Use of a nylon-bag technique for pig feed digestibility studies

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1. The use of a nylon-bag technique for pig feed digestibility determination was studied. Bags, measuring 25 × 40 mm and containing feed samples, were introduced into the pig gastrointestinal tract through a duodenal cannula, and recovered in the faeces between 23 and 69 h later. The disappearance of organic matter and crude protein (nitrogen × 6·25) from the bags was compared with in vivo apparent digestibility, determined by conventional faecal-collection methods, and neutral-detergent-fibre content for eleven feeds. The residues left in the bags after passage through the intestine from whole-crop-pea (*Pisum sativum*) and barley-grain samples were analysed for starch, non-starch polysaccharide residues, Klason lignin, crude protein and ash.

2. Dry matter disappearance of barley or whole-crop peas was not influenced by increasing bag pore size from 10 to 36 μm or sample weight from 250 to 1000 mg. Pepsin (*EC* 3.4.2.1) pretreatment had no effect on the degradation in the bags of the feeds investigated.

3. Organic matter and crude protein disappearance from the bags exceeded in vivo apparent digestibility by up to 0·10 and 0·42 units respectively. In vivo apparent organic matter digestibility could be predicted ($P < 0·001$) by the organic matter disappearance from the bags and the neutral-detergent-fibre content of the feed, while in vivo apparent crude protein digestibility was highly correlated ($P < 0·001$) to all these indices but poorly to crude protein disappearance from the bags.

4. Klason lignin was the least degraded component measured in the whole-crop-pea and barley residues from the bags, while starch was completely digested. Of the non-starch polysaccharide residues, xylose was the most resistant to degradation in both samples whereas other sugars varied in susceptibility to solubilization between samples.

5. Results are discussed in relation to the potential uses of the nylon-bag technique described in the present paper for studies in simple-stomached animals.

The determination of digestibility by conventional methods requires a large quantity of feed, a number of animals, and considerable expenditure on equipment and manpower. Analysis of faecal residues provides little information on the degradation of components of individual feeds due to the number of feed constituents often necessary to provide a nutritionally-adequate diet and to contamination by endogenous and bacterial material during digestion. In ruminant studies several methods, including degradation of small samples of feed in bags placed in the rumen, are widely and routinely used for studying the pattern and extent of feed digestion. However, no equivalent method is currently accepted for investigating digestion in simple-stomached animals. Recent work has shown that the digestion of feed samples, contained in nylon bags and passed through the gastrointestinal tract of pigs, could be used in the rapid determination of feed quality (Petry & Handlos, 1978; Sauer *et al.* 1983). Prolonged retention of the bags in the stomach can be overcome by introduction through a duodenal cannula (Sauer *et al.* 1983). However, discrepancies between these two studies in methods used and results obtained, particularly with regard to bag pore size and extent of crude protein (nitrogen × 6·25) disappearance from the bags relative to in vivo apparent digestibility, require further investigation. The present experiments were also designed to provide information on a nylon-bag method and include chemical analysis of degraded residues from the bags. The suitability of a nylon-bag technique for predicting

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Table 1. *The neutral-detergent fibre (NDF) content, crude protein (CP, nitrogen × 6·25) content and neutral-detergent solubility of crude protein (NDSCP) of the feeds examined, and the number and weight of pigs used in in vivo trials*

Feed	Feed composition			Pigs	
	NDF (mg/g)	CP (mg/g)	NDSCP	No.	Live wt (kg)
Wheat	115	131	0·888	9	15–30
Triticale	130	116	0·849	9	15–30
Rye	139	98	0·802	9	15–30
Barley	164	127	0·864	18	15–30
Distillers' grains 1*	236	247	0·912	8	15–30
Distillers' grains 2†	246	293	0·857	6	40–70
Rapeseed meal (*Brassica napus*)	296	440	0·908	6	40–70
Whole-crop peas (*Pisum sativum*) 1‡	381	203	0·930	7	30–70
Whole-crop peas 2‡	352	152	0·932	8	30–70
Clover (*Trifolium repens*)	337	214	0·896	8	25–65
Grass	599	145	0·619	8	25–65

* Wheat-based and † dehulled barley-based grains.
‡ 1, 2, Early and late harvests respectively.

in vivo apparent digestibility of organic matter (AOMD) and, indirectly, of crude protein is reported.

