Digestion of fibre polysaccharides of pea (*Pisum sativum*) hulls, carrot and cabbage by adult cockerels

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Characterization of the carbohydrates of pea (*Pisum sativum*) hulls, carrot and cabbage using both colorimetric and gas–liquid chromatographic techniques permitted a detailed investigation into the extent of digestion of differing types of fibre. These digestion studies were greatly aided by the development of a rapid bioassay employing starved adult cockerels. Total collection of undigested residues, uncontaminated by food spillage, could be made from trays placed under the cockerels. Chemical analysis showed that pea hulls consisted mainly of fibre with very little available carbohydrate present, whereas more than half of freeze-dried carrot and cabbage consisted of available carbohydrate (sucrose, glucose, fructose, starch) and consequently considerably less fibre was present. The fibre of carrot and cabbage was similarly composed of nearly equal amounts of neutral and acidic polysaccharides, whereas pea-hull fibre had four times as much neutral as acidic polysaccharides. The digestibility of total neutral polysaccharides from all three foodstuffs was extremely low. However, there appeared to be preferential digestion of polysaccharides composed of rhamnose, arabinose and galactose residues, all associated with pectic material, in contrast to the indigestibility of polysaccharides composed of fucose, xylose and glucose. Acidic polysaccharides were digested to a greater extent than neutral ones, and those of carrot and cabbage more so than pea hulls. The polysaccharides which were the most soluble were also the most digestible, but due to the arbitrariness of polysaccharide solubility, quantification of their total digestibility *per se* was considered not possible.

Fibre polysaccharides: Digestion: Cockerels

The increased use of methods for fibre analysis whereby the neutral sugar constituents from the hydrolysis of the fibre polysaccharides are converted to alditol acetate derivatives (Åman & Nordkvist, 1983; Ben-Ghedalia & Miron, 1984; Carre & Leclercq, 1985; Graham *et al.* 1985; Longstaff & McNab, 1986, 1987; Nordkvist & Åman, 1986), which are measured by gas–liquid chromatography, has permitted a more informed discussion on the relationship between polysaccharide structure and its digestion. In this context, fibre represents the sum of the individual neutral sugars together with the uronic acids from pectic substances and acidic xylans. The uronic acids are usually determined separately by either colorimetric (Blumenkrantz & Asboe-Hansen, 1973) or decarboxylation methods (Bylund & Donetzhuber, 1968; Theander & Åman, 1979). These more definitive methods invariably produce lower estimations of fibre compared with the older gravimetric procedures of crude fibre (Williams & Olmsted, 1925), neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) (Goering & Van Soest, 1970; Asp & Johansson, 1981). Discrepancies between the amounts of fibre derived by the newer and older methods are believed to be caused, in part, by losses of the more labile sugars during hydrolysis and incomplete hydrolysis of the more acid-resistant polysaccharide linkages using the newer methods, while the major source of error in the gravimetric methods is the inclusion of non-polysaccharides as fibre. It would seem that many more studies will be required to validate these newer methods, particularly with fibre digestion in simple-stomached animals.

The recent clinical interest in fibre digestion in man (Eastwood & Robertson, 1978; Stephen & Cummings, 1980; Eastwood, 1985) has stimulated numerous studies with simple-stomached animals (Gohl & Gohl, 1977; Low & Rainbird, 1984; Carre & Leclercq, 1985; Johnson & Gee, 1986; Nyman *et al.* 1986) that have implicated fibre in a number of digestive and physiological gut functions. Most of these studies have concentrated on the complex physiological roles of polysaccharides such as their ability to (*a*) delay gastric emptying, (*b*) function as diluents such that absorption is delayed, (*c*) promote fermentation and (*d*) act as chelators or exchange resins. Viscous polysaccharides, both ionic (pectins) and non-ionic (guar gum) are believed to affect lipid metabolism in several ways: by lowering blood cholesterol (Mathe *et al.* 1977; Cerda *et al.* 1985) and by promoting excretion of bile salts (Meittinen & Tarpila, 1977; Robertson *et al.* 1980), or through the production of fermentation products such as propionic acid, the sodium salt of which has been shown to lower blood and liver cholesterol (Chen *et al.* 1984). Viscous polysaccharides have also been shown to reduce blood glucose either by slowing gastric emptying (Tsai & Peng, 1981) or by interfering with absorption (Jenkins *et al.* 1977).

