The effect of heat on amino acids for growing pigs

2. Utilization of ileal-digestible lysine from heat-treated field peas (*Pisum sativum* cultivar Dundale)

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(*Received 6 April 1993 – Accepted 13 October 1993*)

Two growth experiments were conducted to determine the effect of heat on the utilization of ileal-digestible lysine from field peas (*Pisum sativum* cultivar Dundale) fed to growing pigs. Five lysine-deficient diets (0·36 g ileal-digestible lysine/MJ digestible energy (DE)) were formulated using raw field peas, and field peas heated to either 110°, 135°, 150°, or 165° for 15 min respectively in a forced-air dehydrator. Additional diets were formulated with supplements of free lysine to verify that lysine was limiting in the diets containing the raw peas, and peas heated to 150° or 165°. The growth performance and retention of ileal-digestible lysine by pigs given the diets was determined over the 20–45 kg growth phase. Heat had a significant quadratic effect ($P < 0·01$) on growth rate, with responses declining from 543 g/d with pigs given the raw peas, to 407 g/d for those given the peas heated to 165°. Similarly, crude protein deposition declined in a quadratic manner ($P < 0·001$) from 76 to 36 g/d for pigs fed on raw peas and peas heated to 165° respectively. Retention of ileal-digestible lysine was 0·85 in the pigs given the raw field peas and declined in a quadratic manner ($P < 0·001$) with the application of heat to 0·48 in those pigs given the peas heated to 165°. Pigs fed on field peas heated to 165° had increased ($P < 0·05$) liver weights. The results indicate 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. Consequently, ileal digestibility values for lysine in heat-processed meals are unsuitable for diet formulations.

Heat: Ileal digestibility: Lysine utilization: Field peas: Pigs

The ileal digestibility of amino acids has been proposed as a means of estimating amino acid availability for pigs. The assumption is made that if an amino acid is not recovered at the terminal ileum, then it has been absorbed in a form that is available to the pig. This assumption has been questioned by recent studies. For example, Batterham *et al.* (1990*a*) reported that whilst 0·76 of the ileal-digestible lysine was retained by pigs given soya-bean meal, only 0·36 was retained by pigs given cottonseed meal. They suggested that heat applied to the cottonseed-meal protein may result in lysine being absorbed in a form that was not efficiently utilized. Similar results showing apparent ileal digestibility values overestimating lysine utilization in alternative or heat-processed protein concentrates have been reported by Bellaver & Easter (1989), Moughan *et al.* (1991), and Wiseman *et al.* (1991).

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Hurrell *et al.* (1976) reported a substantial decrease in the nutritional value of chicken muscle, as determined by net protein ratio with the rat, when it was heated at 121° for 8 or 27 h respectively. Hurrell & Carpenter (1977) concluded that this decrease was only partly accounted for by the reduced ileal digestibility of the protein, and the reasons for this reduced utilization of protein with heating were unclear. In addition, there is uncertainty as to whether heat alone is responsible for the low utilization of ileal-digestible lysine in cottonseed meal reported by Batterham *et al.* (1990*a*) due to the variable conditions inflicted upon cottonseed meal during processing.

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) reported that the application of graded levels of heat to field peas had little effect on the apparent ileal digestibility of amino acids. Only lysine showed a significant decrease with heating to below that recorded for raw peas (raw, 0·75; 165°, 0·62).

The objective of the work reported in the present paper was to determine the effect of heat on the utilization of apparent ileal-digestible lysine in field peas (*Pisum sativum* cultivar Dundale). This was completed with two experiments. The first determined the utilization of ileal-digestible lysine from raw peas, and peas heated to 110°, 135°, or 150°. The second experiment determined the utilization of ileal-digestible lysine from raw peas and peas heated to 165°.

EXPERIMENTAL

Heat-processed meal

Field peas were used as the protein concentrate (Table 1). Graded levels of heat at 110°, 135°, 150°, or 165° were applied to batches of field peas and maintained for 15 min using a forced-air dehydrator (van Barneveld *et al.* 1994). These levels were chosen because they are indicative of the levels of heat that can be obtained during the processing of commercial meals used in pig diets, and they should produce lysine availabilities varying from 0·90 down to 0·30, similar to those reported in commercial meals by Batterham *et al.* (1984). The digestible energy values of the raw and heat-treated field peas were determined previously using total faeces collection (van Barneveld *et al.* 1994). As discussed by van Barneveld *et al.* (1994), lysine digestibility in field peas was not decreased by heating to 150°, and hence an additional heat treatment of 165° was deemed necessary. As a consequence, the present study has also been completed in two parts.

