Oligosaccharides isolated from goat milk reduce intestinal inflammation in a rat model of dextran sodium sulfate-induced colitis

Federico Lara-Villosladaa,*, Elisabeth Debrasb, Ana Nietoc, Angel Conchad, Julio Gálvez e, Eduardo López-Huertas a, Julio Bozza a, Christiane Obledb, Jordi Xaus a

a Department of Immunology and Animal Sciences. PULEVA BIOTECH, S.A. Camino de Purchil no. 66, 18004 Granada, Spain
b Unité de Nutrition et Métabolisme Protéique, INRA, Centre de Clermont Ferrand/Theix, France
c Health and Progress Foundation, Granada, Spain
d Department of Pathology, Hospital Universitario 'Virgen de las Nieves', Granada, Spain
e Department of Pharmacology, School of Pharmacy, University of Granada, Spain

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Summary
Background and aims: There is increased interest in the study of manipulation of the flora with pro- and prebiotics regarding inflammatory bowel disease. The aim of this work was to evaluate the effect of oligosaccharides from goat milk in a rat model of dextran sodium sulfate- (DSS-) induced colitis.

Methods: Twenty rats were fed the same diet but with different sources of fiber (5% of the diet): cellulose or a mixture of goat’s milk oligosaccharides (GMO) and cellulose. DSS treatment was used to induce a colonic inflammation. Several clinical and inflammatory parameters, as well as intestinal microbiota and gene expression by DNA microarray technology, were evaluated.

Results: DSS induced a decrease in body weight which was not observed in rats fed the GMO (decrease of 21 ± 11% in control rats vs increase of 5.2 ± 8.6 in GMO rats, P < 0.05). DSS also caused an acute colonic inflammatory process which was weaker in rats fed the GMO, as shown by colon myeloperoxidase activity (0.53 ± 0.16 vs 0.14 ± 0.07 U/mg of protein, P < 0.05), as well as clinical symptoms measured by a

Abbreviations: DSS, Dextran sodium sulfate; GMO, Goat’s milk oligosaccharides; IBD, Inflammatory bowel disease; MPO, Myeloperoxidase; PF, Pair-fed; SCFA, Short-chain fatty acids

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Corresponding author. Tel.: +34958240398; fax: +34958240160.
E-mail address: flara@pulevabiotech.es (F. Lara-Villoslada).
Introduction

Inflammatory bowel diseases (IBDs) consist of two related, but clinically and histologically separated diseases: ulcerative colitis and Crohn’s disease. These disorders are characterized by acute exacerbations accompanied by increased influx of neutrophils and increased production of inflammatory mediators.\(^1,2\) Although the exact pathogenesis is poorly understood, there is evidence that it involves interactions among the immune system, genetic susceptibility, and the environment, most notably the diet (intestinal microbiota).\(^3\)

The intestinal flora has a conditioning effect on intestinal homeostasis\(^4\) and there is evidence to suggest that some components of this flora are essential ingredients in the pathogenesis of IBD. For example, it has been shown that germ-free animals do not develop colitis until the gut flora is reconstituted.\(^5\) In this sense there is increasing interest in therapeutic manipulation of the flora and it has been suggested that administration of probiotics can modulate Dextran sodium sulfate (DSS)-induced colitis in rats.\(^6,7\)

However, the administration of probiotics is not the only way to modify the intestinal microflora. Actually there is a great interest in prebiotics which could be defined as non-digestible compounds that selectively stimulate the growth of lactic acid bacteria and bifidobacteria already resident in the colon. Several studies have demonstrated that a fiber-supplemented diet has beneficial effects on rats models of IBD. Thus, lactulose administration ameliorated DSS-induced colitis, improving clinical and inflammatory parameters, such as colonic ulceration, bloody feces and colon myeloperoxidase (MPO) activity.\(^8\) In another study, germinated barley foodstuff feeding attenuated inflammation, in HLA-B27 transgenic rats.\(^9\)

Less is known about the role of other non-digestible carbohydrates, such as oligosaccharides, in intestinal inflammation. However, oligosaccharides from human milk seem to play an important role in the development and differentiation of the intestinal epithelium in newborns. In fact, these carbohydrates are probably responsible for the lower incidence of intestinal infections and necrotizing enterocolitis (NEC) in breastfed children.\(^10,11\)

Among all mammals’ milk, human milk has the greatest content in oligosaccharides (0.7–1.2 g/100 ml). Goat milk has recently been studied as a good source of oligosaccharides, with a much higher content than that of bovine milk (25–30 mg/100 ml vs 2–3 mg/100 ml).\(^12\) In previous works,\(^12\) goat milk was submitted to ultrafiltration and purification processes, to obtain a fraction rich in goat’s milk oligosaccharides (GMO). The aim of the present study was to evaluate the effect of such product in the intestinal inflammatory process caused by DSS treatment, an animal model that resembles human ulcerative colitis.\(^13\) We tested the anti-inflammatory activity of a GMO-enriched diet based on clinical (presence of diarrhea and/or bloody stools) and biochemical (colon MPO activity and colon glutathione concentration) parameters and we evaluated the preventive and healing effects of such prebiotics on intestinal damage.

