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The effect of dietary peptide concentration on endogenous ileal amino acid loss in the growing pig

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The aim of the present study was to determine whether dietary peptide concentration had an effect on endogenous ileal amino acid flow in the growing pig. Eight 33 kg live weight entire male pigs had post-valve T-caecum (PVTC) cannulas surgically implanted for the collection of ileal digesta. The pigs were fed twice daily at 100 g/kg metabolic body weight per d and were given diets containing enzyme-hydrolysed casein (EHC) at 0, 50, 100 and 200 g/kg in a Latin-square design. A basal casein-based diet was fed to the pigs for 6 d periods between receiving the experimental diets. The pigs received the experimental diets for 8 d periods, with continuous collection of digesta for 24 h on each of the fifth and eighth days. The endogenous ileal amino acid flows were determined with reference to recovery of the marker, Cr, directly for pigs receiving the protein-free diet or after centrifugation and ultrafiltration (10 000 Da molecular mass cut-off) for pigs on the EHC-based diets. Mean endogenous ileal N flows were 1753, 1948, 2851 and 5743 μg/g DM intake when the pigs received diets containing 0, 50, 100 and 200 g EHC/kg respectively. There was a significant ($P < 0{\cdot}05$) effect of dietary peptide concentration on the endogenous ileal flows of N and all of the amino acids, with an increase in endogenous ileal amino flow with increasing dietary EHC concentration.

Ileal amino acid flow: Enzyme-hydrolysed protein: Pig

The 'apparent' ileal digestibility of a dietary amino acid is based on a determination of the total flow of amino acids at the terminal ileum. With the apparent measure, no correction is made for the flow of endogenous protein, i.e. protein that is secreted into the digestive tract, including digestive enzymes, mucus and desquamated cells (Fauconneau & Michel, 1970; Snook, 1973). Taking the endogenous component into account results in 'true' digestibility. Theoretically, this is a better measure for representing amino acids absorbed from the gut (Darragh *et al*. 1995) and is important in both animal and human nutrition. Single-stomached animals are often used as nutritional models for man, and the growing pig is a particularly suitable model for protein digestion (Rowan *et al*. 1994).

The traditional approach to determining endogenous protein loss from the small bowel is to feed an animal on a diet devoid of protein, and assume that all of the protein present in the terminal ileal digesta is endogenous. This method has been criticized, however, as being unphysiological. Protein-free feeding may result in a reduction in the amounts of gastric and pancreatic enzymes secreted (Fauconneau & Michel, 1970; Schneeman, 1982) and a general decrease in the rate of protein synthesis in the body and gut (Millward *et al*. 1976).

The enzyme-hydrolysed protein (peptide alimentation) method for determining endogenous protein loss was proposed by Moughan *et al*. (1990) and allows gut endogenous N and amino acid losses to be determined in animals fed on a diet containing peptides. With this method the animal is fed on a semi-purified diet containing an enzyme-hydrolysed protein (usually enzyme-hydrolysed casein, EHC), comprising free amino acids and peptides (molecular mass (MM) < 5000 Da), as the only N source. The EHC is commonly included at a level of 100 g/kg diet. Ileal digesta samples are collected, centrifuged and ultrafiltered. The precipitate plus the high-MM fraction (MM $> 10\,000$ Da) resulting from the ultrafiltration contains the endogenous material. Any unabsorbed dietary amino acids or peptides are discarded in the low-MM fraction. The dietary enzyme-hydrolysed protein is considered to mimic the natural products of gastric digestion with the assumption that the peptides entering the small intestine are similar in size to those that would enter the small intestine if the non-hydrolysed protein had been fed to the animal. An assumption of the

Abbreviations: EHC, enzyme-hydrolysed casein; MM, molecular mass; PVTC, post-valve T-caecum.

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method is that the dietary amino acids and peptides maintain a physiologically normal state in the digestive tract.

Comparison between the protein-free and enzyme-hydrolysed protein methods in the pig and other single-stomached species has shown that endogenous ileal amino acid loss is significantly higher for animals given a peptide-containing diet (Darragh *et al*. 1990; Butts *et al*. 1991, 1993*a*; Moughan *et al*. 1992; Donkoh *et al*. 1995; Hendriks *et al*. 1996; Leterme *et al*. 1996). Higher endogenous ileal amino acid flows when animals receive diets containing peptides or intact protein have also been reported using other methods for the measurement of endogenous ileal amino acid flow, such as the isotope dilution (de Lange *et al*. 1990) and guanidination methods (Moughan & Rutherfurd, 1990). With the enzyme-hydrolysed protein method, however, it is not known whether the dietary concentration of amino acids and peptides influences endogenous ileal amino acid flow.

Furthermore, most comparisons made to date involving the peptide alimentation method have involved a sampling of digesta from the fatally anaesthetized animal. Whereas this is a well-accepted technology, it is possible that such sampling could lead to a bias in results due to possible unrepresentativeness of the sample of digesta collected, as with this approach the sample size is relatively small. Recently, a new method for digesta collection in pigs has been developed (post-valve T-caecum (PVTC) cannulation; van Leeuwen *et al*. 1991) which allows much larger samples of digesta to be collected in conscious pigs.

