



EFFECT OF FERMENTABLE FRUCTO-OLIGOSACCHARIDES ON MINERAL, NITROGEN AND ENERGY DIGESTIVE BALANCE IN THE RAT

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Summary

In the present study, we have assessed the apparent retention of gross energy, nitrogen and Ca, Mg, Fe, Zn and Cu in rats receiving a diet supplemented with fermentable fructo-oligosaccharides with high and low degree of polymerization. Feeding 10% Raftilose® (degree of polymerization : 4.8) or 10% Raftiline® (degree of polymerization : 10) decreased to the same extent (a) the fecal excretion of all the minerals, despite an increase in total fecal mass excretion leading to an improvement of the absorption of Ca, Mg, Fe and Zn; (b) total gross energy absorption; and (c) led to an increase in the faecal excretion and to a decreased urinary excretion of nitrogen, suggesting a displacement of part of nitrogen excretion towards the large intestine. Feeding fermentable fructo-oligosaccharides may thus constitute a good way to counteract syndroms resulting from hyperammonemia or disturbed Fe, Ca, Mg and Zn homeostasis.

Key Words: fructo-oligosaccharides, mineral balance, nitrogen, diet

Fructo-oligosaccharides are a mixture of linear polymers and oligomers of fructose, which have recently been commercialized as dietary fibre and fat or sugar substitutes. They are composed of fructosyl moieties linked by a β 1,2 osidic bond, and differ one from each other by the degree of polymerization (DP). Inulin and its hydrolysis product, which are the subject of the present study, are common natural ingredients (1).

Because of the configuration of their β -osidic bond, such compounds resist the hydrolysis by digestive enzymes, and enter the caecum and the colon where they are highly fermented, mainly by Bifidobacteria, into short chain carboxylic acids (acetate, propionate, butyrate), responsible for a decrease in pH in the intestine. These

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properties, together with their effect on gastric emptying and intestinal motility, allows to classify them as fermentable dietary fibers (2, 3). It has been reported that some dietary fibers may modulate either directly or indirectly the intestinal absorption of nutrients (4, 5).

Many studies both in man and rat report that a diet rich in dietary fibers, like cellulose or hemicellulose, reduces the absorption of some minerals, namely Zn, Cu (6) or Ca (7). However, a recent paper has reported that synthetic fructooligosaccharides, improve the absorption of Ca and Mg in the rat (8). In the present study, we have assessed the apparent retention of Ca, Mg, Fe, Zn and Cu in rats receiving a diet supplemented with fructo-oligosaccharides with high and low degree of polymerization, namely inulin (Raftiline®) and its product of hydrolysis known as Raftilose®. For this experiment, rats from all groups were restricted-fed, in order for all to receive the same amount of minerals in the diet.

Dietary fibre supplementation often leads to an increase in fecal energy excretion resulting from the excretion of non digestible material (like cellulose, for example), but also of protein and lipids from bacterial or metabolic source (4). Our previous experiments have shown that fecal excretion of total lipids is not affected by dietary Raftilose® given at the level of 10% in the diet of rat (9). As suggested by the results obtained by Rémésy et al (2, 10), non digestible oligosaccharides may affect nitrogen fluxes in the digestive tract of rats. Therefore we have also measured, in rats fed ad libitum diets containing Raftilose® or Raftiline® for 50 days, the total energy and nitrogen ingested and excreted in faeces and urines.

Material and Methods

Chemicals

All solvents used were analytical grade and obtained from Merck (Germany) or Union Chimique Belge (Belgium). All chemicals were obtained from Sigma Chemicals, Boehringer (Mannheim, Germany) or Merck and were of the purest grade available. The basal diet given to the animals was a semi-synthetic diet prepared by INRA (Jouy-en-Jouas, France). Raftiline®ST and Raftilose®, used respectively as inulin and its hydrolysate product, were kindly provided by Raffinerie Tirlemontoise (Tienen, Belgium). Raftiline® is almost exclusively composed of glucosyl (fructosyl)_{n-1} fructose (GF_n), with a mean degree of polymerization (n+1) of 11, whereas Raftilose®P95 is a mixture of GF_m (64%) and homooligomers of fructose (F_n) (36%), with a mean degree of polymerization (m or n) of 4.8.

