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The effect of heat on amino acids for growing pigs

1. A comparison of ileal and faecal digestibilities of amino acids in raw and heat-treated field peas (*Pisum sativum* cultivar Dundale)

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Three experiments were conducted to examine the effect of heating field peas (*Pisum sativum* cultivar Dundale) on (1) proximate analysis and total amino acid composition, (2) ileal and faecal digestibilities of amino acids, and (3) digestible energy content. Alternative techniques for assessing ileal and faecal digestibilities and digestible energy respectively, were also investigated. Forced-air dehydrators were used to heat field peas at temperatures of 110°, 135°, 150° or 165°. In the first experiment the apparent ileal and faecal digestibilities of amino acids and the faecal digestibility of energy in the raw and heated field peas were determined using pigs fitted with 'T'-shaped cannulas. In the second, apparent ileal digestibility of amino acids and the faecal digestibility of energy were determined using the direct ileal and rectal sampling technique. This involved a single collection of digesta and faeces from the digestive tract of the pig while it was anaesthetized. The faecal digestibilities of amino acids and energy were determined using total faeces collection in the third experiment. In all experiments the respective field-pea treatments comprised 400 g/kg sugar-based diets and were the only source of amino acids. Heat significantly decreased the lysine (14·6–8·7 g/kg; $P < 0·001$), cystine (3·2–2·6 g/kg; $P < 0·01$) and arginine (16·7–14·5 g/kg; $P < 0·05$) contents of the heated peas. The 'reactive' lysine content of the field peas, as measured using the Silcock technique, was decreased by 0·11 and 0·30 with the application of heat at 150° and 165° respectively. Heat treatments did not alter the ileal digestibility of most amino acids. Only aspartic acid (0·72–0·58), glutamic acid (0·80–0·65) and the basic amino acids, lysine (0·79–0·56) and arginine (0·85–0·75), showed a significant linear decrease ($P < 0·05$) in ileal digestibility over the heat treatments, determined using the ileal cannulation procedure. Heating significantly ($P < 0·05$) decreased faecal digestibility for all amino acids. Faecal digestibility was consistently greater than ileal digestibility for the raw field peas; however, this difference decreased with heat application until faecal digestibility was equal or less than ileal digestibility at the 165° treatment. Heat linearly depressed digestible energy, diet dry-matter digestibility and diet energy digestibility. Losses in lysine, cystine and arginine are likely to be due to early and advanced Maillard reactions. Considerable binding of the remaining lysine also occurred as indicated by a decline in Silcock-reactive lysine. The results indicate that the direct ileal sampling technique is a viable alternative to the cannulation procedure for amino acids, but further method development is required to decrease the variability associated with measurements. The estimation of faecal digestibility using indigestible markers and the partial faeces collection technique was as efficient as total faeces collection. In general, ileal digestibility of amino acids showed little response to heating, however, any changes that were observed were greatest for lysine. In contrast, faecal digestibility of all amino acids was greatly reduced with increasing heat application. This response appeared to be largely due to the effect of heating on microbial degradation and synthesis of

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amino acids in the hind-gut, rather than a reflection of the changes within the protein induced by heating. This variable response makes faecal digestibility an unreliable estimator of amino acid ileal digestibility.

Ileal digestibility: Faecal digestibility: Amino acids: Field peas: Pigs

Despite considerable research into the effect of heat on proteins (Bjarnason & Carpenter, 1970; Carpenter, 1973; Varnish & Carpenter, 1975*a*; Hurrell & Carpenter, 1977; Erbersdobler *et al.* 1989), there is still uncertainty regarding the mechanisms of heat damage and the techniques for assessing the effect of heat on amino acid utilization. Broad investigations have studied the effect of heat on lysine and identified Maillard reactions and protein crosslinks which can result in lysine being digested in a non-utilizable form (e.g. fructoselysine; Erbersdobler, 1977). However, it has proven analytically difficult to quantify these reactions (Hurrell *et al.* 1976). Thus the extent to which amino acids can be apparently absorbed and not utilized is unclear.

Ideally, amino acid availability should be assessed by techniques that examine utilization when an amino acid is limiting. The normal assay is a growth assay where the treatments are designed to measure the slopes of responses to test amino acids relative to the slope of response to a standard (slope–ratio assay; Finney, 1964). Using this assay, Batterham *et al.* (1984) found that lysine availability ranged from 0·30 to 0·95 in conventional protein concentrates. The limitation of this assay, however, is that it requires considerable resources, and only one amino acid can be analysed at a time.

Accordingly, considerable research has been directed towards the ileal digestibility assay for estimating amino acid availability (Low, 1990). This assay has the advantage over faecal digestibility in that influences from microbial degradation of amino acids in the hind-gut are avoided, all essential amino acids can be assessed at one time, and substantially fewer resources are required.

Ileal digestibility of amino acids has been employed as a measure of amino acid availability on the assumption that if an amino acid is not recovered at the terminal ileum, then it has been absorbed in a form that can be utilized by the pig. Recent research suggests that whilst this might apply to good quality proteins, it may not be true for heat-processed, or alternative proteins (Bellaver & Easter, 1989; Batterham *et al.* 1990; Moughan *et al.* 1991; Wiseman *et al.* 1991). For example, Batterham *et al.* (1990) reported that only 0·36 of the ileal-digestible lysine from cottonseed meal was recovered in the empty body compared with 0·75 for soya-bean meal. Furthermore, Wiseman *et al.* (1991) found no advantage of ileal relative to faecal digestibility for diet formulation with severely heat-treated meals. This suggests that heating may influence the extent of microbial utilization of amino acids in the hind-gut.

The ileal digestibility assay is normally conducted with 'T'-piece or re-entrant cannulas inserted in the terminal ileum or by ileo-rectal anastomosis. The use of such techniques is increasingly being questioned on the grounds of animal welfare. An alternative technique is to sample directly from the terminal ileum of pigs before, or at, slaughter (Moughan & Smith, 1987).

The objectives of this series of studies were to define the relationships between the application of heat and total amino acids, the ileal and faecal digestibilities of amino acids, lysine availability, and lysine utilization, and to identify biochemical mechanisms underlying the above responses. The objectives of the work reported in the present paper were to examine the effects of heat on (1) proximate analysis and amino acid composition, (2) ileal and faecal digestibilities of amino acids and (3) digestible energy, and to investigate alternative techniques for assessing ileal and faecal digestibilities and digestible energy.

EXPERIMENTAL

Protein concentrates

Field peas (*Pisum sativum* cultivar Dundale) were used as the protein concentrate. They are a good quality protein source in terms of digestibility and amino acid balance, and are free from most anti-nutritional factors, with the exception of low levels of anti-tryptic factors (Saini & Batterham, 1989). They can therefore be fed raw following coarse crushing and provide an unheated control. Meals such as soya-bean meal, cottonseed meal, fish meal, and meat-and-bone meal are unsuitable for this work as they are all heated during preliminary processing, and thus the extent of heating before feeding is unknown.

Heat treatments

Heat was applied to field peas at temperatures at 110°, 135°, 150°, or 165° using a forced-air dehydrator, and maintained for 15 min. Heating was completed in 140 kg batches with 180, 270, 330 and 450 min required to reach 110°, 135°, 150° and 165° respectively. Any variation between heating batches was eliminated by mixing all batches within a heat treatment in a vertical mixer before rebagging and hammer-milling.

Dry heat was applied so that the effects of heat alone could be investigated, without influences from pressure or moisture associated with autoclaving and similar processes. The selected levels of heat application were chosen because (1) they are indicative of the levels of heat that can be obtained during the processing of commercial meals used in pig diets, and (2) the heat levels should provide field-pea treatments with lysine availabilities ranging from 0·90 down to 0·30, similar to that reported in commercial meals by Batterham *et al.* (1984).

