the experiment. Vitamins supplied in mg/kg diet: thiamin 20, riboflavin 15, pyridoxide 10, nicotinamide 100, pantothenate 70, folic acid 5, biotin 0.3, cyanocobalamin 0.05, retinyl palmitate 1.5, DL-α-tocopheryl acetate 125, cholecalciferol 0.15, menadione 1.5, ascorbic acid 50, myo-inositol 100, choline 1.36 g. Both vitamin and mineral mixes were purchased from UAR (Villemoisson, Epinay-sur-Orge, France).
FF–fiber free. FOS–fructooligosaccharides. PA–phytic acid.

from chicory is composed of β(1–2) fructans with a chain length between 2 and 60. Fructans are present in many plants (g/100 g dry matter): asparagus (30), onion (up to 50), Jerusalem artichoke (20–65), chicory roots (30–70). Thus, in populations consuming a Western-style diet, the intake of inulin-type fructans has been estimated to range between 1 and 4 g/day.[6] Moreover, increased Ca, Mg, and Fe absorption following consumption of inulin-type fructans was observed in humans and animals.[7–11] This increased absorption takes place mainly in the large intestine[12,13] and results in increased bone mineral density.[14]

The aim of the present work was thus to investigate the influence and association of PA and fructooligosaccharides (FOS) such as inulin on the apparent absorption of minerals and the mineral status variables in growing rats. In addition, the extent of the digestive breakdown of PA was measured as well as the influence of FOS on this process.

Methods and materials

Animals and diets

Male Wistar rats weighing about 160 g were used. They were derived from the colony of laboratory animals of the National Institute of Agronomic Research (INRA; Clermont-Ferrand/Theix, France). The animals were fed one of the experimental semipurified diets for 21 days (*Table 1*). All animals were allowed free access to diet and distilled water. During the adaptation period (10 days), the animals were housed 2 per cage (wire-bottomed to limit coprophagy) and maintained in a temperature-controlled room (22°C) with a dark period from 8:00 P.M. to 8:00 A.M. Following the adaptation phase, rats were individually housed for 10 days in metabolic cages for feces collection (experimental period). Daily food consumption and body weight were recorded during the adaptation period, then daily during the last 5 days of the experimental period. During these days, feces and urine were collected for mineral balance determination. Animal handling procedures were approved by Institutional Ethics Committee of the INRA (Clermont-Ferrand/Theix, France).

Sampling procedures

Rats were killed at the end of the dark period (8:00–9:00 A.M.). They were anesthetized with sodium pentobarbital (40 mg/kg) and maintained on a hot plate at 37°C. Blood was drawn into heparinized syringes from the cecal vein and then, from the cecal artery. Blood from each rat was placed in a plastic tube containing heparin and centrifuged at 10,000 *g* for 2 min. For blood flow measurement, bromosulfophtalein in saline (5 mmol/L) was infused into a small vein on the internal curvature of the cecum at a rate of 50 μL/min: Determination of the marker dilution in the vein draining the whole cecum (without collateral circulation to ileum or colon) was used to calculate the cecal blood flow. After blood sampling, liver, tibia, and cecum with content (total cecal weight) were also removed and weighed. Cecal contents were transferred into two microfuge tubes; one was immediately frozen at −20°C and the cecal content pH was measured in the other one. The cecal wall was flushed clean, blotted, and weighed (cecal wall weight).

Analytical procedures

Ca and Mg determinations: Subsamples of tissues, food, and feces samples were dry-ashed (10 hr at 500°C). The resulting residues were extracted with 5 mol/L HCl and made up to an appropriate volume with 1 g/L lanthanum chloride solution.

Fe, Zn, and Cu determinations: Subsamples of liver, tibia, food, and feces, 0.25 to 0.5 g of dried samples were dry-ashed (10 hr at 500°C) and then extracted at 130°C in HNO_3/H_2O_2 (2/1, Merck, Suprapur, Darmstadt, Germany) until decoloration; final dilution was made in 2% HNO_3. Mineral concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer 420, Norwalk, CT USA) in an acetylene-air flame at the following wavelengths: 422 nm (Ca), 285 nm (Mg), 248 nm (Fe), 214 nm (Zn) and 325 (Cu). A nebulizer with high sensitivity was used for trace element determinations. Appropriate quality controls were analyzed with each set of measurements. Transferrin saturation percent and plasma iron were determined using Ferrimat-kit and TIBC additive from BioMérieux (Lyon, France).

