British Journal of Nutrition (2009), **101**, 1171–1177
doi:10.1017/S0007114508056031

Interactive and individual effects of dietary non-digestible carbohydrates and oils on DNA damage, SCFA and bacteria in the large bowel of rats

Michael A. Conlon* and Anthony R. Bird

CSIRO Human Nutrition, PO Box 10041, Adelaide BC, SA 5000, Australia

(Received 8 June 2008 – Revised 16 July 2008 – Accepted 18 July 2008 – First published online 12 September 2008)

Dietary non-digestible carbohydrates (NDC) play an important role in large-bowel health and one form of NDC, resistant starch (RS), can promote low levels of DNA damage and other markers of colonic health. The objective of the present study was to determine whether the ability of dietary RS or other NDC to influence colonic health, particularly DNA damage, is dependent on the type of dietary oil. We compared the effects of diets containing 10 % of NDC from cellulose, wheat bran, high-amylose maize starch (HAS, a rich source of RS type 2) or a retrograded HAS (RHAS, a rich source of RS type 3) on DNA damage, SCFA production and bacterial changes in the large bowel of rats. Each carbohydrate source was combined with 10 % fish oil (FO) or Sunola™ oil (SO; rich in oleic acid). There was a significant interaction between NDC and oil treatments on single-strand DNA breaks in colonocytes isolated from the colon. The damage in rats consuming RHAS was greater for FO consumption than for SO consumption. There was a significant interaction between NDC and oils on caecum weights and treatment effects of NDC and oils were observed for the weights and lengths of other gut tissues. Significant differences were found in colonic SCFA pools and caecal numbers of lactobacilli, bifidobacteria, *Escherichia coli* and *Bacteroides fragilis* with the various NDC and oil treatments. The present results demonstrate that the effects of NDC and oils, particularly on colonic DNA damage, can depend on how they are combined within the diet.

Non-digestible carbohydrates: Resistant starch: Fish oil: Colonic DNA damage

Diet has a large impact on the health of the large bowel. Considerable experimental and epidemiological evidence indicates that dietary complex carbohydrates promote bowel function and are associated with lower risk of diseases such as colorectal cancer (CRC)[1–3]. The non-starch polysaccharides (NSP) and resistant starch (RS), starch which is not digested in the small intestine and reaches the large bowel, play important roles in this regard. These non-digestible carbohydrates (NDC), often regarded as fibre, act largely by increasing faecal bulk and reducing faecal transit time or by increasing fermentation leading to the production of SCFA, especially butyrate. Butyrate is the primary fuel of colonocytes and helps maintain colonic tissue integrity by inducing apoptosis of cells with extensive DNA damage[1,4]. Unrepaired DNA damage can lead to cancer under the right conditions[5,6]. Several recent experiments in rats have shown that dietary RS in the form of high-amylose starch (HAS) can protect against increased colonic DNA strand breaks induced by diets high in protein from various sources[7–10]. However, it has not yet been established whether various forms of dietary RS and NSP differ in their effects on colonic DNA damage and some other markers of bowel health, and importantly whether any differences might be influenced by other dietary factors. As RS and NSP can vary considerably in their capacity to undergo fermentation and hence produce SCFA in the large bowel[1], this may translate to a difference in capacity to prevent or repair DNA damage in the colon.

One of the dietary factors that may influence NDC effects in the large bowel is the source of oil, but few studies have examined the interactions between these components. Previous studies in rats suggest that fish oil (FO), which is rich in *n*-3 PUFA, can influence the actions of fermentable substrates relative to some other oils in terms of their capacity to protect against oxidative DNA damage and colon cancer induced by a chemical carcinogen[11,12]. However, none have examined the interactive effects of these factors, particularly RS, on DNA damage in the colon in the absence of a chemical carcinogen. The objective of the present study was to determine whether different types of dietary RS and NSP, all forms of NDC, have different effects on colonic DNA strand breakage, SCFA production and bacterial populations in a rat model and whether these effects are influenced by the type of oil present in the diet. The present study should increase our understanding of the interactions between dietary components and will produce information important for optimising diets that can potentially lower the risk of large-bowel disease.

Experimental methods

Animals and diets

Male Sprague–Dawley rats (*n* 64) were obtained at 3 weeks of age from the Murdoch University Animal Resource Centre (Perth, WA, Australia), and housed throughout the study in

Abbreviations: FO, fish oil; HAS, high-amylose starch; NDC, non-digestible carbohydrates; RHAS, retrograded high-amylose starch; RS, resistant starch; SO, Sunola™ oil.

