Intestinal metabolism of rye lignans in pigs

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To study the intestinal metabolism of lignans, the concentrations of plant and mammalian lignans
in intestinal digesta sampled along the intestinal tract of pigs were determined by isotope dilution
GC–MS. The pigs were fed rye-bread diets made from either whole rye-grains or rye-grain
milling fractions enriched in pericarp–testa, aleurone or endosperm cells. The content and
characteristics of dietary fibre varied between diets and had been shown to induce different colon
fermentation patterns. As the metabolism of lignans depends on the action of the intestinal flora,
we tested whether the rye-bread diets influence the metabolism of lignans. In the ileum, the
lignans were mainly present as conjugated plant lignans, which were determined only when the
analytical procedure included a hydrolysis step. High recovery of dietary lignans in the ileum
may indicate that the lignans enter the enterohepatic circulation. In addition, two to three times
the intake of lignans were recovered in the faeces when the diets had a high content of dietary
fibre suggesting underestimation of plant lignans in the diet. Most of the plant lignans
disappeared from the intestinal tract between the terminal ileum and the caecum. The intestinal
concentrations and the disappearance of lignans correlated with the content of lignans in the diet,
being highest on the pericarp–testa diet and lowest on the endosperm diet. No effect of
fermentation pattern on the intestinal metabolism of lignans was observed. The lignans were
liberated from the pericarp–testa diet although the plant cell walls remained largely undegraded.

Lignans: Colon fermentation: Dietary fibre

Plant phyto-oestrogens (lignans and isoflavonoids) have received considerable attention in recent years because it has been suggested that they may prevent breast and prostate cancer and perhaps also colon cancer (Adlercreutz, 1995; Knight & Eden, 1996; Adlercreutz & Mazur, 1997; Bingham *et al.* 1998; Murkies *et al.* 1998). Studies have shown that the urinary excretion of phyto-oestrogen meta-bolites is low in breast-cancer patients and high in vegetar-ians and Japanese people, both of which are populations with low risk of hormone-dependent cancers (Setchell & Adlercreutz, 1988; Adlercreutz *et al.* 1995a). Soyabean products and legumes are the main sources of isoflavonoid phyto-oestrogens such as genistein and daidzein (Mazur *et al.* 1998). Plant lignans secoisolariciresinol (Seco) and matairesinol (Mata), the precursors of mammalian derivatives

enterodiol (End) and enterolactone (Enl) respectively, occur as glycosides in wholegrain cereals, seeds, nuts, vegetables, berries and beverages such as tea and coffee (Adlercreutz, 1995; Mazur & Adlercreutz, 1998).

Studies with germ-free rats (Axelson & Setchell, 1981) and human subjects receiving antibiotics (Setchell *et al.* 1981) suggested that in order to exert their biological effects, the plant lignans have to be converted into mam-malian lignans by microbial action (i.e. de-hydroxylation and de-methylation). Borriello *et al.* (1985) showed that viable bacteria from human faeces metabolised Seco and Mata into End and Enl respectively, and that End may be oxidised further to Enl. Nose *et al.* (1992) investigated the structural changes induced when incubating the lignans arctiin and tracheloside with colon content from the rat, and

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concluded that first the glycosidic lignans were deconjugated and then the aglycones were de-methylated. Unknown proportions of the mammalian lignans are absorbed from the lumen of the large intestine to the liver. Presumably the mammalian lignans are re-conjugated to glucuronides and sulfates in the intestinal wall and in the liver and excreted as such (Adlercreutz *et al.* 1995*b*). Axelson & Setchell (1981) showed that in rats, part of the absorbed lignans entered the enterohepatic circulation and were re-excreted in the intestinal tract via the bile. Enterohepatic circulation of isoflavonoids has also been demonstrated by Sfakianos *et al.* (1997) in rats.

The need for conversion of the plant lignans by the colon flora suggests that the colon fermentation processes may play an important role for the metabolism and utilisation of lignans. There is, however, very limited information on the level of plant and mammalian lignans in digesta material, or on how the mode of fermentation in the large intestine influences the conversion from plant to mammalian lignans. In the present investigation we determined, therefore, the concentration of plant and mammalian lignans in digesta from the ileum, the caecum, the middle colon and in faeces from pigs that were fed breads based on whole-rye grains and three rye-grain milling fractions. These diets have previously been shown to vary significantly in composition and characteristics of dietary fibre and subsequently in the mode of fermentation in the large intestine (Glitsø *et al.* 1998; Glitsø & Bach Knudsen, 1999). Animal models allow sampling of material from different parts of the intestinal tract. The pig was chosen as animal model as the morphology and physiology of the gastrointestinal system of the pig is comparable with that of human subjects in many respects (Stevens *et al.* 1986; Miller & Ullrey 1987; Rowan *et al.* 1994).

