Restoration of the integrity of rat caeco-colonic mucosa by resistant starch, but not by fructo-oligosaccharides, in dextran sulfate sodium-induced experimental colitis

Noëlle M. Moreau[1,3], Lucile J. Martin[1,3]*, Claire S. Toquet[3,4,5], Christian L. Laboisse[3,4,5], Patrick G. Nguyen[1,3], Brigitte S. Siliart[1,3], Henri J. Dumon[1,3] and Martine M. J. Champ[2,3]

[1]*Unité de Nutrition et d'Endocrinologie, Ecole Nationale Vétérinaire, Nantes, France*
[2]*Unité des Fonctions Digestives et Nutrition Humaine, Institut National de la Recherche Agronomique, Nantes, France*
[3]*Centre de Recherche en Nutrition Humaine, Nantes, France*
[4]*INSERM U539, Faculté de Médecine, Nantes, France*
[5]*Service d'Anatomie Pathologique, CHU, Nantes, France*

Butyrate is recognised as efficient in healing colonic inflammation, but cannot be used as a long-term treatment. Dietary fibre that produces a high-butyrate level when fermented represents a promising alternative. We hypothesised that different types of dietary fibre do not have the same efficiency of healing and that this could be correlated to their fermentation characteristics. We compared short-chain fructo-oligosaccharides (FOS) and type 3 resistant starch (RS) in a previously described dextran sulfate sodium (DSS)-induced colitis model. Seventy-two Sprague–Dawley rats received water (control rats) or DSS (50 g DSS/l for 7 d then 30 g DSS/l for 7 (day 7) or 14 (day 14) d). The rats were fed a basal diet (BD), or a FOS or RS diet creating six groups: BD-control, BD-DSS, FOS-control, FOS-DSS, RS-control and RS-DSS. Caeco-colonic inflammatory injuries were assessed macroscopically and histologically. Short-chain fatty acids (SCFA) were quantified in caeco-colon, portal vein and abdominal aorta. At days 7 and 14, caecal and distal macroscopic and histological observations were improved in RS-DSS compared with BD-DSS and also with FOS-DSS rats. Caeco-colonic SCFA were reduced in FOS-DSS and RS-DSS groups compared with healthy controls. The amount of butyrate was higher in the caecum of the RS-DSS rats than in the BD-DSS and FOS-DSS rats, whereas distal butyrate was higher in FOS-DSS rats. Partially explained by higher luminal levels of SCFA, especially butyrate, the healing effect of RS confirms the involvement of some types of dietary fibre in inflammatory bowel disease. Moreover, the ineffectiveness of FOS underlines the importance of the type of dietary substrate.

Fructo-oligosaccharide: Resistant starch: Dextran sulfate sodium: Short-chain fatty acids: Butyrate

Acetate, propionate and butyrate, the three major short-chain fatty acids (SCFA) are the endproducts of the colonic anaerobic fermentation of dietary fibre. Luminal SCFA are oxidation substrates for the colonocyte (Clausen & Mortensen, 1994) and have trophic effect on healthy gut epithelial cells (Sakata, 1987). Systemic SCFA also influence the intestinal structure (Tappenden & McBurney, 1998). Among SCFA, butyrate is known to represent the major fuel source for the colonocyte (Roediger, 1980a, 1982; Fleming et al. 1991) and to be essential for mucosa health (Basson & Sgambati, 1998). Indeed, it was shown that a lack of butyrate has deleterious effects on the colonic mucosa (Firmansyah et al. 1989; Roediger, 1993).

Previous studies have demonstrated that SCFA, especially butyrate, have a beneficial trophic effect on inflamed caeco-colonic mucosa of human subjects (Scheppach et al. 1992, 1997; Frankel et al. 1994) or experimental animal colitis (Butzner et al. 1996). Many studies have shown that inflamed rat (Ahmad et al. 2000) or human (Roediger, 1980b; Chapman et al. 1994; Den Hond et al. 1998) colonocytes showed impairment of butyrate oxidation.

Butyrate enemas have shown their benefit for mucosal health (Breuer et al. 1991; Steinhart et al. 1994) in patients suffering from Crohn's disease or ulcerative colitis, which encompass the two major forms of inflammatory bowel disease. However, this positive effect disappears when administered *per os* (Kanauchi et al. 1999). Hence, among therapeutic alternatives, dietary fibre that produces a high butyrate proportion when fermented in the large intestine, and which could be ingested as a functional food, seems a promising and a more realistic treatment

for patients. However, no results are available on what the characteristics of the best dietary substrates to heal inflammatory bowel disease are. In the present paper, in order to assess their healing efficiency, two substrates, well known to yield a large amount of butyrate after fermentation, were tested on a previously described model of colitis based on a dextran sulfate sodium (DSS) administration. In a few days, this continuous DSS treatment was able to induce stable chronic inflammation, predominantly with caecal and distal injuries (Moreau *et al.* 2002). The two fibres were selected according to their fermentation kinetics. When considering the caeco-colon, fructo-oligosaccharides (FOS) are known to be rapidly fermented, whereas resistant starch (RS) is more slowly degraded and can reach the distal part of the colon (Le Blay *et al.* 1999*a,b*).

The objective of the present study was to examine which substrate induced the best recovery of mucosa by looking at morphological macroscopic and histological criteria. Indeed, we made the hypothesis that these fibres, which have different fermentation patterns, would not have the same capacity to restore the inflamed colonic mucosa and that this effect could be partly caused by their ability to be fermented to produce SCFA, especially butyrate.

Methods

Animals and housing

Seventy-two male Sprague–Dawley rats (Harlan, Gannat, France), weighing 310 (SEM 10) g at arrival, were used for the present experiment. They were housed individually in cages designed to avoid coprophagy, and a light–dark cycle (light on 08.00–20.00 hours) was used. The animals were allowed free access to diet and water until killing. The experiment was carried out according to the French animal welfare rules (Decree no. 2002/464, 29 May 2001) and approved by the Nantes Ethics Committee for Laboratory Animals.

