The effects of excessive amounts of protein on lysine utilization in growing pigs

BY STEFAN LANGER* AND MALCOLM F. FULLER

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB

(*Received 11 September 1995 – Revised 22 January 1996 – Accepted 26 February 1996*)

Two experiments were conducted to investigate whether the utilization of lysine in growing pigs is affected by the level of excess protein in the diet. Nine lysine-deficient diets containing 100, 200 or 300 g crude protein/kg and between 1·2 and 6·8 g ileal digestible lysine/kg were prepared. In the first experiment the apparent ileal digestibility of lysine in three of the nine diets was determined using pigs with simple T-cannulas and Cr_2O_3 as an indigestible marker. Ileal digestibility of lysine in the other diets was calculated by interpolation. In the second experiment N retention, as a measure of lysine utilization, was determined in all nine diets using growing pigs over the weight range 30–50 kg. The effect of excess protein on lysine utilization was assessed by comparing the regression of N retention *v.* lysine (ileal digestible) intake at the three levels of protein. Increasing ileal digestible lysine in the diets resulted in a linear increase in N retention with all three protein levels and there was no significant difference amongst the three regressions, indicating that lysine utilization was not affected by the level of protein. Therefore, all data were pooled together to calculate a single regression for all treatments. An increase of 1·0 g ileal digestible lysine led to an increase of 1·43 g N or 8·96 g protein (N × 6·25) retained. Assuming a lysine concentration in the retained body protein of 65–72 mg/g, lysine was utilized with an efficiency of 0·58–0·65.

Protein intake: Lysine: Ileal digestibility: Amino acid imbalance: Pig

The importance of the relative balance among amino acids as a factor in protein quality is firmly established although, in conventional diet formulation, amino acid excesses are normally disregarded. For instance, the chemical score method (Mitchell & Block, 1946), used for estimating protein quality, is based on the premise that the performance of an animal fed on an amino acid-deficient diet is solely dependent on the level of the limiting amino acid, if all other nutrients are supplied in sufficient amounts. Protein concentration and amino acid excesses in the diet seem to play a minor role as long as the minimal requirement for the limiting amino acid is met. However, numerous reports indicate that excesses of amino acids decrease animal performance (D'Mello & Lewis, 1970*a–c*; Harper *et al.* 1970; Benevenga & Steele, 1984) and several papers (Mørup & Olesen, 1976; Berschauer *et al.* 1980; Menke *et al.* 1983; Cieslak & Benevenga, 1986) suggest that the chemical score method should be modified by taking account of the excesses of other amino acids.

In general, the main response to excessive amounts of amino acids is reduced feed intake. However, even at constant intake, metabolic interactions between amino acids can result in a reduction of protein utilization and consequently animal performance (D'Mello, 1994).

Lysine is usually the first-limiting amino acid in cereal-based diets and most other amino acids are in relative excess. Using natural ingredients to meet the requirement for lysine (i.e. without addition of industrially produced lysine), these excesses are unavoidable. This

* Present address: Ralston Purina Europe Inc., European R&T Department, 1 Place Charles de Gaulle, BP 301, 78054 Saint-Quentin-en-Yvelines Cedex, France.

raises the question as to whether such a surplus of amino acids affects the utilization of lysine under the feeding conditions used in practical pig and poultry production where even small changes in lysine utilization would be of great economic importance.

The influence of amino acid excesses on lysine utilization has been investigated in various species over the last decades (Fisher *et al.* 1960; Klay, 1964*a*, *b*; Chamberlain, 1971; Cieslak & Benevenga, 1984*a*, *b*; 1986; Morris *et al.* 1987; D'Mello, 1988; Abebe & Morris, 1990; Henry *et al.* 1992). However, there is very little consistent information about the effects of protein or amino acid excess on lysine utilization in growing pigs. Klay (1964*a*, *b*) investigated the effect of dietary protein (100–260 g crude protein/kg diet) on lysine utilization using lysine concentrations of between 6·3 and 11·1 g/kg diet. Comparing weight gain and feed conversion efficiency (daily weight gain/daily feed intake) of growing pigs he reported a linear decrease of gain and efficiency with increasing levels of dietary protein. Additionally, true digestibility of lysine decreased with increasing level of protein. The author concluded that decreased absorption of lysine might be the reason for the reduced growth and efficiency of pigs fed on diets containing higher levels of protein.