Materials and methods

Rye diets

Rye (*Secale cereale* L., *cv.* Marder, Bornholm, Denmark, 1993) was separated by dry-milling into three fractions enriched in cell tissue from pericarp–testa, the aleurone layer, and endosperm (Glitsø & Bach Knudsen, 1999). Rye breads based on whole rye-grain and rye-grain milling fractions were produced in an industrial bakery (Pandrup Brød, Schulstad A/S, Pandrup, Denmark) (Glitsø & Bach Knudsen, 1999). Wheat-starch, casein, gluten, lard, vegetable oil, vitamins, minerals, yeast and water were added to the rye raw materials. The diets were formulated to give an energy contribution from fat, protein and available carbohydrates of approximately 25, 15 and 60 % respectively. The four breads were balanced with regard to fat, protein, starch, minerals and vitamins but varied in source and hence characteristics of cell walls. The level of dietary fibre (sum of NSP and lignin) was kept constant in the whole rye, pericarp–testa and aleurone diets, but it was not possible to raise the level of dietary fibre in the endosperm bread to that of the others due to the low concentration of dietary fibre in the endosperm milling fraction. Cr_2O_3 was added to the diets as an indigestible marker (see Table 1 for diet ingredients and composition).

Animal experiments

Twenty growing male castrated pigs (five pigs per diet; weight at surgery approximately 30 kg; Danish Institute of Agricultural Sciences Swine herd, Foulum, Denmark) were fitted with simple T-cannulas approximately 15 cm anterior to the ileo-caecal junction and allowed to recover

Table 1. Ingredients (g/kg diet) and composition (g/kg DM) of rye-bread diets

Diet …	Whole rye	Pericarp–testa	Aleurone	Endosperm
Ingredients (g/kg diet)				
Rye raw material*	866	213	591	762
Wheat starch	–	546	277	–
Casein	26	112	28	104
Gluten	9·5	9·6	9·5	9·5
Soyabean oil	35	46	33	49
Lard	29	38	27	41
Baker's yeast	7·1	7·2	7·1	7·1
Vitamin–mineral mixture†	26	27	26	26
Cr_2O_3	0·77	0·87	0·80	0·77
Composition (g/kg DM)				
Protein	133	144	148	174
Fat	94	106	99	103
Starch	519	503	471	559
Non-starch polysaccharides:				
Cellulose	14	31	14	12
Arabinoxylans‡	74 (37)	85 (4)	87 (28)	31 (66)
Arabinose : xylose	0·66	1·04	0·42	0·75
Total non-starch polysaccharides‡	136 (29)	153 (5)	150 (25)	77 (39)
Klason lignin	21	24	27	16
Dietary fibre§	157	177	177	93

* Whole rye, pericarp–testa, aleurone or endosperm, respectively.

† Containing $Ca_2(PO_4)_3$, K_2PO_3, NaCl, $CaCO_3$ and a commercial mixture of vitamins and minerals to pig feed (Solivit Mikro 106, Løvens Kemiske Fabrik, Vejen, Denmark).

‡ Values in parentheses are soluble matter (phosphate buffer pH 7; 100°C, 60 min) expressed as a percentage of total matter.

§ Sum of non-starch polysaccharides and Klason lignin.

for 8–10 d. After an adaptation period of 8–9 d on the experimental diets, the pigs were placed in metabolic cages (balance period) and faeces were collected for 72 h. Following the faecal collection, ileal contents were sampled from the cannulas from 07.00 to 15.00 hours starting at the time of the morning feeding, and this was repeated 2 d later. The ileal collections were performed by attaching small plastic tubes to the cannula. The tubes were emptied either when they were full or they were emptied if they had been attached for 90 min. The pigs rested for 1 week still consuming the experimental diets and were then subjected to a similar second balance period in the metabolic cages. The pigs were slaughtered 3–4 d after the last balance period by an overdose of pentobarbital 4 h post-feeding. The gastro-intestinal tract was immediately removed and intestinal material was collected from the caecum, the middle third of the colon, and rectum. Freeze-dried intestinal samples were analysed for lignans. All aspects of this protocol were approved by the Danish Animal Experiments Inspectorate, Copenhagen, Denmark.

