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Inulin of *Vernonia herbacea* from the Brazilian Cerrado Changes Colon Morphology and Lipid Metabolism in Swiss Mice

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ABSTRACT

The present work describes the influence of inulin obtained from reserve organs of *Vernonia herbacea* on colon morphology and lipid metabolism in Swiss mice. Animals were fed for five weeks either with Nuvilab CR-1 (control diet) or with Nuvilab CR-1 supplemented with 10% *V. herbacea* inulin or with 0.5% cholesterol or 0.5% cholesterol + 10% inulin. Distal colon sections were prepared and stained for morphometrical analysis and histochemical characterisation. Enzymatic assays were used to quantify plasma lipids and lipoproteins. The VLDL fraction and triglycerides were estimated by the Friedwald method. The intake of *V. herbacea* inulin-supplemented diet increased neutral mucins, stimulated sulphomucin production and decreased sialomucin secretion, compared to the control group. It also improved crypt depth and number of goblet cells ($p < 0.05$ vs. control). HDL-cholesterol concentrations tended to be higher with inulin, as was the ratio of HDL to LDL. *V. herbacea* inulin has beneficial effects on mouse colon morphology and lipid metabolism.

Keywords: Asteraceae, cerrado, inulin, lipoproteins, mucins, *Vernonia herbacea*

INTRODUCTION

After starch and sucrose, fructans are the storage carbohydrates with the highest occurrence in plants, being present in approximately 15% of angiosperms, especially in the more derived members of Asterales and Poales (Hendry 1993). Fructans can also be found in several economically important plant species, including cereals (wheat, barley), ornamentals (dahlias, tulips), vegetables (onion, garlic, lettuce) and forage grasses (*Lolium* and *Festuca*) (Carvalho *et al*. 2007).

The use of fructans, especially inulin, in the food industry has increased in the last decade due to its specific functional properties, as it has a mild taste and is easily soluble under heat (Van Loo *et al.* 1995; Kaur and Gupta 2002). The administration of inulin as a prebiotic has been associated with a number of physiologically and biochemical effects, resulting in better health and decreasing the risk of many diseases (Cani *et al.* 2009). A regular intake of low amounts of inulin and oligofructose has bifidogenic and anti-tumour effects, reduce the production of potentially toxic metabolites and may induce important immune-mediated effects (Roberfroid *et al.* 1998; Meyer and Stasse-Wolthuis 2009). Other studies connect a diet with inulin to improved function of the nervous system (Cani *et al.* 2004; Messaoudi *et al.* 2005) and to reduced chronic inflammatory bowel disease (Leenen and Dieleman 2007). Some studies also showed that inulin increases mineral absorption, especially of calcium, decreasing the risk of osteoporosis (Delzenne *et al.* 1995; Abrams *et al.* 2007), and that it is effective at decreasing blood levels of urea and uric acid, maintaining the nitrogen balance in the organism. Almost all of these effects seem to be related to its prebiotic action (Hesta *et al.* 2001). Commercial inulin has been used in kidney function tests, for it is neither toxic nor metabolised when injected in the bloodstream and is therefore considered suitable to determine the rhythm of glomerular filtration (Dias-Tagliacozzo *et al.* 1996).

The addition of inulin in the hypercholesterolemic diet of rats significantly reduced the blood and liver levels of triglycerides and decreased the plasmatic concentration of total cholesterol in these animals (Kaur and Gupta 2002). In hamsters, inulin reduced triglycerides, total cholesterol and VLDL-cholesterol (Trautwein *et al.* 1998). Other studies showed a significant increase in HDL-cholesterol and a tendency to decrease the plasmatic concentration of LDL-cholesterol, resulting in an increased HDL/LDL ratio for animals treated with a diet containing 5% inulin (Kim and Shin 1998). These effects are attributed to changes in absorption and/or in synthesis of cholesterol, mainly induced by the prebiotic effects of inulin (Rozan *et al*. 2008).