Phytic acid was determined using high performance liquid chromatography (HPLC; Dionex, Sunnyvale, CA USA) as described previously.[5] The HPLC system consisted of a gradient pump (Dionex series 4500) equipped with a 25 μL injector loop and an anion-exchange Dionex HPIC AS-11 analytical column (0.5 cm i.d. × 25 cm). An anion-exchange Dionex HPIC AG-11 guard column was used. An anion micromembrane suppressor was used for conductivity detection. Samples in the range 1,500–2,500 mg (routinely 2,000 mg) were extracted with 40 mL HCl 0.65 mol/L under vigorous mechanical agitation (Ika-Werk HS 500, Staufen, Germany) for 4 hr at room temperature. The extracts were centrifuged at 5,000 *g*, and 2 mL of the supernatant were diluted to 10 mL with deionized water (Millipore water system). The diluted supernatant was passed through a 200–400 mesh AG 1-X8 chloride anion exchange column (Bio-Rad, Richmond, CA USA). The columns were washed with 15 mL HCl 0.025 mol/L and phytic acid was eluted from the resin with 15 mL HCl 2 mol/L. The eluates were evaporated to dryness in an evaporator concentrator (Jouan SA, St. Herblain, France) and resuspended in deionized water. Potassium phytate (Sigma, St. Louis, MO USA) was used as the external calibration standard.

Short-chain fatty acids (SCFA) were measured by gas-liquid chromatography on an aliquot of supernatants of cecal contents (20,000 *g* for 10 min at 4°C).

Table 2 Effects of fructooligosaccharides (FOS) and phytic acid (PA) on body weight and cecal variables in rats*,†

	FF	FOS	FF + PA	FOS + PA	ANOVA (*P*-value) FOS	PA	FOS × PA
Body weight (g)	251 ± 20[a]	253 ± 19[a]	262 ± 19[a]	242 ± 18[a]	NS	NS	NS
Cecum weight (g)‡	2.18 ± 0.30[a]	7.67 ± 1.53[b]	2.62 ± 0.59[a]	9.20 ± 1.39[b]	<0.001	NS	NS
Cecal wall weight (g)	0.67 ± 0.14[a]	1.77 ± 0.24[b]	0.75 ± 0.12[a]	2.32 ± 0.29[b]	<0.05	NS	NS
Cecal content weight (g)‡	1.51 ± 0.28[a]	5.90 ± 1.45[b]	1.87 ± 0.53[a]	6.88 ± 1.52[b]	<0.001	NS	NS
Cecal pH	7.01 ± 0.11[b]	5.74 ± 0.07[a]	6.84 ± 0.15[b]	5.51 ± 0.18[a]	<0.01	NS	NS
Cecal SCFA (μmol)‡	179 ± 6[a]	711 ± 75[b]	193 ± 11[a]	686 ± 45[b]	<0.001	NS	NS

*Values are means ± SEM for 8 rats.
†Means in a line not sharing a superscript are significantly different ($P < 0.05$).
‡Data that did not meet the assumption of equal variance were log-transformed before statistical analysis.
FF–fiber-free. NS–not significant ($P > 0.05$). SCFA–short-chain fatty acids. ANOVA–two-way analysis of variance.

Calculations and statistical analysis

The SCFA cecal pool was calculated as follows: cecal pool (μmol) = cecal concentration (μmol/L) × cecal water (L).

For Ca and Mg, the rate of cecal absorption (at the time of the measurement) was calculated using the following formula: cecal absorption (μmol/min) = (cecal vein concentration − cecal artery concentration [μmol/L]) × cecal plasma flow (L/min).

Values are given as the means ± SEM. Results were compared by two-way analysis of variance using the General Linear Models procedure of the SuperANOVA software (Abacus, Berkeley, CA USA). Post-hoc comparisons were done by using Fisher's least significant difference procedures. Differences between groups were considered significant if $P < 0.05$.

Results

Physiological variables

Adding PA and/or FOS in the diet had no influence on final body weight of rats (*Table 2*). The liver and tibia weights were not significantly affected by diets (data not shown). In contrast, dietary treatments had a profound effect on the weight of the cecum contents and the cecal wall. Thus, in rats fed the FOS diets, the cecum was 3–4-fold larger than in rats fed the FF diets ($P < 0.001$). pH conditions were close to neutrality in rats fed the FF diet, whereas they were markedly acidic in rats fed FOS diets (5.51 and 5.74). The FOS diets led to a significant rise in cecal pool of SCFA: In rats adapted to FF diets, the cecal SCFA pool was 180–190 μmol/cecum, whereas this pool was 4–5-fold higher in rats consuming FOS in the diets ($P < 0.001$). Contrary to FOS, PA had no influence on cecal variables.