Increasing the protein concentration from 131 to 158 g/kg in a lysine-deficient diet (approximately 5·6 g lysine/kg diet) had no effect on feed intake but reduced daily weight gain and feed-conversion efficiency of growing pigs (Henry *et al.* 1992). In contrast, the addition of glutamic acid to increase N concentration reduced feed intake and daily weight gain but had no effect on feed-conversion efficiency. The authors suggested that the lower efficiency was caused by the additional energy expended in the catabolism of excess protein. Fuller *et al.* (1987*a*, *b*) compared N retention of pigs fed on lysine-deficient diets with protein concentrations of either 156 or 302 g/kg diet and reported both a positive (1987*a*) and a negative (1987*b*) influence of protein on lysine utilization. However, none of these differences was significant and the experiments were not designed to measure lysine utilization.

Cieslak & Benevenga (1984*a*, *b*) concluded that the effects of protein excesses on growth performance in rats were almost entirely due to changes in voluntary feed intake. Fisher *et al.* (1960) came to a similar conclusion from their investigation of feed intake, growth performance and the efficiency of lysine utilization in growing chicks.

The objective of the present study was to investigate the effect of different levels of protein on lysine utilization based on the amount of apparent ileal digestible lysine. These results were reported briefly to the Nutrition Society (Langer and Fuller, 1995).

MATERIALS AND METHODS

Animals and housing conditions

Expt 1. Six female Cotswold crossbred pigs (25–30 kg body weight) were each provided with a simple T-cannula inserted 100–150 mm anterior to the ileo-caecal junction. After 2 weeks recovery from surgery the animals were gradually adapted to the experimental conditions. During the experiment the animals were housed individually in large, concrete-floored pens without bedding material. Room temperature was kept constant at 22–24°. The initial mean weight of the pigs was 33·9 (SD 2·4) kg and their final weight was 39·2 (SD 2·9) kg.

Expt 2. Eighteen female Cotswold crossbred pigs were used. On the third day of the first period the pigs were moved from open pens to metabolism cages. At the end of the second period there was a rest period in open pens, then the pigs were moved back to the metabolism cages. Room temperature was maintained at 22–24°. At the beginning of the experiment the animals had a mean weight of 31·1 (SD 2·0) kg and at the end 49·1 (SD 3·3) kg.

Table 1. *Composition of basal diet and dilution mixture (g/kg)*

Ingredients	Basal diet	Dilution mixture
Maize	300·0	—
Wheat	299·2	—
Maize gluten	246·4	—
Feather meal	100·0	—
Maize starch	—	572·4
Glucose	—	165·0
Sugar	—	100·0
Cellulose	—	66·0
Oil (vegetable)	20·0	40·0
Mineral and vitamin mixture B*	27·0	—
Mineral and vitamin mixture D†	—	56·6
Threonine	2·7	—
DL-Methionine	1·0	—
Histidine-HCl	1·9	—
Tryptophan	1·8	—

* The basal diet contained (g/kg diet): Pigvite No. 12 (Norvite Feed Supplements, Aberdeenshire) 2·5, dicalcium phosphate (48%) 22·0, sodium chloride 2·0 and limestone 0·5.

† The dilution mixture contained (g/kg diet): Pigvite No. 12 2·5, dicalcium phosphate (48%) 40·0, sodium chloride 1·0, potassium carbonate 7·0, magnesium oxide 0·7, Vitamin B_{12} Customix (Norvite Feed Supplements) 1·5, choline chloride (100%) 1·15 and (mg/kg diet) iron sulphate 96·0, nicotinic acid 9·1, pteroylglutamic acid 2·0, biotin (2%) 2·5, pantothenic acid 6·8, pyridoxine 2·7, thiamin 2·0, riboflavin 0·9, inositol 150·0 and ascorbic acid 12·0.

Table 2. *Composition and analysis of experimental diets*

	Low crude protein				Medium crude protein			High crude protein	
Diet no...	1	2	3	4	5	6	7	8	9
Ingredients (g/kg)									
Basal diet	333·3	333·3	333·3	333·3	666·6	666·6	666·6	1000·0	997·6
Dilution mixture	666·7	664·3	661·9	659·6	333·3	331·0	328·6	—	—
Lysine-HCl*	—	2·37	4·74	7·11	—	2·37	4·74	—	2·37
Analysis (g/kg)									
Dry matter	897·3	899·0	894·5	894·0	891·9	892·8	889·2	888·8	894·8
Neutral-detergent fibre†	78·0	78·0	78·0	78·0	84·5	84·5	84·5	91·0	91·0
Crude protein	102·2	102·4	108·0	111·8	196·3	200·6	199·0	298·8	307·0
Lysine	2·08	4·03	5·52	7·40	4·48	6·46	8·36	6·69	8·22
Digestible energy (MJ/kg)‡	13·43	13·43	13·43	13·43	13·78	13·78	13·78	14·13	14·13

* Free lysine was added as the monochlorohydrate form, taken to contain 0·785 g lysine/g.

