

# The optimum dietary amino acid pattern for growing pigs

## 1. Experiments by amino acid deletion

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A series of four nitrogen-balance experiments was carried out with growing pigs to determine the optimum balance amongst the amino acids in the diet. The reduction in N retention when 20% of a single amino acid was removed from the diet was used to calculate a dietary amino acid pattern in which each amino acid would be equally limiting. A mixture of amino acids simulating the amino acid pattern of casein was used with the same efficiency as casein. From two successive deletion experiments an optimum balance amongst the essential amino acids was derived. Expressed relative to lysine = 100 this had threonine 72, valine 75, methionine + cystine 63, isoleucine 60, leucine 110, phenylalanine + tyrosine 120, tryptophan 18. No estimate was made for histidine. Essential amino acids in this pattern were mixed with non-essential amino acids in ratios of 36:64 up to 57:43. The highest efficiency of N retention was achieved with diets having a ratio of at least 45:55. This included (g/16 g N) lysine 6.5, threonine 4.7, valine 4.9, methionine + cystine 4.1, isoleucine 3.9, leucine 7.2, phenylalanine + tyrosine 7.8, tryptophan 1.2. The N of diets with this amino acid pattern was utilized significantly better than when the pattern proposed by the Agricultural Research Council (1981) was used. The flow of amino acids past the terminal ileum of pigs given the semi-synthetic diet with this amino acid pattern was no greater than that observed with protein-free diets. The proposed pattern thus describes the intrinsic requirements of the growing pig for absorbed amino acids.

**Amino acids: Protein quality: Pig.**

The quality of dietary protein is determined by its content of amino acids, and by their digestibility and availability. Quality can be considered as the degree to which the composition of the absorbed amino acid mixture accords with the balance required by the animal.

An optimum (or ideal) amino acid pattern is needed as a standard profile or reference protein when evaluating the quality of other dietary proteins. Since the nitrogen of essential amino acids can also be used in the synthesis of non-essential amino acids, a protein with essential amino acids in surplus relative to the non-essential amino acids may still support maximum N retention (NR). A more restricted definition of an ideal protein is one which includes the minimum quantity of each essential amino acid compatible with maximum utilization of the protein as a whole.

The question of the dietary amino acid pattern required by growing pigs has been reviewed in the past few years (Cole, 1978; Fuller, 1978; Cole *et al.* 1980; Henry, 1980; Low, 1980; Agricultural Research Council, 1981; Fuller & Chamberlain, 1982; Wiesemüller, 1983; Yen *et al.* 1986). The experiments described here were designed to answer the question 'what is the optimum dietary amino acid balance for growing pigs?'. The optimum dietary amino acid profile is considered to be that which, for a given N intake (NI), results in the highest NR.

The method used is an extension of that published by Bender (1965) with rats. It is based on the concept that the removal of a non-limiting amino acid has no effect on NR. The changes in NR on removing a proportion of each amino acid in turn were used to calculate a dietary amino acid pattern in which all the amino acids were equally limiting.

Table 1. *Expt 1. Composition (g/kg) of diets*

Diet ...	(1) Low-casein (low-control)	(2) High-casein (high-control)	(3) Low-casein + AA	(4) Low-casein + EAA	(5) Protein-free
Amino acid (AA) mixture	—	—	28.59*	13.53†	—
Casein	128.65	160.82	128.65	128.65	—
Maize starch	280.00	265.00	265.06	274.12	300.00
Glucose	297.65	280.48	284.00	290.00	300.00
Sucrose	137.00	137.00	137.00	137.00	150.00
Cellulose	60.00	60.00	60.00	60.00	60.00
Vegetable oil	40.00	40.00	40.00	40.00	40.00
Mineral-vitamin premix‡	56.70	56.70	56.70	56.70	56.70
Nitrogen (g/kg)	17.60	22.00	20.98	19.21	0.21
DE (MJ/kg)	14.10	14.09	14.09	14.09	12.75

EAA, essential amino acids; AA, amino acids; DE, digestible energy.

\* Containing (g): aspartate 2.319, threonine 1.076, serine 1.469, glutamate 5.637, proline 3.174, glycine 0.492, alanine 0.754, valine 1.458, cystine 0.130, methionine 0.591, isoleucine 1.183, leucine 2.349, tyrosine 1.485, phenylalanine 1.351, lysine 2.527, histidine, 1.029, arginine 1.214, tryptophan 0.358.

† As for \*, but omitting aspartate, serine, glutamate, proline, glycine, alanine and arginine.

‡ Supplying (per tonne): retinol 1.5 g, cholecalciferol 25 mg,  $\alpha$ -tocopherol acetate 5 g, phytylmenaquinone 1 g, thiamin 2 g, riboflavin 3.4 g, nicotinic acid 19 g, pyridoxine 2.73 g, vitamin B<sub>12</sub> 21 mg, choline chloride 1150 g, pantothenic acid 12 g, biotin 50 mg, folic acid 2 g, ascorbic acid 12 g, salt 3.5 kg, dicalcium phosphate 40 kg, potassium bicarbonate 7 kg, iron 80 g, zinc 100 g, manganese 40 g, magnesium 420 g, copper 150 g, iodine 2 g, cobalt 0.5 g, selenium 0.15 g.