Ca and Mg status

The Ca and Mg levels were identical in the four experimental diets. However, apparent Ca absorption (i.e., intake − fecal excretion) was significantly greater in rats fed FOS diets than in those consuming FF diets (*Table 3*). ($P < 0.05$). This increase in Ca retention should be due to an increase of cecal absorption: FOS diets elicited a better absorption of Ca in the cecum, whereas PA had no influence on Ca cecal absorption (*Figure 1*). In spite of the enhanced absorption, Ca status did not differ in the different groups and no negative effect of PA was noted (*Table 4*). If PA ingestion had no effect on Mg cecal absorption, the last was strongly and significantly stimulated by the presence of FOS in foods (*Figure 1*). Consequently, apparent Mg absorption was significantly increased by fermentable carbohydrate ingestion (+50% compared to rats fed FF diet). Although PA had moderate influence on apparent absorption ($P < 0.05$) compared to FOS effects ($P < 0.001$), Mg status was altered in rats fed the PA diet: Plasma and

Table 3 Effects of fructooligosaccharides (FOS) and phytic acid (PA) on the daily intake and fecal excretion of calcium and magnesium*,†

	FF	FOS	FF + PA	FOS + PA	ANOVA (*P*-value) FOS	PA	FOS × PA
Calcium (mg/day)							
Intake (I)	96.8 ± 7.5[a]	113.0 ± 9.7[a]	119.7 ± 15.7[a]	128.3 ± 15.8[a]	NS	NS	NS
Fecal excretion (FE)	64.6 ± 5.0[a]	65.6 ± 3.0[a]	77.4 ± 5.6[b]	77.9 ± 3.7[b]	NS	<0.05	NS
I − FE difference	32.2 ± 2.0[a]	47.4 ± 5.2[b]	42.3 ± 3.3[b]	50.4 ± 4.1[b]	<0.05	NS	NS
% apparent absorption	33 ± 2[a]	42 ± 3[b]	35 ± 2[a]	39 ± 1[b]	<0.05	NS	NS
Magnesium (mg/day)							
Intake (I)	9.4 ± 0.9[a]	10.3 ± 0.8[a]	11.6 ± 0.9[a]	11.9 ± 1.5[a]	NS	NS	NS
Fecal excretion (FE)	6.2 ± 0.6[c]	3.2 ± 0.3[a]	7.6 ± 0.6[c]	4.7 ± 0.3[b]	<0.001	<0.01	NS
I − FE differences	3.2 ± 0.3[a]	7.1 ± 0.3[b]	4.0 ± 0.3[a]	7.1 ± 0.3[b]	<0.001	NS	NS
% apparent absorption	35 ± 4[a]	69 ± 1[c]	34 ± 3[a]	60 ± 1[b]	<0.001	<0.005	NS

*Values are means ± SEM for 8 rats. For each rat, the mineral balance was calculated from the analyzed mineral intake and excretion.
†Means in a line not sharing a superscript are significantly different ($P < 0.05$).
FF–fiber-free. NS–not significant ($P > 0.05$). ANOVA–two-way analysis of variance.