Ileal digestibility of lysine in raw and heat-treated field peas

The ileal digestibilities of amino acids in the five field-pea treatments were determined using ileal cannulation and the direct ileal sampling technique (van Barneveld *et al.* 1994). The ileal cannulation experiment was divided into two parts, the first determining the ileal digestibility of amino acids in the raw, 110°, 135°, and 150° treatments, and the second the ileal digestibility of amino acids in the raw and 165° treatments. In the present study, at the time of experimentation to determine the utilization of ileal-digestible lysine from the raw peas and peas heated to 110°, 135°, and 150°, only the digestibility values from the first part of the cannulation study were available. These were 0·80, 0·83, 0·80, and 0·80 for the raw peas, and peas heated to 110°, 135°, and 150° respectively. The final combined values for the ileal digestibility of lysine determined by ileal cannulation reported by van Barneveld *et al.* (1994) differ slightly from these. This is due to an increased number of replicates for the raw peas and higher error degrees of freedom when the two parts of the experiment were combined and analysed statistically.

The diet formulated to determine the utilization of ileal-digestible lysine in the 165°

Table 1. *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				
Component	Raw	110°	135°	150°	165°
Crude protein (N × 6·25)	210	224	221	227	216
Dry matter	913	938	952	961	977
Light petroleum (b.p. 40–60°) extract	21	21	22	15	16
Fibre					
Crude	67	63	65	89	104
Neutral-detergent	81	67	185	467	483
Ash	25	28	27	26	28
Amino acids					
Asp	22·1	24·5	24·9	24·8	24·7
Thr	7·7	8·3	8·5	8·7	8·2
Ser	10·1	11·1	11·3	11·4	10·5
Glu	32·6	37·4	37·7	38·1	38·6
Pro	8·5	9·1	9·2	9·9	9·8
Gly	8·6	9·6	9·7	9·8	9·8
Ala	8·4	9·5	9·6	9·8	9·8
Cys	1·3	1·7	1·6	1·3	1·2
Val	9·4	10·5	10·6	10·8	11·0
Met	1·5	1·4	1·4	1·7	1·1
Ile	8·6	9·5	9·5	9·7	9·9
Leu	13·8	15·4	15·5	15·8	15·8
Tyr	6·4	6·9	7·0	7·2	6·9
Phe	9·2	10·3	10·3	10·6	10·2
Lys	14·3	15·6	15·4	12·8	8·7
His	4·5	5·1	5·1	5·2	4·9
Arg	15·2	19·1	19·3	18·2	15·0
ID values used in diet formulation (proportion of total)	0·80	0·83	0·80	0·80	0·62
Apparent ID of lysine (proportion of total)*	0·75	0·82	0·75	0·75	0·62

ID, ileal digestibility.
* From van Barneveld *et al.* (1994).

treatment was based on a mean digestibility value of 0·62 determined using ileal cannulation and direct ileal sampling, as both were available at the time of experimentation. For comparative purposes, the combined digestibility values determined by ileal cannulation and direct ileal sampling reported by van Barneveld *et al.* (1994; 0·75, 0·82, 0·78, 0·75, 0·62 for raw, 110°, 135°, 150° and 165° respectively) have been used to estimate the ileal-digestible lysine retained in the pig for all treatments, rather than the digestibility values used in diet formulations.

Utilization of ileal-digestible lysine

Diets. Five diets were formulated to contain 0·36 g ileal-digestible lysine/MJ digestible energy (DE; diets 1, 2, 3, 4 and 5; Table 2). This level of lysine is limiting and represents a region of linear growth response by the pig to increasing dietary lysine concentration, while being near the plateau for lysine retention (Batterham *et al.* 1990*b*). To ensure that lysine was the limiting amino acid in the diets, supplements of other essential amino acids were added to provide a surplus of 0·30 relative to lysine, according to estimates of the

Table 2. *Expts 1 and 2. Components (g/kg, air-dry basis) and composition of diets formulated to 0·36 or 0·47 g ileal-digestible lysine/MJ digestible energy (DE)*

