British Journal of Nutrition (1984), **52**, 583–594

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

Modifications to swede (*Brassica napus* L.) anterior to the terminal ileum of pigs: some implications for the analysis of dietary fibre

BY P. MILLARD* AND A. CHESSON

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

(*Received 11 August 1983 – Accepted 22 June 1984*)

1. The degradation of a swede (*Brassica napus* L., cv. Danestone) diet anterior to the terminal ileum was studied in two pigs fitted with T-shaped cannulas 150 mm before the ileo-caecal junction. Digestibility was calculated with reference to chromic oxide and polyethylene glycol.

2. In addition to the total loss of free glucose and fructose, there were substantial modifications to cell-wall material recovered in digesta. These included the apparent loss of 46–50% of uronic acid residues, 72–78% of phenolic material and 10–24% of cellulose initially present in the feed, and a partial solubilization of some hemicellulose components.

3. Since 'fibre' recovered at the terminal ileum differed in a number of important respects from ingested fibre, a number of methods for the analysis of dietary fibre were examined to establish (1) the extent to which analysis of feed represented material recovered at the terminal ileum and (2) whether such methods could be applied directly to digesta samples. Results were compared with a complete analysis of water-soluble (620 g/kg dry matter) and water-insoluble (380 g/kg dry matter) fractions of feed.

4. Chemical fractionation techniques gave more reliable quantitative estimates of fibre than in vitro enzymic digestion methods which overestimated fibre. Acid- and neutral-detergent methods both gave too low an estimate of fibre. As none of the methods could allow for the loss of components (particularly pectic polysaccharides) found by sampling at the terminal ileum, none gave an accurate qualitative or quantitative representation of fibre at this point in the gut.

5. It is suggested that, as vegetable fibre recovered at the terminal ileum has already undergone partial hydrolysis, a more dynamic model of dietary fibre, in which the action of gut micro-organisms is considered, may be required to establish possible physiological roles of fibre or fibre components in the digestive tract. Recovery of digesta from sites of interest may be the only way of reliably estimating fibre or specific fibre components at different levels of the gut. This approach to dietary fibre may be impractical when applied directly to humans but the digestive tract of the pig may be a suitable alternative model.

It is proposed that dietary fibre has an important role in the avoidance of a number of human diseases associated with a highly refined diet (Burkitt & Trowell, 1975). There is, however, conflicting epidemiological evidence for the role of dietary fibre in disease prevention, and there has been little success in identifying those fibre components responsible for the effects noted in clinical trials (Cummings, 1981). A major problem in such studies has been the lack of a consensus on what constitutes fibre in food.

A common definition of dietary fibre, first used by Trowell *et al.* (1976), is the plant polysaccharides (including storage polysaccharides and mucilages) and lignin which are resistant to hydrolysis by the digestive enzymes of man. Others have considered any component of the total diet to be included if it is not digested and absorbed in the small intestine. Indigestible cell-wall-bound proteins and minerals (Spiller *et al.* 1976; Theander & Åman, 1979*a*) have therefore been included in this wider definition.

Methods developed to measure dietary fibre may be considered as falling into one of two categories: (1) sequential chemical extraction; (2) in vitro enzymic digestions.

The chemical extractions used range from simple gravimetric acid (ADF)- or neutral (NDF)-detergent-fibre methods (Van Soest, 1963; Van Soest & Wine, 1967) to complex fractionation schemes based on an understanding of the structure and composition of plant cell walls. These methods provide a measure of the carbohydrate and lignin content of food,

* Present address: Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QT.

the fractions of which may be summed to give a value for dietary fibre (Southgate, 1976; Selvendran *et al.* 1979; Theander & Åman, 1979*b*). In contrast, the enzymic methods require an understanding of digestive physiology rather than plant-cell-wall structure and attempt to mimic the endogenous secretions of the host animal (Hellendoorn *et al.* 1975; Schweizer & Würsch, 1979).

Dietary fibre may be modified substantially during passage through the gut by the non-enzymic effects of digestive secretions and, in particular, as a result of microbial activity. Although fermentation of plant polysaccharides in the hind gut is well recognized, analysis of samples from ileostomy patients has suggested that changes in the composition of fibre may occur before entry into the large intestine (Sandberg, 1982; Holloway *et al.* 1983). Such modifications need to be considered when assessing the possible physiological role of fibre or specific fibre components in the upper digestive tract.