Initially, heat-treatments up to 150° were applied to the field peas and were expected to induce a decrease in the apparent ileal digestibility of lysine based on reports by Jagger (1987) and Batterham *et al.* (1986*a*, *b*, *c*). At the completion of the first ileal digestibility experiment (Expt 1), however, no decrease in lysine digestibility was evident. A further heat treatment of 165° was therefore deemed necessary in an attempt to decrease lysine digestibility and to ensure that heating had induced changes in lysine availability and utilization, which were to be defined in subsequent experiments. For this reason the following experiments have been completed in two parts.

Expt 1. Ileal and faecal digestibilities of amino acids and faecal digestibility of energy by the cannulation technique

The aim of this experiment was to determine the ileal and faecal digestibilities of amino acids and faecal digestibility of energy in raw and heated field peas in pigs fitted with 'T'-piece cannulas.

Diets. Diets were formulated to contain 400 g field peas/kg in a sucrose base (Table 1). This level of field peas supplied approximately 0·36 g ileal-digestible lysine/MJ digestible energy (DE), which was to be the level used in subsequent retention studies. By using a sugar base it was possible to make a direct assessment of the effect of heat on the digestibility of amino acids in the field peas without the complications of amino acids or other nutrients contributed by a cereal base. Cr_2O_3 was included in the diets as an indigestible marker to calculate digestibilities.

Animals and procedures. The ileal digestibilities of amino acids in the raw and heated field peas were determined in an experiment divided into two parts. In the first part of the experiment, the raw, 110°, 135°, and 150° field-pea treatments were assessed using four male pigs of approximately 45 kg live weight. They were fitted with 'T'-shaped cannulas

Table 1. *Expts 1, 2 and 3. Composition of diets (g/kg, air-dry basis) for the ileal digestibility, faecal digestibility and digestible energy studies*

Study...	Expts 1 and 2	Expt 3
Field peas*	400	400
Minerals and vitamins†	5	5
Dicalcium phosphate	30	30
Chromic oxide	2	—
Soya-bean oil	15	15
Sugar	548	549·5
Fuzone 200‡	—	0·5

* Field peas in their raw form, or heated for 15 min at 110°, 135°, 150° or 165° were included in diets 1 to 5 respectively in Expts 1, 2 and 3.

† Contributed the following (/kg diet): Fe 60 mg, Zn 100 mg, Mn 30 mg, Cu 5 mg, I 2 mg, NaCl 2·8 g, Se 0·15 mg, retinol equivalent 960 μg, cholecalciferol 12 μg, α-tocopherol 20 mg, thiamin 1·5 mg, riboflavin 3 mg, nicotinic acid 14 mg, pantothenic acid 10 mg, pyridoxine 2·5 mg, cyanocobalamin 15 μg, menadione 2 mg (as menapthone dimethylpyrimidinol bisulphite; MPB), pteroylmonoglutamic acid 2 mg, choline 500 mg, ascorbic acid 10 mg, biotin 0·1 mg.

‡ Contains 200 g furazolidone/kg.

about 150 mm anterior to the terminal ileum and were housed in plastic-sided metabolism cages in a thermoneutral environment. Before cannulation, the pigs were provided with a highly digestible diet based on spray-dried skimmed-milk powder, maize starch, sugar, minerals, vitamins, and dicalcium phosphate for a period of 7 d. This diet was also given post-cannulation to assist recovery until gradually replaced by the experimental diets. The pigs were allocated to each experimental diet in a 4×4 Latin square design. Rations of 0·70 kg were provided with 1 litre of water at 12 h intervals for a period of 8 d. Water was provided *ad lib.* after ration consumption. At 2 h after feeding, a continuous 6 h collection was completed on days 7 and 8 using plastic bags attached to the cannula, with samples being stored at −20° following collection.

In the second part of the experiment the ileal digestibility of the raw and 165° field-pea treatments was determined. Four male pigs (approximately 45 kg live weight) were cannulated, fed and housed as before, with the exception that water was provided *ad lib.* via 'nipple' drinkers. The four pigs were randomly divided into pairs and given each diet according to a 2×2 Latin square design, to facilitate combined statistical analysis with the first part of the experiment. At the completion of the collections the samples of ileal digesta were frozen, freeze-dried, ground and bulked for each pig, followed by chemical analyses.

In both parts of the experiment the partial collection of faeces was undertaken for the determination of the faecal digestibilities of amino acids and energy. At the time of continuous collection of ileal digesta on the seventh and eighth days, random subsamples of faeces voided during this period were collected, bulked, and stored at −20°. At the end of the collection periods, frozen faeces were thawed, mixed, subsampled, and freeze-dried before chemical analysis.

Expt 2. Ileal digestibility of amino acids and faecal digestibility of energy by the direct ileal and rectal sampling technique

The aim of this experiment was to evaluate the direct ileal sampling technique as a possible alternative to ileal cannulation for the determination of amino acid digestibility. At the same time, grab samples of faeces were taken directly from the rectum to see if suitable digestible energy determination could be made simultaneously.

Diets. The same bath of diets used in Expt 1 (Table 1) was used in this experiment to avoid the possibility of between-mixing batch variation.

Animals and procedures. The five diets were arranged in a randomized block design. Six pigs (mixed sexes) within a weight range of 35–45 kg were allocated to each diet having been blocked on weight and shed position. Pre-experimentally, pigs were trained to a once-daily wet-feeding regime. Experimental diets were introduced over 3 d and then offered for a further 7 d. On the eight day the terminal ileum and the rectum were removed 4 h after the morning feed and the contents collected using the following procedure.

Approximately 3·5 h after consuming its entire ration the pig was pre-medicated using Stresnil (Janssen, Belgium). The animal was then left undisturbed in a calm environment for 0·5 h before total anaesthesia was induced using a 50 mg/g preparation of Biotal (sodium thiamylal; Boehringer Ingelheim, USA) via intravenous injection. A steady plane of anaesthesia was maintained using Halothane (May and Baker, Artarman, NSW, Australia). A 1·5 m section of the ileum anterior to the ileo-caecal valve was then dissected out and the contents removed. The digesta sample collected was stored at −20° before being freeze-dried, ground and analysed.

Faecal samples were collected after dissection of the rectum. The faeces collected were stored at −20° before being freeze-dried, ground and analysed.

Expt 3. Faecal digestibilities of amino acids and energy using total faeces collection

The objective of this experiment was to determine the faecal digestibilities of amino acids and energy in raw and heated field peas by the total collection procedure.

Diets. Diets were as for Expt 1 with the exception that Cr_2O_3 was omitted as a marker, and Fuzone 200 (Rhone Poulenc, Footscray, Australia) was included to guard against a *Campylobacter* burden that was prevalent in this piggery at the time of experimentation (Table 1).

Animals and procedures. Male pigs (20·2 (SD 1·7) kg) were used in this experiment. Four pigs per diet were allocated in a randomized block design. Pigs were housed in metabolism cages, and trained to a once-daily, wet-feeding regime. Experimental diets were introduced over a period of 3 d and then with water in a 2:1 ratio for a further 14 d. On day 11 the pigs were weighed and their daily feeding rate was adjusted to three times maintenance (3 M; $3 \times (0{\cdot}5$ MJ DE/kg body weight$^{0{\cdot}75}$)/diet DE).

On day 14, Fe_2O_3 was added to the diets at a rate of 10 g/kg to mark the beginning of the collection period, and again on day 21 to mark the end of the collection period. Faeces were collected on the appearance of Fe_2O_3, with collection ceasing on its reappearance. Daily samples were bulked and stored at −20°. They were then thawed, mixed, subsampled and freeze-dried before chemical analysis.

