Eur J Nutr (2006) 45:418–425
DOI 10.1007/s00394-006-0610-2

ORIGINAL CONTRIBUTION

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Short-chain fructooligosaccharides, in spite of being fermented in the upper part of the large intestine, have anti-inflammatory activity in the TNBS model of colitis

Received: 31 January 2006
Accepted: 26 June 2006
Published online: 26 July 2006

Abbreviations: DSS: dextran sodium sulfate, FOS: Fructooligosaccharides, GF: β-D-fructofuranosyl-(2,1)-α-D-gluco-pyranose, GF_2: Kestose (β-D-fructofuranosyl-(2,1)-β-D-fructofuranosyl-(2,1)-α-D-gluco-pyranose), GF_3: Nystose (β-D-fructofuranosyl-(2,1)-β-D-fructofuranosyl-(2,1)-β-D-fructofuranosyl-(2,1)-α-D-glucopyranose), GF_4: Fructosyl-Nystose (β-D-fructofuranosyl-(2,1)-β-D-fructofuranosyl-(2,1)-β-D-fructofuranosyl-(2,1)-β-D-fructo-furanosyl-(2,1)-α-D-glucopyranose), IBD: inflammatory bowel disease, LTB4: leukotriene B4., MPO: myeloperoxidase, SCFA: short-chain fatty acids, SC-FOS: short-chain fructooligosaccharides, SEM: standard error medium, TNBS: trinitrobenzenesulfonic acid, TNF-α: tumor necosis factor-α

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EJN 610

Abstract Previous studies have demonstrated the anti-inflammatory effect of fructooligosaccharides (FOS) on intestinal inflammation. The aim of the present study was to elucidate whether the colonic fermentation of these carbohydrates is a prerequisite for this anti-inflammatory activity.

With this aim short chain-FOS (SC-FOS) were used for an in vitro fermentation to elucidate the time of fermentation of these compounds. For the in vivo experiments female Wistar rats were fed several diets with different sources of fibre (5 g/kg): cellulose for control rats (n = 30) or SC-FOS (n = 20) with a high content of kestose (GF_2) for the SC-FOS group. After one month of feeding the different diets 10 rats from each group were sacrificed to analyze cecal and colonic microflora, SCFA production and pH of intestinal contents. A distal colonic inflammation was induced to other 10 rats from each group by the administration of 10 mg of TNBS dissolved in 0.25 ml of 50% ethanol (v/v). The rest of the rats from the control group (n = 10) were rendered healthy. One week after TNBS treatment rats were sacrificed and several inflammatory parameters as well as intestinal microbiota and SCFA contents were analyzed.

In vitro fermentation experiments showed that SC-FOS are fermented during the first 12 h after incorporating the oligosaccharides to intestinal contents, thus suggesting a preferential fermentation of these carbohydrates in the ileum and cecum. In fact, SC-FOS increased cecal lactobacilli and bifidobacteria counts as well as SCFA production in healthy rats. In colitic rats, SC-FOS feeding caused a decrease of MPO activity, leukotriene B4 (LTB4) production and iNOS expression. This anti-inflammatory effect was evidenced macroscopically by a significant reduction in the extent of colonic damage. SC-FOS also promoted a more favorable intestinal microbiota, increasing lactobacilli and bifidobacteria counts.

In conclusion, although oligosaccharides are preferentially fermented in the upper parts of the large intestine, its prebiotic effect is extended to the distal colonic segments, thus exerting a positive effect on colonic inflammation.

Key words
fructooligosaccharides – intestinal inflammation – rat – intestinal microbiota

This work has been supported by Puleva Biotech own founds and by Instituto de Salud Carlos III (PI0121732) with funds from the European Union and Junta de Andalucía (CTS 164).

Introduction

Although the exact pathogenesis of inflammatory bowel disease (IBD) is poorly understood, there is increasing evidence that intestinal microflora play a key role. In fact, the level of antibodies against intestinal bacteria is elevated in patients with IBD [1]. Animal studies also demonstrate that anti-microbial agents specifically active against obligate anaerobes prevent ulceration in guinea pigs [2] and that germ-free animals only develop colitis when repopulated with bacteria [3].

