Eur J Nutr (2010) 49:155–163
DOI 10.1007/s00394-009-0060-8

ORIGINAL CONTRIBUTION

Soybean whey enhance mineral balance and caecal fermentation in rats

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Received: 26 June 2009 / Accepted: 28 September 2009 / Published online: 15 October 2009

Abstract
Background Soybean whey, a by-product of tofu manufacturing, is currently discarded by the food industry. However, it contains valuable compounds such as non-digestible oligosaccharides (NDO), which promote the growth of beneficial lactic acid bacteria in the colon, and are therefore recognized as prebiotics. Acidic fermentation of NDO in the caecum appears to be related with an increase in mineral absorption.
Aim of the study To evaluate the effect of consuming soybean whey containing galacto-oligosaccharides (GOS) on mineral absorption and caecal fermentation in rats.
Methods An in vivo assay was carried out in rats over a period of 4 weeks; previously, the nutritional composition of soybean whey was determined and NDO were measured by high-performance liquid chromatography (HPLC). Faeces and urine were collected weekly throughout the experiment for mineral balance analyses. Animals were killed under anaesthesia, organs were removed and weighed, and short-chain fatty acids in the caecal contents were determined by gas chromatography.
Results and conclusions Non-digestible carbohydrates such as GOS stachyose (318 ± 3 mg/100 mL) and traces of inulin were identified by HPLC. When soybean whey was used as a source of GOS in rats, the consumption of diluted soybean whey (75 mL/day per rat) containing GOS (120 mg/day per rat) exhibited a prebiotic effect and led to an improved mineral balance, especially for calcium and magnesium. In view of its composition and potential health-promoting properties, soybean whey could be used as a valuable ingredient in new functional foods.

Keywords Soybean whey · Galacto-oligosaccharides · Prebiotics · Caecal fermentation · Mineral balance

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Abbreviations

ANOVA	Analysis of variance
COD	Chemical oxygen demand
GLC	Gas liquid chromatography
FOS	Fructo-oligosaccharides
GOS	Galacto-oligosaccharides
HPLC	High-performance liquid chromatography
LMWC	Low-molecular weight carbohydrates
NS	Neutral sugars
NDO	Non-digestible oligosaccharides
SCFA	Short-chain fatty acids
SDF	Soluble dietary fibre
UA	Uronic acids

Introduction

Soybean whey is obtained as a liquid by-product in the manufacturing of tofu from soybean seeds. Regardless of the procedure used for preparing the tofu, the basic principle is that soybean seeds are extracted with water to make soymilk or soy drink, and the soy protein is coagulated with salt or acid to make tofu [16].

The soybean whey obtained after clotting and pressing the tofu is currently discarded by the food industry. Due to the phenomenon of eutrophization and to the increased chemical oxygen demand (COD) in the effluents, disposal poses an industrial and environmental problem [17]. However, soybean whey still contains valuable compounds derived from the soluble fraction of soymilk such as protein and oligosaccharide [6]. Soybean oligosaccharides, mainly stachyose and raffinose, are non-digestible oligosaccharides (NDO) belonging to the raffinose family of oligosaccharides, also known as α-galactosides or galactooligosaccharides (GOS), which are selectively used by bifidobacteria [14].

Non-digestible oligosaccharides promote the growth of beneficial lactic acid bacteria in the colon and are thus recognized as prebiotics [7, 9, 22, 31, 33]. When prebiotics are fermented in the gut, short-chain fatty acids are generated and the pH decreases. Acidic fermentation in the caecum appears to be related with increased absorption of minerals in the large intestine of rats [30, 41].

Most of the studies involving prebiotic oligosaccharides have been done using inulin and its fructo-oligosaccharides (FOS) derivatives [7, 14, 22, 41], and to a lesser extent with various forms of GOS [23, 33]. Consumption of FOS and GOS can have health benefits, particularly as regards their influence on mineral absorption [1, 23, 41] and lipid metabolism, as well as having anticancer properties and anti-inflammatory and other immune effects [7, 22].

On the subject of NDO and mineral intestinal absorption, there are many reports on FOS in the literature which show an enhanced absorption of calcium and magnesium in humans and animals [4, 5, 19, 23–25, 28, 29, 40]. However, there are far fewer reports on the intake of soybean GOS and its role in human health. Some works carried out in animal models demonstrated evidence that prebiotics, particularly GOS, may play a role in increasing mineral absorption, and more specifically in the bioavailability of calcium [2, 33].