Chemical analyses

Techniques used were the methods of the Association of Official Analytical Chemists (1984). Gross energy was determined by adiabatic bomb calorimetry. Cr in diets, ileal digesta and faeces was determined by atomic absorption spectrophotometry (Kimura & Miller, 1957) under nitrous oxide–acetylene having been digested with concentrated HNO_3 and $HClO_4$.

Amino acid analysis. Amino acids in the field peas and ileal digesta were separated by ion-exchange chromatography and measured after reaction with ninhydrin. Norleucine was used as an internal standard with accepted recoveries falling between ±0·025 of the batch mean. Amino acids in the faeces were separated by reverse-phase chromatography and measured after reaction with phenylisothiocyanate (PITC). The internal standard utilized

for this analysis was α-amino butyric acid. Amino acid analysis followed hydrolysis at 110° for 24 h with constant boiling point HCl under N_2.

Analysis of all amino acids in the field peas, except methionine and cystine, was completed in duplicate following hydrolysis. Threonine, serine, valine, and isoleucine values were increased 4, 8, 5 and 7% respectively to account for sub-optimal recovery of these amino acids with 24 h hydrolysis. Methionine and cystine were measured in duplicate in the field peas following pre-oxidation of the samples with performic acid before hydrolysis and subsequent measurement as methionine sulphone and cysteic acid respectively.

The effects of hydrolysing the field peas for 24, 48 or 72 h at 110° with constant boiling point HCl under N_2 was examined, in a single analysis, using reverse-phase chromatography. The influence of heat on the 'reactive' lysine content of the heated peas was measured, in a single analysis, using the Silcock available-lysine assay (Roach *et al.* 1967).

Statistical analysis

Expts 2 and 3: amino acids. The results were analysed by analysis of variance, utilizing a general linear model, and the treatment means were separated by least significant difference (LSD). The effects of heat were statistically analysed in two parts. The first was a comparison of the raw field peas *v.* the 110° treatment. The second examined the linear effects across the four heat treatments only.

Expt 1: amino acids. As this experiment consisted of two parts, a preliminary analysis was required to justify combining the results and subsequent analysis as a single experiment. Data for the raw peas (control) only from each experiment was analysed by analysis of variance utilizing a general linear model, and the treatment means were separated by LSD. There was no significant difference ($P > 0{\cdot}05$) between any variable measured in the two experiments. The data were therefore combined and analysed as a single experiment with five treatments as described for Expts 2 and 3 above.

Comparison of techniques to determine ileal and faecal digestibilities. To facilitate comparison of ileal cannulation and direct ileal sampling and partial faeces collection and total faeces collection respectively, all experiments were regarded as a randomized block design ('pigs' in Expt 1 (cannulation and partial collection) were regarded as blocks). A combined analysis of variance was then performed consisting of the following sources of variation: (1) between methods, (2) between blocks within methods, (3) between diets and (4) experiment × diet interaction. The *F* tests for (3) and (4) were obtained by comparison with the error. The *F* test for (1) was obtained by comparison with (2). The method and diet variables were regarded as fixed rather than random effects during this analysis.

Comparison of ileal and faecal digestibilities. This comparison was restricted to ileal (cannulation) and faecal (partial collection) digestibilities determined in Expt 1. Because an ileal and a faecal digestibility measurement was obtained for each pig, the average difference between each measurement was tested to see if it was different from zero (similar to a paired *t* test). Differences were considered for each diet and block variation eliminated using an analysis of variance.

RESULTS

The effect of heat on the proximate composition and amino acid composition of field peas

A significant linear increase ($P < 0{\cdot}001$) in the dry matter content of the field peas was evident with heat application (Table 2). The application of heat to field peas significantly increased the ash and crude protein contents ($P < 0{\cdot}05$) at the 110° level, consistent with an increase in dry matter. Heat application linearly depressed the content of light-petroleum extract ($P < 0{\cdot}001$) while resulting in a substantial linear increase in the crude and neutral-detergent fibre contents ($P < 0{\cdot}001$).

Table 2. *Proximate composition (g/kg, air-dry basis) of raw field peas* (Pisum sativum *cultivar Dundale) and field peas heated for 15 min at 110°, 135°, 150° or 165° using a forced-air dehydrator*

	Heat treatments					Statistics			
Component	0	110°	135°	150°	165°	Treatment	Raw *v.* 110°	Linear†	SEM
Crude protein (N × 6·25)	210	224	222	227	216	NS	*	NS	3·9
Dry matter	914	939	950	960	975	***	***	***	1·1
Light petroleum extract (bp 40–60°)	21	21	22	15	16	***	NS	***	0·4
Fibre extract:									
crude	67	63	65	89	104	***	NS	***	2·9
neutral-detergent	82	67	184	467	483	***	NS	***	23·1
Ash	25	28	27	26	28	NS	*	NS	0·8

NS, not significant.
* $P < 0·05$, *** $P < 0·001$
† Analysis within heat-treatments (110°, 135°, 150°, 165°) only.

Significant absolute losses of lysine, cystine and arginine ($P < 0·001$; Table 3) were observed with heat application beyond 150°. Heat application resulted in significant ($P < 0·05$) linear decreases of these amino acids. With the exception of glutamic acid, the remainder of the amino acids were not affected by heating. Methionine and cystine were pre-oxidized before analysis, when establishing the total amino acid composition of the field peas, and measured as methionine sulphone and cysteic acid respectively. Pre-oxidation was not completed on any other samples (digesta, faeces), and hence values for methionine and cystine are not considered further.

The effects of extended hydrolysis on amino acid composition were not analysed statistically as the amino acid analysis was not duplicated. Extended hydrolysis of the unheated field peas tended to decrease the content of all amino acids except the branched-chain amino acids valine, isoleucine and leucine (Table 4). Despite showing a decrease with extended hydrolysis in the raw field peas, lysine content appeared to increase with extended hydrolysis in the heat-treated peas.

The 'reactive' lysine content of the field peas, as measured using the Silcock technique (Table 5), was decreased by 0·11 and 0·30 with the application of heat at 150° and 165° respectively. Only minor changes were observed at the lower heat applications.

The effect of heat on the ileal digestibility of amino acids

Cannulation. Heat applied at 110° significantly increased ($P < 0·05$) the ileal digestibilities of isoleucine, leucine, phenylalanine, histidine and arginine (Table 6). Within heat treatments there was no effect on the digestibility of the majority of amino acids, except for the non-essential amino acids aspartic acid and glutamic acid, and the basic amino acids, lysine and arginine. Lysine showed the greatest decrease in ileal digestibility with the application of heat at 165°, to 0·19 below that recorded for the raw field peas.

Direct ileal sampling. Heat applied at 110° significantly increased ($P < 0·05$) the ileal digestibility of arginine (Table 7). Using this technique, lysine was the only amino acid to show a significant linear decrease ($P < 0·05$) in ileal digestibility with heat application.