Diets

Three experimental diets with different fibre contents (Table 1) were specifically formulated for the experiment (INRA, Jouy-en-Josas, France). The basal diet (BD) was a low-fibre diet containing 20 g cellulose/kg. The FOS diet was enriched with short-chain FOS (Actilight P; Beghin-Meiji Industries, Paris, France) and the RS diet with RS type 3 (retrograded Hylon 7, high amylomaize starch, Novelose 330; National Starch and Chemical Ltd, Manchester, UK). They were formulated to provide 60 g indigestible carbohydrate + cellulose/kg DM . The metabolisable energy contents (kJ/g DM) were 17·30, 16·76 and 16·34 for the BD and FOS and RS diets respectively. Water, food intake and body weight were recorded daily.

Colitis induction

At the beginning of the experiment, after a 7 d adaptation to the diet and housing conditions, the animals were randomly divided into two groups. The first group received

Table 1. Composition of the three experimental diets (g/kg)

Diet...	Basal	FOS	RS
Short-chain FOS*	0	63	0
RS (type 3)†	0	0	115
Pregelatinised maize starch‡	650	587	535
Casein	204	204	204
DL-Methionine	4	4	4
Refined maize oil	18	18	18
Lard	58	58	58
Cellulose§	20	20	20
Minerals	44	44	44
Vitamins	2	2	2

FOS, fructo-oligosaccharide; RS, resistant starch.
* Actilight P; Beghin-Meiji Industries, Paris, France.
† Novelose 330; National Starch and Chemical Ltd, Manchester, UK.
‡ Cerestar SF 12018; Cerestar, Vilvoorde, Belgium.
§ Arbocel BOO; Arbocel, Marne-la-Vallée, France.

50 g DSS/l in water (DSS group) (DSS molecular mass 36–44 kDa; ICN Pharmaceuticals, Orsay, France) for 7 d (day 0). Then, the concentration of the DSS solution was reduced to 30 g/l for 7 d (day 7) or 14 d (day 14) in order to induce a stable chronic active colitis as previously described (Moreau *et al.* 2002). The second group was free from DSS throughout the experiment and received drinking water (control group). As shown in Fig. 1, during the adaptation period and the 50 g DSS/l period, all the animals (control and DSS groups) received the BD. The rats were then randomly assigned to one of the three experimental diets (BD, FOS and RS diets). The six groups were identified as: BD-control and BD-DSS, FOS-control and FOS-DSS, RS-control and RS-DSS.

Six rats from each of the six groups were killed at day 7 (water or 50 g DSS/l for 7 d then 30 g DSS/l for 7 d), and six rats at day 14 (water or 50 g DSS/l for 7 d then 30 g DSS/l for 14 d).

Collection of digestive tissues and contents

After being anaesthetised by intraperitoneal injection of 15 mg xylazine/kg (Bayer Pharma, Puteaux, France) and intramuscular injection of 55 mg ketamine/kg (Mérial, Lyon, France), the rats were laparotomised and blood samples were collected from the portal vein and abdominal aorta with an infusion set. The animals were then killed by an intra-cardiac injection of sodium pentobarbital (Vétoquinol, Lure, France). The caecum and the colon were resected after ligature of the ileo-caecal junction up to the rectal ampoule. The entire colon length was measured and the three segments (caecum, proximal and distal colon) were then removed and weighed to assess inflammation. Afterwards, each was cut longitudinally and tissues and digestive contents were recovered. The whole procedure occurred between 09.30 and 10.30 hours for all rats.

Macroscopic and histological scores

Caecum, proximal and distal colon were gently cleaned with isotonic solution (9 g NaCl/l; Aguettant, Lyon, France), and colitis activity was scored by visual
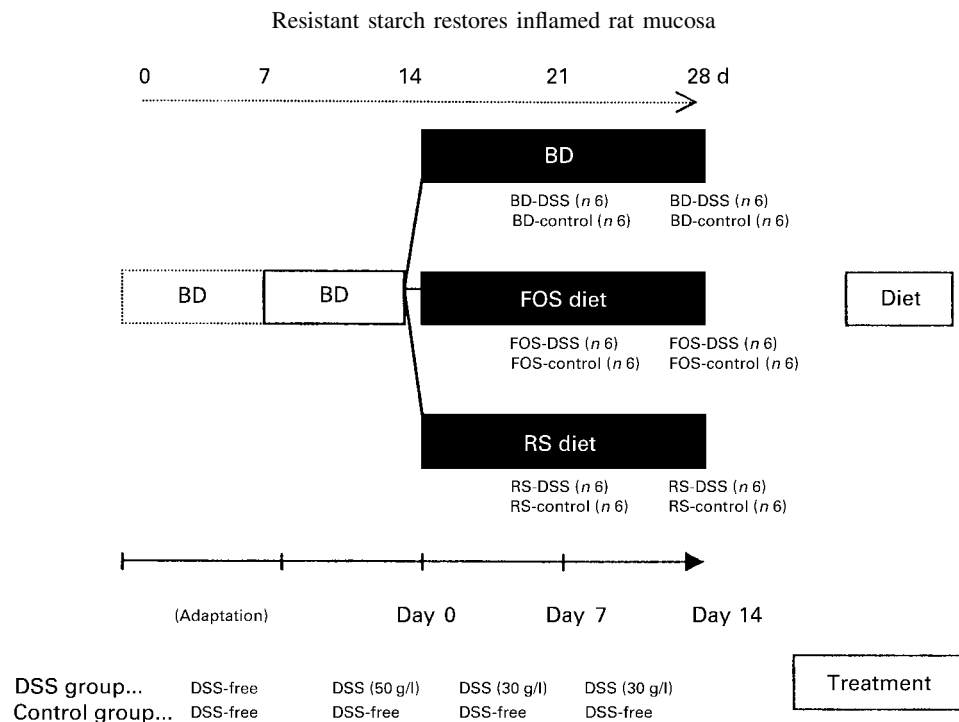
Fig. 1. Experimental protocol. BD, basal diet; FOS, fructo-oligosaccharide; RS, resistant starch; DSS, dextran sulfate sodium. For details of diets, see Table 1.

assessment (assessors were blinded to the treatment group), using a scale described by Appleyard & Wallace (1995) with slight modifications: tissues adhesions (0, none; 1, minor; 2, severe), caecal mucosa (0, normal; 1, hyperaemia; 2, blood in contents; 3, ulcerations), diarrhoea (0, none; 1, mild; 2, severe), blood in the contents (0, none; 1, in one-third of the colon; 2, in two-thirds of the colon; 3, in the entire colon), colonic mucosa thickening (0, normal; 1, moderate; 2, severe), colonic ulcerations (0, any site; 1, focal hyperaemia; 2, limited ulcerations; 3, ulcerations extending more than 10 mm along the length of the colon). The total macroscopic score corresponded to the sum of these partial scores.