† Determined for low- and high-crude-protein diets (1 and 8) and calculated for medium-crude-protein diets by interpolation.

‡ Calculated values.

Diets and feeding

Nine diets containing 100, 200 or 300 g crude protein/kg (low protein (LP), medium protein (MP), high protein (HP)) were formulated using different ratios of a basal diet and a protein-free dilution mixture (Table 1). Free lysine was added (Table 2) to form diets with the same lysine concentration but different levels of protein. The lowest concentration of lysine in the protein (20 g/kg) was the minimum that could be achieved using available

conventional ingredients: the highest (66 g/kg) was the concentration in 'ideal protein' according to the results of Wang & Fuller (1989). Between these limits free lysine was added in equal increments. Both the basal diet and the dilution mixture were calculated to be isoenergetic and to contain similar amounts of fibre (Table 2). The basal diet was supplemented with threonine, methionine, histidine and tryptophan to ensure adequacy of these essential amino acids. The calculation was based on true ileal digestible amino acids according to the recommended 'ideal pattern' of amino acids for growing pigs determined by Wang & Fuller (1989). To examine the effects on lysine utilization unconfounded by variation in the consumption of the diets, animals in both experiments had a daily feed allowance of 85 g/kg body weight $(BW)^{0.75}$, given in three equal meals at 08.00, 12.00 and 16.00 hours. Water was added to the feed in the trough and was also freely available throughout the experiments.

Marker. In Expt 1, Cr_2O_3 mordanted to cellulose, prepared according to the method described by Udén *et al.* (1980), was used as a marker and added to the diets at a level of 20 g/kg. In order to avoid the possibility of particle separation this was added to small batches of diet rather than the entire mix.

Experimental design and sample collection

Expt 1. Amino acid and N digestibilities at the terminal ileum were determined in diets 1, 3 and 8. After a 7 d adaptation period to a diet made of equal amounts of the three diets, the animals and diets were arranged in two 3×3 Latin squares. Each animal received the three diets in three successive 7 d periods. This gave a total of six observations per diet and three observations per animal. After a 5 d adaptation period, ileal digesta were collected during two successive days. Following the first meal of day 6 the cannula was opened and cleaned, a collection bag was attached, and all digesta passing through were collected from 09.00 to 20.00 hours. As soon as digesta appeared in the bag, they were rapidly removed and frozen (< 10 min) to avoid any further digestion or microbial fermentation.

Expt 2. After 4 d adaptation to a mixed diet (as described previously) the pigs received their treatment diets. The experiment included eighteen pigs on each of which measurements were made in three successive periods. Each pig was given a sequence of three diets and each set of three animals received all nine diets. The design was further constrained by arranging that each diet was allocated to only two pigs in each period. The design can therefore be considered as an incomplete block design with animals forming the blocks. Each period consisted of 5 d adaptation to the specific diet and 7 d measurement. To determine N retention, bladder catheters for urine collection were introduced before the measurement as described by Fuller *et al.* (1979). Faeces were collected for 7 d. Urine was collected for two 48 h periods during the last 4 d of each measurement period

Sample storage and chemical analysis

Diet samples were stored at +4°. Determinations of dietary protein content (N × 6·25) and N concentrations in urine, digesta and faeces were carried out after macro-Kjeldahl digestion as described by Davidson *et al.* (1970). Amino acid concentrations in diet and digesta samples were determined by reverse-phase liquid chromatography (Waters Pico Tag® analyser; Waters Ltd, Watford, Herts.). Samples (approximately 10 mg N) were hydrolysed for 18 h in 200 ml 6 M-HCl at about 137°. Norleucine was used as an internal standard. In order to avoid accumulation of Cr_2O_3 in the analyser column, sample hydrolysates were cleaned using a small disposable column (Dowex 50W(X8) in Bio-Rad® Poly Prep 8 × 40 mm).

The digesta samples were stored for 1–2 d in aluminium trays at −20°. Frozen samples

were freeze-dried and ground in a freezer mill for analysis. Samples from 2 d were pooled to give a composite sample for each pig. Freeze-dried digesta samples were stored at +4°. Cr_2O_3 concentrations of digesta samples and diets were analysed as described by Stevenson & Clare (1963).