The primary aim of the present study was to determine whether the concentration of dietary peptides in an EHC-based diet had an effect on the flow of endogenous N and amino acids at the terminal ileum of the pig. An additional aim of the study was to provide, using the PVTC cannulation technique, corroborative evidence for an overall effect of peptide alimentation on endogenous ileal amino acid flow.

Materials and methods

Animals and housing

Eight Large White × (Large White × Landrace) entire male pigs with a common sire, and with an overall mean live weight of 33·3 (SE 1·5) kg at the commencement of the study, were obtained from the Pig Research Unit, Massey University, New Zealand. The animals were housed individually in smooth-walled steel metabolism cages in a room maintained at 22 ± 1°. The Massey University Animal Ethics Committee granted ethical approval for the study.

Surgery

A PVTC cannula was inserted into the caecum of each pig for the collection of ileal digesta, according to the method of van Leeuwen *et al*. (1991). The cannulas were made of medical grade silastic tubing with an i.d. of 25 mm and o.d. of 29 mm. The pigs were not fed for 24 h before surgery. Anaesthesia was induced with an intramuscular injection of Zoletil (Zoletil 50, Techvet Laboratories Ltd, Auckland, New Zealand; 4 mg/kg body weight) and Xylozine (Xylaze 100, Parnell Laboratories, Takanini, New Zealand; 2·2 mg/kg body weight) and maintained via inhalation of halothane (Fluothane, Imperial Chemical Industries Ltd, Cheshire, UK) in O_2. For the first 3 d following surgery, each pig was given an injection of antibiotic (2 ml Engemycin 10 %, Intervet International B.V., Boxmeer, Holland) intramuscularly twice daily and antibiotic powder was dusted on the wound site daily. The site where the cannula was exteriorized was washed with water, and Zn cream was applied daily throughout the experiment. The pigs regained consciousness within 1 h of surgery and were standing 4–5 h after surgery.

Diets and feeding

Five diets were prepared, including a basal diet and test diets that contained 0 (protein-free diet), 50 (EHC5), 100 (EHC10) and 200 (EHC20) g EHC/kg. The dietary ingredient compositions are given in Table 1 and the determined N and amino acid compositions of the 'protein-free' and EHC diets are given in Table 2. The 'protein-free' diet contained 0·63 g N/kg diet, which corresponds to 3·6 g crude protein/kg.

For the first 10 d post-operation, all pigs were fed on a standard barley-based grower diet to appetite. During the following 4 d, meal feeding (08.00 and 17.00 hours) commenced. This feeding regimen was maintained for the remainder of the trial. The basal diet was gradually introduced over these 4 d.

The pigs were then fed on a test diet in two equal portions at a level of 0·10 metabolic body weight/d. Each pig was weighed and the level of food intake adjusted accordingly whenever the animal was introduced to another diet. The diets were mixed with water (1 : 1, w/v) immediately before feeding and water was freely available between meals. Cr_2O_3 was included in each diet as an indigestible marker.

Experimental design

The diets were administered using a Latin-square design (4 × 4 Latin square, duplicated) such that every test diet followed every other test diet once only. The pigs were randomly allocated to the Latin square and received their respective diets for 8 d. At the end of this period, the basal diet was given for a period of 6 d to allow re-equilibration of body N balance for the pigs fed on the protein-free and low-protein diets. Following this, the pigs were given their next test diet. Each pig received each of the four test diets once only.

On the fifth and eighth days of each test period, ileal digesta were collected continuously for 24 h using plastic bags attached to the cannulas. The bung was removed from the cannula 1 h before the collection commenced as suggested by van Leeuwen *et al*. (1991), to allow the ileocaecal valve to move so that it was protruding into the lumen of the cannula instead of the intestinal lumen. The plastic bags were emptied at least hourly, and the digesta were frozen (– 20°) hourly after adjustment to pH 3·5 by the addition of 6 M-H_2SO_4. This procedure was adopted to reduce enzyme and bacterial activity in the digesta.

Chemical analysis

Digesta were thawed and pooled for each diet and pig over

Table 1. Ingredient compositions (g/kg air-dry basis) of the basal and protein-free diets, and the experimental diets containing 50, 100 or 200 g enzyme-hydrolysed casein (EHC)/kg (EHC5, EHC10 and EHC20 respectively)

	Diet				
Ingredient	Basal	Protein-free	EHC5	EHC10	EHC20
Casein	120	–	–	–	–
EHC*	–	–	50	100	200
Soyabean oil	35	35	35	35	35
Cellulose	50	50	50	50	50
Sucrose	70	70	70	70	70
Wheat starch	719	833	783	733	633
Vitamin and mineral mix†	2·5	2·5	2·5	2·5	2·5
Dicalcium phosphate	2·5	2·5	2·5	2·5	2·5
Sodium chloride	0·3	0·3	0·3	0·3	0·3
Potassium carbonate	0·5	0·5	0·5	0·5	0·5
Magnesium sulfate	0·2	0·2	0·2	0·2	0·2
Chromic oxide	–	6	6	6	6

* New Zealand Pharmaceuticals Ltd, Palmerston North, New Zealand. The molecular mass distribution of the EHC was determined using an HPLC gel filtration column (Waters Millipore 625 HPLC system and PSK2000SW 600 mm column). The eluting solvent contained 1 ml trifluoroacetic acid/l and 360 ml acetylnitrile/l, and the sample was detected using a wavelength of 205 nm. All peptides were less than 5000 Da in size, with 0·83 % between 3000 and 5000 Da, 11·3 % in the 1000–3000 Da range and the remaining 87·87 % less than 1000 Da.