Material and methods

Reagents and goat milk oligosaccharides isolation

All chemicals were obtained from Sigma Chemical (St Louis, MO) unless otherwise stated. GMO were obtained as follows. Skimmed goat milk was submitted to an initial filtration process with a 50 kDa membrane. The filtrate was then submitted to a second filtration with a 1 kDa membrane. The product retained on the membrane was finally submitted to a purification process with an anion-exchange chromatography followed anion-exchange chromatography by anion-exchange chromatography and electrodialysis.

Analysis of different oligosaccharides was performed by anion-exchange chromatography with amperometric detection as previously published.\(^12\)
The most abundant oligosaccharides in the final product were N-acetyl-glucosyl-lactose, galactosyl-lactose, N-acetyleneuraminyl-lactose, 3-sialyl-lactose and 6-sialyl-lactose representing 77% of total oligosaccharides.

Animals and diets
Male Sprague-Dawley rats weighing 293 ± 9 g (Charles River, l’Arbresle, France) were housed in individual cages at controlled temperature (22 °C) with a 12-h light:dark cycle (lights on at 21:00 h). They were divided into two groups (n = 20 per group) which were fed the same dry purified diet except from the fiber. One group (control rats, C) received cellulose (50 g/kg of diet) as the unique fiber source, whereas the other group (GMO rats) received cellulose (30 g/kg of diet) plus the GMO fiber sources mentioned above. 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 Vitamine/Mineral mixture (Panlab, Barcelona, Spain), 579 g corn starch (Cerestar, Mechelen, Belgium), 50 g sucrose (Azucarera Ebro, Madrid, Spain) and the fiber sources mentioned above.

Experimental design
After a 7-d adaptation period, control and GMO rats were randomly distributed into two experimental groups. One group, called DSS group (DSS-control, n = 10 and DSS-GMO, n = 10), was treated with DSS (molecular weight 36–44 kDa, ICN Biomedicals, GmbH, Eschwege, Germany) dissolved in drinking water. 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 Vitamine/Mineral mixture (Panlab, Barcelona, Spain), 579 g corn starch (Cerestar, Mechelen, Belgium), 50 g sucrose (Azucarera Ebro, Madrid, Spain) and the fiber sources mentioned above.

During the treatment, body weight and food intake, as well as the presence of blood in the stools, were assessed every day. At the end of the experimental protocol, rats were anesthetized with sodium pentobarbital (6 mg/100 g body weight, Sanofi, Libourne, France). Rats were exsanguinated by sampling blood from abdominal aorta, plasma was separated by centrifugation and kept at −80 °C until analysis. The following tissues were quickly removed, blotted dry, weighed, frozen in liquid nitrogen and stored at −80 °C until analysis: spleen, thymus, liver, lung and gastrocnemius and soleus muscles. The ileum and caecum were removed, rinsed with cold PBS, weighed and stored at −80 °C until analysis. The colon was flushed with cold PBS, dried, weighed and divided into the following sections: 6 cm for mucosal extraction, 0.5 cm for histological evaluation and the rest was stored at −80 °C until analysis. All procedures were performed according to current legislation on animal experiment in France.

Presence of blood in the stools
Since the start of DSS treatment, presence of diarrhea and/or blood in the stools was blindly evaluated following a scoring system: 0 = no blood, no diarrhea; 0.5 = no blood with diarrhea; 1 = little blood with diarrhea; 1.5 = no blood with severe diarrhea; 2 = little blood with severe diarrhea; 2.5 = plenty blood with severe diarrhea.

Blood cell counts
Blood cell counts (white and red cells, platelet and hematocrit) were determined with an automatic hematology counter (Minos, ABX, Montpellier, France) on fresh blood.

Histological analysis
Colonic full-thickness (5 μm) sections were stained with hematoxilin and eosin and analyzed by two pathologists blinded to the experimental groups according to the criteria previously described by Stucchi et al. Microphotographs were taken with a Leika DM 500B microscopy.

Measurement of myeloperoxidase (MPO) activity
MPO activity has been widely accepted as a marker of the infiltration of neutrophils into tissues, and was shown to be correlated with white cells counts and associated with colon histological lesions in DSS-treated rats. Hence, caecum and colon were assayed for MPO activity as described by Krawisz et al.