#### MATERIALS AND METHODS

There were four N balance trials for which, except where otherwise indicated, the experimental procedure was the same. In Expt 5 the digestibilities of amino acids in the experimental diet were estimated.

#### Animals

In Expts 1, 2, 3 and 4, the number of gilts used was eighteen, twenty-four, twenty and twenty respectively. Their starting weights were 25–30 kg and their finishing weights 45–50 kg. All were Large White  $\times$  (Landrace  $\times$  Large White) from the Rowett Institute herd.

#### Housing and cages

At 7 d before the first urine collection the pigs were moved into metabolism cages to adjust to their new environment. The room temperature was controlled at 22–24°. Water was supplied *ad lib.* through nipple drinkers.

#### Experimental diets

*Expt 1.* Of the N in casein 20% was replaced by synthetic amino acids in the same proportions as in casein to confirm that the free amino acids used in this way gave a similar rate of NR to intact protein. The diets used in Expt 1 are shown in Table 1. At the end of the measurement period all animals were fed on a protein-free diet for 11 d (4 d adjustment and 7 d collection of urine and faeces) to measure the obligatory N losses.

*Expt 2.* Casein protein was used to provide the control amino acid pattern. The diets supplied 1.4 g N/kg body-weight (BW)<sup>0.75</sup> per d in the high-control diet (treatment 1) and 1.05 g N/kg BW<sup>0.75</sup> per d in the low-control diet (treatment 12). To provide diets in which

Table 2. *Expt 2. Composition (g/kg) of diets\**

Diet ...	(1) High-control	(2) Threonine	(3) Valine	(4) Methionine + cystine	(5) Isoleucine	(6) Leucine
Amino acid mixture	0	26.61	26.68	26.42	26.52	26.21
Casein	110.91	83.18	83.18	83.18	83.18	83.18
Maize starch	297.39	298.51	298.44	298.70	298.60	298.91
Glucose	300.00	300.00	300.00	300.00	300.00	300.00
Sucrose	130.00	130.00	130.00	130.00	130.00	130.00
Cellulose	65.00	65.00	65.00	65.00	65.00	65.00
Vegetable oil	40.00	40.00	40.00	40.00	40.00	40.00
Mineral-vitamin premix†	56.70	56.70	56.70	56.70	56.70	56.70
Nitrogen (g/kg)	15.17	14.54	14.55	14.54	14.54	14.52
DE (MJ/kg)	14.11	14.11	14.11	14.11	14.11	14.11
Diet ...	(7) Phenylalanine + tyrosine	(8) Lysine	(9) Histidine	(10) Tryptophan	(11) Non- essential amino acids	(12) Low- control
Amino acid mixture	41.52	27.53	27.38	26.59	11.74	0
Casein	66.55	83.18	83.18	83.18	83.18	83.18
Maize starch	300.23	297.59	297.74	298.53	313.38	325.12
Glucose	300.00	300.00	300.00	300.00	300.00	300.00
Sucrose	130.00	130.00	130.00	130.00	130.00	130.00
Cellulose	65.00	65.00	65.00	65.00	65.00	65.00
Vegetable oil	40.00	40.00	40.00	40.00	40.00	40.00
Mineral-vitamin premix†	56.70	56.70	56.70	56.70	56.70	56.70
N (g/kg)	14.18	14.48	14.51	14.54	12.90	11.50
DE (MJ/kg)	14.11	14.11	14.11	14.11	14.13	14.14

DE, digestible energy.

\* Diet (1), high-control, casein-protein only; diet (12), low-control, casein-protein only; diets (2)–(10), the amino acid omitted from the level of amino acids of the high-control pattern to the low-control pattern level (22% of that in high-control); diet (7), omitting 36% of the tyrosine and phenylalanine from the high-control pattern; diet (11), omitting all non-essential amino acids from the supplement.

† For details, see Table 1.

each essential amino acid was reduced from the high-control to the low-control level, diets 2–10 were made by adding to the low-control diet a mixture of synthetic amino acids including all but one of the essential amino acids, together with aspartate, glutamate and alanine, each supplying one-third of the N needed to restore the total N to the concentration in the high-control diet. In treatment 11 these non-essential amino acids were omitted. Because casein protein is high in phenylalanine and tyrosine relative to other amino acids, according to preliminary estimation of an optimal balance (Agricultural Research Council, 1981), 36% of these amino acids were omitted to form treatment 7. The compositions of the diets used in Expt 2 are given in Table 2. From the results the quantity of each amino acid that could be removed without effect on N retention was calculated. These estimates were used to formulate a pattern closer to the ideal than that of casein. For this purpose the simple linear model shown in Fig. 1 was used. In this model it is assumed that (1) removal of the first limiting amino acid (as A in Fig. 1) would reduce NR to the greatest extent; (2) if removal of an amino acid did not reduce NR at all (as C in Fig. 1), then the quantity removed was in excess relative to the first limiting amino acid; (3) if removal of

