

Intestinal degradation in pigs of rye dietary fibre with different structural characteristics

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In order to investigate the effects of dietary fibre (DF) characteristics on carbohydrate degradation and the metabolism in the large intestine, pigs were fed on four rye-bread diets (based on whole rye, pericarp/testa, aleurone or endosperm) with differences in characteristics and amount of DF. The degradability of DF in the large intestine varied greatly between diets. The pericarp/testa DF was hardly degraded in the large intestine, whereas endosperm DF was extensively and rapidly degraded in the caecum. Caecal degradation of aleurone DF was also limited, leaving more material to be degraded in the colon. The undegradable pericarp/testa DF was characterized by high contents of lignin, cellulose, ferulic acids and highly substituted arabinoxylans (the major DF component in rye). Ingestion of this diet resulted in increased faecal bulk and reduced transit time, but with low colonic pH and the lowest concentrations of short-chain fatty acids (SCFA). The aleurone diet, on the other hand, led to a fermentation pattern which may be considered more optimal, with lower colonic pH and higher concentrations of SCFA, in particular butyric acid. Despite the large differences in carbohydrate fermentation only minor significant effects on the presence of protein degradation products and on histological measurements (height and diameter of colonic crypts and thickness of the muscularis externa) were observed.

Rye: Dietary fibre: Fermentation: Pigs

It is well established that many of the beneficial effects of dietary fibre (DF) on human health relate to its action in the large intestine, where DF is partly or completely degraded by the enzymes of the colonic microflora (e.g. Eastwood, 1992). The underlying mechanisms are not fully understood, however, and contradictory results are often reported. One of the suggested mechanisms for the effect of DF is reduced contact between potentially harmful substances and the intestinal environment due to binding to DF, dilution of intestinal components by the increased mass of digesta, or reduced transit time (Bingham, 1990). Other possible mechanisms for the effects of DF relate to mode of fermentation and the production of short-chain fatty acids (SCFA; mainly acetate, propionate and butyrate) resulting from carbohydrate fermentation. SCFA (especially butyrate) have been suggested to have trophic effects on the intestinal mucosa, stimulating cell proliferation (Sakata, 1987), which may protect against colon cancer in rats (McIntyre *et al.* 1993). Another probable effect of enhanced carbohydrate

fermentation is reduced luminal pH, potentially reducing the amount of free secondary bile acids, which are believed to act as cocarcinogens in colorectal cancers (Van Munster & Nagengast, 1993). In addition, fermentation of carbohydrates beyond the proximal part of the large intestine may possibly reduce protein fermentation and thus lower the occurrence of potentially detrimental protein fermentation products (e.g. NH₃, indole compounds, phenols; McBurney *et al.* 1987). Thus, it has been suggested that sources of the resistant types of DF, e.g. wheat bran, are more protective against colon cancer induced in rats than those which are highly fermentable (McIntyre *et al.* 1993).

The contradictory results obtained for the effects of DF may be at least partly caused by the large differences in physico-chemical characteristics between various types of DF (Jacobs, 1986). It seems probable that DF behave differently in the gastrointestinal tract depending on their structural characteristics and, thus, induce different modes of fermentation in the colon of single-stomached animals.

Abbreviations: AX, arabinoxylans; DF, dietary fibre (NSP+Klason lignin); NCP, non-cellulosic polysaccharide; SCFA, short-chain fatty acids.

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There is little information available, however, on the physico-chemical characteristics of DF within the digestive tract environment.

The intake of DF in northern Europe derives largely from bread and other cereal products (Cummins, 1993). In Denmark, and other countries in northern and eastern Europe, rye bread made from wholemeal rye is a common food. The major DF components in rye, as well as in wheat, are arabinoxylans (AX), mixed-linked β -glucans, cellulose and lignin. The exact composition depends on the extraction rate. The structural characteristics of rye AX also vary between different types of rye grain tissue (e.g. bran or endosperm; Vinkx & Delcour, 1996; Glitsø, 1997). The present study was undertaken to characterize the DF components from four types of rye bread, based on whole rye and three milling fractions, during their passage through the large intestine of pigs, and to relate this to physiological variables of potential importance to the health of the colon. The pig provides a practical model to study the intestinal metabolism in single-stomached animals, as it is possible to sample digesta from various segments of the gastrointestinal tract either via cannulation or on slaughtering. The fermentation of AX in relation to their structural characteristics has been reported previously (Glitsø, 1997).

Experimental

Rye milling fractions

Rye (*Secale cereale*, cultivar Marder, Bornholm, Denmark 1993) was separated by dry-milling into fractions enriched

in cells from pericarp/testa, the aleurone layer and endosperm (Glitsø, 1997). The milling fractions are referred to by the botanical names, but this is not intended to indicate that they were pure.

Rye-bread diets

Rye breads based on whole rye and rye milling fractions were baked in an industrial bakery (Pandrup Brød, Schulstad A/S, Pandrup, Denmark). Wheat starch, casein, gluten, lard, vegetable oil, vitamins, minerals, yeast and water were added to the rye raw materials to provide fully adequate diets for the pigs. The energy contributions from fat, protein and available carbohydrate were approximately 25:15:60. The four breads were balanced in regard to fat, protein, starch, minerals and vitamins. The level of NSP was balanced between the whole rye, pericarp/testa and aleurone diets also, but it was not possible to raise the level of NSP in the endosperm bread to that of the others due to the low concentration of NSP in the endosperm milling fraction. Cr₂O₃, serving as a marker of digestibility, was also added to the diets. The ingredients and compositions of the experimental rye-bread diets are given in Table 1.