To preserve the faeces and urine during the collection periods, H_2SO_4 (2 M; 200 ml/d for urine and 500 ml/d for faeces) was added to the containers. Faeces were homogenized and aliquot samples of both urine and faeces were stored at −20°.

Statistical analysis

The marker:N ratio in digesta samples from days 6 and 7 of each period was compared before pooling, using a paired *t* test. The data for ileal lysine and N digestibilities were analysed using REML (Genstat 5 Committee, 1993) with animal and period considered as random effects and diet as a fixed effect. The effect of protein on the regressions of N retention *v.* lysine intake (ileal digestible) was determined by regression analysis using Genstat 5.2. Effects were considered significant when $P < 0.05$.

RESULTS

Expt 1

All pigs recovered quickly from the surgery and there were no signs of illness throughout the experiment. The proportion of digesta sampled was about 45% (calculated from the recovery of Cr_2O_3). Values for the single animals ranged from 23·7–65·2%. There was no difference in the marker:N ratio between the two digesta collections; therefore, it could be assumed that the passage of the marker (days 6 and 7) reached an equilibrium.

Calculation of apparent ileal digestibility. The apparent ileal digestibility (ADTI) of N and lysine was determined according to the following equation:

$$\text{ADTI} = 1 - \frac{m_f \times n_d}{n_f \times m_d},$$

where m_f is the concentration of marker (Cr_2O_3) in feed (g/kg), n_f in the concentration of nutrient (N, lysine) in feed (g/kg), m_d is the concentration of marker in digesta (g/kg), and n_d is the concentration of nutrient in digesta (g/kg).

Apparent ileal nitrogen (protein) digestibility. The estimates of apparent N digestibility at the terminal ileum (ANDTI) are shown in Table 3. Ileal N digestibilities increased slightly from 0·74 to 0·76 in the LP diets to 0·77–0·78 in the HP diets.

Apparent lysine digestibility. Diets 1, 3 and 8 had mean ileal lysine digestibility values of 0·58 (SE 0·015), 0·85 (SE 0·015) and 0·69 (SE 0·015) respectively (Table 4). Addition of free lysine to diet 1 to produce diet 3 led to a higher lysine digestibility. The HP diet (diet 8) had a higher apparent lysine digestibility than the LP diet (diet 1), which was made by 1:2 dilution of diet 8 with a protein-free dilution mixture. Using the results for diets 1, 3 and 8 the ileal digestibility values of lysine in the other diets were calculated (Table 4).

Expt 2

Apparent faecal nitrogen digestibility. Results from two pigs on diet 8, which refused large amounts of feed and had problems with their bladder catheters, were excluded. Apparent (faecal) N digestibility (ANDF) for all nine diets was determined from the difference between N intake and N in faeces (Table 3). Apparent faecal N digestibilities were significantly higher than the ileal N digestibilities determined in Expt 1. Nevertheless, like the ileal digestibilities, the faecal values were slightly greater with higher dietary protein concentration.

Table 3. *Apparent ileal (ANDTI) and faecal (ANDF) digestible nitrogen values for nine diets with differing protein and lysine contents fed to pigs**

Protein level...	Low crude protein				Medium crude protein			High crude protein		
Diet number...	1	2	3	4	5	6	7	8	9	Pooled SED
ANDTI	0·74	0·75†	0·75	0·76†	0·75†	0·76†	0·77†	0·77	0·78†	0·018
ANDF	0·83[a]	0·84[ab]	0·84[ab]	0·84[ab]	0·86[bc]	0·87[c]	0·87[c]	0·88[c]	0·87[c]	0·014

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different ($P < 0·05$).
* For details of diets see Tables 1 and 2.
† Values calculated by interpolation.

Table 4. *Ileal lysine digestibility and concentration (g/kg diet) for nine diets differing in protein and lysine contents fed to pigs**

Protein level...	Low crude protein				Medium crude protein			High crude protein		
Diet number...	1	2	3	4	5	6	7	8	9	Pooled SED
Digestible lysine	1·21	3·14†	4·69	6·56†	2·89†	4·84†	6·74†	4·74	6·34†	—
Lysine digestibility	0·58[a]	0·78†	0·85[b]	0·89†	0·65†	0·75†	0·81†	0·69[c]	0·77†	0·021

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets see Table 1 and 2.
† Values calculated by interpolation.

