

Utilization of ileal digestible amino acids by growing pigs: isoleucine

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Two experiments were conducted to determine the utilization of ileal digestible isoleucine by growing pigs. In the first, the apparent ileal digestibility of amino acids in cottonseed meal, lupin-seed meal and soya-bean meal was determined in pigs fitted with 'T'-shaped cannulas. In the second, three isoleucine-deficient diets were formulated to 0.23 g ileal digestible isoleucine/MJ digestible energy (DE) with the three protein concentrates contributing the only source of isoleucine in sucrose-based diets. An additional three diets were formulated with supplements of isoleucine to confirm that isoleucine was limiting in the first three diets. The growth performance and retention of isoleucine by pigs given the six diets over the 20–45 kg growth phase were then determined. The apparent ileal digestibility of isoleucine in the three protein concentrates (proportion of total) was: cottonseed meal 0.68, lupin-seed meal 0.86, soya-bean meal 0.86. There were no significant differences ($P > 0.05$) in growth rates (g/d) and crude protein deposition rates (g/d) of the pigs given the three diets formulated to 0.23 g ileal digestible isoleucine/MJ DE: cottonseed meal 590, 84; lupin-seed meal 613, 87; soya-bean meal 594, 91 (SEM 13.0, 2.9) respectively. The response of pigs to the addition of isoleucine confirmed that isoleucine was limiting in these diets. The proportion of ileal digestible isoleucine retained by pigs given the cottonseed meal (0.65) was slightly lower than that retained by pigs given soya-bean meal (0.73; $P < 0.05$). These results indicate that values for the ileal digestibility of isoleucine in protein concentrates more closely reflect the proportion of isoleucine that can be utilized by the pig than occurs for other amino acids such as lysine, threonine and methionine.

Ileal digestibility: Isoleucine: Amino acid utilization: Pigs

Previous work has shown that the ileal digestibility assay over-estimates the availability of lysine, threonine, methionine and tryptophan in heat-processed protein concentrates for growing pigs (see Batterham *et al.* 1990a). It appears that a considerable proportion of these amino acids may be absorbed in a form or forms that is inefficiently utilized. However, these amino acids appear to vary in their susceptibility to heat-damage, and lysine appeared more susceptible than the other amino acids. With tryptophan there were also indications that the total amount of tryptophan in the protein concentrates may have been underestimated (Batterham *et al.* 1994).

There appears to be no information in the literature on the relationship between ileal digestibility and availability for the other essential amino acids. Information on the availabilities of all essential amino acids is important for a number of reasons: to formulate diets in terms of the ratio of essential amino acids relative to lysine (or 'ideal' protein, Agricultural Research Council, 1981), to feed more balanced diets to minimize the excretion of excess N in the urine, and to supply the technical information on feedstuffs for computer simulation models of the pig. There is now also greater potential for other essential amino acids to become limiting, as the most often limiting amino acids (lysine,

methionine, threonine and tryptophan) are now produced industrially for inclusion in commercial diets. In particular, isoleucine could become limiting in diets for growing pigs.

The aims of the experiments reported in this paper were to determine the ileal digestibility of isoleucine in three protein concentrates, to assess whether these values were suitable for formulating diets, and to measure the retention of ileal digestible isoleucine from different protein concentrates by growing pigs.

EXPERIMENTAL

Protein concentrates

The three protein concentrates used were a 'prepress' solvent-extracted cottonseed meal, a lupin-seed meal (*Lupinus angustifolius* cv. Gungurra) and a 'prepress' solvent-extracted soya-bean meal (Table 1). These three meals represented the range in estimated availability of lysine in protein concentrates (Standing Committee on Agriculture, 1987). Cottonseed meal represents a meal of estimated low lysine availability (0.40). It contains no anti-nutritional factors for pigs, other than free gossypol, which can be inactivated by the addition of FeSO_4 to the diet, which binds the free gossypol (Tanksley & Knabe, 1981). Pigs can tolerate 100 mg free gossypol/kg in the diet without effect, or at least 500 mg/kg with FeSO_4 (free gossypol-Fe 1:1). This is over twice the level of free gossypol in the meal used in these studies (198 mg/kg). Lupin-seed meal is of medium lysine availability (0.55). It is an unusual protein concentrate in that it has only a medium lysine availability even though it is fed raw following coarse crushing only. Soya-bean meal represents a meal of high lysine availability (0.88) for pigs.