Analytical methods

All analyses were performed at least in duplicate on raw or freeze-dried material. All samples were ground to a particle size < 0.5 mm prior to analysis. The DM content was determined by drying the samples at 105°C for 20 h. Food samples were hydrolysed with an enzyme mixture (containing β-glucuronidase, sulfatase and other minor enzyme activities extracted from the digestive juice of the Burgundy snail (*Helix pomatia*)) and HCl, extracted with diethyl ether and subsequently purified and separated on DEAE- and QAE-Sephadex columns (Pharmacia Five Chemicals, Uppsala, Sweden) according to Mazur *et al.* (1996). Free and hydrolysed conjugates of the plant lignans Seco and Mata were derivatised to form trimethylsilyl ethers and analysed by isotope dilution GC–MS in the selected ion-monitoring mode using synthesised [2]H-labelled internal standards for the correction of losses during the procedure. All raw material and dietary samples were additionally hydrolysed with trifluoroacetic acid ($C_2HF_3O_2$) in a parallel experiment in order to check whether trifluoroacetic-acid hydrolysis would be more efficient in liberating the plant (dietary) lignans Seco and Mata from the matrix compared with the HCl hydrolysis used in the original method (Mazur *et al.* 1996). The modified method consisted of the same steps as the original one, but the conditions at the most critical phase of the method, i.e. the acid hydrolysis, were different (2 M-$C_2HF_3O_2$, 120°C, 2·5 h *v.* 2 M-HCl, 100°C, 2·5 h in the original method). Food samples are not expected to contain End or Enl (W Mazur and H Adlercreutz, unpublished results) and thus these compounds were not included in the analysis on food samples.

Following the addition of [2]H-labelled internal standards for all compounds, the intestinal samples were extracted and purified in several ion-exchange chromatographic steps according to Adlercreutz *et al.* (1995a). Using this methodology, only unconjugated lignans Seco, Mata, End and Enl were extracted from the intestinal samples and, following derivatisation, measured by isotope dilution GC–MS in the selected ion-monitoring mode. The results were corrected for losses during all the purification steps. Ileal samples were additionally hydrolysed with acid and analysed with a combination of both the methods for food samples and faeces. Briefly, the ileum samples were treated, according to Adlercreutz *et al.* (1995a), with distilled water–acetone (1 : 1, v/v), extracted with absolute ethanol and filtered. The samples were then centrifuged and residues were discarded while supernatants were extracted with ether as in the method for dietary samples (free fraction). Subsequently, the water phase after ether extraction was hydrolysed with 2 mol HCl/l (conjugates) and the following steps were identical to those in the method of Mazur *et al.* (1996). Measurements of the plant and mammalian lignans (total conjugates and unconjugated lignans) liberated by this combined methodology were finally performed using isotope-dilution GC–MS in the ion-monitoring mode. [2]H-labelled internal standards of all the compounds were used for the correction of losses during the procedure. Cr_2O_3 was analysed by colorimetry using the method of Schürch *et al.* (1950). Starch was determined enzymatically and the cell-wall monosaccharide components were determined as alditol acetates by GLC as described in details by Bach Knudsen (1997). Arabinoxylans were calculated as the sum of anhydrous arabinose and xylose monomers.

Calculations

The theoretical content of lignans in the diets on the basis of the concentration of lignans in the rye raw materials ($Calc_{lignan}$) was calculated using arabinoxylans as an internal marker for the rye raw materials, and thus assuming that the lignans : arabinoxylans ratio was the same in the rye raw materials and in the corresponding diets:

$$Calc_{lignan} = \frac{Lignan_{raw} \times AX_{diet}}{AX_{raw}},$$

where $Lignan_{raw}$ and AX_{raw} are the concentrations of plant lignans and arabinoxylans in the raw material respectively, and AX_{diet} is the concentration of arabinoxylans in the diet. In order to test this method of calculation, the theoretical content of starch from the rye raw materials to the rye diets ($Calc_{starch}$) was calculated using a similar formula:

$$Calc_{starch} = \frac{Starch_{raw} \times AX_{diet}}{AX_{raw}},$$

where $Starch_{raw}$ is the concentration of starch in the raw material.

Recovery of the dietary plant lignans (R_{diet}; % of intake) as plant and mammalian lignans in the intestinal segments were calculated as shown in the following example for a caecum sample ($R_{diet(caecum)}$):

$$R_{diet(caecum)} = \frac{Lignan_{caecum} \times Cr_2O_{3\,diet} \times 100}{Lignan_{diet} \times Cr_2O_{3\,caecum}},$$

where $Lignan_{caecum}$ is the concentration of plant and mammalian lignans in the caecum sample and $Lignan_{diet}$ is the concentration of plant lignans determined in the diet and $Cr_2O_{3\,diet}$ and $Cr_2O_{3\,caecum}$ are the concentrations of Cr_2O_3 in diet and caecum respectively.