Beneficial changes in the composition and architecture of the colonic epithelium in rats have been ascribed to the addition of inulin in their diet (Kleessen *et al.* 2003). Inulin resists small intestinal digestion but is metabolized by bacteria in the large bowel, resulting in an increase in microbial mass and production of short chain fatty acids (SCFAs) (Roberfroid 1998). Changes in large bowel secretion of mucin were also reported after ingestion of inulin and could play a role in the maintenance of the gut's health (Ito *et al.* 2008).

The main commercial sources of inulin are roots of Asteraceae species such as *Dahlia* sp. and *Cichorium intybus* and tubers of *Helianthus tuberosus* (Van Loo *et al.* 1995). In Brazil, a number of native Asteraceae species from the cerrado, including *Vernonia herbacea*, store high amounts of fructans of the inulin-type in their underground tissues (Carvalho and Dietrich 1993; Carvalho *et al.* 2007). Dias-Tagliacozzo *et al.* (1996) reported the use of *V. herbacea* inulin in determining the rhythm of glomerular filtration, as a substitute of the commercial inulin. *V. herbacea* inulin was also used as an alternative source of fructose-rich syrups, a sweetener that has been indicated for diabetic patients (Pessoni *et al*. 2004).

In the present work we evaluate the potential of inulin extracted from rhizophores of *V. herbacea* in affecting colon morphology, mucin composition and lipid metabolism in Swiss mice. This native species of the cerrado ac-

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cumulates more than 80% of indigestible inulin in the rhizophores and might be considered a new source of health-promoting ingredients.

MATERIALS AND METHODS

Extraction of inulin

Fresh rhizophore samples were obtained from plants of *Vernonia herbacea* (Vell.) Rusby (Asteraceae) harvested in a cerrado area at the Biological Reserve and Experimental Station of Moji-Guaçu (SP, Brazil). These samples were fragmented and immediately immersed in 80% ethanol, homogenised in a blender for 5 min and filtered under vacuum. The residue was extracted in water following Carvalho and Dietrich (1993) and constituted the crude inulin fraction.

Diet preparation and animal experimentation

The basal diet Nuvilab CR-1 (Nuvital Nutrientes S.A., Brazil) contained the following ingredients: crude protein (22%, consisting of equivalent mix of soybean), total carbohydrates (8%, obtained from maize and wheat) and non-digestible carbohydrates, 4.5% crude fat, 10% mineral mixture (1.4% calcium and 0.8% phosphorus), 12.5% moisture and vitamins (U/kg of A 12,000.00 UI; D3 1,800.00 UI, E 30.00 mg, K3 3.00 mg, B1 5.00 mg, B2 6.00 mg, B6 7.00 mg, B12 20.00 µg, nyacin 60.00 mg, pantotenic acid 20.00 mg, folic acid 1.00 mg, biotin 0.05 mg and choline 600.00 mg). These ingredients defined the final composition of the basal diet and were fed to the control group. For the inulin treatments, mice were provided with basal diet supplemented with 10% *V. herbacea* inulin or with 0.5% cholesterol or 0.5% cholesterol and 10% *V. herbacea* inulin, constituting four experimental groups. All supplementations were at the expense of commercial Sol wheat flour (J. Macedo S.A.). For diet preparation, Nuvilab CR-1 pellets were ground and homogenised with the other components to give a paste consistency. Samples were dried in an oven at 70°C as recommended by Vanhoof and De Schrijver (1995) for approximately 14 h. The inulin concentration in the diets was based on Messaoud *et al.* (2005).

The experiment was performed under the approval (n. 195/05 of 14/04/2005) of the Ethics in Research Committee of the Universidade Metodista de São Paulo – CEP-UMESP. Twenty-four 3-month-old male Swiss mice from the Bioterium of the Nucleus of Biological Research of the Universidade Metodista de São Paulo were used, their initial body weight being 35 ± 4 g. The animals were randomly divided into four groups (n = 6/group), and after an acclimation period of 7 days, they were fed the diets already described for 6 weeks.