Colon glutathione concentration
Glutathione (reduced and oxidized) concentrations were assayed by HPLC with fluorimetric detection of oxidized and reduced glutathione, according to Martin and White. Briefly, colon samples (~100 mg) were homogenized at 4 °C in 5 ml of 0.5 mol/l perchloric acid (PCA), supplemented with 100 μl of 180 μmol/l α-phenantroline and 180 μmol/l deferoxamine, 50 mmol/l Hepes, pH 7.4 using a Polytron PT 3100 (16,000 rpm) for 30 s. The

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homogenate was then centrifuged (13,000g, 20 min, 4 °C). Twenty microliters (10 nmol) γ-glutamyl-glutamine, used as internal standard was added to the supernatant (400 μl). Samples were derivatized the same day with iodoacetic acid followed by dansylation of free amino groups to allow fluorimetric detection. Samples were then injected onto a HPLC column (CC25014 Nucleosyl 120-7 NH2, Macherey-Nagel, France) for analysis.

Short-chain fatty acid determination
Colon content was sampled immediately after killing and homogenized with 150 mmol/l NaHCO3 (pH 7.8) (1:5, wt/v) in an argon atmosphere. Samples were incubated for 24 h at 37 °C and stored at −80 °C until the extraction. To extract the short-chain fatty acids (SCFAs), 50 μl of the internal standard 2-methylvaleric acid (100 mmol/l), 10 μl of sulfuric acid and 0.3 ml of ethyl acetate were added to 1 ml of the homogenate and then, centrifuged at 10,000g for 5 min at 4 °C. The supernatants were dehydrated with sodium sulfate and centrifuged at 10,000g for 5 min at 4 °C. Later, 0.5 ml of the sample was inoculated into a gas chromatograph (Varian CP-3800) equipped with an ID (CPWAX 52CB 60 m x 0.25 mm), and connected to a FID detector (Varian, Lake Forest, CA). Helium was used as the carrier and the make-up gas, with a flow rate of 1.5 ml/min. The injection temperature was 250 °C. Acetate, propionate and butyrate concentrations were automatically calculated from the areas of peaks using the Star Chromatography Work Station program (version 5.5), which was on-line connected to the FID detector.

Faecal microbiota
Colon content samples were homogenized in buffered peptone water (100 mg/ml) (AES laboratories). Ten-fold serial dilutions were made in the same medium and aliquots of 0.1 ml of the appropriate dilution were spread onto the following agar media: MRS agar for lactobacilli, MRS agar supplemented with 0.5 mg/l dicloxacillin, 1 g/l LiCl and 0.5 g/l L-cysteine hydrochloride for bifidobacterium; Reinforced Clostridial containing 20 μg/ml of polimixina for Clostridium. All media were obtained from Oxoid (Basingstoke, UK) whereas antibiotics and other supplements were obtained from Sigma Chemical Co. (St Louis, Mo). Culture plates were incubated in absence of oxygen at 37 °C for 24–48 h. Similarly, 1 ml of suitable dilution was spread onto specific Count Plates Petrifilm (3M St Paul, MN) for coliforms, for total aerobes and for Enterobacteriaceae. Plates were incubated at 37 °C for 24 h. After the incubation, the specific colonies grown on the selective culture media were counted and the number of viable microorganism per gram colonic content (CFU/g) were calculated. The mean and standard error per group were calculated from the log values of the CFU/g.

Gene expression analysis using the rat U34 Gene Chip
cRNA preparation. Colon mucosal samples from DSS-control, DSS-GMO and PF control groups (n = 3 per group) were used for RNA isolation following the Trizol Reagent method (Invitrogen, Carlsbad, CA) as described by the manufacturer. RNA samples were hybridized to Affymetrix GeneChips. For all samples, 10 μg of total RNA was the starting material for hybridization to the rat U34 GeneChips.

In all cases, total RNA was converted to biotinylated cRNA, hybridized in the Affymetrix probe array cartridge, stained and then quantified. First and second strand cRNA synthesis was performed using the SuperScript Choice System (Invitrogen AG, Basel, Switzerland), according to manufacturer’s instructions, but using an oligo-dT primer containing a T7 RNA polymerase binding site. Labeled cRNA was prepared with the MEGAscript In vitro Transcription kit (Ambion, Inc., Austin TX). Biotinylated CTP and UTP (Enz Biochem Inc., NY) were used together with unlabeled dNTPs in the reaction, and unincorporated nucleotides were removed with RNeasy columns.

Array hybridization and scanning
cRNA (10 μg) was fragmented at 94 °C for 35 min in buffer containing 40 mmol/l Tris-acetate pH 8.1, 100 mmol/l KOAc, 30 mmol/l MgOAC. Prior to hybridization, fragmented cRNA in a 6 × SSPE-T hybridization buffer (6 × : 1 mol/l NaCl, 10 mmol/l Tris pH 7.6, 0.005% Triton) was heated to 95 °C for 5 min, cooled to 40 °C and loaded onto an Affymetrix probe array cartridge. The probe array was incubated for 16 h at 40 °C at constant rotation (60 rpm), then exposed to 10 washes in 6 × SSPE-T at 25 °C followed by four washes 0.5 × SSPE-T at 50 °C. Biotinylated cRNA was stained with 10 g/ml streptavidin–phycoerytrin conjugate (Molecular Probes) in 6 × SSPE-T for 30 min at 25 °C followed by 10 washes in 6 × SSPE-T at 25 °C. Probe arrays were scanned at 560 nm using a Agilent Gene Array Scanner (Affymetrix). Readings from the quantitative scanning were analyzed with Affymetrix Gene Expression Analysis Software (GeneChip Operating Software 1.1 and Data Mining Tool 3.1).

