An in vitro method for studying digestion in the pig

2. Comparison with in vivo ileal and faecal digestibilities

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1. An in vitro method involving incubation of feed samples with buffered duodenal digesta for 12 h or with buffered ileal and faecal inocula for 48 h was compared with in vivo ileal or faecal apparent digestibilities in pigs.

2. The five diets investigated had crude protein (nitrogen × 6·25) contents from 164 to 185 g/kg, starch contents from 296 to 463 g/kg and dietary fibre contents from 176 to 347 g/kg.

3. In vitro disappearances with duodenal inocula were correlated ($P < 0·05$) with in vivo ileal apparent digestibilities for crude protein, ash, starch, energy and dry matter, but not for dietary fibre.

4. In vitro disappearances with the ileal and faecal inocula were generally correlated ($P < 0·1$) with in vivo faecal apparent digestibilities for dietary fibre, energy and dry matter, but not for ash or crude protein. The patterns of degradability of fibre polysaccharide residues in vitro and in vivo were also similar.

5. Results indicate that this in vitro method could be used to predict the availability of starch and crude protein for digestion in the small intestine, and the degradability of dietary fibre, and thus for comparing the nutritive value of pig feeds.

In recent years several rapid laboratory methods have been developed for the study of digestion and the prediction of digestibility in simple-stomached animals. These include tests in vitro, with duodenal–jejunal fluid or microbial enzyme cocktails (Furuya *et al.* 1979; Dierick *et al.* 1985; Metz & Van der Meer, 1985), and mobile nylon-bag techniques (Petry & Handlos, 1978; Sauer *et al.* 1983; Graham *et al.* 1985*a*, *b*). Both methods have been shown to correlate with faecal apparent digestibilities for organic matter and energy while comparisons with ileal and faecal digestibilities for crude protein (nitrogen × 6·25) are often confounded by the presence of endogenous and microbial contamination in vivo. Both methods have shortcomings in that the former determines only readily available and soluble constituents and thus excludes degradable fibre, while the latter determines only the complete (faecal) digestibility, and cannot be used to estimate the extent to which components such as starch and protein are digested and absorbed in the small intestine. The in vitro method employed in the present study involves determination of readily digestible components by incubation with duodenal enzymes from the pig, essentially as described by Furuya *et al.* (1979), and of total fermentable components by incubation with a microbial culture from the ileal digesta or faeces, essentially as described by Ehle *et al.* (1982). In the previous paper (Löwgren *et al.* 1989) the processes of digestion in vitro were studied, while the present study compares digestion in vitro with ileal and faecal apparent digestibilities in vivo.

METHODS

Diets

Five diets were employed in both in vivo and in vitro studies. The basal diet was a commercial, pelleted pig-grower feed containing (g/kg fresh weight): 266 each of barley, wheat and oats, 60 each of peas (*Pisum sativum* L.) and soya-bean meal, 50 of fishmeal and 32 of a vitamin and mineral supplement (for further details see Graham *et al.* 1986). The

other four diets were prepared by mixing 2 parts of this basal diet with 1 part of feed-grade wheat bran (Kungsörnen AB, Uppsala), sugar-beet pulp (Fibrex; Sockerbolaget, Arlöv) or whole-crop peas (cv Simo) harvested either early (25 July) or late (12 August). For the in vivo studies diets were milled to pass a 3·0 mm screen, while for the in vitro studies a 1·0 mm screen was employed.

In vivo digestibilities

Digestibilities were determined with three 6-month-old crossbred female pigs weighing about 60–80 kg, and details have been published elsewhere (Graham *et al.* 1986; Graham & Åman, 1987). All pigs were fitted with steel, replaceable 'T'-shaped intestinal cannulas just distal to the pancreatic and bile ducts (duodenal) and about 150 mm before the ileo-caecal junction (ileal). Each diet was given to all pigs, with a different random order being employed for each pig. Animals were given 1·2 kg feed daily, mixed with an equal quantity of water and 6 g Cr_2O_3 marker, in two equal meals at 08.00 and 16.00 hours. After a 7 d adaptation period, faeces were collected on days 7–11 and ileal digesta for a 12 h period on day 12. Digesta and faeces were frozen immediately on collection and freeze-dried.