During the experimental period, the animals were kept under standardised bioterium conditions, at 22 ± 2°C, 55% (± 10%) relative air humidity and a 12-h light-dark cycle, with free access to water and food. The food and water intake and body weight of the animals were checked daily to evaluate possible changes due to the diet of the group (Kleessen *et al.* 2003). Clinical signs were monitored regarding the following parameters: urinary frequency, defecation, piloerection, trembling, reflex loss, lachrymation and salivation, as suggested by Carlini (1972).

Blood and tissue samples

At the end of the experimental period, the animals were anesthetized with diethyl ether and killed by decapitation. Blood samples were collected in conical tubes heparinisated with EDTA. The plasma was immediately separated by centrifugation at 3000 rpm for 10 min and stored at -80°C for future analysis of lipids and plasmatic lipoproteins. The distal colon was aseptically removed from the abdominal cavity and separated from fats and mesentery; cuts were taken at 30 mm above the rectum, so that a final sample of approximately 4 cm was obtained. The extremities of the organ were immobilised with pins in a Petri dish previously prepared with paraffin and Bouin's fixing solution in a layer approximately 0.5 cm thick. They were then sectioned longitudinally and processed for histological and histochemical analyses.

Histochemical analysis

Colon tissue samples were fixed in Bouin's solution for 24 h at room temperature. Afterwards, they were dehydrated with decreasing concentrations of ethanol, diaphanised in 3 washes of xylene and embedded in historesin Histosec® (Merck). The colon tissue blocks of each animal were sectioned longitudinally, 5-7 µm thick, by an American Optical Microtome (Model 820, USA) and subjected to the following staining procedures: haematoxylin/eosin/floxin, for the general description of the colon tissues (Drury and Wallington 1980); periodic acid-Schiff (PAS) to identify the presence of neutral mucins; 1% Alcian Blue (AB), pH 2.5, to locate acidic carboxylated mucins (sialomucins); 1% AB, pH 1.0, for selective characterisation of strongly sulphated mucins (sulphomucins) (Romeis 1989). Semi-quantitative histochemical analyses of the mucins secreted by the goblet cells were performed in the slides stained with PAS, 1% AB pH 2.5 and 1% AB pH 1.0. The data were expressed according to staining intensities: 0 – no reaction; 1 – weak intensity; 2 – moderate intensity; 3 – strong intensity; 4 – very strong intensity.

Morphometric analysis of the colonic mucosa was carried out according to Kleessen *et al.* (2003). The morphology of the crypts was assessed in 10 longitudinally-oriented crypts by a light microscope (Nikon–Eclipse E 200, equipped with a camera Motican) in sections stained with haematoxylin/eosin, with the following criteria analysed: (a) crypt depth (µm, measured from the bottom of the crypt to the crypt entrance) and (b) number of goblet cells per crypt (counted in both sides of the crypt). The image analysis system was AV Soft Bio View-4.

Lipid and plasma lipoprotein analyses

Quantification of lipids and plasma lipoproteins was performed by enzymatic kits specific for total cholesterol (Biosystems), HDL-cholesterol (Doles Reagents) and LDL-cholesterol (Wiener lab) assays. The HDL/LDL ratio was calculated, and the VLDL-cholesterol and triglyceride fractions were determined using the method of Friedewald *et al.* (1972).

Quantitative and qualitative analyses of soluble carbohydrates

Crude *V. herbacea* inulin and the diets supplemented with 10% *V. herbacea* inulin or 0.5% cholesterol and 10% *V. herbacea* inulin were analysed for total fructose by the anthrone modified reaction (Jermyn 1956) using fructose as standard. Polydispersity in the chain length of the inulin-type fructan was evaluated qualitatively. Samples of all preparations were deionised in ion exchange columns (10 × 1 cm) containing cationic (Dowex 50X80) and anionic (Dowex 1X8) resins, according to Carvalho and Dietrich (1993). Each sample (400 µg fructose equivalent/ml) was filtered in 0.45 µm filters (Spartan-3, Aldrich) and then analysed by high performance anion-exchange chromatography and pulsed amperometric detection (HPAEC/PAD) in a Dionex model DX-300 system, using a CarboPac PA1 column (4 × 250 mm). The gradient was established according to Shiomi (1993). Eluent A (150 mM NaOH) and eluent B (500 mM sodium acetate in 150 mM NaOH) were mixed as follows: 0-1min, 25 mM; 1-2 min, 25-50 mM; 2-14 min, 50 -500 mM; 14-22 min, 500 mM; 22-30 min, 25 mM. The flow rate through the column was 1 cm^3 min^{-1}. The applied PAD potentials for E1 (300 ms), E2 (120 ms) and E3 (300 ms) were 0.05, 0.60 and -0.60 V, respectively, and the output range was 1 nA.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA), and the means were compared using Tukey's test. The alpha level was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Qualitative analysis of inulin extracted from *Vernonia herbacea* before and after manipulation during diet preparation