Interest in the extent to which fibre is fermented in simple stomached animals by caecal or colonic bacteria has increased in recent years. Evidence of fermentation, however, has been largely by implication, deduced from the difference between the quantity of fibre found in the diet and that in the excreta rather than from direct measurement of volatile fatty acids. The few studies on fibre digestion by chickens has given estimates of implied fermentation of about 15–33% with graminaceous fibre (Bolton, 1954, 1955; Longstaff & McNab, 1986), 20–30% digestion of pea (*Pisum sativum*) fibre (Longstaff & McNab, 1987) and none at all of the insoluble fibre in lupin (*Lupinus luteus*) seeds (Carre & Leclercq, 1985). Pigs, on the other hand, have been reported to derive much more energy from fibre than chickens. Graham *et al.* (1985) reported over 50% degradation of both pea and barley fibre by pigs; Millard & Chesson (1984) reported 46–50% degradation of uronic acids, and 10–24% of cellulose anterior to the terminal ileum in pigs fed on swedes (*Brassica napus*); and other studies have reported 50% digestion of sugar-beet fibre and 20% digestion of wheat-bran fibre (Graham *et al.* 1986). Stanogias & Pearce (1985) reported 2·6–9·3% cellulose digestion and between 1 and 99·9% digestion of hemicelluloses ingested by the pig. Wheat-bran-fibre digestion in the rat was reported to be zero (Mathe *et al.* 1977), while more recent studies by Bach Knudsen *et al.* (1985) showed fibre from barley, barley + aleurone, and barley + husk to be digested by the rat to the extent of 61, 56 and 38% respectively; moreover caecectomy lowered this digestion to 38, 19 and 16% respectively. Nyman *et al.* (1986), who believe that rats can be used as models in human digestion studies, reported about 40% digestion of wheat bran fibre by rats and 34% digestion in humans, and between 75 and 90% digestion of fibre from legumes and fruit in both species.

In the present study when referring to digestibility of fibre, the word is intended to mean its overall disappearance from the gut. Reasons for finding a lower amount of fibre sugar residues in the excreta compared with that in the foodstuff may be due to several factors. The bird's endogenous secretions may aid fibre solubilization, facilitating not only (*a*) its hydrolysis in the crop and hindgut by bacterial enzymic activity, but also (*b*) hydrolysis by previously dormant enzymes intrinsic in the foodstuff and re-activated during passage through the digestive tract. While the former mechanism of fibre degradation would result in the production of volatile fatty acids, the latter might possibly provide a small amount of digestible sugars.

In the present study, pea hulls were chosen as an example of fibre from secondary cell walls, and carrot and cabbage fibre as examples of primary cell walls. All three feedingstuffs

contained water-soluble pectic substances, while their negligible starch content eliminated any risk of inflating cellulose digestion through an inability to remove starch quantitatively from the input.

MATERIALS AND METHODS

Feedingstuffs

Dried peas, var. Progretta, were lightly hammered and the hulls manually separated from the cotyledons. The hulls were ground through a 1 mm sieve.

Fresh carrot and cabbage were finely chopped, frozen, freeze-dried and ground through a 1 mm sieve.

For analytical purposes samples were reground through a 0·12 mm sieve.

Bioassay

Individually caged adult cockerels, which had been starved for 48 h except for the administration of glucose, were tube-fed on each of the three feedingstuffs such that six birds received 50 g and six birds received 25 g (six birds × three feedingstuffs × two doses, i.e. thirty-six birds). The excreta (faeces + urine) voided during the subsequent 72 h were collected from trays placed under the cages, frozen at −20°, freeze-dried, weighed and ground through a 1 mm sieve. A control group of six adult cockerels was given 50 g glucose and these birds provided a measurement of endogenous losses and contribution from bacteria to the excreta. Birds were given access to water and tube-fed on water twice during the 72 h collection period. Further details of the exact protocol followed is given elsewhere (McNab & Blair, 1988). Although the procedure has not previously been used except in this laboratory (Longstaff & McNab, 1986, 1987) to measure the digestibility of the carbohydrate constituents of foods, it has been extensively applied to the determination of the digestibilities of the amino acids in dietary proteins where the same principles apply (Likuski & Dorrell, 1978; Sibbald, 1986). Digestibility of fibre from pea hulls, carrot and cabbage was calculated for each bird. The fibre found in the excreta from the control birds given glucose was subtracted from that found in the excreta from birds given the experimental foods in order to determine true digestibility.

