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Plasma enterolactone or intestinal *Bifidobacterium* levels do not explain adenoma formation in multiple intestinal neoplasia (Min) mice fed with two different types of rye-bran fractions

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The study was designed to evaluate whether two types of rye-bran fractions result in distinct bifidogenic effect or enterolactone production in multiple intestinal neoplasia (Min) mice and whether these parameters are associated with intestinal tumorigenesis in this animal model. The experimental diets were a non-fibre diet (control), a rye-bran diet, and diets containing either the soluble extract or the insoluble fraction prepared from rye bran. The main result on adenoma formation in these experiments was the observation that the soluble extract increased number (P=0·012) and size (P=0·008) of adenomas in the distal small intestine when compared with the non-fibre group. All rye-supplemented diets supported similarly the *in vivo* growth of *Bifidobacterium* (10^8–10^9 colony forming units/g) in Min mice, whereas the non-fibre diet lowered intestinal *Bifidobacterium* below the level of detection. The results show that water solubility does not affect the bifidogenicity of rye bran. Mean plasma enterolactone concentration was highest in the rye-bran group (30·0 nmol/l; P=0·002), which along with the soluble-extract group (16·2 nmol/l; P=0·024) differed significantly from the non-fibre diet group (7·5 nmol/l). Thus, the mice fed with the rye bran were the best enterolactone producers. In conclusion, rye bran and rye fractions influence adenoma formation in Min mice to a varying degree but plasma enterolactone levels or the production of bifidogenic bacteria do not mediate the effect.

Min mice: Rye bran: *Bifidobacterium*: Lignans: Enterolactone

Rye bran with high concentrations of fibre, phyto-oestrogens and phenolic compounds may be beneficial in the prevention of several chronic diseases including colon cancer (Bingham *et al.* 1998; Adlercreutz, 2002). It has been shown to prevent intestinal tumour formation in two studies with different animal models (Davies *et al.* 1999; Mutanen *et al.* 2000), indicating that rye could be a promising candidate as a chemopreventive food component against gastrointestinal tumorigenesis. Several mechanisms may explain the protective action of fibre-rich foods in colon carcinogenesis; for example, bulking effect, adsorption of carcinogens, and presence of phenolic compounds (such as ferulic acid) or other phytochemicals that are released from plant cells and walls inside the gut (Ferguson *et al.* 2001). Particularly in rye bran, the anti-tumour effect may involve the production of enterolactone from plant lignan precursors. This assumption is supported by studies where secoisolariciresinol diglycoside from flaxseed has been found to be protective in rat colon (Jenab & Thompson, 1996) and mammary cancer models (Thompson *et al.* 1996). Similarly, hydroxymatairesinol, which closely resembles matairesinol in rye, had an anti-tumour effect in our earlier experiment with Min mice (Oikarinen *et al.* 2000) and it has also been shown to be protective in a mammary cancer model (Saarinen *et al.* 2000, 2001). Matairesinol and secoisolariciresinol were earlier considered the only plant lignans (Mazur, 1998) that are converted to mammalian lignans in man and animals. Recently novel plant lignans that are putative mammalian lignan precursors have been found especially in rye bran. So far, the *in vitro* conversion of lariciresinol, pinoresinol and syringaresinol to mammalian lignans has been shown (Heinonen *et al.* 2001). The anti-oestrogen properties of lignans and other phyto-oestrogens have been suggested to be part of their cancer-preventive effects (Adlercreutz, 2002). Apart from their precursor role for enterolactone, plant lignans and their mammalian metabolites may act as antioxidants (Kitts *et al.* 1999).

Abbreviations: cfu, colony forming units; Min, multiple intestinal neoplasia.

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In addition, rye carbohydrates may be prebiotic as recently shown with arabinoxylan in an *in vitro* experiment (Crittenden *et al.* 2002).