Chromatographic profiles of samples containing inulin-supplemented diets showed the presence of fructo-oligosaccharides (FOS) and fructo-polysaccharides characteristic of inulin chains. Comparing the profile of crude inulin extracted from rhizophores of *V. herbacea* with those shown in the diet samples, it was possible to demonstrate that exposure to drying temperatures (70°C) during diet preparation did not drastically affect inulin composition, but produced a higher proportion of fructo-oligosaccharides (**Fig. 1**). Similar procedures to those used by Vanhoof and De Schrijver (1995) for diet preparation also did not produce significant changes in the polysaccharide chains regarding the use of drying temperature.

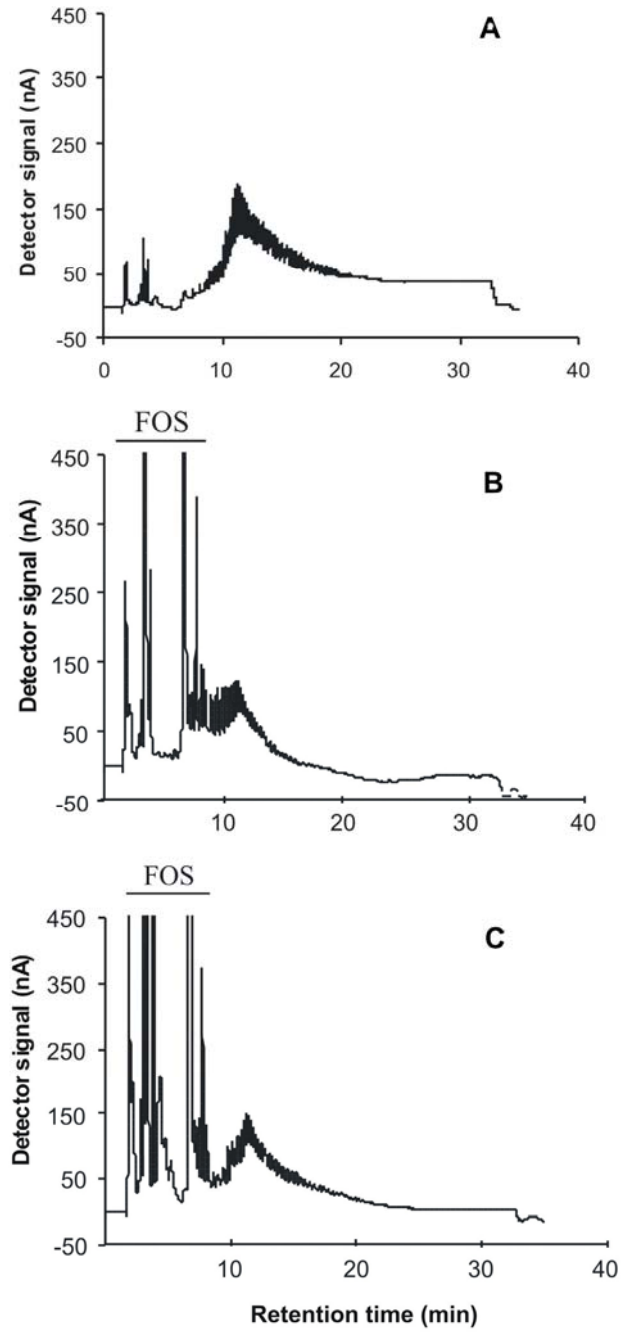

Fig. 1 HPAEC/PAD analysis of inulin present in different diets. Pure inulin from *V. herbacea* (**A**); 10% inulin-supplemented diet (**B**); 0.5% cholesterol +10% inulin-supplemented diet (**C**). FOS = fructo-oligosaccharides.