Tissue was fixed in neutral buffered formalin (100 ml/l), dehydrated, and paraffin-embedded. Sections (5 μm) were stained with haematoxylin and eosin, and scored by considering the loss of mucosal architecture (scored 0–3), cellular infiltration (0–3), muscle thickening (0–3), crypt abcesses (0–1) and goblet cell depletion (0–1) (maximum score 11) (Appleyard & Wallace, 1995; Moreau *et al.* 2002).

Short-chain fatty acid quantification in caeco-colonic contents

A sample of the contents of three gut segments was removed and homogenised in 2 ml mercuric chloride (1 g/l; Merck, Darmstadt, Germany). After centrifugation, the supernatant fraction was frozen immediately at −80°C and stored until later analysis. SCFA concentrations were quantitated by GC (GC-6890; Hewlett Packard, les Ulis, France) using a 1 μm FFAP column (30 m × 0·53 mm) (Hewlett Packard) and flame ionisation detector, using the procedure described by Brighenti (1997), slightly modified, with 2-ethylbutyric acid as internal standard. SCFA

were expressed as molar ratios, μmol per caeco-colonic compartment ('amount' of SCFA per compartment) and μmol/g wet digestive contents ('concentrations' of SCFA in each compartment). In order to verify if DSS inhibits the fermentative intensity, SCFA were expressed as μmol/g food DM intake (mean value of the last 3 d) to take into account the possible differences in food intake between DSS and control rats and between the different diets.

Short-chain fatty acid measurements in blood samples

Total arterial or venous blood was collected in a heparinised tube, centrifuged at 2100 *g* for 10 min, and plasma frozen at −80°C. Plasma SCFA measurements were performed using isovaleric acid (Sigma-Aldrich, St Quentin-Fallavier, France) as internal standard with the procedure described by Brighenti (1997).

Calculations and statistics

Levels of SCFA appearing in the portal blood were calculated by arterio–venous difference (Δ) between concentrations obtained in the portal vein and in the abdominal aorta (Demigné & Rémésy, 1982). The objective of calculating this difference was to subtract the endogenous part of SCFA (which is not of digestive origin) from the portal concentrations, in order to consider only SCFA that *a priori* came solely from caeco-colonic fermentation. Portal SCFA were also expressed as molar ratio ($\Delta C_x/\Delta SCFA$).

Results were expressed as mean values with their standard errors, and *n* refers to the number of rats in each of the six groups. Macroscopic and histological scores were compared using the non-parametric Kruskal–Wallis test. When a

significant effect ($P<0.05$) was observed, diets were compared separately using a Mann–Whitney test. The other variables described in the present paper were analysed by ANOVA with treatment (DSS *v.* control), diet and time (day 7 and day 14) as main factors. When there was no significant interaction effect in the two-way (treatment × diet) or three-way (treatment × diet × time of killing) comparisons, we conducted a one-way comparison, whereas two-way or three-way comparisons were maintained when the *P* value of two-way or three-way interaction effect first was <0.05. When the tested effect was significant, the differences between mean values were assessed by contrasts analysis using Super ANOVA V 1.11 (Abacus Concepts Inc., Berkeley, CA, USA). For all calculations, the accepted level of statistical significance was $P<0.05$.

Results

Daily consumption

No significant difference either in the DSS consumption (Table 2) or in the water:diet consumption ratio was found among BD, FOS and RS groups. In the control group, daily food and water intake were not affected by the type of diet (BD, FOS or RS diet). Diet and DSS consumption of rats fed BD, FOS and RS between days 0 and 7 and between days 7 and 14 (for rats killed at day 14) (see p. 76 and Fig. 1) are indicated in Table 2. In the DSS rats, no difference in food intake appeared between days 0 and 7. Conversely, the FOS-DSS rats had a lower food intake between days 7 and 14 compared with the BD-DSS rats (NS) and RS-DSS rats ($P=0.047$).

Body-weight gain

In the control group, body-weight gain was not influenced by the type of diet (results not shown). In the DSS group, the greatest weight loss was observed in the FOS-DSS group (Table 2). Nevertheless, body weight was stable between days 7 and 14 among the groups.

Length and weight of the colon and weight of the caecum

Macroscopic variables differed significantly according to the treatment DSS *v.* control (length of the colon $P=0.0001$, weight of the caecum $P=0.0002$, weight of the colon $P=0.0001$). Neither the type of diet nor the time of killing (day 7 or 14) had a significant effect. However, at day 14 the colons of RS-DSS rats tended to be longer than FOS-DSS (NS) and BD-DSS rats ($P=0.061$) (Table 3). While the FOS-DSS and BD-DSS rats had significantly heavier caeca and colons than the corresponding

Table 2. Diet intake, dextran sulfate sodium (DSS) intake and body-weight gain of rats fed basal, fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 7 (day 7) or 14 (day 14) d and treated with 50 g DSS/l (7 d) then 30 g DSS/l for 7 (day 7) or 14 (day 14) d*

(Mean values with their standard errors for six rats per group)

	Diet intake (g/d)				DSS intake (g/d)				Body-weight gain†			
	0–7		7–14		0–7		7–14		0–7		0–14	
Day...												
Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Basal	12·0[a]	1·1	14·5[ab]	1·0	15·2[a]	1·0	13·9[a]	0·6	−0·03[a]	0·02	0·02[a]	0·02
FOS	10·3[a]	0·5	12·5[a]	0·6	15·7[a]	0·7	15·7[a]	1·2	−0·12[b]	0·04	−0·09[b]	0·03
RS	11·9[a]	1·1	14·8[b]	1·0	16·5[a]	0·7	15·4[a]	0·8	−0·02[ab]	0·03	−0·01[ab]	0·05

[a,b]Mean values within a column with unlike superscript letters were significantly different ($P<0.05$).
* For details of diets and procedures, see Table 1, Fig. 1 and p. 76.
† Body-weight gain = (weight at day 7 (or 14) − weight at the beginning of the experiment)/weight at the beginning of the experiment.