† Pig grower/finisher vitamin and mineral premix, Danmix, Nutritech, New Zealand, containing (per kg diet): retinol 1376 mg, cholecalciferol 20 mg, α-tocopherol 8040 mg, menadione 800 mg, thiamin 400 mg, riboflavin 1 g, pyridoxine 800 mg, cyanocobalamin 4 mg, pteroylmonoglutamic acid 84 mg, pantothenic acid 4 g, biotin 6 mg, niacin 6 g, choline 20 g, Se 120 mg, Co 200 mg, I 400 mg, Cu 50 g, Fe 40 g, Mn 18 g, Zn 48 g, zinc bacitracin 4 g.

the 24 h collection periods. Each pool of digesta was then weighed and homogenized.

A subsample of approximately 60 g was taken from each digesta pool from the pigs receiving the EHC-based diet, and centrifuged at 7000 ***g*** for 10 min. The supernatant fractions were than ultrafiltered using Centriprep-10 ultrafiltering devices (Amicon Inc., Beverly, MA, USA; 10 000 Da MM cut-off) according to the manufacturer's instructions. The precipitate from the centrifugation step was added to the retentate from the ultrafiltration, and the material freeze-dried and finely ground. A subsample (approximately 60 g) was taken from each digesta pool from the pigs receiving the protein-free diet. This subsample was immediately freeze-dried and finely ground.

Table 2. The amino acid compositions (g/kg DM) of the 'protein-free' diet and the experimental diets containing 50, 100 or 200 g enzyme-hydrolysed casein (EHC)/kg (EHC5, EHC10 and EHC20 respectively)

	Diet			
Amino acid	Protein-free	EHC5	EHC10	EHC20
Lys	0·13	4·69	9·59	19·07
His	0·03	1·69	3·35	6·35
Arg	0·08	2·07	4·37	8·87
Asp	0·19	4·81	10·08	19·79
Thr	0·08	2·62	5·24	10·42
Ser	0·10	3·27	6·41	12·84
Glu	0·31	12·98	26·39	53·09
Pro	0·07	5·30	10·85	20·79
Gly	0·12	1·14	2·23	4·37
Ala	0·12	1·81	3·63	7·23
Cys	0·10	0·32	0·47	0·79
Val	0·10	3·48	7·12	14·34
Met	0·08	1·50	2·80	6·23
Ile	0·08	2·90	5·85	11·96
Leu	0·14	5·18	10·54	21·33
Tyr	0·07	1·52	3·00	6·32
Phe	0·08	2·74	5·57	11·47
Nitrogen	0·63	7·43	13·72	27·99

The diets, non-fractionated digesta samples collected after feeding the protein-free diet, and the digesta precipitate + retentate (MM $>$ 10 000 Da) fractions from pigs fed on the EHC-based diets were analysed for total N, amino acids, Cr and DM.

Total N was determined in duplicate. The samples were combusted at 1050° in O_2 gas. The N was then reduced to N_2 by a catalyst and this was measured using a Leco FP2000 thermal conductivity cell (Leco Corporation, St Joseph, MI, USA).

The amino acid composition of the samples was determined as follows: duplicate samples (5–7 mg) were hydrolysed in 1 ml 6 M-glass-distilled HCl containing 1 g phenol/l in glass tubes sealed under vacuum, for 24 h at 110 $\pm$ 2°. Amino acid concentrations were then measured using a Waters ion exchange HPLC system calibrated against a reference amino acid mixture of known concentrations. The peaks of the chromatograms were integrated using the dedicated software Maxima 820 (Waters, Millipore, Milford, MA, USA) which identifies the amino acids by retention time against a reference amino acid mixture. Norleucine and lysozyme were used as internal and external standards respectively, and the weight of each amino acid was calculated using free amino acid MM values. No corrections were made for losses of amino acids during hydrolysis.

Cysteine and methionine are destroyed during hydrolysis, so were determined after oxidation of duplicate samples (3–4 mg) with 1 ml performic acid (300 ml/l H_2O_2–880 ml/l formic acid, 1 : 9, v/v) for 16 h at 0°. The samples were then

neutralized with 0·15 ml HBr (500 g/l) before hydrolysis. Tryptophan, which is also destroyed during acid hydrolysis, was not determined.

The Cr contents of the diet and ileal digesta were determined using an Instrumentation Laboratory Atomic Absorption Spectrophotometer according to the method described by Costigan & Ellis (1987).

Data analysis

Endogenous N and amino acid flows (related to the ingestion of 1 g DM) were determined using the following equation:

$$\text{endogenous flow} = \frac{\text{concentration of compound in digesta} \times \text{diet Cr concentration}}{\text{digesta Cr concentration}}.$$

The units were μg/g DM intake.

The data were tested for homogeneity of variance using Bartlett's Test (Snedecor & Cochran, 1980). All statistical tests were carried out using the computer programme SAS (version 6.12, 1997; Statistical Analysis Systems Institute Inc., Cary, NC, USA). The data were analysed using a general linear model for a Latin-square design adjusted for the loss of one pig. The model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \varepsilon_{ijk},$$

where α_i, β_j and δ_k represent the effects due to the treatment (diet), collection period and pig respectively.