The physiological effects of inulin obtained from tuberous roots of *Cichorium intybus* processed and commercialised by Orafti (Raftiline) from Belgium have been reported by Trautwein *et al*. (1998) and Kleessen *et al.* (2003). This polysaccharide contained linear chains of up to 60 fructose residues β-2,1 linked (Niness 1999), similar to the inulin from *Vernonia herbacea* rhizophores, which polymerises up to 40 fructose units formed predominantly by linear chains and β-2,1 bonds (Carvalho and Dietrich 1993; Carvalho *et al.* 2007). The biochemical similarities between both polymers reinforce the concept of *V. herbacea* as a new source of inulin for commercialisation and physiological application.

Food and water intake and body weight variation

The animals were maintained in good health during the whole experimental period. There were no significant differences in food and water intake among the experimental groups. No differences were detected in body-weight gain. Furthermore, feeding animals with *V. herbacea* inulin did not result in any alterations of the normal behaviour of the animals. However, dietary 10% inulin and oligofructose from other plant sources has been reported to have an impact on gastrointestinal peptides in rats, increasing the satiety of the animals (Fiordaliso *et al.* 1995; Druce *et al.* 2004), through the reduction of food intake. Messaoudi *et al.* (2005) also reported a relationship between a commercial inulin-supplemented diet and good cognitive performance in rats.

The results obtained in the present work suggest that *V. herbacea* inulin does not produce toxic effects in mice since no alteration was detected in clinical signs such as urinary frequency, defecation, piloerection, trembling, reflex loss, lachrymation and salivation (data not shown). However, specific toxic effects of the polysaccharide and its relation to behavioural and cognitive aspects need investigation.

Morpho-physiological changes in the colon

Mucin is a key component of the gut barrier that prevents potential pathogens from gaining access to the underlying epithelium (Ito *et al*. 2008). Data on the histochemical characterisation and distribution of mucins secreted by goblet cells in mice colon are expressed in **Table 1**.

Analysis of the mucins stained with PAS revealed that goblet cells present in the peripheral area of the crypts show much stronger staining in the 10% inulin group than in other groups. Because the PAS reaction identifies the presence of neutral mucins (Romeis 1989), this result suggests that feeding a 10% inulin-supplemented diet increased the amount of neutral mucins secreted by the goblet cells of the distal colon. In rats, neutral mucins are predominantly found in the jejunum; however, animals fed commercial inulin presented an increase in neutral mucin in the distal colon and altered proportions of acidic mucins (sialomucins and sulphomucins) by stimulating sulphomucin production (Kleessen *et al.* 2003). Data presented in **Table 1** also show that in the group fed 0.5% cholesterol, the colour in the base of the crypts was less intense than in the other experimental groups.

Goblet cells present in the peripheral and basal regions of the crypts were much more strongly stained with AB pH 1.0 in the 10% inulin group than those of the other groups (**Table 1**). The 0.5% cholesterol + 10% inulin group showed a moderate to strong colour intensity, being more reactive than the control and the 0.5% cholesterol groups. The 0.5% cholesterol group showed the least intense reaction. Data obtained from the AB pH 1.0 staining showed that the sulphomucin secretion by basal and peripheral cells of the crypts was greater when inulin was supplemented in the diet.

AB pH 2.5 staining revealed that goblet cells from the periphery and basal regions of the crypts showed a less intense reaction in the 10% inulin group than in the control and 0.5% cholesterol groups. The 0.5% cholesterol + 10%

Table 1 Effects of diet composition on mucin secretion by goblet cells of the distal colon of Swiss mice fed dietary *V. herbacea* inulin.

