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Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats

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Resistant starch and inulin are complex carbohydrates that are fermented by the microflora and known to increase colonic absorption of minerals in animals. The fermentation of these substrates in the large bowel to short-chain fatty acids is the main reason for this increase in mineral absorption. The purpose of the present study was to examine the potential synergistic effect of a combination of these two fermentable carbohydrates. For this purpose, thirty-two adult male Wistar rats weighing 200 g were used in the present study. The rats were distributed into four groups, and fed for 21 d a fibre-free basal purified diet or diet containing 100 g inulin, or 150 g resistant starch (raw potato starch)/kg diet or a blend of 50 g inulin and 75 g resistant starch/kg diet. After an adaptation period of 14 d, the rats were then transferred to metabolic cages and dietary intake, faeces and urine were monitored for 5 d. The animals were then anaesthetized and caecal Ca and Mg absorption were measured. Finally, the rats were killed and blood, caecum and tissues were sampled. Ca and Mg levels were assessed in diets, faeces, urine, caecum and plasma by atomic absorption spectrometry. Our results confirmed that inulin and resistant starch ingestion led to considerable caecal fermentation in the three experimental groups compared with the control group diet. Moreover, both carbohydrates significantly increased the intestinal absorption and balance of Ca and Mg, without altering the plasma level of these two minerals. Interestingly, the combination of the studied carbohydrates increased significantly ($P < 0{\cdot}05$) the caecal soluble Ca and Mg concentrations, the apparent intestinal absorption and balance of Ca, and non-significantly the plasma Mg level. In conclusion, a combination of different carbohydrates showed synergistic effects on intestinal Ca absorption and balance in rats. Further studies with other types of carbohydrate combinations should be carried out to extend these findings.

Fermentable carbohydrates: Inulin: Resistant starch: Short-chain fatty acids: Calcium and magnesium: Intestinal absorption: Rat

Many reports have indicated that Ca can be significantly absorbed and Mg is mainly absorbed from the large intestine, namely the caecum and the colon (Ebel & Gunther, 1980; Allen, 1982; Hardwick *et al*. 1990; Brink & Beynen, 1992; Kayne & Lee, 1993). Fermentable carbohydrates reach the large intestine to be used by the local microflora. They may stimulate bifidobacteria and lactobacilli growth in the intestine, which has been proposed to be connected with health-promoting functions (Gibson *et al.* 1995; Kruse *et al.* 1999). Many other beneficial health effects for fermentable carbohydrates have been already reported, concerning diabetes and lipid metabolism and cancer prevention (de Deckere *et al.* 1993; Younes *et al.* 1995; Jackson *et al.* 1999). Several investigations have demonstrated that rats fed fermentable carbohydrates absorbed more Ca and Mg than control rats, despite an increase in total faecal mass (Ohta *et al.* 1994, 1995, 1996; Delzenne *et al.* 1995).

Carbohydrate fermentation can influence the intestinal absorption of Ca and Mg in many ways. The short-chain fatty acid (SCFA), fermentation products, are responsible for decreasing caecal content pH, which in its turn increases mineral solubility to improve mineral absorption. The SCFA can also directly influence mineral absorption by

Abbreviations: RS, resistant starch; SCFA, short-chain fatty acids.

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complexing with the minerals leading to an increase in their absorption. The caecal enlargement can also increase the surface area exchange to improve mineral absorption (Demigné *et al.* 1989; Rémésy *et al.* 1993; Younes *et al.* 1993). These different aspects are closely linked to the nature of studied fermentable carbohydrates and to mineral concentrations (Ohta *et al.* 1994, 1995, 1996; Younes *et al.* 1996). The enhancing effect of inulin on Ca and Mg absorption has been investigated several times in animals (Levrat *et al.* 1991*a*; Delzenne *et al.* 1995) and human subjects (Coudray *et al.* 1997*a*; Coudray & Fairweather-Tait, 1998; Van den Heuvel *et al.* 1998). Resistant starches (RS) have been examined in animals and also showed enhancing effects on Ca and Mg absorption (Rayssiguier & Rémésy, 1977; Morais *et al.* 1996; Younes *et al.* 1996; Lopez *et al.* 1998). Foods are a complex mixture containing different relative molecular masses and concentrations of fermentable carbohydrates. The complexity and the variety of these later components may allow maintenance of high fermentation activity throughout the large intestine, which can increase their beneficial effects on mineral absorption. Only a few studies have tried to determine the combined effects of different fermentable carbohydrates on SCFA production and hence mineral absorption (Topping *et al.* 1985; Campbell *et al.* 1997).