In the present study two experiments were carried out to examine whether either plant lignan conversion to enterolactone or the quantity of *Bifidobacteria* in the gastrointestinal tract could explain the positive effect of rye bran observed in our previous experiment (Mutanen *et al.* 2000). Two fractions of rye bran were used, i.e. a fraction with a high content of soluble arabinoxylan (pentosan) and fructan and a fraction containing mainly insoluble arabinoxylan to investigate if the production of enterolactone or intestinal *Bifidobacteria* quantity differs between these two types of rye substrates. The animal model used was the multiple intestinal neoplasia (Min) mouse, which is a well-characterized model of intestinal tumorigenesis (Moser *et al.* 1990) having a mutation in the *Apc* gene. The loss of heterozygosity of the *Apc* gene is followed by dysregulation of cellular β-catenin degradation and the development of spontaneous intestinal adenomas with a non-invasive phenotype.

Materials and methods

Preparation and analyses of rye-bran fractions and rye bran

The insoluble fraction was prepared by washing rye bran with water after which it was air-dried. This rye fraction and also rye bran were milled through a 1 mm sieve. For the preparation of the soluble extract, the bran was first extruded and water-soluble components were extracted by the aid of xylanase (Karppinen *et al.* 2003). The soluble extract was then concentrated and freeze-dried. Moisture and ash contents were 7 and 6 % for rye bran, 11 and 4 % for the insoluble fraction, and 6 and 8 % for the soluble extract, respectively. Specific enzymic kits (Megazyme, Bray, Republic of Ireland) were used to analyse total starch, β-glucan and fructan contents of brans. Dietary fibre and pentosan were measured according to Asp *et al.* (1983) and Douglas (1981), respectively, and the protein content by the Kjeldahl method (N×6·25).

The dietary fibre and fructan contents were 34·3 and 6·1 % (on a wet weight basis) for rye bran, and 51·1 and 1·2 % for the water-insoluble fraction, respectively. The calculated amounts of indigestible carbohydrates were thus 40·4 % for rye bran and 52·3 % for the insoluble fraction. The soluble extract contained 21·2 % fructan, 26·7 % pentosan and 4·4 % β-glucan comprising 52·3 % of indigestible carbohydrates. Xylanase treatment depolymerised most of the pentosans.

Plant lignans, matairesinol, secoisolariciresinol, isolariciresinol, lariciresinol, pinoresinol and syringaresinol in the rye bran, rye fractions and diets were determined by GC-MS. The sample pre-treatment method was modified from the method published by Mazur *et al.* (1996) (T Nurmi, S Heinonen and H Adlercreutz, unpublished results). The total lignan level was highest in the soluble extract (240–280 μmol/kg) and hence in the corresponding diet (20 μmol/kg). Median values were found in the rye bran (170–180 μmol/kg) and the rye-bran diet (15 μmol/kg), and lowest in the insoluble fraction (110 μmol/kg) and in the corresponding diet (8 μmol/kg). A trace amount of secoisolariciresinol and isolariciresinol (0·4 μmol/kg) was also found in the non-fibre diet, which might have originated from sunflower-seed or rapeseed oils (Table 1).

Animal treatment and diets

The Laboratory Animal Ethics Committee of the Faculty of Agriculture and Forestry, University of Helsinki approved the study protocol. Animals were housed in plastic cages in a temperature- and humidity-controlled animal facility, with a 12 h light-dark cycle. They had free access to the semi-synthetic diets and tap water for the feeding period of 38 d (experiment 1) or 49 d (experiment 2). The body weights of the animals were recorded weekly. The groups were fed the following high-fat AIN-93G-based diets (Reeves *et al.* 1993): a non-fibre diet, a diet with 10 % (w/w) rye bran, a diet with 7·9 % (w/w) soluble extract or a diet with 7·9 % (w/w) insoluble fraction (Table 1). In all rye diets the amount of indigestible carbohydrates was adjusted to be 4 % of diet (w/w). The fat (40 % energy) used in the diets was a mixture of butter, rapeseed oil, and sunflower-seed oil. The intake of fatty acids corresponded to that in the Western-type diet. The diets were stored at −20°C, and kept at 4°C only when they were to be used within 1 week.

