Utilization of ileal digestible amino acids by growing pigs: threonine

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An experiment was conducted to determine the utilization of ileal digestible threonine by growing pigs. Three threonine-deficient diets (0·22 g ileal digestible threonine/MJ digestible energy (DE)) were formulated using cottonseed meal, meat-and-bone meal and soya-bean meal respectively, as the only source of threonine in the diet. An additional three diets were formulated with supplements of threonine to confirm that threonine was limiting in the first three diets. The growth performance and retention of threonine by pigs given the six diets over the 20–45 kg growth phase was then determined. Growth rates (g/d) of the pigs given the three diets formulated to 0·22 g ileal digestible threonine/MJ DE were significantly different ($P < 0·001$): cottonseed meal 417, meat-and-bone meal 452, soya-bean meal 524 (SED 13·6). The response of pigs to the addition of threonine confirmed that threonine was limiting in these diets. Crude protein (nitrogen × 6·25) deposited by the pigs (g/d) was significantly higher ($P < 0·001$) for those given soya-bean meal (75), relative to meat-and-bone meal (62) and cottonseed meal (47) (SED 3·3). The proportion of ileal digestible threonine retained by pigs given the three protein concentrates was: cottonseed meal 0·44, meat-and-bone meal 0·59, soya-bean meal 0·64 (SED 0·024). These results indicate that values for the ileal digestibility of threonine in protein concentrates are unsuitable in dietary formulations as the assay does not reflect the proportion of threonine that can be utilized by the pig. It appears that, with heat-processed meals, a considerable proportion of the threonine is absorbed in a form(s) that is (are) inefficiently utilized.

Ileal digestibility: Threonine utilization: Protein concentrates: Pig

The ileal digestibility of amino acids is commonly used to estimate the availability of amino acids for the growing pig. However, values for the ileal digestibility of lysine are often considerably higher than values for lysine availability determined by slope-ratio assays (Batterham *et al.* 1990*b*). Furthermore, ileal digestibility values for lysine have been shown to be unsuitable for formulating diets as a considerable portion of the lysine may be absorbed in a form that is inefficiently utilized (Batterham *et al.* 1990*a*). It appears that ileal digestibility values for lysine overestimate availability in heat-damaged meals.

Less is known about the relationship between ileal digestibility and availability of other essential amino acids. Lysine, being di-basic, is thought to be more susceptible to heat damage than other amino acids, due to Maillard-type reactions between the free epsilon amino group of lysine and carbonyl groups of reducing sugars. If this is the reason why some of the ileal digestible lysine is unavailable, then it is possible that only di-basic amino acids are poorly utilized. On the other hand, if other reactions within the protein molecule

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are responsible for the poor retention of ileal digestible lysine, then other essential amino acids may also be affected.

The aim of the present experiment was to determine whether ileal digestibility values for threonine were suitable for formulating diets, and to measure the retention of ileal digestible threonine from different protein concentrates by growing pigs.

EXPERIMENTAL

Protein concentrates

The three protein concentrates used were a 'prepress' solvent-extracted cottonseed meal, a meat-and-bone meal 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 ferrous sulphate 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 ferrous sulphate (free gossypol:iron 1:1). This is over twice the level of free gossypol that was used in this study (182 mg/kg). Meat-and-bone meal is of medium lysine availability (0·70). Provided zinc and Fe levels are adequate, pigs can tolerate the calcium contributed by these meals. Soya-bean meal represents a meal of high lysine availability (0·88) and adequately-processed meal contains no anti-nutritional factors for pigs. The ileal digestibility of amino acids in these concentrates was determined previously with pigs fitted with a T-shaped cannula (Batterham *et al.* 1990*a*).