Animal experimentation

Twenty growing male castrated crossbred pigs of about 30 kg (Danish Institute of Agricultural Sciences swine herd, Foulum, Denmark) were fitted with simple T-cannulas approximately 150 mm anterior to the ileo-caecal junction

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

Diet...	Whole rye	Pericarp/testa	Aleurone	Endosperm
Ingredients (g/kg)				
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 ₂ O ₃	0.77	0.87	0.80	0.77
Chemical composition (g/kg DM)				
Protein (N×6.25)	133	144	148	174
Fat	94	106	99	103
Starch	519	503	471	559
Fructans	19.0	0.8	7.9	16.0
NSP:				
Cellulose	14	31	14	12
β -Glucans	18	2	21	11
Arabinoxylans†	75 (37)	85 (1)	88 (30)	31 (68)
Arabinose	30	44	26	13
Xylose	45	42	63	18
Arabinose:xylose	0.67	1.04	0.42	0.75
Uronic acids	4.3	6.0	5.0	2.2
Total NSP†	135 (29)	152 (3)	153 (27)	78 (40)
Klason lignin	21	25	27	16
Dietary fibre	156	177	180	94
Cr ₂ O ₃	7.3	9.2	9.0	7.3

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

† Values in brackets are soluble matter (phosphate buffer pH7, 100°, 60 min) expressed as a percentage of total.

and allowed to recover for 8–10 d. The pigs were randomly assigned to the experimental diets (five pigs per diet). Following an adaptation period of 8–9 d on the experimental diets, the pigs were placed in metabolism cages (balance period) and faeces were collected for 72 h by means of a dung bag which was changed and frozen two or three times daily. Faecal samples were later thawed, pooled (per pig per balance period) and refrozen as separate portions. Following the faecal collection, ileal contents were sampled from the cannulas on six occasions immediately after feeding. Thus, ileal contents were collected from 07.00 to 15.00 hours; from 23.00 to 07.00 hours and from 15.00 to 23.00 hours on days 1–3 and this sequence was repeated on days 4–6. The ileal collections were performed by attaching small plastic tubes to the cannula. The tubes were emptied when full or after a maximum of 90 min. The ileal samples were frozen and later thawed by careful heating and stirring for minimum time to restrict bacterial degradation, and finally pooled (per pig per balance period per time of day). The pigs were then rested for 1 week while still consuming the experimental diets before starting a similar second balance period in the metabolism cages. At 3–4 d after the last balance period the pigs were slaughtered 4 h post-feeding by an overdose of pentobarbital. The gastrointestinal tract was immediately removed and divided into ten segments: the stomach, the small intestine (separated into three segments of equal length and separating the last third into two segments), the caecum, the colon (separated into three segments of equal length denoted proximal colon, middle colon and distal colon), and the rectum. The intestinal contents were collected, their pH measured and the samples were quickly frozen in portions. One tissue sample was taken for light microscopy from each of the five segments in the large intestine. These were rinsed in PBS (9 g NaCl/l; pH 7.4) and fixed in neutral-buffered formaldehyde (100 ml/l).

Analytical methods

Freeze-dried samples were ground to a particle size of less than 0.5 mm before analysis. DM content was determined by drying the samples at 105° for 20 h and ash was analysed by the method of the Association of Official Analytical Chemists (1990). Protein (N × 6.25) was determined by the Kjeldahl method using a Kjell-Foss 16200 autoanalyser (Foss Electric, Hillerød, Denmark). Fat (HCl-fat) was extracted with diethyl ether, after acid hydrolysis (Stoldt, 1952). Starch was analysed enzymically, neutral NSP and constituent sugars as alditol acetates by GC, uronic acids by colorimetry and Klason's lignin gravimetrically as described in detail by Bach Knudsen (1997). Cellulose was determined as the difference in glucose content when the swelling step with 12 M-H₂SO₄ was included and omitted respectively. Fructans were extracted with acetate buffer, hydrolysed to monosaccharides with 0.037 M-H₂SO₄ and quantified by specific enzymes (Larsson & Bengtsson, 1983). Fructans were calculated as total fructose in hydrolysate corrected for free fructose and fructose from sucrose, and converted to oligosaccharides by the factor 0.92. β-D-Glucans were determined by the enzymic method of McCleary & Glennie-Holmes (1985) (MegaZyme Pty.

Ltd., Co. Wicklow, Ireland). Cr₂O₃ was determined using the method of Schürch *et al.* (1950). SCFA and lactic acid were measured in digesta by capillary GC as described by Jensen *et al.* (1995a), and tryptophan and indole compounds were determined by HPLC (Jensen *et al.* 1995b). The water-holding capacity of ileal effluents was determined by centrifugation (14 000 g, 4°, 20 min), removal of the supernatant fraction and drying of the drained residue, essentially as described by Johansen *et al.* (1996). Most analytical procedures were performed once (SCFA, tryptophan and tryptophan degradation products, NSP) or twice (DM, ash, protein, fat, starch, fructans, Cr₂O₃, water-holding capacity) on each pooled sample (i.e. two faecal samples and six ileal samples per pig). However, for the determination of cellulose, non-cellulosic polysaccharide (NCP)-glucose and water-holding capacity of the ileal material only the samples collected between 07.00 and 15.00 hours were analysed, and for fructan analysis only the ileal samples from the second balance period collected between 07.00 and 15.00 hours were subjected to analysis. In each case, the statistical analyses were performed on the averaged results (per pig). The tissue samples for light microscopy were processed as described by Brunsgaard (1997). The height and diameter of the crypts and the thickness of the muscularis externa were determined using an image analysis system as described by Brunsgaard (1997). The morphological readings were performed at twenty different sites on each tissue sample and the average reported. The thickness of the muscularis externa was not determined on rectal samples due to technical difficulties.

Calculations

Digestibility was calculated using the following formula:

$$Y_X = 1 - \frac{Cr_2O_{3diet} \times X_{GI}}{Cr_2O_{3GI} \times X_{diet}}$$

where Y_X is the digestibility of feed component X in a specific part of the gastrointestinal (GI) tract, e.g. ileum, caecum or faeces; X is the concentration of the component determined in the diet and in the intestinal sample and Cr_2O_3 the concentration of Cr₂O₃ in the diet and in the intestinal sample.

