British Journal of Nutrition (1995), **73**, 259–273

The effect of heat on amino acids for growing pigs

4. Nitrogen balance and urine, serum and plasma composition of growing pigs fed on raw or heat-treated field peas (*Pisum sativum*)

BY R. J. VAN BARNEVELD*, THE LATE E. S. BATTERHAM AND D. C. SKINGLE†

NSW Agriculture, Wollongbar Agricultural Institute, Wollongbar, New South Wales 2477, Australia

AND B. W. NORTON

Department of Agriculture, University of Queensland, St Lucia, Queensland 4072, Australia

(*Received 6 April 1993 – Revised 5 May 1994 – Accepted 14 June 1994*)

Experiments were conducted to determine the effect of heating field peas (*Pisum sativum*) on the N balance and urine, serum and plasma composition of growing pigs. In the first experiment, four diets containing raw field peas (cv. Wirrega) or field peas heated to 150° (cv. Wirrega), 165° (cv. Wirrega) or 150° (cv. Dundale) for 15 min respectively were formulated to contain 1·15 g ileal digestible N/MJ digestible energy (DE) and 0·36 g ileal digestible lysine/MJ DE in a sugar-based diet. Digestibility estimates were based on those for the Dundale cultivar of field peas used in previous experiments. Total urine and faeces collection from eight pigs was conducted over two 7 d collection periods with a 7 d diet change-over period. Serial blood sampling from the external jugular vein was conducted on the final day of each collection period. There was no significant difference ($P > 0·05$) in the N balance or apparent biological value of the field-pea treatments. Pigs fed on diets containing peas heated to 150° (cv. Wirrega) or 165° (cv. Wirrega) had a significantly lower ($P < 0·01$) daily output of urea and uric acid in the urine, and depressed serum protein and serum urea concentrations. Plasma lysine concentration and daily urine lysine output were not significantly different ($P > 0·05$) in pigs fed on heated peas. Protein excretion in the urine of pigs fed on diets containing peas heated to 165° increased 3–7 times (depending on estimation technique) the level observed in pigs fed on diets containing raw peas. A second experiment was conducted to determine the apparent ileal digestibility of N and amino acids in cv. Wirrega field peas. This study revealed that N digestibility (0·44) and lysine digestibility (0·35) in peas heated to 165° were significantly lower than the cv. Dundale estimates (0·57 and 0·62 respectively) used in diet formulations. The depressed serum and urine variables in pigs fed on heated peas were attributed to overestimation of digestibility. The results exemplify the fact that it is not possible to draw general conclusions as to the effects of heat on any particular protein concentrate. Variability in N balance experiments and problems associated with urine analysis are suggested as likely reasons for the current study not reflecting poor utilization of ileal digestible lysine from heat-treated field peas. Despite considerable variation in the results, it is possible that a large proportion of non-utilizable amino acids in heated field peas may be excreted from the pig via the urine in the form of a protein.

Field peas: Heat treatment: Nitrogen: Pigs

Amino acid availability measurements have been shown to reflect the poor utilization of

* Present address: Northfield Pig Research Unit, GPO Box 1671, Adelaide, South Australia 5001, Australia.
† Present address: Centre for Molecular Biology, University of Queensland, St Lucia, Queensland 4072, Australia.

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

lysine from heat-processed proteins (van Barneveld *et al.* 1994*c*) and are hence recommended for use in diet formulations when dealing with heat-processed meals. Before availability values can be used widely in diet formulations, however, a rapid, repeatable and inexpensive assay is required. The slope–ratio analysis of growth experiments is not suitable for routine analysis due to the time and expense involved with such undertakings. By gaining an understanding of the biochemical mechanisms involved with the poor utilization of amino acids from heat-processed proteins, the basis for a suitable alternative assay may evolve.

Van Barneveld *et al.* (1994*b*) reported that only 0·45 of the ileal digestible lysine from field peas (*Pisum sativum*) heated to 165° was retained in the empty body of the pig, and even after correction for maintenance losses, there was still no accounting for 0·30 of the ileal digestible lysine. Since the missing fraction of apparently absorbed lysine was not found in the empty body (van Barneveld *et al.* 1994*b*), it was hypothesized that the poorly utilized lysine may be lost via the urine. In addition, lower utilization of other amino acids as a result of heating lysine would be reflected by increased deamination of the non-utilizable N sources, resulting in elevated levels of urea in the peripheral blood and urine.

As heating induces changes in amino acids other than lysine (Beech *et al.* 1991; Batterham *et al.* 1993), and as total protein deposition is also depressed when heated proteins are fed (van Barneveld *et al.* 1994*b*), the site of loss of these non-utilizable compounds might be more easily identified if overall N metabolism was examined, rather than the fate of a single amino acid.

Measurement of N-contributing components in the urine, serum and plasma of pigs fed on raw and heated peas may provide an insight into the fate of missing lysine. Urine and serum urea can be measured to determine if heated amino acids are highly susceptible to deamination as a result of their poor utilization. Uric acid, another N component of serum and urine, should be examined due to the fact that following a pronounced decrease in the amount of protein metabolized in the body, the absolute quantity of uric acid is also decreased (Hawk, 1965). Creatinine represents a significant proportion of urine and serum N. Unlike humans, the porcine renal tubule absorbs creatinine and hence excretion in the urine is not constant and can be influenced by diet (Pond & Houpt, 1978). Serum and urine protein are important considerations due to the possibility that heating induces the absorption of intact proteins or peptides across the gut wall that are subsequently unable to be metabolized by the growing pig. Poor utilization of plasma amino acids may result in elevated levels in the peripheral blood. Finally, plasma albumin represents a major form of protein synthesized by the liver. Poorly available amino acids may hinder this liver synthesis.

The objectives of the work reported in this fourth paper were to attempt to identify pathways by which poorly utilized lysine from heated peas was lost. This was undertaken by examining (1) the N balance and (2) the urine, serum and plasma composition of growing pigs.

EXPERIMENTAL

Protein concentrates and heat treatments

Field peas (*Pisum sativum* cv. Wirrega) were used as the protein concentrate. Heat treatments of 150° or 165° were applied using forced-air dehydrators as described by van Barneveld *et al.* (1994*a*). The Dundale cultivar of field peas was used in all previous experiments (van Barneveld *et al.*, 1994*a*, *b*, *c*), but as supplies of these peas were diminished, cv. Wirrega peas were substituted in the current study. It was assumed that no major differences in ileal and faecal digestibility existed between these two cultivars, and that the chosen heat treatments would induce similar changes in chemical composition.