Table 3. Changes in the length of the colon, the weight of the caecum and the colon of rats fed basal, fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 7 (day 7) or 14 (day 14) d and treated with water (control group) or 50 g dextran sulfate sodium (DSS)/l (7 d) then 30 g DSS/l for 7 (day 7) or 14 (day 14) d*

(Mean values with their standard errors for six rats per group)

| | | Length of the colon (mm) | | | | | Weight of the caecum (g) | | | | | Weight of the colon (g) | | | | |
| | | Control | | DSS | | Statistical significance of effect: *P*† | Control | | DSS | | Statistical significance of effect: *P*† | Control | | DSS | | Statistical significance of effect: *P*† |
Time	Diet	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
Day 7	Basal	178[a]	9	150[a]	3	0·0171	0·6[a]	0·1	0·8[a]	0·0	NS	1·2[a]	0·1	1·4[a]	0·1	NS
	FOS	166[a]	13	152[a]	7	NS	0·7[a]	0·1	0·9[a]	0·1	NS	1·2[a]	0·1	1·6[a]	0·2	NS
	RS	181[a]	7	152[a]	10	0·0407	0·8[a]	0·2	0·9[a]	0·0	NS	1·3[a]	0·1	1·4[a]	0·1	NS
Day 14	Basal	176[a]	5	147[a]	6	0·0045	0·7[a]	0·0	1·0[a]	0·1	0·0441	1·2[a]	0·1	1·6[a]	0·1	0·0470
	FOS	176[a]	5	154[a]	6	0·0213	0·7[a]	0·0	1·0[a]	0·1	0·0427	1·3[a]	0·1	1·8[a]	0·1	0·0251
	RS	187[a]	3	162[a]	5	0·0025	0·7[a]	0·1	0·9[a]	0·1	NS	1·2[a]	0·1	1·4[a]	0·1	NS

[a,b] Mean values with unlike superscript letters were significantly different ($P<0.05$).
* For details of diets and procedures, see Table 1, Fig. 1 and p. 76.
† DSS *v.* control.

control rats, there was no difference between the RS-DSS and RS-control rats. Moreover, FOS-DSS colons were therefore heavier than RS-DSS ($P=0.0511$) (Table 3).

Macroscopic score

Whatever the time of killing, the control rats' score values were zero. In DSS-treated rats, there was no difference in the score between diets at day 7: 6·6 (SEM 1·1), 7·7 (SEM 1·1) and 6·8 (SEM 1·2) for the BD-, FOS- and RS-DSS groups respectively. However, at day 14 the macroscopic score of the RS-DSS rats (2·7 (SEM 0·6)) was significantly lower than the BD-DSS (6·7 (SEM 0·9), $P=0.0082$) and FOS-DSS rats (6·1 (SEM 1·5), $P=0.0306$). Total score and all the variables included in the macroscopic score at day 14 are detailed in Fig. 2.

Histological assessments

Histological scores of control groups were zero. At day 7 (Fig. 3(a)) and at day 14 (Fig. 3(b)), histological scores measured in the caecum were significantly ($P<0.05$) lower in RS-DSS than in BD-DSS rats and in FOS-DSS rats (at day 7). There was no difference between diets in the proximal colon. In the distal colon, the RS diet significantly ($P<0.05$) decreased the histological score compared with the BD at days 7 and 14.

The total histological score (caecum+proximal+distal scores) was significantly lower for RS-DSS than BD-DSS ($P=0.0008$ at day 7, $P=0.0124$ at day 14) and than FOS-DSS ($P=0.0195$ at day 7, $P=0.0406$ at day 14).

Caeco-colonic fermentations

Effect of dextran sulfate sodium on the caeco-colonic measurements of short-chain fatty acids (μmol or μmol/g wet contents). Whatever the diet, the treatment and the time of killing, the amount of SCFA (μmol) was considerably higher in the caecum than in proximal and distal colon (Table 4). In the caecum, SCFA contents were lower when the rats received the DSS treatment compared with healthy controls. Conversely, proximal and distal amount of SCFA did not vary with DSS (except for RS at day 7).

When results were expressed as concentrations (μmol/g wet contents), except in the caecum at day 7, digestive SCFA of rats fed BD were not modified by the DSS treatment. Conversely, caeco-colonic SCFA concentrations of rats fed the RS- or FOS-enriched diets were significantly ($P<0.05$) reduced with DSS compared with healthy controls (Table 4).

Effect of dextran sulfate sodium on the intensity of fermentation (μmol/g DM intake). The consequences of a DSS-induced inflammation on the intensity of fermentation (SCFA measurements expressed as μmol/g DM intake) differed among diets. DSS treatment did not modify proximal and distal SCFA measurements expressed as μmol/g DM intake. As previously shown (Moreau *et al.* 2002), DSS reduced the fermentative abilities of rats fed for 7 d (day 7) with a low-fibre diet (BD) ($P=0.0249$), but this inhibition disappeared at day 14. In contrast, when DSS-treated rats were fed the diet supplemented with FOS, caecal fermentation was strongly inhibited at day 7 (8·1 (SEM 1·8) *v.* 25·0 (SEM 2·5) μmol SCFA/g DM intake for control, $P=0.0004$) and at day 14 (12·9 (SEM 2·1) *v.* 30·6 (SEM 4·2) μmol/g DM intake for controls, $P=0.0003$). Conversely, for RS rats, no change appeared at day 7 between control (25·4 (SEM 2·6) μmol/g DM intake) and DSS groups (18·9 (SEM 3·6) μmol/g DM intake). At day 14, there was a trend of decrease in caecal SCFA by DSS treatment (13·5 (SEM 4·1) *v.* 22·6 (SEM 2·1) μmol/g DM intake for controls, $P=0.0578$).