Expt 1. Ileal digestibility of amino acids

Diets. Three diets were formulated with cottonseed meal, lupin-seed meal and soya-bean meal as the only source of amino acids in sucrose-based diets (Table 2). The cottonseed meal contained 12240 and 535 mg total and free gossypol/kg respectively and FeSO_4 was added to inactivate any effects that the free gossypol may have on the pigs (Tanksley & Knabe, 1981). Cr_2O_3 was used as an indigestible marker to calculate digestibilities.

Animals and procedures. Four male pigs of approximately 40 kg live weight were fitted with 'T'-shaped cannulas about 300 mm anterior to the terminal ileum. After 7 d the pigs were gradually introduced to the experimental diets. The pigs were given each of the diets in a completely randomized block design. They were given 0.70 kg diet mixed with about 1 litre of water at 12 h intervals. Water was also available between feeds. The diets were offered for 8 d and on days 7 and 8 ileal digesta samples of 70–100 g were collected from the cannulas over the 2–8 h period after feeding.

The samples were stored at -15° until the completion of the collections, then thawed, bulked for each collection period for each diet, freeze-dried and ground, before chemical analysis.

Expt 2. Utilization of ileal digestible isoleucine

Diets. Three diets were formulated to contain 0.23 g ileal digestible isoleucine/MJ digestible energy (DE; diets 1, 2 and 3; Table 3). This level of isoleucine was chosen after considering the relationship between isoleucine and lysine. In previous studies with lysine a level of 0.36 g ileal digestible lysine/MJ DE was used, as it represents a concentration at which the growth rate of the pig responds in a linear manner to lysine concentration but is near the concentration at which lysine retention plateaus (Batterham *et al.* 1990*b*). A similar relationship was assumed for isoleucine and the level of 0.23 g ileal digestible

Table 1. *Composition (g/kg, air-dry basis) of the cottonseed meal, lupin-seed meal and soya-bean meal*

	Cottonseed meal	Lupin-seed meal	Soya-bean meal
Crude protein (N × 6.25)	364	295	453
Dry matter	898	902	888
Light petroleum (b.p. 40–60°) extract	22	56	26
Crude fibre	138	157	75
Ash	64	29	63
Gross energy (MJ/kg)	17.4	18.2	17.7
Gossypol – total	12.2	—	—
free	0.54	—	—
Amino acids			
Asp	34.5	26.7	53.9
Thr	12.6	9.6	18.5
Ser	17.3	14.1	25.3
Glu	74.7	56.3	86.2
Pro	14.9	11.0	24.0
Gly	16.1	11.5	20.3
Ala	15.0	9.2	19.9
Cys	> 3.2	> 1.3	> 4.5
Val	18.3	11.7	23.9
Met	> 4.9	> 1.3	> 5.6
Ile	12.9	11.7	22.9
Leu	22.5	18.4	35.7
Tyr	10.4	9.0	16.4
Phe	20.0	10.5	23.7
Lys	16.6	13.0	28.4
His	9.8	7.0	11.5
Arg	39.9	27.1	33.9

Table 2. *Expt 1. Composition of the diets (g/kg, air-dry basis) for ileal digestibility studies in pigs*

Protein concentrate ...	Cottonseed meal	Lupin-seed meal	Soya-bean meal
Protein concentrate	300	300	300
Minerals and vitamins*	5	5	5
Dicalcium phosphate	30	30	30
FeSO ₄ · 7H ₂ O	1	—	—
Cr ₂ O ₃	2	2	2
Soya-bean oil	15	15	15
Sucrose	647	648	648