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

Table 1. *Expt 1. Components of diets (g/kg, air-dry basis) for the nitrogen balance studies*

Component	Diet 1	2	3	4
Field peas (*Pisum sativum*)				
Raw (cv. Wirrega)	370·00	—	—	—
150° (cv. Wirrega)	—	475·00	—	—
150° (cv. Dundale)	—	—	—	400·00
165° (cv. Wirrega)	—	—	830·00	—
Minerals and vitamins*	5·00	5·00	5·00	5·00
Dicalcium phosphate	30·00	30·00	30·00	30·00
Soya-bean oil	15·00	15·00	15·00	15·00
Sugar	462·01	390·00	111·89	464·58
Chromic oxide	2·00	2·00	2·00	2·00
Fuzone 200†	0·50	0·50	0·50	0·50
Monosodium glutamate	46·20	32·60	—	31·80
Amino acids				
L-Lysine HCl	2·17	2·13	1·85	2·15
L-Cystine	1·79	1·62	1·24	1·68
DL-Methionine	1·68	1·45	0·97	1·56
L-Threonine	3·13	2·13	0·38	2·31
L-Valine	4·48	3·12	—	3·35
L-Isoleucine	4·32	3·07	—	3·38
L-Leucine	6·79	4·81	—	5·18
L-Tyrosine	2·59	1·74	—	1·87
L-Phenylalanine	4·50	3·16	—	3·44
L-Histidine	1·69	1·10	0·09	1·11
L-Tryptophan	0·75	0·57	1·08	0·69
L-Glutamic acid	35·4	25·0	—	24·4

* 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.

† Furazolidone 200 g/kg.

There were sufficient peas to allow a single treatment of cv. Dundale peas heated to 150° to be included for direct comparison with the cv. Wirrega responses.

Expt 1. The effect of heating field peas on the nitrogen balance and urine, serum and plasma composition of growing pigs

Diets. Four diets were formulated to contain equal levels of ileal digestible N (1·15 g/MJ digestible energy (DE)) and ileal digestible lysine (0·36 g/MJ DE; Table 1) based on ileal digestibility estimates for the Dundale cultivar of field peas (van Barneveld *et al.* 1994*a*) and the proximate analysis of each respective cultivar (Table 2). In all diets, 0·25 g ileal digestible lysine/MJ DE was supplied from the respective field-pea treatments, with the remainder supplied as L-lysine monohydrochloride. To ensure lysine was limiting in the diets, all other essential amino acids were added to at least a 0·15 surplus relative to lysine. In addition, the balance of the essential amino acids relative to lysine was made identical for all diets, by estimating the availability of amino acids in the respective field-pea treatments (van Barneveld *et al.* 1994*c*). Monosodium glutamate and glutamic acid were added to balance the essential:non-essential amino acid ratio (46:54; Agricultural Research Council, 1981). Fuzone 200 (Furazolidone, 200 g/kg) was included to guard

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

Table 2. *Expts 1 and 2. Proximate composition and amino acid composition (g/kg, air-dry basis) of raw field peas* (Pisum sativum *cv. Wirrega*) *and field peas heated at 150° (cv. Wirrega), 165° (cv. Wirrega) or 150° (cv. Dundale) for 15 min respectively*

	Heat treatment			
Cultivar...	Raw Wirrega	150° Wirrega	165° Wirrega	150° Dundale
Crude protein (N × 6·25)	178	189	202	227
Dry matter	922	959	958	961
Light petroleum extract (b.p. 40°–60°)	14	17	20	15
Fibre extract				
crude	38	66	122	89
neutral-detergent	124	533	646	467
Ash	25	25	26	26
Amino acids				
Aspartic acid	22·0	21·8	22·0	24·8
Threonine	7·5	7·7	7·5	8·7
Serine	9·7	10·0	9·5	11·4
Glutamic acid	31·0	32·0	32·6	38·1
Proline	7·8	7·7	7·8	9·9
Glycine	8·3	8·6	8·7	9·8
Alanine	8·1	8·6	8·9	9·8
Cystine	1·5	1·2	1·0	1·3
Valine	9·0	9·5	9·7	10·8
Methionine	1·6	1·7	1·7	1·7
Isoleucine	8·3	8·8	8·9	9·7
Leucine	13·4	14·0	14·3	15·8
Tyrosine	6·2	6·3	6·2	7·2
Phenylalanine	9·0	9·4	9·4	10·6
Lysine	14·1	10·6	6·4	12·8
Histidine	4·2	4·3	4·0	5·2
Arginine	14·2	13·1	9·3	18·2
Silcock-reactive lysine	0·99	0·87	0·65	0·89

against a *Campylobacter* burden that was prevalent in the piggery at the time of experimentation. Cr_2O_3 was included in the diets as an indigestible marker to calculate ileal digestibilities.

Animals and procedures. Eight Large White female pigs of approximately 40 g were fitted with jugular catheters inserted via an auricular vein. They were then transferred to metabolism cages and housed in a thermoneutral environment. Water was supplied *ad lib.* via 'nipple' drinkers. Pigs were blocked on weight at the commencement of the experiment and position in the experimental facilities.

Diets were allocated in an incomplete block design (Ott, 1988). Each pig received two diets over the course of the experiment which was divided into two 14 d periods. Diet-adaptation periods (7 d) were allowed before 7 d collection periods. The feed was offered dry, and the daily feeding rate adjusted to three times maintenance (van Barneveld *et al.* 1994*a*) after weighings of the pigs before each collection. The pigs were fed every 3 h with an automatic feeder, to ensure utilization of the available dietary amino acids (Batterham & Murison, 1981).

Total collection of urine and faeces was made over 7 d for each diet. At 2 d before collection, bladder catheters were inserted in the pigs, to facilitate total urine collection. Urine was collected directly into 40 ml 5·5 M-H_2SO_4. Total urine output was measured daily and a 0·10 representative sample taken, bulked, and stored at −20° before chemical

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

analysis. Voided faeces were collected daily, bulked and stored at −20°. At the end of the collection period, faeces were thawed, mixed, subsampled and freeze-dried before chemical analysis.