Differences in endogenous N and amino acid loss between the pigs fed on the protein-free and EHC10 diets were determined by conducting a multiple range test using least square means following Bonferroni adjustment for multiple comparisons.

The endogenous ileal N and amino acid flows were plotted against the EHC concentration in the diet. Linear and curvilinear functions were fitted (SAS version 6.12, 1997) to the data obtained after all four test diets were fed to the pigs, and a linear function was also fitted to the data pertaining to the 50, 100 and 200 g/kg EHC-based diets.

Results

One pig was removed from the trial due to complications with its cannula. The remaining pigs appeared healthy and consumed their diets readily. The mean daily live-weight gains of the pigs determined over the 8 d periods throughout the study were 238 (SE 138), 575 (SE 100), 613 (SE 75) and 925 (SE 75) g/d for pigs fed on the protein-free, EHC5, EHC10 and EHC20 diets respectively. The mean live weight of the pigs at the completion of the experiment was 80·0 kg. There was no leakage of digesta around the cannulas during digesta collections and minimal leakage at other times during the experiment. The Cr recoveries averaged 73·2 (SE 4·4) %.

All variances were found to be homogeneous. The endogenous ileal amino acid and total N flows for the animals given the protein-free and EHC10 diets are shown in Fig. 1. The endogenous flows of total N, aspartic acid, threonine, serine, glutamine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine and methionine were all statistically significantly ($P < 0·05$) higher for pigs receiving the EHC10 diet compared with pigs receiving the protein-free diet.

The mean endogenous ileal N and amino acid flows pertaining to the diets containing different amounts of EHC are shown in Table 3. There was a significant ($P < 0·05$) effect of dietary EHC concentration on total N and amino acid flow for all of the amino acids.

Fig. 2 shows curvilinear and linear relationships between endogenous ileal N flow and dietary EHC concentration. The curve (endogenous N flow $= 1508·6 + e^{0·0665 \times \text{EHC concentration}}$) had an R^2 value of 0·88, whereas the linear relationship pertaining to the 50, 100 and 200 g dietary EHC/kg concentrations (endogenous N flow $= 501·9 + (258·2 \times$ EHC concentration)) had an R^2 value of 0·87. The R^2 value for a linear relationship between the endogenous ileal N flow and 0, 50, 100 and 200 g dietary EHC/kg (endogenous N flow $= 1252 + (208 \times$ EHC concentration)) was 0·82. Only the data for total N are given in Fig. 2, but the shapes of the relationships shown are representative of those found for the individual amino acids.

Table 4 gives linear regression equations for the relationships between endogenous N or amino acid flow and EHC concentration in the diet for pigs receiving the diets containing 50, 100 and 200 g EHC/kg. The R^2 values ranged from 0·06 to 0·87. The slopes were statistically significantly different from 0 for N and all of the amino acids measured except for glycine. The intercepts were also significantly greater than 0 for all amino acids except for glutamine, proline and methionine, and for N.

Discussion

One of the aims of the present study was to determine endogenous ileal amino acid flows to corroborate earlier findings of a significantly higher loss of amino acids and N at the terminal ileum in the growing pig when a mixture of peptides and free amino acids is added to a protein-free diet (Moughan *et al.* 1992; Butts *et al.* 1993*a*; Leterme *et al.* 1996). Most earlier studies relied on the 'slaughter method' for the collection of ileal digesta, whereas the present study used PVTC-cannulated pigs. The slaughter method, which involves removal of a terminal section of small intestine from the anaesthetized animal, with manual collection of the contents, is a straightforward method for the collection of ileal digesta in pigs and other single-stomached animals. The slaughter technique has the advantages of simplicity and ethical acceptability compared with cannulation and anastomosis techniques and there is minimal interference with the animal's digestive tract before sampling. However, because of the relatively small amount of digesta collected with this method, the possibility exists of a bias in results due to an unrepresentativeness of the sample of digesta collected.

PVTC cannulation (van Leeuwen *et al.* 1991) offers a practical alternative that allows a quantitatively greater collection of ileal digesta. The major advantage with the PVTC cannula over several other cannulation methods is