Mean transit time (MTT) in the intestinal segments was calculated as follows:

$$MTT = \frac{Cr_2O_{3GI} \times 24}{Cr_2O_{3day}}$$

where Cr_2O_{3day} is the daily intake of Cr₂O₃.

Statistical analysis

Two kinds of analyses were carried out. One concerned comparison of treatment effects (i.e. diets) in a given intestinal segment. This was accomplished by a simple ANOVA based on the model:

$$Y_{(d,i)} = m + a_{(d)} + \varepsilon_{(d,i)},$$

where $a_{(d)}$ denotes the treatment effect and $\varepsilon_{(d,i)}$ accounts for unexplained variation. The second kind of analysis

concerned investigation of treatment effect over a range of intestinal segments. The analyses were carried out using the following general model:

$$Y_{(d,s,i)} = m + a_{(d)} + b_{(s)} + G_{(d,s)} + U_{(i)} + \varepsilon_{(d,s,i)},$$

where $a_{(d)}$, $b_{(s)}$, $G_{(d,s)}$ are the effects of diet, segment and interaction and i refers to an individual pig. The variance component $U_{(i)} \sim N(0, \tau^2)$ accounts for the repeated measurements that were made on the same individual, whereby these observations are dependent, while the error term $\varepsilon_{(d,s,i)} \sim N(0, \sigma^2)$ represents unexplained variation. If repeated measurements on the same animal were not accounted for, this would lead to rejection of too many hypotheses (i.e. the significances would tend to be too strong). The variance component $U_{(i)}$ is a simple way to include this correlation in the model (Searle *et al.* 1992). The interactions between segment and diet were described as random by assuming $G_{(d,s)} \approx N(0, \omega^2)$. The practical advantage of this approach is that progress in investigating main effects is not barred by a significant interaction. The assumptions underlying this model were checked by a statistician (S. H.) and appeared satisfactory.

Results

Dietary fibre characteristics of the rye-bread diets

The concentrations of DF, NSP and AX were comparable in the whole rye, pericarp/testa and aleurone diets, but lower in the endosperm diet (Table 1). Pericarp/testa AX and NSP were mostly insoluble, whereas endosperm AX were

very soluble. The arabinose:xylose ratio, which is a simple indicator of AX structure, also varied considerably between diets (0.42, 0.67, 0.75 and 1.04 in the aleurone, whole rye, endosperm and pericarp/testa diets respectively). Lignin ranged from 16 g/kg DM in the endosperm diet to 27 g/kg DM in the aleurone diet. The pericarp/testa diet had a considerably higher content of cellulose and lower content of β -glucans than the other diets and, in addition, had the highest concentration of uronic acids and the lowest content of fructans.

Ileal and faecal excretion and digestibility of feed macronutrients

Ileal excretion of wet matter was higher on the pericarp/testa and aleurone diets compared with the others (Table 2). Faecal excretion of wet and dry matter was of the following order: pericarp/testa > aleurone > whole rye > endosperm.

The ileal digestibilities of protein, starch, fructans and NSP and the faecal digestibilities of protein, fat and NSP also differed significantly between diets. While the actual differences in digestibility of protein, fat and starch were small, the digestibility of NSP differed greatly between diets, ranging from -0.10 to 0.19 in the ileum and from 0.14 to 0.87 in the faecal material. Endosperm NSP was most readily and extensively digested in contrast to the pericarp/testa NSP, the majority of which was excreted in the faeces. The pattern of AX digestibility followed that of NSP, whereas NCP-glucose was generally more digestible in the ileum than total NSP and largely degraded in the

Table 2. Ileal and faecal excretion (g/d or g DM/d)* and digestibility of protein, fat, starch, fructans, NSP and various NSP fractions in pigs fed on diets containing different fractions of rye†

(Mean values for five pigs)

Diet...	Whole rye	Pericarp/testa	Aleurone	Endosperm	$\sigma \ddagger$	Statistical significance of effect of diet: $P =$
Ileal excretion (g/d)	3325 ^b	4257 ^a	4538 ^a	2981 ^b	536	0.0007
Ileal excretion (g DM/d)	435 ^b	474 ^b	530 ^a	270 ^c	34	0.0001
Ileal digestibility						
Protein	0.73 ^b	0.74 ^b	0.71 ^b	0.81 ^a	0.047	0.02
Fat	0.83	0.81	0.80	0.86	0.034	0.056
Starch	0.98 ^b	0.98 ^c	0.98 ^{bc}	0.99 ^a	0.002	0.0002
Fructans	0.31 ^c	0.62 ^a	0.36 ^{bc}	0.55 ^{ab}	0.17	0.02
Total NSP	0.07 ^b	-0.10 ^c	0.12 ^{ab}	0.19 ^a	0.056	0.0001
Arabinoxylans	0.10 ^a	-0.07 ^b	0.16 ^a	0.12 ^a	0.059	0.0001
Cellulose	-0.46 ^c	-0.25 ^b	-0.47 ^c	0.10 ^a	0.097	0.0001
NCP-glucose	0.23 ^b	0.20 ^b	0.34 ^a	0.37 ^a	0.077	0.008
Faecal excretion (g/d)	599 ^c	1200 ^a	875 ^b	231 ^d	106	0.0001
Faecal excretion (g DM/d)	180 ^c	331 ^a	232 ^b	74 ^d	18	0.0001
Faecal digestibility						
Protein	0.82 ^b	0.83 ^b	0.78 ^c	0.93 ^a	0.026	0.0001
Fat	0.83 ^b	0.81 ^b	0.80 ^b	0.88 ^a	0.026	0.002
Starch	0.99	0.99	0.99	0.99	0.018	0.190
Total NSP	0.67 ^c	0.14 ^d	0.73 ^b	0.87 ^a	0.030	0.0001
Arabinoxylans	0.65 ^c	-0.01 ^d	0.73 ^b	0.83 ^a	0.024	0.0001
Cellulose	0.28 ^b	0.10 ^c	0.35 ^b	0.84 ^a	0.071	0.0001
NPC-glucose	0.89 ^b	0.74 ^c	0.89 ^b	0.95 ^a	0.016	0.0001

NCP-glucose, non-cellulosic polysaccharide glucose.