Given the implication of commensal bacteria in IBD, the manipulation of the intestinal flora with pre- and pro-biotics has gained interest in the prevention and treatment of this disorder. This beneficial effect has been demonstrated in different animal models of colitis. For example, the administration of *Lactobacillus salivarius* prevented colonic inflammation in a TNBS model of rat colitis [4]. In addition, during the last few years there have been an increasing number of clinical trials demonstrating the beneficial effect of such bacteria in human intestinal inflammation. Recently, the administration of a probiotic preparation, containing bifidobacteria, lactobacilli and a strain of *Streptococcus thermophilus*, in patients with mild to moderate ulcerative colitis, resulted in a combined induction of remission/response rate of 77% [5].

On the other side, the administration of prebiotics, defined as compounds that selectively stimulate the growth of lactobacilli and bifidobacteria already resident in the colon, is also a way to modulate intestinal microflora, which suggest their possible use in the treatment or prevention of intestinal inflammation. In this sense, there are different studies focused on the role of dietary fibre in this inflammatory process. Dietary inulin has been shown to improve distal colitis induced by dextran sodium sulfate (DSS) in the rat [6]. Lactulose have also shown to ameliorate TNBS-induced colitis and it has been suggested that the background of this protective effect may be due to alteration of colonic microflora [7]. In the same way, fructooligosaccharides (FOS), another sort of dietary fibre, have been tested in the TNBS model of rat colitis [8] and in a small open label clinical trial [9] with promising results. In addition, short-chain FOS (SC-FOS) has shown to be preferentially fermented by probiotic bacteria [10]. Thus, the prebiotic characteristics of these oligosaccharides could be of special interest.

Most of the studies reporting the role of dietary fibre in IBD have focused on compounds that reach almost intact the distal colon, where the lesion is normally more evident [11]. In the distal colon, these compounds are fermented by commensal bacteria leading to the production of short chain fatty acids (SCFA) and to the promotion of a beneficial microflora, both contributing to improve colonic lesions. However, whether the distal fermentation is a prerequisite for the beneficial effect of dietary fibre in the intestinal inflammation remains uncertain. In fact, there is some evidence of ileal fermentation of FOS [12] and, as mentioned above, these compounds can have a beneficial effect on distal colonic lesions.

The aim of this work was to evaluate the anti-inflammatory activity of SC-FOS with a high percentage of the compound kestose (GF_2), which could be fermented by commensal bacteria in the upper part of the large intestine. We have evaluated the in vitro fermentation of these compounds and their effect in the TNBS model of distal colonic inflammation.

Material and methods

Reagents

All chemicals were obtained from Sigma Chemical (Madrid, Spain), unless otherwise stated. Glutathione reductase was provided by Boehringer Mannheim (Barcelona, Spain). SC-FOS were obtained by Azucarera Ebro (Madrid, Spain) following a previously described method [13] and the final composition was as follows: 0.85% glucose, 0.6% fructose, 3.8% GF, 50.0% GF_2, 35.0% GF_3, 10.00% GF_4. The GF_2/FOS ratio was 52.6.

In vitro fermentation of SC-FOS

Fresh feces was collected from three healthy female Wistar rats weighed 180–200 g that had consumed a standard diet during their stay in the animal facilities. The feces was homogenized in buffered peptone water (100 mg/ml) and the homogenates thus obtained were incubated in the presence or absence of SC-FOS (10 mg/ml). The incubation was carried out at 37°C with continual shaking in anaerobic conditions during 0, 6, 12, 24 and 48 h. Separate tubes were used for the different fermentation times. Finally samples were stored at −80°C until analysis of SCFA concentration and intestinal counts of lactobacilli and bifidobacteria. Feces from each animal were incubated independently and in duplicate. SCFA and intestinal counts were performed as previously described [4].