The aim of the present study was to investigate the potential synergistic effect of a blend of two different fermentable carbohydrates on fermentation variables and on the caecal as well as the apparent intestinal absorption of Ca and Mg and on their plasma level in rats.

Materials and methods

Animals and diets

Thirty-two male Wistar rats (derived from the colony of laboratory animals of the National Institute of Agronomic Research, Clermont-Ferrand/Theix, France) were fed a commercial pellet diet (U.A.R., Villemoisson s/Orge, France) until body weights reached about 200 g (8 weeks). Groups of eight rats were fed for 21 d a basal (fibre-free) purified diet, or diets containing 100 g inulin/kg, 150 g RS/kg or a blend of 50 g inulin and 75 g RS/kg (Table 1). RS was a crude potato starch supplied by Louis François, St-Maur, France. It is worth noting that up to 750 g/kg starch was RS (Andrieux *et al.* 1989). Inulin (or chicory inulin) was supplied by ORAFTI, Tienen, Belgium. The degree of polymerization of this inulin ranges from 3 to 50, with an average of 9. Inulin contains 1000 g indigestible carbohydrate/kg while crude potato starch contains only 750 g fermentable carbohydrates/kg. That is why we have chosen an inulin:crude potato starch ratio of 1:1·5 to obtain similar levels of fermentations, and to compare then, the effect of their combination. Diet Ca and Mg levels were about 7500 and 900 mg/kg respectively. The rats were provided with fresh food and distilled water daily; these were available *ad libitum*. During the period of adaptation (14 d), the rats were housed two per cage (wire-bottomed to limit coprophagy) and maintained in a temperature-controlled room (22°C) with the dark period from 20.00 hours to 08.00 hours. Following the adaptation phase, rats were individually housed for additional 7 d in metabolic cages fitted with urine–faeces separators to collect faeces and urine (experimental phase). Food consumption and body weight were recorded twice per week during the adaptation phase, then daily during the 7 d experimental phase. Urine (acidified by HCl, final 5 mmol/l) and faeces were collected during the last 5 d of the experiment for determination of mineral balance. The animals were maintained and handled according to the recommendations of the Institutional Ethic Committee of the University of Clermont-Ferrand, France.

Sampling procedures

The rats were killed just after the dark period (between 08.00 and 10.00 hours), because caecal fermentation was still very active. After anaesthesia (40 mg sodium pentobarbital/kg), the rats were maintained on a warming plate at 37°C. The procedure of blood sampling for the measurement of arteriovenous difference across the caecum has been described previously (Demigné & Rémésy, 1985). For blood flow measurement, bromosulfophthalein in saline

Table 1. Composition of experimental diets (g/kg)

Diets…	Fibre-free	Inulin	Resistant starch	Inulin+resistant starch
Ingredients:				
Casein	160	160	160	160
Wheat starch*	720	620	570	595
Inulin	0	100	0	50
Crude potato starch	0	0	150	75
Corn oil	50	50	50	50
AIN salt mix†§	60	60	60	60
AIN vitamin mix‡§	10	10	10	10