^{a,b,c,d} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

* Ileal and faecal excretions were calculated on the basis of the average intake at time of slaughtering (1500 g DM/d).

† For details of diets and procedures, see Table 1 and pp. 458–460.

‡ Estimated standard error on all observations.

faeces (ranging from 0.74 in the pericarp/testa to 0.95 in the endosperm). Only the endosperm cellulose was degraded to a small extent in the ileum. Faecal digestibility of cellulose varied greatly between diets following the pattern of total NSP in the case of the pericarp/testa and endosperm diets, but in the whole rye and aleurone diets it was much lower than that of the NSP.

Table 3 shows the daily disappearance of nutrients in different segments of the intestinal tract. Most of the organic material (948–1213 g/d) was absorbed from the small intestine, but as much as 172 g and 134 g of organic material from the aleurone diet disappeared in the caecum and colon respectively. Almost all of the starch, most of the protein and a large part of the fructans were absorbed from the small intestine, whereas most of the NSP disappeared in the caecum and colon. The largest quantities of NSP and AX disappeared from the pigs fed on the aleurone diet, whereas the disappearance of the carbohydrates in the large intestine was low for those fed on the pericarp/testa diet. Disappearance of NSP components was generally larger in the caecum compared with the colon, the only exception being cellulose which was degraded mainly in the colon.

Mean transit time

Large intestine mean transit times (Table 4) peaked in the proximal or middle colon. The ileum to rectum mean transit time was significantly higher in the pigs fed on the endosperm and whole-rye diets compared with those having the pericarp/testa diet, and the aleurone-fed pigs had intermediate ileum to rectum mean transit time.

Water-holding capacity

The water-holding capacity (water retention by centrifugation) of the ileal material was significantly higher in the pigs fed on the endosperm diet compared with the others (Table 4).

pH of intestinal content

For all diets pH was lowered between the ileum and the caecum and then increased again between the caecum and the rectum (Fig. 1). From ileum to rectum, the intestinal pH was significantly higher in the pigs fed on the pericarp/testa diet compared with the others ($P < 0.003$). Between caecum and rectum, the pH was significantly lower in the pigs fed on the aleurone diet compared with those given the endosperm diet ($P < 0.003$), but not significantly lower than in the pigs fed on whole rye. Only in the middle colon did the aleurone diet induce lower pH than whole rye ($P < 0.002$). Thus, the pH in pigs fed on the pericarp/testa diet ranged from 6.2 to 7.1 compared with 5.4–6.6 in those fed on aleurone.

Short-chain fatty acids

Table 5 shows the concentrations of lactic acid and SCFA in the intestinal samples. Lactic acid was present in the ileum but not in the large intestine. Irrespective of diet, the concentration of SCFA increased markedly between the

ileum and caecum, followed by a gradual decrease towards the rectum. The concentrations of SCFA between the caecum and middle colon were significantly higher in pigs fed on aleurone compared with those fed on endosperm ($P < 0.038$) and pericarp/testa ($P < 0.013$). The caecal SCFA concentration was significantly lower in pigs fed on the pericarp/testa diet compared with the others ($P < 0.014$), but the absolute amounts of SCFA present in the caecum (results not shown) were comparable for all diets due to a large quantity of pericarp/testa caecal material. In the proximal colon, the SCFA content was significantly lower for the endosperm-fed pigs compared with the others ($P < 0.009$). Fig. 2 shows the molar distribution of the SCFA. In all ileum samples acetate comprised 0.92–0.99 of the SCFA present. The SCFA profile in the large intestine of the pigs fed on pericarp/testa differed from the other diets in that acetate made up a significantly larger proportion of SCFA between caecum and faeces ($P < 0.001$). From the caecum onwards, butyrate was significantly higher in the pigs fed on aleurone, compared with those given the endosperm ($P < 0.037$) and pericarp/testa diets ($P < 0.0008$), with the highest proportion (0.11) found in the proximal colon. Isobutyrate was not present in ileal and caecal samples, and in the proximal colon isobutyrate was only present in the pigs fed on the pericarp/testa and endosperm diets. The proportion of isobutyrate increased towards the distal segments peaking at 0.04 in the faeces of pigs fed on the endosperm diet. The differences between diets were not significant, however.

Tryptophan degradation products

For all diets the highest tryptophan concentrations were found in the ileal samples with a marked decrease in the caecum (Table 5). In contrast, the concentration of tryptophan degradation products increased markedly between the ileum and caecum. In the caecum and colon there was no significant difference between diets, but the concentration of tryptophan degradation products in the rectal samples of pigs fed on the pericarp/testa diet was significantly lower than that in the rectal samples of pigs fed on the endosperm ($P < 0.02$) and aleurone ($P < 0.03$) diets.

Histological characteristics

The crypt diameter (Table 6) decreased between the caecum and rectum, but there was no effect of the diet. The crypt height was greater in the rectum than in the other intestinal segments. Overall, between the caecum and the rectum the crypt height of the pigs fed on the pericarp/testa diet was significantly higher compared with that of pigs fed on the whole-rye diet ($P < 0.01$). When examining each intestinal segment this effect was only significant in the caecum ($P < 0.002$) and in the proximal colon ($P < 0.02$). The caecal crypt height in the pigs fed on the aleurone and endosperm diets was also significantly higher than that in pigs fed on the whole-rye diet ($P < 0.04$ and $P < 0.01$ respectively). With regard to muscle layer thickness, there was no significant effect of either diet or intestinal segment.