Animals and diets

Female Wistar rats (180–200 g) were obtained from the Laboratory Animal Service of the University of Granada (Granada, Spain) and maintained in specific pathogen free conditions. The rats were randomly assigned to three groups: non-colitic rats (n = 10), control group (n = 20) and SC-FOS group (n = 20),

that were fed the same diet but with different sources of fibre: cellulose (50 g/kg) for non-colitic and control rats and SC-FOS (50 g/kg) for the third group. The exact composition of diets (g/kg) was as follows: 258 g casein (Arla foods, Videbaek, Denmark), 3 g L-methionine (Sigma, St Louis, Mo), 50 g sunflower oil (Koipe, Madrid, Spain), 10 g a Vitamine/Mineral mixture (Panlab, Barcelona, Spain), 579 g corn starch (Cerestar, Mechelen, Belgium), 50 g sucrose (Azucarera Ebro, Madrid, Spain) and the fibre sources (50 g/kg) mentioned above.

After feeding animals the mentioned diets during one month, half of the rats in the control group ($n = 10$) and in the SC-FOS group ($n = 10$) were sacrificed with an overdose of halothane and their cecum and colon contents were removed in order to analyze the effect of SC-FOS on intestinal microflora in healthy rats. The remaining animals ($n = 30$) were fasted overnight and those from the control and SC-FOS group were rendered colitic by the method originally described by Morris et al. [11]. Briefly, they were anaesthetized with halothane and given 10 mg of TNBS dissolved in 0.25 ml of 50% ethanol (v/v) by means of Teflon cannula inserted 8 cm through the anus. Rats from the non-colitic group were administered intracolonically 0.25 ml of phosphate buffered saline instead of TNBS. After colitis induction, rats were maintained on the same diets for one additional week and then killed with an overdose of halothane. Rats were exsanguined by sampling from abdominal aorta, plasma was separated by centrifugation and kept at −80°C until analysis. The body weight, water and food intake were recorded daily throughout the experiment. This study was carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the National Institute of Health and the Ethic Committee of Animal Experimentation of the Universidad de Granada approved it.

Assessment of colonic damage

Once rats were sacrificed, the colon was removed aseptically and placed on an ice-cold plate, longitudinally opened and luminal contents were collected for the microbiological studies (see below). Afterwards, the colonic segment was cleaned of fat and mesentery, blotted on filter paper and each specimen was weighed and its length measured under a constant load (2 g). The colon was scored for macroscopically visible damage on a 0–10 scale by two observers unaware of the treatment, according to the criteria described by Bell et al. [14] which takes into account the extent as well as the severity of colonic damage. Representative whole gut specimens were taken from a region of the inflamed colon corresponding to the adjacent segment to the gross macroscopic damage and were fixed in 4% buffered formaldehyde. Cross-sections were selected and embedded in paraffin. Equivalent colonic segments were also obtained from the non-colitic group. Full-thickness sections of 5 μm were obtained and scored by two pathologist observers, who were blinded to the experimental groups, according to the criteria described previously by Stucchi et al. [15]. The colon was subsequently divided into four segments for biochemical determinations. Two fragments were frozen at −80°C for myeloperoxidase (MPO) activity and inducible nitric oxide synthase (iNOS) expression, and another sample was weighed and frozen in 1 ml of 50 g/l trichloroacetic acid for total glutathione content determinations. The remaining sample was immediately processed for the measurement of tumor necosis factor-α (TNF-α) and leukotriene B4 (LTB4) levels by ELISA methods. All biochemical measurements were completed within one week from the time of sample collection and were performed in duplicate.

Measurement of biochemical parameters

MPO activity was measured according to the technique described by Krawisz et al. [16]; the results were expressed as MPO units per gram of wet tissue; one unit of MPO activity was defined as that degrading 1 μmol hydrogen peroxide/min at 25°C. Total glutathione content was quantified with the recycling assay described by Anderson [17], and the results were expressed as nmol/g wet tissue.