The aim of the present study was therefore to shed light on the effect of consumption of soybean whey containing GOS on mineral absorption and caecal fermentation in rats. Furthermore, it is also necessary to establish a possible relationship between the main components of soybean whey and the effects observed, in order to raise the value of this by-product.

Materials and methods

Raw material

Soybean whey, a by-product of tofu manufacturing, was kindly provided by a local food industry (Toofu-Ya S.L., Arganda del Rey, Madrid, Spain). For sample homogeneity, soybean whey from three different batches of tofu processing was collected at different times of the year. Raw liquid soybean whey was kept frozen (−20 °C) until analysed or used to feed the rats.

Analysis of soybean whey

The nutritional composition of soybean whey was measured. In order to obtain detectable amounts for analysis, 1,000 mL of soybean whey was concentrated to 500 mL in an R-114 Büchi vacuum rotatory evaporator with a B-480 Büchi water bath and a temperature not exceeding 50 °C. Concentrated soybean whey was kept frozen until the analyses of dry matter, dietary fibre, total protein and ash were performed. Raw liquid soybean whey, unconcentrated, was used for the analyses of low-molecular weight carbohydrates (LMWC).

Dry matter

This was determined by weight loss in 25 mL of concentrated soybean whey. In order to prevent projections, it was first dried at 60 °C in an aerated oven and then oven-dried to constant weight at 105 °C for 16 h.

Low-molecular weight carbohydrates

Soluble sugars and oligosaccharides in soybean whey were analysed directly without any previous extraction step. Liquid samples were passed through 0.22 μm Lida filters for aqueous solutions (Cat. No. 5003-SPA, CA non-sterile, Kenosha, WI, USA), immediately before analysis with high-performance liquid chromatography (HPLC). LMWC (50 μL) were analysed by HPLC, on a Bio-Rad Aminex HPX-87P column (300 × 7.8 mm) with two Bio-Rad micro-guard cartridges (30 × 4.6 mm). The column was eluted isocratically with Milli-Q-filtered (0.45 μm) and degassed water at 85 °C, with a flow rate of 0.6 mL/min [8, 32]. LMWC were identified and quantified by comparison with known carbohydrate standards (inulin, stachyose, raffinose, glucose, fructose, xylose from Sigma; sucrose from Merck). LMWC were expressed as milligram per 100 mL of sample.

The following HPLC instruments were used: Kontron autosampler 360, Kontron ternary pump system 325,

Waters differential refractometer R-401, Jones chromatography thermostatic oven, Kontron data system 450-MT2 and Hewlett-Packard deskjet 600 printer.

Dietary fibre

Soluble dietary fibre (SDF) was analysed in concentrated soybean whey by a modified AOAC enzymatic–gravimetric method [27]. No insoluble dietary fibre residue was obtained after enzymatic treatment and centrifugation. Dialysis was used instead of ethanolic precipitation to avoid losses of SDF as previously reported [21].

Neutral sugars (NS) in SDF hydrolysates were quantified colorimetrically by the anthrone method with glucose as a standard [18]. Uronic acid (UA) was determined spectrophotometrically with galacturonic acid as a standard and 3,5-dimethylphenol as the reagent [35]. SDF was calculated as the sum of NS plus UA, and expressed as milligram per 100 mL of sample.

Protein content

Total nitrogen in concentrated soybean whey was determined with a Leco FP-2000 protein/nitrogen analyzer (Leco Instruments SA, Madrid, Spain). Liquid samples were weighed into ceramic boats and loaded into the analyzer, where they combusted in the pure oxygen environment of the furnace. After passing through a thermoelectric cooler to remove water, an aliquot of the combustion gases was taken. The gases were scrubbed and all nitrogen was reduced to N_2 and detected by a thermal-conductivity cell. A blank without sample was run and the instrument was calibrated with EDTA. Total protein in soybean whey was calculated as nitrogen × 6.25 and expressed as milligram protein per 100 mL of sample.

Ash

25 mL of concentrated soybean whey was first dried at 60 °C in an aerated oven and then incinerated in a digitally controlled Hobersal HD-230 muffle furnace (Barcelona, Spain) at 550 °C for 16 h and weighed.