Table 3. Amounts* (g/d) of selected nutrients disappearing from the small intestine, caecum and colon of pigs fed on diets containing different fractions of rye†
(Mean values and standard deviations for five pigs per diet)

Diet	Organic material‡		Protein		Starch		Fructans		NSP		AX		NCP-glucose		Cellulose		Total carbohydrates§		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Whole rye																			
Small intestine	1050	19	145	6	764	2	9	3	15	13	12	10	11	4	0	–	787	12	
Caecum	129	37	20	6	4	3	20¶	3	72	20	40	13	22	4	0	–	96	20	
Colon	106	20	20	6	9	2	2	2	50	12	21	7	11	4	6	1	59	13	
Pericarp/testa																			
Small intestine	1017	36	160	9	743	11	1	0.2	0	–	0	–	6	3	0	0	737	2	
Caecum	34	12	19	7	9	3	0.5¶	0.2	0	–	0	–	11	1	0	0	12	4	
Colon	81	19	19	7	6	3	3	3	30	3	0	–	6	3	5	0.4	35	5	
Aleurone																			
Small intestine	948	25	153	13	691	1	4	2	27	11	22	6	18	3	0	–	723	13	
Caecum	172	27	20	7	4	3	8¶	2	79	23	44	15	20	3	0	–	94	27	
Colon	134	13	20	7	10	5	5	5	58	37	31	20	11	6	7	2	63	45	
Endosperm																			
Small intestine	1213	37	211	15	826	1	14	4	22	8	5	1	14	2	2	1	861	11	
Caecum	111	34	31	15	5	4	11¶	4	56	13	31	2	12	5	6	6	72	18	
Colon	82	43	31	15	7	4	4	4	24	13	2	2	9	1	6	1	31	16	

AX, arabinoxylans; NCP-glucose, non-cellulosic polysaccharide glucose.

* Calculated using the average intake at time of slaughter (1500 g DM/d), average weight of pigs at slaughter 53 kg. In the case where negative digestibilities occurred, these were not included in the calculations of amounts of nutrients disappearing from the intestinal tract, instead disappearance of 0 g are reported.

† For details of diets and procedures, see Table 1 and pp. 458–460.

‡ Organic material is calculated as DM minus ash.

§ Sum of starch, fructans and NSP.

|| These values include the amount absorbed in the caecum also.

¶ The amount of fructans in the caecum were below the detection limit and fructans were therefore not determined in later intestinal segments.

Table 4. Mean transit time (h) in intestinal segments, time of passage from ileum to rectum, and water-holding capacity (g water/g DM) of ileal effluent of pigs fed on diets containing different fractions of rye* (Mean values for five pigs)

Diet...	Whole rye	Pericarp/testa	Aleurone	Endosperm	σ †	Statistical significance of effect of diet: $P =$
Mean transit time (h)						
Caecum	2.9	2.0	2.1	3.3	1.2	0.27
Proximal colon	10.9 ^{a,b}	7.1 ^c	6.8 ^c	8.3 ^{b,c}	2.3	0.04
Middle colon	8.9	6.2	7.4	7.5	2.5	0.45
Distal colon	5.9	3.8	5.5	6.2	2.4	0.41
Rectum	3.5 ^b	2.1 ^b	3.5 ^b	7.5 ^a	2.2	0.007
Ileum-rectum	32.0 ^{ab}	21.1 ^c	25.3 ^{bc}	32.8 ^{ab}	6.1	0.02
Water-holding capacity of ileal contents (g water/g DM)	3.5 ^a	4.0 ^a	3.6 ^a	6.7 ^b	0.57	0.0001

^{a,b,c} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

* For details of diets and procedures, see Table 1 and pp. 458–460.

† Estimated standard error on all observations in each intestinal segment.

Discussion

Degradation of rye dietary fibre

The four rye-bread diets had comparable chemical compositions except for the lower concentration of DF in the endosperm diet, and the differences in ileal and faecal digestibilities of fat, protein and starch were minor. In contrast, the characteristics of DF varied considerably between diets (Glitsø, 1997), and this resulted in large differences in degradability of DF components. The NSP of the endosperm diet were most extensively fermented and almost 20% were degraded at the ileal stage. Likewise, most of the aleurone and whole-rye NSP were fermented in the large intestine, in contrast to the pericarp/testa NSP that were very resistant to degradation. Bach Knudsen *et al.* (1995) also reported low digestibilities of a wheat pericarp/testa product (faecal digestibility of NSP of 0.24), but these values were still higher than those presented here. The lower digestibilities found in the present study may in part be due to a better isolation of the pericarp/testa cells with the

current milling scheme, as was indicated also by the very low starch content in this milling fraction (35 g/kg DM; Glitsø, 1997). Of the pericarp/testa NSP only NCP-glucose, probably deriving from the readily fermentable β -glucans (Graham *et al.* 1986), was extensively degraded and the actual content of β -glucans was very low in this diet. It was remarkable that most of the material degraded in the large intestine was fermented in the caecum, despite the fact that the caecal transit time only made up about one tenth of the total large intestine transit time.

In a previous study we focused on the structural characteristics of the AX and their modifications as they were degraded along the large intestine (Glitsø, 1997). We found that differences in degradability correlated well with differences in water- and alkali-extractability, which is influenced by the AX structure, degree of lignification and cross-linking to other cell-wall components (Fincher & Stone, 1986; Hromádková & Ebringerová, 1992). The endosperm NSP were very soluble, in contrast to the largely insoluble pericarp/testa NSP, which may explain part of the difference