In vitro disappearances

The in vitro method described in the previous paper (Löwgren *et al.* 1989) was employed. Inocula for in vitro digestions were obtained from two of the cannulated pigs employed in the in vivo study when they were 2-years old and weighed about 170 kg. The pigs were given 1 kg daily of the basal diet (see Table 1) in two equal meals at 08.00 and 16.00 hours, and had free access to water and straw bedding. Duodenal digesta were collected between 09.00 and 10.00 hours, ileal digesta between 12.00 and 13.00 hours, and faeces immediately on defaecation between 10.00 and 12.00 hours. Inocula from the two pigs were pooled, prepared as described previously (Löwgren *et al.* 1989) and diluted 4-fold (w/w) with physiological buffer, and 50 ml of these buffered inocula were incubated at 38° with 0·50 g of the feeds. The incubation times employed were 12 h for the duodenal inocula and 48 h for the other two inocula, and blank incubations were included in every study. Following incubation, the degraded feed residues were recovered by filtration, washed three times with cold water, twice with acetone, and left to air-dry. After weighing, samples from each feed and type of incubation inocula were pooled.

Analytical methods

All samples were milled to pass a 1·0 mm screen before analysis, and all analyses were carried out in duplicate. Dry matter was determined by heating at 105° for 16 h, and all results were calculated on a dry matter basis. Ash and crude protein (Kjeldahl N × 6·25) were determined by standard methods (Association of Official Analytical Chemists, 1975), gross energy determined using an adiabatic calorimeter, and Cr_2O_3 by atomic absorption spectrophotometry (Williams *et al.* 1962). Crude fat was extracted with diethyl ether in a Tecator Soxtec System HT after acid (3 M-hydrochloric acid) hydrolysis (Anon., 1971), while soluble sugars (glucose, fructose, sucrose and fructans) were extracted with 0·05 M-acetate buffer (pH 5·0) at 65° and determined enzymically (Larsson & Bengtsson, 1983). Starch was also determined enzymically (Åman & Hesselman, 1984) and dietary fibre components by the method of Theander & Åman (1979), as modified by Theander & Westerlund (1986), in which Klason lignin is quantified gravimetrically, uronic acid residues by decarboxylation and neutral non-starch polysaccharide (NSP) residues as alditol acetates by gas–liquid chromatography.

Table 1. *The energy content (MJ/kg dry weight) and composition (g/kg dry weight) of the diets investigated*

Diet... Component	Basal	Wheat bran	Sugar-beet pulp	Pea (*Pisum sativum* L.) Early harvested	Pea (*Pisum sativum* L.) Late harvested
Energy	18·6	18·9	18·5	18·6	18·6
Ash	60	57	46	67	56
Crude protein (nitrogen × 6·25)	177	176	164	185	185
Crude fat	34	44	30	31	33
Soluble sugars*	32	40	37	40	34
Starch	463	350	296	364	395
Klason lignin	25	37	22	42	37
NSP residues:†					
Rhamnose	1	—	7	2	2
Arabinose	21	41	77	19	20
Xylose	34	63	28	37	37
Mannose	9	11	12	13	13
Galactose	10	10	25	12	13
Glucose	65	78	104	110	101
Uronic acids	11	14	72	24	22
Dietary fibre‡	176	254	347	259	245

* Glucose, fructose, sucrose plus fructans.
† NSP, non-starch polysaccharide.
‡ Sum of NSP and Klason lignin.

Calculations

Ileal and faecal apparent digestibilities in vivo were calculated relative to Cr_2O_3 content, and means with their standard errors for the three animals reported. In vitro incubations were carried out on three independent occasions with at least four replicates in each run, and the values reported in Fig. 1 are the means with their standard errors for these three determinations.