METHODS

Digestibility studies

Feeds examined were barley, wheat, rye and triticale grains, wheat-based (sample 1) and dehulled barley-based (sample 2) distillers' grains, extracted rapeseed (*Brassica napus*) meal, early (sample 1) and late (sample 2) harvested whole-crop peas (*Pisum sativum*), clover (*Trifolium repens*) and grass (see Table 1).

Three 8-month-old pigs weighing between 80 and 120 kg, and fitted with steel 'T'-shaped duodenal cannulas (Björnhag & Jonsson, 1984), were used for the determination of digestibility in bags. The pigs were given daily 3 kg of a commercial pig diet ((g/kg): 270 barley, 270 oats, 270 wheat, 50 peas, 50 soya-bean meal, 50 fish meal, 40 vitamin and mineral supplement, 175 crude protein) in two equal meals at 08.00 and 16.00 hours, and had access to straw bedding. When bags were being inserted, each meal was divided into three portions with a 30-min interval between feeding each portion. Up to six bags, containing samples, were introduced through the cannula during feeding of both the second and third portions of morning and afternoon feeds. Bags were retrieved immediately after defaecation, extensively washed in cold water to remove contaminating faecal material, frozen and freeze-dried. Bags, measuring 40 × 25 mm, were machine sewn. All feed samples were milled to pass a 1 mm screen before determinations of digestibility using bags.

In vivo digestibilities were determined using at least six growing–finishing pigs (15–70 kg) per feed (see Table 1) and details have been published elsewhere (Håkansson & Malmlöf, 1984; C. W. Newman, S. Lund and M. Rundgren, personal communication). After a 7 d adaptation period the pigs were individually placed in conventional digestion crates and faeces collected twice daily (08.00 and 16.00 hours) for 4 d. Faeces were pooled for each

Table 2. *The in vivo digestibility, and the disappearance from nylon bags passed through pigs, of organic matter and crude protein (nitrogen × 6·25) for eleven pig feeds*

(Mean values with their standard errors; no. of replicates given in parentheses for the in vivo determinations)

	Organic matter				Crude protein			
	in vivo		From bags*		in vivo		From bags*	
Feed	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wheat	0·885	0·005 (9)	0·935	0·005	0·804	0·016 (9)	0·965	0·006
Triticale	0·885	0·005 (9)	0·928	0·005	0·775	0·016 (9)	0·949	0·008
Rye	0·880	0·005 (9)	0·915	0·005	0·713	0·016 (9)	0·937	0·007
Barley	0·831	0·003 (18)	0·889	0·007	0·742	0·011 (18)	0·955	0·008
Distillers' grains 1	0·742	0·012 (8)	0·809	0·007	0·755	0·012 (8)	0·936	0·009
Distillers' grains 2	0·700	0·012 (6)	0·786	0·015	0·673	0·016 (6)	0·956	0·019
Rapeseed meal (*Brassica napus*)	0·671	0·030 (6)	0·754	0·012	0·763	0·035 (6)	0·925	0·009
Whole-crop peas (*Pisum sativum*) 1	0·646	0·039 (7)	0·677	0·008	0·627	0·091 (7)	0·942	0·011
Whole-crop peas 2	0·594	0·038 (8)	0·692	0·020	0·522	0·075 (8)	0·927	0·027
Clover (*Trifolium repens*)	0·614	0·071 (8)	0·686	0·021	0·574	0·088 (8)	0·936	0·030
Grass	0·554	0·070 (8)	0·562	0·023	0·471	0·106 (8)	0·888	0·020

* Six replicates.

pig, weighed and analysed for organic matter and crude protein. The digestibilities of barley, wheat, rye and triticale were determined by feeding with a vitamin and mineral supplement (40 g/kg fresh weight) whereas those of the other feeds were calculated by difference following inclusion (30 g/kg fresh weight) with a standardized diet.

Analytical methods

Dry matter, ash and crude protein were determined by standard methods (Association of Official Analytical Chemists, 1975). Starch was determined enzymically (Åman & Hesselman, 1984), uronic acids by decarboxylation (Theander & Åman, 1979) and neutral-detergent fibre as described by Robertson & Van Soest (1981). Klason lignin was measured gravimetrically as the sulphuric-acid-insoluble fraction, and non-starch neutral sugars were determined by gas–liquid chromatography as alditol acetates following acid-hydrolysis of the starch-free residue (Theander & Åman, 1979).