Results are expressed as fold change between PF control and DSS-control, showing changes caused by DSS treatment, and between DSS-control and
DSS-GMO, showing recuperation with GMO feeding. We focused on immune-related genes and those with intestinal functions.

**Statistical analysis**

Data are means ± standard deviation (SD). Statistical significance was calculated by one-way analysis of variance (ANOVA) and post hoc least significance tests, Fisher test for parametric parameters and Mann–Whitney test for non-parametric parameters. All analyses were performed with the SAS Stat View package (Stat View for windows version 5.0, SAS institute Cary, NC), except those corresponding to DNA microarrays results, which were analyzed with Affymetrix Gene Expression Analysis Software, as mentioned above. Significant differences were defined at \( P < 0.05 \). The main comparison in this work was control-DSS vs GMO-DSS in order to analyze the effects of GMO on intestinal damage caused by DSS treatment. However, comparison between DSS-treated rats and their PF groups is also interesting in order to evaluate whether the effects are due to the decrease in food intake or they are specific from DSS treatment.

**Results**

**Food intake and body weight**

One week before the beginning of DSS treatment animals were fed the different diets. During this period body weight gain and food intake were similar in animals fed the control and the GMO diets (data not shown).

It has been proved that DSS treatment causes anorexia in rats.\(^4\) In DSS-control rats, food intake was reduced during the 8 d of treatment and the following 5 d of recovering. In contrast, food intake of DSS-GMO rats only decreased during the first 2 d of treatment and reached initial values on d 6. Hence, from d 4 until d 11, food intake was higher in DSS-GMO rats than in DSS C rats (Fig. 1A).

Immediately after the beginning of DSS treatment, control rats started to lose weight and did so until d 11 (Fig. 1B). Body weight loss was greater in DSS-control rats compared with their PF since d 5 until d 13. At the end of the experiment, body weight loss of DSS-control rats represented 21% of their initial body weight. Of this loss, 10% could be explained by anorexia. The remaining 11% correspond to specific body weight loss induced by DSS treatment. Body weight loss in DSS-GMO rats started also at the beginning of the treatment (d 0), but it stopped at d 2. Then, it reached initial value at d 5 and from d 6 rats started to gain weight until the end of the experiment. There was no difference in body weight loss between DSS-GMO rats and their PF control. The body weight gain of these two groups of rats from d 2 until the end of the experiment was similar to that of a rat fed ad libitum (data not shown).

**Tissue weight changes and clinical symptoms**

The anorexia caused an important reduction in some tissues weight, as shown by the comparison between PF-control and PF-GMO (Table 1). Thus, gastrocnemius, liver, thymus and ileum showed a lower weight in PF-control rats than in DSS-GMO rats (Fig. 1A).

Independently of anorexia, DSS treatment in rats fed the control diet also caused a decrease in some tissues weight. Thus, gastrocnemius and thymus weights were lower in colitic rats. The GMO diet reduced gastrocnemius and thymus weight loss. Colon weight/length ratio was also lower in this
group, thus indicating a weaker local inflammatory response in rats feeding the GMO diet.

Loose stools were observed in some animals within 3 d after the start of DSS treatment. Diarrhea with bloody stools was first seen for all the DSS-treated rats at d 4, but DSS-control rats had a higher average score than DSS-GMO rats from d 4 until the end of the experiment (Fig. 2). None of the PF rats showed diarrhea nor bloody stools in any moment of the study (data not shown).

Histological analysis

The histological analysis revealed that DSS treatment caused a colonic damage. Figure 3 shows a moderate chronic multifocal inflammatory infiltrate affecting lamina propria and submucosa in DSS-control rats, in spite of the recovering period (Fig. 3B). In contrast, colon samples from DSS-GMO rats showed small size focal infiltrates affecting only lamina propria (Fig. 3C). In spite of the anorexia previously mentioned for PF-control rats, no differences were observed in the histological analysis of colon samples of PF-control and PF-GMO rats (data not shown).

Inflammatory response to DSS treatment

DSS treatment caused an increase in leukocytes in both, control and GMO rats. Among all leukocytes, granulocytes showed the highest increase, specially in the DSS-control group with an increase in the percentage of granulocytes greater than that of the DSS-GMO group (Fig. 4A).

Colon MPO activity was higher in DSS-control rats compared to their PF control (Fig. 4B). In contrast, colon MPO activity in DSS-GMO rats did not statistically differ from that of their PF group.

