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The effect of heat on amino acids for growing pigs

3. The availability of lysine from heat-treated field peas (*Pisum sativum* cultivar Dundale) determined using the slope–ratio assay

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The effect of heat on the availability of lysine in field peas (*Pisum sativum* cultivar Dundale) was determined using the slope–ratio assay with growing pigs. The field peas were heated to 110°, 135°, 150°, or 165° for 15 min using a forced-air dehydrator. Lysine availability was significantly depressed ($P < 0{\cdot}05$) with the application of heat, even at mild temperatures of 110°. Lysine availability values of 0·96, 0·71, 0·77, 0·56, and 0·47 were determined for the raw peas and peas heated to 110°, 135°, 150° or 165° respectively. The effect of dietary protein level on the ileal digestibility of lysine in raw field peas was determined in a second experiment to estimate true ileal digestibility. Five diets were formulated to contain 85, 105, 125, 145 and 165 g protein/kg. Increasing dietary protein from 85 to 105 g/kg resulted in a significant increase in the estimate of apparent ileal digestibility from 0·84 to 0·93. Comparisons were then able to be drawn between the ileal digestibility, availability and utilization of lysine from raw and heat-treated field peas. The results indicate that unlike ileal digestibility values, estimates of lysine availability are sensitive to heat treatment and are a close reflection of lysine utilization in heated protein concentrates. Thus, lysine availability values would be more suitable for use in diet formulations than ileal digestibility estimates when dealing with heat-processed protein concentrates.

Heat: Lysine availability: Field peas: Pigs.

Amino acid availability may be defined as that proportion of an amino acid in a feed actually absorbed from the gastrointestinal tract in a form suitable for utilization (e.g. protein synthesis, metabolism) by the animal (Batterham, 1980; Sauer & Ozimek, 1986). Numerous techniques have been evaluated as a means of estimating amino acid availability. By definition, however, availability can only be measured by determining the animal's utilization of a test amino acid when it is given below the animal's maximum requirement for that amino acid. This criterion is met by applying the slope–ratio analysis (Finney, 1964) to a growth assay where the treatments are designed to measure the slopes of response to a test amino acid relative to the slope of response to a standard. Using this assay, Batterham (1992) found that lysine availability ranged from 0·30 to 0·95 in

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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.

Heated proteins have been shown to have a low amino acid availability (Hurrell & Carpenter, 1974, 1975; Varnish & Carpenter, 1975; Hurrell *et al.* 1976; Batterham *et al.* 1986*b*, 1990). The extent of reduction in amino acid availability with heating, however, varies greatly with the technique used to estimate amino acid availability. As a consequence, based on current literature, it is difficult to quantify the effects of heating proteins on amino acid availability.

The overall objective of this series of studies was to define the relationships between the application of heat to protein concentrates and total amino acids, the ileal and faecal digestibilities of amino acids, lysine availability and lysine utilization. In the first paper, van Barneveld *et al.* (1994*a*) reported that the application of graded levels of heat to field peas (*Pisum sativum* cultivar Dundale) had little effect on the apparent ileal digestibility of amino acids. The second paper (van Barneveld *et al.* 1994*b*) reported that heat applied to protein concentrates, even at mild temperatures, renders lysine in a form that is apparently absorbed but inefficiently utilized by the growing pig.

The objectives of the work reported in the present paper were to determine (1) the effect of heat on the availability of lysine in field peas (*Pisum sativum* cultivar Dundale) using the slope–ratio assay and (2) the effect of dietary protein level on the apparent ileal digestibility of lysine in the raw field peas. This was necessary as the previous estimates (van Barneveld *et al.* 1994*a*) are likely to have underestimated significantly the true ileal digestibility of lysine, due to a low protein level (80 g/kg) in the experimental diets. The effect of dietary protein level on the apparent ileal digestibility of the heated peas was also determined by estimating the true digestibility of lysine using *in vitro* techniques and equations based on endogenous protein and neutral-detergent fibre (Taverner *et al.* 1981; de Lange *et al.* 1989, 1990; Boisen & Fernandez, 1991).

EXPERIMENTAL

Protein sources and heat-processed meal

Wheat and wheat gluten (Table 1) were used to supply the basal level of amino acids in the experimental diets. Field peas were used as the test protein concentrate (Table 1) and were subjected to graded levels of heat at 110°, 135°, 150° or 165° for 15 min as described by van Barneveld *et al.* (1994*a*). The apparent ileal digestibility and utilization of ileal-digestible lysine and digestible energy from the raw and heat-treated field peas were determined previously (van Barneveld *et al.* 1994*a*, *b*).

Expt 1. The effect of heat on lysine availability

The aim of this experiment was to determine the effect of heat on the availability of lysine in field peas using the slope–ratio assay (Finney, 1964). To apply the slope–ratio assay, experimental diets were formulated to contain graded levels of standard (free) lysine and graded levels of test lysine (that is, lysine from raw and heat-treated field peas). The dose levels of lysine from the test proteins were formulated to provide the same total lysine as that from the standard lysine doses, so that the estimates of lysine in the test proteins were an expression of lysine availability. Linear regression coefficients of response (for example, food conversion efficiency on an empty-body basis) to increasing dose level of standard lysine and lysine from the test proteins were calculated. A number of criteria were then tested with the slope–ratio statistical analysis in an attempt to ensure that the responses were due to the test amino acid and not other dietary factors. If these criteria were met for

Table 1. *Expts 1 and 2. Composition (g/kg, air-dry basis) of the wheat, wheat gluten, 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*

Component	Wheat	Wheat gluten	Field pea heat treatments				
			Raw	110°	135°	150°	165°
Crude protein (N × 6·25)	131	738	210	224	221	227	216
Dry matter	921	923	913	938	952	961	977
Light petroleum (b.p. 40–60°) extract	20	31	21	21	22	15	16
Fibre							
Crude	30	67	67	63	65	89	104
Neutral-detergent	ND	81	82	67	185	467	483
Ash	11	25	25	28	27	26	28
Gross energy (MJ/kg)	16·7	22·2	16·9	16·8	17·3	17·6	17·8
Amino acids							
Asp	6·2	30·8	22·1	24·5	24·9	24·8	24·7
Thr	3·7	21·6	7·7	8·3	8·5	8·7	8·2
Ser	6·2	40·7	10·1	11·1	11·3	11·4	10·5
Glu	35·7	238·7	32·6	37·4	37·7	38·1	38·6
Pro	13·3	96·7	8·5	9·1	9·2	9·9	9·8
Gly	5·2	27·4	8·6	9·6	9·7	9·8	9·8
Ala	4·4	21·2	8·4	9·5	9·6	9·8	9·8
Cys	1·8	14·8	1·3	1·7	1·6	1·3	1·2
Val	5·6	32·4	9·4	10·5	10·6	10·8	11·0
Met	1·7	10·8	1·5	1·4	1·4	1·7	1·1
Ile	4·6	30·1	8·6	9·5	9·5	9·7	9·9
Leu	8·3	53·3	13·8	15·4	15·5	15·8	15·8
Tyr	3·7	25·0	6·4	6·9	7·0	7·2	6·9
Phe	5·7	37·4	9·2	10·3	10·3	10·6	10·2
Lys	3·5	15·9	14·3	15·6	15·4	12·8	8·7
His	2·8	15·6	4·5	5·1	5·1	5·2	4·9
Arg	5·6	29·6	15·2	19·1	19·3	18·2	15·0

ND, not determined.

the response, an estimate of lysine availability in the test protein was determined by calculating the linear regression coefficient of lysine in test protein:linear regression coefficient of standard lysine ratio.