Cannulation v. *direct ileal sampling.* Digestibility values determined using direct ileal sampling were generally higher than those achieved using ileal cannulation. Direct statistical comparison revealed a significant ($P < 0·05$) difference between the results

Table 3. *Amino acid composition (g/kg, air-dry basis) of raw field peas* (Pisum sativum *cultivar Dundale*) *and field peas heated for 15 min at 110°, 135°, 150° or 165° using a forced-air dehydrator, and absolute gain or loss (proportion of total) of amino acid with heating relative to the raw peas on a dry-matter basis*

(Values are means with absolute gains or losses as a proportion of the total (%) given in parentheses)

	Heat treatments					Statistics†			
	Raw	110°	135°	150°	165°	Treatment	Raw *v.* 110°	Linear‡	SEM§
Aspartic acid	22·8	24·0 (+0·03)	24·8 (+0·04)	24·6 (+0·03)	24·7 (+0·02)	NS	NS	NS	0·41 (0·011)
Threonine	7·9	8·1 (−0·01)	8·4 (+0·02)	8·5 (+0·02)	8·3 (−0·02)	NS	NS	NS	0·19 (0·021)
Serine	10·3	10·8 (+0·02)	11·2 (+0·04)	11·1 (+0·02)	10·5 (−0·05)	NS	NS	NS	0·24 (0·021)
Glutamic acid	34·3	36·7 (+0·04)	38·0 (+0·06)	37·8 (+0·05)	38·6 (+0·06)	*	*	NS	0·82 (0·011)
Proline	9·0	9·5 (+0·02)	9·7 (+0·03)	10·0 (+0·06)	9·8 (+0·02)	NS	NS	NS	0·34 (0·030)
Glycine	8·9	9·4 (+0·02)	9·6 (+0·04)	9·7 (+0·03)	9·8 (+0·03)	NS	NS	NS	0·18 (0·015)
Alanine	8·7	9·2 (+0·03)	9·6 (+0·06)	9·7 (+0·06)	9·9 (+0·06)	NS	NS	NS	0·19 (0·017)
Cystine	3·2	3·2 (−0·04)	3·1 (−0·07)	2·8 (−0·18)	2·6 (−0·25)	***	NS	***	0·07 (0·018)
Valine	9·7	10·2 (+0·03)	10·8 (+0·07)	10·8 (+0·06)	11·1 (+0·07)	NS	NS	NS	0·20 (0·016)
Methionine	2·1	2·1 (−0·03)	2·2 (−0·02)	2·2 (0·00)	2·1 (−0·06)	NS	NS	NS	0·07 (0·023)
Isoleucine	8·8	9·2 (+0·02)	9·6 (+0·04)	9·6 (+0·03)	9·9 (+0·05)	NS	NS	NS	0·17 (0·017)
Leucine	14·1	14·9 (+0·03)	15·5 (+0·05)	15·5 (+0·05)	15·9 (+0·05)	NS	NS	NS	0·31 (0·020)
Tyrosine	6·4	6·6 (0·00)	7·0 (+0·05)	7·0 (+0·03)	6·9 (+0·01)	NS	NS	NS	0·19 (0·029)
Phenylalanine	9·3	9·8 (+0·03)	10·2 (+0·05)	10·3 (+0·05)	10·3 (+0·03)	NS	NS	NS	0·27 (0·028)
Lysine	14·6	15·2 (+0·01)	15·2 (0·00)	12·6 (−0·18)	8·7 (−0·44)	***	NS	***	0·28 (0·016)
Histidine	4·7	5·0 (+0·03)	5·1 (+0·04)	5·0 (+0·01)	5·0 (−0·01)	NS	NS	NS	0·13 (0·023)
Arginine	16·7	18·9 (+0·10)	19·3 (+0·11)	17·9 (+0·02)	14·5 (−0·18)	***	**	***	0·73 (0·016)

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Analysis of absolute losses only (loss in raw peas assumed to be zero).
‡ Analysis within heat-treatments (110°, 135°, 150°, 165°) only.
§ Standard error of the mean, with standard error of the losses given in parentheses.

Table 4. *Effect of hydrolysis for 24, 48 or 72 h on amino acid composition (g/kg, air-dry basis) of raw field peas* (Pisum sativum *cultivar Dundale*) *and peas heated for 15 min at 110°, 135°, 150° or 165°*

Treatment...	Raw			110°			135°			150°			165°		
Hydrolysis (h)...	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Aspartic acid	23·3	21·9	23·1	21·8	22·4	21·9	20·1	22·0	23·4	22·5	21·7	22·4	22·9	22·0	22·2
Threonine	8·6	7·8	7·6	7·8	8·1	7·4	7·6	7·9	7·9	8·4	8·1	7·8	8·4	8·0	8·0
Serine	11·1	10·4	9·7	10·2	10·6	9·1	9·8	10·4	9·4	11·0	10·7	9·8	10·7	10·4	9·5
Glutamic acid	36·4	33·3	35·5	33·6	34·0	33·7	31·2	33·5	35·7	35·0	33·6	34·6	35·9	34·6	35·1
Proline	9·4	8·6	9·0	8·6	8·9	8·5	8·3	8·9	9·0	9·4	9·0	8·8	9·5	9·3	9·1
Glycine	9·4	8·7	9·2	8·7	9·0	8·8	8·3	8·9	9·6	9·1	9·0	9·3	9·4	9·2	9·4
Alanine	9·7	9·4	9·2	9·0	9·8	8·9	8·7	9·8	9·8	9·7	9·9	9·5	9·8	10·2	9·8
Valine	10·2	10·6	10·6	9·4	11·0	10·1	8·8	10·7	11·0	9·5	10·8	10·6	9·4	10·9	10·8
Isoleucine	9·7	10·2	10·4	8·8	10·6	10·0	8·3	10·4	10·8	9·0	10·5	10·4	8·7	10·5	10·6
Leucine	14·6	15·0	14·4	13·5	15·6	13·8	12·9	15·4	15·2	14·3	15·7	14·7	14·5	16·1	15·2
Tyrosine	7·1	5·8	6·5	6·6	6·0	6·1	6·2	5·7	6·5	7·1	6·0	6·6	7·3	6·1	6·8
Phenylalanine	10·5	10·0	10·2	9·7	10·3	9·7	9·2	10·1	10·5	10·3	10·3	10·3	10·2	10·5	10·5
Lysine	15·2	15·2	14·6	13·9	15·5	13·9	12·7	14·6	14·6	12·0	12·9	12·5	8·5	9·0	9·2
Histidine	6·6	5·0	5·7	6·0	5·2	5·3	5·5	4·8	5·6	5·6	4·8	5·6	5·4	4·6	5·3
Arginine	22·8	20·2	19·3	18·7	21·5	18·7	19·2	20·0	19·7	21·1	20·4	18·3	16·4	18·7	16·0

Table 5. *Total lysine (g/16 g N), and residual (g/16 g N) and 'reactive' lysine (proportion of total) estimated using the Silcock technique*, for raw field peas* (Pisum sativum *cultivar Dundale*) *and peas heated for 15 min at 110°, 135°, 150°, or 165° using a forced-air dehydrator*

Heat treatment	Total lysine	Residual lysine	'Reactive' lysine
Raw	7·1	0·0	1·0
110°	6·7	0·1	0·99
135°	6·9	0·2	0·97
150°	5·6	0·6	0·89
165°	4·0	1·2	0·70

* Roach *et al.* (1967).

obtained for aspartic acid, threonine, serine, alanine, valine, isoleucine, leucine, tyrosine and phenylalanine (Table 8). There were no method × diet interactions for any amino acid.

The effect of heat on the faecal digestibility of amino acids

Partial faeces collection. There was no effect of the 110° heat treatment relative to the raw peas on the faecal digestibility of any amino acid (Table 9). Further heating significantly decreased ($P < 0·001$) faecal digestibility linearly for all amino acids.

Total faeces collection. There was no effect of the 110° heat treatment relative to the raw peas on the faecal digestibility of any amino acid (Table 10). Further heating significantly decreased ($P < 0·05$) faecal digestibility linearly for all amino acids with the exception of proline which was unaffected.

Partial collection v. *total collection.* Direct comparison of these methods revealed no significant differences ($P > 0·05$) for any amino acid and no method × diet interactions (Table 8).