Chemical analysis

1. Colorimetric analysis of free sugars, 'sucrose' and starch. Reducing sugars were quantitatively measured colorimetrically using *p*-hydroxybenzoic acid hydrazide (PAHBAH) which reacts to form a yellow chromogen (Lever, 1972; Hudson & John, 1976; Tawflik & Mardon, 1985). Starch was converted to the reducing sugar glucose by the enzymic action of amyloglucosidase (*EC* 3.2.1.3, Sigma Chemical Co., Poole, Dorset) (Southgate, 1981; Theander & Åman, 1981) and hydrolysis of sucrose to its reducing sugar components, glucose and fructose was achieved using the enzyme invertase (*EC* 3.2.1.26, Sigma Chemical Co.) (Blakeney & Mutton, 1980). The precise details of the procedure are as follows. Feedingstuffs (50 mg) or excreta (50 mg) were weighed into polypropylene tubes. 0·2 M-Sodium acetate buffer, pH 4·5 (9 ml), was added and the capped tubes placed in an oven at 100° for 4 h in order to gelatinize the starch. On cooling to below 50°, 1 ml sodium acetate buffer containing 0·1 mg amyloglucosidase or 1 ml buffer containing 0·1 mg amyloglucosidase + 0·25 mg invertase, or 1 ml sodium acetate buffer (enzyme blank) was added and the contents of the tubes incubated at 50° in a shaking water-bath for 16 h. After cooling, 0·4 ml supernatant fractions were diluted either to 100 ml (feedingstuffs) or 20 ml (excreta) and the free sugars, glucose from starch, and glucose + fructose ('sucrose') were measured colorimetrically after reaction with PAHBAH. The measurement of free sugars

was obtained from the enzyme blank tube, starch by difference from tubes incubated with or without amyloglucosidase, and 'sucrose' by difference from tubes incubated with amyloglucosidase with or without invertase.

2. Analysis of free sugars and 'sucrose' by gas–liquid chromatography. Pea hulls, carrot and cabbage (200 mg) were weighed into polypropylene tubes and destarched with or without the addition of invertase as described previously. An appropriate portion of supernatant fraction was evaporated to dryness and the monosaccharides present were converted to the corresponding alditol acetate and measured by gas–liquid chromatography according to the method of Blakeney *et al.* (1983).

3. Fibre preparation. The fractionation scheme employed here, whereby fibre polysaccharides were separated from starch and free sugars and collected as insoluble and total (soluble + insoluble) polysaccharides was essentially that recommended by Englyst *et al.* (1982) and Englyst & Cummings (1984).

(*a*) *Total polysaccharides (soluble + insoluble).* To the feedingstuff and excreta solutions, prepared as described previously after the removal of 0·4 ml supernatant fraction, were added 30 ml absolute alcohol to make the final solution 75% with respect to alcohol. The tubes were left overnight at 4° to aid precipitation of the water-soluble polysaccharides. Precipitates were washed three times with 30 ml 80% ethanol to ensure removal of free sugars, 'sucrose' and glucose from starch and finally once with 30 ml acetone. Precipitates were magnetically stirred when dry to a fine powder at 40°.

(*b*) *Insoluble polysaccharides.* To a parallel set of tubes containing destarched feedingstuffs and excreta, and after removal of 0·4 ml supernatant fraction, were added 30 ml 0·2 M-sodium phosphate buffer, pH 7·0. The insoluble polysaccharides were washed three times with 30 ml buffer to remove soluble polysaccharides, free sugars, 'sucrose' and glucose from starch and finally once with 30 ml acetone. Insoluble fibre was magnetically stirred when dry to a fine powder at 40°.

4. Hydrolysis of fibre and derivatization of neutral sugars. (*a*) *12 M-sulphuric acid pretreatment followed by 1 M-H_2SO_4 hydrolysis.* To the acetone-dried fibre from 50 mg feedingstuffs or excreta was added 0·25 ml 12 M-sulphuric acid and the tubes were whirled until the acid completely wetted the samples. The tubes were then placed in a water-bath at 40° for 1 h with occasional whirling to aid solubilization. Volumes were diluted to 3 ml 1 M-H_2SO_4 by the addition of 2·75 ml of an aqueous solution of inositol (2 mg/ml). The tubes were then capped and heated for 3 h at 100° in an oven.

(*b*) *1 M-H_2SO_4 hydrolysis of fibre.* To the acetone-dried fibre from 50 mg feedingstuffs or excreta were added 2·75 ml of an aqueous solution of inositol (2 mg/ml), followed by 0·25 ml 12 M-H_2SO_4; the capped tubes were placed in an oven at 100° for 3 h.

After hydrolysis solutions from (*a*) and (*b*) were neutralized with 15 M-ammonia and a 0·2 ml portion taken for determination of alditol acetate formation by the method of Blakeney *et al.* (1983).

5. Measurement of uronic acids. Portions of hydrolysates from the feedingstuffs and excreta from 3(*a*) were diluted appropriately and analysed by the colorimetric method of Blumenkrantz & Asboe-Hansen (1973). D-Galacturonic acid was used as a standard.