Diets

Three diets were formulated to contain 0·22 g ileal digestible threonine/MJ digestible energy (DE; diet nos. 1, 2 and 3; Table 2). This level of threonine was chosen after considering the relationship between threonine and lysine. In previous studies with lysine, a level of 0·36 g ileal digestible lysine/MJ DE was used as it represents an area where the growth rate of the pig responds in a linear manner to lysine concentration, but it is near the area where lysine retention plateaus (Batterham *et al.* 1990*c*). A similar relationship was assumed for threonine and the level of 0·22 g ileal digestible threonine/MJ DE was based on the threonine requirement being approximately 0·6 of lysine needs (Agricultural Research Council, 1981). To ensure that threonine was the limiting amino acid in the diet, supplements of other essential amino acids were added to provide a minimum of at least 0·27 surplus, relative to threonine, according to the estimates of the Agricultural 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.

Diet nos. 4, 5 and 6 were supplemented with threonine to verify that threonine was limiting in diet nos. 1–3. The DE content of the three protein concentrates was determined previously (Batterham *et al.* 1990*a*) and the digestible energy content of the other 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 per diet. The pigs were blocked on 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. The pigs were fed every 3 h, with

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

	Cottonseed meal	Meat-and-bone meal	Soya-bean meal
Crude protein (nitrogen × 6·25)	408	525	463
Dry matter	885	953	883
Light petroleum (b.p. 40–60°) extract	17	95	14
Fibre			
Crude	102	—	43
Neutral detergent	296	—	111
Ash	63	323	66
Amino acids			
Aspartic	39·7	36·0	53·2
Threonine	14·9	16·8	19·2
Serine	20·6	22·4	25·3
Glutamic	86·1	64·2	85·9
Glycine	17·7	77·4	20·1
Alanine	16·7	42·7	20·2
Cystine	8·5	6·3	9·1
Valine	15·5	18·2	16·8
Methionine	6·4	7·7	7·0
Isoleucine	11·7	12·1	17·5
Leucine	25·1	28·9	35·0
Tyrosine	11·8	11·0	16·0
Phenylalanine	21·6	15·8	22·9
Histidine	13·5	13·2	13·9
Lysine	19·7	25·6	26·9
Arginine	47·9	39·5	35·4
Tryptophan	5·3	2·7	6·8
Apparent ileal digestibility of threonine (proportion of total)	0·76	0·72	0·85

an automatic feeder to ensure the utilization of the added free amino acids (Batterham & Murison, 1981). The feed was offered dry and daily feeding rates were adjusted after the weekly weighings of the pigs.

The pigs were slaughtered by electric stunning after reaching a minimum weight of 45 kg. 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, split longitudinally down the middle of the vertebrae and the left-hand side stored at −15°, then ground, mixed, sampled and freeze-dried before chemical analyses. The mixed blood and washed viscera were processed in a similar manner.

In order to determine nutrient retentions, four male and four 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 as the pigs slaughtered at 45 kg live weight.

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; 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, fat and energy deposition:DE intake; protein retention:protein intake; threonine retention:total threonine intake; and threonine retention:apparent ileal digestible threonine intake.

The following factors were used in the previously described calculations: 6·25 to convert nitrogen to protein (Agricultural Research Council, 1981); 0·928 to convert initial live

Table 2. *Composition (g/kg, air-dry basis) of the diets formulated to 0·22 or 0·28 g ileal digestible threonine/MJ digestible energy*

Diet no....	1	2	3	4	5	6
Components						
Cottonseed meal	285	—	—	285	—	—
Meat-and-bone meal	—	278	—	—	278	—
Soya-bean meal	—	—	215	—	—	215
L-Threonine	—	—	—	0·88	0·92	0·86
Amino acids*	9·87	11·33	9·62	9·87	11·33	9·62
Mineral and vitamin premix†	5	10·10	5	5	10·10	5
Dicalcium phosphate	30	—	30	30	—	30
$FeSO_4.7H_2O$	0·95	0·17	—	0·95	0·17	—
Soya-bean oil	15	15	15	15	15	15
Raw sugar (sucrose)	654·18	685·4	725·38	653·3	684·48	724·52
Composition						
DE (estimated) (MJ/kg)	14·62	15·28	15·65	14·62	15·28	15·65
Ileal digestible threonine						
(g/kg)	3·22	3·36	3·48	4·1	4·28	4·34
(g/MJ DE)	0·22	0·22	0·22	0·28	0·28	0·28

DE, digestible energy.

