Introduction

Epidemiological data indicate that diet is probably the most important modulator of human cancer, especially cancer of the alimentary tract. Fat and fibre are thought to be major elements in the concept of dietary factors associated with increased or decreased cancer risk. This is supported by a vast amount of (meta)epidemiological and experimental data, indicating that fat has a promoting effect and fibre an inhibitory effect on carcinogenesis (1–9). Diets rich in fruits and vegetables have decreased cancer risk. This is supported by a vast amount of evidence that dietary fibre, an association of European food manufacturers and inulin, to the group of non-digestible carbohydrates (NDO). NDO may be regarded as soluble dietary fibres as regards their compliance with a generally accepted definition of dietary fibre including both biochemical and nutritional/physiological criteria. This definition, in which the non-digestibility of foodstuffs is particularly stressed, has been proposed and is promoted by the Food Industry ad hoc working group on dietary fibre, an association of European food manufacturers in which also dietary fibre producers participate (10). GOS, along with other soluble fibres, is a fermentable substrate for anaerobic bacteria in the large intestines. The main fermentation products are the short chain fatty acids acetate, propionate and butyrate. Short chain fatty acids reduce the colonic pH, which results in precipitation of bile acids and inhibition of the formation of secondary bile acids. High concentrations of secondary bile acids have been associated with an increased colon cancer risk.

The present paper describes experiments investigating the effects of dietary cellulose and GOS on the development of dimethylhydrazine-induced colorectal cancer in rats fed low-, medium- or high-fat diets. The expected protective effects of the two types of dietary fibre used in this experiment are based on different mechanisms. The objective of this study is to find out which type of fibre confers the most effective protection against colorectal cancer and whether the protective effect is influenced by the level of dietary fat.

Materials and methods

Animals and diets

Four-hundred-and-sixty-eight male specific-pathogen-free Wistar UW rats [Crl: (WI)WU BR; Charles River Wiga, Sulzfeld, Germany], 8 weeks old, were divided into 12 groups of 39 animals each. The different groups were fed an AIN76-based diet containing low (4.51–5.19 wt%) or high (22.55–24.51 wt%) cellulose (LC or HC), or low (8.30–9.54 wt%) or high (26.34–28.63 wt%) GOS (LGOS or HGOS; kindly provided by Borculo Domo Ingredients, Borculo, The Netherlands), and low (3.45–4.51 wt%), medium (6.90–9.02 wt%) or high (14.27–16.24 wt%) fat (LF, MF or HF). The composition of the experimental diets is summarized in Table I. The diets contained about equal amounts of vitamins and minerals per unit of energy, and were prepared freshly every 2 months and stored at −20°C until use. The percentage of linoleic acid (from safflower oil, Adonis Vegetable Oils, Rotterdam, The Netherlands) was kept at a constant level in the different diets. The fat content in the MF and HF diets was increased by adding a high-oleic sunflower oil (Trisun; Contined, Bennekom, The Netherlands) to avoid a possible effect of different levels of linoleic acid on the tumorigenesis. The appearance of GOS was a syrup containing 75 wt% dry substance. The composition of the dry substance was (w/w) 58.8% GOS, 21.3% lactose, 19.3% glucose and 1.1% galactose. The syrup was mixed with water to yield a GOS syrup containing 65 wt% dry substance.

Treatment and housing

All animals were treated with 10 weekly subcutaneous injections with 50 mg/kg body wt 1,2-dimethylhydrazine (DMH; Sigma, Brussels, Belgium). The the diets containing fermentable GOS conferred a greater protection against colorectal cancer than did the diets containing non-fermentable fibre.

Abbreviations: BrdU, bromodeoxyuridine; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; HC, high cellulose; LC, low cellulose; NDO, non-digestible carbohydrates; PBS, phosphate-buffered saline; SCFA, short chain fatty acid.
The number of air changes was ~10/h. Lighting was artificial by fluorescent tubes and time switch controlled at a sequence of 12 h light, 12 h dark.