Similarly, the recovery of ileal lignans (R_{ileum}; % of ileal level) in the large intestine was calculated as shown in the following example for a caecum sample ($R_{ileum(caecum)}$):

$$R_{ileum(caecum)} = \frac{Lignan_{caecum} \times Cr_2O_{3\,ileum} \times 100}{Lignan_{ileum} \times Cr_2O_{3\,caecum}},$$

where $Lignan_{ileum}$ and $Cr_2O_{3\,ileum}$ are the concentrations of lignans and Cr_2O_3 in the ileum respectively.

The daily recovery of dietary lignans in the ileum ($R_{daily(ileum)}$; μmol/d) was calculated as:

$$R_{daily(ileum)} = \frac{Lignan_{daily\,intake} \times R_{diet(ileum)}}{100},$$

where $Lignan_{daily\,intake}$ is the average daily intake of lignans at time of slaughtering (see Table 2).

The disappearance of lignans from the intestinal tract between the ileum and the caecum ($Dis_{ileum-caecum}$) was calculated as:

$$Dis_{ileum-caecum} = R_{daily(ileum)} - \frac{R_{daily(ileum)} \times R_{ileum(caecum)}}{100}.$$

Statistical methods

Values are expressed as means with standard errors of the means. The data were analysed using one-way ANOVA (Table 2) and two-way ANOVA (Table 3). Duncan's multiple range test was used to determine whether mean values were significantly different. Correlation coefficients for relationships between unconjugated lignans and conjugated lignans or recovery of dietary lignans in ileum (Table 2) were assessed using the least squares method (Pearson). Subsequently, the Pearson method was used to test correlation coefficients for plant and mammalian lignans, and recovery of dietary lignans or recovery of ileal lignans (Table 3). The differences were considered significant at $P < 0.05$. All the statistical analyses were performed using the SPSS package program, ver. 6.1J (SPSS Inc., Chicago, IL, USA).

Results

Concentration of plant lignans in rye raw materials and rye diets

In the raw materials, the highest concentration of plant lignans was found in the pericarp–testa sample (5·0 nmol/g DM) (Table 4), the content was intermediate in the aleurone raw material (2·9 nmol/g DM) and lowest in the endosperm (0·5 nmol/g DM). The total concentration of plant lignans was comparable between the whole rye, aleurone and endosperm diets (2·4–3·5 nmol/g DM), but considerably higher in the pericarp–testa diet (10·3 nmol/g DM). Concentrations of lignans obtained using the modified food method (i.e. trifluoroacetic acid hydrolysis) were the same or slightly higher than the results of the standard HCl hydrolysis method (results not shown).

Using the lignan content of the rye raw materials, the theoretical contents of plant lignans in the rye diets were calculated. The calculated contents were considerably lower than the contents of lignans measured in the diets and this was particularly evident for the pericarp–testa diet. The theoretical starch content in the rye diets was also calculated for the whole rye and endosperm diets where the starch originated only from the rye raw materials. The calculated starch concentrations were 530 and 590 g/kg DM for the whole rye and endosperm diets respectively, compared with 530 and 580 g/kg DM as analysed in the two diets.

Concentration of plant and mammalian lignans in intestinal samples

Irrespective of diet, the concentration of total lignans (plant and mammalian) was highest in the ileum samples and at this site, the majority of the lignans were conjugated. Concentration of conjugated lignans thus ranged from

Table 2. Concentration (nmol/g DM) of unconjugated and conjugated lignans in the ileum, daily intake (μmol/d) of plant lignans and the recovery (% and μmol/d) of dietary lignans in the ileum in pigs fed diets containing whole rye or rye milling fractions

(Mean values with standard errors of the mean for five pigs per diet group)

| | Unconjugated lignans* | | | | Conjugated lignans* | | | | Daily intake of plant lignans‡ (μmol/d) | Ileal recovery of dietary lignans | | |
| | nmol/g DM | | % mam* | | nmol/g DM | | % mam* | | | % | | μmol/d |
Diet†	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	Mean	SEM	Mean
Whole rye	2·1[ab]	0·7	21	7	73·0[a]	15	3·1	1·0	3·6	1052	304	38
Pericarp–testa	3·3[b]	0·3	29	6	310·4[b]	78	2·4	0·6	15·5	959	260	149
Aleurone	2·5[b]	0·8	21	9	98·0[a]	38	4·8	1·0	5·1	961	409	49
Endosperm	0·6[a]	0·1	42	15	34·8[a]	7·5	3·7	0·2	3·6	251	51	9
Statistical significance (ANOVA): P	<0.05		NS		<0.05		NS			NS		

mam, mammalian lignans.