* Wheat starch was extensively purified and contains 0 g of resistant starch or NSP/kg.
† AIN salt mixture (g/kg): calcium phosphate dibasic 500, sodium chloride 74, potassium citrate monohydrate 220, potassium sulfate 52, magnesium oxide 24, manganese carbonate (430–480 g Mn/kg) 3·5, ferric sulfate (160–170 g Fe/kg) 6, zinc carbonate (700 g Zn/kg) 1·6, copper carbonate (530–550 g Cu/kg) 0·3, potassium iodate 0·01, sodium selenite 0·01, chromium potassium sulfate 0·55, sucrose, finely powdered 118.
‡ AIN vitamin mixture (mg/kg): thiamine hydrochloride 600, riboflavin 600, pyridoxine hydrochloride 700, nicotinic acid 3000, D-calcium pantothenate 1600, folic acid 200, D-biotin 20, cyanocobalamin (vitamin B_{12}) 1, retinyl palmitate (vitamin A) pre-mix 1600, DL-α-tocopherol acetate 20 000, cholecalciferol (vitamin D_3) 250, menaquinone (vitamin K_2) 50, sucrose, finely powdered 972·9 g.
§ Mineral and vitamin mix were purchased from UAR (Villemoisson, Epinay-sur-Orge, France).

(4·7 mmol/l) was infused into a small vein on the internal curvature of the caecum, at a rate of 50 μl/min. Dilution of the marker in the vein draining the whole caecum (without collateral circulation to ileum or colon) affords determination of the caecal blood flow. Blood was withdrawn from the caecal vein and the abdominal aorta. The blood was placed in microfuge tubes containing heparin and centrifuged at 10 000 ***g*** for 2 min. Plasma samples were stored at 4°C for mineral analysis.

After blood sampling, the caecum, complete with contents, was removed and weighed (total caecal weight). Duplicate samples were collected into 2 ml microfuge tubes that were immediately stored at −20°C. The caecal walls were flushed clean with ice-cold saline, blotted on filter paper and weighed (caecal wall weight). Supernatant fractions of the digestive contents were obtained by centrifuging one of the two microfuge tubes at 20 000 ***g*** for 10 min at 4°C.

Analytical procedures

SCFA were measured by GLC on aliquots of supernatant fractions of caecal contents as previously described (Demigné *et al.* 1980). Ca and Mg were determined on the plasma, caecal supernatant fractions (soluble), urine, and, after mineralization (0·8 M-HCl, 12 h at 800°C), on the untreated caecal samples (total) and faecal materials. After an adequate dilution, mineral concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer 400; Perkin-Elmer, Norwalk, CT, USA) at wavelengths of 422 nm (Ca) and 285 nm (Mg).

Calculation and data analysis

The entire caecal content was calculated as: caecal concentration (μmol/ml) × caecal water (ml); and caecal absorption as (caecal vein) − (artery) difference (μmol/ml plasma) × caecal plasma flow (ml/min). For the determination of digestive balance, food and faeces samples of each rat collected during 5 d were homogenized before mineral analysis. Intake of Ca via the drinking water was considered to be negligible (<0·5 mg/l) compared with that from the diet and was not considered.

Values are given as values with their standard errors and, where appropriate, significance of differences between mean values was determined by ANOVA and multiple range comparisons by Fisher's least-significant difference procedures. Values of $P < 0{\cdot}05$ were considered significant.

Results

Food intake, body weight and caecal fermentation variables

Neither the daily food intake nor the body-weight gain were significantly different between the experimental groups. As expected, the presence of fermentable carbohydrates in the diets resulted in an enlargement of the caecum and lowered caecal pH compared with the control group (Table 2). The results of the rats fed the combination of the carbohydrates were statistically similar to those of the rats fed the carbohydrates separately. With regard to the SCFA, fermentable carbohydrate ingestion increased significantly ($P < 0{\cdot}05$) the molar concentration of SCFA in the caecum, particularly in the RS and inulin + RS groups (Table 3). The effect of fermentable carbohydrates on the production of SCFA became more marked when the results were expressed as the amount of SCFA per caecum, RS group exhibiting the greatest produced amount of SCFA. The profile of SCFA was changed in the caecum in the presence of fermentable carbohydrates. Thus, the molar proportions of propionate and butyrate were raised at the expense of acetate in rats fed inulin or RS respectively. In rats fed the combination of fermentable carbohydrates, both propionate and butyrate molar proportions were increased (Table 3).