Diets. The availability of lysine in the five field-pea treatments was assessed in one experiment. This involved the use of twenty-five diets (Fig. 1): the basal diet (blank), four diets to determine the pig's response to standard lysine, and twenty diets for the five field pea treatments (four/treatment). The wheat used in the basal diet (Fig. 1) was of medium protein content and, in combination with wheat gluten, supplied adequate quantities of all amino acids except threonine and lysine, which were supplemented with free amino acids. L-Lysine was added to bring the basal level up to 5·5 g/kg. The four levels of lysine used to determine the pig's response to standard lysine were in increments of 625 mg L-lysine/kg and were obtained by the addition of L-lysine monohydrochloride (anhydrous, 98% pure) to the basal diet (diets 2–5, Fig. 1). Other essential amino acids were added at the expense of wheat starch to ensure a 0·15 surplus relative to lysine, and hence ensure that lysine was limiting in the diets. The five field pea treatments were incorporated into diets to provide the same four levels of total lysine as the diets containing standard lysine, at the expense of wheat starch (diets 6–25, Fig. 1). When providing a 0·15 surplus of other essential amino acids relative to lysine in these diets, estimates of availability for the essential amino acids

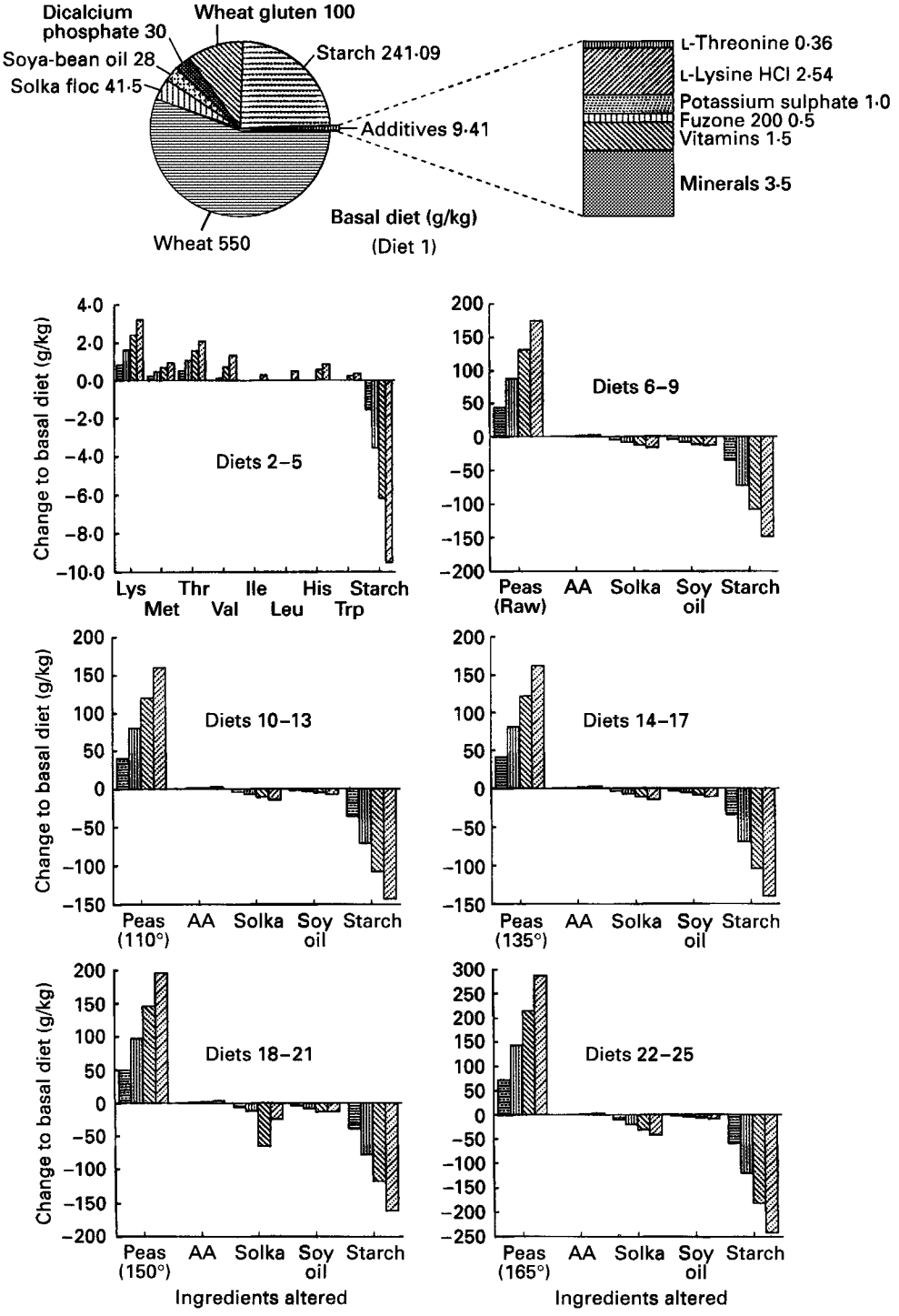

Fig. 1. Expt 1. Composition of the basal diet (g/kg, air-dry basis) and subsequent alterations to provide diets containing total lysine levels of 6·125 ▤, 6·750 ▥, 7·375 ▧, and 8·000 ▨, a constant digestible energy of 14·4 MJ/kg and a crude fibre content of 29·9 g/kg. Peas, field peas (*Pisum sativum* cultivar Dundale); AA, amino acids; Solka, Solka floc (cellulose); soy oil, soya-bean oil.

Table 2. *Expt 2. Components and composition (g/kg, air-dry basis) of diets*

Diet...	1	2	3	4	5
Components					
Field peas (*Pisum sativum* cultivar Dundale) (raw)	405	500	595	690	785
Minerals and vitamins*	5	5	5	5	5
Dicalcium phosphate	30	30	30	30	30
Chromic oxide	2	2	2	2	2
Soya-bean oil	19	25	31	37	43
Sucrose	539	438	337	236	135
Composition					
Crude protein (g/kg)	85	105	125	145	165
Digestible energy (DE) (MJ/kg)	15·5	15·5	15·5	15·5	15·5
Total lysine:DE (g/MJ)	0·37	0·46	0·55	0·64	0·72

* 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 equivalents 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), pteroylmonoglutamic acid 2 mg, choline 500 mg, ascorbic acid 10 mg, biotin 0·1 mg.

in the field-pea treatments were made. Van Barneveld *et al.* (1994*b*) reported that the application of heat to field peas significantly reduced the utilization of ileal-digestible lysine. Similar studies (Beech *et al.* 1991; E. S. Batterham, unpublished results) have reported a similar phenomenon with threonine, methionine, phenylalanine and tryptophan in heat-processed meals. Based on these studies, factors of 1·00, 0·94, 0·89, 0·65, and 0·42 were applied to the ileal digestibilities of all essential amino acids in the raw, 110°, 135°, 150° and 165° field-pea treatments respectively. This was an attempt to account for the reduction in availability of these amino acids due to the application of heat, hence ensuring that lysine was the first limiting amino acid in the experimental diets. In addition, by attempting to maintain an equal amino acid balance across all diets the exaggerated response to the addition of free amino acid resulting from the correction of an amino acid imbalance, as reported by Sato *et al.* (1987), should be minimized.