RESULTS

Composition of diets

The diets employed in the present study had similar gross energy (*c.* 18·6 MJ/kg), ash (*c.* 60 g/kg), crude protein (*c.* 180 g/kg), crude fat (*c.* 35 g/kg) and soluble sugar (*c.* 37 g/kg) contents (Table 1). However, the starch contents varied from 463 g/kg (basal diet) to 296 g/kg (sugar-beet-pulp diet) with a corresponding change in dietary fibre (NSP residues plus Klason lignin; Theander & Åman, 1979) from 176 to 347 g/kg. Glucose, xylose and arabinose were the predominant NSP residues, with uronic acids also important in the sugar-beet-pulp diet.

In vitro and in vivo digestibilities

In the present investigation, in vitro disappearance in duodenal inocula was compared with in vivo ileal apparent digestibility, and in vitro disappearance in ileal and faecal inocula with in vivo faecal apparent digestibility; thus Figs. 1–6 are drawn to facilitate this comparison. The in vivo values are presented, from left to right, in ascending order of digestibility of diets, and the diets are kept in the same order for the corresponding in vitro values, irrespective of disappearance. In Fig. 7 the in vivo ileal and faecal digestibilities of

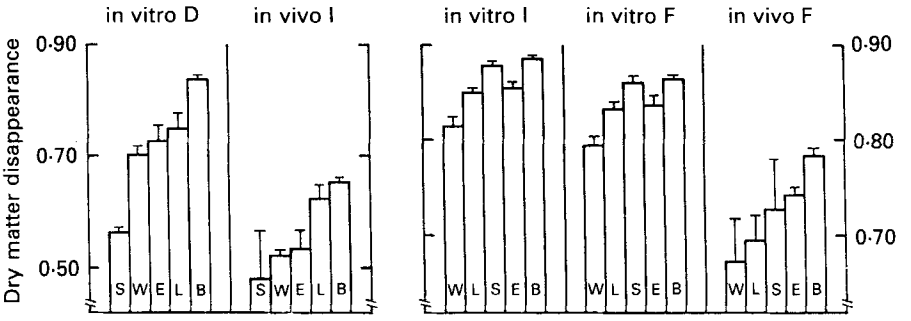

Fig. 1. In vivo ileal (I) and faecal (F) apparent digestibilities and in vitro disappearances, in duodenal (D), ileal (I) and faecal (F) inocula of dry matter in basal (B), wheat-bran (W), sugar-beet-pulp (S), early-harvested-pea (*Pisum sativum* L.) (E) and late-harvested-pea (L) diets. Values are means, with their standard errors represented by vertical bars (*n* 3). For experimental details, see. p. 690.

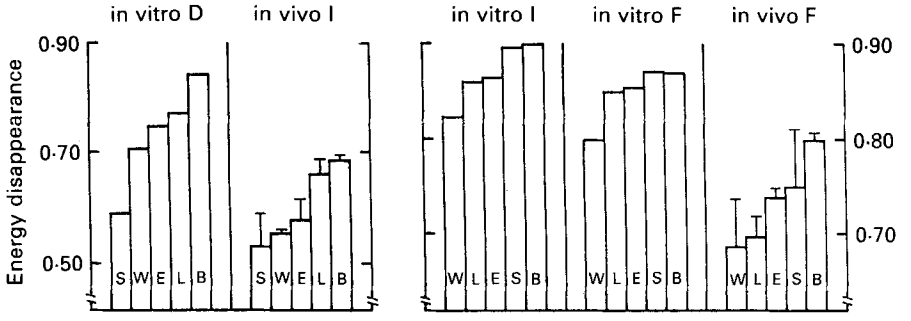

Fig. 2. In vivo ileal (I) and faecal (F) apparent digestibilities and in vitro disappearances in duodenal (D), ileal (I) and faecal (F) inocula of energy in basal (B), wheat-bran (W), sugar-beet-pulp (S), early-harvested-pea (*Pisum sativum* L.) (E) and late-harvested-pea (L) diets. Values are means, with their standard errors represented by vertical bars (*n* 3). For experimental details, see p. 690.