Table 6. *Expt 1. The ileal digestibility of nitrogen (N), dry matter and amino acids in raw field peas* (Pisum sativum *cultivar Dundale*) *and field peas heated for 15 min at 110°, 135°, 150° or 165°, as assessed by cannulation procedures in pigs*†

(Mean values for eight pigs (raw) and four pigs (110°, 135°, 150°, 165°) per dietary group)

	Heat treatments					Statistics				
	Raw	110°	135°	150°	165°	Diet	Raw *v.* 110°	Linear‡	SEM (Raw)	SEM (Heat)§
N	0·60	0·61	0·59	0·64	0·53	NS	NS	NS	0·020	0·034
Dry matter	0·74	0·73	0·70	0·76	0·81	NS	NS	NS	0·020	0·035
Aspartic acid	0·68	0·72	0·66	0·68	0·58	NS	NS	*	0·015	0·026
Threonine	0·43	0·53	0·48	0·58	0·52	NS	NS	NS	0·026	0·045
Serine	0·55	0·63	0·58	0·65	0·56	NS	NS	NS	0·019	0·034
Glutamic acid	0·75	0·80	0·75	0·75	0·65	*	NS	**	0·012	0·021
Proline	0·35	0·44	0·37	0·45	0·44	NS	NS	NS	0·037	0·064
Glycine	0·40	0·49	0·43	0·50	0·45	NS	NS	NS	0·036	0·062
Alanine	0·51	0·61	0·55	0·63	0·58	NS	NS	NS	0·023	0·039
Valine	0·57	0·65	0·60	0·68	0·62	NS	NS	NS	0·019	0·032
Isoleucine	0·62	0·69	0·64	0·72	0·64	NS	*	NS	0·015	0·026
Leucine	0·55	0·65	0·59	0·69	0·62	*	*	NS	0·020	0·034
Tyrosine	0·59	0·65	0·60	0·67	0·63	NS	NS	NS	0·017	0·030
Phenylalanine	0·68	0·74	0·69	0·76	0·67	NS	*	NS	0·014	0·025
Lysine	0·75	0·79	0·74	0·74	0·56	***	NS	***	0·011	0·018
Histidine	0·69	0·75	0·71	0·77	0·68	*	*	NS	0·011	0·019
Arginine	0·79	0·85	0·82	0·84	0·75	**	**	**	0·007	0·012

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see Table 1 and pp. 223–226.
‡ Analysis within heat-treatments (110°, 135°, 150°, 165°) only.
§ SEM of heat treatments (110°, 135°, 150°, 165°) only.

Ileal v. *faecal digestibility of amino acids*

Interactions between diet and faecal digestibilities occurred for all amino acids except aspartic and glutamic acids. Faecal digestibility of all amino acids was consistently greater ($P < 0·05$) than ileal digestibility values for the raw peas and peas heated to 110° or 135° (Table 11). At 150°, however, the difference between ileal and faecal digestibilities began to decrease to the extent that there was no significant difference ($P > 0·05$) between the ileal and faecal digestibilities of alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine. At 165° the faecal digestibilities of lysine ($P < 0·001$) and histidine ($P < 0·01$) were significantly less than their ileal digestibilities. There was no significant difference ($P > 0·05$) between the ileal and faecal digestibilities of the other amino acids.

The effect of heat on the digestible energy of raw and heat-treated field peas

There were no significant differences ($P > 0·05$) between values obtained by the total or partial sampling techniques, in relation to the digestibility of diet dry matter and diet energy or DE content of the respective field-pea treatments. The total and partial sampling techniques produced values that were significantly higher ($P < 0·01$) than the values produced by the rectal collection technique, except at the 165° level of heat application (Table 12). The partial collection technique displayed the lowest variability.

Heat linearly depressed ($P < 0·01$) DE, diet dry-matter digestibility and diet energy digestibility.

Table 7. *Expt 2. The ileal digestibility of nitrogen (N), dry matter and amino acids in raw field peas* (Pisum sativum *cultivar Dundale*) *and field peas heated for 15 min at 110°, 135°, 150° or 165°, as assessed by the direct ileal sampling procedure in pigs*†

(Mean values for six pigs per dietary group)

	Heat treatments					Statistics				
	Raw	110°	135°	150°	165°	Diet	Raw *v.* 110°	Linear‡	SEM (Raw)	SEM (Heat)§
N	0·66	0·71	0·68	0·66	0·60	NS	NS	NS	0·037	0·065
Dry matter	0·81	0·83	0·81	0·80	0·68	NS	NS	NS	0·038	0·066
Aspartic acid	0·71	0·78	0·75	0·72	0·68	NS	NS	NS	0·027	0·046
Threonine	0·64	0·71	0·69	0·68	0·62	NS	NS	NS	0·031	0·054
Serine	0·66	0·73	0·71	0·70	0·65	NS	NS	NS	0.030	0·052
Glutamic acid	0·75	0·83	0·81	0·79	0·75	NS	NS	NS	0·022	0·038
Proline	0·48	0·56	0·58	0·56	0·36	NS	NS	NS	0·064	0·098
Glycine	0·38	0·52	0·44	0·51	0·41	NS	NS	NS	0·060	0·091
Alanine	0·66	0·75	0·73	0·72	0·69	NS	NS	NS	0·029	0·050
Valine	0·67	0·76	0·73	0·74	0·73	NS	NS	NS	0·026	0·046
Isoleucine	0·70	0·78	0·76	0·77	0·77	NS	NS	NS	0·023	0·041
Leucine	0·67	0·75	0·74	0·74	0·73	NS	NS	NS	0·026	0·045
Tyrosine	0·69	0·76	0·74	0·75	0·73	NS	NS	NS	0·025	0·043
Phenylalanine	0·72	0·80	0·79	0·80	0·78	NS	NS	NS	0·023	0·039
Lysine	0·76	0·83	0·80	0·74	0·67	NS	NS	*	0·024	0·042
Histidine	0·71	0·80	0·77	0·76	0·72	NS	NS	NS	0·026	0·045
Arginine	0·75	0·85	0·84	0·82	0·79	NS	*	NS	0·023	0·039

NS, not significant.
* $P < 0·05$.
† For details of diets and procedures, see Table 1 and pp. 223–226.
‡ Analysis within heat treatments (110°, 135°, 150°, 165°) only.
§ SEM of heat treatments (110°, 135°, 150°, 165°) only.

DISCUSSION

The application of heat to field peas had a major effect on chemical composition, but a comparatively small influence on the ileal digestibility of amino acids. Conversely, faecal digestibility was affected by heat, suggesting that micro-organisms in the hind-gut may be limited in their ability to digest and utilize heated amino acids. It is reasonable to suggest that poor amino acid utilization by hind-gut micro-organisms reflects a change in amino acid composition and structure with heating. This change may also result in the poor utilization of digested amino acids by the growing pig fed on heated protein concentrates.

The effect of heat on the proximate composition and amino acid composition of field peas

The loss of volatile compounds with the application of heat to field peas, as indicated by the significant drop in light petroleum extract, justifies expression of the results on an air-dry basis. The most notable change in the proximate analysis of the heated peas was the substantial increase in the content of crude fibre and neutral-detergent fibre. This is consistent with the findings of Wiseman *et al.* (1991) who suggested that plastic-type compounds formed during heat treatment may elevate the fibre content measured in this way.

Losses in lysine, arginine and cystine with the application of heat to field peas are likely to be due to early and advanced Maillard reactions. In the presence of carbohydrates in the field peas, early Maillard reactions would result in the formation of the fructoselysine moiety (Erbersdobler *et al.* 1989) with a subsequent drop in the lysine content. The

Table 8. *Expts 1, 2 and 3. Results of statistical comparison between techniques used to determine ileal and faecal digestibilities of amino acids from field peas* (Pisum sativum *cultivar Dundale*)†

	Ileal digestibility Cannulation *v.* Direct ileal		Faecal digestibility Partial *v.* Total	
	Method	Method × Diet	Method	Method × Diet
N	NS	NS	NS	NS
Dry matter	NS	NS	NS	NS
Aspartic acid	*	NS	NS	NS
Threonine	***	NS	NS	NS
Serine	**	NS	NS	NS
Glutamic acid	NS	NS	NS	NS
Proline	NS	NS	NS	NS
Glycine	NS	NS	NS	NS
Alanine	***	NS	NS	NS
Valine	***	NS	NS	NS
Isoleucine	***	NS	NS	NS
Leucine	***	NS	NS	NS
Tyrosine	**	NS	NS	NS
Phenylalanine	**	NS	NS	NS
Lysine	NS	NS	NS	NS
Histidine	NS	NS	NS	NS
Arginine	NS	NS	NS	NS

NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of procedures, see pp. 223–226.

advanced Maillard reaction, consistent with the formation of brown pigments evident in the peas heated to 150° and 165°, has been shown to lead to the destruction of large proportions of lysine, arginine and to a lesser extent, cystine (Miller *et al.* 1965).