Caecal absorption of calcium and magnesium

Table 4 shows that caecal fermentation of the tested carbohydrates increased significantly the solubility of Ca and Mg (4-fold for Ca and 2-fold for Mg) in the caecum. This has as consequence of increasing the bioavailability of these two minerals. Moreover, rats fed fermentable carbohydrates had a higher caecal blood flow than the control group. Consequently, the caecal Ca and Mg absorption increased significantly in the rats fed fermentable carbohydrates. The rats fed the blend of both fermentable carbohydrates had a higher soluble caecal concentration of both Ca and Mg accompanied by a higher caecal Mg absorption compared with the rats fed with either of the studied fermentable carbohydrates separately (Table 4). However, the difference in the caecal Mg absorption was not statistically significant.

Table 2. Effects of dietary conditions on daily food intake, daily weight gain and variables of caecal development*
(Mean values with their standard errors for eight rats per group)

	Daily food intake (g)		Daily weight gain (g)		Caecum weight (g)		Wall caecum weight (g)		pH	
Diets	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fibre-free	22·2[a]	1·2	6·06[a]	0·44	2·54[a]	0·17	0·64[a]	0·02	7·01[a]	0·10
Inulin	21·0[a]	0·9	5·58[a]	0·36	5·33[b]	0·28	1·21[b]	0·04	5·60[b]	0·07
Resistant starch	22·5[a]	0·9	6·03[a]	0·57	6·03[b]	0·57	1·28[b]	0·09	5·77[b]	0·06
Inulin+resistant starch	22·2[a]	1·0	5·50[a]	0·32	5·12[b]	0·28	1·23[b]	0·04	5·62[b]	0·06

[a,b]Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 480.

Table 3. Effects of dietary conditions on variables of caecal fermentations*
(Mean values with their standard errors for eight rats per group)

Diets	[Acetate] (mM) Mean	SE	[Propionate] (mM) Mean	SE	[Butyrate] (mM) Mean	SE	[Total SCFA] (mM) Mean	SE	Total SCFA (μmol/caecum) Mean	SE	Molar ratio acetate:propionate:butyrate
Fibre-free	71·9[a]	5·5	27·3[a]	1·6	9·8[a]	0·5	109[a]	6	164[a]	9	66:25:9
Inulin	68·5[a]	5·3	48·0[b]	1·9	20·6[b]	0·7	137[b]	6	440[b]	18	50:35:15
Resistant starch	104·2[c]	6·9	42·8[b]	2·0	39·1[c]	1·4	186[c]	9	626[c]	26	56:23:21
Inulin+resistant starch	100·4[c]	6·1	56·0[c]	2·8	36·7[c]	1·7	193[c]	11	575[c]	23	52:29:19

SCFA, short-chain fatty acid.
[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 480.

Table 4. Effects of dietary conditions on caecal concentration and absorption of calcium and magnesium*
(Mean values with their standard errors for eight rats per group)

Diets	Total caecal (mM) Mean	SE	Soluble caecal (mM) Mean	SE	Total caecal pool (μmol) Mean	SE	Soluble caecal pool (μmol) Mean	SE	Caecal solubility (% of total) Mean	SE	Caecal blood flow (ml/min) Mean	SE	Caecal absorption (μmol/min) Mean	SE
Calcium														
Fibre-free	488[c]	28	16·9[a]	0·9	873[a]	39	25·4[a]	2·7	2·91[a]	0·36	1·10[a]	0·06	0·14[a]	0·03
Inulin	230[a]	15	28·0[b]	1·5	1029[a]	68	96·4[b]	6·2	9·37[b]	1·03	1·80[b]	0·08	0·56[b]	0·06
Resistant starch	278[a,b]	20	30·4[b]	2·2	1269[b]	65	102·0[b]	7·3	8·04[b]	0·67	1·90[b]	0·10	0·59[b]	0·07
Inulin+resistant starch	309[b]	18	38·6[c]	2·4	1203[b]	36	115·0[b]	6·8	9·56[b]	1·09	1·92[b]	0·08	0·54[b]	0·06
Magnesium														
Fibre-free	101[b]	5	15·0[a]	0·8	192[a]	9	22·5[a]	1·2	11·7[a]	0·8	1·10[a]	0·06	0·12[a]	0·01
Inulin	49[a]	3	12·2[a]	1·1	224[b]	10	42·0[b]	3·5	18·8[b]	1·6	1·80[b]	0·08	0·43[b]	0·03
Resistant starch	49[a]	2	13·8[a]	1·0	231[b]	9	46·5[b]	4·9	20·1[b,c]	1·7	1·90[b]	0·10	0·34[b]	0·03
Inulin+resistant starch	52[a]	3	18·3[b]	0·9	227[b]	11	54·2[b]	1·5	23·9[c]	2·6	1·92[b]	0·08	0·52[b]	0·04

[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 480.