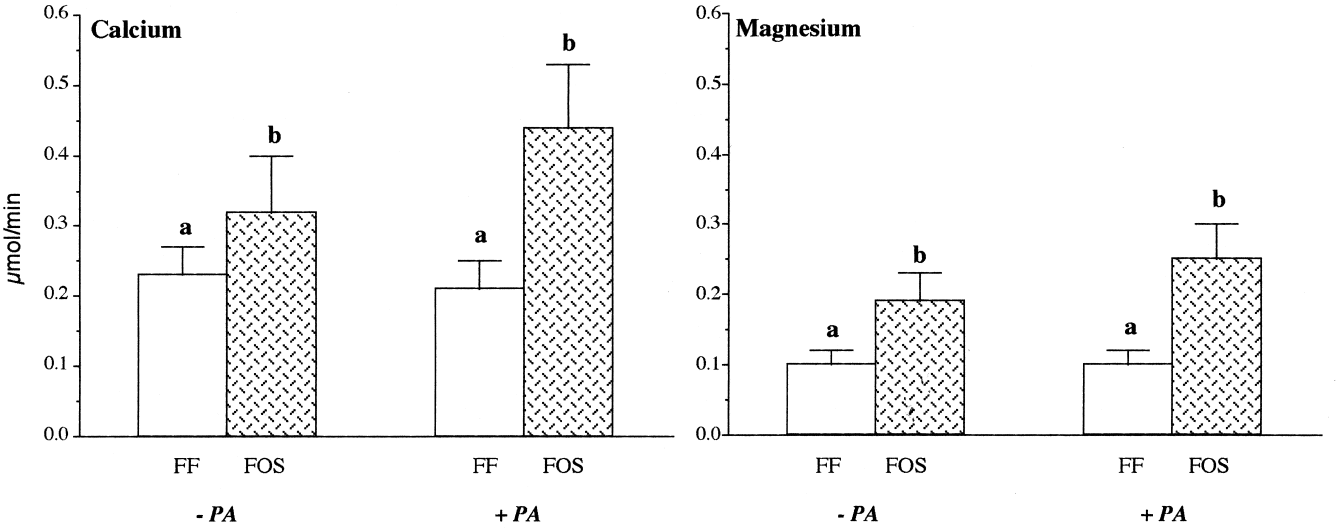

Figure 1 Ca and Mg cecal absorption in rats fed fiber-free (FF) or fructooligosaccharides (FOS) diets with or without phytic acid (PA). Values are means ± SEM; $N = 8$ rats. Means with different letters are significantly different. ($P < 0.05$).

liver Mg were significantly decreased (−15% and −18%, respectively, compared to FF group). Furthermore, daily urinary excretion was reduced in these rats. It must be noted that these inhibitory effects of PA were abolished when FOS was added to PA diet: Mg kidney excretion was stimulated by fiber ingestion, thus showing improved Mg assimilation.

Trace element status (Fe, Zn, Cu)

The dietary levels of these trace elements were adjusted in the four experimental groups and, for each rat, the mineral balance was evaluated from the analyzed mineral intake and excretion. In rats fed the FF diet, 30% of dietary Fe was apparently absorbed, and up to 46% in rats fed FOS alone (*Table 5*). If the Fe fecal excretion was significantly reduced by dietary PA in FF conditions ($P < 0.05$), the incorporation of FOS to the diets significantly stimulated apparent Fe absorption when diet was devoid of PA (+29%) or contained 0.7% PA (+188%, $P < 0.001$). Feeding PA significantly depressed the plasma concentrations of Fe as well as the percentage of transferrin saturation (*Table 6*). Furthermore, Fe liver level was significantly lower in rats adapted to the PA diet than in the other groups (−42% compared to the FF diet). The incorporation of FOS into the PA diet neutralized all these inhibitory effects of PA on Fe status.

Apparently, only 20% of Zn was absorbed in rats fed FF diets, and this absorption was not enhanced in the presence of FOS. However, PA noticeably lowered Zn absorption in rats fed a FF diet (−62%), but not when the diet contained FOS. Although FOS ingestion had a significant effect on apparent absorption ($P < 0.05$), PA seemed to be the major determinant of Zn apparent absorption in this study ($P < 0.01$). It must be noted that the addition of PA into rat diet had repercussions on Zn status: Even if plasma Zn remained constant, liver and bone Zn were significantly depressed by PA ingestion (−24% and −25% compared to FF group, respectively). As for the data observed on apparent absorption, the alteration of Zn status due to dietary PA disappeared when diet contained FOS.

Table 4 Effects of fructooligosaccharides (FOS) and phytic acid (PA) on Ca and Mg status*,†

					ANOVA (*P*-value)		
	FF	FOS	FF + PA	FOS + PA	FOS	PA	FOS × PA
Calcium status							
Plasma (mmol/L)	2.68 ± 0.05^a	2.63 ± 0.06^a	2.70 ± 0.08^a	2.57 ± 0.04^a	NS	NS	NS
Urine (mg/d)	2.01 ± 0.45^a	2.16 ± 0.37^a	2.03 ± 0.28^a	2.23 ± 0.41^a	NS	NS	NS
Tibia (mg/g dw)	248 ± 10^a	265 ± 7^a	240 ± 14^a	263 ± 7^a	NS	NS	NS
Magnesium status							
Plasma (mmol/L)	0.72 ± 0.02^b	0.72 ± 0.03^b	0.61 ± 0.03^a	0.70 ± 0.03^b	NS	<0.05	NS
Urine (mg/d)	3.10 ± 0.33^b	5.45 ± 0.48^c	2.33 ± 0.22^a	5.59 ± 0.61^c	<0.01	<0.05	NS
Liver (µg/g dw)	811 ± 69^b	808 ± 91^b	661 ± 48^a	878 ± 55^b	NS	<0.05	NS
Tibia (mg/g dw)	4.64 ± 0.20^a	5.18 ± 0.13^b	4.47 ± 0.12^a	5.09 ± 0.13^b	<0.05	NS	NS

*Values are means ± SEM for 8 rats.
†Means in a line not sharing a superscript are significantly different ($P < 0.05$).
FF–fiber-free. NS–not significant ($P > 0.05$). ANOVA–two-way analysis of variance.