The mineral and vitamin premix used in the experimental diets has been described by van Barneveld *et al.* (1994*b*; Table 2). Fuzone 200 (Furazolidone 200 g/kg) was included in all diets to guard against a *Campylobacter* burden that was prevalent in the piggery at the time of experimentation.

Dietary energy was maintained at 14·4 MJ digestible energy (DE)/kg using wheat starch and soya-bean oil as non-protein energy sources. Dietary crude fibre was maintained at 29·9 g/kg using Solka floc (cellulose) as an inert fibre source.

Animals and procedures. The twenty-five diets were arranged in a randomized block design. The pigs were blocked on 7-week weight, sex and position in the experimental facilities. There were five blocks, three containing males, and two containing females, all of the Large White breed. Pigs were penned individually and water supplied *ad lib.* via 'nipple' drinkers.

Experimental diets were introduced when the pigs reached 20 kg live weight. The feed was offered dry and the daily feeding rate adjusted to three times maintenance (3M; $3 \times (0{\cdot}5$ MJ DE/kg body weight$^{0{\cdot}75}$)/diet DE) after weekly weighings of the pigs. The pigs were fed every 3 h with an automatic feeder, to ensure utilization of available dietary amino acids (Batterham & Murison, 1981).

After reaching a minimum weight of 45 kg the pigs were slaughtered by electrical stunning. The blood was collected and the viscera washed to remove undigested material.

The blood and washed viscera were then combined and frozen. The carcasses (with hair) were washed clean with water, weighed, split longitudinally down the middle of the vertebrae, and then stored at −20°. They were then ground, mixed, sampled and freeze-dried before chemical analysis. The mixed blood and washed viscera were processed in a similar manner.

Pig response was assessed in terms of daily live-weight gain; food conversion efficiency (FCE); empty-body weight:final live weight ratio; gain/d and FCE on an empty-body-weight basis; protein, fat and energy content in the empty body; energy deposition:DE intake ratio; protein deposition/d; protein deposition:food intake ratio; and estimated lysine retention/d.

The following factors were used in the calculations previously described: 6·25 to convert N to crude protein (Agricultural Research Council, 1981); 0·925 to convert initial live weight to estimated initial empty-body weight; 7·86 to calculate the energy (MJ/kg) and 139 to calculate the protein (g/kg) in the empty bodies of the pigs at the commencement of the experiment (these factors were determined on five males and five females slaughtered at 20 kg live weight as reported by van Barneveld *et al.* (1994*b*)). Energy stored as protein was calculated as protein (kg) × 24·2 (Jordan & Brown, 1970). Fat content was calculated as (total energy (MJ) − protein energy (MJ))/39·6 (Burlacu *et al.* 1973). Estimated lysine retention was determined using a mean carcass lysine value of 6·5 g/16 g N determined in previous studies at this Institute.

Expt 2. Effect of dietary protein level on the apparent ileal digestibility of lysine in raw field peas

The aims of this experiment were (1) to determine the effect of dietary protein level on the apparent ileal digestibility of lysine in raw field peas and (2) to estimate true lysine digestibility in raw peas and peas heated to 110°, 135°, 150° or 165°.

Diets. Five diets were formulated to contain 85, 105, 125, 145 and 165 g protein/kg respectively (Table 2). Diets were sucrose based with raw field peas as the only source of protein. Digestible energy was maintained at 15·5 MJ/kg using soya-bean oil as a non-protein energy source. Cr_2O_3 was included in the diets as an indigestible marker to calculate digestibilities.

Animals and procedures. The five diets were arranged according to a randomized block design. Three pigs (male or female) within a weight range of 35–45 kg were allocated to each diet having been blocked on weight and position in the experimental facilities. Pigs were penned individually with water supplied *ad lib.* via 'nipple' drinkers. The feed was offered dry and the daily feeding rate adjusted to 3M (van Barneveld *et al.* 1994*a*). Pigs were fed every 3 h using automatic frequent feeders. Frequent feeding facilitated a steady flow of digesta through the digestive tract. Experimental diets were introduced over the first 2 d of a 7 d feeding period. On the eighth day the terminal ileum was surgically removed and the contents collected using the direct ileal sampling procedure outlined by van Barneveld *et al.* (1994*a*). The digesta collected was stored at −20° before being freeze-dried, ground and analysed.

Estimation of true lysine digestibility in raw and heated peas. As all diets used by van Barneveld *et al.* (1994*a*) contained protein levels between 80 and 90 g protein/kg it is reasonable to assume that the apparent ileal digestibility of lysine in all treatments was underestimated. Accordingly, an attempt was made to estimate the true (or real) ileal digestibility of lysine in raw field peas and peas heated to 110°, 135°, 150° or 165° using a number of estimation techniques. These included (1) estimation of endogenous protein by the equation: endogenous protein = (0·013 × undigested dry matter − 0·08) × 6·25 (Boisen &

Table 3. *Expt 1. Analysis of variance for slope–ratio analysis*

Source of variation	df
Sex	1
Block (sex)	3
Linearity	
Standard: lysine	1
Test: raw peas	1
Test: 110° peas	1
Test: 135° peas	1
Test: 150° peas	1
Test: 165° peas	1
Blanks	1
Intersection	
Raw–lysine	1
110°–lysine	1
135°–lysine	1
150°–lysine	1
165°–lysine	1
Curvature	
Lysine (quadratic)	1
Raw (quadratic)	1
110° (quadratic)	1
135° (quadratic)	1
150° (quadratic)	1
165° (quadratic)	1
Lysine (cubic)	1
Raw (cubic)	1
110° (cubic)	1
135° (cubic)	1
150° (cubic)	1
165° (cubic)	1
Error	97
Total	125

Fernandez, 1991); (2) *in vitro* estimates (National Institute of Animal Science, Foulum, Denmark); (3) neutral-detergent fibre (NDF) content of the diet using the formula: average output of endogenous amino acids (mg/kg dry-matter intake) = $318{\cdot}8 + 0{\cdot}016 \times$ NDF in diet (mg/kg) (Taverner *et al.* 1981); (4) protein-free estimates of endogenous lysine (de Lange *et al.* 1989); (5) estimates of endogenous lysine using ^{15}N-isotope dilution techniques (de Lange *et al.* 1990).