Losses in cystine and lysine with the application of heat have also been attributed to the formation of cystine-derived crosslinks (Friedman, 1973; Asquith & Leon, 1977). Heat results in the formation of a dehydroalanyl residue from cystine, which is capable of binding with the ϵ-amino group of lysine to form lysinoalanine. In this study, however, lysinoalanine formation is unlikely to have contributed to a reduction in lysine and cystine content as the field peas were heated at a neutral pH (Bjarnason & Carpenter, 1970).

It appears that a considerable proportion of the lysine remaining in the field peas heated to 150° or 165° underwent binding with other compounds as indicated by the sharp reduction in Silcock-available lysine at these temperatures. It has been suggested that binding of lysine occurs due to the formation of amide bonds between the ϵ-amino group of lysine and carboxylic groups of proteins (Bjarnason & Carpenter, 1970). This forms the basis of Carpenter's technique (Carpenter, 1960) and the Silcock technique (Roach *et al.* 1967) for the determination of available lysine. Reductions in the reactive lysine content of heat-processed meals have been shown to overestimate the biological availability of lysine for growing pigs (Batterham *et al.* 1979, 1981, 1986*c*). Hence, the application of heat at 150° and 165° is likely to have induced a reduction in the biological availability of lysine in the field peas to well below 0·89 and 0·70 respectively.

In general, specific compounds formed on heating as a result of lysine binding are difficult to identify using chromatography because the bonds are destroyed on acid hydrolysis before analysis. Increases in the lysine content of the heated field peas subjected

Table 9. *Expt 1. The faecal digestibility of nitrogen (N), dry matter and amino acids in raw field peas* (Pisum sativum *cultivar Dundale*) *and field peas heated for 15 min at 110°, 135°, 150° or 165°, as assessed by partial collection procedures in pigs*†

(Mean values for eight pigs (raw) and four pigs (110°, 135°, 150°, 165°) per dietary group)

	Heat treatments					Statistics				
	Raw	110°	135°	150°	165°	Diet	Raw *v.* 110°	Linear‡	SEM (Raw)	SEM (Heat)§
N	0·79	0·81	0·78	0·75	0·55	***	NS	***	0·012	0·021
Dry matter	0·93	0·93	0·92	0·92	0·88	***	NS	***	0·002	0·004
Aspartic acid	0·86	0·87	0·84	0·82	0·67	***	NS	***	0·008	0·014
Threonine	0·73	0·75	0·73	0·71	0·52	***	NS	***	0·010	0·017
Serine	0·75	0·78	0·76	0·74	0·56	***	NS	***	0·007	0·012
Glutamic acid	0·85	0·87	0·85	0·83	0·67	***	NS	***	0·007	0·013
Proline	0·73	0·75	0·76	0·74	0·57	***	NS	***	0·006	0·011
Glycine	0·73	0·76	0·74	0·71	0·54	***	NS	***	0·009	0·017
Alanine	0·70	0·74	0·71	0·69	0·50	***	NS	***	0·011	0·020
Valine	0·76	0·79	0·76	0·75	0·62	***	NS	***	0·009	0·016
Isoleucine	0·77	0·79	0·76	0·75	0·61	***	NS	***	0·010	0·017
Leucine	0·78	0·80	0·78	0·77	0·63	***	NS	***	0·007	0·012
Tyrosine	0·74	0·76	0·73	0·71	0·53	***	NS	***	0·009	0·015
Phenylalanine	0·79	0·81	0·79	0·78	0·62	***	NS	***	0·007	0·013
Lysine	0·81	0·83	0·80	0·74	0·46	***	NS	***	0·009	0·015
Histidine	0·83	0·85	0·82	0·80	0·64	***	NS	***	0·006	0·011
Arginine	0·86	0·90	0·88	0·85	0·73	***	NS	***	0·006	0·010

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see Table 1 and pp. 223–226.
‡ Analysis within heat treatments (110°, 135°, 150°, 165°) only.
§ SEM of heat treatments (110°, 135°, 150°, 165°) only.

to 48 or 72 h hydrolysis, however, indicate that not all of these bonds may be broken with standard 24 h hydrolysis, hence contributing to the reduction in total lysine content.

The effect of heat on the ileal digestibility of amino acids

The application of heat to a protein concentrate affects the ileal digestibility of its amino acid components to varying degrees. It can be concluded that for those amino acids that show a significant decrease in digestibility with the application of heat, particularly lysine, the heat has modified the protein structure in such a way that the enzymic attack necessarily associated with the digestion of that amino acid is hindered (Varnish & Carpenter, 1975*b*). Similarly, the fact that the ileal digestibility of some amino acids is significantly increased by the application of heat at 110° indicates that this treatment predisposes the amino acids to enzymic attack. The current findings are in contrast with those of Wiseman *et al.* (1991), who showed a significant decrease in the digestibility of all essential amino acids when fish meal was heated to 160°. This difference may be due to the fact that these estimates were based on diets containing pre-processed fish meal. The heat applied to the fish meal before the application of the experimental heat treatments is unknown. The relative proportions of carbohydrate, protein and fibre in a particular protein concentrate may also influence the susceptibility of amino acids to heat damage, making it difficult to draw conclusions on the specific effects of heat on a combination of amino acids within a protein concentrate.

The ileal digestibility of lysine in the raw field peas as assessed by the cannulation (0·75) and direct ileal sampling (0·76) procedures is low compared with previous estimates

Table 10. *Expt 3. The faecal digestibility of nitrogen (N), dry matter and amino acids in raw field peas* (Pisum sativum *cultivar Dundale*) *and field peas heated for 15 min at 110°, 135°, 150° or 165°, as assessed by total collection procedures in pigs*†

(Mean values for four pigs per dietary group)

	Heat treatments					Statistics				
	Raw	110°	135°	150°	165°	Diet	Raw *v.* 110°	Linear‡	SEM (Raw)	SEM (Heat)§
N	0·80	0·75	0·74	0·71	0·53	***	NS	***	0·014	0·024
Dry matter	0·93	0·92	0·92	0·91	0·87	***	*	***	0·004	0·007
Aspartic acid	0·86	0·85	0·81	0·78	0·66	***	NS	***	0·015	0·025
Threonine	0·76	0·72	0·69	0·67	0·53	**	NS	**	0·022	0·038
Serine	0·78	0·74	0·73	0·71	0·58	**	NS	*	0·022	0·039
Glutamic acid	0·86	0·85	0·83	0·80	0·67	***	NS	***	0·015	0·025
Proline	0·63	0·63	0·63	0·61	0·56	NS	NS	NS	0·019	0·033
Glycine	0·72	0·70	0·68	0·65	0·53	**	NS	*	0·022	0·038
Alanine	0·70	0·67	0·63	0·61	0·50	*	NS	*	0·026	0·045
Valine	0·78	0·76	0·72	0·71	0·62	*	NS	*	0·022	0·039
Isoleucine	0·78	0·76	0·72	0·70	0·61	*	NS	*	0·022	0·038
Leucine	0·79	0·77	0·74	0·72	0·62	**	NS	*	0·020	0·035
Tyrosine	0·76	0·73	0·69	0·66	0·53	**	NS	*	0·024	0·041
Phenylalanine	0·80	0·79	0·76	0·73	0·62	**	NS	**	0·019	0·033
Lysine	0·82	0·79	0·75	0·70	0·45	***	NS	***	0·022	0·038
Histidine	0·85	0·83	0·80	0·78	0·64	***	NS	**	0·017	0·029
Arginine	0·87	0·87	0·85	0·82	0·70	***	NS	***	0·013	0·023

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see Table 1 and pp. 223–226.
‡ Analysis within heat treatments (110°, 135°, 150°, 165°) only.
§ SEM of heat treatments (110°, 135°, 150°, 165°) only.