Dietary Group	Neutral Mucin (PAS)[1]		Sulphomucin (AB[2] 1% pH 1.0)		Sialomucin (AB[2] 1% pH 2.5)	
	Base	Periphery	Base	Periphery	Base	Periphery
Basal diet	(1-2)	(3-4)	2	(2-3)	(2-3)	(1-2)
10% Inulin	(1-2)	4	3	3	(1-2)	(1-2)
0.5% Chol[3]	1	(3-4)	(1-2)	(2-3)	(2-3)	2
10% Inulin + 0.5% Chol[3]	(1-2)	(3-4)	(2-3)	(2-3)	(2-3)	1

[1]PAS = Periodic Acid-Schiff; [2]AB = Alcian Blue. [3]Chol = Cholesterol (Sigma). Results expressed according to intensity of reaction: 0 – no reaction; 1 – weak intensity; 2 – moderate intensity; 3 – strong intensity; 4 – very strong intensity. (n = 6)

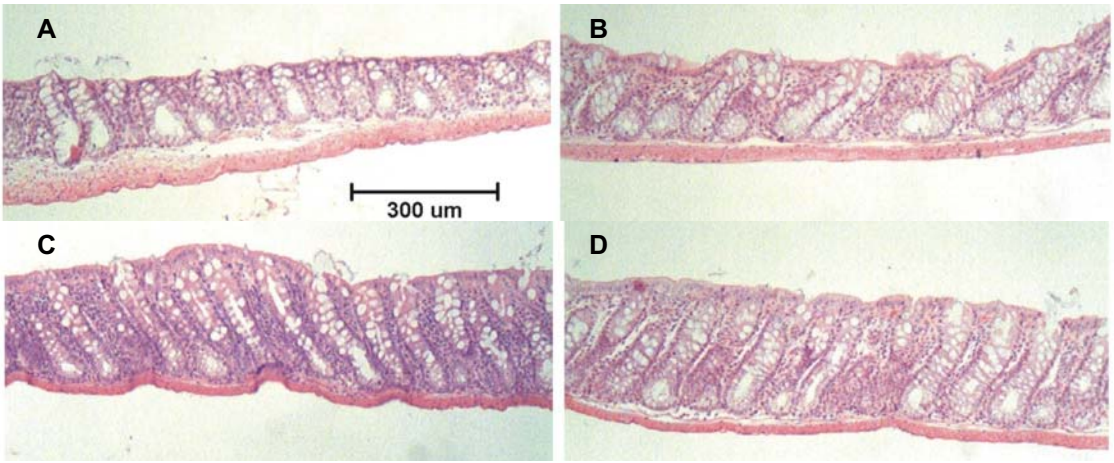

Fig. 2 Longitudinal sections of the distal colon of mice fed several diets. (**A**) Basal diet, (**B**) 0.5% cholesterol, (**C**) 10% inulin and (**D**) 0.5% cholesterol + 10% inulin, displaying differences in the crypt depth. Haematoxylin/eosin/floxin staining. Magnified 100X.

inulin group demonstrated a weaker reaction with AB pH 2.5 staining than the other groups, while the group fed 0.5% cholesterol showed greater colour intensity in the periphery of the crypts. These results indicate that secretion of sialomucin was lower in animals fed 10% inulin.

Like other factors that have activity in the intestinal flora, inulin can affect the dynamics of goblet cells, reflected in the pattern of mucin secretion throughout the gut (Deplancke and Gaskins 2001; Hedemann *et al.* 2009). Goblet cells predominantly secrete sulphomucins (Shah and Shrikhande 1989), which are considered a protective factor in normal colonic mucosa, related to colon cancer prevention (Jenab *et al.* 2001). Increases in sialomucin secretion and decreases in sulphomucin are indicative of the presence of neoplasic cells (Shah and Shrikhande 1989). Data obtained in this work indicated that intake of polydispersed chains of inulin from *V. herbacea* results in benefits for colon physiology of Swiss mice due to increased production of sulphomucins and neutral mucins, and to reduced sialomucin secretion. Recently, Ito *et al.* (2008) reported greater amounts of mucin in rats fed different degree of polymerization (DP 4 – 23) fructans than in those fed the control diet. The authors also showed that the fermentation capacity of fructose polymers decrease with the increasing DP of the fructan ingested. Therefore, it is reasonable to expect that the increases in the secretion of mucin in Swiss mice fed high DP inulin from *V. herbacea* might be related to changes in the distal colon, leading to benefits to the animals. However, differences in the intestinal microflora of different animal systems must be considered for a better understanding of the relationship between mucin production and the type of fructans ingested (Ito *et al.* 2008).