Experimental procedure

In Expt 1, 250, 500 or 1000 mg barley or whole-crop-pea samples were digested in triplicate in precision-woven nylon-cloth bags (ZBF AG, CH-8803 Rüschlikon) with pore sizes of 10, 20 or 36 μm, and dry matter disappearance (DMD) determined. In Expt 2, 20-μm pore-size bags containing 500 mg whole-crop-pea, clover and grass samples or 1000 mg of the grain and rapeseed-meal samples were employed. Triplicate samples were either introduced into the duodenum directly, or following incubation with pepsin (*EC* 3.4.2.1) (3 h, 37°, 460 units/ml in 0·01 M-hydrochloric acid). All residues were dried, weighed and analysed for organic matter and N. In Expt 3, barley (1000 mg, twenty replicates) or whole-crop peas 2 (500 mg, fourteen replicates) were digested in 20-μm pore bags. Residues were dried and pooled for analysis.

Table 3. *First-order regression analysis between in vivo apparent digestibility, and disappearance from nylon bags of organic matter (OM) and crude protein (nitrogen × 6·25; CP), neutral-detergent solubility of crude protein (NDSCP) and neutral-detergent fibre (NDF) content, for eleven pig feeds*

Independent variable	Dependent variable	Intercept	Coefficient of regression	Coefficient of determination
in vivo OM	Bags OM	0·08	0·97	0·95***
	NDF	1·00	−1·01	0·82***
in vivo CP	Bags CP	0·85	0·13	0·50*
	NDSCP	0·64	0·32	0·16 NS
	in vivo OM	0·09	0·94	0·70***
	Bags OM	0·13	0·97	0·76***
	NDF	1·04	−1·14	0·78***

NS, not significant.
* $P < 0·05$, *** $P < 0·001$.

In all experiments, samples of each feed were replicated between pigs and introduction (i.e. morning or afternoon) time.

RESULTS

Expt 1

The mean DMD from the bags of barley and whole-crop peas was 0·881 (SE 0·009, range 0·873–0·888) and 0·703 (SE 0·016, range 0·675–0·730), and was not influenced by changing bag pore-size between 10 and 36 μm or by increasing sample weight from 250 to 1000 mg.

Expt 2

Pepsin pretreatment did not influence digestion in bags, therefore values were pooled. Organic matter disappearance (OMD) from bags for all feeds exceeded in vivo AOMD by 0·01–0·10 units while crude protein disappearance from bags was greater than 0·88 for all feeds and exceeded that of in vivo apparent digestion by 0·14–0·41 units (Table 2). Linear regression of these results demonstrated that in vivo AOMD could be predicted ($P < 0·001$) from both OMD from bags and neutral-detergent-fibre content (Table 3), although the coefficient of determination was higher for the bag method. The latter two indices were also closely correlated (R^2 0·95). Crude protein in vivo apparent digestibility was poorly related with disappearance from bags and neutral-detergent solubility of N (Table 3) but was correlated ($P < 0·001$) with in vivo AOMD, OMD from bags and neutral-detergent-fibre content.

Expt 3

The DMD from bags of the barley and whole-crop-pea feeds were 0·879 (SE 0·010) and 0·704 (SE 0·023) respectively. Starch was almost completely degraded, and non-starch polysaccharides and Klason lignin were the major components of both residues (Table 4). Klason lignin was the least-degraded constituent with a more than six-fold increase in relative content during digestion of the barley sample. Glucose and xylose were the predominant polysaccharide constituents in the residues and xylose was the least-degradable polysaccharide residue in both samples. The other neutral-sugar residues varied in susceptibility to solubilization between the two feeds, while uronic acids accumulated in the

Table 4. *Composition (mg/g) of undigested feed and residues of barley and whole-crop peas* (Pisum sativum) *from nylon bags passed through pigs, and the disappearance from the bags of each component*

	Barley			Whole-crop peas		
Component	Undigested feed	Residue from bags	Disappearance	Undigested feed	Residue from bags	Disappearance
Ash	26	76	0·662	59	45	0·803
Crude protein	127	45	0·957	152	33	0·936
Starch	575	< 5	> 0·999	162	< 5	> 0·991
Klason lignin	30	195	0·220	81	169	0·387
Total NSP:	155	551	0·573	352	610	0·491
Rhamnose	Trace	Trace	—	4	3	0·780
Arabinose	20	71	0·574	17	6	0·896
Xylose	43	204	0·431	57	150	0·226
Mannose	3	6	0·760	7	10	0·580
Galactose	3	8	0·680	14	9	0·811
Glucose	79	230	0·651	182	376	0·393
Uronic acids	7	32	0·451	71	56	0·768

NSP, non-starch polysaccharides.