* 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, pteroylmonoglutamic acid 2 mg, menadione (as menadione dimethylpyrimidinol bisulphite) 2 mg, choline 500 mg, ascorbic acid 10 mg and biotin 0.1 mg.

isoleucine/MJ DE was based on the isoleucine requirement being approximately 0.63 of lysine needs (Fuller & Wang, 1987). To ensure that isoleucine was the limiting amino acid in the diet, supplements of other essential amino acids were added to provide a minimum surplus of at least 0.3, relative to isoleucine, according to the estimates of the Agricultural

Table 3. *Expt 2. Composition (g/kg, air dry-basis) of the diets formulated to 0.23 or 0.3 g ileal digestible isoleucine (Ile)/MJ digestible energy (DE)*

Diet no. ...	1	2	3	4	5	6
Components						
Cottonseed meal	370	—	—	370	—	—
Lupin-seed meal	—	350	—	—	350	—
Soya-bean meal	—	—	180	—	—	180
L-Isoleucine	—	—	—	0.99	1.08	1.08
Amino acids*	13.87	25.68	23.52	13.87	25.68	23.52
Monosodium glutamate	—	10	20	—	10	20
Glutamic acid	—	7.8	27.5	—	13	31
Mineral and vitamin premix†	5	7.5	7	5	7.5	7
Dicalcium phosphate	30	30	30	30	30	30
FeSO ₄ · 7H ₂ O	1	—	—	1	—	—
Fuzone 200	0.5	0.5	0.5	0.5	0.5	0.5
Soya-bean oil	15	15	15	15	15	15
Sucrose	564.63	553.52	696.48	563.64	547.24	691.90
Composition						
DE (estimated; MJ/kg)	14.2	15.4	15.6	14.2	15.4	15.6
Ileal digestible Ile: g/kg	3.26	3.54	3.55	4.24	4.61	4.62
g/MJ DE	0.23	0.23	0.23	0.3	0.3	0.3
Essential:non essential amino acids	44:56	46:54	46:54	44:56	46:54	46:54

* Contributed the following (g/kg) to the cottonseed, lupin-seed meal and soya-bean meal diets respectively: L-threonine 2.12, 2.72, 2.70, DL-methionine 2.07, 4.03, 3.13, L-valine 1.32, 3.35, 3.13, L-leucine 2.7, 3.79, 3.79, L-tyrosine 0, 1.83, 2.01, L-phenylalanine 0, 1.8, 1.28, L-histidine 0.4, 1.37, 1.69, L-tryptophan 0, 0.87, 0.46, L-lysine hydrochloride 5.26, 5.92, 5.33.

† As in Table 2 but with supplements of K₂SO₄ (diets 2 and 5, 2 g/kg, diets 3 and 6, 1.5 g/kg) and MgSO₄ · 7H₂O (diets 2, 3, 5 and 6, 0.5 g/kg).

Research Council (1981) and Fuller & Wang (1987), and as estimated by computer simulation studies using the 'Auspig' model (Black *et al.* 1986) for the Wollongbar genotype.

Diets 4, 5 and 6 were supplemented with isoleucine to verify that isoleucine was limiting in diets 1, 2 and 3. The DE content of the ingredients was estimated from previous determinations at this Institute.

Animals and procedures. The six diets were arranged in a randomized block design. Ten Large White pigs (five male, five female) were allotted to each diet. The pigs were all allotted and blocked on the basis of their 7-week weight, sex and position in the experimental facilities. The pigs were penned individually and water was supplied by 'nipple' drinkers.

Dietary treatments were introduced when the pigs reached 20 kg live weight. The diets were offered at a feeding scale of three times maintenance (estimated as 0.5 MJ DE/kg body weight^{0.75}). The pigs were fed every 3 h, with an automatic feeder, to ensure the full utilization of the added free amino acids (Batterham & Murison, 1981). The feed was offered dry and daily feeding rates were adjusted after the weekly weighing of the pigs.