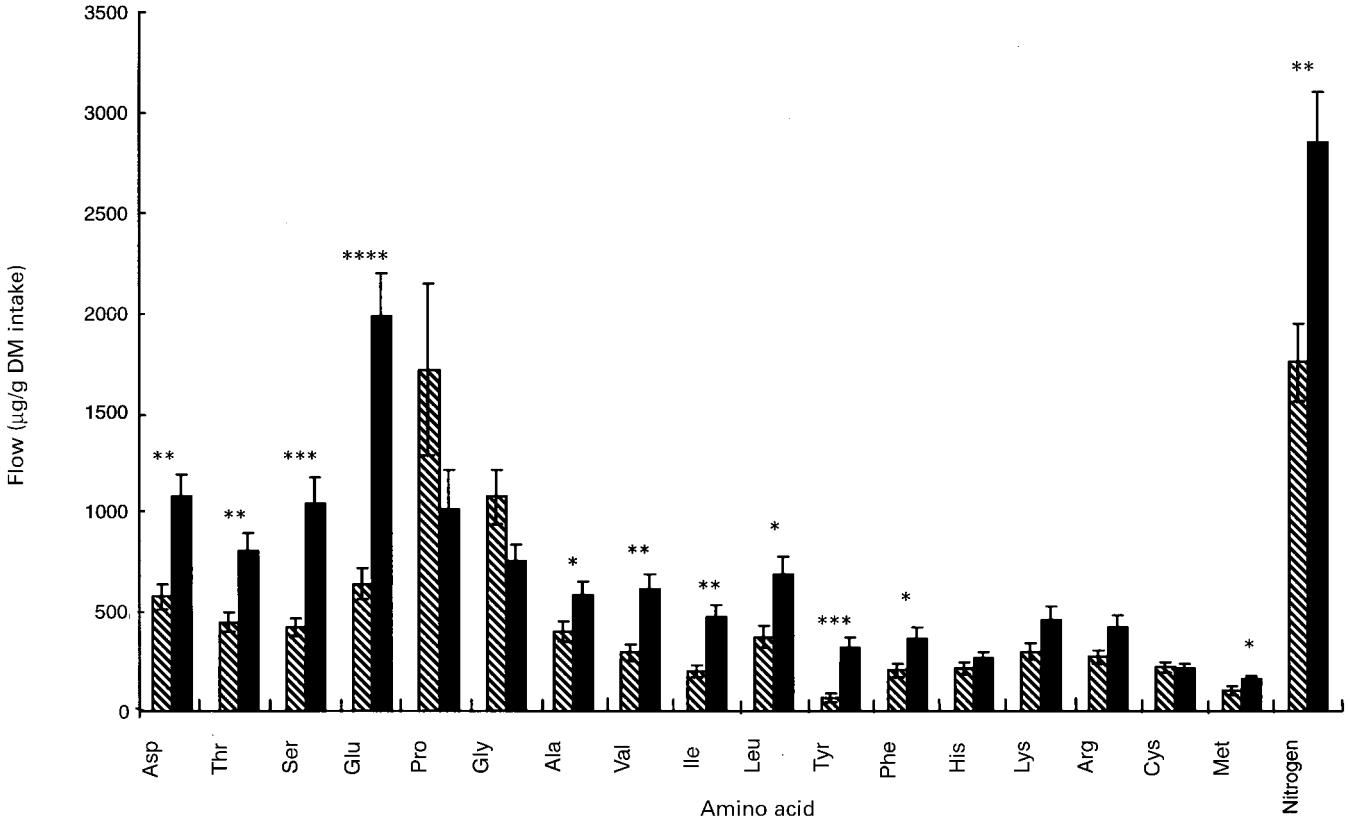

Fig. 1. Endogenous ileal amino acid flows in pigs fed on a protein-free (▨) diet or a diet containing 100 g enzyme-hydrolysed casein/kg (■). Values are means for seven pigs, with their standard errors indicated by vertical bars. Mean values were significantly different from those for the protein-free diet: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$, **** $P < 0{\cdot}0001$.

Table 3. Endogenous flows of amino acids (μg/g DM intake) in pigs receiving semi-purified diets containing 50, 100 or 200 g enzyme-hydrolysed casein (EHC)/kg (EHC5, EHC10 and EHC20, respectively)†

(Mean values for seven pigs, with their pooled standard errors)

	Diet				
Amino acid	EHC5	EHC10	EHC20	Overall SE	Statistical significance of effect of diet
Lys	262	456	523	74·4	****
His	141	264	379	56·1	****
Arg	232	419	456	74·5	***
Asp	502	1078	1509	182·7	****
Thr	382	807	1097	168·7	****
Ser	502	1040	1288	204·6	****
Glu	820	1983	3000	392·1	****
Pro	462	1009	1253	628·8	*
Gly	477	749	696	248·2	**
Ala	302	583	674	106·0	****
Cys	130	217	254	47·3	****
Val	308	610	760	109·4	****
Met	88	162	267	50·6	***
Ile	237	469	540	85·8	****
Leu	377	686	804	104·5	****
Tyr	164	319	353	70·1	****
Phe	201	363	406	61·2	****
Nitrogen	1948	2851	5743	524·7	****

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$, **** $P < 0{\cdot}0001$.

† For details of diets and procedures, see Tables 1 and 2 and pp. 422–423.