Minerals in soybean whey (as well as in commercial feed, faeces and urine) were analysed as described below ("Mineral content in feed and biological samples").

In vivo assay

A total of 20 female Unilever Wistar rats, 6 weeks old, were used in the experiment. The animals were divided into control and treated groups, each with ten rats. Both groups had free access to the commercial feed (Table 1). The control group had free access to drinking water and the treated group to soybean whey diluted in water (1:1, v:v). 100 mL of diluted soybean whey afforded approximately 160 mg of GOS stachyose. The rats were individually caged, in a room with controlled light (12 h, 8 am–8 pm), temperature (22 ± 1 °C) and humidity (60–65%). The animals were kept in accordance with the guidelines for the care and use of laboratory animals (Facultad de Biología, Universidad Complutense, Madrid, Spain). The body weight of each rat was recorded on a weekly basis. For balance studies, food intake and volume of liquid drunk were controlled, and faeces and urine were collected weekly throughout the duration of the experiment.

The animals were fed for 28 days and then killed following ethical procedures under anaesthesia (diethyl ether). The organs (liver, heart, kidneys, spleen, caecum and intestine) were removed, weighed and inspected in order to rule out possible alterations on organ development. Moisture of caecal contents was determined by oven drying to constant weight at 105 °C for 16 h.

pH and short-chain fatty acids in caecum

A portion of the caecal content was diluted in water (1:3, w:v) immediately after sampling; the pH was measured using a microelectrode (Crison, micro pH 2001) and the

Table 1 Composition of commercial feed (g/kg)

Moisture	120
Protein	154
Fat and oil	29
Carbohydrates	605
Starch	443
Total sugar	25
Dietary fibre (cellulose)	39
Additives	
Vit A (U/kg)	15,000
Vit D3 (U/kg)	1,500
Vit E	0.020
Ash	53 ± 1.75
Calcium[a]	9.26 ± 0.75
Magnesium[a]	1.68 ± 0.13
Sodium[a]	2.21 ± 0.27
Potassium[a]	4.02 ± 0.22
Iron[a] (ppm)	83.62 ± 3.39
Copper[a] (ppm)	13.02 ± 1.95
Manganese[a] (ppm)	29.7 ± 2.17
Zinc[a] (ppm)	95.52 ± 8.42
Phosphorus	5.9
Energy (kcal/kg)	3173

Mean values of triplicate determinations ± standard deviation

[a] Mineral content from experimental data

diluted sample was stored at −80 °C until the analysis of short-chain fatty acids (SCFA). To measure SCFA, the diluted samples were thawed and centrifuged (9,000×*g*, 15 min, 4 °C), and the supernatants used for gas liquid chromatography (GLC) analysis. A 0.4 mL sample with 0.5 mL internal standard, in 12% formic acid (4-methyl valeric acid, 2 μmol/mL) and made up to 1 mL with 12% formic acid, was centrifuged as above, and 1 μL of supernatant was injected into a gas liquid chromatograph (5890 Hewlett-Packard) equipped with a flame ionization detector, and a fused silica column (Carbowax 20 M, 10 m × 0.53 mm × 1.33 μm film thickness). The carrier gas was nitrogen with a flow rate of 15 mL/min. The injector and detector temperature was 250 °C and the column temperature was isothermal at 120 °C [37].

Mineral content in feed and biological samples

Commercial rat feed, soybean whey, faeces and urine were analysed for mineral composition [12]. Faeces and urine were collected daily; weekly samples were pooled for each animal, and frozen until analysis. Feed and faeces samples were ashed at a temperature increased linearly to 550 °C for 1 h and then at 550 °C for 20 h in a microwave muffle furnace (Milestone MLS-1200 Pyro). The ashed samples were dissolved in 2 mL of 12 M HCl:14.5 M HNO_3 (1:1, v:v) and then diluted to 10 mL with distilled water. Urine was appropriately diluted with a solution of HNO_3 (2 mL/L) and directly subjected to atomization.

Calcium, magnesium and trace element (Cu, Fe, Mn and Zn) concentrations in commercial feed, soybean whey, faeces and urine samples were measured using a Perkin Elmer Analyst 200 atomic absorption spectrophotometer. Calcium and magnesium samples were previously diluted to 0.1% (w/v) with a lanthanum oxide solution.