* **Corresponding author:** Dr Michael A. Conlon, fax +61 8 8303 8899, email michael.conlon@csiro.au

wire-based cages in a room maintained at 23°C and with a 12 h– 12 h light and dark cycle. The rats were acclimatised to their environment for approximately 1 week before the commencement of the experimental diets. The rats had free access to food and water at all times. The use of animals in the present study was approved by the CSIRO Human Nutrition Animal Ethics Committee.

The composition of experimental diets, which are modifications of the AIN-93 diet[13], is detailed in Table 1. The diets were prepared in a powdered form and kept at 4°C until use and contained 14 mg/kg *tert*-butylhydroxyquinone as an antioxidant. The levels of fibre, protein, simple sugars, total starch and moisture in the cellulose, wheat bran, Hi-maize™ and Novelose™260 were evaluated using previously described methods[14,15] and are presented in Table 2. The diets contained equivalent amounts (w/w or v/w) of protein (20 %), digestible starch (45 %), oil (10 %), sugars (10 %) and NDC (10 %). The diets were formulated to contain 10 % (w/w) of NDC by inclusion of cellulose (Sigma-Aldrich Co., St Louis, MO, USA), wheat bran (Ben Furney Flour Mills, Dubbo, NSW, Australia), Hi-maize™ (HAS, classified as RS type 2, from Starch Australasia, Tamworth, NSW, Australia) or Novelose™260 (retrograded HAS (RHAS), classified as RS type 3, obtained as a gift from National Starch and Chemical Co., Manchester, UK). The diets contained 10 % (v/w) oil as either tuna FO (HiDHA®; Clover Corporation Ltd, Sydney, NSW, Australia) or Sunola™ oil (SO, 80 % oleic acid, 10 % linoleic acid and 10 % stearic acid; Goodman Fielder Food Services Ltd, Macquarie Park, NSW, Australia). The four NDC treatments were tested with FO as well as with SO, a total of eight dietary treatments. The diets all contained equivalent amounts of components. The rats with an average weight of 92 ± 11 g were randomly distributed into eight treatment groups (*n* 8) and given the experimental diets for 6 weeks, after which they were killed by an intraperitoneal injection of pentobarbitone sodium (Nembutal; Rhone Merieux Australia Pty Ltd, Pinkenba, QLD, Australia) at 60 mg/kg body weight. The tissues and contents of the small intestine, caecum and large intestine were removed immediately for analyses.

Analysis of colonic DNA damage

A 6 cm segment of the colon was removed from each rat at a point 3 cm from the most distal end of the colon, and colonocytes were isolated immediately for the measurement of DNA strand breaks using the single-cell gel electrophoresis (comet) assay as described previously[7]. The viability of the colonocytes was measured using the trypan blue exclusion method and 100 cells per slide were counted on a haemocytometer. Colonocyte viability was shown to be greater than 85 %. During electrophoresis under alkaline conditions, a DNA 'tail' emanates from cells embedded in agarose coated on slides. The length of the tail is related to the extent of DNA fragmentation. Comet tail moment is the product of the tail length and the fraction of DNA in the tail and was calculated for fifty cells from each of the three slides per rat using Scion Image Beta 4.02 image processing and analysis software (Scion Corp., Frederick, MD, USA) utilising a public domain macro[16]. Apoptotic and necrotic cells were excluded from analysis based on their morphology.

SCFA and bacterial analyses

The pools of acetate, butyrate, propionate and the total SCFA (including minor SCFA) were determined in the colonic digesta, as described previously[17]. Caecal numbers of selected bacterial types were determined from a 0·5 g aliquot of caecal content that was diluted to 5 ml with sterile distilled water and stored at −20°C until analysis by the culture methods described previously[18].