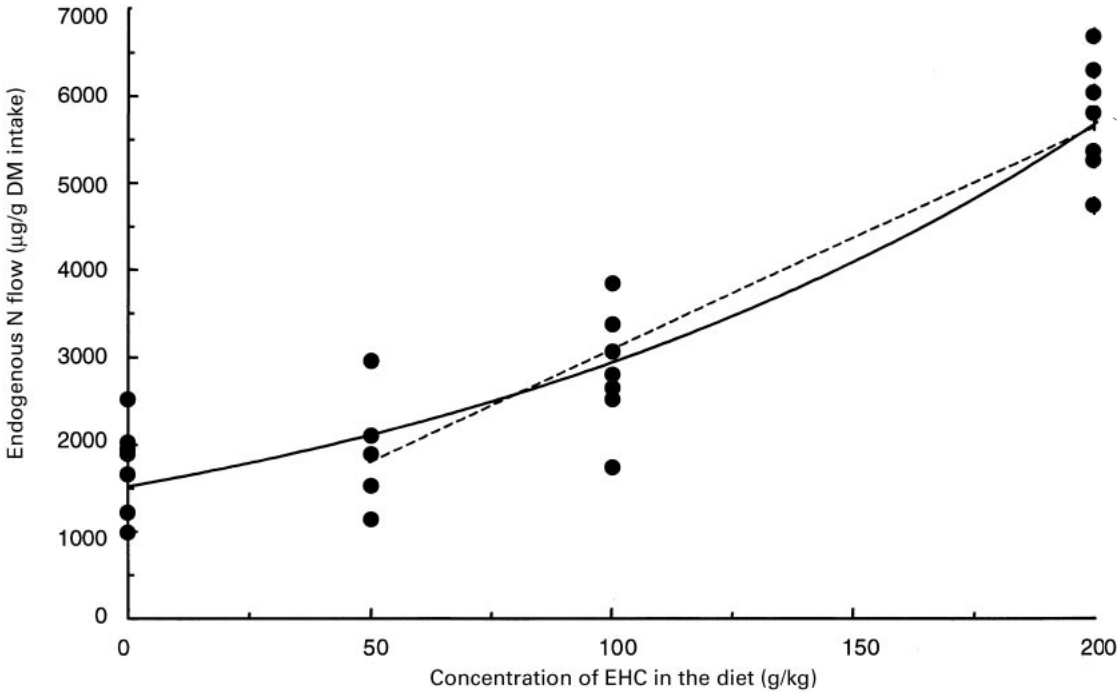

Fig. 2. Endogenous nitrogen flow in growing pigs given diets containing 0, 50, 100 or 200 g enzyme-hydrolysed casein (EHC)/kg. (—), Nonlinear regression for data from all diets. The relationship is described by the equation: endogenous N flow = $1508{\cdot}6 + e^{0{\cdot}0665 \times \text{EHC concentration}}$ (R^2 0·88). (- - -), linear regression for data from diets containing 50, 100 or 200 g EHC/kg (R^2 0·87).

that the small intestine is not transected, so there are minimal effects on ileal muscle function. Higher and more representative recoveries of digesta are expected in comparison with simple T-cannulation. The effect on the animal of long-term PVTC cannulation (12 weeks) has been examined by Köhler *et al.* (1992*a*,*b*) who concluded that the PVTC cannula does not significantly alter the metabolism of the pig and is suitable, therefore, for the collection of digesta in metabolic studies. In the present study, the PVTC-cannulated pigs appeared healthy and grew normally. At post-mortem there were no signs of any adverse effects of the cannulation procedure. Theoretically, a complete collection

Table 4. Linear regression relationships between endogenous ileal amino acid or nitrogen flows and dietary concentration of enzyme-hydrolysed casein (EHC)

	Regression equation*	R^2†	$P_{intercept}$‡	P_{slope}§
Lys	y=228 (SE 61)+16 (SE 5)x	0·35	0·05	0·01
His	y=83 (SE 32)+15 (SE 2)x	0·65	0·05	0·0001
Arg	y=213 (SE 58)+13 (SE 4)x	0·29	0·01	0·01
Asp	y=286 (SE 112)+64 (SE 8)x	0·73	0·05	0·0001
Thr	y=236 (SE 92)+45 (SE 7)x	0·67	0·05	0·0001
Ser	y=378 (SE 131)+49 (SE 10)x	0·54	0·01	0·0001
Glu	y=312 (SE 212)+139 (SE 16)x	0·79	NS	0·0001
Pro	y=339 (SE 176)+49 (SE 13)x	0·38	NS	0·01
Gly	y=504 (SE 103)+12 (SE 8)x	0·06	0·0001	NS
Ala	y=257 (SE 70)+23 (SE 5)x	0·46	0·01	0·001
Cys	y=111 (SE 23)+8 (SE 2)x	0·49	0·0001	0·001
Val	y=233 (SE 76)+28 (SE 6)x	0·53	0·01	0·001
Met	y=36 (SE 29)+12 (SE 2)x	0·58	NS	0·0001
Ile	y=201 (SE 62)+18 (SE 5)x	0·42	0·01	0·001
Leu	y=318 (SE 90)+26 (SE 7)x	0·41	0·01	0·01
Tyr	y=146 (SE 45)+11 (SE 3)x	0·34	0·01	0·01
Phe	y=179 (SE 51)+12 (SE 4)x	0·32	0·01	0·01
Nitrogen	y=501 (SE 298)+258 (SE 23)x	0·87	NS	0·0001

* Where y is the endogenous ileal flow (μg/g DM intake) and x is the concentration of EHC in the diet (50, 100 or 200 g/kg).
† Adjusted for the number of variables in the model.
‡ The probability that the intercept is equal to 0.
§ The probability that the slope is equal to 0.

of digesta is possible with the PVTC cannula. Although this was not found in the present work, the Cr recoveries were relatively high and in line with values reported following continuous collections of digesta for 24 h periods from PVTC-cannulated pigs. Mean Cr recoveries of 71·4 % (Köhler *et al*. 1991), 71·6, 71·9, 90·8, and 106·4 % (den Hartog *et al*. 1988; Köhler *et al*. 1990) have been reported previously. The mean Cr recovery of 73·2 % found in the present work is within this range of reported values. As Cr_2O_3 is believed to be quantitatively recovered in pig faeces (Ehle *et al*. 1982; Mroz *et al*. 1996) it appears that some of the digesta bypassed the cannula and entered the large intestine. The degree of recovery of digesta in the present study was, however, considered to be sufficient to provide representative digesta samples. The representativeness of digesta collection can be tested by determining whether there is a correlation between marker recovery and apparent ileal crude-protein digestibility when the same diet is fed to the animals (van Leeuwen *et al*. 1996). A correlation between Cr recovery and apparent ileal crude-protein digestibility would signify that the digesta collections are not representative. These correlations were calculated using the results from the present study for each diet, and no significant correlation was found, which supports the contention that the digesta collected were representative of ileal digesta.