Animals and samples

Experiment 1 : effect of fructo-oligosaccharides on mineral balance

30 male Wistar rats (Iffa Credo, France) weighing 100 ± 5 g at the beginning of the experiment were divided in 3 groups. The animals of the control group (n = 10) received 15 g/day of a semi-synthetic diet composed of sucrose 220 g/kg, starch 440 g/kg, casein 220g/kg, peanut oil 25g/kg, colza oil 25g/kg, cellulose 20 g/kg, mineral mix 45g/kg, vitamin mix 10g/kg, DL methionine 1.5 g/kg. The caloric value of the diet was 4 kcal/g. The concentration in minerals in this diet was 7g/kg for Ca, 1g/kg for Mg, 82 mg/kg for Zn, 78 mg/kg for Fe, 11 mg/kg for Cu. The animals of the group Raftilose (n=10) were given 16.5g/day of the semi synthetic diet containing 10% of Raftilose® for 24 days in order to have the same intake of minerals as in the control group. The animals of the group Raftiline (n=10) were given 16.5g/day of the diet containing 10%

Raftiline®. On day 18 of the treatment, the animals were put into metabolic cages for 8 days. Faeces and urine were collected every 24h and weighed, but only the samples obtained during the last four days were frozen at -30°C and used for further analysis. Samples of diet and tap water were taken for mineral analysis. Diet and water consumption was also measured daily during this period. Blood samples were taken from the tail vein at the end of the period in metabolic cage. All the material used to collect the samples was rinsed with an aqueous solution containing 0.1% ethylene diamine tetracetic acid before use. At the end of this experiment, rats were sacrificed and enlargement of caeco-colon was assessed by weighing caecum and colon content and tissues.

Experiment 2: assessment of caloric and nitrogen balance

30 male Wistar rats were divided in 3 groups. Control rats were fed with the diet described above; the animals of the Raftilose-group were given the semi-synthetic diet containing 10% of Raftilose®; the animals of the Raftiline-group received a diet containing 10% Raftiline®. The animals were given free access to the diet and tap-water for 50 days. On day 42, the animals were placed in metabolic cages for 8 days and fecal, urinary, diet samples collected the last four days were frozen and used for energy and nitrogen measurements. Blood samples were taken from the tail vein at the end of the period in metabolic cage.

Treatment of the samples and analysis

Measurement of Ca, Mg, Fe, Zn and Cu concentration

Faeces, diet, urine and serum samples were mineralized as follows: 30 ml of a mixture of concentrated nitric and perchloric acids (1:1) were added to 1g of lyophilized powdered faeces or diet or to 5 ml urines, and were heated in a sand bath until complete disappearance of organic material. The same procedure was applied for serum analysis but 100 µl serum were treated with 8 ml of the HNO₃:HClO₄ mixture. The residues were dried, dissolved in concentrated HCl, and then diluted with distilled water to obtain a final concentration of 0.1 N HCl. Samples containing only distilled water were treated the same way to obtain blank values. The concentration of ions was measured by inductively coupled plasma atomic emission spectrometry (11), using a plasma source (Philips PV8490) coupled to a simultaneous spectrometer (Philips PV8210) and a computer (HP9816-Software Philips ES-16). The analytical conditions were: cross flow pneumatic nebulizer (Jarrell-Ash), plasma power 1.1 kW, observation zone 14 mm, sample aspiration rate (2.5 ml/min); plasma-argon flow 17.5 l/min, carrier-argon flow 0.6 l/min; auxiliary argon flow 0.4 l/min. Wavelengths: 315.89 nm for Ca, 324.75 nm for Cu, 259.94 nm for Fe, 383.83 nm for Mg, 213.86 nm for Zn. Multi-element calibration standards were prepared from Titrisol MERCK 1000 mg/l single element standards and contained the same amount of HCl and comparable levels of sodium and potassium as the samples. Emission intensities were background corrected.

Measurement of caloric balance

Direct calorimetry was performed on lyophilized faeces, diet and urinary samples, previously acidified with 1N HCl.

Measurement of protein and urea

The Kjeldahl method was used for the mineralization of faeces, diet and urines. The content in nitrogen was calculated from a standard curve performed using (NH₄)₂SO₄. Kit reference 003-0322-000 from Sopachem (Sopar, Belgium) was used for the measurement of the concentration in urea in the serum.