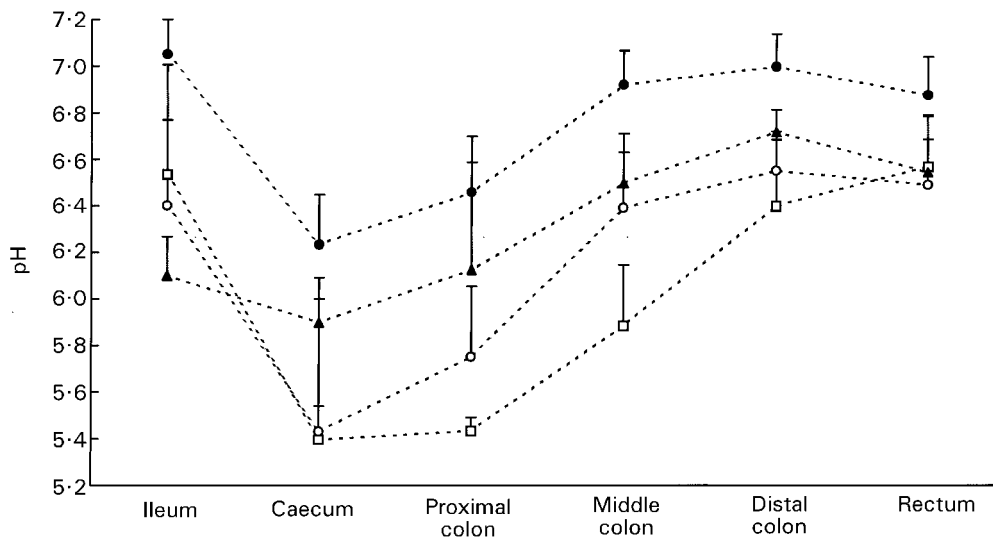


Fig. 1. pH in intestinal samples from pigs receiving diets containing whole rye (○), rye pericarp/testa (●), rye aleurone (□) or rye endosperm (▲). Values are means for five pigs, with standard deviations represented by vertical bars. For details of diets and procedures, see Table 1 and pp. 458–460.

Table 5. Concentrations (mmol/kg or $\mu\text{mol/kg}$) of lactic acid (LA), short-chain fatty acids (SCFA), tryptophan (TRP) and tryptophan degradation products (TDP) in intestinal samples from pigs fed on diets containing different fractions of rye*

(Mean values and standard deviations for five pigs per dietary group)

Diet	LA (mmol/kg)		SCFA (mmol/kg)		TRP ($\mu\text{mol/kg}$)		TDP ($\mu\text{mol/kg}$)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Whole rye								
Ileum	28	16	20	4	265	99	2	2
Caecum	1	2	156	27	65	37	174	112
Proximal colon	0	–	123	24	34	14	152	62
Middle colon	0	–	70	25	41	35	142	94
Distal colon	0	–	54	22	33	10	166	80
Rectum	0	–	57	14	30	11	233	100
Pericarp/testa								
Ileum	5	3	20	12	220	70	3	4
Caecum	0	–	112	18	55	34	108	75
Proximal colon	0	–	107	7	56	21	208	81
Middle colon	0	–	77	13	38	11	165	87
Distal colon	0	–	70	16	39	9	140	88
Rectum	0	–	73	15	38	6	146	102
Aleurone								
Ileum	49	38	19	8	214	37	2	2
Caecum	5	7	164	21	30	11	127	74
Proximal colon	0	–	160	19	35	5	137	33
Middle colon	0	–	122	23	39	18	145	51
Distal colon	0	–	74	30	37	12	166	53
Rectum	0	–	68	20	41	32	271	97
Endosperm								
Ileum	25	11	23	6	414	119	6	7
Caecum	1	2	151	10	86	44	131	118
Proximal colon	0	–	104	18	91	69	201	117
Middle colon	0	–	82	24	81	44	249	141
Distal colon	0	–	57	16	65	37	252	148
Rectum	0	–	67	14	47	35	249	175

* For details of diets and procedures, see Table 1 and pp. 458–460.

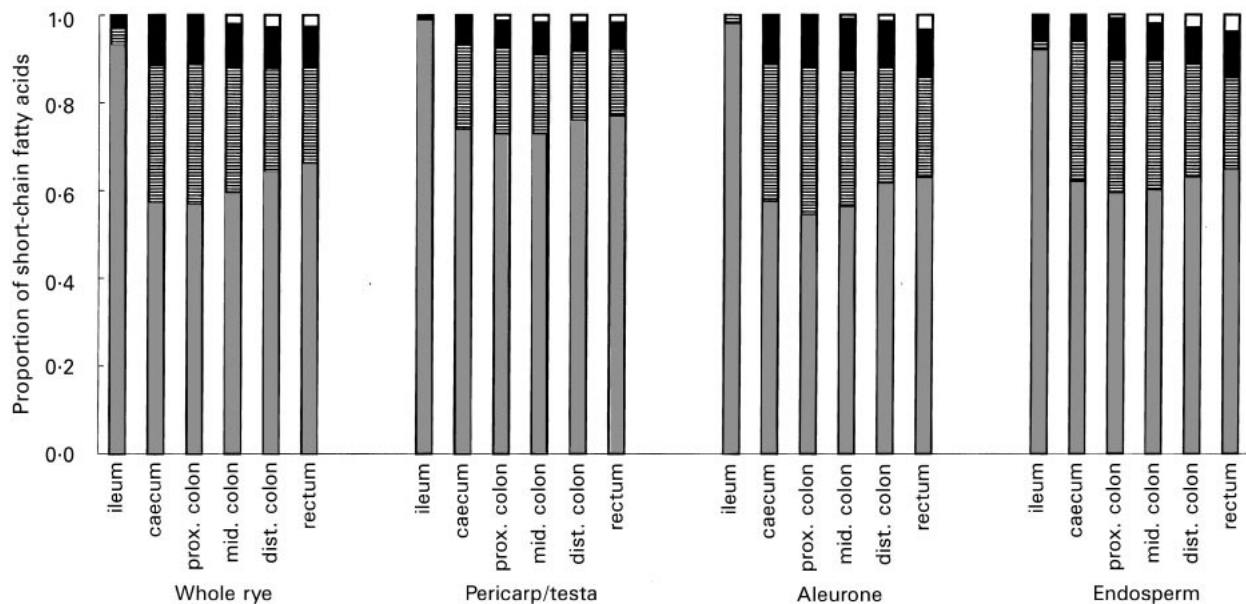
**Fig. 2.** Molar distribution of short-chain fatty acids (acetate, propionate, butyrate and isobutyrate) in intestinal contents from pigs fed on diets containing different fractions of rye. Prox, proximal; mid, middle; dist, distal. For details of diets and procedures, see Table 1 and pp. 458–460.