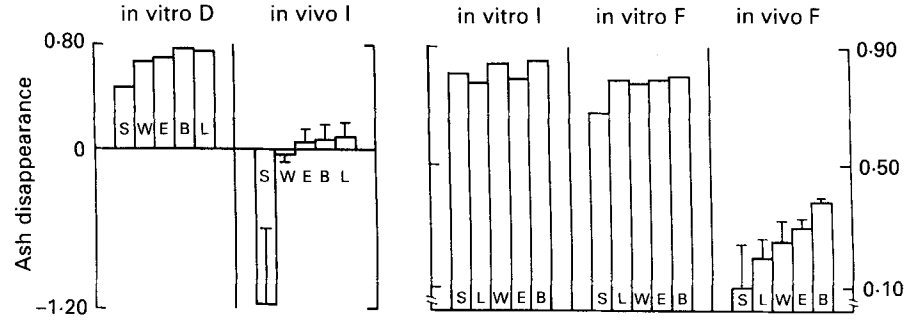

Fig. 3. In vivo ileal (I) and faecal (F) apparent digestibilities and in vitro disappearances in duodenal (D), ileal (I) and faecal (F) inocula of ash in basal (B), wheat-bran (W), sugar-beet-pulp (S), early-harvested-pea (*Pisum sativum* L.) (E) and late-harvested-pea (L) diets. Values are means, with their standard errors represented by vertical bars (*n* 3). For experimental details, see p. 690.

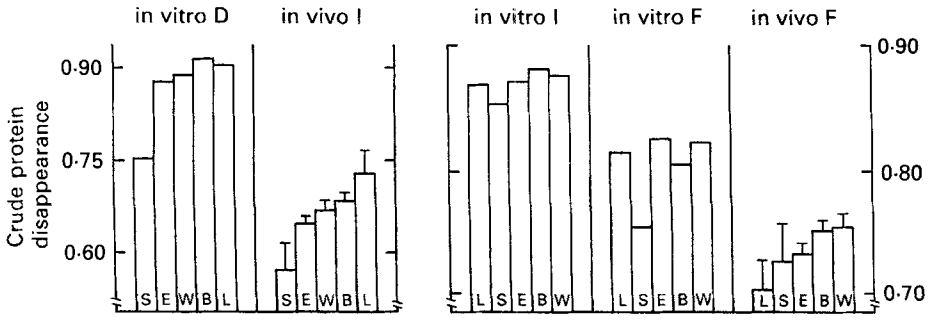

Fig. 4. In vivo ileal (I) and faecal (F) apparent digestibilities and in vitro disappearances in duodenal (D), ileal (I) and faecal (F) inocula of crude protein (nitrogen × 6·25) in basal (B), wheat-bran (W), sugar-beet-pulp (S), early-harvested-pea (*Pisum sativum* L.) (E) and late-harvested-pea (L) diets. Values are means, with their standard errors represented by vertical bars (*n* 3). For experimental details, see p. 690.