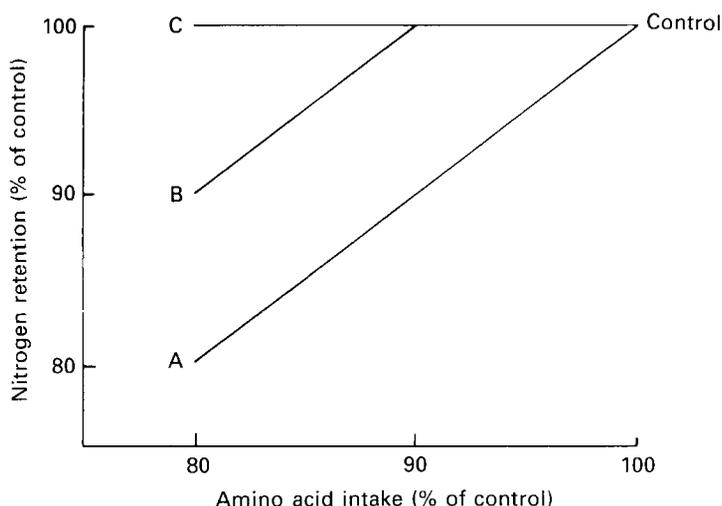


Fig. 1. Principle of the method of determining amino acid requirements by deduction. A is the first limiting amino acid; B and C are amino acids which are respectively 10 and > 20% in excess relative to A.

an amino acid resulted in a reduction in NR intermediate between 1 and 2 (as B in Fig. 1) then the proportion that could have been removed without reducing NR could be interpolated proportionately.

The resulting estimates were used to form a provisional 'ideal' pattern. Because the quantity of some amino acids removed from the control pattern in Expt 2 did not affect NR, it was not possible to calculate from the results a pattern of equally limiting amino acids. Thus the provisional ideal pattern was tested in the third experiment.

*Expt 3.* In this experiment another 20% of each essential amino acid was removed in turn from the new pattern. The compositions of the diets used in Expt 3 are given in Table 3. The results of the third experiment were again used to make a second estimate of the ideal amino acid pattern, i.e. in which each essential amino acid would be equally limiting. This new pattern was used as the basis of the fourth experiment.

*Expt 4.* Four diets were formulated in which the essential amino acids were in the pattern derived from Expt 3 and in which the ratio of their sum: the sum of the non-essential amino acids was varied from 36:64 to 57:43, corresponding to 5, 6, 7 and 8 g lysine/16 g N. The pattern proposed as 'ideal protein' by the Agricultural Research Council (1981), which contained 7 g lysine/16 g N, was also included in this experiment. All these diets supplied the same amount of N. The compositions of the diets in Expt 4 are given in Table 4.

#### Feeding

The animals were given the diets at the rate of 93 g/kg  $BW^{0.75}$  per d in three equal meals each with 0.8 litres water. The feeding times were 08.30, 12.30 and 17.30 hours. All pigs received their experimental diets for 7 d, made up of a 3 d preliminary period and 4 d of collection, except that the preliminary period before the collection of urine in the first period was 7 d.

#### Collections and measurement

Before the first collection bladder catheters were introduced as described by Fuller *et al.* (1979). In each period urine was collected continuously into acid (250 ml 2 M-sulphuric

Table 3. *Expt 3. Compositions (g/kg) of diets*

Diet ...	(1) High-control	(2) Threonine	(3) Valine	(4) Methionine + cystine	(5) Isoleucine
Amino acid mixture*	80.21	80.33	80.36	80.19	80.25
Casein	43.15	43.15	43.15	43.15	43.15
Maize starch	288.94	288.82	288.79	288.96	288.90
Glucose	295.00	295.00	295.00	295.00	295.00
Sucrose	136.00	136.00	136.00	136.00	136.00
Cellulose	60.00	60.00	60.00	60.00	60.00
Vegetable oil	40.00	40.00	40.00	40.00	40.00
Mineral-vitamin premix†	56.70	56.70	56.70	56.70	56.70
Nitrogen (g/kg)	14.52	14.52	14.52	14.52	14.52
DE (MJ/kg)	14.11	14.11	14.11	14.11	14.11
Diet ...	(6) Leucine	(7) Phenylalanine + tyrosine	(8) Lysine	(9) Histidine	(10) Tryptophan
Amino acid mixture*	80.29	80.01	80.85	80.80	80.27
Casein	43.15	43.15	43.15	43.15	43.15
Maize starch	288.86	289.14	288.30	288.35	288.88
Glucose	295.00	295.00	295.00	295.00	295.00
Sucrose	136.00	136.00	136.00	136.00	136.00
Cellulose	60.00	60.00	60.00	60.00	60.00
Vegetable oil	40.00	40.00	40.00	40.00	40.00
Mineral-vitamin premix†	56.70	56.70	56.70	56.70	56.70
N (g/kg)	14.52	14.52	14.52	14.52	14.52
DE (MJ/kg)	14.11	14.11	14.11	14.11	14.11

\* Diets (2)–(10) were formed by isonitrogenous replacement of amino acids by aspartate, glutamate and alanine (see p. 79 for details).