RESULTS

The free sugar, 'sucrose' and starch contents (the available carbohydrates) of pea hulls, carrot and cabbage measured colorimetrically are shown in Table 1. The small quantity of starch detected in pea hulls probably arose from contamination of the hulls with cotyledon. Carrot and cabbage contained large amounts of free sugars but very little starch. Carrot contained the largest amount of 'sucrose'. The available carbohydrate contents of carrot

Table 1. *Free sugars, 'sucrose' and starch contents of pea* (Pisum sativum) *hulls, carrot and cabbage analysed colorimetrically (g/kg dry matter)*

(Mean values and standard deviations)

	Free sugars		'Sucrose'		Starch		Available carbohydrate	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pea hulls	8·35	3·90	4·86	3·06	17·74	3·23	30·95	5·63
Carrot	305·98	24·30	238·98	10·58	8·47	8·73	553·43	17·77
Cabbage	485·34	33·11	63·88	6·22	21·12	24·41	570·33	19·46

and cabbage were similar, 553·43 and 570·33 g/kg respectively, comprising slightly more than half the dry matter content of these dicotyledons. In contrast, the available carbohydrate content of pea hulls was extremely low, 30·95 g/kg.

The monosaccharide compositions of the available carbohydrates of the three feedingstuffs are shown in Table 2. With each feedingstuff fairly good agreement was found between values derived by the gas–liquid chromatographic method (Table 2) and those from the colorimetric method (Table 1). Because the gas–liquid chromatographic method was performed on samples that had been treated with amyloglucosidase, the free sugar component contained a small amount of glucose released from starch and, in the case of pea hulls, it is most likely that all this free glucose arose from contaminating starch. Although the alditol acetate of mannose was detected by gas–liquid chromatography, both in the free sugar and 'sucrose' components of carrot and cabbage, it is most unlikely that mannose occurred as a monosaccharide in its own right, but arose from the borohydride reduction of fructose (a keto-sugar) in the derivatization procedure. On reduction and acetylation, fructose gives equimolecular amounts of mannitol hexa-acetate and glucitol hexa-acetate. Therefore, from the free sugar component of carrot, it can be deduced that there was 157·19 g fructose/kg (78·59 g mannitol hexa-acetate/kg + 78·59 g glucitol hexa-acetate/kg) leaving only 105·56 g glucose/kg present as a free sugar (184·15 g total glucitol hexa-acetate/kg, 78·59 g glucitol hexa-acetate/kg formed from fructose). Galactose occurred as a free sugar in carrot and cabbage, but not in pea hulls. By the same reasoning the fructose content of the 'sucrose' component of carrot can also be deduced. In this instance, however, fructose occurs, not as a monosaccharide but linked to glucose as half the 'sucrose' molecule before invertase treatment. Thus, carrot contained only 191·02 g true sucrose/kg (95·51 g glucose/kg + 95·51 g fructose/kg) leaving 62·95 g fructose/kg and 3·50 g galactose/kg having arisen from some other source. Similar deductions were also made for pea hulls and cabbage.

The monosaccharide compositions of the hemicellulotic polysaccharides and the combined hemicellulotic and cellulotic polysaccharides are shown in Table 3. In the case of the latter, analysis was extended to include insoluble as well as total (soluble + insoluble) polysaccharides. It can be seen that pea hulls contained the largest amount of hemicellulotic polysaccharides, composed mainly, although not exclusively, of xylose. Carrot and cabbage contained much less hemicellulose and only a very small amount of xylose-containing polysaccharides, typical of primary cell wall material. The mannose content of carrot and cabbage was higher than that of pea hulls. As a result of 12 M-H_2SO_4 pretreatment, glucose from cellulose contributed most to the increase in the sum of neutral sugars and to the greatest extent with pea hulls, typical of secondary cell wall material. The stronger acid

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Table 2. *Free sugar and 'sucrose' contents of pea* (Pisum sativum) *hulls, carrot and cabbage* (*g/kg dry matter*)

(Mean values and standard deviations)

	Free sugar + starch			'Sucrose'				
				Total			Total	Available carbohydrate
Pea hulls:				*Analysed as alditol acetate*				
		Gluc HA			Man HA	Gluc HA		
Mean		22·27			2·45	4·40		
SD		1·14			0·30	0·23		
				22·27			6·86	29·12
				1·14			0·25	1·39
				Deduced as monosaccharide				
		Glucose			Fructose	Glucose		
Mean		22·27			4·91	1·95		
SD		1·14			0·57	0·36		
Carrot:				*Analysed as alditol acetate*				
	Man HA	Gal HA	Gluc HA		Man HA	Gal HA	Gluc HA	
Mean	78·59	5·56	184·15		79·23	3·50	174·73	
SD	2·56	0·07	1·90		6·00	0·56	16·32	
				268·30			257·46	525·77
				4·52			22·63	22·19
				Deduced as monosaccharide				
	Fructose	Galactose	Glucose		Fructose	Galactose	Glucose	
Mean	157·19	5·56	105·56		158·46	3·50	95·51	
SD	5·19	0·07	0·67		11·99	0·56	7·65	
Cabbage:				*Analysed as alditol acetate*				
	Man HA	Gal HA	Gluc HA		Man HA	Gal HA	Gluc HA	
Mean	125·46	9·50	344·46		19·84	1·63	57·31	
SD	3·92	0·97	8·42		2·77	0·21	3·38	
				479·40			78·78	558·18
				13·30			6·38	19·68
				Deduced as monosaccharide				
	Fructose	Galactose	Glucose		Fructose	Galactose	Glucose	
Mean	250·88	9·50	219·00		39·67	1·65	37·47	
SD	7·84	0·97	4·50		5·54	0·25	0·61	