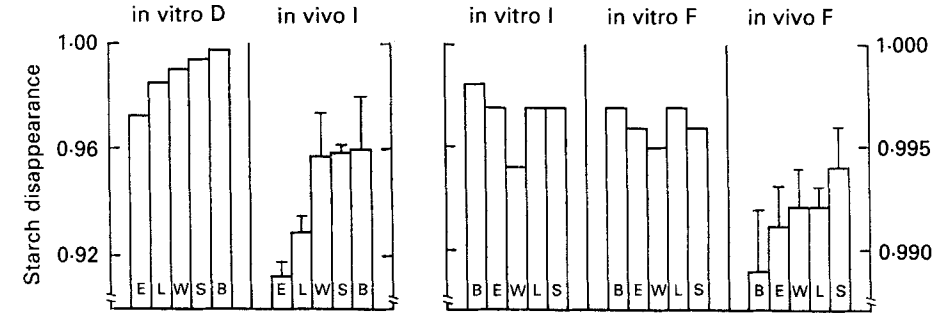

Fig. 5. In vivo ileal (I) and faecal (F) apparent digestibilities and in vitro disappearances in duodenal (D), ileal (I) and faecal (F) inocula of starch in basal (B), wheat-bran (W), sugar-beet-pulp (S), early-harvested-pea (*Pisum sativum* L.) (E) and late-harvested-pea (L) diets. Values are means, with their standard errors represented by vertical bars (*n* 3). For experimental details, see. p. 690.

major NSP residues are again presented in ascending order (from left to right) and in vitro ileal and faecal solubilities are also in this order.

Dry matter. Following 12 hours incubation with duodenal inocula, between 0·56 and 0·84 of the dry matter had been solubilized (Fig. 1). These disappearances were on average 0·18 greater than in vivo ileal digestibilities; however, the ranking of digestibility of the diets was the same in vitro as in vivo, and the two methods were closely correlated (R^2 0·79; $P <$ 0·05). Incubation with ileal or faecal inocula gave similar dry matter disappearances, on average 0·13 (ileal) and 0·11 (faecal) higher than in vivo faecal digestibility (Fig. 1). With the exception of the sugar-beet-pulp diet, which was relatively more soluble in both ileal and faecal inocula, the order of degradability was similar between in vitro and in vivo studies, with R^2 values of 0·71 ($P < 0·1$) for the correlation between in vivo faecal digestibility and in vitro ileal solubility, and 0·65 ($P < 0·1$) for that between in vivo faecal digestibility and in vitro faecal solubility. The mean standard errors for duodenal, ileal and faecal in vitro dry matter disappearances were 0·016, 0·004 and 0·007 respectively, compared with 0·035 for ileal in vivo digestibility and 0·028 for faecal in vivo digestibility.

Energy. Duodenal in vitro energy disappearances ranged between 0·59 (sugar-beet-pulp diet) and 0·85 (basal diet) and were on average 0·13 higher than in vivo ileal digestibilities (Fig. 2). In vitro ileal and faecal solubilities were also about 0·13 higher than in vivo faecal values, with in vitro ileal solubilities about 0·03 higher than in vitro faecal values. The order

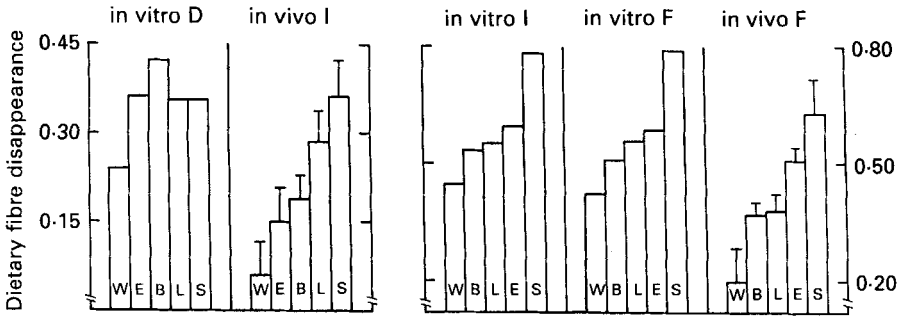

Fig. 6. In vivo ileal (I) and faecal (F) apparent digestibilities and in vitro disappearances in duodenal (D), ileal (I) and faecal (F) inocula of dietary fibre in basal (B), wheat-bran (W), sugar-beet-pulp (S), early-harvested-pea (*Pisum sativum* L.) (E) and late-harvested-pea (L) diets. Values are means, with their standard errors represented by vertical bars (*n* 3). For experimental details, see p. 690.