On the final day of each collection period, serial blood sampling was conducted over 6 h (i.e. two 3 h feeding periods), with 10 ml samples taken via the auricular-external jugular vein catheter at 30 min intervals. The first blood sample was collected immediately before the first morning feed. Following collection the samples were allowed to clot at room temperature before being centrifuged at 2000 rev./min for 15 min. A portion of serum (5 ml) was then drawn off and stored at 4° before chemical analysis on the same day.

Plasma for amino acid and albumin analysis was collected at a set interval after feeding. At the completion of each 7 d collection period, before diet changes or the completion of the experiment respectively, a 10 ml blood sample was collected via the auricular-external jugular vein catheter, exactly 15 min after feeding 0·125 of the pigs' daily ration. This sample was placed in a heparinized tube and centrifuged at 2000 rev./min for 15 min. Plasma was drawn off, and the samples stored at −70° before amino acid analysis.

Following the final collection period, a sample of ileal digesta was collected from each pig using the direct ileal sampling procedure described by van Barneveld *et al.* (1994*a*).

The following factors were used to estimate the N contribution from components of the urine: 0·4665 to convert urea to urea N; 0·3715 to convert creatinine to creatinine N; 0·3333 to convert uric acid to uric acid N (Merck, 1976); 0·16 to convert protein to protein N (Agricultural Research Council, 1981).

Expt 2. The effect of heat on the ileal digestibility of nitrogen, dry matter and amino acids in field peas (cv. Wirrega)

As it was assumed that field peas of cv. Wirrega and cv. Dundale were affected equally by the chosen heat treatments, the aim of this experiment was to determine the effect of heat on the ileal digestibility of amino acids in cv. Wirrega field peas, and to compare these with the digestibility values for cv. Dundale (van Barneveld *et al.* 1994*a*), which were used for diet formulations in Expt 1.

Diets. Three diets were formulated to contain 1·5 g total N/MJ DE (Table 3). The raw peas (cv. Wirrega), and peas (cv. Wirrega) heated to 150° or 165° represented the only source of amino acids as the diets were sugar based. High inclusion of field peas was necessary to maintain lysine and crude protein levels in the diets. Chromic oxide was included as an indigestible marker to calculate digestibilities.

Animals and procedures. The three diets were allocated according to 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 position in the experimental facilities. Feed was offered dry for a period of 7 d, which included a 2 d diet-adaptation period. The daily feeding rate was adjusted to three times maintenance (3M) ($3 \times (0{\cdot}5$ MJ DE $W_{kg}^{0{\cdot}75})$/diet DE) and the pigs were fed every 3 h using automatic frequent feeders. On the eighth day, the terminal ileum was surgically removed and the contents collected using the direct ileal sampling technique described by van Barneveld *et al.* (1994*a*). The digesta collected was stored at −20° before being freeze-dried, ground and analysed.

Chemical analysis

Expt 1. Proximate composition, amino acid composition and Silcock-reactive lysine of the field peas (cv. Wirrega) were determined using techniques as described by van Barneveld *et al.* (1994*a*), with the exception that methionine and cystine were not pre-oxidized before hydrolysis.

N content of the urine and faeces was determined using the macro-Kjeldahl technique (Association of Official Analytical Chemists, 1984). Serum urea, uric acid, creatinine and

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

Table 3. *Expt 2. Components and composition of diets (g/kg, air-dry basis) for ileal digestibility determination in field peas* (Pisum sativum *cv. Wirrega*)

Diet no....	1	2	3
Component			
Field peas (cv. Wirrega)			
Raw	845·0	—	—
150°	—	750·0	—
165°	—	—	660·0
Sugar	102·5	197·5	272·5
Soya-bean oil	15·0	15·0	30·0
Minerals and vitamins*	5·0	5·0	5·0
Fuzone 200†	0·5	0·5	0·5
Dicalcium phosphate	30·0	30·0	30·0
Chromic oxide	2·0	2·0	2·0
Composition			
Crude protein	150·5	141·9	134·1
Digestible energy (DE) (Mj/kg)	16·07	15·05	14·25
Nitrogen: DE (g/MJ)	1·5	1·5	1·5
Total lysine: DE (g/MJ)	0·70	0·53	0·30

* For composition, see Table 1.
† Furazolidone 200 g/kg.

total protein were measured on a Beckman CX-5 autoanalyser using Synchron CX® reagents (Beckman Instruments (Australia) Pty Ltd., Gladesville, NSW, Australia). Urea was measured by an enzymic-rate method. Urea was hydrolysed by urease (*EC* 3.5.1.5) to NH_3 and CO_2. Glutamate dehydrogenase (*EC* 1.4.1.2) catalysed the condensation of NH_3 and α-ketoglutarate to glutamate with a concomitant oxidation of NADH to NAD and change in absorbance measured at 340 nm over a fixed time interval. Uric acid was measured by a timed-endpoint method. Uric acid was oxidized by uricase (*EC* 1.7.3.3) to produce allantoin and H_2O_2. The H_2O_2 reacts with 4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzene sulphonate in a reaction catalysed by peroxidase (*EC* 1.11.1.7) to produce a coloured product. Creatinine was measured by a modified-rate Jaffé method. Creatinine combines with picrate in an alkaline solution to form creatinine–picrate complex. Total serum protein was measured by a timed-endpoint biuret method. Peptide bonds in the protein sample bind to Cu^{2+} ions in an alkaline medium to form coloured peptide–copper complexes. Plasma albumin was determined after reaction with Bromocresol Green dye and analysis on a Beckman CX-5 Autoanalyser.

Urine urea, uric acid and creatinine were measured using similar techniques to those described for serum with the exception that the analysis was completed on a COBAS MIRA autoanalyser (Roche Products, Frenchs Forest, NSW, Australia) which automatically buffered samples before analysis. Urine total protein was determined using three different techniques. The first technique measured protein after reaction with Modified Coomassie® Brilliant Blue (Biorad, Hornsby, NSW, Australia) and analysis on a COBAS-MIRA autoanalyser. The second method measured urine protein after reaction with Coomassie® Protein Assay Reagent (Pierce, Illinois, USA) on a Shimadzu UV-160A spectrophotometer (Shimadzu Corporation, Kyoto, Japan) read at 595 nm within 90 min. The third method measured urine protein after samples were adjusted to neutral pH (7·0) and reacted with bicinchoninic acid (BCA; Pierce, Illinois, USA) reagent.