Gluc HA, glucitol hexa-acetate; Man HA, mannitol hexa-acetate; Gal HA, galacitol hexa-acetate.

Table 3. *Monosaccharide composition of pea* (Pisum sativum) *hulls, carrot and cabbage fibre after 1 M-sulphuric acid hydrolysis with or without 12 M-H_2SO_4 pretreatment (g/kg dry matter)*

(Mean values and standard deviations)

	Pea hulls				Carrot				Cabbage			
	Insoluble		Soluble + insoluble		Insoluble		Soluble + insoluble		Insoluble		Soluble + insoluble	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 M-H_2SO_4 hydrolysis												
Rhamnose	—	—	9·05	2·30	—	—	4·07	1·26	—	—	4·34	0·81
Fucose	—	—	2·87	0·38	—	—	0·60	0·16	—	—	1·34	0·17
Arabinose	—	—	32·22	7·35	—	—	13·38	2·13	—	—	22·33	6·21
Xylose	—	—	80·10	3·91	—	—	2·69	0·11	—	—	9·25	0·54
Mannose	—	—	0·94	0·10	—	—	1·41	0·20	—	—	2·19	0·55
Galactose	—	—	14·22	1·67	—	—	20·91	0·78	—	—	12·51	1·51
Glucose (H)	—	—	14·10	3·84	—	—	2·47	0·87	—	—	4·32	2·81
Sum of neutral sugars	—	—	153·26	8·47	—	—	45·54	0·79	—	—	55·57	4·31
12 M-H_2SO_4 pretreatment and 1 M-H_2SO_4hydrolysis												
Rhamnose	2·51	0·92	7·12	0·30	0·68	0·15	3·04	0·63	0·63	0·39	2·21	0·50
Fucose	1·74	0·35	2·83	0·30	0·22	0·13	0·41	0·06	0·92	0·32	1·26	0·80
Arabinose	12·04	4·20	32·25	3·80	4·23	0·55	12·70	1·05	9·43	1·82	19·28	3·08
Xylose	99·20	4·36	112·26	9·34	4·74	2·02	4·26	0·45	9·65	1·09	11·30	1·88
Mannose	1·84	0·09	2·45	0·18	4·35	0·39	4·66	0·28	4·05	0·87	4·80	0·73
Galactose	9·98	0·76	21·02	3·54	6·64	0·27	20·83	0·84	6·79	0·60	12·97	1·10
Glucose (H + C)	349·96	60·70	429·95	39·72	68·30	0·46	72·97	4·09	59·77	16·07	74·66	8·99
Sum of neutral sugars	436·23	51·80	607·88	49·24	88·48	1·83	118·92	1·20	91·37	18·81	128·16	15·88
Uronic acids	83·79	25·30	145·62	10·45	17·82	4·13	111·64	6·35	20·27	2·64	88·62	5·00

H, hemicellulotic; C, cellulotic.

treatment increased the contributions from xylose and mannose, and decreased slightly those from rhamnose and arabinose, presumably because of their degradation. Although pea hulls contained the largest amount of acidic polysaccharides, measured as uronic acids, in proportion to their neutral polysaccharides the amount was small, whereas carrot and cabbage possessed almost equal amounts of neutral and acidic polysaccharides. Total (soluble + insoluble) polysaccharides and insoluble polysaccharides were measured directly, but soluble polysaccharides were derived by difference. It can be seen that polysaccharides containing fucose, xylose, mannose and glucose from cellulose were only sparingly soluble, while those containing rhamnose, arabinose and galactose, characteristic of pectic substances, were more readily soluble.