The present study was undertaken: (1) to measure the extent of digestion of dietary fibre components in the small intestine of the pig given a vegetable diet with a high soluble fibre content; (2) to compare several methods of dietary fibre analysis in both feed and ileal digesta, and to determine the extent to which methods developed for the analysis of feeds could be applied directly to digesta samples; (3) to consider the suitability of the pig as a model for human digestion.

MATERIALS AND METHODS

Animals and housing

The experiments were carried out on two Large White × (Landrace × Large White) female pigs weighing 165 kg (pig A) and 160 kg (pig B), each fitted with a T-shaped cannula 150 mm before the ileo-caecal junction. Such cannulation does not appear to affect the efficiency of digestion anterior to the ileo-caecal junction, in spite of some disruption of the digesta flow (Wenham & Wyburn, 1980). No formal proof of digestive efficiency equivalent to that found in the intact animal can, however, be readily obtained. The animals were housed individually in smooth-walled pens with a floor area 2·3 × 2·0 m. The ambient temperature was maintained at about 20° and water was freely available from drinking bowls. Bedding material was not provided during periods of experimental work.

Diets

Swedes (*Brassica napus* L., cv. Danestone) were prepared by maceration in a Wolfking mincer. The experimental diet consisted of macerated swede supplemented with minerals, trace elements and vitamins (g/kg dry matter; 13·2 dicalcium phosphate, 0·3 vitamin B_{12} supplement, 2·5 vitamin–trace element supplement). In addition, 5 g chromic oxide and 10 g polyethylene glycol 4000 (PEG) were added per kg dry matter, as insoluble and soluble markers respectively. Feeds were thoroughly mixed and individual portions weighed into polyethylene bags and stored at 4°. The pigs were given 2·5 kg feed four times daily at 08.00, 11.00, 14.00 and 17.00 hours. A gradual loss of appetite occurred after feeding this diet for more than 5 d so between periods of digesta collection, animals were given a feed comprising (g/kg) 500 swede and 500 cereal feed (based on barley, weatings and extracted soya-bean meal) on a dry matter basis.

Collection of digesta

Animals were given the experiment diet for 3 d before digesta samples were collected. A PVC tube (300 × 50 mm) was attached to each cannula from 09.30 to 16.30 hours on the day of collection, and emptied as necessary to collect all forthcoming digesta (2–5 litres). The length of digesta collection was sufficient to minimize any sampling errors due to a circadian rhythm of digestibility (Livingstone *et al.* 1980). Feed samples were also taken

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

on the day of collection, and both digesta and feed samples were freeze-dried, milled and stored at −20°.

Chemical analysis

Samples were separated into water soluble or insoluble fractions by suspending 5·0 g of material in 80 ml water, stirring for 30 min at room temperature, then centrifuging at 17000 ***g*** for 30 min. The supernatant fraction was retained and the pellet re-extracted twice as described. The pellets and combined supernatants were freeze-dried and the weight of each fraction measured.

Monomeric glucose, fructose and mannose levels in soluble samples were determined by the u.v. enzymic methods (Boehringer Mannheim) using hexokinase (*EC* 2.7.1.1), β-fructofuranosidase (*EC* 3.2.1.26) and phosphomannose isomerase (*EC* 5.3.1.8) respectively. For the determinations of other neutral sugars, soluble samples (60 mg) were hydrolysed with 3 ml 0·5 M-sulphuric acid at 100° for 3 h and neutralized with 15 M-ammonia solution. Insoluble samples (15 mg) were hydrolysed in 0·3 ml 12 M-H_2SO_4 at room temperature for 1 h, diluted with water to give 1·0 M-H_2SO_4 then heated to 100° for 5 h before neutralization as described previously. Monosaccharides released from samples by acid-hydrolysis were determined as their alditol acetates, prepared by the method of Blakeney *et al.* (1983) and separated by gas–liquid chromatography (GLC) using the method of Bacon & Gordon (1980). The method of Kintner & Van Baren (1982) was used for the determination of uronic acids in the acid-hydrolysates. Methylation analysis of polysaccharides used the preparative methods of Lomax *et al.* (1983) and the separation and identification procedures of Lomax & Conchie (1982).

Volatile fatty acids (VFA) were analysed by the GLC method of Supelco Inc. (1975) for acetic, propionic and butyric acids. Lactic acid was determined by the microdiffusion technique described by Conway (1957). An estimate of the total phenolic content of samples was made using the acetyl bromide method described by Morrison (1972), against calibrations prepared with known amounts of ferulic acid. The cellulose content of water-insoluble fractions was determined by the method of Updegraff (1969). Ash was estimated by the method of the Association of Official Agricultural Chemists (1965), insoluble nitrogen by a microKjeldahl method, and soluble protein by the technique of Read & Northcote (1981) with bovine serum albumin as a standard.