Total lysine in the urine and amino acids in the faeces were determined using reverse-phase chromatography and measured after reaction with phenylisothiocyanate (PITC).

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

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. Free urine lysine and plasma lysine were determined using reverse-phase chromatography following filtering of the urine and plasma through ultra-free filters (Waters ultra-free UFC30HV00; Millipore, Australia Pty Ltd, Lane Cove, NSW, Australia).

Expt 2. Techniques used to measure Cr, N, dry matter and amino acids were as described by van Barneveld *et al.* (1994*a*).

Statistical analysis

Expts 1 and 2. The results were analysed by analysis of variance, utilizing a general linear model, with the treatment means separated by least significant difference (LSD).

RESULTS

The effect of heat on the proximate analysis and chemical composition of field peas (cv. Wirrega)

The application of heat at 150° or 165° to field peas (cv. Wirrega) increased the crude protein, dry matter and light petroleum extract content (Table 2). Substantial increases in crude and neutral-detergent fibre were also evident. Ash content was unaffected by heat application.

Heating field peas (cv. Wirrega) to 150° or 165° resulted in a substantial decrease in the total lysine content (Table 2). Cystine and arginine levels were also depressed, while the remainder of the amino acids were comparatively unaffected by heating. There was also a substantial decrease in the quantity of Silcock-reactive lysine in peas heated to 165°.

Expt 1. The effect of heating field peas on the nitrogen balance and urine, serum and plasma composition

Results for cv. Wirrega treatments (diet nos 1, 2 and 3 only). Despite a significant increase ($P < 0.01$) in total N intake of pigs fed on diets containing heated peas (Table 4) there was a substantial decrease in the urine N output of these pigs, although this difference was not statistically significant ($P > 0.05$). There was a substantial decrease in the mean daily urine output of pigs fed on heated peas, but again this difference was not significant ($P > 0.05$; Table 4). There was a significant increase ($P < 0.001$) in the quantity of N excreted in the faeces of pigs fed on heated peas. There was no significant difference ($P > 0.05$) in the N balance or apparent biological value of the field-pea treatments. Heating resulted in a significant decrease ($P < 0.01$) in apparent net protein utilization.

Pigs fed on diets containing field peas heated to 150° or 165° had a significantly lower ($P < 0.01$) daily output of urea and uric acid in the urine (Table 5). Despite no significant change ($P > 0.05$) in the daily creatinine output, the proportion of creatinine N in the urine increased from 0·066 with the diet containing the raw peas, to 0·14 with the diet containing peas heated to 165°. Estimates of daily N output measured as the sum of urea, creatinine, uric acid and protein were higher than estimates of total N output determined using the macro-Kjeldahl procedure (Table 4).

There was substantial variation in the estimate of total protein content of the urine, depending on the technique used for estimation (Table 5). Despite this variation, all techniques revealed a general trend for the total protein content of the urine to increase with diets containing peas heated to 165°. Total protein determined using both Coomassie procedures revealed significant increases ($P < 0.05$) in daily urinary protein output in pigs fed on diets containing peas heated to 165°.

There was no significant difference ($P > 0.05$) in the daily output of total lysine or free

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

Table 4. *Expt 1. Nitrogen intake (g/d), urinary faecal nitrogen output (g/d), nitrogen balance, apparent net protein utilization (NPU), and apparent biological value (BV) for pigs fed on diets containing raw field peas* (Pisum sativum *cv. Wirrega*) *or field peas heated to 150° (cv. Wirrega), 165° (cv. Wirrega) or 150° (cv. Dundale)*†

	Heat treatment				Statistics	
Cultivar...	Raw Wirrega	150° Wirrega	165° Wirrega	150° Dundale	Treatment	SEM
Intake						
N intake	36·33	39·74	51·75	40·06	**	2·402
Urine						
Daily urine (g)	4010	3685	2291	2987	NS	880·55
Urine N	10·40	9·29	6·18	12·54	*	1·378
Faeces						
Daily faeces (g)	77·4	123·1	469·8	147·4	***	16·40
Faeces N	2·95	6·18	26·17	7·37	***	0·947
N balance						
Balance	22·98	24·26	19·40	20·15	NS	2·092
Apparent NPU‡	0·63	0·62	0·37	0·50	**	0·040
Apparent BV§	0·69	0·72	0·75	0·61	NS	0·047

NS, not significant.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
† For details of diets and procedures, see Tables 1 and 2 and pp. 260–263.
‡ (N intake − (urine N + faeces N))/N intake.
§ (N intake − (urine N + faeces N))/(N intake − faeces N).

Table 5. *Expt 1. Urea, creatinine, uric acid and protein nitrogen output (g/d) in urine of pigs fed on diets containing raw field peas* (Pisum sativum *cv. Wirrega*) *or field peas heated to 150° (cv. Wirrega), 165° (cv. Wirrega) or 150° (cv. Dundale)*†

	Heat treatment				Statistics	
Cultivar...	Raw Wirrega	150° Wirrega	165° Wirrega	150° Dundale	Treatment	SEM
Urea N	34·45	25·18	8·04	23·72	**	3·369
Creatinine N	2·47	1·97	1·32	1·87	NS	0·330
Uric acid N	0·061	0·026	0·014	0·021	***	0·006
Protein N						
Coomassie 1‡	0·025	0·033	0·080	0·025	*	0·013
Coomassie 2§	0·003	0·012	0·024	0·005	*	0·004
BCA reagent	0·83	1·15	2·20	1·22	NS	0·496

NS, not significant; BCA, bicinchoninic acid.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
† For details of diets and procedures, see Tables 1 and 2 and pp. 260–264.
‡ Determined using Modified Coomassie® Brilliant Blue Total Protein Assay (Biorad, Hornsby, NSW, Australia).
§ Determined using Coomassie® Protein Assay Reagent (Pierce, Illinois, USA).

lysine in the urine of pigs fed on raw or heated peas (Table 6) despite the fact that daily urinary total lysine output was almost halved in pigs fed on peas heated to 165°. The free urinary lysine:total urinary lysine ratios were 0·21, 0·53 and 0·66 in the raw peas and peas heated to 150° and 165° respectively. This increase was not significant ($P > 0·05$).