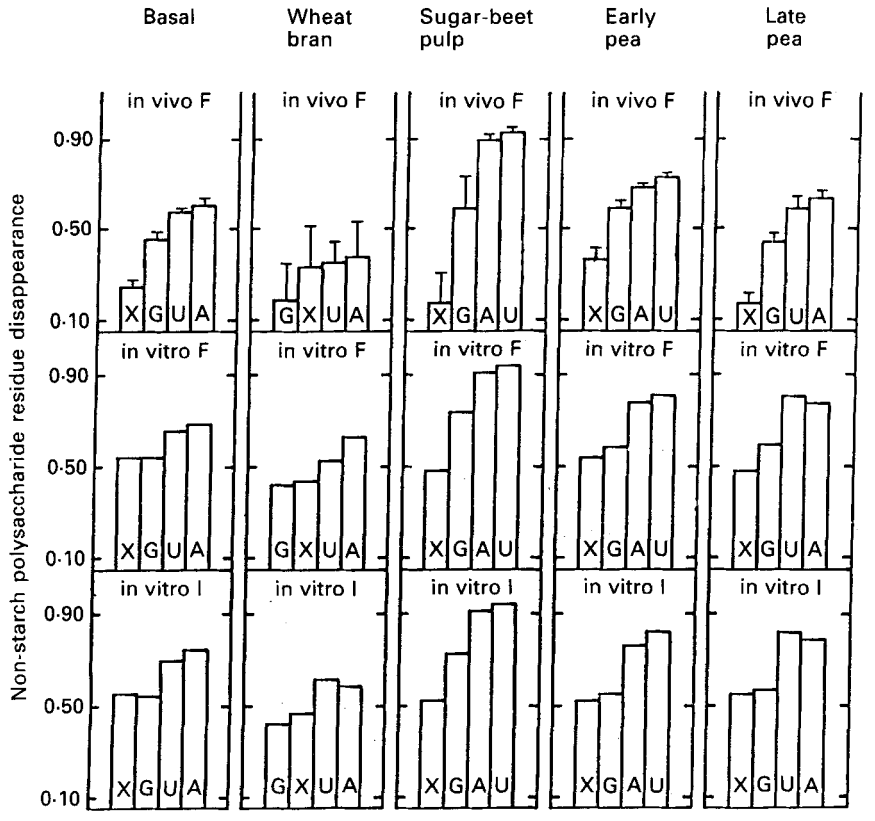

Fig. 7. In vivo faecal (F) apparent digestibilities and in vitro disappearances in ileal (I) and faecal (F) inocula of non-starch arabinose (A), xylose (X), glucose (G) and uronic acid (U) residues in basal, wheat-bran, sugar-beet-pulp, early-harvested-pea (*Pisum sativum* L.) and late-harvested-pea diets. Values are means with their standard errors represented by vertical bars (*n* 3). For experimental details, see. p. 690.

of degradabilities was similar between in vitro duodenal incubations and in vivo ileal digestibilities, and between in vitro ileal and faecal incubations and in vivo faecal digestibilities, with R^2 values of 0·78 ($P < 0·05$), 0·73 ($P < 0·1$) and 0·65 ($P < 0·1$) respectively.

Ash. In vitro duodenal solubilities of ash were between 0·49 (sugar-beet-pulp diet) and 0·79 (basal diet), and were on average 0·84 greater than in vivo ileal digestibilities which ranged between −1·16 (sugar-beet-pulp diet) and 0·10 (late pea diet; Fig. 3). However, the in vitro values were closely correlated with those in vivo (R^2 0·88; $P < 0·02$). Both ileal and faecal in vitro values for ash were on average 0·55 higher than in vivo faecal digestibilities, with no correlation between in vitro and in vivo values.