Diet...	1	2	3	4	5	6	7	8
Components								
Field peas (*Pisum sativum* cultivar Dundale)								
Raw	480	—	—	—	—	480	—	—
110°	—	430	—	—	—	—	—	—
135°	—	—	435	—	—	—	—	—
150°	—	—	—	520	—	—	520	—
165°	—	—	—	—	860	—	—	860
Minerals and vitamins*	5	5	5	5	5	5	5	5
Dicalcium phosphate	30	30	30	30	30	30	30	30
Soya-bean oil	15	15	15	15	15	15	15	15
Fuzone 200†	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Monosodium glutamate	27·2	38·0	10·8	—	—	32·6	—	—
Sucrose	420·5	461·4	484·7	417·2	86·4	413·0	415·1	84·6
Amino acids								
L-Thr	3·24	2·94	2·76	1·96	0·18	3·24	1·96	0·18
L-Val	3·14	2·84	2·63	1·63	—	3·14	1·63	—
DL-Met	3·79	3·69	3·63	3·43	2·90	3·79	3·43	2·90
L-Ile	1·79	1·59	1·53	0·63	—	1·79	0·63	—
L-Leu	4·13	3·63	3·44	1·84	—	4·13	1·84	—
L-Tyr	2·31	2·31	2·16	1·46	—	2·31	1·46	—
L-Phe	1·24	1·00	0·89	—	—	1·24	—	—
L-Lys hydrochloride	—	—	—	—	—	2·15	2·09	1·81
L-His	1·43	1·33	1·22	0·72	—	1·43	0·72	—
L-Trp	0·78	0·78	0·74	0·64	0·05	0·78	0·64	0·05
Composition								
DE (estimated (MJ/kg)	15·47	15·17	15·16	15·01	13·02	15·47	15·01	13·02
Ileal-digestible lysine								
(g/kg)	5·57	5·46	5·46	5·40	4·69	7·27	7·05	6·12
(g/MJ DE)	0·36	0·36	0·36	0·36	0·36	0·47	0·47	0·47

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

† Contains 200 g furazolidone/kg.

Agricultural Research Council (1981), Fuller & Wang (1987), and estimations determined with the 'Auspig' computer simulation model (Black *et al.* 1986) for the Wollongbar genotype. Diets 6, 7 and 8 (Table 2) were supplemented with free lysine to contain 0·47 g ileal-digestible lysine/MJ DE, to verify that lysine was limiting in diets 1, 4 and 5. It was assumed that if a response to added lysine was obtained with diets containing the raw peas and the most severely heated field peas (diets 1 and 5), then lysine was also limiting in diets 2 and 3. The DE content of ingredients other than the field peas was estimated from previous determinations at this Institute.

Animals and procedures. The utilization of ileal-digestible lysine in the five field-pea treatments was determined in two experiments.

Expt 1. In the first experiment, the raw, 110°, 135°, and 150° field-pea treatments (using diets 1, 2, 3 and 4 respectively) were assessed. Diets 6 and 7 were given in this experiment to verify that lysine was limiting in diets 1 and 4. The six diets were arranged according to

a randomized block design. Ten pigs of the Large White breed (five males and five females) were allocated to each diet. The pigs were blocked on 7-week weight, sex and position in the experimental facilities. Pigs were penned individually and water was 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.

In order to determine nutrient retentions, five male and five female pigs were slaughtered at the commencement of the experiment (20 kg live weight) and the chemical composition of the blood plus washed viscera and whole carcasses determined in a similar manner to the pigs slaughtered at 45 kg live weight.

Amino acid composition of the empty bodies of the pigs was determined using carcass and mixed blood and washed viscera samples recombined in proportions resembling that of the pig before slaughter. Recombination was based on the N and energy contents of the samples. Recombinations were bulked for each treatment, giving one sample for amino acid analysis for each diet. This process was completed in duplicate.

Pig response was assessed in terms of daily live-weight gain; food conversion ratio (FCR); backfat thickness (P_2); empty-body weight:final live-weight ratio; gain/d and FCR on an empty-body weight basis; protein, fat and energy contents in the empty body; protein, fat and energy deposition/d; protein, fat and energy deposition:DE intake ratios; protein retention:ileal-digestible protein intake ratio; lysine retention:total lysine intake ratio; and lysine retention:apparent ileal-digestible lysine intake ratio.

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 the five males and five females slaughtered at 20 kg live weight). Energy stored as protein was calculated as protein (kg) $\times$ 24·2 (Jordan & Brown, 1970). Fat content was calculated as (total energy (MJ) − protein energy (MJ))/39·6 (Burlacu *et al.* 1973).