[a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

* Conjugated and unconjugated lignans as sum of plant (secoisolariciresinol and matairesinol) and mammalian (enterodiol and enterolactone) lignans, and mammalian lignans as a percentage of total lignans.

† For details of diets see Table 1.

‡ Calculated using the average feed intake at time of slaughtering (1500 g DM/d; average weight of pigs at slaughter 53 kg).

Table 3. Concentration (nmol/g DM) of unconjugated plant* and mammalian† lignans and recovery (%) of dietary and ileal lignans in segments of the large intestine in pigs fed diets containing whole rye or rye milling fractions

(Mean values with standard errors of the means for five pigs per diet group)

Diet‡	Intestinal segment	n	Plant lignans (nmol/g DM)		Mammalian lignans (nmol/g DM)		Recovery of dietary lignans (%)§		Recovery of ileal lignans (%)	
			Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Whole rye	Caecum	3	4·9[b]	1·4	32[a]	14	306[b]	105	42	18
	Middle colon	5	0·3[a]	0·1	46[a]	2·8	288[b]	15	36	9
	Faeces	5	0·1[a]	0·1	55[a]	8·6	306[bc]	55	37	10
Pericarp–testa	Caecum	4	4·9[b]	1·7	42[a]	3·9	131[a]	6	21	8
	Middle colon	5	0·6[a]	0·2	85[b]	21	210[b]	52	33	13
	Faeces	5	0·3[a]	0·1	99[b]	25	216[c]	52	35	14
Aleurone	Caecum	5	8·5[c]	1·4	16[a]	3·7	176[a]	22	31	6
	Middle colon	6	1·2[a]	0·5	48[a]	6·9	275[b]	45	36	8
	Faeces	6	0·2[a]	0·2	72[a]	6·9	360[b]	34	51	13
Endosperm	Caecum	2	4·5[b]	0·1	14[a]	8·6	86[a]	24	29	3
	Middle colon	5	0·2[a]	0·1	12[a]	3·6	42[a]	10	16	1
	Faeces	4	0·1[a]	0·1	19[a]	4·8	49[a]	13	19	2
Statistical significance of effect (two-way ANOVA): P										
Main effect										
Diet (D)			<0·01		<0·001		<0·001		NS	
Intestinal segment (S)			<0·001		<0·01		NS		NS	
Interaction D×S			NS		NS		NS		NS	

Mean values within a column with unlike superscript letters were significantly different (P < 0·05).
* Secoisolariciresinol and matairesinol.
† Enterodiol and enterolactone.
‡ For details of diets see Table 1.
§ Statistical difference analysed for the same segment of different diets.

34·8 nmol/g DM in the ileum of the endosperm-fed pigs to 310·4 nmol/g DM in the ileum of the pericarp–testa-fed pigs compared with a range of 0·6 to 3·3 nmol unconjugated lignans/g DM (Table 2). More than 95 % of the conjugated lignans in the ileum were plant lignans, while the mammalian lignans constituted a greater percentage of the unconjugated lignans (21–42 %).

In the samples from the large intestine only the unconjugated lignans were determined. The concentration of plant lignans decreased markedly from the ileum to the caecum, whereas the concentration of mammalian lignans increased in the caecum samples and further in the middle colon or faeces for the pigs fed the pericarp–testa or the aleurone diets respectively (Table 3). The faecal concentration of

Table 4. Plant lignans* (nmol/g DM), starch and arabinoxylans in raw materials and rye diets, and theoretical content of lignans and starch in the diets based on the content in the rye raw materials†

	n	Plant lignans (nmol/g DM)				AX (g/kg DM)	Starch (g/kg DM)
		Seco	Mata	Lignans	SEM lignans		
Raw materials							
Whole rye	2	0·9	0·4	1·3	0·2	90	630
Pericarp–testa	2	2·2	2·9	5·0	0·7	395	–
Aleurone	2	1·8	1·1	2·9	0·2	163	–
Endosperm	2	0·3	0·1	0·5	0·2	42	790
Diet (analysed content)							
Whole rye	4	1·5	0·9	2·4	0·7	75	530
Pericarp–testa	10	9·1	1·1	10·3	3·5	85	–
Aleurone	4	2·6	0·8	3·4	0·9	88	–
Endosperm	4	2·1	0·2	2·4	0·8	31	580
Diet (theoretical content)‡							
Whole rye		0·8	0·3	1·1	–	–	530
Pericarp–testa		0·5	0·6	1·1	–	–	–
Aleurone		1·0	0·6	1·6	–	–	–
Endosperm		0·2	0·1	0·3	–	–	590

Seco, secoisolariciresinol; Mata, matairesinol; AX, arabinoxylans.
* Secoisolariciresinol and matairesinol.
† For details of diets see Table 1.
‡ Theoretical content in the diets based on the content in the rye raw materials (calculated using the AX content as an internal marker).