Table 5. *The disappearance of dry matter of barley or whole-crop peas* (Pisum sativum) *from nylon bags passed through three pigs*

(Mean values with their standard errors; no. of replicates in parentheses)

	Pig 1		Pig 2		Pig 3	
	Mean	SE	Mean	SE	Mean	SE
Barley	0·884	0·007 (8)	0·879	0·010 (10)	0·879	0·006 (10)
Whole-crop peas	0·697	0·019 (8)	0·702	0·021 (8)	0·695	0·018 (8)

barley and diminished in the whole-crop peas during digestion. Protein loss was high in both samples, and about 60% of the residual N and 14% of the dry matter were neutral-detergent soluble.

General

The results obtained from the nylon-bag method were more reproducible than those from the in vivo study and the average standard errors of means for the dry matter and crude protein losses from the bags for the eleven feeds examined (six replicates for each) were 0·012 and 0·015 respectively (Table 2). The variability tended to increase as potential degradability decreased, but little between-pig variation was apparent in the present study (Table 5).

The mean gastrointestinal retention time of the bags placed in the tract at afternoon feeding (43·7 h, SE 11·9, seventy-eight samples) exceeded that of those introduced in the morning (37·0 h, SE 10·1, seventy-six samples). Bags were retained for between 23 and 69 h and, while 66% of the bags introduced in the morning were recovered the following day, only 20% of the samples administered in the afternoon were retrieved the next evening. Samples were not recovered in the order that they were introduced into the pig, and the

retention times of bags placed simultaneously in the duodenum differed by up to18 h. Retention time did not influence the extent of barley degradation but DMD from the bags of the whole-crop-pea sample 2 increased by 0·002 units/h with retention time ($P < 0·001$; DMD at zero time of 0·599).

DISCUSSION

Choice of bag pore size for digestibility studies is essentially a compromise between minimizing feed particle exchange and maximizing liquid and bacterial flow between the bag contents and its environment. A pore size of 20 μm permits bacterial exchange (Lindberg *et al.* 1984) and did not hinder degradation of barley or whole-crop peas in the present study. That increasing bag pore size from 10 to 36 μm did not influence DMD from either sample examined would suggest that the feed particles lost through the larger pores consisted of potentially-degradable material. However, particle loss from the bags, which depends on pore size and feed-particle size distribution, could be a source of error in this method.

Up to 1 g barley or whole-crop peas did not impede digestion. This sample size relative to bag surface area greatly exceeds that recommended for rumen studies (see Lindberg, 1983) and this difference may be due to the relative physiology of the respective digestive organs or to the rapid reduction of effective sample size following the solubilization of readily-degradable components from the samples in the pig duodenum. That pepsin pretreatment did not influence N disappearance from the bags is not surprising considering the capacity of the postduodenal intestinal tract, and in particular the caecum–colon, to degrade protein (Just *et al.* 1981; Zebrowska, 1982).

The extensive degradation in bags of the non-starch polysaccharides and lignin in the feeds investigated (Table 4) demonstrates the importance of microbial activity in the gastro-intestinal tract. The pattern of solubilization of non-starch polysaccharide residues in the whole-crop peas from the bags was similar to that observed in in vitro ruminant studies (P. Åman, 1984; unpublished results) and in vivo pig studies (Graham *et al.*1985). The accumulation of uronic acid and arabinose residues during degradation of the barley indicates that these sugars are primarily present as relatively indigestible xylans (Bacic & Stone, 1981; Van Soest, 1982). In the pea crop, however, these sugar residues are also found in degradable pectins (Van Soest, 1982). The relatively high solubilization of non-starch glucose in the barley was probably due to the presence of mixed-linked β-glucans which are susceptible to microbial degradation (Bacic & Stone, 1981). As previously shown for faecal digestibilities of a cereal-based diet and whole-crop peas in pigs (Graham *et al.*1985), xylans were the least-degradable polysaccharide in both feeds investigated. The increase in DMD from the bags of the whole-crop peas, but not the barley, with retention time in the pigs is probably indicative of the more resistant lignocelluloses found in the hulls of the latter sample.