### In-life measurements

Food intake and body weight of all animals were recorded on a regular basis. The faeces production of animals fed an HC diet was markedly increased.

### Autopsy, histology and histopathology

Nine months after the start of the experiment all animals were killed by exsanguination from the abdominal aorta under ether anaesthesia. A thorough autopsy was performed. The colon was removed, cut open longitudinally, rinsed with saline and examined for the presence of neoplastic changes. The number, size and distance from the anus of all colorectal tumours were recorded. The remaining parts of the colon were collected as Swiss rolls. The weight and pH of the caecum content were recorded. The collected tissues were preserved in a neutral aqueous phosphate-buffered 4% solution of formaldehyde, embedded in paraffin wax, sectioned at 5 µm, and stained with haematoxylin and eosin. Serial sections were made whenever necessary to expose the stalk, if present, of a tumour. The collected tissues were examined microscopically and the type of the tumours (benign or malignant) was established and recorded. Microscopic classification of the tumours was done according to the criteria described by Whiteley et al. (11).

### Labelling index

Ten intraperitoneal injections with bromodeoxyuridine (BrDU; Sigma), 25 mg/kg body wt, 1 h prior to killing for future determination of the BrDU labelling index (LI). Swiss rolls of the colon and colorectal adenomas from animals treated with BrDU were stained with a monoclonal antibody against BrDU (Beckton Dickinson, Mountain View, CA) and examined by light microscopy. The BrDU staining protocol included incubation of the slides in a solution of formaldehyde, embedded in paraffin wax, sectioned at 5 µm, and stained with haematoxylin and eosin. Serial sections were made whenever necessary to expose the stalk, if present, of a tumour. The collected tissues were examined microscopically and the type of the tumours (benign or malignant) was established and recorded. Microscopic classification of the tumours was done according to the criteria described by Whiteley et al. (11).

### Statistical analysis

The multiplicity, size and distance from the anus of the colorectal tumours, absolute and relative caecum weights, pH (for statistical analysis expressed as H⁺ concentration) of the caecum content, and BrDU LI were analysed using two-way analysis of variance (ANOVA) with factors fat and fibre.
Effect of diet on colorectal cancer development

Table IV. Absolute and relative weights (g ± SE) and pH of the caecum content

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<tbody>
<tr>
<td>Absolute caecum</td>
<td>2.8 ± 0.2</td>
<td>3.4 ± 0.1**</td>
<td>2.9 ± 0.2</td>
<td>3.2 ± 0.1**</td>
<td>2.0 ± 0.1</td>
<td>2.5 ± 0.1**</td>
<td>2.9 ± 0.1</td>
<td>5.5 ± 0.2**</td>
<td>2.8 ± 0.1</td>
<td>5.0 ± 0.3**</td>
<td>2.2 ± 0.2</td>
<td>4.6 ± 0.4**</td>
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<tr>
<td>content weight (g)</td>
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<tr>
<td>Relative caecum content weight (g/kg body wt)</td>
<td>5.2 ± 0.3</td>
<td>6.7 ± 0.3**</td>
<td>5.6 ± 0.6</td>
<td>6.4 ± 0.3**</td>
<td>3.6 ± 0.2</td>
<td>5.1 ± 0.4**</td>
<td>5.3 ± 0.3</td>
<td>10.4 ± 0.4**</td>
<td>5.2 ± 0.4</td>
<td>9.2 ± 0.4**</td>
<td>4.1 ± 0.3</td>
<td>9.0 ± 0.6**</td>
</tr>
<tr>
<td>pH</td>
<td>6.5</td>
<td>6.5</td>
<td>6.6</td>
<td>6.4</td>
<td>6.4</td>
<td>6.3</td>
<td>5.8**</td>
<td>6.2</td>
<td>5.8**</td>
<td>6.2</td>
<td>5.8**</td>
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</tr>
</tbody>
</table>

LF, MF and HF, low, medium and high fat; LC and HC, low and high cellulose; LGOS and HGOS, low and high galacto-oligosaccharide.