The pigs were slaughtered by electric stunning after reaching a minimum weight of 45 kg. The blood was collected and the gastrointestinal tract was washed to remove undigested material. The blood and washed viscera were then combined and frozen. The carcasses (with hair) were washed clean with water, split longitudinally down the middle of the vertebrae and the left-hand side was stored at -15°, 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 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 content in the empty body, protein, fat and energy deposition/d, protein and energy deposition:DE intake ratio, protein retention:ileal digestible protein intake ratio, isoleucine retention:total isoleucine intake ratio and isoleucine retention:apparent ileal digestible isoleucine intake ratio.

The following factors were used in the calculations previously described: 6.25 to convert N to 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), 139 to calculate the protein (g/kg) and 3.6 g isoleucine/16 g N to calculate the isoleucine in the empty bodies of the pigs at the commencement of the experiment (these factors were previously determined on the five male and five female pigs 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-protein energy) \div 39.6 (Burlacu *et al.* 1973).

The results were analysed by analysis of variance and treatment means separated by least significant difference (LSD).

Chemical analyses

The techniques used were as reported by Batterham *et al.* (1990*b*). Total amino acids (except tryptophan) were determined using reflux hydrolysis under N_2 with constant boiling point HCl (110° for 72 h) and separation of amino acids by ion-exchange chromatography after reaction with ninhydrin (in the protein concentrates and ileal samples in Expt 1) or by reverse-phase chromatography and reaction with phenylisothiocyanate (recombined empty-body samples in Expt 2). Recoveries of amino acids in the empty-body samples were adjusted to 0.95 of Kjeldahl N (Association of Official Analytical Chemists, 1984).

RESULTS

Expt 1. Ileal digestibility of amino acids

The apparent ileal digestibility of isoleucine was significantly lower in pigs given cottonseed meal (0.68) relative to lupin-seed meal (0.86) and soya-bean meal (0.86) ($P < 0.001$; Table 4). The apparent ileal digestibilities of a number of other amino acids were also significantly lower for pigs given cottonseed meal relative to the other two meals; these included threonine, valine, leucine, tyrosine and histidine. Apparent ileal digestibilities of amino acids were similar for the lupin-seed and soya-bean meal diets, except for proline and methionine, which were lower in the lupin-seed meal.

There were no significant differences in N or dry matter digestibilities ($P > 0.05$).

Expt 2. Utilization of ileal digestible isoleucine

There were no significant differences ($P > 0.05$) in the growth rates of the pigs given the three protein concentrates in the diets formulated to 0.23 g ileal digestible isoleucine/MJ DE (Table 5). The addition of isoleucine to the three diets increased growth rates and lowered the FCR ($P < 0.001$). There were also no significant differences ($P > 0.05$) in crude protein deposition or in the composition of the empty bodies of the pigs given the diets formulated to 0.23 g ileal digestible isoleucine/MJ DE (Table 6).

The concentrations of isoleucine in the protein of the empty bodies of the pigs ranged from 3.6 to 3.8 g/16 g N (Table 7). Retention of ileal digestible isoleucine was slightly lower for pigs given cottonseed meal (0.65) relative to those given soya-bean meal (0.73) ($P < 0.05$), whilst that for lupin-seed meal (0.69) was in between (Table 8).

Table 4. *Expt 1. Apparent ileal digestibility of amino acids, N and dry matter in cottonseed meal, lupin-seed meal and soya-bean meal fed to pigs†*

(Mean values for four pigs)

Protein concentrate ...	Cottonseed meal	Lupin-seed meal	Soya-bean meal	Statistical significance of difference	SEM (edf 6)
Asp	0.73	0.85	0.83	*	0.020
Thr	0.59	0.76	0.79	**	0.024
Ser	0.68	0.81	0.83	*	0.025
Glu	0.83	0.91	0.87	*	0.016
Pro	0.58	0.68	0.80	*	0.031
Gly	0.62	0.62	0.75	NS	0.087
Ala	0.65	0.74	0.82	*	0.032
Cys	0.79	0.84	0.88	NS	0.030
Val	0.72	0.83	0.85	**	0.017
Met	0.76	0.78	0.91	**	0.018
Ile	0.68	0.86	0.86	***	0.015
Leu	0.69	0.83	0.84	**	0.020
Tyr	0.74	0.86	0.86	**	0.017
Phe	0.81	0.86	0.86	NS	0.016
Lys	0.61	0.85	0.86	***	0.020
His	0.77	0.86	0.87	*	0.021
Arg	0.87	0.92	0.91	NS	0.019
N	0.71	0.77	0.81	NS	0.037
Dry matter	0.76	0.81	0.83	NS	0.018