Statistical analyses

Data are presented as the mean with its standard error. The data were analysed by two-way ANOVA followed by Tukey's test to compare the differences and interactions between treatments, or by regression analysis, using a SigmaStat statistical software program (SigmaStat 2.0 for Windows; SPSS Inc.,

Table 1. Composition of experimental diets*†

	Sunola™ oil				Fish oil			
Ingredient	C	W	H	N	C	W	H	N
Sunola™ oil	100	100	100	100	–	–	–	–
Fish oil	–	–	–	–	100	100	100	100
α-Cellulose	100	–	–	–	100	–	–	–
Wheat bran	–	238	–	–	–	238	–	–
Hi-maize™	–	–	223	–	–	–	223	–
Novelose™	–	–	–	202	–	–	–	202
Maize starch	449·5	373·5	326·5	347·5	449·5	373·5	326·5	347·5
Casein	200	150	200	200	200	150	200	200
Sucrose	100	88	100	100	100	88	100	100
Mineral mix	35	35	35	35	35	35	35	35
Vitamin mix	10	10	10	10	10	10	10	10
L-Cystine	3	3	3	3	3	3	3	3
Choline	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5

* Ingredients are expressed as g/kg of diet and based on the AIN-93 formulation.
† Abbreviations denoting dietary non-digestible carbohydrate components are as follows: C, cellulose; W, wheat bran; H, Hi-maize™ (HAS); N, Novelose™260 (RHAS).

Table 2. Composition of dietary sources of non-digestible carbohydrates*

Component	Cellulose	Wheat bran	Hi-maize™ (HAS)	Novelose™ (RHAS)
Digestible starch	0·0	17·1	44·3	41·2
Sugars	0·0	5·0	0·0	0·0
Protein	0·0	17·3	0·0	0·0
Fat	0·0	3·2	0·0	0·0
Total dietary fibre	96·4	42·0	44·7	49·5
Ash	0·0	5·0	0·0	0·0
Moisture	3·6	10·4	11·0	9·3

HAS, high-amylose starch; RHAS, retrograded high-amylose starch.
* Components are expressed as a percentage of the dietary source.

Chicago, IL, USA). The SCFA data underwent logarithmic transformation. Significance was deemed as $P < 0{\cdot}05$.

Results

Body and tissue weights

Table 3 shows the body weights at kill and the weights and lengths of gut tissues. A significant interaction between NDC and oil treatments was observed only for the weight of the caecum. The caecum weight was also significantly affected by NDC treatment, with lower weights for cellulose relative to each of the other NDC treatments. Although there was no oil treatment effect, the weight of the caecum was significantly higher for FO relative to SO when wheat bran was consumed. This effect of oil was not seen for the other NDC treatments. Indeed, the reverse trend of a higher caecum weight (not significant) with SO consumption for the HAS and, particularly, RHAS groups explains why a significant interaction between NDC and oils has occurred.

There were significant treatment effects of both NDC and oils on the SI weight. The HAS diets resulted in significantly lower SI weights than those for cellulose and wheat bran. The oil effect was due to the SO diets causing significantly lower SI weights than for FO.

The SI length was significantly affected by oil treatment but not by NDC treatment. The length of the SI was significantly shorter for the SO diets relative to the FO diets. This was especially evident for animals fed RHAS.

An effect of NDC treatment, but not oil treatment, was observed for the length of the colon. The length of the colon was significantly shorter for the HAS diets relative to the wheat bran diets. A significant difference in the colon length between the FO and SO diets was only observed when wheat bran was consumed, with FO giving the greater length.

There were no significant individual or interactive effects of treatment on the body weight or the colon weight.

Colonic genetic damage

There was a significant interaction between NDC and oil treatments on the comet assay tail moment of the colonocytes isolated from the colon ($P=0{\cdot}03$), but the separate NDC and oil effects were not significant. The tail moments of the colonocytes isolated from the rats consuming RHAS were significantly higher ($P=0{\cdot}01$) in the FO consumers relative to those eating SO (Fig. 1).

Table 3. Effects of diets varying in the sources of non-digestible carbohydrates (NDC) and oils on body weights (at kill) and the weights and lengths of the small intestine and large-bowel tissues weight described is at kill*
(Mean values with their standard errors for six to eight rats per group)