Before analysis of PEG in total feed or digesta samples, 1·5 g was hydrated with 10 ml water, shaken at 4° for 16 h, a further 10 ml water added and the samples centrifuged at 17000 ***g*** for 30 min. The pellet was washed twice with 20 ml water and a portion of the combined supernatant fractions used for the determination of PEG by the technique of Malawar & Powell (1967). Analysis of Cr_2O_3 in total feed and digesta samples was by the method of Hesford & Buhrer (1978).

Dietary fibre preparations

Feed and digesta samples were fractionated by various methods used to determine dietary fibre. Methods used were the enzymic digestions of Schweizer & Würsch (1979) and Dovell & Harris (1982); extractions of Theander & Åman (1979*b*) and Chen & Anderson (1981) and ADF and NDF methods of Van Soest (1963) and Van Soest & Wine (1967). All soluble and insoluble fibre-fractions produced were freeze-dried and the individual components analysed by the methods described here, rather than those used by the various authors.

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

Table 1. *Composition of the swede* (Brassica napus *L.*) *diets and the major constituents recovered and the components lost anterior to the terminal ileum*

(Values are expressed as g/kg dry matter content of the feed and are the means with their standard errors for six replicate experiments for pig A and three for pig B)

	Feed dry mater				Feed dry matter recovered in digesta				Loss of each component (%)	
	Soluble		Insoluble		Soluble		Insoluble			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Carbohydrate:										
Polysaccharide residues										
Rhamnose	1·7	0·3	2·7	0·2	1·9	0·3	1·1	0·2	30·53	4·27
Fucose	Trace		1·3	0·1	0·7	0·1	0·7	0·1	0	0
Arabinose	4·7	0·4	7·8	0·2	3·7	0·3	3·6	0·4	41·60*	3·26
Xylose	0·4	0·2	9·0	0·4	0·8	0·1	8·4	0·5†	2·53	0·60
Mannose	0	0	6·8	1·2	2·9	0·7	4·4	0·4††	1·73	0·08
Galactose	8·7	0·5	7·7	0·4†	4·1	0·2	4·4	0·3	48·07	2·45
Cellulose	—	—	121·5	4·5	—	—	98·3*	3·1	19·20	2·79
Uronic acid	41·6	4·3	103·8	4·3	40·3	4·0	35·0**	1·6	48·23	2·32
Free sugars										
Fructose	159·9	6·9	0	0	1·2	0·4†	0	0	99·23	0·28
Glucose	173·7	11·1	0	0	3·4*	0·4	0	0	97·90	0·50
Non-carbohydrate:										
Phenolics	74·7	6·3	15·9	1·6†	10·6*	1·0†	1·25	1·1	74·13	2·71
Soluble protein	2·6	0·2	0	0	4·4	0·6	0	0	0	0
Nitrogen	—	—	8·0	1·4	—	—	2·1	0·3	71·33*	3·55
Ash	59·2	2·9	47·4	3·8	68·0	5·6†	55·5	3·7†		
Total 1‡	527·2		331·9		142·0		226·0			
Total 2§	620·2	22·2	379·8	17·9	227·7**	4·8††	253·2	11·1		

Significant difference in means between animals: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.
Significant difference in variability between animals: † $P < 0{\cdot}05$, †† $P < 0{\cdot}01$.
‡ Total of components identified.
§ Total of components measured gravimetrically.

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

Table 2. *The apparent loss of water-insoluble components from a swede diet recovered from the terminal ileum calculated using cellulose as an internal marker*

(Values are means with their standard errors for six replicate experiments for pig A and three for pig B)

	Loss of feed dry matter (%)			
	Pig A		Pig B	
Component	Mean	SE	Mean	SE
Rhamnose	0·12	0·04	0·13	0·03
Fucose	0·01	0·01	0	
Arabinose	0·33	0·07	0·22	0·06
Xylose	0·22	0·03	−0·15	0·04
Mannose	0·12	0·12	−0·12	0·04
Galactose	0·20	0·08	0·15	0·09
Cellulose	0		0	
Uronic acids	5·97	0·54	4·79	1·60
Phenolics	−0·03	0·16	−0·45	0·06

Table 3. *(1 → 4)-Linked glucose present in the feed and recovered from the digesta of pigs*