Nitrogen retention. Increasing ileal digestible lysine concentration resulted in a linear increase in N retention at all three protein levels. There was no significant difference amongst the three regressions of N retention on lysine intake, indicating that excess protein had no effect on lysine utilization. Therefore, all data were pooled together to calculate one single regression line for all treatments. N retention (y, g/d) was related to ileal digestible lysine intake (x, g/d) by the equation:

$$y = 1·43 \text{ (SE } 0·078)\, x + 2·37 \text{ (SE } 0·515) \quad (r^2\ 0·87).$$

Fig. 1 summarizes the effect of increasing intake of ileal digestible lysine on N retention at three levels of dietary protein.

Assuming that 1 g body protein has 160 mg N, an increase of 1 g ileal digestible lysine would increase protein deposition by about 8·96 g. The equation for the relationship between protein retention (y, g/d) and ileal digestible lysine intake (x, g/d) was thus:

$$y = 8·96\, x + 14·78 \quad (r^2\ 0·87).$$

At any intake of digestible lysine N retention was the same for diets with free lysine as with protein-bound lysine, suggesting that the lysine in the basal diet was fully available.

Efficiency of lysine utilization. Assuming that 1 g body protein contains about 65 mg lysine (the mean value from Batterham *et al.* 1990*b*), the efficiency of utilization of the absorbed lysine could be calculated. The relationship between the rate of lysine retention (y, g/d) and ileal digestible lysine (x, g/d) was described by the equation:

$$y = 0·58\, x + 0·96 \quad (r^2\ 0·87).$$

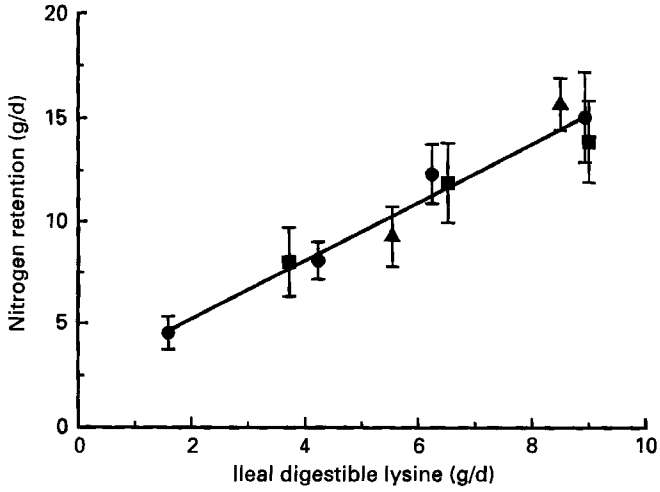

Fig. 1. Nitrogen retention in relation to ileal digestible lysine at three levels of protein: 100 g crude protein/kg (●); 200 g crude protein/kg (■); 300 g crude protein/kg (▲). Values are means for six pigs, with standard deviations indicated by vertical bars.

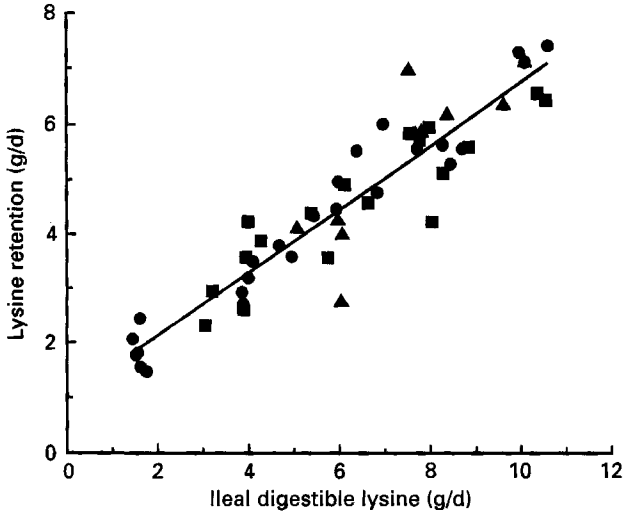

Fig. 2. Lysine retention as a function of ileal digestible lysine intake at different levels of protein intake, calculated from nitrogen retention assuming 65 mg lysine/g body protein (N × 6·25) gained. Diets contained 100 g crude protein/kg (●); 200 g crude protein/kg (■); 300 g crude protein/kg (▲). Individual values for each animal.