Colon and liver glutathione concentrations

In our model of intestinal inflammation, liver and colon glutathione content in DSS-treated rats did not statistically differ from that of the PF controls. It must be noticed that we did not measure glutathione content at the end of DSS treatment, but after a 5-d recovering period.

In contrast, comparing glutathione content in both groups of DSS-treated rats, DSS-GMO rats had higher colon glutathione content, both total and reduced, compared to DSS-control rats, although

Table 1 Effect of dextran sodium sulfate (DSS) treatment and diet on tissue weights in rats.*,†

<table>
<thead>
<tr>
<th>Organ</th>
<th>PF-control</th>
<th>DSS-control</th>
<th>PF-GMO</th>
<th>DSS-GMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius</td>
<td>1.966±0.089</td>
<td>1.469±0.287†</td>
<td>2.104±0.086§</td>
<td>2.065±0.183§</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.128±0.013</td>
<td>0.120±0.008</td>
<td>0.133±0.014</td>
<td>0.141±0.012</td>
</tr>
<tr>
<td>Liver</td>
<td>8.755±0.925</td>
<td>12.360±1.187§</td>
<td>11.677±1.565§</td>
<td>15.314±1.658§</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.660±0.072</td>
<td>0.765±0.222</td>
<td>0.724±0.104</td>
<td>1.022±0.162</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.300±0.071</td>
<td>0.163±0.111†</td>
<td>0.557±0.092§</td>
<td>0.460±0.097§</td>
</tr>
<tr>
<td>Illeum</td>
<td>1.481±0.160</td>
<td>2.041±0.644†</td>
<td>2.305±0.223§</td>
<td>2.527±0.340</td>
</tr>
<tr>
<td>Caecum</td>
<td>0.775±0.07</td>
<td>1.660±0.375†</td>
<td>2.280±0.312§</td>
<td>2.611±0.494§</td>
</tr>
<tr>
<td>Colon</td>
<td>0.961±0.145</td>
<td>2.106±0.326†</td>
<td>1.582±0.256§</td>
<td>1.692±0.194§</td>
</tr>
<tr>
<td>Colon weight/length ratio</td>
<td>0.063±0.006</td>
<td>0.156±0.036†</td>
<td>0.08±0.009</td>
<td>0.103±0.01†</td>
</tr>
</tbody>
</table>

*Values are means (g)±SD or means (g/cm)±SD for colon weight/length ratio.
†One-way ANOVA and Fisher test were used to calculate statistical significance (P<0.05).
§Different from pair-fed controls.
Differs from control rats.
††Difference between PF-control and PF-GMO.
†††Different from DSS-control.

Figure 2 Presence of diarrhea and/or blood in the stools. Since the start of dextran sodium sulfate (DSS) treatment the presence of diarrhea and/or blood in the stools was evaluated daily by a scoring system from 0 (no symptoms) to 2.5 (plenty of blood with severe diarrhea). Values are mean ± SD. One-way ANOVA and Mann–Whitney U-test were used to determine statistical significance (P<0.05). DSS-C: DSS-control rats. DSS-GMO: DSS-goat milk oligosaccharides rats. †P<0.05 vs DSS-C.
no effect was observed for the oxidized form of glutathione (Table 2).

No differences were observed in liver total, oxidized or reduced glutathione concentration between experimental groups.

Faecal microbiota and SCFA content

In PF rats, the colonic content of lactobacilli and bifidobacteria was higher with the GMO diet than with the C diet (Table 3). No changes were observed in bacteroides, enterobacteria and coliforms between both PF groups.

DSS treatment caused an increase in enterobacteriaceae and coliforms. The GMO diet, although did not increase the probiotic (lactobacilli and bifidobacteria) content of DSS-treated rats, did cause a decrease of bacteroides, enterobacteria and coliforms compared to DSS-control rats.

The concentration of SCFA in the colon content, although not statistically significant, tended to be higher in PF-GMO rats compared to PF-control (50.10 ± 10.56 vs 70.01 ± 10.89 mg/g of colon content, \( P = 0.08 \)). However, in contrast to previously published results, \(^{19}\) we did not observe a decrease in SCFA concentration in DSS rats, probably due to the recovering period. The percentage of butyric acid, a major fuel source for colonocytes, \(^{20}\) tends to be lower in DSS-treated rats compared to PF groups, but no significant effect was observed for GMO diet (21.4 ± 2.75% and 21.9 ± 6.97% for PF-control and PF-GMO vs 19.2 ± 2.46% and 18.8 ± 1.49 for DSS-control and DSS-GMO, respectively).