(Values are expressed as g/kg feed dry matter and are means with their standard error for six replicate experiments in the case of pig A and three replicates in the case of pig B)

	g/kg Feed dry matter			
	Pig A		Pig B	
	Mean	SE	Mean	SE
Glucose in feed	112·7	4·5	105·0	10·3
Glucose in digesta	85·8	7·0	80·7	10·0
Loss	26·9	3·3	24·3	17·7

RESULTS

Modification of a swede diet by passage through the small intestine

A dual-phase marker system was used throughout and the ratio, Cr_2O_3: PEG in feed and digesta samples compared. The mean (with SE) values for feed and digesta respectively were 1·81 (SE 0·09) and 1·94 (SE 0·20) for pig A and 1·97 (SE 0·04) and 2·52 (SE 0·18) for pig B. When calculating the quantity of each soluble fibre component an arithmetic correction was made to allow for discrepancy between the marker ratios in the appropriate feed and digesta samples. The fractions of the major swede constituents recovered from the terminal ileum are shown in Table 1 together with the original composition of the feed.

Two distinct modifications to the swede diet were detected in digesta recovered from the terminal ileum. Firstly, there was a loss of virtually all soluble glucose, fructose and phenolic material, and an approximately 50% loss of uronic acids, galactose and arabinose. In addition, there was a partial loss from the insoluble fraction of the feed of each of the neutral sugars, uronic acids and phenolic material, suggesting that some solubilization of cell-wall material had taken place.

The levels of cellulose found in the digesta were also lower than those in the diets for both animals (Table 1). To confirm that this loss was not an artefact due to possible errors associated with the insoluble-phase marker used (for example Cr_2O_3 not passing at the same

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

Table 4. *Organic acids recovered in digesta from the terminal ileum*

(Values are expressed as g/kg digesta dry matter and are means with their standard errors for six replicate samples for pig A and three for pig B)

	g/kg Digesta dry matter			
	Pig A		Pig B	
Acid	Mean	SE	Mean	SE
Acetic	35·1	6·4	11·7	0·9
Propionic	17·3	5·4	8·0	0·3
Butyric	7·6	0·6	0·7	0·2
Lactic	16·4	2·8	27·6	2·4

rate as the insoluble phase of digesta, or not being closely associated with that phase) the values for the water-insoluble components were recalculated using cellulose as an internal marker, thereby assuming no loss (Table 2). This reduced the apparent losses of phenolic material, uronic acids and galactose, while xylose was recovered from the digesta of both pigs in amounts greater than those consumed. This strongly suggested that an absolute loss of cellulose had occurred. The phenomenon was further studied by methylation analysis of insoluble polysaccharides of feed and digesta to measure specifically (1 → 4)-linked glucose, a more accurate determination of cellulose than the colorimetric assay used. Again, results indicated an absolute loss of cellulose anterior to the terminal ileum (Table 3).

Analysis of organic acids recovered from digesta

VFA and lactic acid were identified in the digesta samples recovered from both pigs (Table 4). However, measurements were made on freeze-dried material. Although the pH of digesta was near neutrality, subsequent checks on the effect of freeze-drying on VFA recovery indicated that the values shown in Table 4 are underestimated by approximately 10% overall.

Analysis of dietary fibre

Results shown in Table 1 indicated that when ash and residual free sugars were excluded a loss of approximately 40% of the dry matter from the swede diets occurred before digesta passed into the hind gut.

Comparative analysis of a feed sample

A single feed and corresponding digesta sample from pig A, in which the recovery of swede dry matter was 219 g/kg of that consumed, were selected to evaluate a number of representative methods of dietary fibre analysis. The selected methods were used to prepare fractions of both feed and digesta. For comparative purposes, analysis of each fraction was by the methods described here rather than those selected by the authors.

The extraction techniques of Theander & Åman (1979*b*) and Chen & Anderson (1981) both include fractionations of an alcohol-insoluble food residue into water soluble or insoluble components, after enzymic removal of any starch present with an amylase (*EC* 3.2.1.1 and 3.2.1.2) or amyloglucosidase (*EC* 3.2.1.3) preparation. These methods gave dietary fibre values (Table 5) of 243 g/kg (Theander & Åman, 1979*b*) and 225 g/kg (Chen & Anderson, 1981) diet compared with 219 g/kg swede dry matter recovered in the digesta. Both methods overestimated soluble uronic acids and insoluble cellulose in the diet compared with the ileal digesta analysis while giving low recoveries of soluble phenolic material (Table 5). The chief quantitative difference between the prepar-