edf, Error degrees of freedom; NS, not significant ($P > 0.05$).* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets and procedures, see Tables 1 and 2, and p. 532.

DISCUSSION

The results indicate that isoleucine is less susceptible to the effects of heat processing than the other amino acids previously examined. In earlier studies the formulation of diets on an equal ileal digestible lysine (Batterham *et al.* 1990*a*), threonine (Beech *et al.* 1991), methionine (Batterham *et al.* 1993) and tryptophan (Batterham *et al.* 1994) basis resulted in large differences in growth response. In contrast, ileal digestible isoleucine was used with similar efficiency for growth from the three protein sources.

Ileal digestibility of amino acids

The ileal digestibility of amino acids in soya-bean meal was similar to that in the sample used previously (Batterham *et al.* 1990*a*) but there were differences in the digestibilities of amino acids in cottonseed meal. Isoleucine was only 0.68 digested in the current cottonseed sample, relative to 0.85 in the previous sample. Lower digestibilities were also recorded for a number of other amino acids including lysine, threonine, valine, leucine, tyrosine and phenylalanine. The ileal digestibilities of amino acids in lupin-seed meal were slightly lower than the true digestibilities reported by Taverner *et al.* (1982).

Utilization of ileal digestible isoleucine

The significant responses in growth and protein deposition of the pigs to supplements of isoleucine in diets 4–6 confirmed that isoleucine was the limiting amino acid in diets 1–3. The results indicate that ileal digestible isoleucine from the three protein sources was used with similar efficiency for growth and protein deposition, whilst there were small differences

Table 5. Expt 2. The effect of formulating diets on an apparent ileal digestible isoleucine (Ile) basis on the growth performance of pigs over the 20-45 kg growth phase†

Diet no. ... Protein source ... Apparent ileal digestible Ile content (g/MJ DE) ...	1						2						3						4						5						6						Statistical analysis						
	Cot		Lupin		Soya		Cot + Ile		Lupin + Ile		Soya + Ile		Cot + Ile		Lupin + Ile		Soya + Ile		Cot + Ile		Lupin + Ile		Soya + Ile		Cot + Ile		Lupin + Ile		Soya + Ile		Cot + Ile		Lupin + Ile		Soya + Ile		Protein (Pr)		Ile		Pr × Ile		SEM (45 edf)
Gain (g/d)	590	613	594	613	594	613	656	649	649	673	594	613	656	649	649	673	594	613	656	649	649	673	594	613	656	649	649	673	594	613	656	649	649	673	NS	***	NS	***	NS	***	13.0		
Food conversion ratio	2.41	2.13	2.13	2.13	2.13	2.13	2.14	2.02	2.02	1.90	2.13	2.13	2.14	2.02	2.02	1.90	2.13	2.13	2.14	2.02	2.02	1.90	2.13	2.13	2.14	2.02	2.02	1.90	2.13	2.13	2.14	2.02	***	***	***	***	***	***	0.043				
Empty-body wt: live wt (kg/kg)	0.913	0.909	0.929	0.909	0.929	0.909	0.909	0.906	0.906	0.927	0.929	0.909	0.909	0.906	0.906	0.927	0.929	0.909	0.909	0.906	0.906	0.927	0.929	0.909	0.909	0.906	0.906	0.927	0.929	0.909	0.909	0.906	***	***	***	***	***	***	0.0046				
Gain (g/d) (empty-body-wt basis)	533	550	553	550	553	550	588	578	578	624	553	550	588	578	578	624	553	550	588	578	578	624	553	550	588	578	578	624	553	550	588	578	*	***	***	***	***	***	11.2				
Food conversion ratio (empty-body-wt basis)	2.66	2.37	2.29	2.37	2.29	2.37	2.38	2.26	2.26	2.05	2.29	2.37	2.38	2.26	2.26	2.05	2.29	2.37	2.38	2.26	2.26	2.05	2.29	2.37	2.38	2.26	2.26	2.05	2.29	2.37	2.38	2.26	***	***	***	***	***	***	0.046				
Backfat (P ₂ ; mm)	13.8	13.4	14.7	13.4	14.7	13.4	13.1	12.6	12.6	13.0	14.7	13.4	13.1	12.6	12.6	13.0	14.7	13.4	13.1	12.6	12.6	13.0	14.7	13.4	13.1	12.6	12.6	13.0	14.7	13.4	13.1	12.6	NS	*	NS	*	NS	*	0.54				