* Contributed the following (g/kg) to the cottonseed, meat-and-bone meal and soya-bean meal diets respectively: DL-methionine 1·31, 2·05, 2·28, L-valine 1·57, 1·50, 2·41, L-isoleucine 0·87, 1·13, 0·45, L-leucine 1·21, 0·81, 0·41, L-phenylalanine 0, 1·56, 0, L-histidine 0·81, 1·65, 1·30, L-tryptophan 0, 0·59, 0·06, L-lysine 4·10, 2·04, 2·71.

† Contributed the following (mg/kg diet): iron 60, zinc 100, manganese 30, copper 5, iodine 2, sodium chloride 2·8 g, selenium 0·15, retinol equivalent 960 μg, cholecalciferol 12 μg, α-tocopherol 20, thiamin 1·5, riboflavin 3, nicotinic acid 14, pantothenic acid 10, pyridoxine 2·5, cyanocobalamin 15 μg, pteroylmonoglutamic acid 2, choline 500, ascorbic acid 10 and biotin 0·1. Additional supplements of zinc oxide (100 mg/kg) and potassium sulphate (5 g/kg) were added to diet nos. 2 and 5.

weight to estimated initial empty-body-weight; 7·75 to calculate the energy (MJ/kg) and 140 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 four males and four females slaughtered at 20 kg live weight). Energy stored as protein was calculated as protein (kg) × 24·2 (Jordan & Brown, 1970). Fat content was calculated as (total energy − protein energy)/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*c*). The total amino acid contents of composite samples of blood plus viscera and carcasses of the pigs fed on each diet and for those slaughtered at 20 kg live weight were determined and used to calculate the amino acid content of the pigs.

RESULTS

Three pigs given diet no. 2 (meat-and-bone meal), two given diet no. 5 (meat-and-bone meal) and one given diet no. 2 (cottonseed meal) died with post-mortem symptoms of slow blood clotting and haemorrhaging from gastric ulcers or from a ruptured abdominal vessel. These pigs were treated as missing plots in the statistical analyses. All other pigs remained healthy during the experiment.

Growth rates (g/d) of the pigs given the three diets formulated to 0·22 g ileal digestible threonine/MJ DE were significantly different ($P < 0·001$): cottonseed meal 417, meat-and-bone meal 452, soya-bean meal 524 (SED 13·6) (Table 3). The addition of threonine to the three diets increased growth rates and lowered the FCR ($P < 0·001$).

Table 3. *The effect of formulating diets on an apparent ileal digestible threonine basis*† *on the growth performance of pigs over the 20–45 kg growth phase*

Diet no....	1	2	3	4	5	6	Statistics			
Protein source...	Cot	Meat	Soya	Cot + Thr	Meat + Thr	Soya + Thr	Protein (Pr)	Thr	Pr × Thr	SED (37 edf)
Apparent ileal digestible threonine content (g/MJ DE)....	0·22	0·22	0·22	0·28	0·28	0·28				
Gain (g/d)	417	452	524	495	523	557	***	***	NS	13·6
Food conversion ratio	3·2	2·8	2·4	2·7	2·5	2·2	***	***	***	0·06
Empty-body-wt: live wt (kg/kg)	0·94	0·95	0·95	0·93	0·95	0·95	*	NS	NS	0·007
Gain (g/d) (empty-body-wt basis)	396	435	504	461	507	541	***	***	NS	13·1
Food conversion ratio (empty-body-wt basis)	3·35	2·88	2·46	2·90	2·53	2·31	***	***	**	0·065
Backfat (P_2, mm)	16	15	14	14	14	12	NS	*	NS	1·3

Cot, cottonseed meal; Meat, meat-and-bone meal; Soya, soya-bean meal; NS, not significant ($P > 0·05$); DE, digestible energy.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets, see Tables 1 and 2.