Table 5. Effects of dietary conditions on the digestive and total balances of calcium and magnesium*
(Mean values with their standard errors for eight rats per group)

Diets	Intake (mg/d) Mean	SE	Faecal excretion (mg/d) Mean	SE	Absorption (mg/d) Mean	SE	Absorption (% intake) Mean	SE	Urinary excretion (mg/d) Mean	SE	Net balance (mg/d) Mean	SE
Calcium												
Fibre-free	164[a]	10	126[d]	5	38[a]	2	23[a]	2	4·69[a]	0·53	33·3[a]	2·1
Inulin	162[a]	9	102[b]	3	60[b]	3	37[b]	2	9·00[b]	0·76	51·0[b]	3·2
Resistant starch	166[a]	12	114[c]	4	52[b]	3	31[b]	4	9·18[b]	0·98	42·8[b]	2·4
Inulin+resistant starch	160[a]	9	75[a]	4	85[c]	5	53[c]	2	12·71[c]	0·87	72·3[c]	4·1
Magnesium												
Fibre-free	20·8[a]	1·1	13·6[b]	0·4	7·2[a]	0·4	35[a]	2	3·80[a]	0·27	3·4[a]	0·2
Inulin	22·4[a]	1·2	9·7[a]	0·9	12·7[b]	0·7	57[b]	3	7·52[b]	0·28	5·2[b]	0·3
Resistant starch	23·0[a]	1·0	11·1[a]	0·7	11·9[b]	0·6	52[b]	5	7·52[b]	0·53	4·9[b]	0·2
Inulin+resistant starch	21·9[a]	1·1	7·9[a]	0·6	14·0[b]	0·7	64[b]	3	8·10[b]	0·60	5·9[b]	0·8

[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 480.

Intestinal absorption, balance and plasma level of calcium

As shown in Table 5, the daily intake of Ca was similar, whereas daily faecal excretion of Ca was significantly different between the four studied groups. This is because intestinal absorption of Ca (mg/d) was significantly increased in the groups fed the fermentable carbohydrates compared with the control group. Moreover, this increase was significantly higher in the group fed the blend of both fermentable carbohydrates than that observed in rats fed one of the studied fermentable carbohydrates, separately (Table 5). Urinary Ca excretion was also increased in the

Table 6. Effects of dietary fibre conditions on the plasma content of calcium and magnesium*

(Mean values with their standard errors for eight rats per group)

Diets	Plasma Ca (mM)		Plasma Mg (mM)	
	Mean	SE	Mean	SE
Fibre-free	2·77[a]	0·05	0·74[a]	0·04
Inulin	2·68[a]	0·04	0·78[a]	0·03
Resistant starch	2·83[a]	0·07	0·84[a,b]	0·04
Inulin+resistant starch	2·92[a]	0·08	0·95[b]	0·05

[a,b]Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and p. 480.

rats fed fermentable carbohydrates separately and this increase was significantly more important in the group fed the blend of both fermentable carbohydrates than in the other groups. In spite of this increase in Ca urinary excretion, Ca retention (overall balance) significantly increased in the rats fed fermentable carbohydrates and this increase was the most important in the group fed the blend of both fermentable carbohydrates (Table 5). Finally, the ingestion of the tested carbohydrates, separately or in combination, was without effect on plasma Ca level (Table 6).