Analysis of gene expression by DNA microarray technology

Table 4 resumes changes observed in gene expression, showing those induced by DSS treatment and recuperation in the expression of these genes in rats fed the GMO diet. No changes were observed between both groups of PF rats in the expression of the analyzed genes (data not shown).
We have focused on genes involved in inflammation and intestinal function. Hence, DSS treatment caused a reduction in the expression of growth factors with important functions in intestinal maturation and repair, such as epidermal growth factor related protein, which has been shown to play key roles in the mucosal defence and repair.21 GMO diet increased the expression of this gene up to normal values. Other growth factor involved in angiogenesis regulation,22 placenta growth factor, was also decreased by DSS treatment, and recuperation was evident in DSS-GMO rats. A group of genes encoding mucines (MUC-3, MUC-5, MUC-1, MUC-2) were down-regulated by DSS treatment and their expression tended to be normal in DSS-GMO rats. In the latter genes there was no significant differences but a strong trend similar in all mentioned genes (*P* = 0.07).

Alkaline phosphatase, a marker of IBD,23 was induced by DSS treatment and its expression returned to normal values in DSS-GMO rats. Genes involved in inflammation were also modified. IL-1β, a T_h1 cytokine with pro-inflammatory effects, was strongly induced by DSS treatment. GMO diet showed a decrease in the expression of this inflammatory cytokine.

**Discussion**

The main aim of this work was to study the effects of GMO in the early recovery of experimental induced colitis by DSS in rats. The results of our work suggest an important role of GMO in recovering from colonic damage.

| Table 2 | Effect of dextran sodium sulfate (DSS) treatment and diet on total, reduced (GSH) and oxidized (GSSG) glutathione concentrations in liver and colon of rats. *|† |
| --- | --- | --- | --- | --- |
| **Liver** | PF-control | DSS-control | PF-GMO | DSS-GMO |
| Total (µmol/g) | 6.04±0.35 | 7.36±1.73 | 6.99±1.32 | 8.87±0.52 |
| GSH (µmol/g) | 5.71±0.39 | 7.07±1.17 | 6.54±1.28 | 8.40±0.50 |
| GSSG (µmol/g) | 0.16±0.03 | 0.15±0.03 | 0.22±0.02 | 0.23±0.02 |
| **Colon** | PF-control | DSS-control | PF-GMO | DSS-GMO |
| Total (µmol/g) | 2.10±0.10 | 1.90±0.30 | 2.90±0.20 | 2.60±0.30† |
| GSH (µmol/g) | 1.98±0.08 | 1.77±0.31 | 2.08±0.12 | 2.47±0.27 |
| GSSG (µmol/g) | 0.06±0.01 | 0.05±0.0 | 0.09±0.01 | 0.07±0.00† |

*Values are means (µmol/g of tissue)±SD.
†One-way ANOVA and Fisher test were used to calculate statistical significance (*P*<0.05).
‡Different from DSS-control.
DSS is a macromolecule with epithelial toxicity that induces crypt destruction in the distal colonic mucosa and diffuse inflammation with crypt abscesses.\(^{13}\) This model was chosen because genetic susceptibility and specific immunity do not seem to play important roles in the inflammatory process induced by DSS.\(^3\) Therefore, the model used is one of the most suitable to study environmental components of IBD, especially the diet. In addition, we decided to modify the pattern of DSS-colitis induction, in order not to produce a chronic inflammation, but an acute one, and then, to study the effects of a particular component of the diet in the early recovery of the disease.

As previously published,\(^{14}\) DSS treatment caused anorexia in rats. This effect should be considered as an important symptom in this model, since it is partially responsible for body weight loss and gastrocnemius and thymus weight loss observed in DSS-control rats. GMO diet improved the anorexia caused by DSS treatment, and as a consequence body weight loss was lower in DSS-GMO rats. This effect was also evident for tissue weight loss, since in DSS-GMO rats most of the tissues had a weight similar to those of rats fed ad libitum. In contrast, colon and caecum weights were increased in rats fed the GMO diet, probably due to a slight hypertrophy caused by gas production during oligosaccharides fermentation.

GMO not only protect rats from the anorexia, but also from toxicity of DSS since rats fed the GMO showed less severe clinical symptoms (diarrhea and bloody stools) than DSS-control rats during DSS treatment and the recovering period, since MPO activity was lower than that of rats on the cellulose diet. The higher food intake could probably play a key role in the improvement observed in rats fed the GMO diet. However, specific roles of oligosaccharides from goat milk are likely to contribute to this beneficial effect.