Table 4. *The effect of formulating diets on an apparent ileal digestible threonine basis*† *on the concentrations, deposition rates and efficiency of retentions of protein, fat and energy in growing pigs (20–45 kg live weight)*

Diet no....	1	2	3	4	5	6				
Protein source...	Cot	Meat	Soya	Cot + Thr	Meat + Thr	Soya + Thr	Statistics			
Apparent ileal digestible threonine content (g/MJ DE)....	0·22	0·22	0·22	0·28	0·28	0·28	Protein (Pr)	Thr	Pr × Thr	SED (34 edf)
Composition (empty-body-wt basis)										
Protein (kg/kg)	0·127	0·139	0·144	0·145	0·150	0·150	***	***	*	0·0027
Fat (kg/kg)	0·295	0·254	0·244	0·241	0·214	0·215	***	***	NS	0·0096
Energy (MJ/kg)	14·8	13·4	13·1	13·1	12·1	12·1	***	***	NS	0·34
Deposition rates										
Protein (g/d)	47	62	75	70	80	87	***	***	NS	3·3
Fat (g/d)	174	158	169	157	142	152	NS	**	NS	7·5
Energy (MJ/d)	8·0	7·7	8·5	7·9	7·5	8·1	*	NS	NS	0·28
Retentions										
Protein retained:protein intake (g/kg)	0·29	0·31	0·57	0·42	0·40	0·65	***	***	NS	0·020
Protein retained:DE intake (g/MJ)	2·4	3·2	3·9	3·6	4·1	4·4	***	***	*	0·15
Fat retained:DE intake (g/MJ)	9·1	8·2	8·7	8·0	7·3	7·8	NS	***	NS	0·40
Energy retained:DE intake (MJ/MJ)	0·42	0·40	0·44	0·40	0·39	0·42	*	NS	NS	0·015

Cot, cottonseed meal; Meat, meat-and-bone meal; Soya, soya-bean meal; NS, not significant ($P > 0·05$); DE, digestible energy.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets, see Tables 1 and 2.

Table 5. *Amino acid composition (g/16 g nitrogen, empty-body-weight basis) of pigs slaughtered at 20 kg and at 45 kg live weight when given diet nos. 1–6**

	20 kg pigs	Diet no. 1	2	3	4	5	6
Threonine	3·6	3·8	3·8	3·7	3·8	3·8	3·8
Valine	4·3	3·9	4·1	3·9	4·0	4·2	4·1
Cystine	1·3	1·1	1·1	0·7	1·1	1·3	1·1
Methionine	1·6	1·8	1·8	1·7	1·9	1·9	1·8
Isoleucine	3·1	3·1	3·2	3·1	3·2	3·5	3·4
Leucine	6·6	6·7	6·6	6·4	6·5	7·0	6·7
Tyrosine	2·9	2·5	2·6	2·5	2·5	2·9	2·8
Phenylalanine	3·8	3·5	3·5	3·6	3·4	3·8	3·6
Histidine	3·1	3·1	3·3	3·1	3·5	3·5	3·3
Lysine	6·2	6·4	6·6	6·4	6·5	6·9	6·5
Other†	56·5	57·1	58·4	57·9	57·6	58·2	56·9
Proportion of N recovered	0·93	0·93	0·95	0·93	0·94	0·97	0·94

* For details of diets, see Tables 1 and 2.

† Includes: aspartic acid, serine, glutamic acid, glycine, alanine, arginine, proline, hydroxyproline, tryptophan and ammonia. Proline, hydroxyproline and tryptophan were not detected by high-performance liquid chromatography analysis; values of 6·1, 2·7 and 0·8 g/16 g N respectively were assumed from Campbell *et al.* (1988).

Crude protein deposition (g/d) was greater in the pigs given soya-bean meal (75) relative to those given meat-and-bone meal (62) and cottonseed meal (47; SED 3·3; $P < 0{\cdot}001$; Table 4).

Threonine content of the pigs was uniform and ranged from 3·7 to 3·8 g/16 g N (Table 5).

Retention of ileal digestible threonine was 0·44, 0·59 and 0·64 (SED 0·024) for pigs given cottonseed meal, meat-and-bone meal and soya-bean meal respectively (Table 6).