Statistical analysis

Expt 1. The analysis of variance (Table 3) for the slope–ratio assay (Finney, 1964) was completed in distinct sections. An analysis of variance using a general linear model was first applied to test for linearity, the component for blanks, the component for interception, quadratic curvature and cubic curvature respectively. The 'linearity' term tests whether the four levels of standard lysine give a linear response. The 'blanks' term tests whether the response is curved at low lysine levels. Significance for either of these terms indicates statistical invalidity which corresponds to failure of the model and so requires modifications in the analysis. The 'intersection' term tests whether the regression lines for the lysine in the test protein meet at the basal lysine level while the 'curvature' term tests whether the four levels of lysine from the test proteins give a curvilinear response. Significance for either

of these terms indicates fundamental invalidity, which means that the test lysine is not simply a dilution of the standard and hence cannot be assayed against it.

The regression of the responses *v.* lysine dose level was then performed, yet only valid responses by the above analysis were considered. Availability estimates were determined by calculating linear regression coefficient for lysine in test protein: that for standard lysine. Covariances were also generated to allow estimation of the standard deviation of these estimates (Finney, 1964).

The regressions were then performed for empty-body weight: final live weight and energy retention *v.* lysine dose level for each field pea treatment. These analyses were conducted to determine whether there was any effect of inclusion level of field peas on these variables.

The regressions of daily empty-body gain and estimated lysine retention *v.* daily lysine intake were performed and the results were analysed by the method of Campbell (1966). This analysis was conducted for comparative purposes as many availability studies are conducted under *ad lib.* feeding conditions and it is not appropriate to calculate regression *v.* dose level. Standard deviations were determined according to Finney (1964).

Expt 2. The results were analysed by analysis of variance, utilizing a general linear model, and the treatment means separated by least significant difference (LSD).

Chemical analyses

Expt 1. The techniques used were as reported by van Barneveld *et al.* (1994*b*) with the exception that amino acids were not determined in the fat-extracted carcass or blood and washed viscera.

Expt 2. The techniques used were as reported by van Barneveld *et al.* (1994*a*) with the exception that amino acids in the ileal digesta were separated by reverse-phase chromatography and measured after reaction with phenylisothiocyanate. 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.

RESULTS

Expt 1. The effect of heat on lysine availability

Performance results of the pigs are presented in Figs 2–4. All regression lines for all responses intersected at the basal lysine level indicating fundamental validity of the assay. All responses exhibited significant quadratic curvilinearity ($P < 0{\cdot}001$) to lysine doses supplied from peas heated to 165°, and hence availability estimates for lysine in these peas were calculated but are fundamentally invalid.

The level of inclusion of protein concentrate depressed the empty-body weight ($P < 0{\cdot}05$) of pigs given the diets containing peas heated to 165° (Table 4). The proportion of energy retained in the empty bodies decreased for those pigs given diets containing the field peas heated to 150° or 165° ($P < 0{\cdot}05$; Table 4).

Crude protein content of the empty body (Table 5) increased ($P < 0{\cdot}05$) in pigs given diets containing free lysine or lysine from raw peas or peas heated to 110°, 135°, or 150°. Energy and fat content of the empty body (Table 5) decreased ($P < 0{\cdot}05$) in all pigs with increasing lysine dose level.

Using FCE on an empty-body basis as the criterion of response, lysine availability was depressed with the application of heat to 150° (0·96–0·56; Table 6). Estimates of availability assessed on a protein deposited/d or protein deposited: food intake basis were higher than the estimates based on empty-body data for all treatments, with estimates of availability for the raw peas being greater than 1·0.

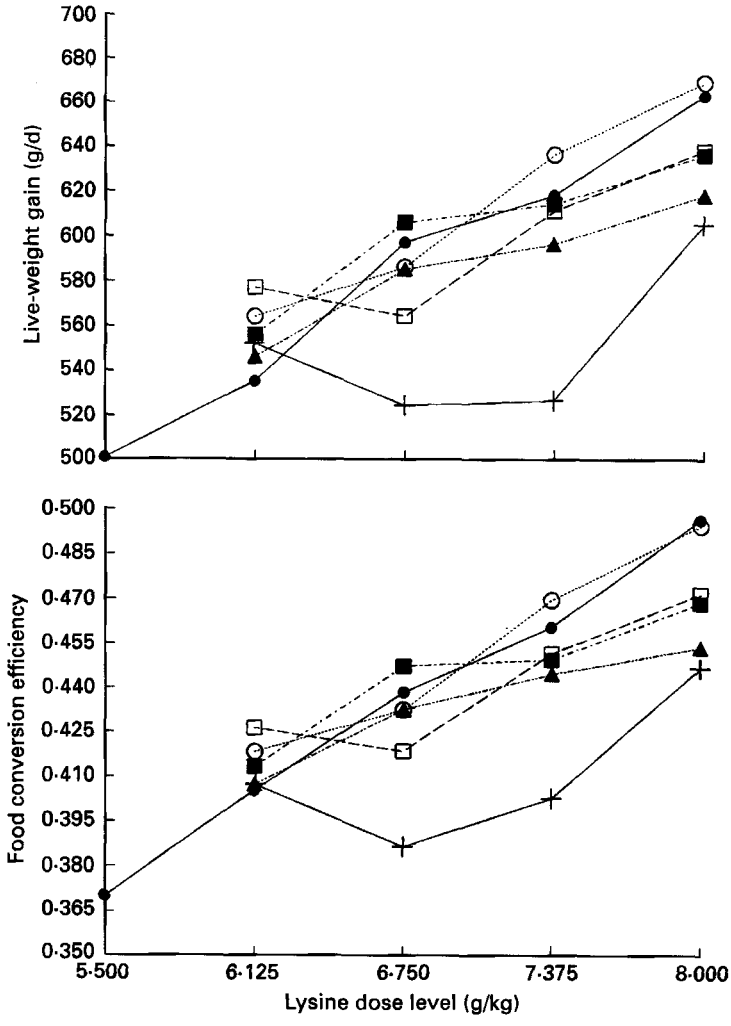

Fig. 2. Expt 1. Live-weight gain (SEM 15·26) and food conversion efficiency (SEM 0·0099) of pigs during the 20–45 kg growth phase when given diets for the slope–ratio assay for free lysine (●——●) and lysine in raw field peas (*Pisum sativum* cultivar Dundale) (○···○) and field peas heated to 110° (□–––□), 135° (■–·–■), 150° (▲–···–▲) or 165° (+——+). Food conversion efficiency = live-weight gain (kg):food intake (kg).

The regression of estimated lysine deposition *v.* lysine intake (Fig. 5) revealed a significant deviation from the point of intersection for the basal lysine level for pigs given diets containing peas heated to 165°. The availability estimate using this response for lysine in peas heated to 165° was therefore invalid. Availability estimates determined using the regression of daily empty-body gain *v.* daily lysine intake (Table 7) exhibited no intersection or curvilinearity for the 165° treatment and gave the only valid estimate of 0·47. The availability estimates for all treatments determined using this technique were generally higher than those determined using the slope–ratio analysis.