Although the mechanisms of the protective effects of oligosaccharides need further investigations, intestinal microflora might play a key role. As we have pointed out in the introduction, the commensal bacteria colonizing the intestinal surface are an essential driving factor for the inflammatory process.\(^{24}\) This is the approach that has been followed by several researchers who have shown that probiotic bacteria, modulating the gut flora, are useful in the prevention and control of IBD.\(^{25}\) The mechanism of action is uncertain but could be due to competitive metabolic interactions with potential pathogens\(^5\) or positively alter the intestinal barrier function.\(^{26}\)

In contrast to probiotics, prebiotics affect the host by selectively stimulating the growth of beneficial bacteria already established in the colon and by pH decrease, inhibiting the growth of pathogenic bacteria. This has been the aim of different studies with respect to the role of fiber, inulin or fructo- and galacto-oligosaccharides in the prevention or treatment of intestinal diseases involving inflammation. Interestingly, in rats suffering from ulcerative colitis after DSS oral administration, inulin intake (400 mg/d) prevented inflammation, as evidenced by lower colonic lesion scores, a lower release of inflammatory mediators and lower MPO activity in colonic tissue than in control rats. Inulin also induced an acid pH in colon and increased counts of lactobacilli.\(^{27}\) Resistant starch has also been shown to restore the integrity of rat caeco-colonic mucosa in DSS-induced colitic rats.\(^{28}\)

### Table 3

<table>
<thead>
<tr>
<th>Faecal Microbiota</th>
<th>PF-control</th>
<th>DSS-control</th>
<th>PF-GMO</th>
<th>DSS-GMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacilli</td>
<td>9.52 ± 0.07</td>
<td>9.59 ± 0.26</td>
<td>10.66 ± 0.06(^{\dagger})</td>
<td>9.71 ± 0.14</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>9.35 ± 0.06</td>
<td>9.58 ± 0.09</td>
<td>10.63 ± 0.07(^{\dagger})</td>
<td>9.93 ± 0.17</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>9.76 ± 0.19</td>
<td>9.70 ± 0.14</td>
<td>9.75 ± 0.34</td>
<td>9.51 ± 0.08(^{\ddagger})</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>7.08 ± 0.27</td>
<td>9.21 ± 0.40(^{\star})</td>
<td>6.33 ± 0.33</td>
<td>7.09 ± 0.28(^{\ddagger})</td>
</tr>
<tr>
<td>Coliforms</td>
<td>7.09 ± 0.35</td>
<td>9.31 ± 0.42(^{\star})</td>
<td>7.04 ± 0.52</td>
<td>6.96 ± 0.38(^{\ddagger})</td>
</tr>
<tr>
<td>Total aerobes</td>
<td>9.23 ± 0.23</td>
<td>10.08 ± 0.22(^{\star})</td>
<td>10.26 ± 0.23(^{\ddagger})</td>
<td>9.47 ± 0.10(^{\star\ddagger})</td>
</tr>
</tbody>
</table>

\(^{\star}\) Values are means (log cfu/g of colon content) ± SD.

\(^{\dagger}\) Different from DSS-control.

\(^{\ddagger}\) One-way ANOVA and Fisher test were used to calculate statistical significance (\(P < 0.05\)).

\(^{\star}\) Different from pair-fed controls.

\(^{\ddagger}\) Different from DSS-control.

\(^{\star\ddagger}\) Difference between PF-control and PF-GMO.
DSS-treated rats, GMO did not increase the number of lactobacilli or bifidobacteria but decreased the number of bacteriodes, enterobacteria and coliforms. In spite of this effect on intestinal microbiota, butyrate production was not significantly increased by GMO at the end of the experiment. With these data the role of butyrate in the anti-inflammatory effect of GMO remains unclear. This could be contradictory with other studies where healing effects of dietary fiber on intestinal epithelium are related to a higher luminal butyrate concentration.\(^{28,29}\) However, there are also studies showing that the beneficial effect of fructo-oligosaccharides is more probably due to microflora modification rather than only to butyrate production.\(^{30}\)

In addition, milk oligosaccharides in contrast to other prebiotics, such as inulin or shorter length chain fructo-oligosaccharides, are able to bind to specific pathogen bacteria and inhibit their ability to bind to the host cells.\(^{10}\) These milk oligosaccharides can therefore act as soluble homologues to the epithelial cell surface receptors, which are the targets of specific pathogens. In essence, the glycosyltransferases that make human oligosaccharides are similar to the glycosyltransferases that synthesize the oligosaccharide moieties of mammalian cell surface receptors synthesized by goblet cells.\(^{31}\) This inhibition of the adhesion of pathogens could partially explain the decrease in the colon content of bacteriodes, coliforms and enterobacteria observed in DSS-GMO rats.

It has been reported in different animal models that intestinal inflammation is associated with impairment of the mucus protective layer. Thus, chronic colitis developed by HLA-B27 transgenic rats is associated with mucin alterations all along the gut.\(^{32}\) DSS is also directly cytotoxic to the colonic epithelium, inducing crypt damage and altering mucoprotein synthesis and secretion.\(^{33,34}\) Unfortunately, we did not measure mucoprotein synthesis, but our results are in alignment with those previously mentioned\(^ {32-34}\) since in our model DSS treatment caused a down-regulation in the expression of some genes involved in gut repair as well as genes encoding mucoproteins. GMO diet was able to improve this down-regulation as compared to the control diet containing cellulose, as the sole non-digestive carbohydrate source.