Experiment 1

Male C57BL/6J-Min/+ (Min) mice were obtained from the Jackson Laboratory (Bar Habor, ME) at the age of 42–49 d. The animals were stratified by body mass and assigned randomly to the experimental diets, ten to twelve mice per group, with initial body mass of 21–22 g. The diet groups were non-fibre, 10 % (w/w) rye bran, 7·9 % (w/w) soluble extract, and 7·9 % (w/w) insoluble fraction. The feeding period was 38 d.

Experiment 2

The Min pedigree was maintained at the Animal Centre, University of Helsinki by mating wild type C57BL/6J-+/+ females with Min males originally obtained from the Jackson Laboratory (Bar Habor, ME). Mice were genotyped after weaning by allele-specific polymerase chain reaction (Dietrich *et al.* 1993). Male and female Min mice were assigned randomly to the experimental diets, four male and seven female mice per group, at the age of 35–43 d. The diet groups were non-fibre, 10 % (w/w) rye bran, and 7·9 % (w/w) soluble extract. The feeding period in this experiment was 49 d. Five Min mice were fed with standard chow (Altromin 1314; Altromin GmbH, Lage, Germany) from weaning at the age of 42 d and used as controls for *Bifidobacterium* analysis.

Samples and intestinal polyp scoring

At the end of the feeding periods, the mice were killed by CO_2 asphyxiation at the age of 80–87 and 84–91 d in

Table 1. Nutrient composition of experimental diets (g/kg diet)*

Ingredient	Diet			
	Non-fibre	Rye-bran	Soluble-extract	Insoluble-fraction
Casein	236·2	213·4	222·0	215·5
Dextrose	479·0	425·8	436·3	443·8
Butter	148·9	134·4	135·7	135·0
Sunflower-seed oil	13·3	12·0	12·1	12·1
Rapeseed oil	62·2	56·2	56·7	56·4
AIN-93 mineral mix	41·6	39·9	39·9	39·9
AIN-93 vitamin mix	11·8	11·3	11·3	11·3
L-Cystine	3·6	3·5	3·5	3·5
Choline chloride	3·6	3·5	3·5	3·5
Tertiary butylhydroxyquinone	0·014	0·014	0·014	0·014
Rye supplement	–	100·0	79·0	79·0
Diet composition				
Dietary fibre and non-digestible oligosaccharides	–	40	41	41
Plant lignans (μmol/kg diet)†				
Total‡	0·4	15·4	20·5	7·5
Matairesinol	0·0	0·6	0·4	0·2
Secoisolariciresinol	0·1	0·4	0·6	0·1
Isolariciresinol	0·3	1·7	1·4	1·1
Lariciresinol	0·0	0·3	0·5	0·0
Pinoresinol	0·0	1·3	1·5	0·2
Syringaresinol	0·0	10·9	16·0	6·0

* Casein was obtained from Kainuun Osuusmeijeri (Sotkamo, Finland), dextrose from Six Oy (Helsinki, Finland), mineral and vitamin mixes from Harlan Teklad (Madison, WI), L-cystine, choline chloride and tertiary butylhydroxyquinone from Yliopiston Apteekki (Helsinki, Finland), and rye bran from Melia (Raisio, Finland). The soluble extract and insoluble fraction were prepared as described on p. 120 in VTT (Espoo, Finland). Butter, sunflower-seed oil, and rapeseed oil were from a local market.

† Analysed from the experimental diets fed to mice.