Table 6. Crypt diameter (μm), crypt height (μm) and thickness of muscle layer (μm) in large-intestine tissue samples from pigs receiving diets containing different fractions of rye*
(Mean values and standard deviations for five pigs per dietary group)

Diet...	Whole rye		Pericarp/testa		Aleurone		Endosperm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Crypt diameter								
Caecum	139	15	128	14	134	19	139	4
Proximal colon	140	21	147	23	132	9	135	5
Middle colon	118	5	122	9	115	9	114	11
Distal colon	118	8	114	11	115	16	119	15
Rectum	104	10	105	15	107	5	109	4
Crypt height								
Caecum	374	31	467	18	430	46	442	51
Proximal colon	384	34	438	35	401	20	420	36
Middle colon	381	33	461	84	417	75	398	65
Distal colon	394	45	447	29	417	53	411	67
Rectum	515	83	570	104	514	45	576	54
Muscle layer thickness								
Caecum	415	113	411	90	422	114	333	26
Proximal colon	333	88	414	72	366	61	289	59
Middle colon	442	154	357	73	284	85	318	160
Distal colon	348	119	348	69	274	123	317	159

* For details of diets and procedures, see Table 1 and pp. 458–460.

in digestibility. In addition, the endosperm and pericarp/testa milling fractions and diets had the lowest and highest concentrations respectively, of lignin, cellulose, ferulic acid and uronic acid (Glitsø, 1997). Lignin is highly undegradable (Leisola & Garcia, 1989) and probably impedes degradation of other cell-wall components, either by physical encapsulation or chemical binding (Cornu *et al.* 1994). The actual differences in (Klason) lignin concentration were not large between the four diets, whereas the concentration of cellulose was considerably higher in the pericarp/testa diet compared with the three others. In the diets with low digestibility of cellulose this might lower the digestibility of AX also, as it has been suggested that unbranched segments of AX can bind to cellulose microfibrils (Carpita & Gibeau, 1993). The pericarp/testa AX were very branched, however, which may rule out this possibility. The concentration of alkali-labile bound ferulic acid, estimated from the concentrations in the raw materials (Glitsø, 1997), was also lower in the endosperm compared with the pericarp/testa (0.85, 1.56, 1.16 and 0.14 g ferulic acid/kg DM of the whole rye, pericarp/testa, aleurone and endosperm diets respectively). Ferulic acid may bind to the arabinose-substituents of AX, and thus provide another possibility of cross-linking to other polymers, lignin or proteins (Iiyama *et al.* 1994) that may hinder degradation of the pericarp/testa DF. It should be noted, though, that only the ferulic acids that could be liberated by alkali hydrolysis were determined, and thus the actual extent of cross-binding with ferulic acid may be considerably larger (Iiyama *et al.* 1990). The highly substituted and complex structure of the pericarp/testa AX (Glitsø, 1997), including the higher concentration of uronic acid, possibly also adds to the difficulties of degrading pericarp/testa DF, in agreement with other studies on degradation of (glucurono)-AX (Verbruggen, 1996; Schooneveld-Bergmans, 1997). The fermentability of DF has also been positively correlated

with its hydration properties, as these determine the accessibility of water and hence the ease with which enzymes can reach the substrate (Auffret *et al.* 1993). The current study showed that the water-holding capacity of ileal material was highest in pigs fed on the endosperm diet, and hence the ease of NSP fermentation in this sample may also, in part, be explained by better access for the enzymes. On the other hand, the water-holding capacity was comparable between the pericarp/testa and the two remaining diets despite large differences in digestibility between these dietary treatments. As the water-holding capacity was determined on intact intestinal material, components other than the DF may have affected its hydration properties.

Thus, no single cell-wall component or DF feature was associated unambiguously with the range of digestibilities observed in the four rye-bread diets, and it is possible that the degradability of rye DF is determined by the action and interaction of a range of factors. In discussing how various cell-wall components may influence degradability, it should be kept in mind also that concentrations of cell-wall components do not yield information on the actual conformations or interactions between the components.

The physiological impact of rye dietary fibre degradation

The differences in DF characteristics influenced metabolism in the large intestine. For instance, there was a marked difference in faecal excretion between diets. Bulking can be achieved by an increase in bacterial mass caused by fermentable DF, or more importantly by the physical presence and ability to absorb water, which is characteristic of poorly degradable DF (Stephen & Cummings, 1980; Wisker *et al.* 1996). In accordance, faecal bulk was largest in the pigs fed on the pericarp/testa diet and intermediate in those fed on the whole-rye and aleurone diets. The increased faecal mass induced by the pericarp/testa diet reduced

transit time in agreement with previous results (Van Dokkum *et al.* 1983), although the ileal–rectal transit time of the pigs fed on the aleurone diet was not significantly higher than that of pigs fed on the pericarp/testa diet. Increased faecal bulking as well as reduced transit time may act to reduce the contact between potentially harmful substances and the intestinal environment (Bingham, 1990).