Protein. In vitro duodenal solubilities of crude protein varied between 0·78 (sugar-beet-pulp diet) and 0·92 (basal diet), and were on average 0·21 greater than the corresponding in vivo ileal values (Fig. 4). The order of digestibility was similar between these two methods, with a close correlation (R^2 0·78; $P < 0·05$). In vitro ileal solubilities were about 0·10 greater than the in vitro faecal values, which in turn were approximately 0·10 greater than the in vivo faecal values. There was no correlation between in vivo faecal digestibilities and in vitro ileal and faecal solubilities for crude protein.

Starch. In vitro duodenal solubilities of starch ranged from 0·973 to 0·997 and were about 0·04 higher than the corresponding in vivo ileal values to which they were closely correlated (R^2 0·92; $P < 0·01$; Fig. 5). In vitro ileal and faecal starch solubilities were greater than 0·995 and were not related to in vivo faecal values.

Dietary fibre. In vitro disappearances of dietary fibre in duodenal inocula varied from 0·24 (wheat-bran diet) to 0·42 (basal diet), and were not related to in vivo ileal digestibilities (Fig. 6). In vitro ileal and faecal dietary fibre solubilities were similar, and were about 0·16 higher than in vivo faecal values, to which they were correlated (R^2 0·88; $P < 0·05$).

NSP residues. As with dietary fibre, the in vitro duodenal disappearances of NSP residues were not related, either in pattern or extent, to in vivo ileal values, and are not presented here. In vitro ileal and faecal solubilities of the major NSP residues (arabinose, xylose, glucose and uronic acids) were very similar in both extent and pattern (Fig. 7). In vivo faecal digestibilities were about 0·15 lower than in vitro values, but the patterns of digestibility were very similar. Xylose and glucose were the least degraded of the main NSP residues in all samples, both in vitro and in vivo, while arabinose and uronic acids were degraded to a greater, and approximately equal, extent.

DISCUSSION

General

An incubation period of 12 h was chosen for in vitro duodenal studies as enzymic degradation would be nearing completion at this time, while microbial degradation would be limited (Löwgren *et al.* 1989). This is considerably longer than the transport time between the duodenum and ileum in vivo, but allows for the dilution of digesta in the buffer and the lag period in degradation, and should reduce the variability of results. Nevertheless, the standard errors of duodenal in vitro values were almost three times those of ileal and faecal values. A shorter incubation period could be chosen to give in vitro values closer to those observed in vivo. The in vitro ileal and faecal incubation period of 48 h was chosen as degradation has reached a plateau after this time and will, therefore, give more reproducible results, relatively independent of activity in the inocula (Löwgren *et al.* 1989).

In vitro disappearance is inevitably an overestimate of digestibility due to the loss of soluble and particulate matter inherent in the method. Compared with in vivo samples, in vitro residues are relatively free from endogenous and microbial contamination, and the

smaller particle size of the feeds employed in the in vitro study could also lead to a higher digestion. However, feed particle size should not significantly affect in vitro digestibilities at the longer incubation times employed for the ileal and faecal inocula (Löwgren *et al.* 1989).

Comparison of in vitro duodenal solubility with in vivo ileal digestibility

The in vitro duodenal solubilities of all major dietary components exceeded the observed in vivo ileal digestibilities, especially for ash (0·84 greater) and crude protein (0·21 greater). Although taking a shorter in vitro incubation period would reduce this difference, the lower in vivo values for these two components would primarily be due to the presence of endogenous and microbial material in the ileal digesta. This is particularly true for the ash, where ileal recovery in vivo was between 0·90 and 2·16 of the dietary intake. Dierick *et al.* (1985), using an enzyme–duodenal fluid inoculum, have also observed similar differences between in vivo ileal digestibility of crude protein and in vitro solubility and attributed this to the presence of endogenous N compounds in vivo. Nevertheless, the in vitro duodenal incubation method was closely correlated ($P < 0·05$) with the in vivo ileal digestibilities of starch, protein and ash, and consequently of dry matter and energy.