The absolute and relative weight of the caecum content were inversely related to the fat content of the diets (P < 0.01). The fat content of the diets did not influence the pH of the caecum content. The caecum pH in animals fed the HGOS diets was statistically significantly decreased (P < 0.01). Statistics: two-way analysis of variance (ANOVA) with factors fat and fibre.

Table V. Incidence (% tumour-bearing animals) of colorectal tumours after 9 months

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</thead>
<tbody>
<tr>
<td>Adenomas</td>
<td>49</td>
<td>68</td>
<td>67</td>
<td>71</td>
<td>79*</td>
<td>92*</td>
<td>92*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinomas</td>
<td>60</td>
<td>46*</td>
<td>69</td>
<td>39*</td>
<td>79</td>
<td>97*</td>
<td>94*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total tumours</td>
<td>77</td>
<td>86</td>
<td>92</td>
<td>79</td>
<td>97*</td>
<td>100</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

LF, MF and HF, low, medium and high fat; LC and HC, low and high cellulose; LGOS and HGOS, low and high galacto-oligosaccharide.

The incidence of adenomas is the percentage of adenoma-bearing animals with or without carcinoma. The incidence of carcinomas is the percentage of carcinoma-bearing animals with or without adenoma. The incidence of total tumours is the percentage of animals bearing adenoma or carcinoma or both. In the cellulose groups the incidences of adenomas and total tumours were significantly increased with an increasing fat content of the diet (P < 0.05). In the HGOS groups an HF diet resulted in an increased incidence of carcinomas (P < 0.05). A HC diet increased the incidence of adenomas and resulted in a significant decrease of the carcinoma incidence (P < 0.05), whereas no effect on total tumour incidence occurred. The incidence of tumours was generally decreased in the HGOS-groups when compared with the LGOS-groups, although the differences were not statistically significant. Statistics: Pearson \( \chi^2 \) test.

Levene’s test was used to test whether variances among the groups were homogeneous. If Levene’s test indicated homogeneous variances, the groups were compared by a one-way ANOVA for equal variances, followed if significant by pooled variance \( t \)-tests. If Levene’s test indicated heterogeneous variances, the groups were compared by a one-way ANOVA for unequal variances, followed if significant by separate variance \( t \)-tests. Tumour incidences were analysed using Pearson \( \chi^2 \) test. All statistical tests were performed using BMDP Statistical Software (12). A probability value of \( P < 0.05 \) (two-tailed) was used as the critical level of significance.

Results

Food consumption/energy intake/terminal body weight

The experimental diets had different energy contents. Consequently, the food consumption slightly varied among the groups, since rats eat in accordance with their caloric needs (13). Although the food consumption, as anticipated, was inversely related to the energy content of the different diets, the calculated energy intake still showed marginal differences between the groups, ~10% or less (Table II). As a result, the terminal body weights were also slightly different and in accordance with the calorie consumption (Table II).

Faeces production

The mean faeces production per animal was markedly increased in the animals fed HC (Table III). The lowest production was measured in the HF/HGOS group, whereas the other groups produced intermediate amounts of faeces. The faeces resulting from the cellulose diets was light coloured, whereas the faeces of the HGOS-fed animals was dark.

It has previously been reported that feeding oligosaccharides or non-starch polysaccharides may lead to diarrhoea (14). In the present study, the consistency of the faeces was slightly softer in the HGOS groups and normal in all other groups. Diarrhoea did not occur.

Caecum weight and pH

The caecum weights and pH are summarized in Table IV. The absolute and relative weight of the caecum content were inversely related to the fat content of the diets (P < 0.01). HC- and HGOS-fed animals showed a markedly enlarged caecum in comparison with LC- and LGOS-fed animals, respectively (P < 0.01). The fat content of the diets did not influence the pH of the caecum content. The pH in animals fed the cellulose-diets varied from 6.4 to 6.6. The HGOS-diets resulted in a slightly lower pH, whereas the caecum pH in animals fed the HGOS diets was statistically significantly decreased (P < 0.01).