The mode of carbohydrate fermentation was also reflected in the pH values and in the concentration of SCFA along the large intestine. Carbohydrate fermentation yields of SCFA, which may decrease pH, was apparent in the caecal samples. Restriction of carbohydrate fermentation, due either to the undegradable pericarp/testa DF or the low amounts of DF in the endosperm diet, resulted in high pH values in all pericarp/testa samples and, for the endosperm diet, a smaller decrease in caecum and higher values in the colon compared with the whole-rye and aleurone diets. The aleurone diet resulted in the lowest pH values along the large intestine, even if the value was only significantly lower than the whole-rye diet in the middle colon. It is possible that the pH pattern of the aleurone diet is beneficial, as lower colonic pH may be associated with lower risk of cancer, because it restricts the bacterial conversion from primary to secondary bile acids as well as the solubility of bile acids (Thornton, 1981; Bruce, 1987; Andrieux *et al.* 1989). Therefore, the presence of slowly fermented polysaccharides reaching the distal large intestine, and thus maintaining a low pH may play a special role in the protection against colon cancer (McDougall *et al.* 1996). Compared with the pericarp/testa and endosperm diets, the aleurone diet provided more AX to be degraded in the colon, possibly due to the complete, but slow, fermentation of an AX with a low degree of substitution (Glitsø, 1997). Thus, this structure could be important for maintaining a low pH in the distal large intestine.

In accordance with the pH values, the concentration of SCFA was generally lowest in the intestinal samples of the pigs fed on the pericarp/testa diet and highest in those fed on the aleurone diet, at least as far as the middle colon. This may be of interest as SCFA are believed to be beneficial to colonic health and may act to protect against disorders such as diversion colitis, ulcerative colitis and colorectal cancer (Brøbech Mortensen & Rye Clausen, 1996). Moreover, the proportion of butyrate was highest in the large intestine samples of the aleurone-fed pigs, and it is believed that butyrate provides the best protective effects of the SCFA (Brøbech Mortensen & Rye Clausen, 1996). Bach Knudsen *et al.* (1993) reported that oat AX increased the proportion of butyrate in pigs compared with oat β -glucans, and attempts to link monosaccharide composition of DF with fermentation products suggested that xylose was the most suitable sugar for the production of butyrate (Salvador *et al.* 1993). These observations are in agreement with the present study as the aleurone diet contained more AX with larger proportions of xylose compared with the endosperm diet. It has been suggested that the influence of substrate on the composition of fermentation products relates to rate of fermentation, microflora growth rate or differences in bacterial activities (Cummings & MacFarlane, 1991; Bourquin *et al.* 1992). McIntyre *et al.* (1993) were able to relate increased concentration of butyrate along the length

of the colon with reduced tumour incidence in rats fed on a diet based on a commercially produced wheat bran. Assuming similarities between wheat and rye, the current study suggests that this effect of wheat bran may be ascribed to the aleurone rather than the pericarp/testa. The theoretical SCFA production values calculated from the disappearance of carbohydrate in the large intestine were (mmol/d): 1185, 377, 1208 and 800 for the whole rye, pericarp/testa, aleurone and endosperm diets respectively (assuming that 1 mg polysaccharide yields 0.5 mg SCFA as estimated by Cummings *et al.* (1989) and assuming an average molecular mass of the SCFA of 65 g/mol). These calculated differences in daily production of SCFA indicate the extent of physiological differences induced by diets with such different modes of carbohydrate fermentation.

Carbohydrates are the preferred substrates, compared with proteins, for the colonic flora, and it has been suggested that prolonging carbohydrate fermentation will reduce protein fermentation, and thus the presence of potentially toxic protein degradation products (NH_3 , phenols, indoles) in the distal colon (Cummings *et al.* 1979; McBurney *et al.* 1987). Isobutyrate and tryptophan degradation products (indole acetic acid, indole propionic acids, indole and skatole) were the protein degradation products measured in the present study. The concentrations of these components increased between the caecum and the faeces as expected, but did not differ significantly between diets, despite the large differences in carbohydrate fermentation. More animals may be needed to detect differences between the dietary treatments as there were large variations between pigs receiving the same diet. Although it was not possible to demonstrate an effect of carbohydrate fermentation on protein degradation, there were trends which may indicate such effects. Isobutyric acid was thus present in the proximal colon of the pigs fed on the pericarp/testa and endosperm diets where carbohydrate fermentation was limited, but only in later intestinal segments for the pigs fed on the aleurone diet, which provided more carbohydrates to be fermented in the colon. Similarly, the concentration of tryptophan degradation products increased markedly between the caecum and proximal colon for the pericarp/testa and endosperm diets, whereas a marked increase was observed more distally (between the distal colon and the rectum) for the aleurone diet.

Previous studies have shown effects on intestinal cellular metabolism when feeding rye-based products to rats (Lund *et al.* 1993; Lundin *et al.* 1993). The histological characteristics of the large intestine that were determined in the present experiment did not vary to any large extent between the four rye-bread diets, however. An effect of the diet on the intestinal tissue occurred mainly in the upper part of the large intestine, where the crypt height in the proximal colon was significantly greater in the pigs fed on the pericarp/testa diet compared with those fed on the whole-rye diet. The significance of this difference in crypt height is not clear, but it may suggest a relatively low proliferative activity in the epithelium of these animals where the DF was highly undegradable and the nutrient supply to the epithelium restricted. Several investigations have shown that SCFA stimulate cellular proliferation in the epithelium of the large intestine (Sakata, 1987; Frankel

et al. 1994). The higher crypts and the suggested lower proliferative activity of the epithelium may have an impact on the secretion of mucin as discussed by Brunsgaard (1997). Although the morphology of the large intestine was limited to simple characteristics, the present investigation indicated that the differences in colonic metabolism induced with the rye-bread diets may have an impact on the mucosa.

In conclusion, the present study showed how differences in characteristics and amount of rye DF in otherwise similar diets led to large variations in mode of carbohydrate fermentation. It was shown that mode of carbohydrate fermentation affected physiological variables such as faecal bulking, transit time, pH and SCFA production. Despite the large differences in carbohydrate fermentation, however, only minor significant differences in the presence of protein degradation products and on the histological measurements were observed. The pericarp/testa diet led to the highest faecal bulking and the lowest transit time, whereas the aleurone diet resulted in the lowest pH values and the highest concentrations of SCFA, including butyrate. It should be noted that the fermentation pattern of the whole-rye diet generally resembled that of the aleurone diet, but the effects of the aleurone diet may have been even more pronounced if it had been possible to purify the aleurone layer further.

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