In the present study the endogenous flows of total N, aspartic acid, threonine, serum, glutamine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine and methionine were statistically significantly ($P < 0·05$) higher for the EHC10-fed pigs compared with those fed on the protein-free diet. This is in agreement with previous studies involving a controlled comparison of peptide alimentation and the protein-free approach in the growing pig (Moughan *et al*. 1992; Butts *et al*. 1993*a*; Leterme *et al*. 1996) and it would appear that dietary peptides exert a positive influence on endogenous ileal protein loss.

An advantage of the peptide alimentation method is that it allows the endogenous flows of total N and all of the amino acids to be determined, as opposed to the homoarginine (Hagemeister & Erbersdobler, 1985; Rutherfurd & Moughan, 1990) and ^{15}N methods (Souffrant *et al*. 1982; Souffrant, 1991) where only the lysine or N flows respectively are determined. With the peptide alimentation method, endogenous free amino acids or peptides that are less than 10 000 Da in size are discarded with the ultrafiltrate leading to some underestimation of the endogenous flows determined with this method. Attempts have been made to quantify the extent of this underestimation by ultrafiltering digesta collected from pigs receiving a protein-free diet, and determining the amino acids present in the ultrafiltrate. Moughan & Schuttert (1991) in a carefully controlled study showed that amino N present in the ultrafiltrate comprised only 11 % of the N in the total ileal digesta. In contrast, Butts *et al*. (1992) found that the ultrafiltrate (MM $< 10\,000$ Da) of digesta obtained after feeding rats on a protein-free diet contained some 13–24 % of each amino acid in the total ileal digesta, except for glycine (46 %), histidine (36 %) and lysine (28 %) where higher amounts were found. Leterme *et al*. (1996) also ultrafiltered the soluble fraction of digesta obtained after feeding pigs on a protein-free diet and found that some 22 % of the total digesta N was present in the $< 10\,000$ Da fraction. In the latter two studies, however, the pH of the digesta was not adjusted to prevent autolysis in the digesta, thus these values may be overestimates. Further, it needs to be recognized that the feeding of peptides may affect the composition as well as the amount of endogenous excretion, and higher than normal amounts of free amino acids and small peptides may occur with protein-free feeding. Physiologically, it seems unlikely that there would be a high proportion of unabsorbed small peptides in the digesta at the terminal ileum and this is reflected by the observations of Moughan & Schuttert (1991). The MM profile of the EHC used in the present study showed that only 0·83 % of the total peptides had a MM greater than 3000 Da. The peptide alimentation method could, therefore, be modified by ultrafiltering the digesta using ultrafiltration devices with a MM cut-off of 3000–5000 Da as opposed to 10 000 Da. This would be expected to substantially decrease the proportion of endogenous N that may be discarded with the ultrafiltrate, thereby increasing the accuracy of the method. To conclude, the removal of endogenous material in the ultrafiltrate will lead to some underestimation of endogenous amino acid loss with the peptide alimentation method, but such underestimation only serves to make the protein-free–EHC diet comparison more conservative.

The ultrafiltration devices used in the present study have been shown to be effective for the ultrafiltration of purified protein, peptide and amino acid solutions. Butts *et al*. (1991) demonstrated that only negligible amounts of proteins that are smaller than 10 000 Da remain in the retentate and equally small amounts of proteins that are larger than 10 000 Da move through the filter during ultrafiltration. The effectiveness of the ultrafiltration devices that were used in the present study has also been tested by adding increasing amounts of EHC to digesta, ultrafiltering the digesta and quantifying the N and amino acids in the fractions of digesta (SM Hodgkinson and PJ Moughan, unpublished results). In the latter work the precipitate plus retentate fraction of digesta was found to have a relatively constant concentration of amino acids even when large amounts of EHC were added to the digesta. This demonstrates that any binding of EHC to the retentate of the ultrafiltered digesta is negligible and that the ultrafiltration devices are effective for the ultrafiltration of digesta which contain EHC.

The main aim of the present study was to determine whether the concentration of peptides in the diet had an effect on endogenous ileal amino acid flow in the growing pig. As shown in Table 3, increasing the EHC concentration of the diet from 50 to 200 g/kg significantly increased the endogenous ileal flows of N and all of the amino acids. The endogenous flows of N and amino acids for pigs given the EHC10 diet were numerically similar to those found in previous studies where pigs were fed on a diet containing 100 g EHC/kg (Moughan *et al*. 1992; Butts *et al*. 1993*a*,*b*). Schulze *et al*. (1995) fed growing pigs on a diet containing 180 g EHC/kg and the endogenous amino acid flows were generally greater than those determined in the studies by Butts *et al*. (1993*a*,*b*) and Moughan *et al*. (1992). The flows reported by Schulze *et al*. (1995) are very similar to those recorded here for the EHC20 diet. The present finding

confirms the preliminary result of Butts *et al*. (1998), who examined the effect of dietary peptide intake on endogenous ileal lysine flow using the enzyme-hydrolysed protein method in the growing rat, and found a statistically significant effect of dietary peptide concentration on endogenous ileal lysine flow. In contrast, Souffrant *et al*. (1997) have reported preliminary results using the homoarginine and isotope dilution methods in piglets, where there was no difference in endogenous ileal N flows dependent on dietary casein concentration.