† For details, see Table 1.

Table 4. *Expt 4. Composition (g/kg) of diets*

Diet ...	(1)	(2)	(3)	(4)	(5)*
EAA:NEAA ...	36:64	43:57	50:50	57:43	40:60
Lysine (g/16 g N) ...	5.0	6.0	7.0	8.0	7.0
Amino acid mixture	63.17	46.77	30.37	13.97	52.27
Maize starch	292.96	298.13	303.30	308.46	296.95
Casein	56.17	67.40	78.63	89.86	63.08
Glucose	295.00	295.00	295.00	295.00	295.00
Sucrose	136.00	136.00	136.00	136.00	136.00
Cellulose	60.00	60.00	60.00	60.00	60.00
Mineral-vitamin premix†	56.70	56.70	56.70	56.70	56.70
Vegetable oil	40.00	40.00	40.00	40.00	40.00
Nitrogen (g/kg)	14.52	14.52	14.52	14.52	14.52
DE (MJ/kg)	14.11	14.11	14.11	14.11	14.11

EAA, essential amino acids; NEAA, non-essential amino acids; DE, digestible energy.

\* Pattern proposed by the Agricultural Research Council (1981).

† For details, see Table 1.

Table 5. *Expt 5. Composition (g/kg) of diet*

Amino acid mixture*	43.83	Vegetable oil	40.00
Maize starch	298.90	Mineral-vitamin premix†	56.70
Casein	69.57	Nitrogen (g/kg)	14.52
Glucose	295.00	DE (MJ/kg)	14.12
Sucrose	136.00		
Cellulose	60.00		

DE, digestible energy.

\* Containing (g): aspartic acid 15.17, threonine 1.63, monosodium glutamate 19.29, alanine 1.06, valine 0.48, cystine 0.16, methionine 1.31, isoleucine 0.14, leucine 0.82, lysine hydrochloride 1.72, histidine 0.60, arginine 1.20, tryptophan 0.26.

† See Table 1 for details.

acid) for 48 h. The faeces were collected and preserved in acid in the last 10 d of each balance. Total N was analysed in the faeces and urine samples by the Kjeldahl method of Davidson *et al.* (1970). The amino acid composition of casein was measured by ion-exchange chromatography (Spackman *et al.* 1958). A sample of casein was first treated with performic acid to oxidize methionine and cystine to methionine sulphone and cysteic acid (Moore, 1963). Both this and an unoxidized sample were hydrolysed in 6 M-hydrochloric acid for 24 h. A sample of casein was hydrolysed in 4 M-lithium hydroxide for 4 h for tryptophan analysis (Lucas & Sotelo, 1980).

#### Design

*Expt 1.* Eighteen animals were formed into two blocks of nine on the basis of BW. Within each block four different treatments were tested in two periods, giving a total of nine observations with each treatment.

*Expts 2 and 3.* Two animals were given each treatment in three sequential periods, ensuring that no animal was on the same treatment twice. This gave a total of six observations per treatment.

*Expt 4.* Twenty animals were used for two periods to test five different treatments. Four animals were allocated to each treatment ensuring that no animal was on the same treatment twice. This resulted in eight observations per treatment.

*Expt 5.* Eight gilts, weighing 45 kg, fitted with simple 'T' cannulas in the terminal ileum were used to measure the digestibility of amino acids. The diet was based on a mixture of synthetic amino acids added to casein to make the balanced amino acid pattern derived from the results of Expt 4. Measurements were also made on a protein-free diet (as in Table 1) and the values for daily amino acid flow were used to calculate the true digestibility of the amino acids in the test diet. Chromic oxide (3 g/kg) was used as an indigestible marker. The composition of the diet is given in Table 5. At 7 d after the animals were introduced to the diet, an 8 h collection of digesta was made. Samples were frozen and then freeze-dried for analysis.

#### Statistical analysis

In each experiment, a regression analysis was made to determine the relation between NR and amino acid intake, using each set of amino acid values for animals with limiting intakes and those on the high-control diet, adjusting for the effects of animal, period and N intake. GENSTAT (Lawes Agricultural Trust, 1982) was used in all the statistical analyses.