Effect of dextran sulfate sodium on short-chain fatty acid molar ratio. Table 4 also summarises the qualitative disturbances of caeco-colonic fermentation induced by DSS administration. For the BD-DSS rats, the caecal butyrate molar ratio was higher at day 7 compared with control groups. With FOS diet, the acetate molar ratio was reduced in DSS groups at day 7 in the caecum and the proximal colon, whereas the propionate molar ratio of DSS rats
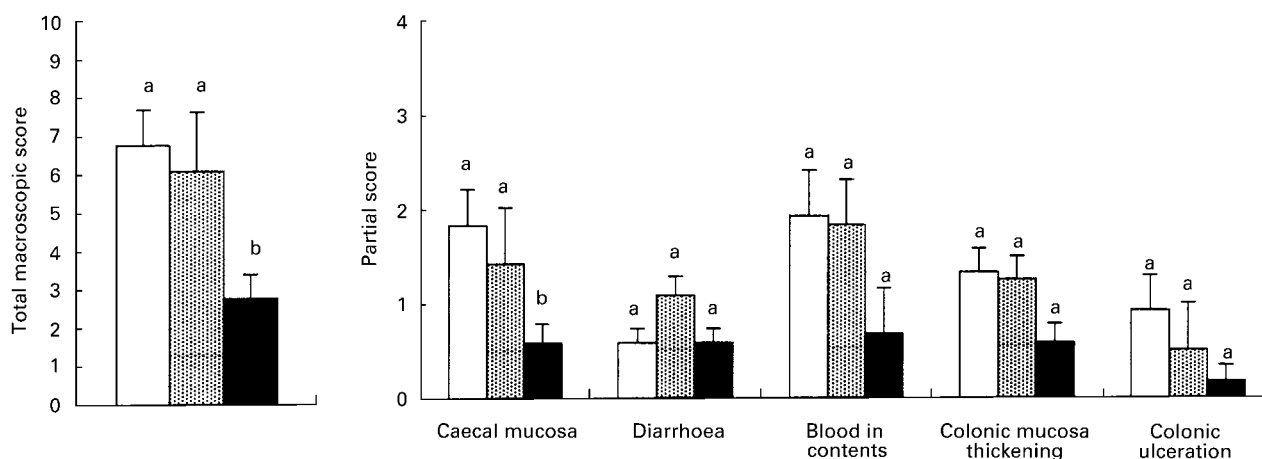
Fig. 2. Changes in the total and detailed macroscopic score of rats fed basal (BD), fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 14 d and treated with 50 g dextran sulfate sodium (DSS)/l (7 d) then 30 g DSS/l for 14 d. □, BD-DSS; ▨, FOS-DSS; ■, RS-DSS. For details of diets, procedures and scoring system, see Table 1, Fig. 1 and p. 77. Values are means for six rats per group with their standard errors shown by vertical bars. [a,b]Mean values with unlike superscript letters were significantly different: $P<0.05$.
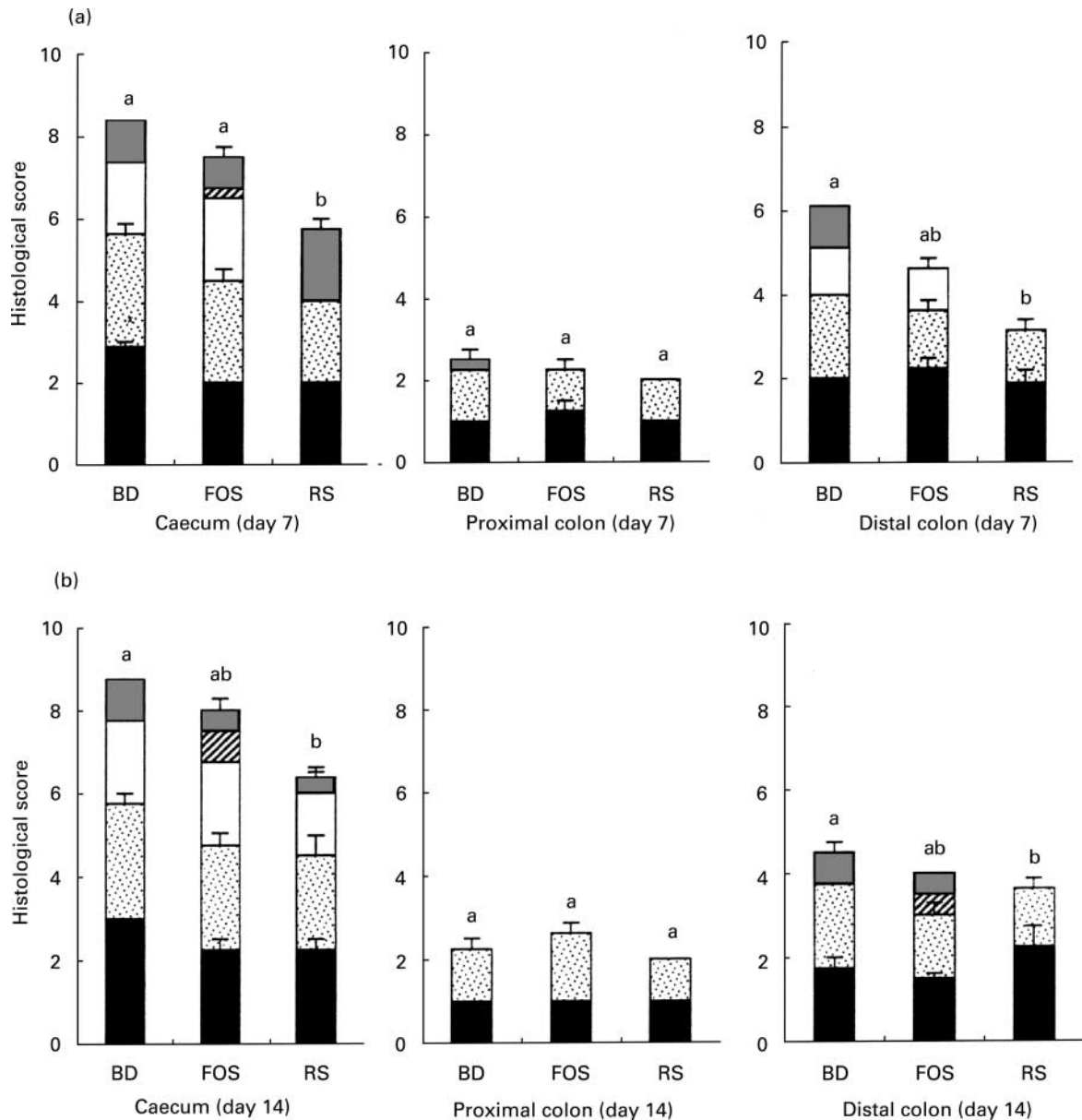
Fig. 3. Changes in the variables of the histological score of rats fed basal (BD), fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 7 (day 7) (a) or 14 (day 14) (b) d and treated with 50 g dextran sulfate sodium (DSS)/l (7 d) then 30 g DSS/l for 7 (day 7) (a) or 14 (day 14) (b) d. ▨, Goblet cell depletion; ▨, crypt abcesses; □, muscle thickening; ▨, cellular infiltration; ■, loss of mucosal architecture. For details of diets, procedures and scoring system, see Table 1, Fig. 1 and p. 77. Values are means for four rats per group. The standard errors shown by vertical bars correspond to each variable included in the histological score. [a,b]Mean values of total histological score with unlike superscript letters were significantly different: $P < 0.05$.