Apart from their prebiotic and protective roles, oligosaccharides could also have anti-inflammatory effects. Thus, it has been reported that oligosaccharides from human milk inhibit leukocyte infiltration, by the inhibition of monocyte, lymphocyte and neutrophil adhesion to endothelial cells.\(^ {35}\)

Although this anti-inflammatory action has not been evaluated in this work, our results, concerning

### Table 4

<table>
<thead>
<tr>
<th>Gene</th>
<th>PF-control vs DSS-control</th>
<th>DSS-control vs GMO-control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immune-related genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>−2.0</td>
<td>1.7</td>
</tr>
<tr>
<td>IL-1/β</td>
<td>−0.8</td>
<td>−1.6</td>
</tr>
<tr>
<td>IL-6 receptor</td>
<td>−0.8</td>
<td>−1.8</td>
</tr>
<tr>
<td>IL-2 receptor-α chain</td>
<td>−0.7</td>
<td>−1.8</td>
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<tr>
<td>Lymphotoxin</td>
<td>−0.2^z</td>
<td>3.2^z</td>
</tr>
<tr>
<td>IL-1 receptor type 2</td>
<td>−0.9</td>
<td>1.4</td>
</tr>
<tr>
<td>IL-1 receptor antagonist</td>
<td>−1.4</td>
<td>0.5</td>
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<tr>
<td>IL-1β converting enzyme</td>
<td>2.1</td>
<td>−1.3</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.2</td>
<td>3.1</td>
</tr>
<tr>
<td>IL-13</td>
<td>−1.4</td>
<td>1.9</td>
</tr>
<tr>
<td>TNF-α receptor</td>
<td>1.5</td>
<td>−1.5</td>
</tr>
<tr>
<td>TGF-β receptor</td>
<td>−1.0</td>
<td>4.2</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>−1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>3.7</td>
<td>−1.6</td>
</tr>
<tr>
<td>TGF-β1/2</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>IL-15</td>
<td>−1.7^z</td>
<td>1.3^z</td>
</tr>
<tr>
<td>MHCIId D-α chain</td>
<td>3.2^z</td>
<td>−1.9^z</td>
</tr>
<tr>
<td><strong>Growth factors</strong></td>
<td></td>
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</tr>
<tr>
<td>Placenta growth factor</td>
<td>−3.6^z</td>
<td>7.5^z</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>−1.7^z</td>
<td>1^z</td>
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<tr>
<td>related protein</td>
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<td></td>
</tr>
<tr>
<td><strong>Intestinal markers of inflammation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1.5^z</td>
<td>−1.8^z</td>
</tr>
<tr>
<td><strong>Mucins</strong></td>
<td></td>
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</tr>
<tr>
<td>MUC-1</td>
<td>−1.5</td>
<td>1.9</td>
</tr>
<tr>
<td>MUC-3</td>
<td>−1.8</td>
<td>1.3</td>
</tr>
<tr>
<td>MUC-5</td>
<td>−1.5</td>
<td>3.2</td>
</tr>
<tr>
<td>MUC-2</td>
<td>−2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Intestinal mucin</td>
<td>−4.9</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Values represent fold change between pair-fed control and DSS-control (first column) and between DSS-control and DSS-GMO (second column). 
^z_Affymetrix Gene Expression Analysis Software was used to determine statistical significance (P < 0.05).
^z_Represents changes statistically different (P < 0.05). The others changes had a P value between 0.05 and 0.07, not statistically different but strongly tend to be different.

Milk oligosaccharides can also be considered as prebiotics, since they are not digested in the upper part of the intestine, being partially degraded by Bifidobacteria and lactic acid bacteria and, hence, stimulating the growth of beneficial bacteria in the colon. The comparison of the two PF groups clearly shows an increase in lactobacilli and bifidobacteria in rats fed the GMO diet. Comparing both groups of DSS-treated rats, GMO did not increase the number of lactobacilli or bifidobacteria but decreased the number of bacteriodes, enterobacteria and coliforms. In spite of this effect on intestinal microbiota, butyrate production was not significantly increased by GMO at the end of the experiment. With these data the role of butyrate in the anti-inflammatory effect of GMO remains unclear. This could be contradictory with other studies where healing effects of dietary fiber on intestinal epithelium are related to a higher luminal butyrate concentration.\(^{28,29}\) However, there are also studies showing that the beneficial effect of fructo-oligosaccharides is more probably due to microflora modification rather than only to butyrate production.\(^ {30}\)
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a lower MPO activity in rats fed the GMO, are in agreement with this report.

In conclusion, our results suggest that oligosaccharides from goat milk play important roles in intestinal protection and repair after a damage caused by DSS-induced colitis. Further studies are needed to elucidate the mechanisms involved in the beneficial effects of these compounds in intestinal function and their implication in human intestinal inflammation.

Acknowledgments

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References