The difference between organic matter degradation in the bags and in vivo may be partly due to particle losses from the bags, the smaller feed-particle size in the bag method relative to in vivo studies, and the presence of endogenous and microbial material in faeces. The use of mature pigs, with a more-developed microflora and large intestine (see Mason, 1980), in the bag studies probably also contributed to this difference. However, the use of mature pigs greatly extends the experimental life of surgically-altered animals and should increase reproducibility both within and between experiments. Although Petry & Handlos (1978) could detect no influence of pig diet on feed degradation in bags, diet can influence microbial activity in the caecum–colon of pigs (Ehle *et al.* 1982). Further investigations into the effect of dietary composition, particularly of degradable fibres or heat-damaged proteins, on the extent and pattern of degradation in bags are necessary.

Petry & Handlos (1978), using bags of 5-μm pore size, found that organic matter and protein digestibilities were about 3% lower and up to 17% higher respectively than in vivo values, whereas Sauer *et al.* (1983), with bags of 50-μm pore size, reported that protein degradabilities determined by both methods were similar. Both investigations were carried out with growing pigs (30–80 kg), which could partly account for the lower degradability of the feeds in bags in these studies compared with the present experiment. However, as the bags were not washed on retrieval in the two investigations discussed, the major cause of this discrepancy is probably the contamination of residues in the bags by faecal material. This contamination, which will be greater when larger bag pore sizes are employed, will particularly influence the apparent N content of the residues and result in a large variation in degradability measured in bags between experiments, depending on the method used to isolate the bags from the faeces. The high neutral-detergent solubility of the N in the residues from bags in the present study suggested some microbial contamination (see Robertson & Van Soest, 1981) despite careful washing, and this was confirmed by microscopic examination.

The high protein-disappearance values from bags for all feeds could have resulted from the loss of feed particles and the solubilization of nitrogenous compounds which are not digested in vivo. However, as previously discussed, most particles lost would seem to be potentially degradable and, assuming uniform content of N in the undegraded particles, such losses would result in an equal disappearance of organic matter. Also, water-soluble nitrogenous compounds found in pig faeces account for only about 25% of faecal N irrespective of the fibre content of the diet, and consist mainly of partially-degraded endogenous secretions (Mason *et al.* 1982). The low N content of the residues from the bags and of the neutral-detergent fibre of the original feeds in this investigation concur with earlier observations that low in vivo apparent crude-protein digestibilities of many high-fibre feeds are not due to the presence of non-degradable fibre-bound N (Ehle *et al.*1982; Mason *et al.* 1982). Assuming that 13% of the N in pig faeces is undigested dietary N (Mason *et al.* 1982), it can be estimated that the disappearance of crude protein from the bags in the present study was an underestimation of in vivo actual digestibility of N in all feeds, possibly because of microbial contamination of the degraded residues in the bags. The difference between N degradation measured in vivo and in bags would be narrowed by up to 0·05 units by recalculating in vivo values as 'true' protein digestibility by traditional methods (see Eggum, 1973). However, Mason (1980) has shown that estimates of 'true' protein digestibility by the conventional approach can differ by up to 0·1 units depending on the N content of the diet and on the value accepted for metabolic faecal N excretion, and will be an underestimate of the actual digestibility of highly digestible proteins contained in natural feeds.

The nylon-bag technique described may be used to predict the in vivo apparent digestibility of pig feeds and would be particularly useful for a rapid comparison of a large number of similar samples. The inclusion of standardized feeds, preferably similar in composition and physical characteristics to the test samples, and the use of mature pigs would increase continuity between experiments. Providing that several cannulated pigs with similar feed retention times are available, a large number of samples may be investigated at one time; for example, in the present experiment with three pigs, up to twelve feeds, each with six replicates, could be examined daily. Analysis of partially degraded residues from the bags could provide valuable information on the feed components resistant to digestion and on the influence of treatments on feed degradation in simple-stomached animals.

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