This regression, shown in Fig. 2, implies a marginal efficiency of utilization of absorbed lysine of 0·58. However, the value of 65 mg lysine/g body protein may be too low. Reviewing a number of publications, Susenbeth (1995) calculated a mean lysine concentration per g body protein of 68 mg for pigs of 20–25 kg body weight and 71 mg for pigs of 40–60 kg body weight. Interpolating these values gives a mean of 70 mg lysine/g body protein for the pigs (30–50 kg) in the present experiment. Accordingly, the relationship between lysine retained (y, g/d) and the ileal digestible lysine intake (x, g/d) was recalculated using this value, which gave the following equation:

$$y = 0{\cdot}63\,x + 1{\cdot}04 \quad (r^2\ 0{\cdot}87).$$

The use of this slightly higher body lysine concentration in the calculation resulted in a higher efficiency of 0·63.

DISCUSSION

Expt 1

It is generally accepted that, because the amino acid pattern of digesta is modified by the microflora during passage through the large intestine (Tanskley & Knabe, 1984; Sauer & Ozimek, 1986), amino acid digestibility determined at the terminal ileum gives a more accurate indication than faecal digestibility of the amount of amino acids absorbed by the animal. Various authors have shown that pigs cannot utilize amino acids 'disappearing' from the large intestine for N retention (Zebrowska, 1973; Just *et al.* 1981; Wünsche *et al.* 1982). Consequently, in the present experiment, ileal digestible lysine rather than total lysine concentration or faecal digestible lysine was used as a measure of lysine absorption. However, N is absorbed in the large intestine (Just *et al.* 1980; Deguchi & Namioka, 1989) so faecal N digestibility was also determined and used in the calculation of N retention.

Apparent nitrogen (protein) digestibility. Digestibility of N at the end of the ileum was lower than the digestibility at the end of the gastrointestinal tract in all treatments, probably because of digestion and absorption of N in the large intestine (Sauer & Ozimek, 1986; Deguchi & Namioka, 1989). The difference between apparent ileal and faecal N digestibility was about 10 percentage units. Increasing dietary crude protein from 100 g/kg (diet 1) to 300 g/kg (diet 8) increased both apparent ileal and apparent faecal digestibility of N by about 4 and 6 percentage units respectively. This was presumably caused by a higher proportion of endogenous N in the digesta with the lower protein diets as shown by Fan *et al.* (1994).

Apparent lysine digestibility. It can be concluded that the aim of obtaining diets with similar amounts of ileal digestible lysine at different protein concentrations was achieved. However, diet 8 had approximately 11 percentage units higher ileal lysine digestibility than diet 1. The large difference between diet 1 and diet 8, using the same source of protein (diet 1 contained 333 g diet 8/kg and 667 g of a protein-free dilution mixture/kg), could be caused by a relatively high proportion of endogenous lysine in the digesta of pigs fed on a low-protein diet compared with pigs fed on diets containing larger amounts of protein (Furuya & Kaji, 1989). Nevertheless, the change in lysine digestibility was much more pronounced than the change in N digestibility.

Fan *et al.* (1994) showed how apparent ileal lysine digestibility increased with dietary lysine to reach a plateau value of 0·86 with 8·5 g lysine/kg diet. This is above the higher level used in the current experiment and that could explain the difference between diet 1 and diet 8.

At higher dietary protein (lysine) concentrations the influence of endogenous lysine appears to be negligible. Li *et al.* (1993) did not find any effect of protein concentration on lysine digestibility, using diets ranging from 165 to 255 g crude protein/kg diet (i.e. 14·8 to 20·5 g lysine/kg diet), produced by different ratios of soyabean meal and maize starch. A similar observation was made by Just *et al.* (1980). Nevertheless, at lower dietary protein (lysine) concentrations the higher proportion of endogenous lysine should be considered when comparing the results on an apparently-digestible-lysine basis.

The difference in lysine digestibility between diet 1 and diet 3 can be completely explained by the addition of lysine in free form, taken to be 100% digestible (Baker, 1994).

Expt 2

Some animals on the HP diet with the highest lysine deficiency (diet 8) refused some of their feed. A reduction of feed intake appears to be a protective mechanism against severe effects

of amino acid imbalance (Leung & Rogers, 1969; Tews *et al.* 1979). However, on all other diets the pigs ate all their feed so the aim of equalizing feed intake was largely achieved.

Effects of lysine intake on protein retention. From his review of a large number of experiments, Susenbeth (1995) calculated that, with diets first limiting in lysine, an increase of 1 g in lysine intake led to an increase of about 7·5 g in protein retention (based on slaughter experiments) in growing pigs weighing between 20 and 90 kg. A similar result for pigs between 25 and 55 kg live weight can be derived from Yen *et al.* (1986) using diets based on cereals and soyabean meal. Assuming approximately 0·75–0·80 (Tanksley & Knabe, 1984) ileal digestibility of lysine in these diets, between 9·38 and 10·00 g protein would be retained per g ileal digestible lysine. These values agree with the estimate of 8·96 g protein retained per g ileal digestible lysine intake determined in the present experiment.