(Standing Committee on Agriculture, 1987). The diets used to determine the ileal digestibility of amino acids contained only 400 g raw field peas/kg (in order to provide approximately 0·36 g ileal-digestible lysine/MJ DE, and based on recommended maximum inclusion levels of raw peas in grower pig diets by the Standing Committee on Agriculture, 1987) and hence the dietary protein level was 80 g/kg. Apparent protein digestibility may be markedly influenced by the protein level in the diet (Eggum, 1973; de Lange *et al.* 1990) and therefore apparent protein digestibilities of feedstuffs should only be compared under standardized conditions. Dietary protein contents of at least 150 to 160 g/kg will minimize increases in apparent digestibility with increasing protein intake (Sauer *et al.* 1989).

The effect of heat on the faecal digestibility of amino acids

Faecal digestibility estimates for amino acids in the raw field peas are all higher than the ileal digestibility estimates. This is probably due to further digestion of amino acids by the microbial flora of the hind-gut (Sauer & Ozimek, 1986). Unlike the ileal digestibility values, all faecal digestibilities, except proline determined using total collection procedures, showed a significant linear decrease with increasing heat application. This difference can be attributed to the heat rendering the N and amino acids less susceptible to microbial attack in the hind-gut in the same way as heat can render proteins undegradable in ruminant feeding regimes (Chalupa, 1975; Broderick & Craig, 1980).

Faecal digestibility values for tyrosine and lysine were significantly lower than ileal digestibility with the 165° treatment, suggesting a net synthesis of these amino acids by the

Table 11. *Expts 1, 2 and 3. Comparison of the differences (proportion of total; faecal minus ileal) between ileal and faecal digestibilities of amino acids from raw and heat-treated field peas* (Pisum sativum *cultivar Dundale) determined by ileal cannulation and partial faeces collection respectively in pigs*†

	Heat treatment					Statistics‡							
	Raw	110°	135°	150°	165°	Diet	Raw	110°	135°	150°	165°	SEM (Raw)	SEM (Heat)§
Aspartic acid	0·18	0·15	0·18	0·14	0·09	NS	***	***	***	***	*	0·017	0·029
Threonine	0·30	0·22	0·24	0·13	0·00	**	***	***	***	*	NS	0·028	0·050
Serine	0·21	0·16	0·18	0·09	0·00	**	***	***	***	*	NS	0·020	0·035
Glutamic acid	0·10	0·07	0·11	0·08	0·02	NS	***	*	***	**	NS	0·014	0·025
Proline	0·39	0·31	0·39	0·29	0·13	*	***	***	***	***	NS	0·034	0·060
Glycine	0·34	0·27	0·31	0·20	0·09	*	***	***	***	**	NS	0·033	0·057
Alanine	0·19	0·12	0·15	0·05	−0·08	***	***	**	**	NS	NS	0·022	0·040
Valine	0·19	0·14	0·16	0·07	−0·01	**	***	**	***	NS	NS	0·021	0·036
Isoleucine	0·14	0·09	0·11	0·03	−0·04	**	***	*	**	NS	NS	0·020	0·034
Leucine	0·23	0·15	0·18	0·08	0·01	***	***	**	***	NS	NS	0·022	0·038
Tyrosine	0·15	0·11	0·13	0·04	−0·10	***	***	**	**	NS	**	0·018	0·031
Phenylalanine	0·11	0·07	0·10	0·02	−0·05	***	***	**	**	NS	NS	0·016	0·027
Lysine	0·07	0·04	0·06	0·00	−0·09	***	***	*	**	NS	***	0·011	0·019
Histidine	0·14	0·10	0·12	0·02	−0·04	***	***	**	**	NS	NS	0·016	0·027
Arginine	0·07	0·04	0·06	0·02	−0·02	**	***	*	**	NS	NS	0·010	0·017

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see Table 1 and pp. 223–226.
‡ Analysis for significant difference between ileal and faecal digestibility.
§ SEM for heat treatments (110°, 135°, 150°, 165°) only.

Table 12. *Expts 1, 2 and 3. The digestible energy (DE; MJ/kg dry matter), of raw field peas* (Pisum sativum *cultivar Dundale) and peas heated for 15 min at 110°, 135°, 150° or 165°, and dry-matter digestibility (DMD) and energy digestibility (ED) of diets containing these peas as assessed by total, partial or rectal collection of faeces in pigs*†

	Heat treatments					Statistics				
	Raw	110°	135°	150°	165°	Diet	Raw *v.* 110°	Linear‡	SEM (Raw)	SEM (Heat)§
Field-pea DE										
Total (T)	16·37[a]	14·85[a]	15·24[a]	10·04[a]	13·21[a]	***	***	**	0·216	0·306
Partial (P)	15·78[a]	15·59[a]	15·81[a]	15·39[a]	12·73[a]	***	NS	***	0·200	0·283
Rectal	14·79[b]	14·10[b]	13·75[b]	13·84[b]	12·23[a]	***	NS	**	0·248	0·351
SEM: T+P	0·256	0·362	0·362	0·362	0·362					
Rectal	0·209	0·295	0·295	0·295	0·295					
Diet DMD										
Total (T)	0·93[a]	0·92[a]	0·92[a]	0·91[a]	0·87[a]	***	*	***	0·0045	0·0064
Partial (P)	0·93[a]	0·93[a]	0·93[a]	0·92[a]	0·87[a]	***	NS	***	0·0035	0·0050
Rectal	0·90[b]	0·90[b]	0·88[b]	0·88[b]	0·86[a]	**	NS	**	0·0061	0·0087
SEM: T+P	0·006	0·008	0·008	0·008	0·008					
Rectal	0·005	0·007	0·007	0·007	0·007					
Diet ED										
Total (T)	0·94[a]	0·92[a]	0·92[a]	0·91[a]	0·86[a]	***	*	***	0·0045	0·0064
Partial (P)	0·93[a]	0·94[a]	0·94[a]	0·93[a]	0·86[a]	***	NS	***	0·0043	0·0061
Rectal	0·91[b]	0·90[b]	0·89[b]	0·89[b]	0·85[a]	***	NS	***	0·0059	0·0083
SEM: T+P	0·006	0·008	0·008	0·008	0·008					
Rectal	0·005	0·007	0·007	0·007	0·007					

NS, not significant.

[a,b] Within a measurement (DE, diet DMD, diet ED), means within a column for different collection methods bearing unlike superscript letters were significantly different ($P < 0·01$).

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details of diets and procedures, see Table 1 and pp. 223–226.

‡ Analysis within heat treatments (110°, 135°, 150°, 165°) only.

§ SEM for heat treatments (110°, 135°, 150°, 165°) only.

micro-organisms in the hind-gut. Net bacterial synthesis of amino acids in the hind-gut, including cystine, methionine, tyrosine, lysine and arginine have all been shown previously to be sufficiently high that faecal digestibility values underestimate their absorption (Holmes *et al.* 1974; Zebrowska & Buraczewski, 1977; Mason, 1984; Sauer & Ozimek, 1986). The decrease in the difference between ileal and faecal digestibilities with increasing heat application may also be due to an increase in the proportion of fermentable carbohydrate in the field peas with heating. The peas subjected to the 165° heat treatment showed a substantial increase in the proportion of crude fibre and neutral-detergent fibre. Dietary fibre acts as a predictor of nutrient supply to the caecum–colon, which in turn influences the microbial activity, and thereby the relationship between degradation and synthesis of amino acids (Jorgensen *et al.* 1985). That is, as the proportion of fermentable carbohydrate reaching the large intestine increases the net disappearance of N and amino acids as a result of microbial degradation decreases. Hence the faecal digestibility estimate is closer to the ileal digestibility value (Sauer & Ozimek, 1986).