Results

Body weight growth was similar, for both experiments, in all 3 experimental groups (data not shown).

Along the treatment, except for the period in metabolic cage, in experiment 1 the control and treated groups ate their whole meal (15g for controls, 16,5 g for Raftilose and Raftiline groups). Spoiling represented less than 1% of the amount of given diet.

TABLE I

Amount of minerals (Ca, Mg, Fe, Zn, Cu) ingested (water and food) and excreted (urines, faeces) from days 21 to 25 in control rats, and rats receiving 10% Raftilose® or 10% Raftiline® in the diet.

	Controls	Raftilose 10%	Raftiline 10%
Calcium (mg/4 days)			
Ingested	407 ± 7	374 ± 8*	387 ± 8
Faecal excretion	301 ± 11	209 ± 10**	228 ± 8.5*
Urinary excretion	2.5 ± 0.90	2.4 ± 0.35	3.0 ± 0.57
Magnesium (mg/4 days)			
Ingested	58 ± 0.99	53 ± 1.1*	55 ± 1.1
Faecal excretion	32 ± 1.6	13 ± 0.8**	13 ± 0.9**
Urinary excretion	7.9 ± 1.3	6.6 ± 0.6	8.6 ± 1.7
Iron (mg/4 days)			
Ingested	4.5 ± 0.08	4.1 ± 0.08*	4.2 ± 0.09*
Faecal excretion	3.7 ± 0.16	2.7 ± 0.15**	2.9 ± 0.13**
Urinary excretion	0.048 ± 0.007	0.038 ± 0.010	0.032 ± 0.006
Zinc (mg/4 days)			
Ingested	4.7 ± 0.08	4.33 ± 0.09*	4.5 ± 0.09
Faecal excretion	2.6 ± 0.15	1.9 ± 0.12**	2.05 ± 0.07*
Urinary excretion	0.052 ± 0.008	0.035 ± 0.01	0.029 ± 0.006*
Copper (µg/4 days)			
Ingested	688 ± 17	615 ± 12*	640 ± 17*
Faecal excretion	570 ± 32	440 ± 33*	480 ± 29*
Urinary excretion	14 ± 1.2	8 ± 0.9*	11 ± 1.8

Mean ± SEM of the amount of minerals ingested and excreted during the 4 days-period from day 21 to 25.

During the period in metabolic cage, diet and tap water intake, faecal, and urinary excretion were monitored daily. The quantity of diet eaten during this period was lower than before being placed in metabolic cages (56.6 ± 1.0 g/4 days vs 60g/4 days expected for controls; 57.2 ± 1.2 (Raftilose) and 59.0 ± 1.0 g/4 days (Raftiline) vs 66 g/4 days for treated rats). Such a reduction in food intake was probably due to the stress conditions in metabolic cages.

The fecal mass excreted during the period of 4 days was significantly ($p < 0.05$, t test student) higher in Raftilose®- (7.3 ± 0.7g) or Raftiline®- (6.9 ± 0.3g) fed rats than in controls (5.7 ± 0.4); this results mainly from an increase in fecal excretion of water, as there is no significant difference in dry mass excretion between groups (3.3 ± 0.1; 3.4 ± 0.1; 3.6 ± 0.4g for control, Raftilose® and Raftiline® fed rats, respectively; $p > 0.05$, t test student).

The concentration of Ca, Mg, Zn, Cu and Fe was measured in diets and tap water, in fecal samples, and urinary samples collected between days 21 and 25. The amounts of each mineral ingested (food + drinking water) and excreted in the faeces and in the urines are presented in table 1 which shows that feeding a diet enriched in Raftilose® or Raftiline® decreased the fecal excretion of all the minerals, despite an increase in total fecal mass excretion.