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

Table 6. *Expt 1. Total lysine (mg/g) and free lysine (mg/d) excreted in the urine of pigs fed on diets containing raw peas* (Pisum sativum *cv. Wirrega; diet 1) or peas heated to 150° (cv. Wirrega; diet 2), 165° (cv. Wirrega; diet 3) or 150° (cv. Dundale; diet 4)*†

	Heat treatments				Statistics	
Cultivar...	Raw Wirrega	150° Wirrega	165° Wirrega	150° Dundale		
Diet...	1	2	3	4	SEM	Diet
Total lysine	32	34	20	38	9·1	NS
Free lysine	7	13	12	11	2·8	NS
Free lysine: total lysine	0·22	0·38	0·60	0·29		

NS, not significant.
† For details of diets and procedures, see Tables 1 and 2 and pp. 260–265.

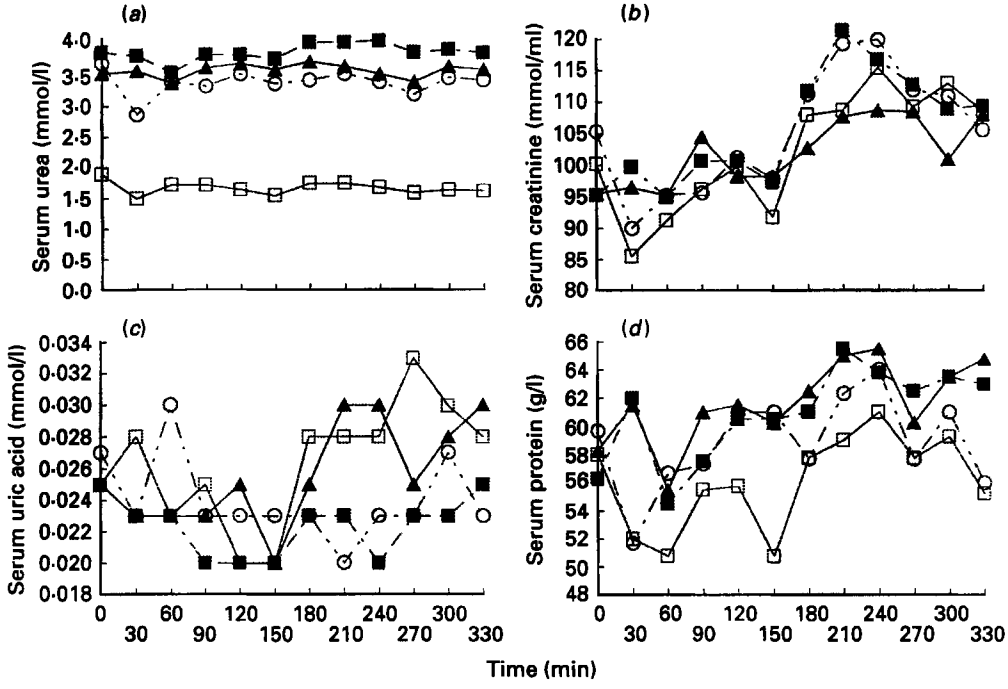

Fig. 1. Expt 1. Serum levels of (*a*) urea, (*b*) creatinine, (*c*) uric acid and (*d*) protein in pigs fed on raw peas (*Pisum sativum* cv. Wirrega: ▲—▲), or peas heated to 150° (cv. Wirrega; ○···○), 165° (cv. Wirrega; □—□) or 150° (cv. Dundale; ■–·–■) measured at 30-min intervals. Feeding was at 0 and 180 min. Statistical analysis revealed the following: serum urea, pooled SEM 0·224, diet effect $P < 0·001$, time effect not significant (NS), diet × time effect NS; serum creatinine, pooled SEM 7·501, diet effect NS, time effect $P < 0·001$, diet × time effect NS; serum uric acid, pooled SEM 0·0035, diet effect $P < 0·05$, time effect NS, diet × time effect NS; serum protein, pooled SEM 3·214, diet effect $P < 0·001$, time effect $P < 0·01$, diet × time effect NS.

Serum urea and serum protein levels were significantly lower ($P < 0·001$) in pigs fed on diets containing peas heated to 165° (Fig. 1). Serum creatinine and serum uric acid levels were not affected by heat treatment. In addition, no significant changes ($P > 0·05$) were observed in the plasma lysine or plasma albumin levels of pigs fed on raw or heated field peas (Table 7).

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

Table 7. *Plasma lysine (mg/l) and plasma albumin (g/l) concentrations of pigs fed on diets containing raw peas* (Pisum sativum *cv. Wirrega; diet 1) or peas heated to 150° (cv. Wirrega; diet 2), 165° (cv. Wirrega; diet 3) or 150° (cv. Dundale; diet 4)*†

	Heat treatment				Statistics	
Cultivar...	Raw Wirrega	150° Wirrega	165° Wirrega	150° Dundale		
Diet...	1	2	3	4	SEM	Diet
Plasma lysine (mg/l)	24·09	16·42	22·40	14·75	2·762	NS
Plasma albumin (g/l)	28·50	29·33	29·75	29·00	2·025	NS

NS, not significant.
† For details of diets and procedures, see Tables 1 and 2 and pp. 260–265.

Table 8. *Expt 1. The faecal digestibility† of nitrogen, dry matter and amino acids in raw field peas* (Pisum sativum *cv. Wirrega) and field peas heated to 150° (cv. Wirrega), 165° (cv. Wirrega) or 150° (cv. Dundale) for 15 min respectively assessed using total collection techniques*‡

	Heat treatment				Statistics	
Cultivar...	Raw Wirrega	150° Wirrega	165° Wirrega	150° Dundale	Treatment	SEM
N	0·83	0·75	0·46	0·70	***	0·027
Dry matter	0·95	0·93	0·74	0·91	***	0·007
Aspartic acid	0·92	0·85	0·56	0·80	***	0·018
Threonine	0·83	0·77	0·49	0·70	***	0·023
Serine	0·85	0·80	0·55	0·75	***	0·020
Glutamic acid	0·91	0·86	0·59	0·82	***	0·016
Proline	0·87	0·80	0·49	0·70	***	0·023
Glycine	0·85	0·78	0·49	0·71	***	0·022
Alanine	0·79	0·73	0·46	0·67	***	0·028
Cystine	—	—	—	—	—	—
Valine	0·87	0·81	0·59	0·75	***	0·021
Methionine	—	—	—	—	—	—
Isoleucine	0·86	0·80	0·58	0·75	***	0·022
Leucine	0·86	0·80	0·56	0·75	***	0·021
Tyrosine	0·83	0·76	0·47	0·69	***	0·024
Phenylalanine	0·87	0·81	0·56	0·76	***	0·020
Lysine	0·89	0·78	0·38	0·74	***	0·021
Histidine	0·90	0·85	0·56	0·79	***	0·017
Arginine	0·92	0·86	0·57	0·83	***	0·016

*** $P < 0·001$.
† Digestibility calculated assuming free amino acids and monosodium glutamate added to experimental diets were fully digested.
‡ For details of diets and procedures, see Tables 1 and 2 and pp. 260–263.