Expt 2. In the second experiment the retention of lysine in the raw and 165° peas (using diets 1 and 5 respectively) by growing pigs was determined. Diet 8 was fed in this experiment to verify that lysine was limiting in diet 5. The raw diet was included in this experiment to allow a direct comparison of responses with Expt 1. The three diets were arranged in a randomized block design. Five male and five female pigs were allotted to the raw diet, while four male and four female pigs were allotted to both diets 5 and 8. The high inclusion of field peas in diets 5 and 8 restricted the quantity of diet mixed, and only eight pigs per diet could be supported over the experimental period. Blocking, housing, and feeding regimes were as for Expt 1. Slaughtering procedures were also as for Expt 1 with the exception that the liver was removed from the viscera and weighed before recombination and freezing.

Statistical analysis

The results were analysed by analysis of variance, utilizing a general linear model, and the treatment means separated by least significant difference (LSD). The statistical analysis was divided into a number of distinct sections. First, a direct comparison of the responses to the raw diet given in Expts 1 and 2 was made. There was no significant difference ($P > 0.05$) between the two experiments, and accordingly the data were combined and the two experiments analysed simultaneously. The effects of heat on lysine utilization were then statistically analysed in two parts. The first was a comparison of the raw *v.* the 110° treatment. The second examined the linear and quadratic effects across the four heat treatments only. Finally, responses to the addition of lysine in diets 1, 4 and 5 were analysed.

Chemical analyses

Techniques used were as described by van Barneveld *et al.* (1994) except that N and gross energy in the carcass samples and mixed blood and washed viscera samples were determined by near infra-red reflectance spectrophotometry using previously established regression equations (George *et al.* 1987). Amino acids in the recombined empty-body samples were separated by reverse-phase chromatography and measured after reaction with phenylisothiocyanate following fat extraction. The internal standard used 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

Growth rates (g/d) of the pigs given five diets formulated to 0·36 g ileal-digestible lysine from raw and heat-treated field peas/MJ DE were significantly different ($P < 0.001$; Table 3). Across the heat treatments, growth rates, on a live-weight and empty-body-weight basis, showed a quadratic ($P < 0.01$) decrease while FCR increased quadratically ($P < 0.001$). Backfat showed a significant quadratic decrease ($P < 0.01$) over the heat treatments. The addition of lysine to the diets containing the raw peas and peas heated to 150° and 165° respectively increased growth rates and lowered the FCR ($P < 0.001$).

Crude protein deposition was significantly lower in pigs fed on peas heated to 110° (69 g/d) relative to those fed on raw peas (76 g/d; $P < 0.001$; Table 4). Across the heat treatments, crude protein deposition showed a significant quadratic decrease ($P < 0.001$) down to 36 g/d in the pigs fed on peas heated to 165°. Fat deposition and energy deposition showed significant quadratic decreases ($P < 0.01$ and $P < 0.001$ respectively) across the heat treatments (Table 4).

Retention of dietary protein and the proportion of protein retained to DE intake were significantly lower ($P < 0.001$ and $P < 0.01$ respectively) in the pigs fed on peas heated to 110° relative to the pigs fed on the raw peas (Table 5). Across the heat treatments, retention of dietary protein and the proportion of protein retained to DE intake showed a significant quadratic decrease ($P < 0.001$). There was a significant quadratic decrease ($P < 0.01$) in the retention of DE across the heat treatments from 0·38 in pigs fed on peas heated to 110° down to 0·30 in the 165° treatment. There was a significant increase ($P < 0.05$) in the weight of the liver in the empty body of pigs fed on peas heated to 165° compared with those fed on raw peas (Fig. 1).

Lysine content in the pigs varied from 6·3 to 7·2 g/16 g N (Table 6). This difference in amino acid content was not significant ($P > 0.05$) with no apparent change due to feeding on heated field peas.

Retention of ileal-digestible lysine was significantly reduced with the application of heat at 110° ($P < 0.001$; Fig. 2). Across the heat treatments, retention of ileal-digestible lysine

Table 3. *Expts 1 and 2. The effect of formulating diets containing raw and heat-treated field peas* (Pisum sativum *cultivar Dundale*) *on an apparent ileal-digestible* (*ID*) *lysine basis on the growth performance of pigs over the 20–45 kg growth phase*†

(Mean values for eight to twenty pigs per dietary group)