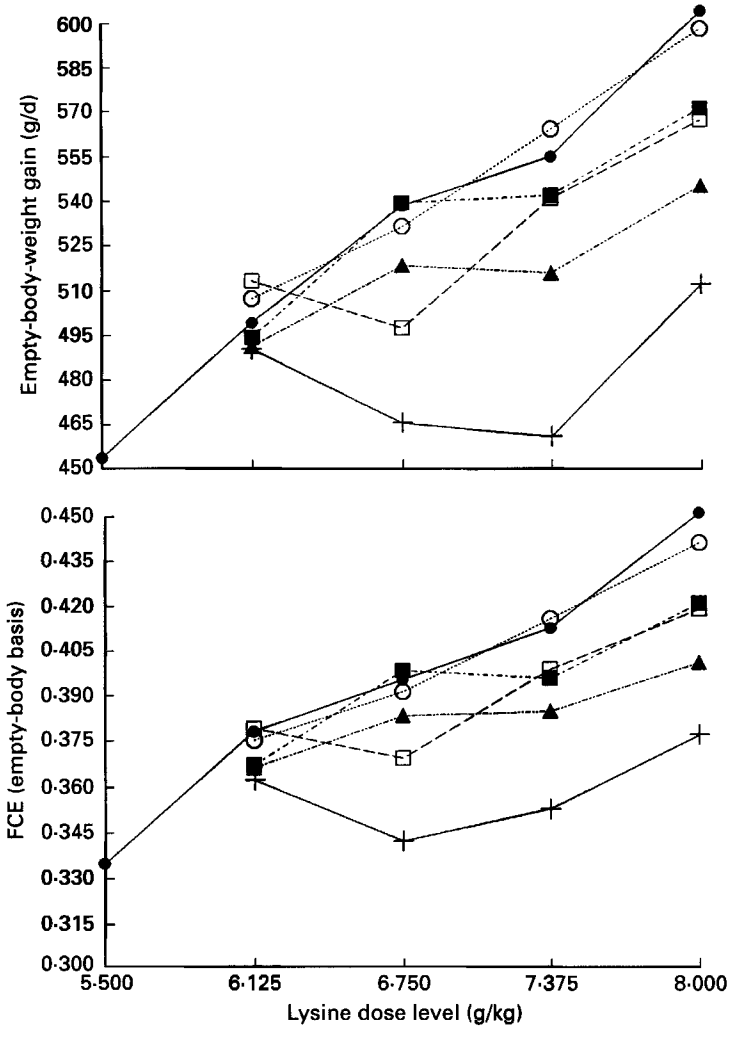

Fig. 3. Expt 1. Daily carcass gain (SEM 12·85) and food conversion efficiency (FCE) on an empty-body basis (SEM 0·0085) of pigs during the 20–45 kg growth phase when given diets for the slope–ratio assay for free lysine (●——●) and lysine in raw field peas (*Pisum sativum* cultivar Dundale) (○···○) and field peas heated to 110° (□---□), 135° (■-·-■), 150° (▲-··-▲) or 165° (+——+). Daily empty-body-weight gain = ((hot carcass weight (kg) + blood (kg) + washed viscera (kg)) − (initial live weight (kg) × 0·925): period (d) on experiment × 1000. FCE (empty-body basis) = (hot carcass weight (kg) + blood (kg) + washed viscera (kg)) − (initial live weight (kg) × 0·925): food intake (kg).

Expt 2. Effect of dietary protein level on the apparent ileal digestibility of lysine in raw field peas

The increase in dietary crude protein level from 85 to 105 g/kg resulted in a significant increase ($P < 0·01$) in the apparent ileal digestibility of lysine in the raw field peas from 0·84 to 0·93 (Fig. 6). There was no significant difference ($P > 0·05$) in the apparent ileal digestibility of lysine using diets containing 105 to 165 g protein/kg, with a mean digestibility of 0·92 over this range.

Depending on technique and heat treatment, estimates of true digestibility (Table 8) were 0·04–0·19 higher than those determined by van Barneveld *et al.* (1994*a*). All estimation

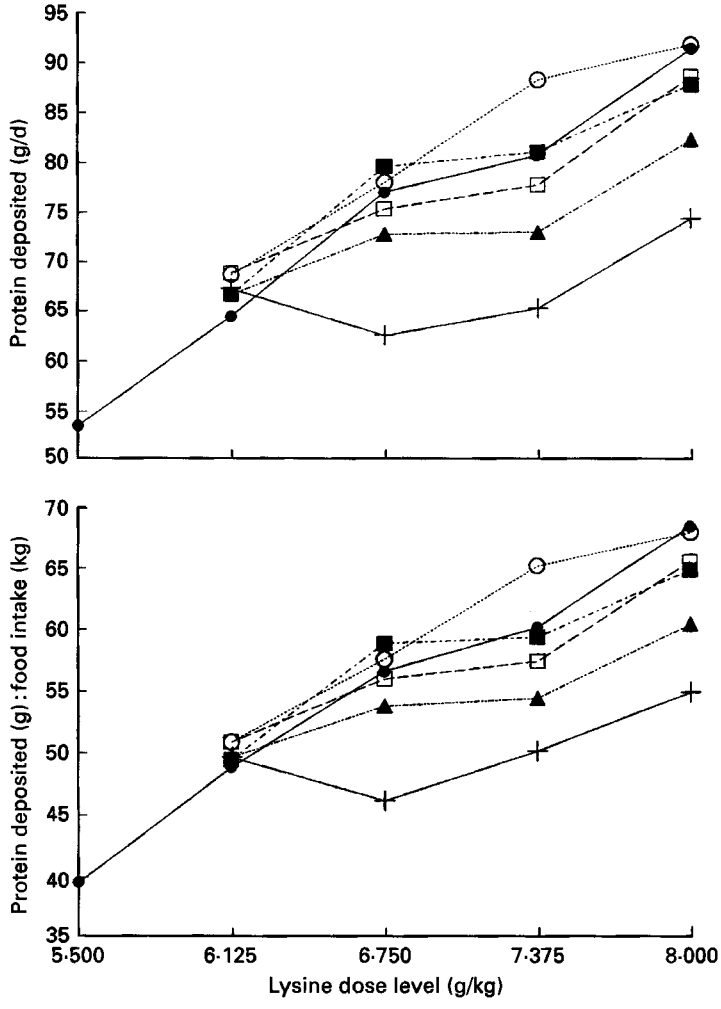

Fig. 4. Expt 1. Daily protein deposited (SEM 2·46) and protein deposited:food intake ratio (SEM 1·72) of pigs during the 20–45 kg growth phase when given diets for the slope–ratio assay for free lysine (●——●) and lysine in raw field peas (*Pisum sativum* cultivar Dundale) (○···○) and field peas heated at 110° (□–––□), 135° (■–·–■), 150° (▲–···–▲) or 165° (+——+). Daily protein deposited = (protein in carcass (g) + protein in blood and washed viscera (g)) − ((initial live weight (g) × 0·925) × 0·139):period (d) on experiment. Protein deposited:food intake ratio = (protein in carcass (g) + protein in blood and washed viscera (g)) − ((initial live weight (g) × 0·925) × 0·139):food intake (kg).

techniques gave similar estimates of true digestibility for the respective treatments. The proportion of the digestibility estimates for heat-treated field peas:raw peas was also constant regardless of estimation technique. The digestibility of lysine in the 110°, 135°, 150° and 165° treatments was 1·05, 1·00, 1·01 and 0·91 times the digestibility of lysine in the raw peas. By applying these factors to the value of 0·92 for lysine digestibility in raw peas determined in the present study, adjusted apparent ileal digestibility values for lysine in heated field peas were obtained (Table 8).