Table 6. *Expt 1. Effect of replacing 20% casein-nitrogen by synthetic amino acids on N balance in pigs*

Dietary treatment	NI	UN*	NR*	ABV*	BV*
High-casein	1.988	0.658 <sup>a</sup>	1.233 <sup>a</sup>	0.655 <sup>a</sup>	0.738 <sup>a</sup>
Low-casein (LC)	1.592	0.439 <sup>b</sup>	1.056 <sup>b</sup>	0.713 <sup>b</sup>	0.813 <sup>b</sup>
LC + amino acids	1.898	0.600 <sup>a</sup>	1.201 <sup>a</sup>	0.665 <sup>a</sup>	0.753 <sup>a</sup>
LC + essential amino acids	1.737	0.465 <sup>b</sup>	1.176 <sup>a</sup>	0.720 <sup>b</sup>	0.812 <sup>b</sup>
Pooled SE	0.0020	0.0175	0.0177	0.0104	0.0097

<sup>a, b</sup> Means with different superscript letters differ significantly ( $P < 0.05$ ).

NI, N intake (g/kg body-weight (BW)<sup>0.75</sup> per d); UN, urinary N excretion (g/kg BW<sup>0.75</sup> per d); NR, N retention (g/kg BW<sup>0.75</sup> per d); ABV, apparent biological value; BV, biological value.

\* Results adjusted for body-weight, period and animal.

Table 7. *Expt 2. Nitrogen metabolism of pigs given diets with partial deletion of individual amino acids\**

Treatment	Diet	NI	UN†	NR†	ABV†	BV†
(1)	High-control	1.36	0.28	0.89	0.75	0.88
(2)	Threonine	1.32	0.45	0.72	0.62	0.75
(3)	Valine	1.31	0.31	0.86	0.73	0.85
(4)	Methionine + cystine	1.31	0.47	0.70	0.60	0.73
(5)	Isoleucine	1.29	0.30	0.87	0.74	0.86
(6)	Leucine	1.30	0.30	0.87	0.74	0.86
(7)	Phenylalanine + tyrosine	1.26	0.30	0.87	0.74	0.86
(8)	Lysine	1.29	0.27	0.90	0.76	0.88
(9)	Histidine	1.31	0.24	0.93	0.79	0.91
(10)	Tryptophan	1.32	0.27	0.90	0.76	0.88
(11)	Non-essential amino acids	1.17	0.30	0.87	0.77	0.87
(12)	Low-control	1.32	0.45	0.72	0.64	0.75
	Pooled SE	0.016	0.016	0.016	0.013	0.012

NI, N intake (g/kg body-weight<sup>0.75</sup> per d); UN, urinary N (g/kg body-weight<sup>0.75</sup> per d); NR, N retention (g/kg body-weight<sup>0.75</sup> per d); ABV, apparent biological value; BV, biological value.

\* Diet (1), high-control, casein-protein only; diet (12), low-control, casein-protein only; diets (2)–(10), the amino acid omitted from the level of amino acids of the high-control pattern to the low-control pattern level (22% of that in high-control); diet (7), omitting 36% of the tyrosine and phenylalanine from the high-control pattern; diet (11), omitting all non-essential amino acids from the supplement.

† Means adjusted for the effects of period and N intake.

## RESULTS

### *Expt 1*

Mean results showing a comparison of the four treatments in Expt 1 are given in Table 6. These show the N intake (NI), urinary N excretion (UN), NR, apparent biological value (ABV) and biological value (BV). The UN and faecal N (FN), of pigs given the protein-free diet, were 0.132 (SE 0.0172) and 0.112 (SE 0.0246) g/kg BW<sup>0.75</sup> per d respectively. There was a slight difference in NI between treatments 2 (high-casein) and 3 (low-casein + free

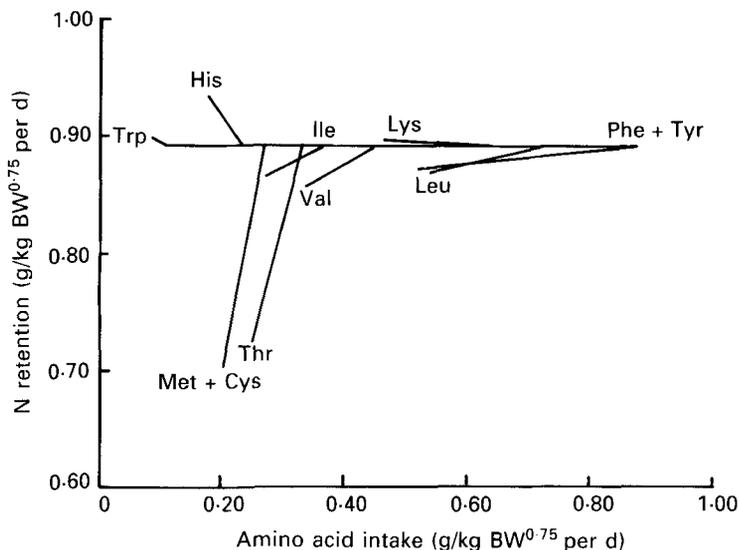


Fig. 2. Expt 2. The effects on nitrogen retention of deleting 22% (36% for phenylalanine (Phe) and tyrosine (Tyr)) of each essential amino acid. Trp, tryptophan; His, histidine; Met, methionine; Cys, cystine; Thr, threonine; Ile, isoleucine; Val, valine; Lys, lysine; Leu, leucine. BW, body-weight.