Crypt morphology

The group that received the 10% inulin-supplemented diet showed crypt depths significantly higher ($P < 0.05$) than the control and the 0.5% cholesterol groups (**Table 2**). The 0.5% cholesterol +10% inulin group tended to have crypts deeper than the 0.5% cholesterol group, indicating that when inulin is added to a cholesterol-containing diet, crypt depth increases. Morphological aspects of the distal colon of animals fed 10% inulin and 0.5% cholesterol are shown in **Fig. 2**, confirming that an inulin-supplemented diet increased crypt depth. Therefore, changes found in the distal colon, similar to other animal systems (Kleessen *et al.* 2003), might be stimulated by dietary intake of fructans.

Table 2 Crypt depth and number of goblet cells per crypt in the distal colon of Swiss mice fed dietary *V. herbacea* inulin.

Dietary Group	Crypt Depth (µm)	Goblet Cells (Number/Crypt)
Basal diet	187.20 ± 48.58 a	12.16 ± 1.96 a
10% Inulin	250.45 ± 38.79 b	17.13 ± 2.94 b
0.5% Chol	209.65 ± 50.72 a	12.88 ± 4.14 ab
10% Inulin + 0.5% Chol	234.44 ± 56.74 ab	13.29 ± 2.50 ab

Data presented as the mean ± standard deviation, n = 6. Letters indicate groups with significant differences ($P < 0.05$). Chol = Cholesterol (Sigma).

As shown in **Table 2**, the 10% inulin group presented more goblet cells per crypt ($P < 0.05$) than the control and the 0.5% cholesterol groups. Although animals that received the 0.5% cholesterol + 10% inulin diet also had a higher average number of these cells than the control and the 0.5% cholesterol groups, no significant differences were observed between them ($P > 0.05$).

The increase in the number of goblet cells in the mice distal colon was also reported by Klessen *et al.* (2003) when commercial inulin was supplied. This increase was due to the production of SCFAs by bifidobacteria, associated with the inulin-supplemented diet. These SCFAs are used by colonic cells – including the goblet cells – as a source of energy and stimulus for cellular differentiation (Mahida 2004) to protect against colon cancer and contribute to increase the elasticity of the intestinal wall, improving intestinal traffic (Niba and Niba 2003). Our results suggest that inulin from *V. herbacea* might act in the same way; however, for a thorough understanding of the mechanism by which this natural product improves gastrointestinal metabolism in Swiss mice further investigation is required. According to Delzenne *et al.* (2005) and Cani *et al.* (2007) and based on animal models, SCFAs create a gut-liver axis

Table 3 Plasmatic concentrations of triglycerides, cholesterol and lipoproteins (mmol L^{-1}) in animals fed dietary *V. herbacea* inulin.

Dietary Group	Triglycerides	VLDL	HDL-LDL	HDL	LDL	Cholesterol
Basal diet	1.49 ± 0.81 a	0.30 ± 0.16 a	0.80 ± 0.40	0.40 ± 0.07	0.64 ± 0.34	1.47 ± 0.47
10% Inulin	2.76 ± 1.17 ab	0.55 ± 0.23 ab	1.60 ± 1.18	0.46 ± 0.08	0.41 ± 0.23	1.48 ± 0.37
0.5% Chol	4.21 ± 1.33 b	0.84 ± 0.27 b	0.58 ± 0.34	0.28 ± 0.16	0.49 ± 0.16	1.54 ± 0.53
0.5% Chol +10% Inulin	3.48 ± 1.26 ab	0.70 ± 0.25 ab	0.56 ± 0.17	0.35 ± 0.09	0.65 ± 0.16	1.70 ± 0.42