Incidence of colorectal tumours

The tumour yield was high with a mean incidence of 89%. The incidence of tumours per diet group is presented in Table V. The incidence of adenomas is the percentage of adenoma-bearing animals with or without carcinoma. The incidence of carcinomas is the percentage of carcinoma-bearing animals with or without adenoma. The incidence of total tumours is the percentage of animals bearing adenoma or carcinoma or both. In the cellulose groups the incidences of adenomas and total tumours were significantly increased with an increasing fat content of the diet (P < 0.05). In the HGOS groups an HF diet resulted in an increased incidence of carcinomas (P < 0.05). A HC diet increased the incidence of adenomas and resulted in a significant decrease of the carcinoma incidence (P < 0.05), whereas no effect on total tumour incidence occurred. The incidence of tumours was generally decreased in the HGOS-groups when compared with the LGOS-groups, although the differences were not statistically significant. Statistics: Pearson \( \chi^2 \) test.
Fig. 1. The multiplicity of the tumours, expressed as the mean number (± SEM) of tumours per tumour-bearing animal. The HC diets resulted in an increase of the multiplicity of adenomas (P < 0.01) and total tumours (P < 0.01), but in a decrease of the carcinoma multiplicity (P < 0.01). In the HGOS-fed animals the multiplicity of adenomas, carcinomas (P < 0.01) and total tumours (P < 0.01) was significantly decreased. LF, MF and HF, low, medium and high fat; LC, HC, low, high cellulose; LGOS, HGOS, low, high galacto-oligosaccharide. Statistics: analysis of variance with factors fat and fibre.

Table VI. Size (mm ± SE) of colorectal tumours

<table>
<thead>
<tr>
<th>Diets</th>
<th>Adenomas</th>
<th>Carcinomas</th>
<th>Total tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF/LC</td>
<td>2.8 ± 1.0</td>
<td>3.3 ± 1.6</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>MF/LC</td>
<td>7.6 ± 1.4</td>
<td>5.4 ± 0.6*</td>
<td>8.2 ± 1.9</td>
</tr>
<tr>
<td>HC/LC</td>
<td>4.9 ± 0.9</td>
<td>4.4 ± 1.3*</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>LF/LGOS</td>
<td>2.8 ± 1.0</td>
<td>3.3 ± 1.6</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>MF/LGOS</td>
<td>7.6 ± 1.4</td>
<td>5.4 ± 0.6*</td>
<td>8.2 ± 1.9</td>
</tr>
<tr>
<td>HGOS/HGOS</td>
<td>4.9 ± 0.9</td>
<td>4.4 ± 1.3*</td>
<td>6.0 ± 1.4</td>
</tr>
</tbody>
</table>

LF, MF and HF, low, medium and high fat; LC and HC, low and high cellulose; LGOS and HGOS, low and high galacto-oligosaccharide.
The mean size of carcinomas and total tumours was significantly (P < 0.05) decreased in the HC- and HGOS-fed animals when compared with the LC- and LGOS-fed animals, respectively. Statistics: two-way analysis of variance (ANOVA) with factors fat and fibre.

adenoma. The incidence of total tumours is the percentage of animals bearing adenoma or carcinoma or both. In the cellulose groups the incidences of adenomas and total tumours were significantly increased with an increasing fat content of the diet (P < 0.05). The carcinoma incidence was also elevated, but the increase did not reach the level of statistical significance. An HC diet increased the incidence of adenomas and resulted in a significant decrease of the carcinoma incidence (P < 0.05), whereas no effect on total tumour incidence occurred. In the GOS groups an HF diet resulted in an increased incidence of carcinomas (P < 0.05), but not of adenomas or total tumours. The incidence of tumours was generally decreased in the HGOS-groups when compared with the LGOS-groups, although the differences were not statistically significant.
Effect of diet on colorectal cancer development