Because no significant differences in the digestibility of fibre were found between birds that had been fed on either 50 g or 25 g feedingstuffs the results were pooled to obtain twelve birds per feedingstuff. Digestibility of total (soluble + insoluble) hemicellulotic polysaccharides is shown in Table 4. The overall digestibilities, calculated from the sum of neutral sugar residues, were very low, 6·1, 7·0 and 5·0% for pea hulls, carrot and cabbage respectively. The most digestible polysaccharides were those composed of rhamnose, arabinose, galactose and mannose. The extremely small amount of mannose-containing polysaccharide in pea hulls made accurate assessment of mannose in the excreta very difficult and, because more mannose was found in the excreta of control birds given glucose, all digestibilities were over 100%. The least digestible polysaccharides were those consisting of fucose and xylose in all three feedingstuffs and those consisting of glucose from pea hulls and carrot. A hemicellulotic polysaccharide in cabbage containing glucose was quite well digested. Differences in digestibility of polysaccharides were found between the three feedingstuffs.

The digestibility of total hemicellulotic and cellulotic polysaccharide (soluble + insoluble) is shown in Table 5. The overall digestibilities, calculated from the sum of neutral sugars, were extremely low, on average 4·2, 0·8 and −3·6% for pea hulls, carrot and cabbage respectively. The decrease in digestibility after 12 M-H_2SO_4 pretreatment was a consequence of the greater contribution made by indigestible cellulose. The most digestible polysaccharides were those belonging to the pectic substances, composed of rhamnose, arabinose, galactose and galacturonic acid. The least digestible polysaccharides were those composed of fucose, xylose and glucose from cellulose. The uronic acid-containing polysaccharides of carrot and cabbage were much more digestible than those of pea hulls. Differences in polysaccharide digestibilities were found between the three feedingstuffs.

Table 6 shows the influence of sample weight on the quantification of total and insoluble acidic polysaccharides as measured by their uronic acid contents. It can be seen that a consistent recovery of total acidic polysaccharides in vitro was possible because of their complete precipitation after three washings with 30 ml of 80% ethanol when sample weights of 50–200 mg feedingstuff (carrot) and 25–100 mg excreta (from two carrot-fed birds) were taken. On the other hand the quantity of insoluble acidic polysaccharides recovered after three washings with 30 ml water increased with increasing sample weight both in feedingstuff and excreta. Hence acidic polysaccharide solubility decreased with increased sample weight:solvent ratio.

DISCUSSION

The amounts of free sugars and 'sucrose' measured by the colorimetric method using PAHBAH were in close agreement with those measured by gas–liquid chromatography. The latter method, however, revealed that the sucrose content had been overestimated, its value inflated by the presence of sugars most likely obtained from the invertase hydrolysis

Table 4. *Digestibility* (%) *of total hemicellulotic fibre* (*soluble* + *insoluble*) *of pea* (Pisum sativum) *hulls, carrot and cabbage after 1* M*-sulphuric acid hydrolysis*

(Mean values and standard deviations)

	Pea hulls		Carrot		Cabbage			Statistical significance
	Mean	SD	Mean	SD	Mean	SD	SED	*P*
Rhamnose	14·8	14·8	2·2	8·4	9·7	10·5	4·74	< 0·05
Fucose	2·7	16·1	−42·7	36·3	−29·2	28·9	11·60	< 0·001
Arabinose	21·6	10·8	13·7	8·6	16·8	6·6	3·61	NS
Xylose	0·8	10·8	−55·4	64·2	−30·7	47·3	18·70	< 0·05
Mannose	> 100*	—	20·6	11·0	27·5	10·9	4·46	NS
Galactose	19·2	8·3	13·4	7·0	5·8	9·2	3·35	= 0·001
Glucose (H)	−5·9	15·9	0·3	23·5	34·2	23·1	8·63	< 0·001
Sum of neutral sugars	6·1	10·0	7·0	9·6	5·0	12·5	4·40	NS

H, hemicellulotic; SED, standard error of the difference; NS, not significant.
* Not included in statistical analysis.

Table 5. *Digestibility* (%) *of total hemicellulotic and cellulotic fibre* (*soluble* + *insoluble*) *of pea hulls* (Pisum sativum), *carrot and cabbage after 12* M-*sulphuric acid pretreatment followed by 1* M-H_2SO_4 *hydrolysis*

(Mean values and standard deviations)

	Pea hulls		Carrot		Cabbage			Statistical significance:
	Mean	SD	Mean	SD	Mean	SD	SED	*P*
Rhamnose	23·6	10·4	3·8	6·8	17·5	18·4	5·25	< 0·002
Fucose	1·2	8·9	−119·8	58·6	−57·5	55·5	19·14	< 0·001
Arabinose	22·5	7·1	11·6	6·2	5·2	19·5	5·10	< 0·01
Xylose	1·3	6·0	−22·3	52·4	−26·0	55·9	18·01	NS
Mannose	23·4	4·9	−9·6	7·7	3·4	18·5	4·81	< 0·001
Galactose	19·4	10·6	11·0	6·2	9·0	16·2	4·79	NS
Glucose (H + C)	2·8	9·2	−1·3	7·3	−3·4	13·1	4·10	NS
Sum of neutral sugars	4·2	7·7	0·8	6·5	−3·6	16·0	4·38	NS
Uronic acids	5·3	11·5	24·2	9·8	27·5	13·7	4·80	< 0·001

H + C, hemicellulotic + cellulotic; SED, standard error of the difference; NS, not significant.