Data presented as the mean ± standard deviation, $n = 6$. Letters indicate groups with significant differences ($P < 0.05$), Chol = Cholesterol (Sigma).

that causes decreases in lipogenic gene expression, hepatic lipogenesis and hepatic and seric triglycerides. These findings can explain why inulin and oligofructose can have beneficial impacts on dyslipidaemia. Fermentation of fructans in the colon promotes incretin production such as glucagon-like peptide 1, which is involved in regulating insulin production in rats (Cani *et al.* 2005). Short-chain fructans from *Agave* species also promote production of satietogenic/incretin peptides in the lower part of the mouse gut (Urias-Silvas *et al.* 2008).

Lipids and plasma lipoproteins

The 0.5% cholesterol group showed significant increases in triglycerides and VLDL-cholesterol concentrations ($P < 0.05$) compared with the control group (**Table 3**), as expected when cholesterol is added to the diet (Trautwein *et al.* 1998). Although our experiments showed no significant differences in plasmatic VLDL-cholesterol and triglyceride levels, the 0.5% cholesterol + 10% inulin group showed a tendency of decreases of these levels compared to the 0.5% cholesterol group. Rozan *et al.* (2008) found in rats significant reduction of cholesterol and triacylglycerol due to a diet composed of oligofructose-enriched inulin. The authors suggested that the high propionate concentrations in the portal vein could explain the lipid-modulating actions of inulin and oligofructose since propionate is well known to be involved in lipid metabolism.

In our analysis of the HDL-c/LDL-c ratio (**Table 3**), there was a marginal difference ($P = 0.07$), with the 10% inulin group showing the highest values. A significant increase of the HDL-c/LDL-c ratio was also induced by diets containing aqueous extract of roots of *Cichorium intybus* (Kim and Shin 1998). This increase might be due mainly to increased plasmatic concentrations of HDL-c. Vanhoof and De Schrijver (1995) also reported an increased HDL-c/LDL-c ratio in mice fed a diet containing commercial inulin. However, a similar increase was not observed when 6% inulin was supplemented to the diet containing 1% cholesterol (Vanhoof and De Schrijver 1995).

The 10% inulin group tended to have slightly higher levels of HDL-c; however, statistical analysis did not indicate significant differences among the studied groups (**Table 3**), which is consistent with results described by Trawtwein *et al.* (1998). Similarly, no differences in total cholesterol were observed in our experiments. This result may be explained by the Friedewald principle (Friedewald *et al.* 1972) by which, depending on the way the plasmatic concentrations of lipoproteins vary, the total cholesterol is not affected. In spite of this, the pattern of changes observed in this work due to the intake of inulin from *V. herbacea* indicates that this natural product shows beneficial effects on mice. As known, high values of the HDL-c/LDL-c ratio are related to longevity and inverse correlations between plasmatic concentration of HDL-c and risk of cardiovascular diseases are frequently reported. In addition, high levels of triglycerides and VLDL-c are usually associated with the development of premature atherosclerosis (Kaur and Gupta 2002).

The effects of *V. herbacea* inulin on colon morphology and lipid metabolism open a new alternative for the high DP fructans as health-promoting food ingredients. However, other studies are needed for the use of this inulin from a species native of the Brazilian cerrado as an alternative to commercial species in a global perspective. Furthermore, a long-term experiment is also needed for further understanding of the potential health benefits of *V. herbacea* inulin.

CONCLUSIONS

Inulin extracted from *Vernonia herbacea* modifies the colonic morpho-physiology of Swiss mice, increasing the amount of neutral mucins and sulphomucin and decreasing sialomucin secreted by goblet cells of the distal colon. When inulin is supplemented to the cholesterol-containing diet, the depth of crypts and the number of goblet cells per crypt of the distal colon increase. In addition, inulin from *V. herbacea* modifies lipid metabolism in Swiss mice and shows a pattern of increasing HDL-cholesterols and the HDL/LDL ratio. Supplementation of inulin to a diet that does not contain cholesterol showed a tendency to reduce VLDL-cholesterol and triglyceride values. This natural product did not present toxic effects neither promoted behavioural changes in the animals.

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