Protein source			Measurement					
Diet	Heat treatment	ID lysine (g/MJ DE)	Gain (g/d)	FCR	EBW:LW (kg/kg)	Gain (g/d) (EBW basis)	FCR (EBW basis)	Backfat (P_2, mm)
Heat								
1	Raw	0·36	543	2·4	0·92	498	2·6	14
2	110°	0·36	546	2·4	0·90	482	2·7	14
3	135°	0·36	539	2·4	0·90	477	2·7	14
4	150°	0·36	509	2·6	0·90	450	2·9	15
5	165°	0·36	407	3·5	0·85	315	4·5	10
Lysine								
6	Raw + lysine	0·47	609	2·1	0·91	548	2·3	13
7	150° + lysine	0·47	587	2·3	0·91	522	2·5	14
8	165° + lysine	0·47	562	2·6	0·86	452	3·2	14
Statistics								
Diet			***	***	***	***	***	***
Heat								
Raw *v.* 110°			NS	NS	NS	NS	NS	NS
Linear (analysis of diets 2, 3, 4, 5 only)			***	***	**	***	***	**
Quadratic (analysis of diets 2, 3, 4, 5 only)			**	***	**	***	***	**
Lysine								
Lysine (Diets 1, 4, 5 *v.* 6, 7, 8)			***	***	NS	***	***	NS
Treatment × lysine			**	***	NS	***	***	***
SEM (52 edf)								
Diet 1	(*n* 20)		7·2	0·05	0·005	6·1	0·05	0·4
Diet 2, 3, 4, 6, 7	(*n* 10)		12·4	0·08	0·008	10·6	0·09	0·6
Diet 5, 8	(*n* 8)		14·3	0·09	0·009	12·2	0·10	0·7

DE, digestible energy; FCR, food conversion ratio; EBW, empty-body weight; LW, live weight; NS, not significant ($P > 0·05$); SEM, standard errors of the means; edf, error degrees of freedom.

** $P < 0·01$; *** $P < 0·001$

† For details of diets and procedures, see Table 2 and pp. 244–248.

Table 4. *Expts 1 and 2. The effect of formulating diets containing raw and heat-treated field peas* (Pisum sativum *cultivar Dundale*) *on an apparent ileal-digestible (ID) lysine basis on the concentrations and deposition rates of protein, fat and energy in growing pigs (20–45 kg live weight)*†

(Mean values for eight to twenty pigs per dietary group)

Protein source			Composition (empty-body weight basis)			Deposition rates		
Diet	Heat treatment	ID lysine (g/MJ DE)	Protein (kg/kg)	Fat (kg/kg)	Energy (MJ/kg)	Protein (g/d)	Fat (g/d)	Energy (MJ/d)
Heat								
1	Raw	0·36	0·147	0·196	11·3	76	132	7·1
2	110°	0·36	0·141	0·218	12·1	69	149	7·5
3	135°	0·36	0·141	0·230	12·5	68	157	7·9
4	150°	0·36	0·138	0·242	12·9	61	159	7·8
5	165°	0·36	0·127	0·225	12·0	36	112	5·3
Lysine								
6	Raw + lysine	0·47	0·147	0·189	11·0	85	135	7·4
7	150° + lysine	0·47	0·143	0·222	12·3	77	164	8·3
8	165° + lysine	0·47	0·136	0·208	12·0	60	134	6·8
Statistics								
Diet			***	***	***	***	**	**
Heat								
Raw *v.* 110°			***	*	*	***	NS	NS
Linear (analysis of diets 2, 3, 4, 5 only)			***	NS	NS	***	*	***
Quadratic (analysis of diets 2, 3, 4, 5 only)			**	NS	*	***	**	***
Lysine								
Lysine (diets 1, 4, 5 *v.* 6, 7, 8)			***	*	NS	***	NS	**
Treatment × lysine			*	NS	NS	**	NS	NS
SEM (52 edf)								
Diet 1	(*n* 20)		0·0009	0·0052	0·20	1·2	5·0	0·20
Diet 2, 3, 4, 6, 7	(*n* 10)		0·0015	0·0089	0·34	2·1	8·7	0·35
Diet 5, 8	(*n* 8)		0·0018	0·0103	0·40	2·4	10·0	0·40

DE, digestible energy; NS, not significant ($P > 0·05$); SEM, standard errors of the means; edf, error degrees of freedom.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
† For details of diets and procedures, see Table 2 and pp. 244–248.