Faecal digestibility of the field peas contained in the experimental diets (Table 1) was estimated assuming that the free amino acids and monosodium glutamate added to the diet were fully digested. Heat application resulted in a significant decrease ($P < 0·001$) in the faecal digestibility of N, dry matter and all amino acids (Table 8). Ileal digestibility of N, dry matter and amino acids determined using collection from the eight pigs at the end of

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

Table 9. *Expt 2. The ileal digestibility of nitrogen, dry matter and amino acids in raw field peas* (Pisum sativum *cv. Wirrega*) *and field peas* (*cv. Wirrega*) *heated to 150° or 165° for 15 min respectively assessed using the direct ileal sampling technique*†

	Heat treatment			Statistics	
	Raw	150°	165°	Treatment	SEM
N	0·73	0·63	0·44	***	0·036
Dry matter	0·54	0·59	0·60	NS	0·032
Aspartic acid	0·79	0·62	0·43	***	0·043
Threonine	0·72	0·61	0·42	**	0·044
Serine	0·75	0·62	0·49	**	0·039
Glutamic acid	0·84	0·70	0·49	***	0·036
Proline	0·74	0·63	0·47	**	0·038
Glycine	0·68	0·56	0·37	**	0·044
Alanine	0·70	0·61	0·43	**	0·041
Cystine	0·68	0·62	0·49	*	0·039
Valine	0·74	0·67	0·49	**	0·040
Methionine	0·79	0·69	0·48	**	0·049
Isoleucine	0·77	0·70	0·50	**	0·041
Leucine	0·75	0·68	0·50	**	0·043
Tyrosine	0·80	0·73	0·51	**	0·038
Phenylalanine	0·82	0·74	0·55	**	0·039
Lysine	0·82	0·64	0·35	***	0·043
Histidine	0·82	0·72	0·53	***	0·035
Arginine	0·87	0·80	0·63	***	0·028

NS, not significant.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
† For details of diets and procedures, see Tables 1 and 2 and pp. 260–263.

Expt 1 were not meaningful due to the small number of replications, and hence the results have not been presented.

Comparison of results between cv. Wirrega peas heated to 150° and cv. Dundale peas heated to 150°. There was no significant difference between urine N output, daily faeces N output, N balance, apparent net protein utilization, urinary and serum N components, plasma lysine and albumin for the two cultivars subjected to the 150° treatment with the exception of serum uric acid which was significantly lower ($P < 0·05$) in pigs fed on cv. Dundale peas.

The faecal digestibility of N, dry matter and amino acids was consistently lower in cv. Dundale peas heated to 150° compared with cv. Wirrega peas heated to 150°. There was close agreement between the faecal digestibility of amino acids determined in the current experiment for cv. Dundale peas, and those determined by van Barneveld *et al.* (1994*a*). In addition, cv. Wirrega peas heated to 165° produced faecal N, dry matter and amino acid digestibilities that were substantially lower than those reported for cv. Dundale subjected to the same heat treatment by van Barneveld *et al.* (1994*a*).

Expt 2. The effect of heat on the ileal digestibility of nitrogen, dry matter and amino acids in field peas (*cv. Wirrega*)

The application of heat at 165° resulted in a significant decrease in the ileal digestibility of N ($P < 0·001$), and all amino acids in field peas of the Wirrega cultivar (Table 9). Dry matter digestibility was unaffected by the application of heat. Based on these values, N and lysine digestibility estimates used to formulate diet nos 1, 2 and 3 in Expt 1 were significant overestimates for cv. Wirrega. Using the determined digestibility values for cv. Wirrega

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

Table 10. *Comparison of nitrogen retained (g/d), nitrogen retained: ileal digestible (ID) nitrogen intake, and urine nitrogen: ID nitrogen intake estimated using nitrogen digestibility used in diet formulations or nitrogen digestibility estimated using direct ileal sampling*

	Heat treatment				Statistics	
Cultivar...	Raw Wirrega	150° Wirrega	165° Wirrega	150° Dundale	Treatment	SEM
N digestibility (estimated)†						
ID N intake (g/d)	29·94	31·18	29·57	31·09	NS	1·825
N retained‡	19·53	21·89	23·40	18·56	NS	2·033
N retained:ID N intake	0·65	0·70	0·79	0·59	NS	0·049
Urine N: ID N intake	0·35	0·30	0·21	0·41	NS	0·049
N digestibility (determined)§						
ID N intake (g/d)	31·53	30·40	23·52	31·09	*	1·811
N retained‡	21·12	21·11	17·34	18·56	NS	2·006
N retained:ID N intake	0·67	0·70	0·73	0·59	NS	0·050
Urine N:ID N intake	0·33	0·30	0·27	0·41	NS	0·050

NS, not significant.
* $P < 0·05$.
† Digestibility values for cv. Dundale used in diet formulations.
‡ N retained = (ileal digestible N − urine N).
§ Digestibility values determined for the respective treatments.

field peas, N retention, ileal digestible N intake, the proportion of ileal digestible N retained, and the proportion of ileal digestible N excreted in the urine were recalculated, and are presented in Table 10. With the exception of ileal digestible N, there was no significant difference ($P > 0·05$) between these values.