Table 8. Expt. 3. Nitrogen metabolism of pigs given diets with partial deletion of individual amino acids

Treatment	Diet	NI	UN*	NR*	ABV*	BV*
(1)	High-control	1.42	0.51	0.81	0.61	0.73
(2)	Threonine	1.43	0.61	0.71	0.54	0.66
(3)	Valine	1.43	0.56	0.75	0.57	0.69
(4)	Methionine +cystine	1.43	0.60	0.71	0.54	0.67
(5)	Isoleucine	1.44	0.55	0.77	0.58	0.70
(6)	Leucine	1.42	0.54	0.77	0.59	0.71
(7)	Phenylalanine +tyrosine	1.44	0.66	0.67	0.50	0.63
(8)	Lysine	1.42	0.59	0.72	0.55	0.67
(9)	Histidine	1.43	0.52	0.80	0.61	0.72
(10)	Tryptophan	1.43	0.59	0.73	0.55	0.67
	Pooled SE	0.006	0.017	0.016	0.012	0.011

NI, N intake (g/kg body-weight<sup>0.75</sup> per d); UN, urinary N (g/kg body-weight<sup>0.75</sup> per d); NR, N retention (g/kg body-weight<sup>0.75</sup> per d); ABV, apparent biological value; BV, biological value.

\* Means adjusted for the effects of period and N intake.

amino acids); the lower NR in treatment 3 compared with treatment 2 was due to the low NI. NR was expressed as a proportion of the absorbed N (ABV) or, with correction for UN and FN losses on the protein-free diet, as BV. Expressed in these terms, the results demonstrated that the synthetic amino acid mixture with the amino acid composition of casein could replace casein protein without significant difference in N utilization.

#### Expt 2

The mean value of FN excretion was 0.105 (SE 0.0022) g N/kg BW<sup>0.75</sup> per d. As there were no significant differences between treatments in FN excretion, this value was used for the

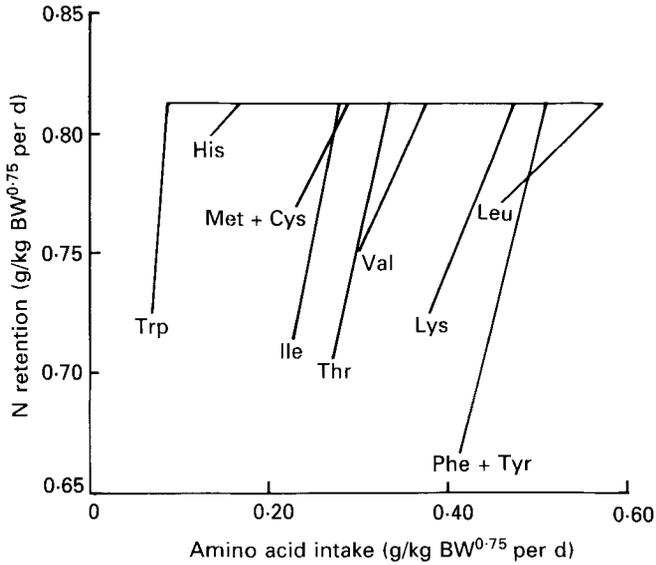


Fig. 3. Expt 3. The effects on nitrogen retention of deleting 20% of each essential amino acid. Trp, tryptophan; His, histidine; Met, methionine; Cys, cystine; Thr, threonine; Ile, isoleucine; Val, valine; Lys, lysine; Leu, leucine. BW, body-weight.

calculation of NR, BV and ABV. The results for each treatment are shown in Table 7. The responses to the deduction of individual amino acids were expressed in terms of NR. Fig. 2 shows these responses to reductions in the daily amino acid intake. Only the omissions of methionine+cystine and threonine gave significantly ( $P < 0.001$ ) lower NR than the high-control treatment. In the other treatments NR was either unchanged or improved in response to the deduction of essential amino acids. The simple linear model shown in Fig. 1 was used to calculate the proportion of each essential amino acid which was surplus to an ideally balanced pattern.

#### Expt 3

The mean value of FN excretion was 0.115 (SE 0.0202) g N/kg BW<sup>0.75</sup> per d. The values of NR, ABV and BV for each treatment are shown in Table 8. The responses of NR to daily amino acid intake are shown in Fig. 3. For each essential amino acid except histidine, a 20% deduction was sufficient to make it in turn the first limiting amino acid in the diet. It was, therefore, possible to calculate a pattern in which each essential amino acid would be equally limiting. Expressed relative to lysine = 100 this had threonine 72, valine 75, methionine+cystine 63, isoleucine 60, leucine 110, phenylalanine+tyrosine 120, tryptophan 18. No estimate was made for histidine.