Colonic LTB4 determination was performed as previously described [18]. Briefly, samples were immediately weighed, minced on an ice-cold plate and suspended in a tube with 10 mM sodium phosphate buffer (pH 7.4) (1:5 w/v). The tubes were placed in a shaking water bath (37°C) for 20 min and centrifuged at 9,000*g* for 30 s at 4°C; the supernatants were frozen at −80°C until assay. LTB4 was determined by enzyme-immunoassay (Amersham Pharmacia Biotech, Buckinghamshire, UK) and the results expressed as ng/g wet tissue.

Determination of inducible nitric oxide synthase (iNOS) by Western blotting

iNOS expression was analyzed by western blotting as previously described [19]. Control of protein loading and transfer was conducted by detection of the β-actin levels.

Statistical analysis

All results are expressed as the mean ± SEM. Differences between means were tested for statistical sig-

nificance using a one-way analysis of variance (ANOVA) and post hoc least significance tests. Non-parametric data (score) are expressed as the median (range) and were analyzed using the Mann–Whitney U-test. Differences between proportions were analyzed with the chi-squared test. All statistical analyses were carried out with the Statgraphics 5.0 software package (STSC, Maryland), with statistical significance set at $P < 0.05$.

Results

In vitro fermentation of SC-FOS

In order to determine the time of fermentation of SC-FOS, we used an in vitro simulation of intestinal conditions, using fresh feces as a source of intestinal microorganisms. As shown in Fig. 1, SC-FOS were early fermented since 6 h after the beginning of fermentation, SCFA concentration was significantly higher than in the control samples ($P = 0.04$ vs basal). The highest SCFA concentration in the SC-FOS sample was reached at 12 h, then it started to decrease and at 24 h there was no difference between control and SC-FOS samples ($P = 0.1$). The analysis of intestinal microflora in these samples showed significantly higher counts of lactobacilli and bifidobacteria ($P = 0.03$ vs basal) in the SC-FOS samples after 24 h of incubation (table 1). After 48 h the number of bifidobacteria was also significantly higher in the SC-FOS samples compared to control samples ($P = 0.04$), but in this case the lactobacilli count was not statistically different ($P = 0.1$).

Effect of SC-FOS on SCFA concentration and intestinal microflora of healthy rats

We evaluated the impact of SC-FOS fermentation on the cecal and colonic microflora of healthy rats. After

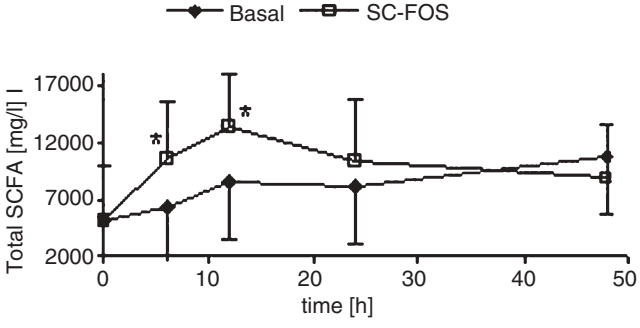

Fig. 1 Total SCFA concentration (mg/l) in batch cultures of feces from healthy rats incubated in the absence (basal) or in the presence of SC-FOS (10 mg/ml) during 0, 6, 12, 24 and 48 h. Values are the mean of three independent experiments. Bars represent the SEM. * $P < 0.05$ vs basal (Fisher test)

Table 1 Lactobacilli and Bifidobacteria counts (log cfu/g) in the rat feces incubated 24 and 48 h in the absence (basal) or presence of SC-FOS (10 mg/ml)[a]

	Basal		SC-FOS	
	24 h	48 h	24 h	48 h
Lactobacilli	9.51 ± 2.3	10.18 ± 2.6	10.91 ± 3.1*	10.88 ± 4.4
Bifidobacteria	7.70 ± 2.6	7.60 ± 2.4	8.43 ± 3.5*	8.58 ± 4.1*

[a] Values are mean (log cfu/g) of three independent experiments ± SEM. One way ANOVA and Fisher test were used to determine statistical significance. * $P < 0.05$ vs basal