DISCUSSION

The effect of heating field peas on the nitrogen balance and urine, serum and plasma composition

Diets were formulated using digestibility values determined using cv. Dundale field peas (van Barneveld *et al.* 1994*a*), and it was assumed that these would be similar in cv. Wirrega field peas based on similar Silcock-reactive lysine estimates. In Expt 2, however, it was revealed that heat applied to cv. Wirrega peas at 165° lowered lysine digestibility from 0·82 to 0·35, almost half the value of 0·62 determined for cv. Dundale peas used in diet formulations. Hence, ileal digestible N and lysine intakes of pigs fed on cv. Wirrega peas heated to 165° were greatly reduced. Estimates for the raw peas and peas heated to 150° were similar for both cultivars and, hence, these values can still be considered.

By accounting for the overestimation of N digestibility in the cv. Wirrega peas (Table 10) there was still no significant difference between apparent N retention, the proportion of ileal digestible N retained and the proportion of ileal digestible N excreted in the urine. The original hypothesis suggested that the proportion of ileal digestible N in urine may increase with the excretion of poorly utilized amino acids. In addition, the overestimation of lysine digestibility in cv. Wirrega peas heated to 165° meant that a greater imbalance of amino acids existed in these diets, and hence the proportion of ileal digestible N excreted in the urine would be expected to be further increased.

An explanation for the above contrast is that heat did not affect the utilization of ileal digestible lysine from the cv. Wirrega peas. However, as the N balance of the cv. Dundale

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

peas heated to 150° was also unaffected, this is highly unlikely. Instead, it appears that variability associated with N balance may have reduced the sensitivity of comparison. In the study of van Barneveld *et al.* (1994*b*), significant decreases in daily protein deposition and the retention of ileal digestible protein in pigs fed on heated peas were detected using growth assays and empty-body analysis. Coefficients of variation for these variables were in the vicinity of 6–7, and the decrease was of the order of 0·15. The coefficients of variation of 6–7 for protein deposition and energy retention may be slight underestimates of the true variability, as all pigs were assumed to have the same protein content at the start of the experiment (139 g/kg; based on the composition of ten similar pigs killed at 20 kg live weight). In the current N balance experiment there was a difference in apparent N retention of 0·18 between pigs fed on raw peas (cv. Wirrega) and pigs fed on peas heated to 165° (cv. Wirrega). A 0·12 decrease in the retention of ileal digestible N existed between pigs fed on raw peas (cv. Wirrega) and cv. Dundale peas heated to 150°. These differences were not significant as the coefficients of variation for these estimates were in the range of 13–30. This exemplifies the variability associated with N balance experiments and clearly shows why no significant difference was detected with only four replicates. Based on these coefficients of variation, approximately 15–30 replicates would be required to detect a 0·15 decrease in N retention with a 0·90 probability (Cochran & Cox, 1957).

The N contribution from urea in pigs fed on raw peas exceeded the total N estimate using the macro-Kjeldahl technique by 24·05 g/d. Despite the mean urea concentration for this diet (317 mmol/l) falling well within the normal range of 163–597 mmol/l for growing pigs with a mean daily urine output of 2–6 litres (Pond & Houpt, 1978), urea values appear to be substantial overestimates. This is exemplified if we consider that pigs fed on diets containing raw peas consumed an average of 36·33 g N/d but apparently excreted 34·45 g/d as urea N. It appears that a normal constituent of pig urine interferes with urea analysis using human pathology techniques. In addition, this interfering factor appears to be substantially reduced in the urine of pigs fed on heated peas. Assuming that 0·70 of urine N is in the form of urea N (Hawk, 1965), there was a far greater overestimate in the urine of pigs fed on raw peas (473 %) than in the urine of pigs fed on peas heated to 165° (185 %). There is a need to identify the cause of this overestimation.

There was wide variation in the estimates of urine protein content. This difference may be due to Coomassie stain being very irregular and exhibiting wide variation in response to proteins. Some proteins do not bind to Coomassie stain at all (Rawn, 1991), while the amount of stain bound can vary considerably with other proteins (Kley & Hale, 1977; Pierce & Suelter, 1977). Discrepancies between values obtained using the Coomassie techniques may be due to samples analysed using the Coomassie no. 2 technique not being adjusted to neutral pH.

Regardless of technique used, and despite a significantly lower ileal digestible N and lysine intake, daily urine protein excretion was at least three times, and up to eight times, greater in urine from pigs fed on diets containing peas heated to 165°. The lack of statistical difference between the urinary protein in the raw peas and peas heated to 150° or 165° determined using the BCA reagent is likely to be due to the small number of replicates (two per diet) for this analysis. Due to time and resource constraints, only urine samples from the second collection period were analysed in this way. The high protein levels obtained using the BCA technique are acceptable if we consider that proteinuria appears to occur in normal pigs (Pond & Houpt, 1978). The glomerular capillary basement membrane in pigs appears to be permeable to protein molecules. If urinary protein excretion levels determined using the BCA technique are correct, it suggests that some of the poorly utilized protein from heat-processed protein concentrates (Batterham *et al.* 1990; Beech *et al.* 1991; van Barneveld *et al.* 1994*b*) may be excreted as intact protein in urine.

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

The trend for the proportion of free lysine to increase in the urine of pigs fed on heated peas may reflect some degree of lysine racemization during heating (Schwass & Finley, 1984) resulting in poor utilization and subsequent excretion in the urine. The lack of statistical difference between the levels of free and total lysine in the urine most likely reflects the inaccuracies and difficulties associated with chemical analysis of urine.

The overestimation of ileal digestible N and lysine in the diets of pigs fed on peas heated to 165° is likely to account for the depressed serum urea and protein levels. Other variables, however, are comparable. It appears that serum variables are not affected by diets containing heated proteins. This is supported by the fact that there was no difference in serum urea, protein or creatinine in pigs fed on raw peas (cv. Wirrega; in which ileal digestibility of N and lysine was actually overestimated), cv. Wirrega peas heated to 150° and cv. Dundale peas heated to 150°. The lack of difference in plasma lysine levels may reflect inaccuracies associated with sampling at a site other than the portal vein or the fact that an equilibrium in plasma amino acid levels is rapidly achieved by the body, and therefore, dietary effects are difficult to detect.