Table 4. *Expt 1. Empty-body weight:final live weight ratio and energy deposited:digestible energy intake ratio by pigs during the 20–45 kg growth phase when given diets containing free lysine or heat-treated field peas* (Pisum sativum *cultivar Dundale*) *for the slope–ratio assay for lysine**

(Mean values for five pigs per dietary group)

Lysine dose level (g/kg)	Form of lysine addition					
	Free lysine	Field pea heat treatment				
		Raw	110°	135°	150°	165°
Empty-body weight:final live weight (kg/kg)†						
5·50	0·915	—	—	—	—	—
6·125	0·929	0·911	0·906	0·905	0·911	0·905
6·75	0·912	0·914	0·902	0·906	0·904	0·904
7·375	0·909	0·903	0·902	0·901	0·892	0·899
8·00	0·916	0·907	0·905	0·909	0·902	0·880
Pooled SEM 0·0064						
Energy deposited:digestible energy intake‡						
5·50	0·435	—	—	—	—	—
6·125	0·461	0·453	0·440	0·427	0·438	0·425
6·75	0·450	0·419	0·403	0·445	0·417	0·405
7·375	0·450	0·423	0·417	0·413	0·408	0·364
8·00	0·452	0·424	0·414	0·399	0·389	0·384
Pooled SEM 0·0124						

* For details of diets and procedures, see Table 2 and pp. 258–264.

† Calculated as (hot carcass weight (kg) + blood (kg) + washed viscera (kg)):final live weight (kg).

‡ Calculated as (energy in carcass (MJ) + energy in blood and washed viscera (MJ)) − ((initial live weight (kg) × 0·925) × 7·86):digestible energy intake (MJ).

DISCUSSION

The effect of heat on lysine availability

The application of heat to field peas resulted in a significant decrease in lysine availability determined using the slope–ratio assay. Regardless of the response used, lysine availability in the raw peas decreased by 0·21–0·27 with the application of heat at 110°. Heating field peas to 135° resulted in little change in lysine availability (0·77–0·97) from that observed at 110° (0·71–0·91). However, further decreases were evident when peas were heated to 150° (0·56–0·70) and 165° (0·47). Possible mechanisms responsible for this reduction in available lysine with the application of heat have been discussed previously by van Barneveld *et al.* (1994*a*).

All responses and regression techniques (that is, *v.* lysine dose level or *v.* lysine intake) produced valid estimates for lysine in raw peas and peas heated to 110°, 135°, or 150°. Estimates based on FCE on an empty-body basis appear to be the most appropriate as they had the lowest standard deviation and coefficient of variation, and were the best reflection of lysine utilization determined by van Barneveld *et al.* (1994*b*). The availability estimate for lysine in the 165° peas (0·47) determined by calculating the regression of daily empty-body gain *v.* lysine intake had the lowest coefficient of variation and was the only estimate that was fundamentally valid. It was, therefore, the only estimate that could be considered for this treatment.

When results were expressed on a protein deposition basis, estimates of availability for all treatments were higher. This is consistent with results reported by Batterham *et al.*

Table 5. *Expt 1. Crude protein, energy and fat contents of the empty body of pigs grown over the 20–45 kg growth phase when given diets containing free lysine or raw or heat-treated field peas* (Pisum sativum *cultivar Dundale*) *for the slope–ratio assay for lysine**

(Mean values for analysis of samples from five pigs per treatment)

Lysine dose level (g/kg)	Form of lysine addition: Free lysine	Field pea heat treatment: Raw	110°	135°	150°	165°
Crude protein (g/kg, empty-body basis)†						
5·50	128	—	—	—	—	—
6·125	134	137	136	137	137	138
6·75	141	143	145	144	140	136
7·375	142	149	141	145	140	141
8·00	146	147	148	147	145	142
Pooled SEM 1·78						
Energy (MJ/kg, empty-body basis)‡						
5·50	13·7	—	—	—	—	—
6·125	13·2	13·0	12·7	12·7	12·9	12·7
6·75	12·5	11·9	12·1	12·3	12·1	12·8
7·375	12·1	11·6	11·8	11·7	11·8	11·6
8·00	11·5	11·2	11·3	11·0	11·1	11·5
Pooled SEM 0·20						
Fat (g/kg, empty-body basis)§						
5·50	269	—	—	—	—	—
6·125	251	245	236	238	243	237
6·75	229	213	216	223	219	240
7·375	219	201	211	208	214	208
8·00	202	192	194	188	192	203
Pooled SEM 5·24						

* For details of diets and procedures, see Table 2 and pp. 258–264.

† Calculated as ((N in carcass (g) + N in blood and washed viscera (g)) × 6·25)/(hot carcass weight + blood and washed viscera (kg)).

‡ Calculated as (energy in carcass (MJ) + energy in blood and washed viscera (MJ))/(hot carcass weight (kg) + blood and washed viscera (kg)).

§ Calculated as (fat in carcass (g) + fat in blood and washed viscera (g))/(hot carcass weight (kg) + blood and washed viscera (kg)).

(1990). Estimates based on protein deposition/d and the protein deposited:food intake ratio generally had higher coefficients of variation (4·8–11·1 and 3·5–7·7 respectively) than FCE on a carcass basis (1·9–8·9). As shown by Campbell (1966), larger coefficients of variation and smaller correlation coefficients result in higher estimates of availability. The same is true for estimates based on daily empty-body gain and regression of estimated lysine retention *v.* lysine intake. As dietary energy was equalized, energy intake was controlled and lysine was limiting, the difference between estimates determined by the regression of response *v.* dose level and lysine intake should not be influenced by these factors.

Invalid estimates result when curvilinearity is significant due to the fact that curvilinearity usually reflects a factor other than lysine dose level influencing response. The curvilinearity exhibited in the responses to lysine doses from peas heated to 165°, however, appears to be due to random effects. These curves exhibit a quadratic increase below the responses to standard lysine, which suggests that an anti-nutritional factor is not present. Batterham

Table 6. *Expt 1. Availability of lysine (proportion of total) in raw and heat-treated field peas* (Pisum sativum *cultivar Dundale*) *as assessed by the slope–ratio analysis*†

(Mean values, standard deviations and coefficients of variation)

Field pea heat treatment	Daily empty-body gain			Empty body gain: food intake			Protein deposited/d			Protein deposited: food intake		
	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
Raw	1·01	0·032	3·168	0·96	0·018	1·875	1·13	0·054	4·778	1·10	0·038	3·455
110°	0·74	0·029	3·919	0·71	0·016	2·253	0·91	0·049	5·385	0·89	0·035	3·932
135°	0·83	0·030	3·614	0·77	0·016	2·078	0·97	0·050	5·154	0·93	0·035	3·763
150°	0·59	0·027	4·576	0·56	0·015	2·679	0·70	0·045	6·429	0·68	0·032	4·706
165°	0·16*	0·027	16·875	0·18*	0·016	8·889	0·38*	0·042	11·05	0·39*	0·030	7·692

* Quadratic curvature significant ($P < 0·05$) in statistical analysis.