Table 5. Faecal excretion relative to intake of dietary fibre (μmol/g dietary fibre) in pigs (*n* 5 per diet group) fed the rye-bread diets and in human subjects (omnivorous and vegetarian)*

Diet†		Faecal excretion of lignans (μmol/d)	Stool weight (g/d)	Intake of dietary fibre (g/d)	Faecal excretion of lignans per intake of dietary fibre (μmol/g dietary fibre)
Pigs	Whole rye	11	599	236	0·05
	Pericarp–testa	33	1200	266	0·12
	Aleurone	18	875	266	0·07
	Endosperm	1·8	231	140	0·01
Human subjects	Omnivorous	1·7	148	19‡	0·09
	Vegetarian	3·9	223	23	0·17

* Data on human subjects adapted from Adlercreutz *et al*. (1986, 1995*a*).
† For details of diets see Table 1.
‡ Average intake over 1 year (intake in winter 18 g/d and in summer 20 g/d).

mammalian lignans was thus significantly higher for the pigs fed the pericarp–testa or the aleurone diets compared with the other pigs (72–99 *v*. 19–55 nmol/g DM).

Recovery of plant lignans in intestinal samples

The daily intake of lignans at the time of slaughtering was 15·5 μmol/d with the pericarp–testa diet and 3·6–5·1 μmol/d with the other diets (Table 2). The ileal recovery of dietary lignans ranged from 251 % (in pigs fed the endosperm diet) to 1052 % (in the pigs fed the whole-rye diet), which means that at least twice as many lignans as had been ingested were recovered in the ileum of the pigs. These recoveries corresponded to a daily recovery in the ileum of 9–149 μmol/d.

Table 3 shows the recovery of both dietary and ileal lignans in the large intestine. Approximately half of the ingested endosperm lignans were recovered in faeces, whereas two to three times as many lignans as had been ingested were recovered in the faeces of the pigs fed the three other diets (i.e. recoveries ranged from 216 to 360 %). In the caecum, the recovery of ileal lignans ranged from 21 % in the pericarp–testa sample to 42 % in the whole rye, which may indicate that the majority of ileal lignans disappeared from the lumen before the caecum. There was no significant effect of diet on the recovery of ileal lignans in the colon.

Discussion

Plant lignans in rye raw materials and rye diets

Nilsson *et al*. (1997) determined the concentrations of lignans in rye milling fractions and showed that the highest content was present in the bran. This was also the case in the present study, which further showed that the concentration of lignans was higher in the pericarp–testa than in the aleurone. In the rye diets, the lignan concentrations were two to nine times higher than expected on the basis of the content in the rye raw materials; and the good agreement between the calculated and measured starch concentrations in the whole-rye and endosperm diets showed that this was not merely due to the insecurities of the calculation. The largest difference between the theoretical lignan concentration and the actual concentration was observed in the pericarp–testa diet, where the lignan concentration was

nine times higher than the theoretical concentration. To confirm the analysis of the first four subsamples of the pericarp–testa diet samples, the measurements were repeated on another six subsamples and the results remained high.

A higher concentration in the diets relative to the raw materials was expected as other of the rye-bread ingredients (Table 1) may contain lignans too. The other diet ingredients were not analysed for lignans, but based on our previous studies wheat flour contains approximately 0·22 nmol lignans/g and soyabean oil approximately 0·03 nmol/g (Adlercreutz & Mazur, 1997). Assuming a similar concentration in the wheat starch as in the wheat flour, this would account for approximately 0·12 nmol/g DM for the pericarp–testa diet and 0·06 nmol/g DM for the aleurone diet. This corresponds to approximately 10 % and 3 % of the lignans derived from the pericarp–testa and aleurone raw materials (i.e. of the theoretical contents of lignans) respectively. However, the contributions of lignans from the wheat flour are small compared with the discrepancies between the lignans in the rye raw materials and the rye diets. The contribution from the soyabean oil is even smaller and also cannot explain the discrepancy. The reason for this large increase in lignan concentration from the pericarp–testa raw material to the pericarp–testa bread is not known, but it can be speculated that it is due to the cell wall structure and the linkages between cell wall components in the high-fibre raw materials, such as pericarp–testa, which may not be broken during acid hydrolysis. Like other cell-wall esterified phenolic compounds (Eraso & Hartley, 1990), dimers, trimers and higher oligomers of the lignans may occur (Anderegg & Rowe, 1974; Ayres & Loike, 1990) and these probably contain ether bonds not broken in the method used. However, the activity of yeast or the baking procedure itself may potentially have made a bigger fraction of the lignans in the pericarp–testa raw material available for the acid hydrolysis. High temperatures applied at the baking process (reaching 260°C in the oven) may have destroyed bonds in possible poly- and/or oligomeric lignans or liberated yet unknown mammalian lignan precursors from the aleurone or pericarp–testa cell matrix. H Härkönen, M Nilsson, P Åman, G Hallmans KE Bach Knudsen and H Adlercreutz (unpublished results) did not observe changes in the lignan content during the baking procedure of rye bread, however, but only whole-rye bread was tested in that experiment.