Mineral balance

Apparent mineral absorption and balance were calculated as follows: Apparent mineral absorption (%) = [(mineral intake − faecal excretion)/mineral intake] × 100; whereas Apparent mineral balance (mg/day) = [(mineral intake − faecal mineral excretion) − urinary mineral excretion] and efficiency of mineral retention (%) were calculated according to the following equation: Mineral balance (%) = 100 × [(apparent mineral balance in mg/day)/mineral intake].

Statistical analysis

Statgraphics Plus version 5.0 was used. All determinations were performed at least in triplicate and are reported as mean values ± standard deviation. The in vivo study was designed with two factors: time and treatment. The factor time had four levels corresponding to the weekly measurements (1, 2, 3 and 4 weeks). The factor treatment had two levels [control ($n = 10$) and treated rats ($n = 10$)]. Effects of time and diet were analysed by an analysis of variance (ANOVA). Differences of $P < 0.05$ were considered statistically significant. Duncan's multiple range test was used to determine whether mean values were significantly different between weeks ($P < 0.05$).

Results

Composition of soybean whey

Dry matter of soybean whey amounted to 2.46 ± 0.01 g/100 mL. Major components are LMWC and protein, as shown in Table 2. Non-digestible oligosaccharides (328 ± 3 mg/100 mL) were identified by HPLC as GOS stachyose and traces of inulin. A considerable amount of the total protein content determined for commercial soymilk (3,610 ± 10 mg/100 mL) was found to remain in soybean whey (530 ± 5 mg/100 mL). Soybean whey also

Table 2 Composition of soybean whey (mg/100 mL)

Component	
Total LMWC[a]	821 ± 5
Soluble sugars	
Sucrose	434 ± 14
Glucose	31 ± 7
Fructose	6 ± 1
Xylose	21 ± 1
Oligosaccharides	
Inulin	10 ± 1
Stachyose (GOS)	318 ± 3
Soluble dietary fibre	
Neutral sugars	45 ± 6
Uronic acids	13 ± 5
Total protein	530 ± 5
Ash	246.2 ± 5.9
Calcium	14.6 ± 0.5
Magnesium	5.7 ± 0.6
Sodium	6.2 ± 0.8
Potassium	49.1 ± 3.2
Iron	0.073 ± 0.009
Copper	0.076 ± 0.001
Manganese	0.003 ± 0.001
Zinc	0.024 ± 0.007

Mean values of triplicate determinations ± standard deviation. Soybean whey given to treated rats was diluted (1:1, v:v) in water

[a] *LMWC* Low-molecular weight carbohydrate

Table 3 Effect of feed intake and soybean whey drinking on body weight gain, growth rate and feeding efficiency

	Control	Treated	Sign. level
Initial weight (g)	225.1 ± 12.48	228.9 ± 10.42	NS
Final weight (g)	239.0 ± 7.87	234.1 ± 8.53	NS
Growth rate (g/day)	0.60 ± 0.12	0.24 ± 0.19	***
Feeding efficiency	0.02 ± 0.004	0.01 ± 0.008	**
Water or soybean whey (mL/day)	26.10 ± 5.26	74.79 ± 12.80	***

Mean values ($n = 10$) ± standard deviation

Feeding efficiency = body weight gain (g/day)/food intake (g/day)

Analysis of variance: *NS* non significant effect. Values marked with asterisks differ significantly (** $P < 0.01$; *** $P < 0.001$)

Table 4 Moisture, pH and short-chain fatty acids in the caecal content of rats

	Control	Treated	Sign. level
Moisture % (w/w)	83 ± 4	85 ± 6	NS
pH	6.72 ± 0.30	6.38 ± 0.14	*
Total SCFA, μmol/g (molar proportions, %)	97.4 ± 16.8 (100)	154.5 ± 54.3 (100)	*
Acetic acid	46.1 ± 14.1 (47.4)	68.5 ± 27.2 (44.3)	*
Propionic acid	14.8 ± 3.1 (17.5)	23.2 ± 7.5 (15.6)	NS
Butyric acid	30.8 ± 13.2 (31.6)	60.1 ± 26.5 (38.9)	*

Mean values ($n = 10$) ± standard deviation; Traces (less than 2%) of iso-butyric, iso-valeric and valeric acids in all rats; Analysis of variance: *NS* non significant effect. Values marked with asterisks differ significantly (* $P < 0.05$)

SCFA Short-chain fatty acids

contained, as minor components, ash and small amounts of soluble dietary fibre, mainly composed of NS and UA from residual soybean pectins (Table 2). Regarding the mineral fraction of soybean whey (Table 2), potassium content was the highest followed by calcium; however, the amounts were clearly lower than in the commercial feed where the major mineral was calcium (Table 1).