	Cellulose				Wheat bran				Hi-maize™ (HAS)				Novelose™ (RHAS)				Main effects (*P* values)		
	SO		FO		SO		FO		SO		FO		SO		FO				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	NDC	Oil	NDC × oil
Body weight (g)	404	11	396	38	383	23	370	10	367	26	329	13	377	9	396	13	0·078	0·586	0·555
SI weight (g)	$10{\cdot}32^{a,c}$	0·50	$11{\cdot}36^{a,c}$	0·71	$9{\cdot}84^{a}$	0·63	$11{\cdot}95^{b,c}$	0·35	$9{\cdot}24^{a}$	0·62	$9{\cdot}00^{a,d}$	0·36	$9{\cdot}79^{a,c}$	0·51	$10{\cdot}14^{a,c}$	0·45	0·004	0·034	0·156
Caecum weight (g)	$0{\cdot}81^{a}$	0·05	$0{\cdot}95^{a}$	0·19	$1{\cdot}14^{a}$	0·08	$1{\cdot}87^{b,c}$	0·22	$2{\cdot}60^{c,d}$	0·24	$2{\cdot}51^{c}$	0·22	$2{\cdot}43^{b,d}$	0·07	$2{\cdot}05^{b,c}$	0·18	0·001	0·409	0·016
SI length (mm)	$120{\cdot}6^{a,b}$	3·6	$123{\cdot}3^{a,b}$	3·9	$117{\cdot}3^{a}$	5·1	$123{\cdot}4^{a,b}$	1·3	$115{\cdot}8^{a}$	5·1	$120{\cdot}6^{a,b}$	3·5	$116{\cdot}8^{a}$	1·7	$131{\cdot}9^{b}$	1·1	0·256	0·002	0·205
Colon weight (g)	1·86	0·15	2·02	0·24	1·84	0·20	2·30	0·14	1·91	0·24	1·42	0·15	1·97	0·28	1·86	0·12	0·236	0·954	0·119
Colon length (mm)	$20{\cdot}1^{a,b}$	0·79	$20{\cdot}1^{a,b}$	0·85	$19{\cdot}3^{a}$	0·56	$21{\cdot}5^{b}$	0·39	$17{\cdot}7^{a}$	0·8	$18{\cdot}3^{a}$	0·77	$19{\cdot}3^{a,b}$	0·65	$19{\cdot}4^{a,b}$	0·59	0·005	0·145	0·340

SO, Sunola™ oil; FO, fish oil; SI, small intestine; HAS, high-amylose starch; RHAS, retrograded high-amylose starch.
^{a,b,c,d}Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
* Data were analysed by two-way ANOVA and *post hoc* analysis by Tukey's test.

https://doi.org/10.1017/S0007114508056031 Published online by Cambridge University Press

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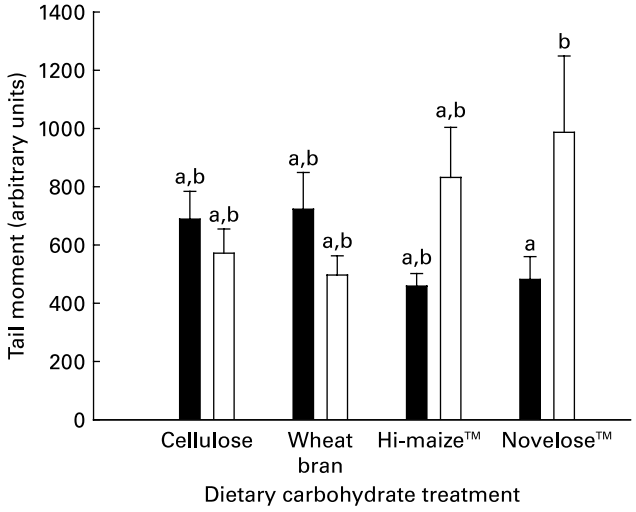

Fig. 1. Effects of diets varying in the sources of non-digestible carbohydrates and oils (▭, Sunola™ oil; ▬, fish oil) on colonic DNA damage in rats. The comet assay was used to determine the number of single-strand DNA breaks in the colonocytes extracted from the colon and the resulting comet tail moments (comet tail length × per cent DNA in the tail) are presented. Values are presented as the mean with its standard error (n 8). [a,b] Mean values with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

Colonic SCFA

Acetate, butyrate, propionate and the total SCFA pools were measured in the colonic digesta and the results are presented in Table 4. There were no interactions between NDC and oil treatments on any of the SCFA measured, nor were there any effects of oil treatment. However, there was a significant effect of NDC treatment on propionate and butyrate pools in the colon. When the rats consumed FO, the addition of RHAS to the diet resulted in significantly larger colonic butyrate pools compared to cellulose and HAS. When the rats consumed SO, butyrate pools were not significantly different between dietary NDC treatments. The propionate pool was substantially larger in the rats fed RHAS relative to the other NDC treatments.