Table VII. BrdU labelling index in normal colonic crypts and adenomas in animals fed GOS-diets

<table>
<thead>
<tr>
<th>Groups</th>
<th>Labelling index (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal crypts</td>
</tr>
<tr>
<td>LF/LGOS</td>
<td>13.88 ± 1.30a</td>
</tr>
<tr>
<td>LF/HGOS</td>
<td>14.17 ± 1.64a</td>
</tr>
<tr>
<td>HF/LGOS</td>
<td>16.47 ± 1.23b</td>
</tr>
<tr>
<td>HF/HGOS</td>
<td>21.70 ± 3.37b</td>
</tr>
</tbody>
</table>

LF and HF, low and high fat; LGOS and HGOS, low and high galacto-oligosaccharide.
The labelling index in normal crypts and in adenomas was increased in animals fed a HF diet.

a,b Values with different superscripts are statistically significantly different (P < 0.05). Statistics: two-way analysis of variance (ANOVA) with factors fat and fibre.

**Multiplicity of colorectal tumours**
The multiplicity of the tumours, expressed as the mean number of tumours per tumour-bearing animal, is presented in Figure 1. When compared with the LF diets, the HF diets resulted in increased multiplicity of adenomas (P < 0.01) and total tumours (P < 0.01) in the cellulose-fed animals. The HC diets resulted in a significant increase of the multiplicity of adenomas (P < 0.01) and total tumours (P < 0.01), and in a significant decrease of the carcinoma multiplicity (P < 0.01). In the HGOS-fed animals the multiplicity of adenomas, carcinomas (P < 0.01) and total tumours (P < 0.01) was significantly decreased.

**Size of colorectal tumours**
The mean size of adenomas, carcinomas and total tumours are presented in Table VI. The amount of cellulose or GOS in the diets did not influence the mean size of the colorectal adenomas found at autopsy. However, the mean size of carcinomas and total tumours was significantly (P < 0.05) decreased in the HC- and HGOS-fed animals when compared with the LC- and LGOS-fed animals, respectively. The amount of dietary fat did not affect the size of the tumours.

**Location of colorectal tumours**
Most tumours were found in the distal two-thirds of the colon. The location of carcinomas was slightly more al than the location of the adenomas in all groups. In general, the location of the tumours was not influenced by the amount of dietary fat, nor by the type or amount of dietary fibre.

**Labelling index (LI)**
The LI measured in normal colonic crypts and in adenomas are presented in Table VII. The LI in the adenomas was considerably higher than in the normal colonic crypts. The LI in normal colon crypts was slightly higher in animals fed an HGOS diet compared with animals fed an LGOS diet, but the difference was not statistically significant. An HF diet resulted in a statistically significantly increased LI in colon crypts when compared with an LF diet. In the adenomas the LI in the HF groups was higher than in the LF groups, and in the HGOS groups lower than in the LGOS groups, but these differences were not statistically significant.

**Discussion**
This paper describes the effect of dietary cellulose and GOS on the development of chemically induced colorectal cancer in rats fed diets with different levels of fat. The most important finding was that GOS was highly protective against the development of colorectal tumours, as was demonstrated by an inhibitory effect on tumour incidence, multiplicity and size, irrespective of the fat content of the diet.