In vivo assay

Weight gain, feed and drink consumption

Both groups of rats received an equivalent caloric content as they had a similar amount of food intake (data not shown). All rats increased their body weight; at the end of the experiment, however, the increase in the growth rate was three times higher in control than in treated rats, with significant differences ($P \leq 0.005$). Regarding drink consumption, nearly three times more diluted soybean whey (75 mL/day) was drunk by the treated rats, than water by the control group (Table 3). No significant differences were found in organ weights (heart, liver, spleen, kidneys) between both groups. A visual inspection of the organs showed no macroscopic alterations. Moreover, no renal calcification was observed on kidney sections in either group of rats.

Caecal fermentation

Moisture, pH and SCFA in the caecal content of rats are shown in Table 4. In vivo colonic fermentation resulted in a slightly lower pH in the caecal contents of GOS-fed rats, as compared to controls ($P < 0.05$). Total SCFA content was nearly 1.5 times higher in the treated group than in the control group. As regards molar proportions, there appeared to be a reduction in acetic acid in treated rats (47.4% in controls vs. 44.3% in the treated group). However, the average amount of this acid was 46.2 μmol/g in control rats versus 68.5 μmol/g in treated rats, and consequently the proportion of acetic acid in the total amount was altered due to the significant increase in butyric acid (two times higher) in the GOS-treated group; no differences in propionic acid levels were found between both groups of animals.

Mineral absorption

Table 5 shows the results of faecal mineral and urine excretion. Mineral intakes did not significantly differ between control and GOS groups for each mineral (data not shown), but faecal excretions of calcium, magnesium and iron were significantly lower in the group receiving the GOS diet. In contrast, when GOS were included in the diet, urinary mineral excretions were significantly higher for calcium,

Table 5 Mineral elements in faeces (mg/day) and urine (μg/day)

	Week 1		Sign. level	Week 2		Sign. level	Week 3		Sign. level	Week 4		Sign. level
	Control	Treated		Control	Treated		Control	Treated		Control	Treated	
Ca												
Faeces	147.50 ± 2.28	126.85 ± 1.43	***	145.26 ± 3.76	122.31 ± 3.72	***	136.81 ± 1.87	114.03 ± 3.47	***	131.37 ± 1.61	115.38 ± 1.77	***
Urine	2.40 ± 0.79	4.73 ± 0.42	**	2.96 ± 0.39	4.37 ± 0.85	**	3.94 ± 0.26	5.59 ± 0.89	**	3.94 ± 0.37	4.94 ± 0.43	**
Mg												
Faeces	25.45 ± 1.80	19.13 ± 2.87	*	22.22 ± 1.96	18.01 ± 0.65	**	19.21 ± 3.32	14.55 ± 1.47	*	19.16 ± 1.06	15.32 ± 0.53	***
Urine	1. 61 ± 0.41	5.87 ± 0.77	***	2.11 ± 0.40	4.36 ± 1.27	*	4.34 ± 0.01	6.88 ± 1.34	**	5.38 ± 0.39	6.09 ± 0.49	**
Fe												
Faeces	1.49 ± 0.04	1.31 ± 0.10	*	1.51 ± 0.08	1.29 ± 0.10	**	1.23 ± 0.06	1.13 ± 0.07	NS	1.32 ± 0.06	1.07 ± 0.08	**
Urine	1.05 ± 0.25	2.41 ± 0.57	**	7.95 ± 0.005	13.17 ± 6.64	NS	8.45 ± 2.25	16.15 ± 5.24	*	3.01 ± 0.11	7.02 ± 3.46	NS
Cu												
Faeces	0.22 ± 0.02	0.16 ± 0.01	**	0.24 ± 0.01	0.17 ± 0.02	*	0.15 ± 0.01	0.14 ± 0.02	NS	0.18 ± 0.04	0.13 ± 0.01	NS
Urine	0.74 ± 0.35	0.89 ± 0.32	*	2.49 ± 1.82	3.65 ± 0.74	***	1.83 ± 0.18	2.98 ± 1.05	NS	0.62 ± 0.09	0.97 ± 0.36	NS
Zn												
Faeces	1.40 ± 0.02	1.11 ± 0.09	**	1.24 ± 0.22	1.33 ± 0.07	NS	1.19 ± 0.07	1.18 ± 0.09	NS	1.19 ± 0.29	1.12 ± 0.15	NS
Urine	4.50 ± 1.4	7.38 ± 1.17	*	8.15 ± 0.17	9.7 ± 1.66	NS	8.55 ± 0.40	8.75 ± 0.65	NS	4.15 ± 0.06	6.95 ± 1.86	*