Table 2 Effect of SC-FOS on cecal and colonic SCFA concentrations (mg/g), pH and lactobacilli and bifidobacteria counts of healthy rats[a]

	Control		SC-FOS	
	Cecum	Colon	Cecum	Colon
SCFA				
Acetate	38.81 ± 1.15	33.30 ± 1.64	37.05 ± 1.2	47.76 ± 3.60*
Propionate	7.93 ± 0.50	8.61 ± 0.55	13.30 ± 0.88*	18.79 ± 0.8*
Butyrate	2.08 ± 0.04	2.17 ± 0.08	4.78 ± 0.49*	5.18 ± 0.27*
PH	6.33 ± 0.39	6.26 ± 0.23	5.56 ± 0.43*	5.57 ± 0.36*
Microflora				
Lactobacilli	7.79 ± 0.82	7.69 ± 0.91	8.81 ± 0.93*	8.13 ± 0.72*
Bifidobacteria	7.44 ± 0.86	7.99 ± 0.95	7.97 ± 1.0*	8.43 ± 0.98*

[a] Values are mean ($n = 10$) (mg/g) ± SEM. One-way ANOVA and Fisher test were used to calculate statistical significance * $P < 0.05$ vs control ($P < 0.05$)

one month of feeding rats the SC-FOS enriched diet, half of the animals were sacrificed and intestinal microflora and SCFA production were analyzed. SC-FOS feeding resulted in an increase of SCFA concentration in intestinal contents. As shown in table 2, propionic and butyric acid concentrations were significantly higher ($P = 0.03$) in the cecum and colon contents of rats from the SC-FOS group compared to control group. Acetic acid concentration was also higher in the SC-FOS group, but it reaches statistical significance only in colon content ($P = 0.09$ vs control cecum content and $P < 0.05$ vs control colon content). According to these results, pH of cecum and colon contents was significantly lower ($P = 0.04$) in rats fed the SC-FOS diet (table 2). In addition, feeding rats with these oligosaccharides during one month resulted in a significant increase of lactobacilli and bifidobacteria both in the cecum and in the colon contents ($P = 0.03$), compared to rats feeding cellulose (table 2). There were no significant differences ($P > 0.1$) in other components of intestinal microflora such as coliforms or enterobacteria (data not shown).

Anti-inflammatory role of SC-FOS in the rat model of TNBS-induced colitis

To further investigate the effect of SC-FOS in the colon, we evaluated the role of these oligosaccharides

Table 3 Effect of SC-FOS on intestinal damage and alteration of biochemical parameters caused by TNBS treatment[a]

	Non-colitic	TNBS-control	TNBS SC-FOS
Intestinal damage			
Colon weight/length ratio (mg/cm)	70.32 ± 6.22	243.08 ± 42.22*	168.16 ± 17.79*†
Macroscopic damage score	–	7.0 ± 1.2	5.5 ± 1.1†
Extent of damage (cm)	–	3.53 ± 0.44	2.65 ± 0.24†
Microscopic damage score	–	20.3 ± 4.8	11.5 ± 0.9†
Biochemical parameters			
MPO (UI/g of tissue)	125 ± 10	188 ± 15*	158 ± 10*†
GSH (mmol/g of tissue)	2035 ± 289	1307 ± 262*	1580 ± 331*†
LTB4 (ng/g of tissue)	1.30 ± 0.72	3.40 ± 1.10*	2.32 ± 1.24*†

[a] Values are mean (n = 10) ± SEM. One way ANOVA and Mann–Withney U test (damage score) or Fisher test were used to calculate statistical significance.
* $P < 0.05$ vs non-colitic. † $P < 0.05$ vs TNBS-control

in an experimental model of distal colonic inflammation. SC-FOS administration during one month did not induce any symptoms of diarrhea or affected weight evolution (data not shown). However, once colitis was induced, the SC-FOS treated rats showed an overall lower impact of TNBS-induced colonic damage compared to the TNBS control group. Thus, the percentage of animals suffering from diarrhea was significantly lower ($P = 0.04$) in rats from the SC-FOS group compared to control rats from day 2 (60 vs 90%, $P < 0.05$) until the end of the experimental protocol (10 vs 40%, $P = 0.03$).