‡ The expected values of total lignans, calculated from the plant lignan analysis of rye bran and both rye fractions, were 17–18 μmol/kg for the rye-bran diet, 19–22 μmol/kg for the soluble extract diet and 8 μmol/kg for the insoluble-fraction diet.

experiment 1 and 2, respectively. Blood samples were collected from the abdominal aorta, centrifuged at 6000 ***g*** for 1 min, after which plasma was stored at −70°C for enterolactone analysis. The small intestine, caecum and colon were removed, and then opened along the longitudinal axis. Intestinal contents were collected from the caecum and distal colon and kept at −20°C before bacterial analysis. Intestinal tissues were rinsed with ice-cold saline (9 g NaCl/l) and the small intestine was divided into five sections. The scoring of adenomas was done as described by Mutanen *et al.* (2000). Most of the small-intestinal adenomas (60–70 %) were found in the distal part, which represents 40 % of the small-intestinal area.

Bifidobacterium *analysis*

The contents of the caecum and distal colon were taken under anaerobic conditions, weighed and pooled into two to four samples per experimental group. The pooled samples were then suspended in 10 ml of peptone saline containing 0·5 g cysteine hydrochloride/l in a plastic bag. A series of ten-fold dilutions were prepared and plated onto Beerens (1990). The plates were incubated under anaerobic conditions for 3 d at 37°C. Major colony types from the plates were further investigated for microscopic cell morphology and for growth under aerobic and anaerobic conditions. The detection limit was 10^4 colony forming units (cfu)/g wet weight.

Enterolactone analysis of plasma samples

Time-resolved fluoroimmunoassay was used to analyse plasma enterolactone samples (Adlercreutz *et al.* 1998; Stumpf *et al.* 2000). The plasma samples (50 μl) were incubated overnight at 37°C with hydrolysis reagent (50 μl) containing sulfatase and β-glucuronidase. After hydrolysis, 0·5 % (w/v) bovine serum albumin-tris(hydroxymethyl)-aminomethane buffer (150 μl; pH 7·8) was added to the samples to obtain the optimal pH and protein concentration for analysis. The analyses of samples (20 μl) were performed in duplicate on anti-rabbit antiserum-coated microtitration strips. Enterolactone concentrations were measured with the Victor 1420 multilabel counter (Wallac Oy, Turku, Finland).

Statistical analysis

Data were analysed using SPSS 9·0 (SPSS Inc., Chicago, IL). The results from female and male mice did not differ (except in body weight) in the second experiment and the results were combined. With regard to the non-fibre, rye-bran and soluble-extract groups the adenoma data of both experiments were also pooled. Data were analysed using the non-parametric Mann-Whitney U test for pair-wise comparisons between the control non-fibre group and the experimental groups. Differences were considered significant at the $P < 0{\cdot}05$ level.

Results

Intestinal adenoma formation

Intestinal adenoma data of both experiments separately as well as the pooled data from the non-fibre, rye-bran and soluble-extract groups are shown in Tables 2 and 3. The major differences between the experimental groups were found in the distal small intestine, where most of the adenomas were. The trend for increasing number of adenomas in the distal part of the small intestine in the soluble-extract group (P=0·156 for experiment 1 and P=0·032 for experiment 2) became significant when the data were pooled (P=0·012) (Table 2). In both experiments differences in adenoma growth between the diets were also found in the distal small intestine (Table 3). The insoluble fraction decreased adenoma size in the distal small intestine when compared with the control diet (P=0·008) in experiment 1, while the soluble extract increased adenoma size both in the distal (P=0·001) and the total small intestine (P=0·003) in experiment 2. The pooled data also showed a growth-promoting effect of the soluble extract in the distal small intestine (P=0·008).

Almost all the adenomas found in the colon were in the distal part. In experiment 1, there were no significant

Table 2. Number of intestinal adenomas in multiple intestinal neoplasia mice fed with the non-fibre diet, or diets containing 10 % (w/w) rye bran, 7·9 % (w/w) soluble extract or 7·9 % (w/w) insoluble fraction*

(Mean values and standard derivations)