#### Expt 4

The mean value of FN excretion was 0.086 (SE 0.0151) g N/kg BW<sup>0.75</sup> per d. The values for NR, ABV and BV for each treatment are shown in Table 9. The responses of NR to the ratio, essential:non-essential amino acids (and to daily lysine intake) are shown in Fig. 4. Maximum NR was achieved when the ratio, essential:non-essential amino acids was 50:50 or 57:43, with lysine at 7 or 8 g/16 g N, and these treatments gave a significantly ( $P < 0.05$ ) higher rate of NR than the 'ideal protein' pattern recommended by the Agricultural Research Council (1981). A broken line model was fitted to these values and from this the optimal ratio, essential:non-essential amino acids was estimated to be 45:55.

Table 9. *Expt 4. Nitrogen metabolism of pigs given diets with various ratios of essential: non-essential amino acids (EAA:NEAA). The pattern of amino acids proposed by the Agricultural Research Council (1981) is also given (diet 5)*

Diet	EAA:NEAA	Lysine (g/16 g N)	NI	UN*	NR*	ABV*	BV*
(1)	36:64	5.0	1.31	0.34	0.88	0.72	0.84
(2)	43:57	6.0	1.30	0.25	0.96	0.79	0.90
(3)	50:50	7.0	1.30	0.21	1.00	0.83	0.93
(4)	57:43	8.0	1.31	0.23	0.99	0.81	0.92
(5)	45:55	7.0	1.30	0.26	0.95	0.78	0.89
Pooled SE			0.004	0.015	0.014	0.012	0.011

NI, N intake (g/kg body-weight<sup>0.75</sup> per d); UN, urinary N (g/kg body-weight<sup>0.75</sup> per d); NR, N retention (g/kg body-weight<sup>0.75</sup> per d); ABV, apparent biological value; BV, biological value.

\* Means adjusted for the effects of period and N intake.

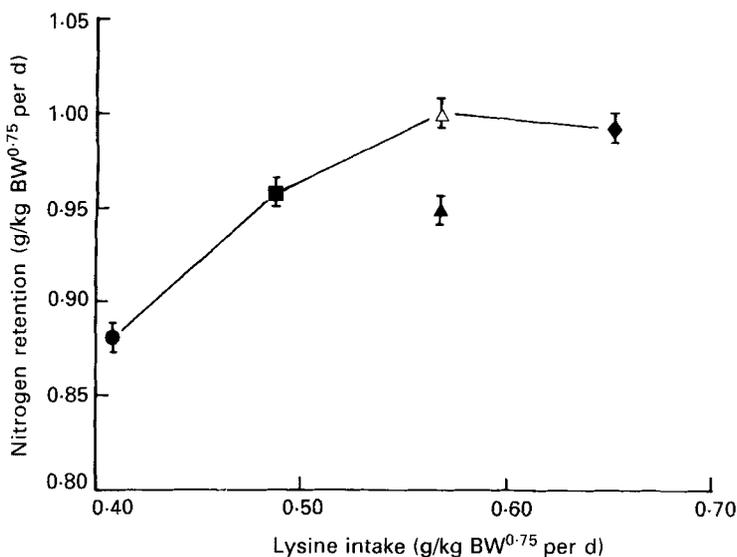


Fig. 4. The effects of varying the ratio; essential amino acids (EAA): non-essential amino acids (NEAA). The EAA were in the proportion deduced from the results of Expt 3 (see p. 85). The ratio, EAA:NEAA was (●), 36:64; (■), 43:57; (△), 50:50; (◆), 57:43. The amino acid pattern proposed by the Agricultural Research Council (1981) was also included (▲). All diets supplied 1.3 g N/kg body-weight (BW)<sup>0.75</sup> per d.

#### Expt 5

The results are shown in Table 10. There were no significant differences in daily amino acid flow between the animals fed on the protein-free and those fed on the test diet suggesting that the amino acids in these semi-synthetic diets were absorbed completely before the terminal ileum.

#### DISCUSSION

Amino acids surplus to the animal's requirement are degraded and their N is excreted. When dietary energy is limiting, amino acids are oxidized and used as an energy source. In

Table 10. *Expt 5. Amino acid digestibility and daily amino acid flow at the terminal ileum in pigs given the semi-synthetic diet*

Amino acid	Digestibility		Amino acid flow (g/d)	
	Apparent	True	Semi-synthetic diet	Protein-free diet
Threonine	0.850	1.018	1.41	1.41
Valine	0.886	1.022	0.92	0.73
Isoleucine	0.878	0.988	0.74	0.45
Leucine	0.920	1.090	0.98	1.00
Tyrosine	0.911	1.077	0.57	0.56
Phenylalanine	0.910	1.088	0.54	0.58
Histidine	0.881	1.094	0.49	0.66
Lysine	0.939	1.101	0.63	0.61
Arginine	0.846	1.089	0.53	0.73
Methionine	0.968	1.116	0.20	0.18
Cystine	0.390	0.593	0.44	0.55
SED	0.0385	0.0514		

SED, standard error of difference.

the present experiments non-protein energy was supplied generously to prevent energy being a limiting factor for protein accretion.