Cot, cottonseed meal; Lupin, lupin-seed meal; Soya, soya-bean meal; DE, digestible energy; edf, error degrees of freedom; NS, not significant ($P > 0.05$), * $P < 0.05$, *** $P < 0.001$.

† For details of diets and procedures see Tables 1 and 3, and pp. 532-535.

Table 6. *Expt 2. The effect of formulating diets on an apparent ileal digestible isoleucine (Ile) basis on the concentrations, deposition rates and efficiency of retentions of protein and energy in growing pigs (20–45 kg live weight)†*

Diet no. ...	Apparent ileal digestible Ile content (g/MJ DE) ...						Statistical analysis			
	1	2	3	4	5	6	Protein (Pr)	Ile	Pr × Ile	SEM (45 edf)
Protein source ...	Cot	Lupin	Soya	Cot+Ile	Lupin+Ile	Soya+Ile				
	0.23	0.23	0.23	0.3	0.3	0.3				
Composition (empty-body basis)										
Protein (kg/kg)	0.149	0.147	0.149	0.151	0.150	0.151	NS	*	NS	0.0012
Fat (kg/kg)	0.185	0.191	0.198	0.167	0.168	0.162	NS	***	NS	0.0052
Energy (MJ/kg)	10.9	11.1	11.4	10.3	10.3	10.1	NS	***	NS	0.210
Deposition rates										
Protein (g/d)	84	87	91	96	94	102	NS	***	NS	2.9
Fat (g/d)	128	140	151	125	124	127	NS	**	NS	5.9
Energy (MJ/d)	7.1	7.7	8.2	7.3	7.2	7.5	NS	NS	NS	0.27
Retentions										
Protein retained: ileal digestible protein intake (kg/kg)	0.55	0.57	0.60	0.62	0.61	0.68	***	***	NS	0.013
Protein retained: DE intake (g/MJ)	4.2	4.2	4.4	4.7	4.6	5.0	**	***	NS	0.10
Energy retained: DE intake (MJ/MJ)	0.35	0.37	0.40	0.36	0.35	0.37	*	NS	NS	0.010

Cot, cottonseed meal; Lupin, lupin-seed meal; Soya, soya-bean meal; DE, digestible energy; edf, error degrees of freedom; NS, not significant ($P > 0.05$).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets and procedures see Tables 1 and 3, and pp. 532–535.

Table 7. *Expt 2. Amino acid composition (g/16g N, empty-body-weight basis) of pigs slaughtered at 45 kg live weight when given diets based on cottonseed meal, lupin-seed meal or soya-bean meal**†