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

Table 5. *Comparison of the composition of dietary fibre fractions prepared from a feed sample, with the appropriate digesta sample from pig A*

(Values are given as g dry matter/kg feed, and are means from duplicate preparations; ash values and amounts of free sugars are omitted)

	Unfractionated feed		Unfractionated digesta		Fibre fractions prepared from feed by the method of: Theander & Åman (1979*b*)		Chen & Anderson (1981)		Schweizer & Würsch (1979)		Dovell & Harris (1982)	Van Soest & Wine (1967)	Van Soest (1963)
Component	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Insoluble
Rhamnose	1·8	2·4	1·8	1·2	1·5	1·5	3·5	0·9	2·4	2·4	2·0	0·5	0·1
Fucose	Trace	0·5	0·5	0·6	0·6	Trace	0·2	0·6	0·4	0·7	0·2	0·5	0
Arabinose	5·4	8·3	3·7	4·2	2·5	4·5	4·7	2·7	2·1	4·0	1·7	2·2	0·2
Xylose	Trace	10·2	0·6	9·6	1·2	8·4	0·9	7·4	0·3	10·8	0·5	6·8	2·1
Mannose	0	5·6	0·9	4·1	1·5	2·8	0·7	4·0	0·5	5·6	0·3	3·2	2·3
Galactose	9·0	7·2	3·1	4·6	2·9	5·0	4·8	5·0	3·1	7·0	3·1	2·5	0
Cellulose	0	105·9	0	85·4	0	107·8	0	94·4	0	126·1	0	90·4	101·7
Uronic acid	33·2	113·2	28·0	38·2	32·8	60·7	74·6	15·2	41·1	72·2	37·1	6·1	9·4
Phenolics	62·8	17·3	12·7	14·0	1·6	4·4	1·1	3·5	0·2	4·3	1·0	2·3	2·0
Soluble protein	1·8	0	4·8	0	Trace	0	0·9	0	1·9	0	1·8	0	0
Nitrogen	0	15·1	0	1·1	0	2·9	0	1·5	0	2·5	0	0·4	0·5
Total (dietary fibre)	114·0	286·2	56·1	163·0	44·6	198·0	91·4	133·7	51·3	238·8	47·7	114·9	118·3

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

ations was in the recovery of uronide: insoluble uronide was overestimated by the method of Theander & Åman (1979*b*) compared with the digesta sample, and underestimated by the method of Chen & Anderson (1981).

Both these methods compared favourably with the in vitro enzymic digestion technique used by Schweizer & Würsch (1979), which attempts to mimic the effects of passage of food through the small intestine on dietary fibre. This gave an estimate of 288 g dietary fibre/kg (Table 5) and overestimated all the main fibre components, particularly insoluble uronic acid, galactose and mannose. Their value for insoluble fibre in the diet was therefore 46% higher than that recovered from the digesta. It is unclear why more cellulose was recovered from digesta than was present in the feed, but this was not due to retrograde starch.

The method of Dovell & Harris (1982) produces a soluble fibre fraction extracted by ion-exchange resin (to remove soluble pectins) and an insoluble residue which is treated with enzyme preparations similar to those used by Schweizer & Würsch (1979). However, incomplete separation of the resin from the insoluble fraction meant that only analysis of the soluble fraction could be achieved (Table 5). This method overestimated soluble uronide compared with recoveries from digesta, and underestimated soluble fucose, arabinose, xylose and mannose as the method did not allow for the solubilization of the hemicellulose found at the terminal ileum. The overall recovery of soluble fibre was only 84% of those components identified in the soluble digesta.

The detergent extraction methods developed by Van Soest and his co-workers (Van Soest, 1963; Van Soest & Wine, 1967; Goering & Van Soest, 1970) give an estimate of cellulose, hemicellulose and lignin by NDF extraction or cellulose and lignin by ADF extraction. The recovery of insoluble fibre was low compared with the insoluble feed residues found in the digesta, and there were substantial losses of uronic acid, hemicellulose and phenolic material.