was higher compared with control rats. No molar ratio modifications were found in the RS-DSS rats compared with the RS-control rats. Concerning butyrate, it was interesting to note that its molar ratios in caecal, proximal and distal contents were not modified by the introduction of DSS when the animals were fed a fibre-enriched diet.

Effect of dextran sulfate sodium on the amount of butyrate. Because of the lack of time effect (and time × diet interaction, two-way ANOVA, $P = 0.79$) on the amount of digestive butyrate (μmol), results obtained at days 7 and 14 were pooled. The amount of caecal butyrate observed in RS-DSS (38.8 (SEM 8.2) μmol) rats was significantly higher than in BD-DSS (9.6 (SEM 1.1) μmol,

$P = 0.0002$) and in FOS-DSS (12.5 (SEM 2.5) μmol, $P = 0.0006$). No difference among diets was observed in the proximal colon. Conversely, in the distal colon, the greatest amount of butyrate was measured in FOS-DSS rats (4.4 (SEM 0.8) μmol) compared with the RS-DSS (3.9 (SEM 0.9) μmol) (NS) and BD-DSS animals (2.3 (SEM 0.4) μmol, $P = 0.0198$).

Short-chain fatty acids in portal blood

Arterio–venous differences in SCFA are detailed in Table 5. Whatever the diet, DSS rats had a lower Δacetate than controls, whereas Δpropionate and Δbutyrate

Table 4. Short-chain fatty acid (SCFA) measurements, weight of the wet contents and amounts of the major SCFA in the caecum, proximal and distal colon of rats fed basal, fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 7 (day 7) or 14 (day 14) d and treated with water (control group) or 50 g dextran sulfate sodium (DSS)/l for 7 (day 7) or 14 (day 14) d‡

(Mean values with their standard errors for six rats per group)

Diet…	Day 7												Day 14											
	Basal				FOS				RS				Basal				FOS				RS			
Group…	Control		DSS		Control		DSS		Control		DSS		Control		DSS		Control		DSS		Control		DSS	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Caecum																								
SCFA (μmol)	193·7	28·5	87·7**	16·5	373·9	53·0	79·4***	27·1	411·8	42·4	223·5*	59·3	143·6	20·5	90·8†	19·8	432·0	50·9	147·4***†	28·3	361·9	32·3	201·6**†	59·3
SCFA (μmol/g wet contents)	80·1	6·0	51·9*	1·9	108·5	12·1	45·8***	4·7	109·2	11·7	83·5*	10·7	65·0	6·1	49·7	4·5	108·2	12·6	65·2***	4·5	104·0	9·5	70·4**	7·7
wet content (g)	2·4	0·2	1·7	0·3	3·4	0·3	1·6***	0·5	3·9	0·3	2·5**	0·4	2·2	0·2	1·8	0·3	4·0	0·1	2·2***	0·3	3·5	0·2	2·6	0·6
C_2 (% $C_2 + C_3 + C_4$)	76	1	71†	3	82	1	68**†	2	63	4	66†	4	74	2	72	3	70	5	70	3	62	4	68	2
C_3 (% $C_2 + C_3 + C_4$)	16	1	18†	2	9	1	22**†	2	19	3	15†	2	17	1	17	2	16	4	18	3	18	2	17	2
C_4 (% $C_2 + C_3 + C_4$)	8	1	11**	0	9	1	10	1	18	3	18	2	8	1	11	1	14	1	12	1	20	4	15	2
Proximal colon																								
SCFA (μmol)	19·2	5·6	20·5	4·3	42·1	8·2	27·8	5·2	29·5	2·9	15·9*†	2·7	16·9	2·2	12·7	2·2	14·8	2·9	22·7	3·7	18·8	3·7	28·9	8·5
SCFA (μmol/g wet contents)	37·7	5·0	37·7†	3·1	108·2	12·7	43·7***†	12·8	74·5	12·8	39·9*†	2·5	38·6	3·8	31·8	2·0	62·1	6·0	36·5***	4·0	54·2	6·6	37·1*	2·6
wet content (g)	0·5	0·1	0·6	0·1	0·4	0·1	0·8*	0·2	0·4	0·0	0·4	0·1	0·4	0·1	0·4†	0·0	0·2	0·0	0·6**†	0·0	0·3	0·0	0·8**†	0·2
C_2 (% $C_2 + C_3 + C_4$)	79	1	71**†	2	83	1	72*†	2	69	5	76†	2	78	0	74	2	72	5	76	4	69	4	73	3
C_3 (% $C_2 + C_3 + C_4$)	13	1	18†	2	9	1	19*†	2	16	3	13†	1	15	1	17	2	16	4	14	3	16	2	16	2
C_4 (% $C_2 + C_3 + C_4$)	8	1	10	0	8	1	8	1	14	4	11	1	7	1	9	1	11	2	10	1	14	3	11	2
Distal colon																								
SCFA (μmol)	10·4	1·3	17·7	3·7	45·3	8·0	33·6	8·1	36·1	9·2	16·8	3·5	7·0	1·4	24·6	8·3	30·7	6·4	37·0	6·4	47·3	2·2	41·1	7·4
SCFA (μmol/g wet contents)	29·6	9·0	33·1	8·4	56·1	7·9	30·7**	3·8	47·0	4·5	27·6*	2·0	13·9	2·0	25·2†	3·0	43·0	4·8	32·3†	2·4	49·1	6·4	29·0**†	4·3
wet content (g)	0·5	0·1	0·7	0·2	0·8	0·1	1·2	0·3	0·7	0·1	0·6	0·1	0·5	0·1	0·9	0·3	0·8	0·2	1·1	0·2	0·9	0·1	1·4	0·2
C_2 (% $C_2 + C_3 + C_4$)	80	1	69*	4	78	1	64**	3	72	8	72	3	78	2	66**	2	70	3	72	4	76	4	70	3
C_3 (% $C_2 + C_3 + C_4$)	9	1	21***	2	11	1	26**	3	12	5	14	3	9	1	22***†	2	15	2	15†	3	9	2	18*†	3
C_4 (% $C_2 + C_3 + C_4$)	11	1	10	2	10	1	10	1	16	4	14	2	12	1	12	1	15	2	13	1	15	4	12	1