According to these results the colonic damage score was significantly lower ($P = 0.04$) than that of control rats, with a significant reduction in the extent of colonic necrosis and/or inflammation induced by the administration of TNBS/ethanol (table 3). This anti-inflammatory effect was associated with a significant reduction in the colonic weight/length ratio in SC-FOS rats compared to control group ($P = 0.04$), an index of colonic edema, which increased significantly as a consequence of the inflammatory process (table 3). The histological studies confirmed the beneficial role of SC-FOS on intestinal damage. Colonic samples from the control group showed disruption of the normal architecture and extensive ulceration and inflammation involving all the intestinal layers of the colon. However, histological analysis of the colonic specimens from SC-FOS treated rats revealed a more pronounced recovery in the intestinal architecture, giving a microscopic damage score significantly lower than that from TNBS-control rats ($P = 0.02$) (table 3).

The presence of leukocyte infiltration was also assessed biochemically by the measurement of colonic MPO activity. As shown in table 3, TNBS treatment caused a significant increase in MPO activity comparing non-colitic rats to TNBS-treated rats ($P = 0.01$). However, rats fed the SC-FOS diet showed a significantly lower MPO activity than TNBS-control rats ($P = 0.04$). TNBS-treated rats also showed a significantly lower ($P = 0.01$) level of intestinal glutathione (table 3). However, in colitic rats fed SC-FOS, a partial significant restoration in colonic glutathione levels was observed ($P = 0.04$).

The colonic inflammation induced by TNBS was also characterized by increased levels of colonic LTB4 (table 3) as well as higher colonic iNOS expression (Fig. 2), comparing control rats with non-colitic rats. SC-FOS treated rats showed a significantly lower LTB4 ($P = 0.04$) and a reduction of colonic iNOS expression compared to control rats.

The analysis of the intestinal contents revealed that administration of TNBS also caused a significant decrease in the colonic concentration of butyric acid (4.2 ± 2.8 mg/g for non-colitic rats vs 1.4 ± 0.5 mg/g for TNBS-control rats, $P = 0.03$). In contrast, rats fed SC-FOS showed no significant changes in the colonic concentration of butyric acid compared to non-colitic rats (4.2 ± 2.8 mg/g vs 3.1 ± 0.7 mg/g, $P > 0.1$). This was accompanied by changes in the intestinal microbiota, especially in the beneficial bacteria (table 4) both in the cecum and colon. Thus, the colonic lactobacilli and bifidobacteria counts were significantly lower in the control colitic group compared to non-colitic rats ($P = 0.04$). However, SC-FOS increased the number of these bacteria up to values not statistically different from the non-colitic group ($P = 0.1$). The analysis of the microbiota in the cecum also showed higher contents of lactobacilli in rats fed the SC-FOS diet ($P = 0.04$). No significant differences were observed for the enterobacteria and coliforms counts neither in the colon nor in the cecum contents.

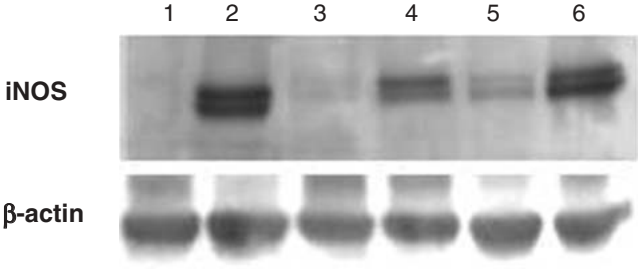

Fig. 2 Colonic expression of iNOS in non-colitic and colitic rats measured by Western blot. Lane 1: non-colitic; lane 2: TNBS-control; lanes 3–6: TNBS SC-FOS

Table 4 Effect of SC-FOS on the intestinal microflora (log cfu/g of content) of non-colitic and TNBS-induced colitic rats[a]