Table 5. *Expts 1 and 2. The effect of formulating diets containing raw and heat-treated field peas* (Pisum sativum *cultivar Dundale*) *on an apparent ileal-digestible (ID) lysine basis on the retentions of protein, fat and energy in growing pigs (20–45 kg live weight)*†

(Mean values for eight to twenty pigs per dietary group)

	Protein source		Retentions			
Diet	Heat treatment	ID lysine (g/MJ DE)	Protein retained:ID protein intake (kg/kg)	Protein retained:DE intake (g/MJ)	Fat retained:DE intake (g/MJ)	Energy retained:DE intake (MJ/MJ)
Heat						
1	Raw	0·36	0·63	3·9	6·7	0·36
2	110°	0·36	0·54	3·5	7·5	0·38
3	135°	0·36	0·64	3·4	8·0	0·40
4	150°	0·36	0·53	3·1	8·2	0·40
5	165°	0·36	0·26	2·0	6·4	0·30
Lysine						
6	Raw + lysine	0·47	0·71	4·3	6·9	0·38
7	150° + lysine	0·47	0·66	3·9	8·3	0·43
8	165° + lysine	0·47	0·40	3·2	7·1	0·36
Statistics						
Diet			***	***	*	*
Heat						
Raw *v.* 110°			***	**	NS	NS
Linear (analysis of diets 2, 3, 4, 5 only)			***	***	NS	*
Quadratic (analysis of diets 2, 3, 4, 5 only)			***	***	*	**
Lysine						
Lysine (diets 1, 4, 5 *v.* 6, 7, 8)			***	***	NS	**
Treatment × lysine			NS	*	NS	NS
SEM (52 edf)						
Diet 1	(*n* 20)		0·010	0·06	0·26	0·011
Diet 2, 3, 4, 6, 7	(*n* 10)		0·018	0·11	0·45	0·018
Diet 5, 8	(*n* 8)		0·021	0·13	0·52	0·021

DE, digestible energy; NS, not significant ($P > 0·05$); SEM, standard error of the mean; edf, error degrees of freedom.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
† For details of diets and procedures, see Table 2 and pp. 244–248.

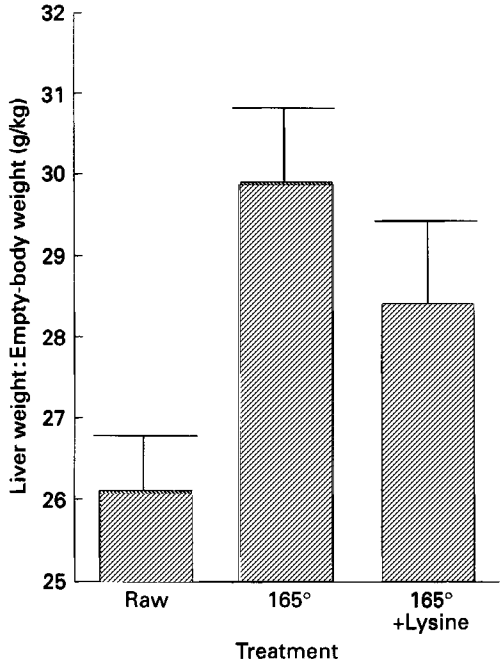

Fig. 1. Expt 2. The weight of the liver as a proportion of empty-body weight in pigs fed on raw field peas (*Pisum sativum* cultivar Dundale) and peas heated to 165° over the 20–45 kg growth phase. (Statistics: least significant difference 1·44).

Table 6. *Expts 1 and 2. Amino acid composition (g/16 g N, empty-body-weight basis) of pigs slaughtered at 20 kg, and at 45 kg live weight when given diets 1–8**

(Values are the means of two analyses performed on recombined carcasses derived from the number of pigs per treatment)

Diet...	1	2	3	4	5	6	7	8		
Treatment...	Raw	110°	135°	150°	165°	Raw + Lys	150° + Lys	165° + Lys	20 kg Pigs	Pooled SD
n...	20	10	10	10	8	10	10	8	10	
Asp	8·3	7·8	7·8	8·0	8·6	8·8	8·8	8·5	7·9	0·78
Thr	4·1	3·9	4·0	3·7	4·1	4·2	4·3	4·1	3·7	0·22
Ser	4·3	4·3	4·2	4·1	4·6	4·6	4·5	4·5	4·3	0·28
Glu	13·8	13·1	13·1	12·9	14·9	15·7	14·1	14·0	12·9	0·96
Pro	6·1	6·3	5·9	8·2	6·5	6·4	6·2	8·5	8·4	1·19
Gly	8·2	8·5	8·0	9·2	8·8	8·7	8·1	9·8	9·0	0·82
Ala	6·3	6·3	6·1	6·3	6·7	6·7	6·5	6·9	6·2	0·44
Cys	0·7	0·7	0·7	0·5	0·7	0·6	0·6	0·7	0·6	0·12
Val	4·8	4·5	4·4	4·5	5·0	5·0	5·0	5·0	4·4	0·53
Met	2·4	2·3	2·4	1·9	2·4	2·4	2·4	2·3	1·7	0·12
Ile	3·9	3·8	3·8	3·5	4·0	4·0	4·1	3·9	3·4	0·28
Leu	7·5	7·4	7·4	6·7	7·7	7·7	7·9	7·5	6·9	0·40
Tyr	3·0	2·9	2·9	2·7	3·1	3·1	3·1	3·0	2·7	0·19
Phe	3·7	3·6	3·5	3·4	3·8	3·8	3·9	3·7	3·5	0·25
Lys	6·9	6·6	6·6	6·5	6·9	7·2	7·2	7·2	6·3	0·46
His	3·8	3·7	3·7	3·2	3·2	3·9	3·8	3·4	3·0	0·18
Arg	7·6	7·5	7·3	7·4	8·0	8·0	7·8	8·0	7·2	0·49