Table 5 Effects of fructooligosaccharides (FOS) and phytic acid (PA) on the daily intake and fecal excretion of trace elements (Fe, Zn, Cu)*,†

					ANOVA (*P*-value)		
	FF	FOS	FF + PA	FOS + PA	FOS	PA	FOS × PA
Iron (μg/day)							
Intake (I)	885 ± 32[a]	1031 ± 82[a]	1063 ± 81[a]	1158 ± 115[a]	NS	NS	NS
Fecal excretion (FE)	615 ± 57[a]	608 ± 43[a]	891 ± 62[b]	613 ± 41[a]	NS	<0.05	<0.01
I − FE difference	270 ± 44[b]	423 ± 67[c]	171 ± 35[a]	545 ± 64[c]	<0.001	NS	<0.05
% apparent absorption	31 ± 5[b]	40 ± 5[c]	16 ± 4[a]	46 ± 4[c]	<0.001	NS	<0.005
Zinc (μg/day)							
Intake (I)	873 ± 71[a]	990 ± 90[a]	1020 ± 89[a]	1111 ± 91[a]	NS	NS	NS
Fecal excretion (FE)	690 ± 59[a]	773 ± 32[a]	935 ± 54[b]	893 ± 42[b]	NS	<0.05	NS
I − FE difference	183 ± 37[b]	217 ± 57[b]	85 ± 29[a]	218 ± 51[b]	<0.05	<0.01	NS
% apparent absorption	21 ± 4[b]	22 ± 5[b]	8 ± 3[a]	19 ± 4[b]	NS	<0.01	NS
Copper (μg/day)							
Intake (I)	123 ± 10[a]	143 ± 11[a]	147 ± 11[a]	160 ± 14[a]	NS	NS	NS
Fecal excretion (FE)	103 ± 8[a]	101 ± 4[a]	130 ± 8[b]	120 ± 5[a,b]	NS	<0.05	NS
I − FE difference	20 ± 6[a]	42 ± 8[b]	17 ± 3[a]	40 ± 7[b]	<0.01	NS	NS
% apparent absorption	16 ± 6[a,b]	29 ± 4[c]	11 ± 2[a]	24 ± 3[a,b,c]	<0.05	NS	NS

*Values are means ± SEM for 8 rats. For each rat, the mineral balance was calculated from the analyzed mineral intake and excretion.
†Means in a line not sharing a superscript are significantly different ($P < 0.05$).
FF–fiber-free. NS–not significant ($P > 0.05$). ANOVA–two-way analysis of variance.

In rats fed the FF diet, 16% of dietary Cu was absorbed in rats fed the control diet, whereas this absorption was significantly stimulated when FOS fiber was introduced into food. PA significantly decreased Cu absorption when the diet was fiber free. The addition of FOS into a PA diet abolished the inhibitory effects of PA on Cu retention. Thus, only FOS feeding had a significant and positive effect on Cu apparent absorption ($P < 0.05$). However, the different diets did not change Cu status in growing rats: Whatever the nutritional conditions, plasma and liver Cu levels were not affected.

PA fate

For a same intake of PA (150 mg/day), fecal excretion was significantly higher in rats fed the FF diet than those fed FOS (*Figure 2*). The breakdown of PA (i.e., intake − fecal excretion) was higher when the diet contained FOS.

Discussion

PA and FOS such as inulin are two food components that influence mineral absorption. Because humans lack appropriate endogenous phytate degrading enzymes,[15] phytate is considered antinutritional, liable to cause negative effects on the absorption of essential dietary minerals.[2] In contrast to PA, stimulatory effects of inulin-type fructans on Ca, Mg, or Fe absorption were observed in humans and animals.[7–11] This increased intestinal absorption is mainly reflective of greater absorptive capacities in the large intestine.[12,13] In parallel, the present study indicates that FOS ingestion improved apparent mineral absorption and mineral status in the rats, whereas the antinutritional effects of PA were minor compared to the stimulatory effects of FOS. Enhanced fermentation in the cecum due to FOS feeding apparently promotes a better hydrolysis of PA and, thus, enhanced cecal mineral absorption.