Intestinal absorption, balance and plasma level of magnesium

As shown in Table 5, daily intake of Mg was similar, but daily faecal excretion of Mg was significantly decreased in the three fermentable carbohydrate groups compared with the control group. In accordance with this, the intestinal absorption of Mg (mg/d), the excretion of Mg in urine and the retention of Mg (overall balance) were significantly increased ($P < 0·05$) in the groups fed the fermentable carbohydrates compared with the control group. However, these increases were not significantly different between the group fed the blend of both fermentable carbohydrates and those fed one of the studied fermentable carbohydrates separately (Table 5). In addition, plasma Mg level in the rats fed the fermentable carbohydrates singly was not different from that of the control group, whereas it was significantly increased ($P < 0·05$) in the rats fed the blend of both fermentable carbohydrates, compared with the control and inulin groups (Table 6).

Discussion

It has often been reported that vegetable products, rich in indigestible dietary carbohydrates, may affect the bioavailability of minerals. Impairment of Ca and Mg absorption by dietary fibre is traditionally ascribed to the phytic acid content or to the uronic acid content of fibre fractions (McCance & Widdowson, 1942; Reinhold *et al.* 1976). However, when fermentable carbohydrates like RS and inulin were ingested, there was no decrease, but an increase in Ca and Mg absorption. The beneficial effects of fermentable carbohydrates on mineral absorption in rats were reported for the first time in our laboratory in 1977 (Rayssiguier & Rémésy, 1977). This beneficial effect is now well documented with different fermentable carbohydrates in many nutritional conditions in animals (Demigné *et al.* 1980; Ohta *et al.* 1994, 1995, 1996; Delzenne *et al.* 1995; Campbell *et al.* 1997; Yanahira *et al.* 1997) and in some studies with human subjects (Van den Heuvel *et al.* 1999; Coudray *et al.* 1997*a*, Tahiri *et al.* 2001). However, such effects are dependent on the type of fermentable carbohydrates (dose, structure and relative molecular mass) and on the duration of the experiment (Lajvardi *et al.* 1993; Campbell *et al.* 1997). The aim of the present study was to investigate the potential synergistic effect of a blend of two different fermentable carbohydrates on the fermentation variables and on the caecal and the apparent intestinal absorption of Ca and Mg in rats. Our results showed clearly that a combination of different carbohydrates had synergistic effects on intestinal Ca and Mg absorption and on plasma Mg level in rats.

The caecum of the rat is the main site of degradation of fermentable carbohydrates. As expected, our present results, obtained in a relatively short-term experiment lasting 3 weeks, indicate that both inulin and RS are extensively fermented in the rat caecum leading to significant changes in caecum content and wall weights, SCFA production and caecal lumen pH. The ingestion of fermentable carbohydrates induced caecal development and caecal weight rise was at least 2-fold greater than in the control group, resulting in a greater exchange surface area in the caecum. This development of the caecal wall is due to a combination effect of both hypertrophy and hyperplasia (Rémésy *et al.* 1993; Levrat *et al.* 1991*a*). Caecal SCFA concentration was the most important in the group fed the blend of inulin and RS, which may reflect higher activity of fermentation in these rats than in those received one of the studied fermentable carbohydrates singly. Similar results have been reported by Topping *et al.* 1985. They observed that concentrations of SCFA in caecal fluid did not differ between rats fed gum arabic or cellulose, but were raised in rats fed the mixture. SCFA play an important role in the enhancement of caecal mineral absorption (Scharrer & Lutz, 1990, 1992; Trinidad *et al.* 1999). The acidic caecal pH resulting from ingestion of the fermentable carbohydrate diets is caused by the greater level of total SCFA production (Younes *et al.* 1996; Le Blay *et al.* 1999). This reduction in caecal pH leads to greater solubilization of Mg and Ca so that the biologically available concentration of these minerals is increased (Lutz *et al.* 1991; Rémésy *et al.* 1993). Indeed, it was shown that the reduction in the ileal pH was inversely related to apparent absorption of Mg (Heijnen *et al.* 1993). Another possible hypothesis is that SCFA contribute directly to the enhancement of Mg and Ca absorption via a cation exchange mechanism (Trinidad *et al.* 1996, 1999). SCFA in a protonated form are absorbed across the apical membrane and undergo dissociation within the intracellular environment. The increased intracellular H^+ are then secreted from the cell into the lumen in exchange for Mg^{2+} or one Ca^{2+}. SCFA may also directly influence intestinal mineral absorption by forming a lower charge complex with Mg or Ca which can across the cell membrane (Lutz *et al.* 1991; Trinidad *et al.* 1996, 1999). Finally, SCFA

may also responsible for the rise in caecal blood flow and may thus increase the overall mineral input, as reported in the colon (Kvietys & Granger, 1981).