DISCUSSION

The significant responses in growth and protein deposition of the pigs to supplements of threonine in diet nos. 4–6 confirmed that threonine was the limiting amino acid in diet nos. 1–3. The results indicate that there are considerable differences in the utilization of ileal digestible threonine. For cottonseed meal, only 0·44 of the ileal digestible threonine was retained in the pig, compared with 0·59 for meat-and-bone meal and 0·64 (SED 0·024) for soya-bean meal. Growth rate, FCR, and protein deposition were all markedly inferior for pigs given cottonseed meal relative to meat-and-bone meal and soya-bean meal. This indicates that a proportion of the ileal digestible threonine in cottonseed meal was absorbed in a form that was not efficiently utilized.

These results are similar to those reported previously for lysine. It appears that, as with lysine, heat damage to threonine can induce changes which, although not affecting ileal digestibility, depress utilization. These results indicate that the nature of the heat damage in cottonseed meal is not specific to the free epsilon group of lysine but most probably involves reactions between amino acids within the protein molecule. This supports earlier work (Batterham *et al.* 1984) where it was reported that the chemical estimate for 'available lysine' in cottonseed meal, which was based on reaction of 1-fluoro-2,4-dinitrobenzene with the free epsilon amino group of lysine, indicate high 'availability' (0·87), even though the availability for pigs was very low (0·39). Although differences in growth performances of the pigs given the three diets were somewhat similar to those given lysine-deficient diets

Table 6. *The effect of formulating diets on an apparent ileal digestible threonine basis† on the retention of threonine by growing pigs over the 20–45 kg growth phase*

Diet no.…	1	2	3	4	5	6	Statistics			
Protein source…	Cot	Meat	Soya	Cot + Thr	Meat + Thr	Soya + Thr	Protein (Pr)	Thr	Pr × Thr	SED (34 edf)
Apparent ileal digestible threonine content (g/MJ DE)	0·22	0·22	0·22	0·28	0·28	0·28				
Threonine retention: total threonine intake (g/g)	0·34	0·42	0·55	0·41	0·44	0·54	***	NS	*	0·019
Threonine retention: ileal digestible threonine intake (g/g)	0·44	0·59	0·64	0·51	0·58	0·61	***	NS	*	0·024

Cot, cottonseed meal; Meat, meat-and-bone meal; Soya, soya-bean meal; NS, not significant ($P > 0.05$); DE, digestible energy.
* $P < 0.05$, *** $P < 0.001$.
† For details of diets, see Tables 1 and 2.

(Batterham *et al.* 1990*a*), overall differences in threonine retention were less (threonine retention 0·44–0·64; lysine retention 0·36–0·75). It is possible that the changes to threonine availability may be less severe than those to lysine, where availability varies from approximately 0·40 for cottonseed meal to 0·88 for soya-bean meal (Standing Committee on Agriculture, 1987).

The deaths of the pigs in the present experiment appeared to be due to a vitamin K deficiency in the diet. No vitamin K supplement was included in the current experiment as, in earlier experiments with sugar–soya-bean-meal diets (Beech *et al.* 1990), no health problems had been encountered. Presumably, the level of vitamin K in meat-and-bone meal (where the majority of the deaths occurred), was lower than in the other vegetable protein concentrates, or intestinal synthesis was reduced, or both, necessitating dietary supplementation. In subsequent experiments (E. S. Batterham, L. M. Andersen & D. R, Baigent, unpublished results), a vitamin K supplement has been included in the premix and no further haemorrhaging has been encountered.

That the maximum retention of ileal digestible threonine was only 0.64 (SED 0·024) indicates that approximately 0·36 threonine was used in maintenance or, a portion of the ileal digestible threonine was absorbed in a form that was not fully utilizable. Thus, maximum retention of threonine was slightly lower than for lysine (0·73–0·75, Batterham *et al.* 1990*ac*).

Overall, the results indicate that values for the ileal digestibility of threonine in protein concentrates are unsuitable in dietary formulations. It appears that, as with lysine, a proportion of the ileal digestible threonine may be absorbed in a form(s) that is inefficiently utilized. Thus, the assay is not a reliable indicator of threonine availability in heat-processed meals. However, the results also indicate that the differences in threonine availability between the three meals may be slightly less than the differences in lysine availability. There is a need to determine separate estimates of the availability of threonine in protein concentrates.

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