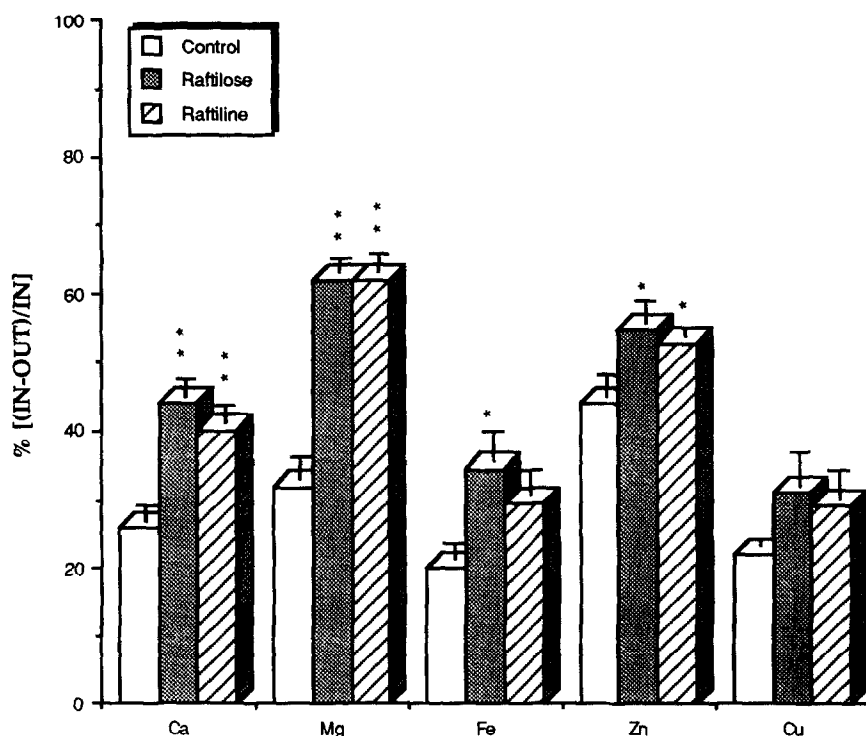


Fig. 1

Apparent retention of Ca, Mg, Fe, Zn and Cu calculated as follows :

$$\frac{[(\text{mineral intake (diet + water)} - \text{mineral excretion (fecal + urinary)}) / \text{mineral intake}] \times 100}{m \pm \text{sem} (n = 10)}$$

* $p < 0.05$ vs control (t test student), ** $p < 0.01$ vs control (t test student)

A decrease in dietary intake of minerals was also observed for Ca, Mg, Fe, Zn and Cu in animals receiving Raftilose®, but only for Fe and Cu in Raftiline®-treated rats. Nevertheless, the apparent absorption $[(\text{IN}-\text{OUT})/\text{IN}]$; IN= diet + water intake; OUT = fecal + urinary excretion] calculated for all minerals shows that both Raftilose® and Raftiline®, at the same extent, significantly improve the absorption of Ca, Mg, Fe and Zn (Fig 1). Raftiline® and Raftilose® supplementation almost doubled the apparent retention of Mg, whereas a more modest increase of retention was observed for the other minerals. Free Ca, Mg, Zn and Cu concentrations have been measured in the serum of rats at the end of the experiment : as compared to controls, no significant modifications occurred in Raftiline®- or Raftilose®-treated animals (Table 2).

TABLE II
Ca, Mg, Zn and Cu concentration in the serum

	Control	Raftilose 10%	Raftiline 10%
Calcium (mg/l)	98 ± 3.2	107 ± 5.0	100 ± 3.2
Magnesium (mg/l)	23 ± 0.6	27 ± 0.8	25 ± 10.9
Zinc (mg/l)	1.55 ± 0.11	1.54 ± 0.09	1.47 ± 0.10
Copper (mg/l)	0.84 ± 0.03	0.89 ± 0.01	0.89 ± 0.03

Concentration of free Ca, Mg, Zn and Cu measured at day 25 of the protocol in the serum taken from tail vein of control rats, or rats receiving 10% Raftilose® or 10% Raftiline®. Mean ± SEM (n = 10)

Caecal and colon tissues and content were weighed in animals sacrificed at the end of the experiment. Caecal content (in g) was 2.22 ± 0.26 , $5.56 \pm 0.53^{**}$ and $5.89 \pm 0.19^{**}$ in control, Raftilose®- and Raftiline®- fed rats, respectively; the colon contents (in g) were not significantly different, namely 0.73 ± 0.22 , 1.05 ± 0.21 and 1.02 ± 0.27 , respectively. The weight of the caecal tissue was 2.2 ± 0.2 g, $5.5 \pm 0.5^{**}$ g and $5.9 \pm 0.2^{**}$ g in control, Raftilose®- and Raftiline®- fed rats, respectively, reflecting an hyperplasia in fructo-oligosaccharides-fed animals. No modification of colon tissues weight was observed.