Caecal bacteria

Effects of diets on caecal bacteria are detailed in Table 5. There were no significant interactions between NDC and oils on the numbers of caecal bacteria. However, there were significant individual effects of NDC and oil treatments. Significant effects of dietary NDC treatment were found for caecal numbers of *Escherichia coli*, *Bacteroides fragilis*, lactobacillus, bifidobacteria and total anaerobes. The effects of NDC treatment on *E. coli* and *B. fragilis* were primarily a result of significantly lower numbers in the rats given wheat bran. However, for lactobacillus, the wheat bran, HAS and RHAS treatments significantly increased the numbers relative to cellulose. For bifidobacteria and total anaerobes, both HAS and RHAS increased the numbers relative to both cellulose and wheat bran. Significant effects of oil treatment were found for *B. fragilis* and total anaerobes. For *B. fragilis*, FO significantly lowered bacterial numbers compared to SO in cellulose and wheat bran groups, whereas for total anaerobes the numbers were significantly lower in the FO diets of the HAS and RHAS groups.

Table 4. Effects of diets varying in the sources of non-digestible carbohydrates (NDC) and oils on colonic SCFA pools (mM) in rats*
(Mean values with their standard errors for six to eight rats per group)

	Cellulose				Wheat bran				Hi-maize™ (HAS)				Novelose™ (RHAS)				Main effects (P values)		
	SO		FO		SO		FO		SO		FO		SO		FO				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	NDC	Oil	NDC × oil
Acetate	21·0[a]	6·7	23·9[a]	5·3	23·1[a]	2·9	36·6[a]	7·7	21·0[a]	3·2	41·7	16·8	32·4[a]	8·2	59·3[a]	19·4	0·307	0·209	0·529
Propionate	5·5[a]	2·3	6·1[a]	1·1	4·2[a]	0·4	5·0[a]	0·6	8·5[a]	3·0	5·7[a]	0·9	12·4[a]	3·7	14·8[a]	3·2	0·048	0·154	0·782
Butyrate	2·8[a]	0·8	3·8[a]	0·6	10·8[a,b]	2·1	5·3[a,b]	1·3	8·3[a,b]	2·7	2·6	0·6	8·5[a,b]	1·8	10·3[b]	1·8	< 0·001	0·254	0·360
Total SCFA	30·1[a]	10·4	33·8[a]	7·0	38·5[a]	2·5	50·7[a]	10·3	56·2[a]	21·4	28·7[a]	4·2	53·4[a]	14·1	83·8[a]	23·2	0·134	0·282	0·498

SO, Sunola™ oil; FO, fish oil; HAS, high-amylose starch; RHAS, retrograded high-amylose starch.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
*Data were analysed by two-way ANOVA and *post hoc* analysis by Tukey's test.

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Table 5. Effects of diets varying in the sources of non-digestible carbohydrates (NDC) and oils on the populations of caecal bacteria (log_{10} colony-forming units per g caecal content) in rats*
(Mean values with their standard errors for six to eight rats per group)

	Cellulose				Wheat bran				Hi-maize™ (HAS)				Novelose™ (RHAS)				Main effects (*P* values)		
	SO		FO		SO		FO		SO		FO		SO		FO				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	NDC	Oil	NDC × oil
Escherichia coli	7·1[a,c]	0·2	6·4[b,c]	0·3	5·7[b]	0·2	5·9[b]	0·3	7·1[a]	0·2	6·9[a,c]	0·2	6·7[a,c]	0·2	6·7[a,c]	0·3	<0·001	0·264	0·250
Bacteroides fragilis	6·4[a]	0·3	5·4[b]	0·2	5·5[b]	0·3	4·5[c]	0·2	6·5[a,b]	0·3	6·2[a,b]	0·2	6·0[a,b]	0·3	6·1[a,b]	0·3	0·006	0·004	0·127
Lactobacilli	8·1[a]	0·1	8·3[a]	0·1	8·7[a,b]	0·1	8·5[a,b]	0·1	8·7[a,b]	0·1	8·7[a,b]	0·1	8·9[b]	0·1	8·9[b]	0·1	<0·001	0·955	0·324
Bifidobacteria	7·4[a]	0·2	7·8[a]	0·2	7·9[a]	0·3	8·5[a]	0·2	9·2[b,c]	0·1	8·9[a,c]	0·3	9·4[b,c]	0·2	8·7[a,c]	0·4	<0·001	0·282	0·498
Total aerobes	7·4	0·2	8·3	0·2	8·3	0·2	7·4	0·3	7·9	0·3	7·7	0·1	7·1	0·2	8·1	0·2	0·763	0·649	0·268
Total anaerobes	9·5[a,c]	0·1	9·2[a,c]	0·1	9·3[a]	0·1	9·1[a]	0·1	10·0[b]	0·1	9·6[c]	0·2	9·9[b]	0·1	9·6[c]	0·1	<0·001	<0·001	0·872