It should be noted, in addition, that there were large variations in the determinations of plant lignans in the diets. This may in part be due to the difficulty of taking a small sample (20–50 mg) representatively from a rye bread, even when it is freeze-dried and ground.

Lignan metabolism in the intestinal tract of pigs

In contrast to the method for food material (Mazur *et al.* 1996), conjugated lignans (glucuronides, glucosides and sulfates) were not measured in the faeces method (which was used to analyse all the intestinal samples) as more than 90 % are believed to be deconjugated in the faeces (Adlercreutz *et al.* 1995*a*) and consequently the method did not include a hydrolysis step. In the present study, the ileal samples were also analysed by a combined method, which included the acid (HCl) hydrolysis step (originally in the food method only) in the analysis of the ileal content, thus making it possible to determine both unconjugated and conjugated lignans present in the ileum. The results clearly showed that the majority (> 95 %) of lignans present in the ileum were conjugated, and hence hydrolysis is needed to determine the content of lignans in ileal material. In the ileum of pigs, the microbial activity is low compared with that in the caecum (Bach Knudsen *et al.* 1993), and bacterial growth rates and activities (e.g. reductive, oxidative and hydrolytic reactions) are also greatest in the right colon of human subjects (Cummings & MacFarlane, 1991). It was therefore assumed that the samples from the caecum and middle colon would be deconjugated by the microbial enzymes. The results on the ileal samples suggested that the initial deconjugation of plant lignans carried out by the microflora (Nose *et al.* 1992) had not yet occurred in the ileum. The lignans are therefore probably not biologically available at this site. It is unlikely that plant lignan glycosides are absorbed, because there are to the best of our knowledge no glycosidases present in human (or pig) organs except in the gut. It is probable that the lignan glycosides behave like oestrogen conjugates, which are not absorbed from the intestine without previous hydrolysis (Adlercreutz *et al.* 1976). In accordance, the majority of the conjugated ileal lignans, in particular, were plant lignans in contrast to the caecum, where the lignans were mostly mammalian. This confirmed the need for microbial action to convert the plant lignans into the biologically active mammalian lignans (Borriello *et al.* 1985). The low concentration of mammalian lignans in the ileum was in agreement with the very low urinary excretions of mammalian lignans from ileostomy patients consuming rye breads as described by Pettersson *et al.* (1996). Overall, the intestinal concentrations of lignans were highest in the samples derived from the pericarp–testa diet, which had the highest content of dietary lignans.

Using the Cr_2O_3 marker, it was possible to estimate the quantitative recoveries of lignans in the intestinal segments. The recovery of dietary lignans in the ileum was considerably higher than the intake of lignans. Axelson & Setchell (1981) showed that a proportion of lignans absorbed from the intestinal lumen of rats were re-excreted with the bile via the enterohepatic circulation. Lignans are closely related to phenolic oestrogens (Adlercreutz & Mazur, 1997), and Adlercreutz and co-workers have studied the enterohepatic

circulation of oestrogens in human subjects (Adlercreutz, 1970; Adlercreutz & Martin, 1980). They estimated that about 50 % of the conjugated oestrogens in the liver are excreted into the bile and re-enter the human intestine (Adlercreutz, 1970; Adlercreutz & Martin, 1980). It seems likely, therefore, that enterohepatic circulation of lignans explains at least part of the high recoveries of dietary lignans in the ileum of pigs. As the ileal lignans were mostly plant lignans, the questions remain whether the lignans entering the enterohepatic circulation are plant lignans absorbed as such and/or absorbed mammalian lignans are re-converted into plant lignans before re-excretion into the small intestine. Plant lignans were recovered in the urine of ileostomy patients (Pettersson *et al.* 1996) suggesting that these are absorbed as such, whereas the re-conversion of lignans may be supported by the study from Nose *et al.* (1992) which indicated that the lignans arctiin and tracheloside were re-methylated in the liver of rats.