† For details of diets and procedures, see Table 2 and pp. 258–264.

et al. (1981, 1986*a*) have also reported invalid availability estimates due to random curvature and intersection in meals of low lysine availability.

The decrease in energy retention in the empty bodies of those pigs fed on diets containing peas heated to 150° or 165° may be due to a greater demand on dietary energy to catabolize excess or non-utilizable amino acids in these diets. Lower energy retention was also observed in pigs fed on diets containing these peas by van Barneveld *et al.* (1994*b*).

The significant decrease in lysine availability with the application of heat at 110° is an important consideration when examining commercial pelleting and processing conditions of protein concentrates. The extent of heat damage will vary depending on the physical composition of the protein concentrate, the heating time, and the pressure and moisture conditions during heating. The current study used peas subjected to dry heat with prolonged time periods required to reach the desired temperatures. Under commercial processing conditions, heating time is often reduced, yet other factors such as pressure and moisture may amplify the effects of heating for short periods. Van Barneveld *et al.* (1994*a*) reported that heating at 110° significantly improved the apparent ileal digestibility of lysine despite a significant drop in lysine utilization (van Barneveld *et al.* 1994*b*). Mild heating of soya-bean meal also improves digestibility (Vandergrift *et al.* 1983; Hancock *et al.* 1990), hence it is reasonable to assume that availability of lysine in soya-bean meal may also be depressed under these conditions. Wright (1981) reported that many levels of moisture and temperature, at given times, will adequately reduce trypsin inhibitor to a safe limit and denature protein to achieve a good digestibility in soya-bean meal. Temperatures of 100° or above were recommended for use in processing. Batterham *et al.* (1979, 1984, 1990) reported a range of lysine availability estimates for soya-bean meal from 0·84–0·98. Part of the variation in these estimates may have been due to variation in processing conditions of the soya-bean meal batches used in these experiments. Hence, despite improving amino acid digestibility and inactivating anti-nutritional factors, current heat processing of protein concentrates may be significantly reducing the availability of amino acids.

Wettstein & Wild (1991) investigated the use of expanders in combination with standard pellet mills, and reported improved pellet quality and digestibility. The expander allowed the processor to attain higher temperatures (up to 140°) and pressures. Results from the current study indicate that this practice may increase the possibility of reduced amino acid availability.

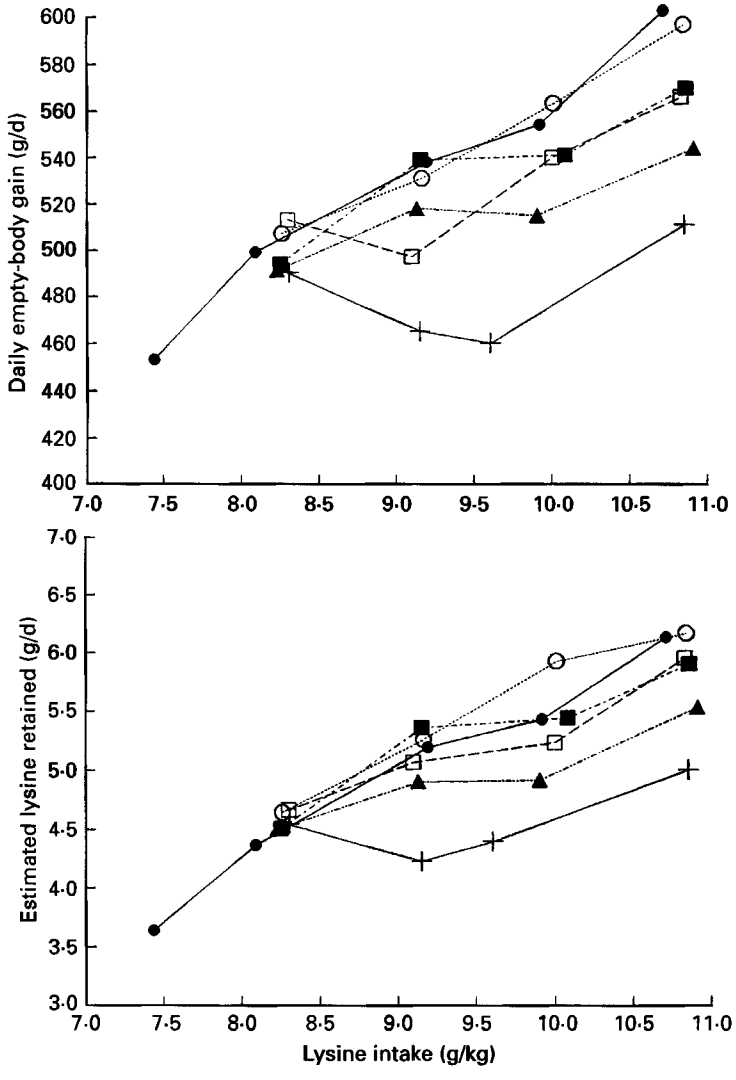

Fig. 5. Expt 1. Daily carcass gain (SEM 12·85) and estimated daily lysine deposition (SEM 0·156) of pigs during the 20–45 kg growth phase when given diets for the slope–ratio assay for free lysine (●——●) and lysine in raw field peas (*Pisum sativum* cultivar Dundale) (○···○) and field peas heated to 110° (□–––□), 135° (■–·–■), 150° (▲–··–▲) or 165° (+——+). Estimated daily lysine deposition = ((protein in carcass (g) + protein in blood and washed viscera (g) × 6·5) − (initial live weight (g) × 0·925) × 0·139) × 6·2): period (d) on experiment.

Effect of dietary protein on the apparent ileal digestibility of lysine

The increase in the apparent ileal digestibility of lysine in raw field peas with increasing dietary protein levels confirms that the previous estimate (0·75) determined by van Barneveld *et al.* (1994*a*) significantly underestimated true digestibility. This is consistent with Eggum (1973) and de Lange *et al.* (1990) who showed that apparent protein digestibility can be markedly influenced by protein level in the diet. Low dietary protein levels resulted in a significant difference between apparent and true digestibility.

There was a substantial difference between the lysine digestibility estimates for diets containing 85 g protein/kg in the current study and the lysine digestibility estimates

Table 7. *Expt 1. Availability of lysine (proportion of total) in raw and heat-treated field peas (Pisum sativum cultivar Dundale) as assessed by regression of daily empty-body gain and estimated lysine retention v. lysine intake (g/d)*†

(Mean values, standard deviation and coefficient of variation.

Field pea heat treatment	Daily empty-body gain (g)			Estimated lysine retained (g/d)		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Raw	0·99	0·026	2·626	1·07	0·050	4·673
110°	0·83	0·024	2·892	0·95	0·047	4·947
135°	0·88	0·025	2·841	0·98	0·048	4·898
150°	0·74	0·024	3·243	0·83	0·045	5·422
165°	0·47	0·026	5·532	0·64*	0·046	7·188

* Point of intersection significantly different ($P < 0·05$) from that of the basal diet.
† For details of diets and procedures, see Table 2 and pp. 258–264.