When functions were fitted to the data overall (Fig. 2), a curve (endogenous N flow $= 1508{\cdot}6 + e^{0{\cdot}0665 \times \text{EHC concentration}}$) gave the best overall fit (R^2 0·88). However, if it is assumed that pigs given the protein-free diet are effectively in a different physiological state compared with those receiving protein-containing diets, it may be more appropriate to fit functions over the range of 50–200 g EHC/kg inclusion. When this was done (Fig. 2) a linear function gave a similar fit to the data (R^2 0·87).

The slopes of the linear regression equations for ileal amino acid flow related to dietary EHC concentration were positive and generally significantly different from zero (Table 4). The present result demonstrates that the stimulatory effect of dietary peptides on endogenous amino acid flows is not an 'all or nothing' effect, but is rather 'dose-dependent'. This has important implications for the practical determination of true digestibility coefficients in diet formulation.

Previous studies have demonstrated that increasing dietary protein concentration at a set DM intake results in an increase in apparent ileal amino acid digestibility in the growing pig (Sauer *et al*. 1980; Bell *et al*. 1983; Furuya & Kaji, 1989). It has been suggested that the endogenous ileal amino acid losses remain constant with increasing dietary protein concentration and, therefore, constitute a greater proportion of the protein present at the terminal ileum at lower dietary protein concentrations (Taverner, 1979; Sauer *et al*. 1980). Studies that have investigated the relationship between dietary protein concentration and true ileal digestibility at a constant level of dietary DM intake have assumed a constant value for endogenous ileal amino acid flows in their calculations (Furuya & Kaji, 1989; Donkoh & Moughan, 1994), and have found no difference in true digestibility with increasing dietary protein concentration. Donkoh & Moughan (1994) examined the effect of protein concentration in the diet on true ileal digestibility coefficients for meat-and-bone meal. If the endogenous N and amino acid flows determined in the present study are used with the values of Donkoh & Moughan (1994), it can be shown that the true digestibility of meat-and-bone meal increases with increasing inclusion of peptides in the diet. There is no obvious explanation for this observation. When true digestibility coefficients are to be compared among different protein sources, the coefficients may need to be determined at a standard inclusion level of dietary protein.

There is now a considerable body of work available on endogenous ileal N and amino acid flows in the growing pig determined using the protein-free and enzyme-hydrolysed protein approaches. Table 5 presents a summary of selected published results for endogenous ileal lysine and total N flows determined using these two methods in the growing pig. The data presented in Table 5 are taken only from studies where growing pigs were fed on semi-purified diets containing 30–50 g cellulose/kg as the only source of fibre. In spite of this basis for comparability, the reported endogenous ileal N and lysine flows determined under protein-free alimentation are highly variable, ranging from 1360 to 3168 and 250 to 630 mg/kg DM intake respectively. The

Table 5. Published endogenous ileal flows of nitrogen (mg/kg DM intake) and lysine (mg/kg DM intake) in the growing pig (live weight 10–115 kg) determined using the protein-free and peptide alimentation methods

		Flow		
Method		Nitrogen	Lysine	Reference
Protein-free alimentation*		1753	298	Present study
		1790	284	Souffrant *et al.* (1997)
		1500	252	Butts *et al.* (1993*a*)
		2970	530	Furuya & Kaji (1992)
		1360	350	Leterme *et al.* (1992)
		2300	312	Moughan *et al.* (1992)
		3168	530	de Lange *et al.* (1989*b*)
		2960	630	de Lange *et al.* (1989*a*)
		1710	260	Furuya & Kaji (1989)
		1810	250	Taverner *et al.* (1981)
	Mean	2132	370	
Peptide alimentation†		2851	456	Present study
		3700	448	Butts *et al.* (1993*a*)
		2746	591	Butts *et al.* (1993*b*)
		NR	461	Moughan *et al.* (1992)
	Mean	3417	524	

NR, not reported in the publication.
* Diets were protein-free, with N and amino acids present in ileal digesta assumed to be of endogenous origin.
† Pigs fed on diets with 100 g enzyme-hydrolysed casein/kg (molecular mass < 5000 Da) as the only protein source. Ileal digesta ultrafiltered (molecular mass cut-off 10 000 Da) to separate endogenous protein.

mean flows under protein-free alimentation (2132 and 370 mg/kg DM intake for N and lysine respectively) are notably lower than those obtained under peptide alimentation (3417 and 524 mg/kg DM intake for N and lysine respectively).

The EHC-based endogenous ileal amino acid flows determined in the present study were statistically significantly higher for most of the amino acids determined, compared with those found with protein-free alimentation. This confirms, using a different approach to digesta collection, other reported findings that the traditional protein-free method markedly underestimates endogenous loss in the growing pig. Increasing the peptide concentration of the diet had a major effect on endogenous ileal amino acid losses in the growing pig.

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