However, two to three times as much lignan as had been ingested was recovered in the faeces of the pigs fed the high-fibre diets (whole rye, pericarp–testa and aleurone). In contrast, only half of the ingested lignans from the low-dietary-fibre endosperm diet were recovered in faeces. The high faecal recoveries of dietary lignans are in agreement with previous results. Adlercreutz & Mazur (1997) observed higher urinary excretion of lignans compared with the intake in women consuming rye breads, and Rickard *et al.* (1996) showed that the urinary excretion of lignans was five times higher in rats fed a linseed diet compared with rats fed the same amount of Seco determined in the diet. These observations suggest that the amount of lignans in the diets may be underestimated, either because not all precursors or intermediary forms are identified or because not all of the identified precursors are measured with the current methodology. The difference between diets in recovery of dietary lignans indicated an effect of dietary characteristics on the determination of lignans in food material. Dietary lignans may be underestimated if they are protected by the surrounding plant cell wall (which comprise the majority of dietary fibre in cereals) or by another part of the food matrix, and the acid hydrolysis applied during analysis is not strong enough to open up the plant material. However, the lignans not included during analysis of the diet may be detected in the ileum samples where the majority of the food matrix has been digested and the microbial degradation of dietary fibre is initiated. Dietary lignans may also be underestimated due to the presence of particular lignan structures, e.g. the tan polymer isolated from flaxseed by Bakke & Klosterman (1956) which required methoxide treatments to liberate Seco or lignans polymers as suggested by Anderegg & Rowe (1974), which may escape analytical determination and still be degraded and available in the body.

In the large intestine, only the unconjugated lignans were determined, assuming that the active fermentation of the caecum would deconjugate the majority of lignans from this site onwards. As the content of lignans in the diet may be underestimated, the recovery of lignans in the large intestine were also calculated relative to the ileal lignans. The recoveries of ileal lignans showed that only 21–42 % of ileal lignans were recovered in the caecum, and thus indicated that most of the ileum lignans were absorbed in the low

ileum and in the caecum. The daily disappearance of lignans from this part of the intestine amounted to 22, 118, 34 and 6 μmol/d for the whole rye, pericarp–testa, aleurone and endosperm diets respectively. In comparison, the daily urinary excretion of lignans in women ingesting rye breads in the aforementioned study by Adlercreutz & Mazur (1997) was about 1·9–7·3 μmol/d, and values up to 17 or 48 μmol/d have been reported as daily urinary lignan excretion in human subjects consuming vegetarian or high-lignan diets (Adlercreutz *et al.* 1981; Lampe *et al.* 1994). As evident in Table 5, the amount of lignans excreted with faeces is higher in pigs than in human subjects, because the pigs pass larger amounts of stool. If calculated relative to intake of dietary fibre, the pigs excreted approximately 0·05, 0·12, 0·07 and 0·01 μmol/g dietary fibre ingested from the whole rye, pericarp–testa, aleurone and endosperm diets respectively. From our studies (Adlercreutz *et al.* 1986, 1995a) on human subjects we calculated that 0·09 and 0·17 μmol lignans were excreted per g dietary fibre ingested by omnivorous and vegetarian subjects respectively, and thus the level of faecal excretion of lignans relative to dietary fibre intake appeared to be similar in pigs and human subjects.

Fermentation and metabolism of rye lignans

The diets used in the present study behaved quite differently in the large intestine of pigs as a result of differences in dietary fibre characteristics (Glitsø *et al.* 1998). The pericarp–testa dietary fibre was barely degraded, whereas endosperm dietary fibre was extensively degraded in the caecum and aleurone dietary fibre was degraded to nearly the same extent but at a slower rate. These differences in fermentation pattern lead to considerable variations in pH, transit time, bulking effect and production of short-chain fatty acids between the rye-bread diets (Glitsø *et al.* 1998). Despite these differences in colon fermentation, however, the recovery of ileal lignans in the large intestine was largely similar between diets and thus an effect of colon fermentation on the utilisation of lignans was not evident. The current study showed that degradation of plant cell walls is not a prerequisite for lignan metabolism, as the pericarp–testa lignans were converted from plant to mammalian lignans despite the very low degradability of dietary fibre in this diet.

In conclusion, the present study showed that the concentration of lignans in intestinal samples and the amount of lignans disappearing from the intestinal tract was determined by the content of plant lignans in the diet. Most of the rye lignans seemed to disappear in the low ileum and in the caecum, and in this segment the disappearance of lignans was about twenty times higher from the pericarp–testa diet compared with the endosperm diet, and about three to five times higher compared with the whole rye and aleurone diets. Lignan liberation and metabolism in the colon was similar between diets despite large variations in colon fermentation patterns.

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