Diet...	Non-fibre			Rye-bran			Soluble-extract			Insoluble-fraction		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Small intestine												
Experiment 1	33	13	10	43^{NS}	26	12	40^{NS}	19	12	38^{NS}	14	12
Experiment 2	30	9	11	40^{NS}	26	11	49§	27	11			
Pool	31	11	21	42^{NS}	25	23	44‡	23	23			
Distal small intestine†												
Experiment 1	19	10		25^{NS}	17		27^{NS}	16		21^{NS}	8	
Experiment 2	17	7		24^{NS}	15		31¶	18				
Pool	18	9		24^{NS}	16		29**	17				
Colon												
Experiment 1	0·8	1.0		1·1^{NS}	1·3		1·0^{NS}	1·1		0·9^{NS}	0·9	
Experiment 2	0·3	0·6		1·6**	1·7		1·1¶	0·9				
Pool	0·5	0·9		1·3¶	1·5		1·0‖	1·0				

NS, non-significant (P>0·1; Mann–Whitney U test).
* Data were analysed using the non-parametric Mann–Whitney U test for pair-wise comparisons between the control non-fibre group and each experimental diet group. Differences were considered significant when compared with the control non-fibre group at P<0·05.
† 60–70 % of the small-intestine adenomas were found in the distal small intestine, which represents 40 % of the small intestine area.
‡ P=0·063.
§ P=0·056.
‖ P=0·037.
¶ P=0·032.
** P=0·012.

Table 3. Mean size (mm) of the adenomas in the small intestine of multiple intestinal neoplasia mice fed with the non-fibre diet, or diets containing 10 % (w/w) rye bran, 7·9 % (w/w) soluble extract or 7·9 % (w/w) insoluble fraction*

(Mean values and standard derivations)

Diet...	Non-fibre			Rye-bran			Soluble-extract			Insoluble-fraction		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Small intestine												
Experiment 1	1·46	0·14	10	1·34‡	0·12	12	1·38^{NS}	0·10	12	1·33†	0·15	12
Experiment 2	1·06	0·15	11	1·04^{NS}	0·11	11	1·33‖	0·20	11			
Pool	1·25	0·25	21	1·19^{NS}	0·19	23	1·35^{NS}	0·15	23			
Distal small intestine												
Experiment 1	1·28	0·13		1·17^{NS}	0·13		1·27^{NS}	0·11		1·10§	0·14	
Experiment 2	0·85	0·09		0·83^{NS}	0·15		1·24¶	0·21				
Pool	1·06	0·24		1·01^{NS}	0·22		1·26§	0·16				

NS, non-significant (P>0·1; Mann–Whitney U test).
* Data were analysed using the non-parametric Mann–Whitney U test for pair-wise comparisons between the control non-fibre group and each experimental diet group. Differences were considered significant when compared with the control non-fibre group at P<0·05.
† P=0·075.
‡ P=0·048.
§ P=0·008.
‖ P=0·003.
¶ P=0·001.

differences in adenoma number between dietary groups (Table 2). In experiment 2, the rye-bran (P=0·012) and the soluble-extract (P=0·032) groups had significantly more colon adenomas than the non-fibre control group, a result which was confirmed when the adenoma data were pooled (P=0·032–0·037). The size of the colon adenomas in Min mice was not significantly altered between the experimental groups (data not shown).

Intestinal Bifidobacterium *level*

Bifidobacterium levels in the contents of the caecum and distal colon were 10^8–10^9 cfu/g in all Min mice fed with standard chow (Altromin 1314), and in those fed the two rye preparations and the rye-bran diet. Consumption of the non-fibre diet for several weeks in experiments 1 and 2 decreased intestinal *Bifidobacteria* below the detection limit of $<10^4$ cfu/g wet weight.

Plasma enterolactone level

Plasma enterolactone levels of Min mice were measured in experiment 2 and the results were unexpected on the basis of lignan analysis of the diets showing that the soluble-extract diet contained more lignans than the rye-bran diet (Table 1). Mean plasma enterolactone concentration was, however, highest in the rye-bran group (30·0 nmol/l; P=0·002), which together with the soluble-extract group (16·2 nmol/l; P=0·024) differed significantly from the non-fibre diet group (7·5 nmol/l). Thus, the rye bran-fed mice were the best enterolactone producers (Fig. 1).