SO, Sunola™ oil; FO, fish oil; HAS, high-amylose starch; RHAS, retrograded high-amylose starch.
[a,b,c] Mean values with unlike superscript letters were significantly different ($P < 0·05$).
*Data were analysed by two-way ANOVA and *post hoc* analysis by Tukey's test.

Discussion

Relatively few studies have investigated the interaction between dietary NDC and oils, both significant components of the human diet, on gastrointestinal health. One study[12] has shown that the type of oil can influence the capacity of the fermentable fibre pectin to influence oxidative damage in the colon of rats. Another study[19] found no evidence of interactions between dietary NDC (cellulose and HAS) and oils (including FO) on agonist-induced contractility in the ileum of rats, but did find interactions between these components on colonic SCFA concentrations. However, we are not aware of any studies involving RS that have looked at such interactions with respect to colonic DNA damage. In the present study, we have demonstrated that the ability of dietary NDC, especially RS, to modulate colonic DNA damage and other gut-related variables in rats is dependent on the type of oil present.

In the present study, we have used the single-cell gel electrophoresis assay, also known as the comet assay, to measure single-strand DNA breaks. This assay has been increasingly used as a valuable means of detecting and measuring genotoxicity *in vivo* in response to various agents. One extensive study[20] concluded that the assay performed well in detecting a large array of genotoxic carcinogens in the organs of the mouse, and a number of studies have also used the assay for biomonitoring of genotoxic agents in human subjects[21]. Many of these studies have measured single-strand breaks and obtained meaningful measures of genotoxicity in tissues. While it is generally accepted that DNA damage is an important early event in carcinogenesis, the link between single-strand breaks and carcinogenesis is not clear. Other forms of damage to DNA, such as double-strand breaks, may pose more risk[22]. However, increases in the numbers of single-strand breaks can be viewed as an indicator of a more genotoxic environment. In the context of the present study, differences in the numbers of single-strand breaks are likely to reflect the differences in the genotoxicity of the colonic environment and hence the differences in the genotoxic potential of the diets. The comet assay has been used previously to demonstrate that faecal water from human subjects on diets considered a high risk for colorectal cancer can increase the levels of DNA damage when added to cultures of cells derived from the colon[23,24]. The present data, using cells extracted from the colon of rats undergoing dietary intervention, suggest that the genotoxicity of the colonic luminal environment may be significantly influenced by the type of NDC and oils in the diet. Future *in vivo* studies examining other colonic DNA damage events such as double-strand breaks and oxidative damage will be useful in understanding the extent to which these dietary components influence large-bowel health.

We observed significantly lower levels of DNA damage in rats consuming RHAS in combination with SO than in rats consuming RHAS with FO. Previous studies in rats showed that increased levels of dietary HAS lead to reduced levels of DNA damage in the colon, probably via an associated increase in the levels of butyrate due to greater availability of RS for fermentation[7–10]. However, in the present study, it was not possible to demonstrate that the source of oils influenced the amount of RS available to the colon for fermentation. There was no obvious relationship between the DNA damage levels observed and the colonic butyrate pools, suggesting that production of this SCFA alone cannot explain

the differences between DNA damage induced by the various treatments. Nevertheless, it is possible that caecal measures of SCFA, which were not carried out in the present study, may have enabled a more accurate picture of the relationship between butyrate production and DNA damage, as a large percentage of butyrate is likely to have been removed for use by the colon and a remnant remains in the colonic digesta.