Comparative analysis of a digesta sample

It is evident from the previous section that the composition of fibre in feed, as determined by the published methods of analysis, differed in a number of important respects from that of the corresponding digesta sample. These differences were a product of two factors, namely, the inability of any of the methods to take account of microbial or other digestive action in the small intestine, and shortcomings in the analytical methods themselves. To examine the second of these two factors further the preparatory techniques already described were also applied directly to the digesta sample from pig A referred to previously (Table 6). Some 540 g/kg digesta was dietary fibre, while the rest comprised 220 g ash, 80 g VFA and 160 g unidentified components/kg. None of the methods recovered all the dietary fibre components. All gave low yields of soluble and insoluble neutral sugars and insoluble phenolics, and none adequately reflected soluble or insoluble uronide constituents. The extraction methods of Theander & Åman (1979*b*) and Chen & Anderson (1981) gave the most satisfactory quantitative recoveries of 387 and 415 g/kg respectively and provided the best account of insoluble cell wall material.

DISCUSSION

A major criterion in evaluating non-ruminants as models for dietary fibre studies in humans is their ability, relative to that of man, to retain and digest plant fibre. Digestion of fibre in man occurs to a greater extent than in small mammals such as the rabbit or rat, but to a lesser extent than in the pig (Van Soest *et al.* 1983). Such overall comparisons do not, however, provide any indication of the importance of different sites of fermentation, much or all of which is assumed to occur in the hind-gut. The small intestine of pigs is also extensively and permanently colonized by micro-organisms. VFA have been identified in

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

Table 6. *Comparison of the composition of dietary fibre fractions prepared from a digesta sample*

(Values are given as g dry matter/kg digesta, and are means from duplicate preparations; ash values and residual amounts of free sugars are omitted)

Component	Unfractionated digesta		Fibre fractions prepared from digesta by the method of: Theander & Åman (1979*b*)		Chen & Anderson (1981)		Schweizer & Würsch (1979)		Dovell & Harris (1982)	Van Soest & Wine (1967)	Van Soest (1963)
	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Insoluble
Neutral sugars (excluding cellulose)	33	57	13	35	12	35	12	36	13	28	8
Cellulose	0	202	0	183	0	191	0	200	0	171	175
Uronic acid	75	91	44	94	141	22	33	119	45	14	26
Phenolics	34	33	3	11	2	9	1	1	1	6	4
Soluble protein	9	0	Trace	0	2	0	2	0	1	0	0
Nitrogen	0	3	0	5	0	2	0	4	0	1	1
Total	151	386	60	328	157	259	48	369	60	220	214

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

the stomach and small intestine of slaughtered pigs (Friend *et al.* 1963*a*; Argenzio & Southworth, 1975; Clemens *et al.* 1975) and in digesta collected from duodenum, mid-jejunum and terminal ileum (Sambrook, 1979). The chief organic acids found were lactic and acetic acids, with lesser quantities of propionic and butyric acids, as found in the present investigation. Lactic acid, detected in substantial amounts in digesta from the terminal ileum (Table 4), was found as a minor component only of organic acids in the caecum and colon by Friend *et al.* (1963*a*, *b*). This makes it unlikely that the organic acids found represented contamination with material from the large intestine due to a disturbed flow of digesta caused by cannulation. However, VFA can be formed by the fermentation of any carbohydrate, and Mason & Just (1976) have shown that where the diet contains more digestible or fermentable carbohydrate there will be relatively more fermentation in the stomach and less in the large intestine. The identification of VFA in the digesta is therefore indicative only of the presence of microbes anterior to the terminal ileum.

A loss of 'hemicellulose' (measured by the difference between cell wall content and ADF values) of up to 47% before the terminal ileum, was found by Keys & DeBarthe (1974) who gave diets containing 300 g cell wall material/kg diet to 82–90-kg pigs fitted with T-shaped cannulas. For one diet used these workers also recorded a 33% apparent digestion of cellulose anterior to the terminal ileum. Sambrook (1979) also reported a loss of ADF from the diet anterior to the duodenum and between the mid-jejunum and terminal ileum of 30-kg pigs fitted with re-entrant cannulas. Although these results suggest that microbial fermentation of cell wall components can occur anterior to the terminal ileum, the authors of both studies expressed some doubt about the general significance of their findings and both suggested that the loss of cellulose in the small intestine may have been an artefact.

The results obtained in the present investigation point to a substantial loss of pectic polysaccharides, phenolic material and $(1 \rightarrow 4)$-linked glucan (cellulose) from swede residues recovered from the terminal ileum (Table 1). The calculation of the magnitude of these losses was dependent on the recovery of the dual markers used. It is well recognized that the use of Cr_2O_3, in particular, may give rise to biased results (Beever *et al.* 1978). However, recalculating the recoveries of water-insoluble components using cellulose itself as a marker for the insoluble phase (and assuming no loss) showed that xylose was recovered from digesta in amounts greater than that consumed. This strongly suggests that a loss of cellulose, of similar magnitude to that detected by the use of Cr_2O_3, had occurred.