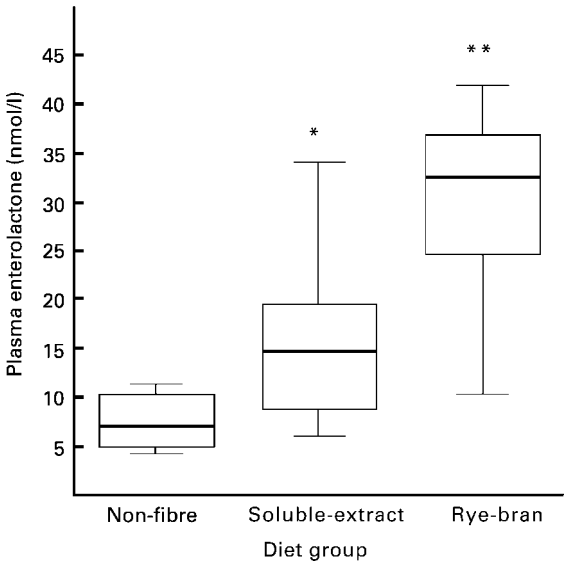

Fig. 1. Plasma enterolactone levels (nmol/l) of multiple intestinal neoplasia mice fed the non-fibre diet (n 10), the diet containing 7·9 % (w/w) soluble extract (n 10) or the 10 % (w/w) rye-bran diet (n 11) for 7 weeks. For each group, the box represents the inter-quartile range, which contains 50 % of values. The whiskers (vertical lines) extend from the box to the highest and lowest values. Medians are indicated by horizontal lines across the boxes. The plasma enterolactone levels of the soluble-extract-fed (* P=0·024) and the rye bran-fed (** P=0·002) mice differed significantly from the non-fibre-fed mice. Differences were considered significant at $P<0·05$.

There was some enterolactone in the plasma of the non-fibre group, which was not expected because the lignan level in the non-fibre diet was very low. The reason for this may be that some consumption of wood-chip bedding of aspen (*Populus tremula*) might have occurred.

Animal growth

The weight gain of the Min mice was similar between the diet groups in both experiments (data not shown).

Discussion

The two experiments reported here were designed to evaluate whether two rye-bran fractions result in distinct bifidogenic effect or enterolactone production in Min mice and whether these parameters are associated in this animal model with intestinal tumorigenesis. The main result was that the soluble extract increased the number and growth of adenomas in the distal small intestine when compared with the non-fibre group. The rye-bran group had a similar number of intestinal adenomas when compared with the non-fibre group; a result similar to that found in the previous study (Mutanen *et al.* 2000). The adenoma result regarding the soluble extract is in line with previous work in experimental animals that has shown that readily fermentable fibres, such as oat bran, guar gum, pectin or inulin, actually increase intestinal tumorigenesis when compared with slowly fermentable fibres (Jacobs & Lupton, 1986; McIntyre *et al.* 1993; Zoran *et al.* 1997; Mutanen *et al.* 2000). Fractionation of rye bran was based on water solubility and it led to fractions with different fibre composition and clearly different *in vitro* fermentation rates. In the study of Karppinen *et al.* (2001), initial rates of fermentation (expressed as the slope of short-chain fatty acid formation as a function of time during the first 2 h) of rye bran, soluble extracts and the insoluble fraction were 88, 109–156, and 82 μmol/h, respectively. The reason for the adenoma growth effect in the small intestine of Min mice fed the soluble-extract diet is unclear. It is possible that caecal bacteria are found already in the distal small intestine (Tannock, 1995), and readily fermentable carbohydrates may be fermented already there. The formation of fermentation products in that way could contribute to the tumour growth. It can also be asked to what extent the presence of adenomas, mainly in the distal small intestine, affects the flow of digesta, which in turn could change colonization of microbes in this area.