* For details of diets see Table 2.

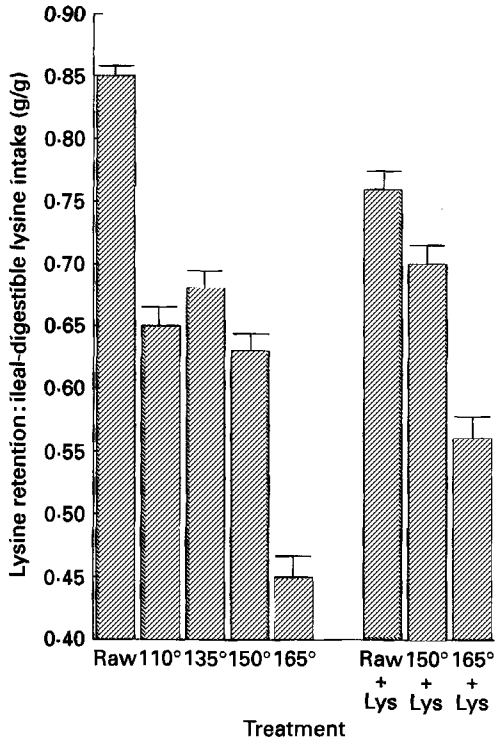

Fig. 2. Expts 1 and 2. The retention of ileal-digestible lysine in raw and heat-treated field peas (*Pisum sativum* cultivar Dundale) fed to growing pigs over the 20–45 kg growth phase. Statistical significance of effects: diet, $P < 0{\cdot}001$; raw *v.* 110°, $P < 0{\cdot}001$; linear (analysis of diets 2, 3, 4 and 5 only), $P < 0{\cdot}001$; quadratic (analysis of diets 2, 3, 4 and 5 only), $P < 0{\cdot}001$; lysine (diets 1, 4 and 5 *v.* 6, 7 and 8), not significant; treatment × lysine interaction, $P < 0{\cdot}001$.

showed a significant quadratic decrease ($P < 0{\cdot}001$) from 0·65 in pigs fed on peas heated to 110° down to 0·48 in pigs fed on peas heated to 165°.

DISCUSSION

The results indicate that ileal-digestible lysine is apparently absorbed but poorly utilized by growing pigs fed on heated protein concentrates. Mild heating of the field peas to 110° resulted in a significant decrease in the retention of ileal-digestible lysine, from 0·85 to 0·67. Much greater reductions in the retention of ileal-digestible lysine were observed with the 150° (0·59) and 165° (0·48) heat treatments. The significant responses in growth and protein deposition of the pigs to supplements of lysine in diets 6, 7 and 8 verified that lysine was the limiting amino acid in diets 1, 4 and 5. It is therefore reasonable to assume that lysine was also the limiting amino acid in diets 2 and 3.

Hurrell & Carpenter (1977) concluded that when proteins are severely heated, in the presence or absence of sugars, the fall in nutritional value (i.e. protein utilization) appears to be largely, although not completely, explained by reduced protein digestibility. The current results suggest that the fall in nutritional value not reflected by decreased digestibility is of more nutritional significance than the fall in digestibility. With only mild heat application there was an improvement in the apparent ileal digestibility of lysine relative to the raw peas, yet there was a significant drop in lysine utilization.

There are a number of possible mechanisms which may explain the above phenomenon. (1) The application of heat to proteins may induce changes within the protein molecule that have little effect on digestibility but render the absorbed amino acids less available. These amino acids may be subsequently deaminated and excreted by the pig. (2) Following digestion, amino acids altered by heating may be metabolized in the gut wall and, hence, not absorbed in the portal blood.