Table 6 Effects of fructooligosaccharides (FOS) and phytic acid (PA) on trace elements status (Fe, Zn, Cu)*,†

					ANOVA (*P*-value)		
	FF	FOS	FF + PA	FOS + PA	FOS	PA	FOS × PA
Iron status							
Plasma (μmol/L)	39.7 ± 3.0[b]	38.2 ± 1.7[b]	35.1 ± 2.4[a]	40.8 ± 4.5[b]	NS	<0.05	NS
Transferrin saturation (%)	48.7 ± 2.3[b]	46.5 ± 2.8[b]	33.4 ± 4.8[a]	49.2 ± 5.8[b]	NS	<0.01	NS
Liver (μg/g dw tissue)	259 ± 31[b]	283 ± 16[b]	169 ± 17[a]	278 ± 22[b]	NS	<0.05	NS
Zinc status							
Plasma (μmol/L)	20.9 ± 1.5[a]	21.5 ± 1.8[a]	19.7 ± 1.6[a]	22.0 ± 2.1[a]	NS	NS	NS
Liver (μg/g dw tissue)	114 ± 10[b]	133 ± 19[b]	86 ± 5[a]	106 ± 5[a]	NS	<0.05	NS
Tibia (μg/g dw tissue)	199 ± 9[b,c]	211 ± 8[c]	150 ± 6[a]	182 ± 5[b]	<0.001	<0.001	NS
Copper status							
Plasma (μmol/L)	15.1 ± 0.5[a]	15.6 ± 0.6[a]	14.8 ± 0.3[a]	15.3 ± 0.3[a]	NS	NS	NS
Liver (μg/g dw tissue)	14.1 ± 1.5[a]	15.6 ± 2.0[a]	12.2 ± 2.6[a]	16.5 ± 2.7[a]	NS	NS	NS

*Values are means ± SEM for 8 rats.
†Means in a line not sharing a superscript are significantly different ($P < 0.05$).
FF–fiber-free. NS–not significantl ($P > 0.05$). ANOVA–two-way analysis of variance.

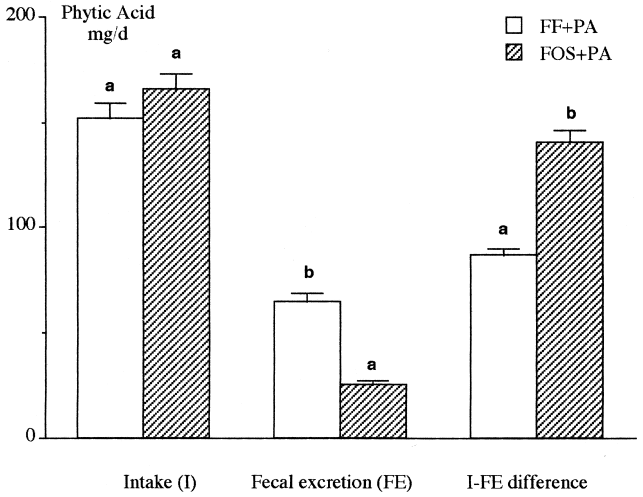

Figure 2 Ingestion, excretion, and difference between ingestion and excretion of phytic acid (PA) in rats fed fiber-free (FF) or fructooligosaccharides (FOS) diets with phytic acid. Values are means ± SEM; $N = 8$ rats. Means for a variable (intake, fecal excretion, or I − FE difference) not sharing the same letter are significantly different ($P < 0.05$).

Because an adequate supply and a good bioavailability of Ca are essential to attain maximum bone mass on which adult bone status depends, it was important to know the respective effects of PA and FOS on Ca absorption. Ca absorption takes place by two routes. The nonsaturable paracellular route of Ca occurs throughout the small intestine and is thought to be driven by passive diffusion. The saturable active transcellular transport is the major absorptive pathway in the proximal intestine (duodenum and jejunum). The large intestine may represent a major site of Ca absorption. In this view, Hylander et al.[16,17] showed that the colon plays an important role for the absorption of Ca after small-intestinal resection: In patients with ileostomy, preservation of at least half of the colon improves Ca fractional accumulation in the skeleton. Moreover, rat experiments have shown that fermentable oligosaccharides improve colorectal and cecal absorption of Ca.[11,13,18,19] Several hypotheses to elucidate these stimulatory effects of fibers on Ca absorption could be suggested. Because FOS is not hydrolyzed by enzymes in the small intestine of monogastrics, it reaches the colon intact. FOS is fully metabolized by colonic microflora. End products of carbohydrate fermentation are gases, lactate, and SCFA such as acetate, propionate, and butyrate. The high concentration of organic acids in the cecum leads to a decrease of cecal pH that raises the concentration of soluble Ca. In parallel, in rats fed fermentable carbohydrate diets, hypertrophy of the cecum is observed (increase of cecal wall weight, crypt column height, and cell number per crypt), leading to a greater exchange surface area. Thus, the enlargement of cecum and the elevation of Ca solubility allow a better cecal absorption of Ca in rats adapted to FOS diets. It is also possible that SCFA can directly stimulate Ca absorption in the rat colon[20] and Ca could pass through the cell membrane more readily in the form of a less charged complex (Ca acetate) by a passive pathway.[21] However, a high rate of passive calcium absorption in the large intestine could trigger a feedback mechanism involving inhibition of duodenal active absorption as a consequence of a change in endocrine factors.[22,23] In spite of this feedback, FOS ingestion improved Ca retention, involving a slight increase of bone Ca content in the present study. In humans, Coudray et al.[7] showed that FOS improves Ca absorption by more than 50%, whereas Van den Heuvel et al.[24] reported that FOS had no effect on Ca absorption. Nevertheless, this last team recently found that oligofructose stimulates Ca absorption in adolescents.[25] Indeed, the applied methodologies are of great importance.[26] Ohta et al.[14] observed a highly significant correlation between bone mass density and apparent Ca absorption. Thus, FOS feeding significantly suppressed the decrease in bone mass density of the femur and tibia in gastrectomized rats. They concluded that FOS feeding completely prevented osteopenia in rats.