Mean values ($n = 10$) ± standard deviation

Analysis of variance: *NS* non significant effect. Values marked with asterisks differ significantly (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

magnesium and iron, but not for the remaining trace elements. Table 6 shows the metabolism of minerals in rats fed control and GOS diets. Apparent absorption of Ca and Mg was significantly higher for rats consuming the GOS-containing diet than for the control group. Apparent balance did not show significant differences between both groups. However, when apparent balance was expressed as a percentage of retention, it was significantly higher—especially for Ca—in GOS-fed rats throughout the assay. Percentage of Mg retention showed moderately significant differences from the third week. Regarding the metabolism of trace elements, mean values for absorption and balance of Fe and Cu were higher in the GOS-treated group than in the control group. However, no significant differences ($P > 0.05$) were observed between the treatments; consequently there was no clear evidence that trace element metabolism was enhanced by the presence of GOS in the diet.

Discussion

Soybean oligosaccharides can be used as a sweetener as they are lower in calories than sucrose. They are relatively stable at a low pH and fairly resistant to high temperatures [14]. These properties make oligosaccharides especially suitable for sweetening products with a low pH such as yoghourt, carbonated beverages, fruit-based and pickled products, etc. Soybean oligosaccharides can also provide potential health-promoting properties to the products to which they are added. Soybean whey, a by-product of the tofu manufacturing industry, still contains appreciable amounts of soybean oligosaccharides, as was shown by the HPLC analyses (Table 2) in three different batches of this by-product; moreover, no significant differences in composition or amounts were found between batches of sample in the LMWC analyses. Although stachyose and raffinose have been reported in raw soybean seeds [14], their concentration could be affected during processing [10]. For this reason, only stachyose and traces of inulin were found in the HPLC analysis of soybean whey. The scant variability found in the HPLC analyses from one batch to another highlighted the standardization of the industrial process. Soybean whey was therefore considered for this study as a source of soybean GOS, and tested in rats.

Both groups of rats received an equivalent caloric content and no differences in body weight gain were found (Table 3). However, the feed intake and consumption of soybean whey during the experiment led to differences in growth rates and feeding efficiency. At the doses used, all the animals remained in good health throughout the experiment and showed no signs of side effects, such as diarrhoea; and the rats behaved normally throughout the study. Regarding the internal organs and caecum, no