	Cecum			Colon		
	Non-colitic	TNBS-control	TNBS SC-FOS	Non-colitic	TNBS-control	TNBS SC-FOS
Enterobacteria	7.33 ± 0.20	7.75 ± 0.26	7.72 ± 0.23	7.82 ± 0.07	7.86 ± 0.19	7.50 ± 0.28
Coliforms	7.22 ± 0.20	7.67 ± 0.31	7.37 ± 0.23	7.66 ± 0.04	7.78 ± 0.20	7.23 ± 0.31
Lactobacilli	8.13 ± 0.16	8.10 ± 0.26	8.68 ± 0.23*†	8.30 ± 0.0	7.69 ± 0.35	8.37 ± 0.32
Bifidobacteria	8.11 ± 0.12	7.54 ± 0.23*	7.87 ± 0.30	7.49 ± 0.32	6.00 ± 0.27*	6.94 ± 0.38

[a] Values are mean (log cfu/g) (n = 10) ± SEM. One way ANOVA and Fisher test were used to determine statistical significance. * $P < 0.05$ vs non-colitic. † $P < 0.05$ vs TNBS-control

Discussion

The beneficial effect of different prebiotics on IBD has been widely described in previous works [8, 9, 20] most probably due to fermentation by colonic microbiota. However, it has been reported that the effect of prebiotics depends on the chemical composition. Thus, it has been proposed that easily and rapidly fermented prebiotics, such as FOS, are devoid of any beneficial effect in a model of experimental colitis, compared to other compounds more slowly fermented throughout the colon [21]. In contrast, FOS have been shown to ameliorate inflammation in the TNBS model of rat colitis [8]. These contradictory results could be due to both, different composition of FOS and the differences in the two experimental models used. In this work, we used FOS rich in short-chain compounds, namely GF_2 and GF_3, which are supposed to be preferentially fermented in upper parts of the intestine, to elucidate whether the colonic fermentation of FOS is a pre-requisite for their anti-inflammatory activity.

Our in vitro experiment showed that SC-FOS are rapidly fermented, because at 6 h of fermentation SCFA concentration was already significantly increased compared to basal samples. In addition, 12 h after the beginning of fermentation, SCFA production started to decrease and reached basal values at 24 h. Hence these oligosaccharides could be preferentially degraded by microbiota in the distal small intestine or upper parts of the large intestine. The importance of substrate size in fermentation of FOS had been already established by previous works, which demonstrated that GF_2 and GF_3 were preferentially fermented by Lactobacillus paracasei and that GF_4 was not consumed even after the GF_2 and GF_3 fraction had been mostly depleted [22, 23]. With these data it can be supposed that a preparation rich in GF_2, as the one used in the present study, would be easier to degrade by microflora than other preparation rich in longer compounds. Our results are in agreement with those previously published [12] suggesting that FOS could be degraded by microbiota from the distal small intestine of piglets. In the same way, a nearly complete degradation in the terminal ileum of piglets was reported for FOS included at a level of 40 g/kg [24]. With these data ileal and cecal fermentation of FOS, especially those rich in short chain compounds, seem to be not negligible and thus it should be taken into account when analyzing the prebiotic role of FOS.

Once in vitro experiments had shown that SC-FOS was theoretically fermented in upper parts of the intestine, we decided to analyze whether this early fermentation was also evident in vivo. Our results show that feeding SC-FOS to healthy rats during one month resulted in a significantly higher content of cecal lactobacilli and bifidobacteria. An increase in SCFA concentration and a decrease in pH were also evident in cecal contents. These data suggest that SC-FOS could have a prebiotic effect in the cecum of healthy rats, thus promoting a more favorable intestinal microbiota and a beneficial intestinal environment. Similar effects were demonstrated by Campbell et al. [25] in rats fed FOS at the 6% dietary level. These authors reported increased cecal concentration of SCFA and higher cecal contents of bifidobacteria in rats fed the SC-FOS diet. In contrast, they did not report changes in cecal lactobacilli counts. In a recent report, the effect of chain length of FOS in SCFA production has been analyzed. The results demonstrate that feeding rats a preparation containing 5% of FOS rich in GF_2 increased the cecal production of SCFA compared to other preparation containing 5% of FOS rich in GF_3 [26]. Unfortunately these authors did not study changes in the counts of cecal microbiota, but their data suggest that shorter chain FOS are easier to degrade by cecal microbiota. To our knowledge our results are the first showing significant changes in cecal lactobacilli and bifidobacteria in healthy rats, thus suggesting that the cecal fermentation of FOS promote a more favorable intestinal microbiota even in healthy rats.