In contrast, it is clear that dietary PA can have an inhibitory effect on Ca absorption[27,28] and the reduction of PA may significantly increase Ca availability.[29] As growing rats need an important Ca dietary supply, the ratio of PA to Ca was probably too low to observe a significant effect of PA on Ca absorption in the present study. In any case, the chelating effects of PA seem minor compared to the nutritional potentialities of FOS for Ca status.

The major route of Mg absorption in the distal part of the digestive tract is the passive paracellular pathway, whereas active transcellular transport is considered as relatively minor. Thus, increasing mineral solubility and enlarging the intestinal surface exchange area are probably the principal determinants of Mg absorption. The possibility of a negative feedback in response to a highly effective absorption in the large intestine seems less likely for Mg than for Ca. It has been previously shown that fermentable carbohydrates stimulate Mg absorption.[9,13,30] Chicory inulin raised the cecal pool of soluble Mg by acidifying digestable contents (data not shown). The increase of the cecal absorption may arise from cecal hypertrophy, Mg solubilization and, possibly, a specific effect of SCFA. In particular, SCFA absorption at acidic pH would supply more protons to the exchangers, resulting in a higher transport rate.[31] Fermentable carbohydrates may play a noticeable role by increasing mineral absorption in the large intestine, and this effect may be of particular interest when the overall process of absorption is inefficient such as in elderly subjects. In the present study, the introduction of FOS into the rat diets led to an improved apparent Mg absorption as well as a better Mg bone status (+50% and +11% compared with rats fed the FF diet, respectively). Paradoxically, although PA ingestion did not affect apparent absorption, it must be noted that PA significantly decreased plasma Mg together with hepatic Mg. It has been reported that diets rich in Ca, marginal in Mg, and supplemented with PA decrease Mg bioavailability dose dependently, and thereby affect the dietary Mg requirement.[32] In human nutrition, this situation is not common: In general, plants rich in PA (such as whole cereal products, legumes, or oilseeds) also contain large amounts of Mg as well as fermentable carbohydrates. Under these conditions, unrefined foods remain the major source of Mg: A recent study[33] showed that the amount of absorbed Mg daily is significantly enhanced in rats fed a whole-flour diet compared with those fed a white-flour diet. Moreover, in this

study,[33] plasma, liver, and tibia levels of Mg are significantly increased in rats fed the unrefined product.

For Fe nutrition, the source of iron as well as composition of the meal is of great importance because dietary factors play an important role in iron absorption. There are two kinds of dietary iron: heme Fe and nonheme Fe. If heme Fe is poorly affected by other components in the diet, nonheme Fe, which comprises the main part of the Fe intake, is absorbed in ionic form by receptors on the mucosa cells and its bioavailability varies depending on the Fe status of the subjects and different dietary factors. There is evidence that PA has a very marked inhibitory effect on the absorption of nonheme Fe in man.[34] It is of interest to note that only small amounts of PA (5–10 mg phytates) in a meal are sufficient to halve Fe absorption.[35] In our study, the presence of 0.7% PA in the diet altered Fe status. The latter can be restored to normal values when a PA diet also contains FOS. Stimulatory effects of FOS have been already reported by Delzenne et al.[8] Ohta et al.[10] also suggested that FOS might have a stimulatory effect on Fe absorption in the large intestine because FOS feeding was found to increase the soluble fraction of iron in the cecal contents. FOS ingestion can also activate phytases in the large intestine, which then degrade PA and improve Fe status. Even if there is a strong inverse correlation between Fe absorption and PA content of foods, the ability of a prebiotic such as FOS to overcome the inhibitory effect of PA on Fe absorption is an interesting result.