Numerous experimental studies have been carried out in animals to gain an understanding of the effects of the diet on the health of the gut, especially in relation to CRC initiation and treatment. These studies have generally used the carcinogen azoxymethane to induce tumours. The present data are consistent with one such study by Coleman *et al.* [11], who demonstrated that a FO/HAS combination resulted in a 27 % larger number of aberrant crypt foci (tumour precursors) than for a FO/cellulose combination in the azoxymethane-treated rats. In the present study, there was a 45 % greater comet tail moment of the extracted colonocytes when compared to the same dietary treatments. This suggests that diet-induced changes in the colonic environment may have similar effects on factors influencing tumour initiation and progression by a chemical carcinogen as they do on factors influencing DNA damage in the absence of a chemical carcinogen. Another study of particular relevance to the present study is that by Sanders *et al.* [12] They compared the effects of dietary cellulose and pectin (a fermentable fibre) with either FO or maize oil on oxidative DNA damage, apoptosis and reactive oxygen species (ROS) in the colonic cells of rats treated with azoxymethane. The FO/pectin combination appeared most effective in increasing ROS and lowering oxidative DNA damage, and this was linked to an increase in apoptosis levels. Our highly fermentable substrates were HAS and RHAS. There was a significantly higher level of DNA damage in the colonocytes from the RHAS/FO combination in the present study than that of the RHAS/SO combination. This suggests that FO has hindered the ability of RHAS to reduce DNA damage. This is not what might have been predicted from the Sanders *et al.* study. However, the effects of a pectin/FO combination need to be used to make a true comparison. A measure of apoptosis and ROS in future studies would also reveal whether the same mechanisms are at work.

The pattern of increases in the weight of the caecum and the length of the colon that we observed in the present study reflects the response to the greater large-bowel digesta masses and fermentation products that are generated as dietary fermentable substrate increases[1]. That is, these weights and lengths were lowest with dietary cellulose, a poorly fermented substrate, and highest with dietary HAS and RHAS, which are the most fermentable. Neither of these variables was significantly affected by oil. Increased colonic fermentation associated with substrates such as RS also has significant effects on the composition of the gut microflora, tending to increase bacteria associated with a healthy bowel and tending to decrease those that are potentially harmful. In the present study, we have shown that lactobacilli and bifidobacteria, which are generally considered beneficial, are increased in number in the caecum with the RS diets relative to those of cellulose and wheat bran. However, *E. coli*, often implicated in gastrointestinal infections, was higher in number for the RS treatments and cellulose compared to wheat bran. A similar effect was seen for *B. fragilis*. Overall, oils had little effect on the specific types of bacteria examined, although for *B. fragilis*, FO caused a significant lowering of numbers when cellulose and wheat bran were consumed. Nevertheless, FO caused an overall lowering of total anaerobe numbers. Further studies will be needed to clarify the specific effects that FO is having on the large-bowel bacterial populations, and given the likelihood that FO is protective against CRC, this will be important in helping to understand the mechanisms behind that protection.

Interactions between dietary NDC and oils were observed for colonic DNA damage and also the weight of the caecum. The means by which oil influences the bowel health effects of dietary NDC are not known. Indeed, our understanding of the physiological processes important for maintaining colorectal health is still generally poor. The different responses to oils we have observed with the various NDC could relate to their different physico-chemical characteristics. When the broad pattern of effects of the diet on DNA damage is considered (Fig. 1), the HAS and RHAS diets were similar in the way they interacted with FO and SO, but different from the way cellulose and wheat bran interacted. The latter diets deliver less fermentable material to the large bowel and the effects of oils on colonic DNA damage could be altered by the acidic environment induced by fermentation or by changes in the physical properties of the digesta. A recent study has demonstrated that a combination of dietary HAS and FO results in higher ileal contractility than cellulose and FO, and combinations of SO with HAS and cellulose, in rats. The changes in the contractility of the gastrointestinal tract are expected to substantially alter transit of the digesta and the contact between toxic agents and colorectal tissues. It is possible that interactions between oils and NDC on DNA damage in the present study might be explained, at least in part, by effects stemming from differences in the digesta transit rate and consequent delivery of dietary substrates to the large bowel.

In summary, we have shown that the capacity of NDC to induce DNA single-strand breaks and influence other markers of health in the colon of rats is dependent on the type of oil included in the diet. It will be important to gain a deeper understanding of these interactions given that DNA damage or modification is an accepted prerequisite for the development of cancer.

Acknowledgements

The authors wish to thank Corinna Bennett, Thelma Bridle, Rhys Bushell and Michelle Vuaran for their excellent technical assistance. Both of the authors contributed to the design, management and analysis of the study presented as well as to the writing of the manuscript. The authors have no conflicts of interest to declare.

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British Journal of Nutrition