There has been extensive research investigating the changes that occur within a protein molecule with the application of heat (Bjarnason & Carpenter, 1970; Varnish & Carpenter, 1975; Erbersdobler, 1977; Hurrell & Carpenter, 1977). Research has also shown that some of the compounds formed in proteins with the application of heat, such as Maillard compounds (e.g. fructoselysine) or isopeptides formed by protein–protein crosslinking, can be digested, but are inefficiently utilized (Bjarnason & Carpenter, 1969; Ford & Shorrock, 1971; Hurrell *et al.* 1976). Some of these compounds may be contributing to the phenomenon of reduced utilization of ileal-digestible lysine in the present study. There is a need to identify these compounds and quantify their contribution to reduced utilization of ileal-digestible lysine. This would confirm that the application of heat to field peas induced changes within the protein molecule that rendered absorbed lysine less available. It is clear, however, that the heating conditions, the carbohydrate content of the meal, and its initial protein structure will have a large influence on the severity of the heat damage and the compounds formed following heating. As a consequence, the identification of non-utilizable compounds that result from heating in one protein meal may not tell us much about the utilizable amino acids in another. This stresses the need to identify a specific animal response sensitive to reduced amino acid availability.

Rérat (1990) suggested that dietary nutrients may be metabolized in the gut wall during absorption. As a consequence, ileal digestibility may significantly overestimate absorption of amino acids. It is reasonable to suggest that amino acids from heated proteins may diffuse from the intestinal lumen into the epithelial cells. However, due to the requirements of amino acids for active transport mechanisms through the epithelium, they may not progress to the portal blood if their structure has been altered and cannot be recognized by any specific carrier system. Hence, through diffusion, the amino acid may be removed from the intestinal lumen, and therefore apparently digested. Failure to conform to a specific site on an active carrier, however, might result in accumulation and subsequent catabolism of the altered amino acid in the gut wall without further progression.

As well as resulting in the poor utilization of ileal-digestible lysine, the application of heat to protein concentrates may have a significant effect on liver size. In the present study a reduction in available amino acids may have resulted in a significant reduction in plasma protein concentration. Prolonged reduction in plasma protein concentration causes mitosis of the hepatic cells, and actual growth of the liver to a larger size, with increased output of plasma proteins from the enlarged liver maintaining the equilibrium between plasma proteins, amino acids in the blood, and the tissue proteins (Guyton, 1986).

The increased retention of dietary digestible energy observed with diets 3 and 4 may be due to a slightly better balance of amino acids in these diets. Smaller additions of free amino acids to these diets were required to bring the essential amino acids to a surplus of 0·30 relative to lysine. The decrease in the retention of dietary digestible energy with diet 5 may be due to the high inclusion of field peas (0·86) in this diet. The high field-pea inclusion levels increase the level of dietary fibre and hence the proportion of dietary energy derived from the absorption of volatile fatty acids from the hind-gut. Consequently, the dietary energy utilization is less efficient than with diets 2, 3 and 4 which have sucrose as their primary energy source. In addition there may be greater demand on dietary energy to catabolize excess or non-utilizable amino acids in diet 5.

In the pigs fed on raw peas the retention of ileal-digestible lysine was high compared with previous estimates for similar meals (e.g. soya bean, 0·75, Batterham *et al.* 1990*a*). This may be due to the soya bean being processed before feeding. Despite improving digestibility and removing anti-nutritional factors, the mild processing of soya bean may be significantly reducing the utilization of ileal-digestible lysine.

The conclusion that values for the ileal digestibility of lysine in heat-processed meals are unsuitable for dietary formulations (Batterham *et al.* 1990*a*) is supported by these results. Similar conclusions were drawn by Wiseman *et al.* (1991) who showed that diets containing heat-processed fish meal, formulated on the basis of ileal digestibility, could not produce performance responses equivalent to those with diets containing untreated fish meal.

Overall, 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. Consequently, ileal digestibility values for lysine in heat-processed meals are unsuitable for dietary formulations. Thus, there is a need to assess alternative ways of estimating amino acid utilization. One alternative is the use of the slope–ratio analysis of growth responses of pigs to estimate amino acid availability.

The authors are grateful to Messrs R. C. Wilson and A. W. Davis, and Ms L. M. Andersen for management of pigs and skilled technical assistance, the late Mr R. L. Davies (South Australian Department of Agriculture), Dr D. C. Skingle and Ms W. E. Peasley for amino acid analysis, and Ajinomoto Inc. in Japan for the supply of free amino acids used in experimental diets. R. J. V. B. was in receipt of an Australian Pig Research and Development Corporation Research Fellowship and the work was supported by financial grants from the Pig Research and Development Corporation.

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