Ca and Mg are absorbed from both the small and the large intestine, which includes the caecum. According to our results, the fermentable carbohydrates ingestion resulting in the lowering of lumen pH led to an increase in the soluble mineral fractions in the caecum (4-fold for Ca and 2-fold for Mg). Again, the combination of both studied fermentable carbohydrates was more effective in increasing soluble Ca and Mg concentrations in the caecum than each fermentable carbohydrates fed singly. This is very important, because the solubility of a mineral is a major determinant for its absorption. Moreover, the caecal blood flow was significantly increased in the rats receiving the fermentable carbohydrates compared with the control group. This phenomenon has been already observed (Demigné *et al.* 1989). Consequently, the process of caecal absorption of Ca and Mg was enhanced in parallel with the dietary intake of fermentable carbohydrates, and large amounts of Ca and Mg were absorbed via the caecum in the rats fed the fermentable carbohydrate diets (3- to 4-fold) compared with the control diet. This is in agreement with the increase in SCFA production and the increase in the higher solubility of Ca and Mg observed in the rats fed the fermentable carbohydrates in the present experiment. The rats fed the blend of fermentable carbohydrates presented the higher Ca and Mg soluble caecal concentration with a high Mg caecal absorption rate, which reflects a potential synergistic effect between the two fermentable carbohydrates.

The results of the present study showed that apparent intestinal absorption of Ca and Mg increased significantly ($P < 0{\cdot}05$) in rats fed the fermentable carbohydrates separately. Interestingly, the increase in apparent intestinal absorption of Ca and Mg was more important in the rats fed with the blend of two studied fermentable carbohydrates, which reached a significant threshold when the Ca absorption was expressed in both mg/d and in percentage terms. This is in agreement with the results of caecal mineral absorption. In the present study, feeding rats with fermentable carbohydrates had a comparable effect on the intestinal absorption of Ca and Mg. Thus, increasing divalent cation absorption in the large intestine did not seem to be accompanied by a lower absorption of these cations in the small intestine under the conditions of the present study.

The improvement of apparent intestinal Ca absorption by the fermentable carbohydrates singly and in combination was not accompanied by significant modifications in plasma Ca levels. Such results are in accordance with previous observations (Levrat *et al.* 1991*a*; Delzenne *et al.* 1995; Lopez *et al.* 1998, 2000). Moreover, the increase in apparent intestinal Mg absorption by the fermentable carbohydrates, fed singly, was not accompanied by significant modifications in plasma Mg levels, but fermentable carbohydrates in combination led to significantly ($P < 0{\cdot}05$) higher plasma Mg levels than in the control or the inulin groups. Indeed, Ca blood homeostasis is efficiently controlled, whereas that of Mg is less tightly controlled. In keeping with this, many reports in the literature indicate that a high intake of dietary Mg or an improvement in intestinal Mg absorption are generally accompanied by an increase in plasma Mg levels (Rayssiguier & Rémésy, 1977; Navas & Cordova, 1996; Coudray *et al.* 1997*b*).

In conclusion, the feeding of inulin or RS significantly ($P < 0{\cdot}05$) increased the apparent intestinal absorption of Ca and Mg in rats without significant change in their plasma levels. Interestingly, the combination of these different fermentable carbohydrates showed significant synergistic effects on intestinal Ca absorption and on plasma Mg levels in rats. These results are relevant because foods are very complex and may contain many types of fermentable carbohydrates in each meal. The non-purified vegetable products have the double interest of being both rich in minerals and an important source of fermentable carbohydrates which can enhance the intestinal absorption of these minerals. Further studies with other types of fermentable carbohydrates deserve to be carried out to extend these findings.

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