C_2, acetate; C_3, propionate; C_4, butyrate.

Mean values were significantly different from those of the control group at days 7 and 14: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Interactions of diet × treatment were significantly different for each variable (two-way ANOVA): †$P<0.05$.

‡ For details of diets and procedures, see Table 1, Fig. 1 and p. 77.

Table 5. Plasma short-chain fatty acid (SCFA) concentrations (portal − arterial) and plasma molar ratios for rats fed basal, fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 7 or 14 d and treated with water (control group) or 50 g dextran sulfate sodium (DSS)/l for 7 d then 30 g DSS/l for 7 (day 7) or 14 (day 14) d‡

(Mean values with their standard errors for six rats per group)

Diet...	Basal				FOS				RS			
	Control		DSS		Control		DSS		Control		DSS	
Group...	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Day 7												
ΔC_2 (μM/l)	212·8	28·3	118·1*	7·2	271·2	53·9	141·6**	10·6	277·5	25·4	145·8**	17·2
ΔC_3 (μM/l)	51·5	9·9	39·6†	5·0	52·0	9·6	91·7*†	14·8	117·6	18·0	39·5***†	8·3
ΔC_4 (μM/l)	15·6	3·4	21·3†	2·6	30·4	9·6	31·5†	5·4	90·0	22·2	41·9**†	12·8
C_2 (%$C_2 + C_3 + C_4$)	76	2	66*†	1	77	1	55***†	3	58	5	66*†	3
C_3 (%$C_2 + C_3 + C_4$)	18	2	22†	1	15	1	33***†	3	24	3	17*†	1
C_4 (%$C_2 + C_3 + C_4$)	5	1	12*	1	8	1	12	2	18	4	17	2
Day 14												
ΔC_2 (μM/l)	164·0	9·5	126·4	6·5	262·9	36·4	154·8**	16·0	274·9	36·6	166·5**	30·2
ΔC_3 (μM/l)	37·4	5·0	58·9	12·0	82·2	23·5	53·9	9·1	119·6	36·6	64·7	14·0
ΔC_4 (μM/l)	7·7	2·5	32·5	5·9	63·3	26·5	34·5	5·3	66·5	14·1	51·8	18·3
C_2 (%$C_2 + C_3 + C_4$)	79	2	60**†	4	67	4	64†	3	60	4	61†	5
C_3 (%$C_2 + C_3 + C_4$)	17	1	26*	3	20	2	22	2	23	4	23	2
C_4 (%$C_2 + C_3 + C_4$)	3	1	14**	0	13	3	14	1	16	4	16	4

ΔC_2, arterio−venous difference for acetate; ΔC_3, arterio−venous difference for propionate; ΔC_4, arterio−venous difference for butyrate.
Mean values were significantly different from those of the control group for each diet: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
Interactions of diet × treatment were significantly different for each variable (two-way ANOVA): †$P < 0.05$.
‡ For details of diets and procedures, see Table 1, Fig. 1 and p. 77.

appeared to be less affected by the DSS treatment. Major changes concerned RS-DSS rats at day 7: this group had a significantly ($P < 0.05$) lower Δacetate, Δpropionate and Δbutyrate than their corresponding control group. Interestingly, butyrate molar ratios did not vary with DSS when rats were fed the FOS- and RS-enriched diet.

Discussion

The effect of butyrate on the health of the mucosa is well known and its role appears to be multi-factorial. Indeed, its role in the colonic immune system has been shown recently and appears to be increasingly well documented (for example via interactions with cytokines (Nancey *et al.* 2002), lymphocytes (Chapman, 2001), neutrophils (Liu *et al.* 2001) or nuclear factor-κB (Segain *et al.* 2000) etc.). The trophic effect of butyrate on the inflamed colonic mucosa has been described in several studies. Most of them used butyrate enemas in patients with ulcerative colitis (Breuer *et al.* 1991; Scheppach *et al.* 1992; Steinhart *et al.* 1994) or in animals (Butzner *et al.* 1996). But, interestingly, there was no effect when butyrate was given to inflamed rats by *per os* administration (Kanauchi *et al.* 1999). As colonic butyrate administration is too invasive for human therapy, an interesting alternative for colonic treatment could be dietary fibre. Some fibres, such as those in *Plantago ovata* seeds in patients with ulcerative colitis (Fernandez-Banares *et al.* 1999) and pectin (Andoh *et al.* 1999) and germinated-barley foodstuff in DSS-induced colitis (Kanauchi *et al.* 1999; Araki *et al.* 2000a,b; Kanauchi *et al.* 2001), showed promising results that are, according to the authors, partly explained by the trophic action of butyrate.