Table 6 Mineral metabolism in control and GOS-supplemented rats

Element	Week	Apparent absorption (mg/day)		Sign. level	Mineral balance (mg/day)		Sign. level	Mineral balance (%)		Sign. level
		Control	Treated		Control	Treated		Control	Treated	
Ca	1	24.53 ± 1.17^{a}	28.72 ± 0.80^{a}	**	45.54 ± 1.77^{a}	46.38 ± 1.43^{a}	NS	23.30 ± 0.91^{a}	26.06 ± 0.81^{a}	**
	2	25.68 ± 1.92^{a}	31.28 ± 2.09^{b}	**	47.23 ± 3.60^{a}	51.29 ± 4.17^{b}	NS	24.16 ± 1.84^{a}	28.82 ± 2.34^{b}	*
	3	30.00 ± 0.95^{b}	35.93 ± 1.95^{c}	**	54.69 ± 1.99^{b}	58.35 ± 2.88^{c}	NS	27.98 ± 1.02^{b}	32.78 ± 1.62^{c}	**
	4	32.78 ± 0.82^{c}	35.17 ± 0.99^{c}	**	60.13 ± 1.38^{c}	57.64 ± 1.57^{c}	NS	30.77 ± 0.70^{c}	32.39 ± 0.88^{c}	*
Mg	1	28.13 ± 5.07^{a}	42.78 ± 8.57^{a}	*	8.35 ± 2.03^{a}	8.77 ± 3.79^{a}	NS	23.57 ± 5.74^{a}	26.22 ± 11.35^{a}	NS
	2	37.26 ± 5.54^{b}	46.10 ± 1.95^{a}	*	11.08 ± 2.30^{a}	11.05 ± 1.89^{a}	NS	31.29 ± 6.49^{a}	33.04 ± 5.66^{a}	NS
	3	36.86 ± 4.62^{b}	56.47 ± 4.41^{b}	***	8.71 ± 1.64^{a}	11.99 ± 1.23^{a}	*	24.61 ± 4.62^{a}	35.88 ± 3.68^{a}	*
	4	45.88 ± 2.99^{c}	54.18 ± 1.68^{b}	**	10.86 ± 0.97^{a}	12.02 ± 0.80^{a}	NS	30.69 ± 2.74^{a}	35.94 ± 2.39^{a}	*
Fe	1	15.55 ± 2.18^{a}	15.70 ± 6.88^{a}	NS	0.27 ± 0.038^{a}	0.24 ± 0.11^{a}	NS	15.49 ± 2.17^{a}	15.54 ± 6.91^{a}	NS
	2	14.56 ± 5.50^{a}	17.10 ± 6.74^{a}	NS	0.25 ± 0.097^{a}	0.25 ± 0.10^{a}	NS	14.11 ± 5.50^{a}	16.25 ± 6.41^{a}	NS
	3	28.78 ± 4.94^{b}	27.09 ± 4.50^{b}	NS	0.45 ± 0.15^{b}	0.41 ± 0.07^{b}	NS	25.76 ± 8.32^{b}	26.05 ± 4.72^{b}	NS
	4	25.45 ± 3.49^{b}	30.98 ± 4.87^{b}	NS	0.45 ± 0.062^{b}	0.47 ± 0.08^{b}	NS	25.28 ± 3.50^{b}	30.53 ± 4.96^{b}	NS
Cu	1	30.47 ± 6.76^{a}	44.39 ± 4.81^{ab}	*	0.096 ± 0.02^{a}	0.12 ± 0.01^{ab}	NS	30.14 ± 6.70^{a}	43.83 ± 4.88^{ab}	*
	2	31.25 ± 7.21^{a}	38.98 ± 5.51^{a}	NS	0.10 ± 0.02^{a}	0.10 ± 0.01^{a}	NS	31.00 ± 7.22^{a}	35.81 ± 5.26^{a}	NS
	3	54.14 ± 2.24^{a}	51.07 ± 9.58^{b}	NS	0.16 ± 0.005^{a}	0.14 ± 0.03^{b}	NS	51.50 ± 1.69^{a}	48.56 ± 9.47^{b}	NS
	4	42.66 ± 16.83^{a}	52.48 ± 1.61^{b}	NS	0.13 ± 0.05^{a}	0.15 ± 0.00^{b}	NS	41.72 ± 16.8^{a}	51.30 ± 1.44^{b}	NS
Zn	1	47.91 ± 0.84^{a}	53.55 ± 4.65^{a}	NS	1.28 ± 0.02^{a}	1.27 ± 0.11^{a}	NS	47.74 ± 0.88^{a}	53.24 ± 4.63^{a}	NS
	2	54.03 ± 9.33^{a}	49.12 ± 3.89^{a}	NS	1.44 ± 0.25^{ab}	1.16 ± 0.09^{a}	NS	53.73 ± 9.33^{a}	48.71 ± 3.92^{a}	NS
	3	55.71 ± 2.89^{a}	50.25 ± 4.48^{a}	NS	1.42 ± 0.12^{a}	1.19 ± 0.11^{a}	NS	52.98 ± 4.44^{a}	49.88 ± 4.49^{a}	NS
	4	55.91 ± 12.66^{a}	52.75 ± 7.44^{a}	NS	1.50 ± 0.34^{a}	1.25 ± 0.18^{a}	NS	55.76 ± 12.66^{a}	52.46 ± 7.39^{a}	NS

Mean values ($n = 10$) ± standard deviation

Values in the same column with different letters differ significantly; $P < 0.05$

Analysis of variance: *NS* non significant effect. Values marked with asterisks differ significantly (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

significant differences were found between both groups (data not shown). However, in the treated group the mean weight of liver, kidneys and spleen was slightly lower, while the mean length of the intestine was slightly greater than in the control group. No stones were observed in the kidneys of treated rats. The data provided evidence that the consumption of soybean whey was not harmful to rats, and several benefits were observed.