Comparison of the effect of heat on the proximate composition, chemical composition and ileal digestibility of nitrogen and lysine in cv. Wirrega and cv. Dundale field peas

The effect of heat on the proximate composition of cv. Wirrega peas appears different to the effects described for cv. Dundale peas by van Barneveld *et al.* (1994*a*). The most notable of these differences is the increase in the light petroleum extract content in cv. Wirrega peas compared with a significant linear decrease in cv. Dundale. This suggests that the loss of volatile compounds from the cv. Wirrega was not as great as that from cv. Dundale. Both cultivars exhibited a marked increase in the crude and neutral-detergent fibre content most likely attributable to reasons discussed by van Barneveld *et al.* (1994*a*).

Changes in amino acid composition were similar for both cultivars; however, the loss of total lysine was far more pronounced in cv. Wirrega. As described by van Barneveld *et al.* (1994*a*), losses in lysine, cystine and arginine in cv. Wirrega are likely to be due to early and advanced Maillard reactions. Silcock-reactive lysine for cv. Wirrega peas was 0·65 compared with 0·70 for cv. Dundale peas heated to the same level. Hence, there was no reason to assume that the lysine remaining in the cv. Wirrega peas would be significantly different to that remaining in the cv. Dundale peas.

The difference in the digestibility of N and lysine between cv. Wirrega and cv. Dundale peas shows that heat can induce variable effects on ileal digestibility depending on the starting nature of the protein source before heating. The greater reduction in total lysine content suggests that the cv. Wirrega peas were more susceptible to heat damage than cv. Dundale. If binding, other than the Maillard reactions, was more prolific between the remaining amino acids of the cv. Wirrega peas, the protein may have been modified so that it was more resistant to enzymic attack during digestion (van Barneveld *et al.* 1994*a*). There is no basis to suggest, however, that lysine utilization in the cv. Wirrega peas was not reduced to a greater extent than digestibility, and was thus the reason for a lack of significant difference between the N balance of pigs fed on the respective heat treatments.

Overall, the results exemplify the fact that it is not possible to draw general conclusions as to the effects of heat on any particular protein concentrate. The application of identical heat treatments to similar cultivars of field peas resulted in significant differences in the proximate analysis, amino acid composition, and ileal and faecal digestibility of N and amino acids. The current study did not reflect the poor utilization of ileal digestible lysine from heated proteins reported previously (van Barneveld *et al.* 1994*b*). This was attributed to the variability in N balance and problems associated with urine analysis. However, despite considerable variation in the results, it is possible that a large proportion of non-

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press

utilizable amino acids in heated field peas may be excreted from the pig via the urine in the form of a protein. This is based on the excretion of up to 13·76 g protein/d in the urine of pigs fed on peas heated to 165° (cv. Wirrega) compared with 5·20 g/d in the urine of pigs fed on raw peas (cv. Wirrega) as determined using BCA reagent.

The authors are grateful to Messrs R. C. Wilson, J. Savins and A. W. Davis for pig management and skilled technical assistance, Ms J. Baker (South Australian Department of Agriculture) for Silcock-reactive lysine analysis and amino acid analysis, Ms W. E. Peasley for amino acid analysis, Dr J. A. Fernandez (National Institute of Animal Science, Denmark) for technical assistance with bladder catheter placement, Mr B. Beshaw and the staff of the Lismore Pathology Laboratories for analysis of blood and urine, Dr R. O. Ball (University of Guelph, Canada) for protein analysis of urine and technical advice, 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.

REFERENCES

Agricultural Research Council (1981). *The Nutrient Requirements of Pigs*. Slough: Commonwealth Agricultural Bureaux.

Association of Official Analytical Chemists (1984). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th ed. Washington DC: Association of Official Analytical Chemists.

Batterham, E. S., Andersen, L. M. & Baigent, D. R. (1993). Utilization of ileal digestible amino acids by growing pigs: methionine. *British Journal of Nutrition* **70**, 711–720.

Batterham, E. S., Andersen, L. M., Baigent, D. R., Beech, S. A. & Elliott, R. (1990). Utilization of ileal digestible amino acids by pigs: lysine. *British Journal of Nutrition* **64**, 679–690.

Batterham, E. S. & Murison, R. D. (1981). Utilization of free lysine by growing pigs. *British Journal of Nutrition* **46**, 87–92.

Beech, S. A., Batterham, E. S. & Elliott, R. (1991). Utilization of ileal digestible amino acids by growing pigs: threonine. *British Journal of Nutrition* **65**, 381–390.

Cochran, W. G. & Cox, G. M. (1957). *Experimental Design*, 2nd ed. New York: John Wiley and Sons.

Hawk, P. B. (1965). *Hawk's Physiological Chemistry*, 14th ed. [B. L. Oser, editor]. New York: McGraw-Hill.

Kley, H. V. & Hale, S. M. (1977). Assay for protein by dye-binding. *Analytical Biochemistry* **81**, 485–487.

Merck (1976). *The Merck Index: An Encyclopaedia of Chemicals and Drugs*, 9th ed [M. Windholz, editor]. New Jersey: Merck and Co. Inc.

Ott, L. (1988). *An Introduction to Statistical Methods and Data Analysis*, 3rd ed. Boston: PWS-Kent Publishing Company.

Pierce, J. & Suelter, C. H. (1977). An evaluation of the Coomassie Brilliant Blue G-250 dye-binding method for quantitative protein determination. *Analytical Biochemistry* **82**, 478–480.

Pond, W. G. & Houpt, K. A. (1978). *The Biology of the Pig*. Ithaca: Comstock Publishing Associates.

Rawn, J. D. (1991). *Proteins, Energy and Metabolism*. North Carolina: Neil Patterson Publishers.

Schwass, D. E. & Finley, J. W. (1984). Heat and alkaline damage to proteins: racemization and lysinoalanine formation. *Journal of Agricultural and Food Chemistry* **32**, 1377–1382.

van Barneveld, R. J., Batterham, E. S. & Norton, B. W. (1994*a*). The effect of heat on amino acids for growing pigs. 1. A comparison of apparent ileal and faecal digestibilities of amino acids in raw and heat-treated field peas (*Pisum sativum* cultivar Dundale). *British Journal of Nutrition* **72**, 221–241.

van Barneveld, R. J., Batterham, E. S. & Norton, B. W. (1994*b*). 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). *British Journal of Nutrition* **72**, 243–256.

van Barneveld, R. J., Batterham, E. S. & Norton, B. W. (1994*c*). 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. *British Journal of Nutrition* **72**, 257–275.

Printed in Great Britain

https://doi.org/10.1079/BJN19950028 Published online by Cambridge University Press