Subsequent work with unmodified animals, in which the entire digestive tract was removed, sectioned and each section examined for microbial activity, has shown that both cellulolytic and pectinolytic bacteria are present in substantial numbers in swede-fed animals. In contrast, no cellulolytic organisms were isolated from bran-fed animals and numbers of pectinolytic bacteria were much reduced (P. Millard and A. Chesson, unpublished results).

Micro-organisms in the human ileum may be fewer in number and may be more transitory than is typical of the pig (Drasar & Hill, 1974). Nonetheless, studies with ileostomy patients have shown significant losses of 'dietary fibre' components in the upper digestive tract. Analysis of the ileostomy contents of Swedish patients receiving bran or pectin showed maximum losses of approximately 20% of ingested bran cell walls and 30% of ingested pectin (Sandberg, 1982). In a similar study in New Zealand, Holloway *et al.* (1983) also detected a loss of 15–45% of ingested pectic substances in the small intestine. Although these values are lower than the 46–50% loss of pectic substances found for the pig (Table 1), the patients examined were, in both cases, from industrial-type societies. Residents of non-industrial-type societies, whose diets generally are richer in carbohydrate and hence dietary fibre, have been shown to support an ileal flora in which the numbers of anaerobes

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

approach that of the pig (Bhat *et al.* 1972). Thus the microbial modification of fibre anterior to the terminal ileum may be greater in these subjects.

The results from this and other studies with surgically modified pigs and those obtained with ileostomy patients indicate that, in the case of vegetable fibre at least, dietary fibre at the terminal ileum has already been hydrolysed to some extent. Although methods of fibre analysis have been developed which reflect the composition and structure of plant cell walls, they cannot predict modification of these structures in the small intestine and beyond (Table 5). Useful information from clinical trials or epidemiological studies may be obtained by correlation of dietary fibre intake (often calculated from information provided in food tables) with effects noted in small or large bowel to indicate the value or otherwise of a high-fibre diet. However, when considering the possible physiological role of fibre or fibre components in the diet, a more dynamic model of dietary fibre, in which the effects of gut micro-organisms are considered, may be more appropriate. In this case perhaps the only valid measure of dietary fibre would be the recovery and analysis of digesta from the site of interest. Clearly this approach poses considerable ethical and practical problems when applied directly to human digestion and points to the need for a suitable model, such as the pig digestive tract, as an alternative.

The authors would like to thank Dr R. M. Livingstone and Mr C. A. Simpson for the surgical preparation of the pigs and Ms Sandra Bain for her skilled assistance.

The results of the research, the contents of which are reported in the document, are the property of the Ministry of Agriculture, Fisheries and Food and are Crown Copyright.

REFERENCES

Argenzio, R. A. & Southworth, M. (1975). *American Journal of Physiology* **228**, 454–460.

Association of Official Agricultural Chemists (1965). *Official Methods of Analysis.* Washington DC: Association of Official Agricultural Chemists.

Bacon, J. S. D. & Gordon, A. H. (1980). *Journal of Agricultural Science, Cambridge* **94**, 361–367.

Beever, D. E., Kellaway, R. C., Thomson, D. J., MacRae, J. C., Evans, C. C. & Wallace, A. S. (1978). *Journal of Agricultural Science, Cambridge* **90**, 157–163.

Bhat, P., Shantatumari, S., Rajan, D., Mathan, V. I., Kapadia, C. R., Swarnabi, C. & Baker, S. J. (1972). *Gastroenterology* **62**, 11–21.

Blakeney, A. B., Harris, P. J., Henry, R. J. & Stone, B. A. (1983). *Carbohydrate Research* **113**, 291–299.

Burkitt, P. P. & Trowell, H. C. (1975). *Refined Carbohydrate Foods and Diseases.* London: Academic Press.

Chen, W. L. & Anderson, J. W. (1981). *American Journal of Clinical Nutrition* **34**, 1077–1082.

Clemens, E. R., Stevens, C. E. & Southworth, M. (1975). *Journal of Nutrition* **105**, 759–768.

Conway, E. J. (1957). *Microdiffusion Analysis and Volumetric Error.* London: Crosby Lockwood.

Cummings, J. H. (1981). In *Nutrition and Health: a Perspective*, pp. 35–48 [M. R. Turner, editor]. London: MTP Press Ltd.

Dovell, C. J. & Harris, N. D. (1982). *Journal of the Science of Food and Agriculture* **33**, 185–193.