An intake of 2–10 g/day of indigestible oligosaccharides is currently considered to provide a prebiotic effect in humans [14, 36, 39]. This would imply an average intake of approximately 29–143 mg NDO/day per kg for a person weighing 70 kg. Considering that 100 mL of diluted soybean whey afforded approximately 160 mg of GOS stachyose, the intake of GOS in the treated group was about 120 mg GOS/day per rat.

The prebiotic effect and other health-promoting properties of okara from soybean, a solid by-product composed mainly of dietary fibre, have recently been reported in rats [12, 26]. SCFA are fermentation products of non-digestible carbohydrates that are responsible for the decrease in pH in the caecum. The increase in total SCFA obtained in GOS-fed rats, as a result of an increase in butyric and acetic acid levels in caecal contents, agreed with previous results [37]. Whole protein was probably digested, as only a trace of branched-chain fatty acids was found. The results for caecal fermentation clearly showed an in vivo prebiotic effect of soybean GOS in treated rats.

Although the treated rats drank three times more soybean whey (Table 3), the amount of minerals afforded by the feed (Table 1) was much higher than that afforded by the drink (Table 2), and therefore both groups of rats received an equivalent amount of minerals. The increase in Ca and Mg retention for GOS intake could be due to an increase in the efficiency of caecal absorption [19, 42]. The absorption of Ca and Mg is stimulated by NDO that escape digestion in the small intestine. NDO act as a substrate for SCFA production in the large intestine, leading to a reduction in caecal pH, and increased weight in caecum and caecal digesta. This would produce an increase in the absorption of these minerals as a consequence of increased solubility, hypertrophy of the cells of the mucosa, and an increase in the absorption surface [15, 23, 41].

Several authors reported that indigestible dietary carbohydrates lower luminal intestinal pH and thereby increase the solubility of calcium and magnesium [25, 30, 34]. In this work, caecal pH was also observed to be

slightly lower (Table 4) in GOS-fed rats. Different mechanisms are proposed [3] to explain the favourable effects of fermentable carbohydrates on Mg absorption: a substantial increase in the production of SCFA with the consequent acidification of the luminal content may improve magnesium solubility; and SCFA may also directly contribute to the enhancement of magnesium absorption via a cation exchange mechanism. Marcfalane et al. [22] reported that quantitatively and in significant terms in host physiology, SCFA—particularly acetate, propionate and butyrate—are the main end products of the microbial fermentation process in the large gut. Kashimura and co-workers [13] observed that butyric acid is more effective in Mg absorption than acetic or propionic acid, and Trinidad et al. [38] reported the contribution of acetic acid to the absorption of Ca in the form of calcium acetate. SCFA probably represent an important stimulus for the colonic cell proliferation frequently observed after feeding fibre [20]; it is conceivable that this trophic effect might result in a more effective absorption of Ca.

Calcium may interact with Mg, leading to decreased Mg absorption [11]. Accordingly, Younes et al. [41] detected that a high dietary Ca supply consistently led to an inhibition of Mg absorption in the caecum, and to a reduction in Mg digestibility. Coudray et al. [4] reported that soluble caecal Mg levels were inversely related to Ca intake. In the present work, the high levels of calcium intake might explain the major significant difference found in the absorption and retention of calcium in relation to the magnesium balance in GOS-fed animals.

Coudray et al. [3] reported that the endogenous faecal excretion of Mg is directly proportional to Mg intake. A high Mg content in the diet might contribute to an endogenous loss of Mg.

In summary, soybean whey, a by-product of tofu manufacturing, has been used as a source of non-digestible GOS in rats. GOS from soybean whey exhibited a prebiotic effect in vivo and an improved mineral balance. The composition and properties of soybean whey could make it a valuable waste product, with useful applications in the pharmaceutical and food industry.

Acknowledgments The Spanish Ministry of Science and Innovation is acknowledged for financial support through research projects AGL2005-02447 ALI and AGL2008-0998 ALI. Thanks are given to Mr. M. Takazumi from Toofu-Ya S.L. for the soybean whey provided and also to the Danone Institute for the scholarship granted to one of the authors (I. E.-M.).

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