The animals in the present experiment responded to an increase in lysine intake with a similar linear increase in protein retention, up to the maximum of about 11 g ileal digestible lysine, equivalent to about 13 g total lysine/d, at all three protein levels. It can be concluded that the daily lysine requirement was above these values.

From the present results it seems unnecessary, at least within the range of 100–300 g crude protein/kg, to consider the effects of protein excess in lysine-deficient diets. For such diets chemical score appears to give a satisfactory estimate of protein quality.

Efficiency of lysine utilization. Taking a constant value of 65 mg or 70 mg lysine/g protein gave efficiency values of 0·58 or 0·63 respectively. However, results from Batterham *et al.* (1990*b*) indicate that body lysine concentration may depend on the deficiency of lysine in the diet and that the lysine concentration in the body protein gained, rather than in the final body protein, should be used for the calculation of efficiency of lysine utilization. From the data of Batterham *et al.* (1990*b*) the regression of lysine concentration in the retained body protein (y, g/16 g N) *v.* ileal digestible lysine intake (x, g/d) for the linear part of the response (3·49–10·84 g ileal digestible lysine intake; see Batterham *et al.* (1990*b*)) gave the following equation:

$$y = 0{\cdot}15\,x + 5{\cdot}27 \quad (r^2\ 0{\cdot}99).$$

Recalculating the present data values derived from this equation, the relationship between lysine retention (y, g/d) and ileal digestible lysine intake (x, g/d) was given by the equation:

$$y = 0{\cdot}65\,x + 0{\cdot}41 \quad (r^2\ 0{\cdot}90).$$

Batterham *et al.* (1990*a*) determined the efficiency of lysine retention in pigs from 20 kg to 45 kg live weight using wheat–soyabean-meal diets. The lysine retained:ileal digestible lysine intake value was 0·63. Based on available lysine intake rather than ileal digestible lysine intake the result was 0·64. These values are in good agreement with the efficiency of 0·58–0·65 estimated in the current experiment. However, in another study from the same group (Batterham *et al.* 1990*b*) an efficiency of lysine retention per g ileal digestible lysine intake in soyabean meal of 0·86 was estimated using regression analysis. Excluding the treatment with the lowest lysine intake, which caused problems with the growth of the animals (E. S. Batterham, 1993; personal communication), results in a much lower efficiency of 0·77. Bikker (1994) calculated a similar value of 0·74 for lysine utilization.

There could be several reasons for these differences in the estimates of the marginal efficiency of lysine utilization. First, the estimates depend critically on the correct estimation of digested lysine. The use of different methodologies for both amino acid analysis and digestibility measurements could give rise to discrepancies in the values deduced. Second, experiments using N balance might be expected to give different results from those based on comparative slaughter. On one hand, it is well established that balance methods tend to overestimate true rates of N retention; on the other hand, N balance is

usually, as here, a short-term measurement in contrast with comparative slaughter experiments which last weeks or months and which consequently allow time for metabolic adaptations which may not be apparent in the shorter term. Third, the estimate is likely to be affected by the range of intakes over which it is made. At very low intakes greater conservation of the limiting amino acid, through more complete suppression of its oxidation, may lead to higher estimates of efficiency than are measured at intakes closer to the animal's requirement. Finally, variation in the efficiency of utilization of limiting amino acids may be an important component of genetic differences in performance. It is noteworthy that in the experiment of Batterham *et al.* (1990*b*) intact male pigs had a considerably higher efficiency than females. It may, therefore, be inappropriate to expect a constant value for all animals.

We are grateful to Dr M. Franklin and Mr D. Hirst for statistical advice. This work was supported by the Scottish Office Agriculture and Fisheries Department and the financial support (SL) of Eurolysine SA and Degussa AG is gratefully acknowledged.

REFERENCES

Abebe, S. & Morris, T. R. (1990). Note on the effects of protein concentration on responses to dietary lysine in chicks. *British Poultry Science* **31**, 255–260.

Baker, D. H. (1994). Utilization of precursors for L-amino acids. In *Amino Acids in Farm Animal Nutrition*, pp. 37–61 [J. P. F. D'Mello, editor]. Wallingford: CAB International.