GOS is readily fermented in the caecum. The main fermentation products are short chain fatty acids (SCFA), such as acetate, propionate and butyrate, which are responsible for the observed decreased caecal pH. At a low pH the formation of secondary bile acids, which are cytotoxic and are thought to enhance carcinogenesis, is inhibited and their solubility is decreased (15–17). Butyrate has been shown to suppress colorectal tumour formation (18), to protect the colonic epithelium from dysplastic change, and to stimulate cell differentiation, induce apoptosis, and decrease proliferation of colonic cells in vivo and in vitro (19–24). The BrdU LI in normal colonic crypts was slightly increased in animals fed an HGOS diet. However, in adenomas from HGOS-fed animals the LI was lower when compared with those from LGOS-fed animals, which points to an inhibitory effect of GOS on proliferation of tumour cells, most probably via the formation of short chain fatty acids. Gibson et al (22) also has found that normal colonic cells may react differently to butyrate than colonic tumour cells. Other investigators have reported that oligosaccharides may promote the growth of beneficial gut microflora, such as bifidobacteria and lactobacillus (25,26). This phenomenon may also, at least in part, be responsible for the protective effect of GOS. In this study, GOS has been shown to behave like a fibre and perfectly fits in the broader definition of dietary fibre.

The faeces production was markedly increased in the animals fed HC diets, when compared with the other groups. This can be explained by the fact that cellulose leaves the body practically unaltered. Moreover, since cellulose contributes very little to the energy content of the diet, the animals have to eat more to meet their calorific requirements. Therefore, diets with an HC content result in enlargement of the caecum and faeces bulking. This is clearly demonstrated by comparison of the mean food consumption and faeces production in the LF/LC group with those of the LF/HC group (Tables II and III). In the LF/HC group both the mean daily food consumption and the daily faeces production were 3 g higher than in the LF/LC group. Faeces bulking is one of the mechanisms thought to have a protecting effect in colorectal carcinogenesis. The protecting effect is ascribed to binding and dilution of carcinogens, and by shortening of the faecal transit time (27). Although faeces bulking was a convincing result in the present study, it did not seem to have a protecting effect on the development of colorectal tumours. This was rather surprising since previous studies have shown an inhibitory effect of cellulose on the development of colorectal cancer (28–30). The multiplicity data, however, indicate that the development of carcinomas was inhibited in the HC-fed rats. Some investigators state that most carcinomas arise de novo from the mucosa (31), the work of others support the hypothesis of an adenoma–carcinoma sequence (32). Carcinomas probably develop in both ways. Nevertheless, the fact that in the present study the multiplicity of adenomas in the HC fed animals was increased suggests that the decreased multiplicity of carcinomas may have been due to an inhibited formation of carcinomas from adenomas. The decreased size of carcinomas and total tumours in HC-fed animals is also indicative for an inhibitory effect on the growth of these tumours.

Unlike in HC-fed animals, the enlargement of the caecum in HGOS-fed animals most probably occurs mainly because of...
water-binding properties of GOS. Resorption of the water in the colon results in normal amounts of faeces in these rats.

The promotional effect of dietary fat on carcinogenesis has been widely recognized and was confirmed in the present study. This effect is partly ascribed to the high caloric density of fat, because animal experiments have clearly shown a significant reduction of tumour formation (33,34) and development of pre-neoplastic colonic aberrant crypt foci (35) in animals kept on a calorie restricted diet. Other mechanisms may also play a role, for instance, dietary fat may modulate the activation of protein kinase C (36) or elevate the levels of secondary bile acids (37). Although the differences in caloric intake between the experimental groups were quite small, they cannot completely be discounted as factors responsible for the lower cancer rate in the HGOS fed animals. However, the animals fed a HC diet had the same reduced caloric intake, but in these animals the cancer rate was unaffected or even slightly enhanced. Therefore, the reduced cancer rate in the HGOS fed animals cannot be solely ascribed to a reduced caloric intake.

From the results of the present study it may be concluded that dietary cellulose, despite marked faeces bulking, either had no effect or an enhancing effect on the formation of colorectal tumours, although the development of carcinomas was decreased, possibly through an inhibited formation of carcinomas from adenomas. Furthermore, it can be concluded that GOS is highly protective against the development of colorectal tumours, as was demonstrated by an inhibitory effect on tumour incidence, multiplicity and size, regardless of the fat content of the diet. GOS may be a valuable functional food with a high potency to decrease the risk to develop colorectal cancer.

Acknowledgement

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References


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