Colon adenoma number of the rye-bran group was similar in experiment 1 and increased in experiment 2 when compared with the non-fibre group. No difference was found in colon adenoma number between the rye-bran and control non-fibre group in our previous study (Mutanen *et al.* 2000). However, together with the facts that colon adenomas are rare in Min mice, and even very young Min mice (at the age of 5 weeks) can already have an adenoma in the colon, a better end-point marker in the colon might be needed. Microadenomatous lesions, less than 300 μm in size, have been found to be abundant in the colon of Min mice (Yamada *et al.* 2002) and it would be

interesting to evaluate these as an end point in a diet experiment.

Processing of rye bran into two fractions did not affect the bifidogenicity of the substrate. A considerable amount (10^9 cfu/g) of *Bifidobacteria* was found in mice fed the standard chow before the feeding experiments. Removal of fibre in the non-fibre diet decreased the level of *Bifidobacteria* below the level of detection (less than 10^4 cfu/g). However, 6–7 weeks of feeding with three different rye diets sustained *Bifidobacteria* at the same level of 10^8–10^9 cfu/g. The results clearly show that water solubility does not affect the bifidogenicity of rye bran; even the insoluble fraction sustained bifidobacteria growth, as has been shown for soluble preparations *in vitro* (Crittenden *et al.* 2002).

The role of prebiotics and/or probiotic bacteria in colon carcinogenesis has mainly been studied using aberrant crypt foci formation (Arimochi *et al.* 1997; Challa *et al.* 1997; Onoue *et al.* 1997; Reddy *et al.* 1997; Rowland *et al.* 1998) or tumours (Singh *et al.* 1997) as end-point markers in the colon of carcinogen-treated rats. The results of these studies are not consistent and show how strong the influence of a background diet, strain of the bacteria or source of prebiotic as well as the end-point parameters measured can be. Furthermore, the number of final tumours may be different from the number of aberrant crypt foci as shown in some studies with cereal fibre and fish oil (Hardman *et al.* 1991; Good *et al.* 1998). In one study with Min mice (Pierre *et al.* 1997) where short-chain fructo-oligosaccharides were used as a prebiotic substrate for *Bifidobacterium* a decreased number of colon adenomas was found without any change in the number of adenomas in the small intestine. In the second study the authors concluded that T cells and not bifidogenicity participate in a mechanism of colon tumour initiation in Min mice fed with short-chain fructo-oligosaccharides (Pierre *et al.* 1999). In the present study the non-fibre group had a concomitant decrease in the intestinal *Bifidobacterium* level during the feeding period, and still this group did not have an increased adenoma number in the colon. It seems that the growth of *Bifidobacteria* in Min mice does not regulate colon adenoma formation.

Plasma enterolactone level did not explain adenoma growth in the present study. Adenoma sizes in the non-fibre and rye-bran groups were significantly smaller than in the soluble-extract group. Plasma enterolactone level in the soluble-extract group was, however, in between the other two groups. Another interesting observation was that plasma enterolactone levels did not reflect straightforwardly the analysed lignan contents of the diets. The discrepancy may be that the rye-bran matrix can be more resistant to analytical hydrolysis than the water-soluble fraction (Mazur, 1998), but still be degraded extensively inside the colon, and bound lignan structures in this way will be available for absorption. The possibility may also exist that slowly fermentable rye bran favours enterolactone formation over the easily fermentable soluble extract. Intestinal bacterial adaptation (other than *Bifidobacteria*) to experimental diets can also take place during the feeding period of 7 weeks, which could lead to differences in plasma enterolactone levels.

The present study showed that the soluble extract, which was enzymically partly hydrolysed and easily fermentable, contained large amounts of mammalian lignan precursors, supported the growth of *Bifidobacterium* and also promoted adenoma growth in Min mice. However, it was not shown that any of these factors was a cause for an enhanced growth pattern during intestinal tumorigenesis. Further studies are needed to resolve the mechanism through which adenoma growth in Min mice is regulated, and the role of dietary components in this regulation.

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