Table 6. *Influence of sample concentration on the in vitro solubility of acidic polysaccharides*

(Mean values and standard deviations)

		Total uronic acids (g/kg DM)		Insoluble uronic acids (g/kg DM)		
		Mean	SD	Mean	SD	Solubility
Carrot wt (mg):	50	101·4	7·1	16·6	1·9	0·836
	75	99·4	12·0	21·7	3·3	0·782
	100	100·3	11·4	32·3	2·2	0·678
	150	101·6	6·2	41·9	4·7	0·587
	200	89·4	7·8	51·4	2·3	0·425
Excreta wt (mg):	25	152·0	22·7	54·3	7·1	0·643
	50	146·7	24·0	84·8	20·5	0·422
	75	151·5	10·9	101·9	7·1	0·327
	100	160·6	19·4	112·6	6·6	0·299

DM, dry matter.

of galactosyl sucrose oligosaccharides. Invertase is known to hydrolyse the sucrose component of raffinose, stachyose and verbascose to release fructose, and any contaminating α-galactosidase activity present in the invertase preparation may have hydrolysed the galactose linkages. Although it was expected that 80% ethanol would have achieved complete solubilization of these higher oligosaccharides, a small amount may have been precipitated along with the fibre fraction. If this was the case, then the fructose portion of the molecule may be the source of the readily digestible mannose observed after 1 M-H_2SO_4 treatment of the fibre. The mannose detected after 12 M-H_2SO_4 pretreatment is more likely to be a mannan polysaccharide closely associated with cellulose and, being more indigestible, would reduce the overall digestibility of mannose as seen with the stronger acid treatment.

Measurement of monosaccharides released after 1 M-H_2SO_4 hydrolysis shows that the digestion of total (soluble + insoluble) fibre by adult cockerels was very low, on average 6·1, 7·0 and 5·0% for pea hulls, carrot and cabbage respectively. After 12 M-H_2SO_4 pretreatment to solubilize cellulose, digestion of fibre was even lower, on average 4·2, 0·8 and −3·6% for pea hulls, carrot and cabbage respectively, because of the increased proportion of indigestible cellulose present. However, digestion expressed as the sum of neutral sugars concealed the greater digestibility of the pectic polysaccharides which were the source of rhamnose, arabinose, galactose and galacturonic acid to the hydrolysis medium. These pectic polysaccharides (rhamnogalacturonans, arabinans and galactans) were digested to a greater extent in all three feedingstuffs (on average from both types of hydrolysis 11·9, 15·2 and 12·9% respectively) than polysaccharides composed of fucose, xylose and glucose from cellulose. This greater digestion of pectic polysaccharides in dicotyledons compared with xylans and cellulose is in agreement with findings from other simple stomached animals (Graham *et al.* 1986; Longstaff & McNab, 1986), ruminant studies (Dekker *et al.* 1972; Chesson & Monro, 1982; Ben-Ghedalia & Miron, 1984) and in vitro studies using rumen fluid (Åman & Nordkvist, 1983; Nordkvist & Åman, 1986).

Polysaccharides containing uronic acids from pea hulls were only digested to a small extent (5·3%) in contrast to their greater digestibility in carrots (24·2%) and cabbage (27·5%). Unfortunately there is no suitable simple colorimetric method available which will

distinguish between glucuronic acid, associated with acidic xylans, and galacturonic acid, a constituent of pectic polysaccharides. Although pea hulls are known to contain pectic substances, they are also rich in acidic xylans and cellulose (Selvendren, 1985). Ben-Ghedalia & Miron (1984) showed that the uronic acid associated with the insoluble cell wall material of lucerne (*Medicago sativa*) (glucuronic acid) was digested by sheep to the extent of 40%, whereas the soluble uronic acid (galacturonic acid) from pectic substances was digested 86·5%.