Drasar, B. S. & Hill, M. J. (1974). *Human Intestinal Flora.* London: Academic Press.

Friend, D. W., Cunningham, H. M. & Nicholson, J. W. G. (1963*a*). *Canadian Journal of Animal Science* **43**, 174–181.

Friend, D. W., Cunningham, H. M. & Nicholson, J. W. G. (1963*b*). *Canadian Journal of Animal Science* **43**, 156–168.

Goering, H. K. & Van Soest, P. J. (1970). *Forage Fiber Analyses. US Department of Agriculture. Agricultural Handbook* no. 379. Washington DC: US Department of Agriculture.

Hellendoorn, E. W., Nordohoff, M. G. & Shagman, J. (1975). *Journal of the Science of Food and Agriculture* **26**, 1461–1468.

Hesford, F. & Buhrer, M. (1978). *Clinica Chemica Acta* **82**, 225–228.

Holloway, W. D., Tasman-Jones, C. & Maher, K. (1983). *American Journal of Clinical Nutrition* **37**, 253–255.

Keys, J. E. & DeBarthe, J. V. (1974). *Journal of Agricultural Science, Cambridge* **39**, 53–56.

Kintner, P. K. & Van Baren, J. P. (1982). *Journal of Food Science* **47**, 756–759, 764.

Livingstone, R. M., Baird, B. A., Atkinson, T. & Crofts, R. M. J. (1980). *Journal of Agricultural Science, Cambridge* **94**, 399–405.

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press

Lomax, J. A. & Conchie, J. C. (1982). *Journal of Chromatography* **236**, 385–394.
Lomax, J. A., Gordon, A. H. & Chesson, A. (1983). *Carbohydrate Research* **122**, 11–22.
Malawar, S. J. & Powell, D. W. (1967). *Gastroenterology* **53**, 250–256.
Mason, V. C. & Just, A. (1976). *Zeitschrift für Tierphysiologie, Tierenährung und Futtermittelkunde* **36**, 301–310.
Morrison, I. M. (1972). *Journal of the Science of Food and Agriculture* **23**, 455–463.
Read, S. M. & Northcote, D. M. (1981). *Analytical Biochemistry* **116**, 53–64.
Sambrook, I. E. (1979). *British Journal of Nutrition* **42**, 279–287.
Sandberg, A.-S. (1982). *Dietary Fibre – Determination and Physiological Effects*. Göteborg, University of Göteborg, Sweden.
Schweizer, T. F. & Würsch, P. (1979). *Journal of the Science of Food and Agriculture* **30**, 613–619.
Selvendran, R. R., Ring, S. G. & DuPont, M. S. (1979). *Chemistry and Industry* 225–230.
Southgate, D. A. T. (1976). In *Fiber in Human Nutrition*, pp. 73–107 [G. A. Spiller and R. J. Amen, editors]. New York and London: Plenum Press.
Spiller, G. A., Fassett-Cornelius, G. & Briggs, G. M. (1976). *American Journal of Clinical Nutrition* **29**, 934–935.
Supelco Inc. (1975). *Technical Bulletin* 749D. Bellefonte: Supelco Inc.
Theander, O. & Åman, P. (1979*a*). In *Dietary Fibers: Chemistry and Nutrition*, pp. 215–244 [G. E. Inglett and S. I. Falkehag, editors]. New York and London: Academic Press.
Theander, O. & Åman, P. (1979*b*). *Swedish Journal of Agricultural Research* **9**, 97–106.
Trowell, H., Southgate, D. A. T., Wolevar, T. M. S., Leeds, A. R., Gassull, M. A. & Henkins, D. J. A. (1976). *Lancet* **i**, 967.
Updegraff, D. M. (1969). *Analytical Biochemistry* **32**, 420–424.
Van Soest, P. J. (1963). *Journal of the Association of Official Agricultural Chemists* **46**, 829–835.
Van Soest, P. J., Feraci, J. & Foose, T. (1983). In *Fibre in Human and Animal Nutrition*, pp. 75–80 [G. Wallace and L. Bell, editors]. Wellington: Royal Society of New Zealand.
Van Soest, P. J. & Wine, R. H. (1967). *Journal of the Association of Official Analytical Chemists* **50**, 50–55.
Wenham, G. & Wyburn, R. S. (1980). *Journal of Agricultural Science* **95**, 539–546.

Printed in Great Britain

https://doi.org/10.1079/BJN19840125 Published online by Cambridge University Press