TABLE III
Effect of fructo-oligosaccharides on gross energy absorption in experiment 2

	Control	Raftilose 10%	Raftiline 10%
Ingested energy (kcal/24h)	89 ± 8	84 ± 8	88 ± 3
Fecal dry mass excretion (g/24h)	0.90 ± 0.10	1.07 ± 0.05	1.17 ± 0.10*
Fecal excretion of energy (kcal/24h)	3.0 ± 2.0	4.4 ± 0.5*	5.0 ± 0.4**
% of absorption of energy	96 ± 0.5	94 ± 0.6*	94 ± 0.5

*Mean ± SEM (n = 10) of fecal excretion and dietary intake of gross energy measured from day 46 to 50 in experiment 2 (see Material and methods). * p < 0.05 vs control (paired student t test)*

*** p < 0.01 vs control (paired student t test)*

The calorimetric analysis of diet and faeces in the experiment 2 allowed to calculate the percentage of gross energy absorbed (Table 3). It is significantly decreased in Raftilose® and Raftiline® groups as compared to controls; this was mainly due to an increase in faecal excretion of gross energy.

The total amount of nitrogen absorbed in the diet and excreted in the faeces and in the urine are presented in table 4. As compared to controls, Raftilose® and Raftiline® in the diet lead to an increase in the faecal excretion of nitrogen, and to a decreased urinary excretion of nitrogen. The total nitrogen balance was not affected by the treatment. The uremia was significantly lower in Raftilose® and Raftiline®- fed rats (Table 4). Uremia level in the serum is more depressed with Raftiline® whilst the effect of two oligosaccharides on fecal nitrogen is very similar.

TABLE IV
Influence of dietary fructo-oligosaccharides on nitrogen balance

	Control	Raftilose 10%	Raftiline 10%
Ingested N (mg/4 d)	1876 ± 76	1858 ± 98	1738 ± 107
Fecal N excreted (mg/4 d)	143 ± 11	197 ± 17*	207 ± 12**
Urinary N excreted (mg/4 d)	453 ± 26	330 ± 11**	306 ± 25**
Nitrogen balance (%)	71.0 ± 2.3	71.0 ± 2.7	70.0 ± 3.7
Uremia (mg/dl)	20.0 ± 0.1	17.0 ± 0.6*	13.5 ± 0.2**

Mean ± SEM (n = 10) of fecal excretion and dietary intake of nitrogen from day 46 to 50 in experiment 2. Nitrogen balance has been calculated as follow : [(ingested N - (fecal + urinary excreted N))/ingested N] X 100
Uremia has been measured at day 50.

** p < 0.05 vs control (paired student t test)*

*** p < 0.01 vs control (paired student t test)*

Discussion

Malabsorption of calcium and magnesium by dietary fibers is traditionally ascribed to the phytic acid content or to the uronic acid content of fiber fractions (12). But when fibers, like pectin for example, are largely fermented in the large intestine, there is no decrease, and even an increase in calcium absorption (13).

Our results, obtained in a relatively short-term experiment (25 days of treatment) indicate that dietary supplementation with Raftilose® or Raftiline®, which are fructo-oligosaccharides which have fermentable dietary fiber-like effects, leads to an increase in the apparent retention of Ca and Mg, as previously shown by Ohta et al (8, 14), but also of Fe and Zn.

Knowing that fructo-oligosaccharides reach the colon, without being transformed by digestive enzymes, and are highly fermented by Bifidobacteria in the large intestine, it is important to consider the lower part of the digestive tract as the putative site of their effect on mineral absorption. The development of caecal fermentations upon fibers intake is generally accompanied by an accumulation of cations in the large bowel, which helps to neutralize the organic acids generated in situ via fermentation (10). Caecum and colon are both important sites for the absorption of Mg (15) and Ca (16), even in rats fed a fiber-free diet. The process of caecal absorption of Ca and Mg is enhanced (almost linearly) in parallel with the dietary inulin level in the rat (17). Ohta et al (14) have recently shown using caeectomized rats in a mineral balance study, that stimulatory effect of fructo-oligosaccharides on Mg absorption occurred mainly in the colon whereas the stimulatory effect of fructo-oligosaccharides on the absorption of Ca occurred mainly in the caecum. In our study, we show that the increase of apparent retention of Mg is more important than that of Ca.