Conversely, fermentation of short-chain FOS (Le Blay *et al.* 1999b) and RS (Le Blay *et al.* 1999a) are known to yield high levels of butyrate. Results obtained in models of inflammatory bowel disease (trinitrobenzene sulfonic acid-induced) indicated that FOS (Cherbut *et al.* 2003) and RS (granulated pea-starch) (Jacobasch *et al.* 1999) are good candidates for the treatment of the inflamed epithelial mucosa. FOS (Gallaher *et al.* 1996; Pierre *et al.* 1997; Perrin *et al.* 2001) and RS (Perrin *et al.* 2001) have also shown beneficial properties against colon carcinogenesis, even if the impact of RS seems controversial (Sakamoto *et al.* 1996; Pierre *et al.* 1997).

Our results presented here focus on the effect of FOS and RS on the digestive mucosa inflamed by DSS. Histopathological characteristics of injuries observed in this model concern the distal colon and predominantly the caecum (Moreau *et al.* 2002). Macroscopic and histological results showed no caeco-colonic mucosal repair with FOS neither at day 7 nor at 14 compared with the BD. Conversely, macroscopic and histological results indicated that RS diet can provide beneficial effects on the chronic inflamed caecal and distal mucosa. This seemed to appear as soon as 7 d (day 7) and persisted throughout the experiment. This RS healing effect against DSS damage was significant compared with the fibre-free BD, but also compared with the FOS-supplemented diet. As proximal injuries induced in this DSS model were few (Moreau *et al.* 2002), it is not surprising that dietary manipulations had a minor effect on the proximal colon.

The differing histopathological consequences of a FOS or RS supplementation could be suspected to be linked with some possible bias, such as food consumption differences. It appeared that this effect cannot be explained by

differences either in diet or in DSS consumption. We observed identical food consumption for the three DSS-treated groups between days 0 and 7, whereas food consumption of FOS-DSS rats was lower than the RS-DSS group between days 7 and 14 (Table 2). Simultaneously, histological analysis indicated that the first signs of mucosal healing appeared at day 7 for the RS-DSS groups, whereas the inflammation observed in FOS-DSS rats was always more severe. This suggests that the lower food consumption observed in FOS-DSS rats could be a consequence of the changes in the inflammation process compared with RS-DSS rats. It is probably not a causal event explaining the lack of healing effect of FOS diet on the injured mucosa. Moreover, the water:diet intake ratio remained constant among groups.

Many hypotheses could be proposed to explain, at least in part, the difference of effect between FOS and RS on the inflamed mucosa. Among them, differences in caeco-colonic SCFA profiles and concentrations yielded after fermentation of the tested diets could be proposed. Considering only DSS-treated rats, our present results underlined discrepancies in SCFA measurements between diets. The amount of caecal SCFA (μmol) measured at day 7 in FOS-DSS rats was considerably lower than in RS-DSS rats (results were equivalent at day 14). Moreover, at day 7 as well as 14, there was proportionally less butyrate in the caecum of FOS-DSS rats than in RS-DSS rats, which could partially explain the lack of benefit of FOS observed in the caecal mucosa. Nevertheless, it is interesting to note that in FOS-DSS rats, despite a higher level of butyrate in the distal colon compared with the other diets, no improvement of distal histological scores was observed. Consequently, the caecal fermentation appears to be essential for the healing process.

After 7 and 14 d of chronic colitis, our present results indicated that the DSS treatment inhibits the fermentation process, SCFA measurements (amount or concentrations) being lower in DSS rats than in controls (except for BD rats at day 14). Consequently, to be efficient, dietary fibre-containing diets must yield sufficient butyrate to counteract the inhibition of fermentation caused by DSS inflammation. These results underline the fact that the efficiency of dietary fibre against DSS injuries seems to be partly related to their fermentation characteristics.

Another possible explanation of the difference between FOS and RS could concern the luminal composition and particularly sulfate-reducing bacteria. They have been reported to colonise the large intestine in ulcerative colitis (Pitcher et al. 1995; Roediger et al. 1997) and to generate high levels of sulfides, sulfites and mercaptans (Roediger & Nance, 1990; Roediger et al. 1997). Some of these compounds may inhibit the butyrate uptake by the colonocytes (Stein et al. 1995; Jacobasch et al. 1999) and the colonic butyrate oxidation (Roediger et al. 1993a,b, 1996; Pitcher & Cummings, 1996) leading to an alteration of the mucosal integrity (Roediger, 1995).

Sulfate-reducing bacteria prefer near neutral pH conditions and sulfate reduction is optimal at alkaline pH (Widdel, 1988). By decreasing caeco-colonic pH with the production of SCFA, FOS and RS could inhibit the action of sulfate-reducing bacteria. Moreover, RS (Brown

et al. 1997; Kleessen et al. 1997; Cresci et al. 1999) and FOS (Djouzi & Andrieux, 1997; Le Blay et al. 1999b; Marx et al. 2000) are available to stimulate the growth of lactic-acid bacteria such as Bifidobacteria and Lactobacilli. On one hand, they contribute to a decrease in digestive pH, and on the other hand, Lactobacteria have been reported to inhibit sulfate-reducing bacteria. These reasons may indicate that both RS and FOS may be of great interest for the inflamed mucosa. However, our present results showed that only the RS-supplemented diet was effective, suggesting that this effect cannot be exclusively attributable to its interaction with the luminal microflora. To explain the RS effect, it could be hypothesised that the digestive pH obtained after RS ingestion could rapidly reach a steady state, while pH obtained after FOS ingestion would be more unsteady. Moreover, it was previously shown that colonic mucin synthesis increased with butyrate (Finnie et al. 1995) and lower pH may also stimulate mucus production (Bustos-Fernandez et al. 1978). As the amount of butyrate in the caecum with the RS diet is higher than with FOS, the higher production of mucus should be one of the hypothetical factors of mucosa protection.

In conclusion, a dietary RS supply induced significant improvement in macroscopic and histological characteristics of caeco-colonic inflammatory lesions due to DSS in rats. This could therefore confirm the interest in substrates generating high levels of butyrate during their colonic fermentation in inflammatory bowel disease. According to our present results, the effect of RS could be partially explained by higher amounts of caecal SCFA and especially butyrate. The ineffectiveness of the FOS-supplemented diet underlines the importance of the type of dietary substrate. Obviously, all the substrates susceptible to be fermented producing a high proportion of butyrate are not equivalent as far as their healing properties on DSS inflammation are concerned.

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