The large negative digestibility coefficients obtained for fucose and xylose are difficult to explain. Negative xylose digestibilities have occurred in findings from other studies with sugar-beet pulp, but not with wheat bran (Graham *et al.* 1986) and negative xylose, fucose and glucose digestibilities have occurred after guar gum ingestion by human volunteers (Nyman *et al.* 1986). Negative rhamnose, fucose, xylose, mannose and glucose digestibilities were found after giving a basal diet containing insoluble lupin-seed polysaccharides as well as after giving the low-fibre basal diet itself to adult cockerels (Carre & Leclercq, 1985). After correction for polysaccharide excretion from birds fed on the basal diet, these latter authors found that negative digestibilities only remained in the case of xylose and only to a small extent. Negative digestibility coefficients for xylose were not observed after arabino-xylan ingestion from wheat (Longstaff & McNab, 1986) nor in the present study with pea hulls. This could arise because the fucose and xylose contents of pea hulls and the xylose content of wheat are much higher than those in carrot and cabbage and so digestibilities are less affected by endogenous, bacterial or previous dietary residues containing these constituents. Microscopic examination of excreta from birds given carrot and cabbage revealed the presence of aleurone cell walls, which undoubtedly arose from food consumed before the start of the balance experiment, and aleurone cell walls were also detected in excreta from control birds fed on glucose. Carrot and cabbage may also encourage more bacterial proliferation (Eastwood, 1985) or induce excretion of more endogenous material for which control birds cannot properly compensate. The excess fucose-polysaccharide material appeared to be mainly soluble. Fucose-containing polysaccharides from dietary fibre were only sparingly soluble and it seems likely that the fucose in excreta had arisen from endogenous material.

An interesting aspect of the present work was the solubility characteristics of polysaccharides, and one of the questions to be answered appears to be whether or not water-soluble polysaccharides are more digestible than insoluble polysaccharides by simple-stomached animals. Soluble polysaccharides such as pectins are believed to be readily fermented in man and pigs (Graham *et al.* 1986; Nyman *et al.* 1986; Longland & Low, 1988). When sugar beet was fed to pigs, Graham *et al.* (1986) found an increased concentration of soluble polysaccharides in the ilea of the pigs compared with that found in the feedingstuff, and this suggested to them that pectins were being solubilized during passage down the digestive tract. This could explain the apparently large negative digestibility coefficient observed for soluble polysaccharides with ileal digesta samples. They also reported a subsequent decrease in the concentration of soluble polysaccharides in the excreta and implied that the disappearance was caused by fermentation. They did not, however, give precise details of the method used to quantify both soluble and total polysaccharides, such as weights of feedingstuff, digesta and excreta samples taken, nor the amount of solvent used for extraction. The present study also suggests that the most readily digested polysaccharides appear to be the most soluble. The difficulty lies, however, in quantifying soluble polysaccharides. While it may be reasonable to conclude that some polysaccharides such as cellulose and acidic xylans may remain sparingly soluble in any amount of solvent, the solubility of others appears to be arbitrary, and it is this very arbitrariness which makes a quantitative investigation into their digestibility exceedingly difficult.

It was the initial intention in the present study to investigate the digestibilities of insoluble (measured) and soluble (by difference from total) polysaccharides separately, but results have not been reported as subsequent findings (Table 6) of the influence of sample weight and, hence, fibre concentration on solubility, made sensible conclusions difficult. To illustrate this point, the results of digestibilities with carrot are given now. Total and insoluble fibres were prepared in the same manner from 50 mg of feedingstuff and 50 mg excreta (from twelve birds), and uronic acids measured. The amounts of soluble uronic acid ingested by birds fed on 50 or 25 g carrot were 4388 and 2193 mg respectively, and the average amounts excreted by the two groups of six birds were 1985 mg and 1204 mg respectively, giving an overall average digestibility of 49·9%. Similarly, the amounts of insoluble uronic acids ingested by birds fed on 50 or 25 g carrot were 833 mg and 416 mg respectively and the amounts excreted were 1878 mg and 821 mg respectively, giving a large negative digestibility of −111·4% overall. Cabbage values gave similar results. This might have led to the conclusion that pectins previously soluble in the feedingstuff were being precipitated in the gut. However, birds fed on pea hulls did not show this apparent increase in insoluble acid polysaccharides in excreta. These findings have most likely arisen because the concentration of fibre in the excreta from birds fed on carrot and cabbage was much greater than that in the feedingstuff, whereas the fibre concentration in excreta from birds fed on pea hulls was much closer to that in the feedingstuff. It is not possible to calculate in advance this fibre difference and so weigh out feedingstuff and excreta samples of equivalent fibre content, since it necessitates an *a priori* knowledge of its concentration in excreta, which is the precise issue under examination.

In conclusion, the small amount of fibre digestion found in the present study with adult cockerels is in contrast to the large amounts which are purported to be digested by the pig, rat and man. This difference may be partly due to the stringent control of fibre intake and quantitative collection of output with this bioassay. Such feeding protocols have rarely been used with other species. On the other hand, poultry may lack an extensive bacterial population in their hindgut and, together with the shorter residence time, this may limit the extent of fermentation. Nevertheless, a similarity between chickens and other species has been demonstrated, in that they appear preferentially to digest water-soluble pectic substances.

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