An increased surface of resorption, resulting from the important proliferation of caecal tissue in animals fed with fermentable carbohydrates has been proposed as a mechanism to explain an increase in cation retention. In the present experiment, we have shown that both Raftilose®- and Raftiline®- led to caecal tissue proliferation, but no modification of the weight of colon was observed. Thus, other mechanisms may be proposed as (a) an increased solubilization of the cations. Since, as suggested by Levrat (17), the organic acids (acetate, propionate, butyrate) produced by fermentation

of the carbohydrates in the large intestine, could solubilize part of the calcium phosphate, a reaction involved in the steady-state for a pH value near to 6; the Ca^{++} resulting from this reaction should be strongly absorbed by the caecal tissue. In the rumen, short-chain fatty acids may stimulate Mg absorption, but there is an antagonism with potassium and ammonia (18); or (b) an increased absorption by an osmotic effect : this phenomenon has largely been described in lactose-fed animals, and could lead to an increase of the transport of Ca by a non saturable paracellular system (19);

The effect of fermentation on Zn, Cu and Fe bioavailability is less documented. In our experiments, about 20% of the iron ingested in control rat is absorbed. It increases to 35% and 31% in Raftilose®- and Raftiline®- fed rats, respectively. An increase in the bioavailability of iron has been observed in rats fed a diet containing 8% of highly esterified pectin (20) but it seems that the mechanism proposed is specific for pectin which is able, via its carboxylic acids groups, to form soluble complexes with iron, that facilitates its absorption through cell membrane. It is not the case of fructo-oligosaccharides. Raftilose® and Raftiline® also improve the apparent resorption of Zn. Other dietary fermentable compounds like polymers of glucose and lactose stimulate the intestinal absorption of Zn, but the mechanism has not been elucidated yet (19, 21).

Wapnir and Balkman (22) have previously shown that dietary polymers of glucose increased the intestinal absorption of Cu. In the present experiment, fructo-oligosaccharides-feeding decreased the fecal excretion of copper, without affecting the apparent retention of the cation.

The improvement of ion apparent absorption by Raftilose® and Raftiline® dietary supplementation, was not accompanied by significant modifications of the serum levels of these micronutrients. Such a result is in accordance with observation by Levrat et al (17), who have also shown that, despite an increase in Ca and Mg caecal absorption, the plasma concentration of Mg and Ca was not affected.

Beside an effect on mineral homeostasis, we have shown, in a longer experiment (50 days) that fructo-oligosaccharides feeding led to an increase in the fecal excretion of gross energy. The increase in fecal energy excretion is only partly explained by the increase in total dry mass excretion : for example, in Raftiline® group, the fecal energy excretion is increased by about 70% of the control, whereas the increase in total dry mass excretion is only 30%. We may thus postulate that, besides an effect on the quantity of faeces excreted, qualitative modifications of composition of faeces also occur. At least until 50 days, it seems that this phenomenon has no consequences on body weight evolution.

The increase in fecal mass excretion results mainly from microbial proliferation, which is strongly stimulated in dietary fructo-oligosaccharides fed rats (3, 23). This needs a nitrogen supply to allow protein synthesis. Both sources of nitrogen exist : the nitrogen present in situ, consisting of non digested dietary protein, digestive enzymes, and cells produced by desquamation. Another important source is blood urea. The decrease in urea following dietary fructo-oligosaccharides supplementation had already been observed by Rémésy et al (2). Bacteria have a strong urease activity, which creates a gradient favouring the transfert of urea from blood to the large intestine. The presence of oligosaccharides in the caecum increases the total urease activity (2). The decrease in uremia, the increase in faecal excretion and the decrease in the urinary excretion of nitrogen by Raftiline® and Raftilose® observed in the present experiment, are in accordance with the displacement of part of nitrogen excretion towards the large intestine. The lower level of uremia measured in Raftiline® fed rats, as compared to Raftilose® fed rats, could be partly explained by the lower

dietary intake of nitrogen in the former group.

As feeding fermentable fructo-oligosaccharides displaces nitrogen excretion and leads to a decrease in uremia, the effect is rather similar to the one generated by lactulose (24). The effect of dietary Raftilose®/Raftiline® is confirmed in human, it could thus maybe constitute a good nutritional way to counteract hepatic encephalopathy.

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