In Expts 2 and 3 the changes in NR when dietary essential amino acids were deducted from the control pattern were used to calculate the optimum balance between the essential amino acids. The optimum pattern calculated includes the amino acids required to meet the obligatory N losses. There is in any case some doubt as to the correctness of subtracting the obligatory N losses in these calculations. Obligatory N losses were measured as the N output of animals given a protein-free diet. As protein is essential for both growth and maintenance, NR is affected not only by the quality of the dietary protein, but also by the level of protein intake. Although all animals had approximately the same daily NI, it was not possible to ensure that every animal had exactly the same amount of N. First, although the feeding scale was based on body-weight, the animals were not weighed daily during the experiments, and their mean weights were somewhat different. Second, the animals did not always eat all the food they were offered. There were, therefore, some small differences in NI between animals. The results were accordingly adjusted for differences in NI by regression analysis.

The amino acids in the diets were mainly from casein, which is highly digestible by pigs of this body-weight (Kies *et al.* 1986). In all treatments, apart from those with amino acid deletions, all other dietary conditions (e.g. dietary energy and fibre) were the same. As the period used for urine collection was not long enough for faeces collection (because of the error arising from the variable retention time in the gut of the small quantity of faeces with these diets) faeces were collected for the last 10 d of each balance, and a mean value of FN for all pigs was used in the calculations.

Synthetic amino acids were used to vary the amino acid composition of the experimental diets. The efficiency of utilization of free amino acids by the animal can be affected by the frequency of feeding. Lysine was the first free amino acid to be examined in this context (Walz, 1972; Batterham, 1979). Compared with protein-bound amino acids, free amino acids pass more rapidly through the stomach and are absorbed in the small intestine (Rolls *et al.* 1972; Buraczewska *et al.* 1978). Protein synthesis requires that all necessary amino acids are available to the tissues at the same time. Batterham (1979) suggested that the use

of free amino acids in a conventional diet to estimate amino acid requirements could lead to an overestimate especially when animals were fed only once daily. The efficiency of utilization of the free lysine could be improved by increasing the frequency of feeding (Batterham, 1974, 1979; Batterham & O'Neill, 1978; Buraczewska & Buraczewski, 1980). However, the errors arising from the use of free amino acids in these experiments are thought to be small because (1) compared with other protein sources, casein is one of the most fully and rapidly digested (Kies *et al.* 1986); (2) not only one, but almost all the essential amino acids were used in each experimental diet so that a balanced mixture was being supplied; (3) instead of feeding the animals once daily, the animals were fed three times daily. M. F. Fuller, H. S. Willcox and A. Cadenhead (unpublished results) used intragastric infusion to study the efficiency of replacing casein with its constituent amino acids and showed that the two diets were equally utilized when NR per unit N apparently absorbed was used as the criterion of response.

As shown in Fig. 2, there were improvements in NR when certain essential amino acids were removed from the amino acid mixture in the casein protein pattern, suggesting that imbalances between essential amino acids depressed NR; even with casein protein the imbalance between the essential amino acids was sufficient to depress NR. These results suggest that the use of only the first limiting amino acid (as in chemical score) does not describe accurately enough dietary protein quality. The amino acids which, when removed from the casein protein pattern, improved NR were tryptophan, lysine, and phenylalanine + tyrosine. The deduction of isoleucine, leucine or valine had little effect. The lowest NR in response to deduction was with methionine + cystine, suggesting that this pair of amino acids was first limiting in casein protein, whilst the second limiting amino acid was threonine.

In the second experiment, deduction of histidine improved NR; in the third experiment there was still no significant response in NR to a further 20% deduction in histidine. Whether this means that the histidine requirement is even lower, or that there is a bigger body reserve of histidine than of other amino acids, is not clear.

As shown in Fig. 3, all deductions, except for histidine, were sufficient to make the amino acid deducted first limiting. When calculated in relation to the first limiting amino acid (phenylalanine + tyrosine) in the control pattern the calculated optimum balance amongst the essential amino acids was, relative to lysine = 100, methionine + cystine 63, threonine 72, tryptophan 18, valine 75, isoleucine 60, leucine 110, phenylalanine + tyrosine 120.

The surpluses of some essential amino acids relative to the ideally balanced pattern were used as sources of N for non-essential amino acids. The results of Expt 4 (Fig. 4) clearly show this. Based on these results the optimum ratio, essential:non-essential amino acids was at least 45:55, and the optimum balance of amino acids (g/16 g N) was thus lysine 6.5, methionine + cystine 4.1, threonine 4.7, tryptophan 1.2, valine 4.9, isoleucine 3.9, leucine 7.2, phenylalanine + tyrosine 7.8.

The dietary ideal protein determined in these experiments is based on the assumption that casein and the synthetic amino acids used are completely absorbed in the small intestine, and thus describes the intrinsic needs of the pig. To use the results as a reference pattern in evaluating the quality of dietary proteins, and for practical diet formulation, amino acid digestibility (measured at the terminal ileum) must also be considered. The pattern describes the total requirements of the pig, that is, its requirements for both maintenance and tissue accretion.

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