That the duodenal in vitro incubation could not be used to predict either the order or pattern of in vivo ileal dietary fibre digestibilities is neither surprising nor a particular failing of the method. As shown here and in the previous study (Löwgren *et al.* 1989), the microbial population found in the ileal digesta has a fibre-degrading capacity similar to that of the faecal population, whereas the microbiota of duodenal digesta are considerably less active. The pattern of degradation of fibre constitutents at the ileum is very similar to that observed in the faeces of the pig (Graham *et al.* 1986). Thus the fibre degradation observed in the small intestine of the pig (Millard & Chesson, 1984; Graham *et al.* 1986; Graham & Åman, 1987) probably occurs close to the ileum where pH and passage rate would be more favourable, while that observed with the short duodenal incubation period employed here would be primarily due to the loss of soluble and particulate matter. However, as all fibre degradation in the pig is due to microbial activity, the energy value to the animal of the organic acids produced is probably similar whether this degradation occurs in the small or large intestine. Thus faecal degradability values would be sufficient for estimating the availability of energy from fibre in a pig diet.

Comparison of in vitro ileal and faecal solubilities with in vivo faecal digestibility

In vitro solubilities determined by incubation with ileal or faecal inocula were very similar for all components except crude protein, where the former exceeded the latter by approximately 0·1. As discussed in the preceding paper (Löwgren *et al.* 1989), the faecal microflora may adhere more closely with the undegraded residue, thus decreasing crude protein apparent solubility. However, this did not seem to influence the extent or pattern of degradation of other components. Caecal and faecal inocula have also been shown not to differ in fibre-degrading ability (Ehle *et al.* 1982).

As for ileal digestibilities the in vitro methods gave higher values than faecal digestibilities observed in vivo for all main dietary components. That the digestibilities of dry matter and energy of the sugar-beet-pulp diet for both in vitro methods were greater than would be predicted from in vivo values (Figs. 1 and 2) suggests that the in vivo results, which had a wide between-pig variation, were an underestimate. Nevertheless, as previously shown both in vitro and in nylon bag studies (Graham *et al.* 1985*a*, *b*), solubilities with both ileal and faecal inocula in vitro were generally correlated ($P < 0·1$) with in vivo faecal digestibilities of dry matter and energy. This correlation was also found for dietary fibre, and the patterns of degradability of NSP residues were similar for both in vivo and in vitro investigations.

Neither in vitro incubation was correlated with in vivo faecal ash or crude protein digestibilities, indicating that endogenous and microbial material significantly affected the faecal content of these components. Similar high values for crude protein digestibilities, and a lack of any correlation with in vivo faecal digestibilities, have also been observed previously in both in vitro and mobile nylon bag studies (Graham *et al.* 1985*a*, *b*), suggesting that actual N digestibility in normal pig diets could approach or exceed 0·95. As shown by both in vitro and in vivo methods, starch can be considered as completely degradable in the pig intestine.

In conclusion, in vitro digestion methods generally have several inherent drawbacks including the loss of soluble and particulate matter, and the dilution and subsequent reduction in the potency of anti-nutritional compounds. The effects of time of passage, endogenous secretions and microbial biomass on in vivo apparent digestibilities of different diets also confound in vitro comparisons. Nevertheless, the present study has shown that ileal digestibilities of starch and crude protein could be predicted from a short incubation with duodenal digesta, and faecal digestibility of dietary fibre from incubation with ileal or faecal inocula. Thus, by combining data from these in vitro incubations it should be possible to estimate both the dietary components that are readily digested and absorbed in the small intestine and those that are degraded by microbes in the whole intestine. Although further studies are necessary before in vitro methods can be generally applied to the prediction of the nutritive value of feeds for pigs and other simple-stomached animals, the method described here could be employed for the rapid comparison of similar feeds and, for example, examining the effects of processing on digestibility.

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