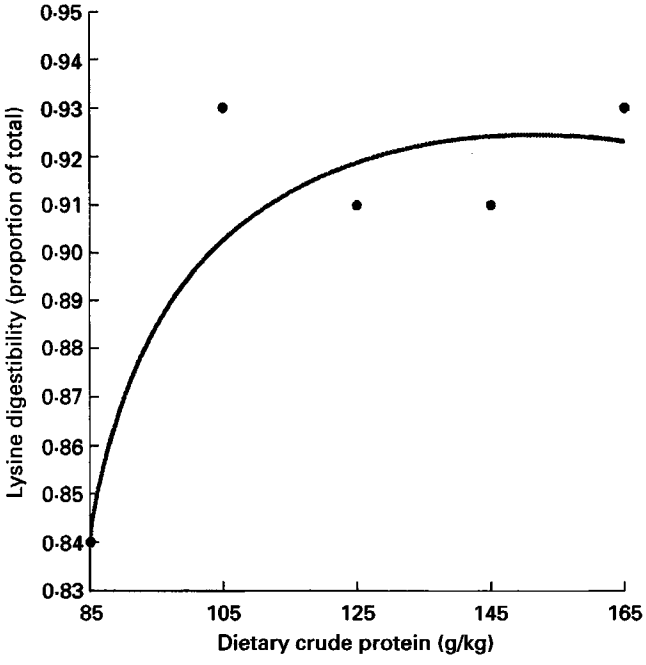

Fig. 6. Expt 2. Effect of dietary protein level on the apparent ileal digestibility of lysine in raw field peas (*Pisum sativum* cultivar Dundale) fed to growing pigs (SEM 0·010). For details of diets and procedures see Table 2 and pp. 258–264.

reported by van Barneveld *et al.* (1994*a*), despite similar protein levels. This is likely to be due to the large difference in the number of replicates in the respective experiments. In the current study, only three replicates were used. However, all estimates were well within the range of 0·55–0·88 determined by van Barneveld *et al.* (1994*a*) who utilized twelve replicates to obtain a mean lysine digestibility value of 0·76.

Table 8. *Estimated true ileal digestibility of lysine in raw and heat-treated field peas* (Pisum sativum *cultivar Dundale*) *using different estimation methods*

(Values in parentheses represent the lysine digestibility (heat treatment):lysine digestibility (raw) ratio)

Field pea heat treatment	Apparent ileal digestibility*	Estimation method†					Adjusted apparent ileal digestibility
		A	B	C	D	E	
Raw	0·75	0·84	0·87	0·80	0·83	0·86	0·92‡
110°	0·80 (1·07)	0·88 (1·05)	—	0·84 (1·05)	0·87 (1·05)	0·89 (1·03)	0·97
135°	0·75 (1·00)	0·84 (1·00)	—	0·81 (1·01)	0·85 (1·02)	0·85 (0·99)	0·92
150°	0·75 (1·00)	0·84 (1·00)	—	0·81 (1·01)	0·85 (1·02)	0·87 (1·01)	0·93
165°	0·62 (0·83)	0·73 (0·87)	0·79 (0·91)	0·71 (0·89)	0·77 (0·93)	0·81 (0·94)	0·84

* From van Barneveld *et al.* (1994*a*)

† Estimation methods: (A) endogenous protein = (0·013 × undigested dry matter (DM) − 0·08) × 6·25 (Boisen & Fernandez, 1991); (B) *in vitro* estimate, National Institute of Animal Science, Foulum, Denmark; (C) average output of endogenous amino acids (mg/kg DM intake) = 318·8 + 0·016 × dietary neutral-detergent fibre (mg/kg) (Taverner *et al.* 1981); (D) endogenous protein = 19·8 g/kg DM intake determined using protein-free diets (de Lange *et al.* 1989); (E) endogenous protein = 25·5 g/kg DM intake determined with soya-bean meal using ^{15}N-isotope dilution (de Lange *et al.* 1990).

‡ Determined in Expt 2.

Comparison of lysine digestibility, availability and utilization in heat-processed meals

Using results reported by van Barneveld *et al.* (1994*a*, *b*) a comparison of the effect of heat on lysine digestibility, availability and utilization can be made (Fig. 7). Heat had little effect on ileal digestibility, yet the utilization of ileal-digestible lysine was significantly reduced, even at mild temperatures (110°). Heat applied to protein concentrates appears to render lysine in a form that is apparently absorbed but inefficiently utilized by the growing pig. Ileal digestibility values for lysine in heat-processed meals are consequently unsuitable for diet formulations. In contrast, estimates of lysine availability determined with the slope–ratio assay were a close reflection of the utilization of lysine from heat-treated field peas and would be more suitable for use in diet formulations. The slope–ratio assay, however, is unsuitable for use in routine analysis for estimating amino acid availability due to the time and expense involved. A rapid, inexpensive and efficient amino acid availability assay is required.

Overall, the application of heat to field peas resulted in a significant decrease in lysine availability as determined by the slope–ratio assay. Unlike ileal digestibility values, availability estimates were sensitive to heat treatment and are a close reflection of the utilization of ileal-digestible lysine from heated protein concentrates. Thus lysine availability values would be more suitable for use in diet formulations than ileal digestibility estimates when dealing with heat-processed protein concentrates. Determination of amino acid availability with the slope–ratio assay is not suitable for routine analysis and hence a rapid alternative is required. The basis for such an alternative may be obtained by gaining an understanding of the biochemical mechanisms involved in the poor utilization of ileal-digestible lysine from heated protein concentrates. In the experiments to date, no investigation into the fate of N compounds in the urine of pigs fed on heated peas has been made. Hence, by examining the N balance of pigs fed on heated peas we may gain an insight into these biochemical mechanisms.

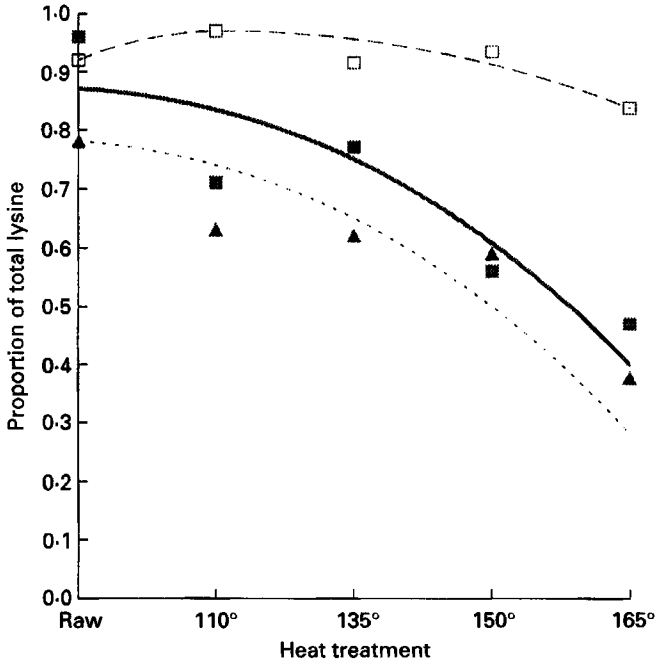

Fig. 7. Relationship between lysine digestibility (□---□), availability (■——■) and utilization (▲····▲) in raw field peas (*Pisum sativum* cultivar Dundale) and field peas heated for 15 min at 110°, 135°, 150° or 165° using a forced-air dehydrator.

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