Although there is no doubt that dietary calcium is a factor influencing skeletal development, focusing on that nutrient alone may be too simplistic. A moderate deprivation of dietary zinc (2 μg Zn/g diet) in monkeys has been shown to retard skeletal growth, maturation, and mineralization.[36] In addition, PA has been shown to alter Zn equilibrium in several monogastric species including man. The major effect of PA on Zn is on the reabsorption of endogenously secreted Zn.[37] The inhibitory effects of PA on Zn homeostasis can be predicted by the molar ratios of PA to Zn in the diet. Molar ratios in excess of 15:1 progressively inhibit Zn absorption and have been associated with suboptimal Zn status in humans. Even molar ratios as low as 5:1 may have some negative impact. Thus, with a molar ratio of 50:1, PA strongly depressed Zn absorption in healthy elderly and young subjects.[38] High levels of Ca may exacerbate the inhibitory effect of PA on Zn absorption in humans by forming a Ca-Zn-PA complex in the intestine that is less soluble than PA complexes formed by either cation alone. Even if the diets contained moderate Ca content and the molar ratio of PA to Zn was 14:1, PA consumption led to a significant decrease in Zn apparent absorption, resulting in lower liver and bone Zn contents. It is noteworthy that the addition of FOS in the diet restored Zn absorption as well as Zn tissue retention to the levels of the control group. As for the Fe case, this beneficial effect of FOS on Zn absorption can be explained by at least 2 ways: Microbial fermentation can enhance zinc bioavailability both via the presence of organic acids (mainly SCFA), which form soluble ligands with Zn, thereby preventing the formation of insoluble Zn phytates, and via PA hydrolysis induced by microbial phytase enzymes derived from colonic microflora.

Results on the effects of PA and fiber on Cu bioavailability are contradictory. Sandstead[39] reported that addition of dietary fiber sources to a mixed Western-type diet increased the Cu absorption and allowed to maintain balance in men. But in this review, some of the fiber sources (e.g., wheat bran) contained PA and some did not. Some authors found that refined fiber did not impair Cu absorption in rats.[40,41] The true effects of PA on Cu absorption are still unknown. Dietary PA had no effect on Cu absorption in men,[42] or decreased whole-body retention of Cu in rats by 57%[43] or enhanced Cu bioavailability.[44] In the present study, FOS fermentation significantly increased Cu bioavailability, whereas negative effect of PA on the absorption of Cu was nonsignificant. In previous research, we reported a negative effect of PA on Cu absorption in rats.[4,5] In these two studies, we noted that the addition of fermentable carbohydrates (resistant starch or inulin) in the PA diets can counteract the chelating effects of PA on Cu absorption.

In humans, hydrolysis of PA is mainly due to dietary plant phytases because mucosal intestinal phytase does not seem to play an important role in PA degradation.[15,45] On the other hand, the contribution of gastrointestinal microflora to PA breakdown remains unclear: Using germfree and conventionalized rats, Miyazawa et al.[46] suggested that the large intestine including the cecum is not a major site of PA hydrolysis, whereas Wise and Gilburt[3] showed the importance of cecal bacteria to degrade PA. Recent studies[4,5] demonstrated that cecal fermentation stimulated by resistant starch leads to an improved PA breakdown in rats. It should be noted that in the colon of pigs, phytate hydrolysis was impaired by addition of calcium carbonate to the diet. The reason for the decreased phytate degradation due to calcium has been suggested to be the formation of insoluble calcium phytate complexes during the passage through the colon.[28] Such complexes are not considered to be readily hydrolyzed by phytase. It is also possible that the high calcium carbonate level decreases microbial phytase activity. However, Jackman and Black[47] observed a drop in solubility of calcium phytate complexes at pH 5.5 to 6. In the present study, the cecal pH was 5.5–5.7 in rats consuming FOS diets. Moreover, the present study showed that microbial phytase activity (EC 3.1.3.8.) occurring in the colon flora was stimulated by FOS ingestion and, in this case, phytate hydrolysis seemed to take place mainly in the distal part of the digestive tract.

In conclusion, the results of this current study emphasize the importance of dietary PA and prebiotic foods such as FOS on mineral and trace element bioavailability. Further, they verify a depressive effect of PA addition on mineral absorption and status. In contrast to PA, FOS fermentation can stimulate mineral absorption in the distal part of the digestive tract through decreasing pH, increased mucosal mass, and bacterial hydrolysis of PA. Thus, the lowering of mineral bioavailability due to PA can be totally offset by soluble fiber ingestion. Even though a direct extrapolation of present results to humans may be questionable due to differences in digestive tract structure and in colonic microflora, these results show that PA-rich foods within fermentable carbohydrate complexes may even improve mineral absorption and status. Human studies are still needed to validate these results obtained with rats.

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