In addition, our data demonstrate that, in spite of the fact that SC-FOS seem to be preferentially fermented in the upper part of the large intestine, the prebiotic effect could also be extended to the colon, since these carbohydrates have also a positive role on the distal colonic microflora, pH and SCFA concentration. Although more studies will be needed, this

effect on the colon could be due to both colonic fermentation of the remaining SC-FOS or dragging of SCFA and bacteria from cecal content to colonic content. To further demonstrate the beneficial effect of SC-FOS in the colon we used an animal model of distal colonic inflammation, the TNBS-induced colitis. We decided to use this model since it has been described that the administration of TNBS in ethanol causes ulceration and thickening of the bowel wall, which is more serious in the distal part of the colon [11].

The results obtained in the present experiment reveal the efficacy of feeding a 5% of SC-FOS in this model of rat intestinal inflammation. Thus, SC-FOS rats showed a significant reduction in the extent and severity of colonic damage, with lower macroscopic and microscopic scores. The beneficial effect was also stated biochemically by a decrease in colonic MPO activity, a marker of neutrophil infiltration that has been previously described to be up-regulate in experimental colitis [16]. The anti-inflammatory effect shown in rats fed SC-FOS can justify the inhibition of the synthesis and/or release of other mediators in the inflammatory process, such as LTB4. A reduction in NO production could also be expected since iNOS expression was reduced in SC-FOS rats.

The prebiotic role of SC-FOS was also evident, since the decrease in colon lactobacilli and bifidobacteria caused by TNBS treatment was not observed in colitic rats fed the SC-FOS diet. The selective stimulation of lactobacilli and bifidobacteria growth could be involved in the anti-inflammatory activity, since previous works have demonstrated the efficacy of probiotic therapy in experimental and human intestinal inflammation [3, 27].

The anti-inflammatory effect of the SC-FOS used in the present study is very similar to those previously published for longer-chain FOS. Thus, Cherbut C et al. reported that oral administration of oligofructose to rats significantly reduced pH of the colon contents, macroscopic lesion score and colon MPO activity [8]. These authors stated that the capacity of oligofructose to increase the lactobacilli counts was the main mechanism explaining its anti-inflammatory effect, rather than the increment in SCFA production. Dietary inulin, a long-chain polymer of fructose, have also been tested with positive results in an animal model of distal colitis [6], decreasing the release of inflammatory mediators and MPO activity and increasing the colonic counts of lactobacilli. The reduction in inflammatory parameters and the improvement of intestinal microflora showed in the above mentioned studies are very similar to those obtained in the present work, in spite of the fact that the compounds used differ in the chain length.

In conclusion, SC-FOS, in spite of being preferentially fermented in the upper part of the large intestine, seem to be as effective as other longer chain FOS in the reduction of distal colonic inflammation. Although the distal colonic fermentation of SC-FOS could play a role, our results suggest that it is not a pre-requisite for the anti-inflammatory effect of these carbohydrates.

Acknowledgments The authors are grateful to Ana Nieto from Health and Progress Foundation (Granada, Spain) and Angel Concha from the hospital 'Virgen de las Nieves' (Granada, Spain) for their kind help in the microscopic histological analysis. We would also like to thank Dr. Arjan Geerlings for the English revision of the manuscript. Monica Comalada is a recipient of a grant from the Juan de la Cierva Program of Spanish Ministry of Science and Technology. Desirée Camuesco is a recipient of a grant form the Junta de Andalucía (Spain).

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