The decrease in the difference between ileal digestibility and faecal digestibility with increasing heat treatment is consistent with the results of Tanksley *et al.* (1981). In this study the heat treatment associated with commercial processing of direct solvent and screw-press cottonseed meals resulted in a net increase in the appearance of alanine, isoleucine, leucine, tyrosine, phenylalanine, and lysine in the hind-gut.

Comparison of techniques used to determine ileal and faecal digestibilities

The direct ileal sampling technique and ileal cannulation procedure revealed highly comparable trends for the effect of heat on amino acid digestibility. Direct comparison of the techniques amino acid by amino acid, however, revealed some significant method differences. These differences were greatest for measurements made with diets containing raw peas or peas heated to 110° or 135°.

It became evident that the amount of stress imparted on the animal at the time of surgery, surgical proficiency, diet composition, and feeding regimen, largely influenced the success of the direct ileal sampling technique. Highly digestible diets, such as those containing the raw field peas, given once daily, allowed only a small amount of digesta to be collected when the time between feeding and surgical removal of the ileum exceeded 3·5 h. Frequent feeding up to eight times daily, as suggested by Moughan & Smith (1987), should provide a constant flow of digesta through the digestive tract and may minimize this problem and contribute to a reduction in experimental variability. The fact that the digestibility values determined using direct ileal sampling were generally higher than those determined using ileal cannulation indicates that sampling up to 1·5 m anterior to the ileo-caecal junction in the direct ileal sampling procedure has minimal effect on digestibility determinations.

The fact that some differences were found between amino acids should not discount the direct ileal sampling procedure as an alternative to cannulation. Alteration of the technique as suggested above may improve its accuracy. In addition, the ileal dissection technique is less labour intensive and may be preferred on animal welfare grounds, further promoting its development.

Partial collection of faeces for the determination of amino acid digestibility is a viable alternative to total faeces collection. The lower variability associated with the partial collection technique may be due to the pigs in this experiment consuming their entire daily ration. Feed refusal was substantial in Expt 3 due to diets appearing to be less palatable to the 20 kg pigs. These results are consistent with those of Just *et al.* (1985) who concluded that faecal digestibility estimates using Cr_2O_3 as a marker were as accurate as quantitative collection. The present results suggest that a 2 d partial collection is suitable, in contrast to

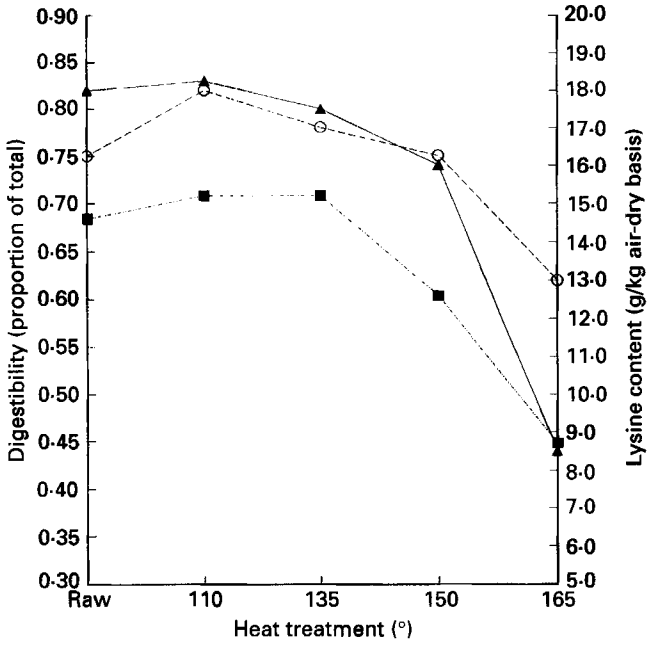

Fig. 1. Expts 1, 2 and 3. The relationship between the ileal (○---○; combined results from cannulation and direct ileal sampling) and faecal (▲——▲; combined results from partial and total faeces collection) digestibility of lysine in raw and heat-treated field peas (*Pisum sativum* cultivar Dundale) fed to growing pigs and the effect of heat on the total lysine (■-·-·-■) content of field peas. For details of diets and procedures, see Table 1 and pp. 223–226.

Moughan *et al.* (1991) who found it necessary to collect daily grab samples for at least five consecutive days to form a suitable composite grab sample. The difference may be due to a larger proportion of the faeces voided over the 2 d collection period being collected in the current trial.

As there were no significant technique differences for the determination of ileal digestible lysine using cannulation or direct ileal sampling and faecal digestible lysine determined using partial or total faeces collection respectively, these results have been combined to produce best estimates for use in subsequent research into the effects of heat on lysine availability and utilization (Fig. 1).

The effect of heat on the digestible energy of raw and heat-treated field peas

The significant linear reduction in DE with the application of heat, revealed by all techniques, is consistent with a reduction in dry matter digestibility and energy digestibility. Heating inevitably induces pyrolysis and Maillard reactions within the peas, reducing the susceptibility of energy sources to digestion.

Comparison of techniques used for the determination of DE

As with the faecal digestibility of amino acids, the partial collection technique is equivalent to total faeces collection for the determination of DE, diet dry-matter digestibility, and diet energy digestibility. The rectal collection technique produced consistently lower results (DE for raw peas (MJ/kg dry matter): total, 16·37; partial, 15·78; rectal, 14·79), however, these display closer agreement with previous estimates for raw field peas. Taverner & Curic (1983) determined the DE and energy digestibility of raw field peas to be 14·4 MJ/kg dry

matter and 0·872 respectively. Despite this, the results obtained from the total and partial collections of faeces are probably a more accurate representation of the DE values in these field peas, due to the fact that they utilize a larger and more representative faecal sample.

Conclusions

The application of heat to field peas resulted in marked increases in dry matter and fibre contents while significantly depressing the content of light petroleum extract. Heat application resulted in a decrease in the lysine, arginine and cystine contents of the field peas. This is likely to be due mainly to early and advanced Maillard reactions. Considerable binding of the remaining lysine in field peas heated to 150° or 165° was also evident, as indicated by a decline in the level of Silcock-reactive lysine.

The results indicate that the direct ileal sampling technique is a viable alternative to the cannulation procedure although some method adjustments, such as the incorporation of a frequent-feeding regimen, are needed to reduce the variability associated with the smaller ileal samples collected with direct ileal sampling. The estimation of faecal digestibility using indigestible markers and partial faeces collection is as efficient as total faeces collection. A limitation of the direct ileal sampling technique is that simultaneous rectal collections of faeces do not allow accurate DE determinations. Partial faeces collection is highly desirable because accurate DE determinations can be completed simultaneously with ileal digestibility determinations using ileal cannulation.

In general, ileal digestibility of amino acids showed little response to heating. However, any changes that were observed were greatest for lysine. In contrast, the faecal digestibility of amino acids was greatly reduced with increasing heat application. This response, however, appeared to be due largely to the effect of heating on the microbial degradation and synthesis of amino acids in the hind-gut, rather than being a reflection of changes within the protein induced by heating. This variable response makes faecal digestibility an unreliable estimator of ileal amino acid digestibility.

There is a need now to determine the utilization of ileal-digestible lysine from heat-treated field peas, to assess how well ileal digestibility estimates reflect lysine availability and utilization. There is a subsequent need to assess direct measurements of lysine availability in heated field peas as indicators of lysine utilization.

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