Diet no. ...	1	2	3	4	5	6	Pooled SD
Asp	8.3	8.5	8.2	7.9	8.5	8.2	0.42
Glu	13.0	13.3	12.9	13.1	13.2	13.2	0.29
Ser	5.0	5.0	4.6	4.6	4.6	4.6	0.11
Gly	9.2	8.3	8.9	8.3	8.4	10.1	0.50
His	3.4	3.5	3.4	3.5	3.5	3.1	0.07
Arg	7.6	7.5	7.4	7.6	7.4	7.8	0.17
Thr	4.1	4.2	3.9	4.1	4.0	3.9	0.08
Ala	6.5	6.2	6.4	6.4	6.4	6.7	0.11
Pro	6.7	6.2	6.8	6.7	6.5	7.4	0.45
Tyr	2.4	2.6	2.5	2.5	2.5	2.2	0.17
Val	4.6	4.6	4.7	4.9	5.0	4.7	0.13
Met	1.2	1.3	1.8	1.3	1.4	1.1	0.46
Ile	3.6	3.7	3.7	3.8	3.8	3.6	0.08
Leu	7.0	7.2	7.2	7.3	7.2	6.8	0.10
Phe	3.5	3.7	3.5	3.6	3.3	3.5	0.10
Lys	5.6	5.8	5.5	5.9	6.0	5.6	0.12

* Hydroxyproline and tryptophan were not detected by high-performance liquid chromatographic analysis; values of 2.7 and 0.8 g/16 g N respectively were assumed from Campbell *et al.* (1988).

† For details of diets and procedures see Tables 1 and 3, and pp. 532–535.

in the efficiency of isoleucine retention. These results indicate that isoleucine differs from the other amino acids previously examined in that it appears that there is little difference in the utilization of absorbed isoleucine from the different heat-processed proteins. As such, the ileal digestibility values appear to reflect availability more closely.

This difference is probably due to the different chemical structures of the amino acids. Isoleucine is a branched-chain amino acid and appears to be less susceptible to the effects of heat. For example, isoleucine concentration increased slightly from 4.2 to 4.6 g/16 g N in field peas subjected to graded levels of dry heat, whereas lysine declined from 7.1 to 4.0 g/16 g N (Van Barneveld *et al.* 1991). The metabolism of isoleucine is also different from that of lysine (and the other amino acids previously examined) as it is metabolized predominantly in the muscle tissue, whereas the other amino acids are metabolized by the liver.

The similar utilization of the isoleucine from lupin-seed meal, compared with the other two protein sources, also indicates that there is no apparent problem with the availability of isoleucine in this meal. This is in contrast to earlier work with lysine, where the availability ranged from 0.37 to 0.65 in four samples of lupin-seed meal (Batterham *et al.* 1984). However, recent results at Wollongbar with the current sample of lupin-seed meal (E. S. Batterham and J. A. Fernandez, unpublished results) indicate that the availability of lysine in this sample is high. The reason for the difference in lysine availability between samples of lupin-seed meal is currently being investigated.

Overall, the results indicate that isoleucine, a branched-chain amino acid, is less susceptible to the effects of heat than other amino acids such as lysine, threonine, methionine and tryptophan. As such, ileal digestibility values for isoleucine appear more suitable than the above amino acids for formulating diets from heat-processed protein concentrates.

Table 8. *Expt 2. The effect of formulating diets on an apparent ileal digestible isoleucine (Ile) basis on the retention of Ile in pigs over the 20–45 kg growth phase†*
(Mean values for ten pigs per dietary group)

no. in source ... apparent ileal digestible Ile content (g/MJ DE)...	1	2	3	4	5	6	Statistical analysis		
	Cot 0.23	Lupin 0.23	Soya 0.23	Cot + Ile 0.3	Lupin + Ile 0.3	Soya + Ile 0.3	Protein (Pr)	Ile	Pr × Ile
Retention: total Ile intake (g/g)	0.45	0.60	0.62	0.46	0.54	0.54	***	***	***
Retention: ileal digestible Ile intake (g/g)	0.65	0.69	0.73	0.62	0.60	0.61	NS	***	*

Cot, cottonseed meal; Lupin, lupin-seed meal; Soya, soya-bean meal; DE, digestible energy; edf, error degrees of freedom; NS, not significant ($P > 0.05$), *** $P < 0.001$.

† For details of diets and procedures, see Tables 1 and 3, and pp. 532–535.

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