Batterham, E. S., Andersen, L. M., Baigent, D. R., Darnell, R. E. & Taverner, M. R. (1990*a*). A comparison of the availability and ileal digestibility of lysine in cottonseed and soyabean meals for grower/finisher pigs. *British Journal of Nutrition* **64**, 663–677.

Batterham, E. S., Andersen, L. M., Baigent, D. R. & White, E. (1990*b*). Utilization of ileal digestible amino acids by growing pigs: effect of dietary lysine concentration on efficiency of lysine retention. *British Journal of Nutrition* **64**, 81–94.

Benevenga, N. J. & Steele, R. D. (1984). Adverse effects of excessive consumption of amino acids. *Annual Review of Nutrition* **4**, 157–181.

Berschauer, F., Gaus, G., Cornelius, H., Kühl, J. & Menke, K. H. (1980). Prediction of protein retention in rats and pigs from amino acid pattern, protein energy ratio and feed intake. In *Proceedings of the 3rd EAAP-Symposium on Protein Metabolism and Nutrition*, vol. 2. *EAAP Publication* no. 27, pp. 672–677 pH. J. Oslage and K. Rohr, editors]. Braunschweig: European Association for Animal Production.

Bikker, P. (1994). Protein and lipid accretion in body components of growing pigs: effects of body weight and nutrient intake. PhD Thesis, University of Wageningen, The Netherlands.

Chamberlain, A. G. (1971). Protein requirements of the growing pig. In *Pig Production*, pp. 203–223 [D. J. A. Cole, editor]. London: Butterworths.

Cieslak, D. G. & Benevenga, N. J. (1984*a*). The effect of amino acid excess on utilization by the rat of the limiting amino acid – lysine. *Journal of Nutrition* **114**, 1863–1870.

Cieslak, D. G. & Benevenga, N. J. (1984*b*). The effect of amino acid excess on utilization by the rat of the limiting amino acid – lysine and threonine at equalized food intakes. *Journal of Nutrition* **114**, 1878–1883.

Cieslak, D. G. & Benevenga, N. J. (1986). Response of rats to diets of equal chemical score: effect of lysine or threonine as the limiting amino acid and of an amino acid excess. *Journal of Nutrition* **116**, 969–977.

Davidson, J., Matheson, J. & Boyne, A. W. (1970). The use of automation in determining nitrogen by the Kjeldahl method, with final calculation by computer. *Analyst* **95**, 181–193.

Deguchi, E. & Namioka, S. (1989). Synthesis ability of amino acids and protein from non-protein nitrogen and role of intestinal flora on this utilization in pigs. *Bifidobacteria and Microflora* **8**, 1–12.

D'Mello, J. P. F. (1988). Dietary interactions influencing amino acid utilisation by poultry. *Worlds Poultry Science Journal* **44**, 92–102.

D'Mello, J. P. F. (1994). Amino acids imbalances, antagonisms and toxicities. In *Amino Acids in Farm Animal Nutrition*, pp. 63–97 [J. P. F. D'Mello, editor]. Wallingford: CAB International.

D'Mello, J. P. F. & Lewis, D. (1970*a*). Amino acid interactions in chick nutrition. 1. The interrelationship between lysine and arginine. *British Poultry Science* **11**, 299–311.

D'Mello, J. P. F. & Lewis, D. (1970*b*). Amino acid interactions in chick nutrition. 2. Interrelationships between leucine, isoleucine and valine. *British Poultry Science* **11**, 313–323.

D'Mello, J. P. F. & Lewis, D. (1970*c*). Amino acid interactions in chick nutrition. 3. Interdependence in amino acid requirements. *British Poultry Science* **11**, 367–385.

Fan, M. Z., Sauer, W. C. & Lien, K. A. (1994). Effect of dietary amino acid level on the determination of apparent

ileal amino acid digestibility in pigs. In *Proceedings of the VIth International Symposium on Digestive Physiology in Pigs*, vol. 1. *EAAP Publication* no. 80, pp. 25–27 [W. B. Souffrant and H. Hagemeister, editors]. Dummerstorf: Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere.

Fisher, H., Griminger, P., Leveille, G. A. & Shapiro, R. (1960). Quantitative aspects of lysine deficiency and amino acid imbalance. *Journal of Nutrition* **71**, 213–220.

Fuller, M. F., Cadenhead, A., Mollison, G. & Sève, B. (1987*a*). Effects of the amount and quality of dietary protein on nitrogen metabolism and heat production in growing pigs. *British Journal of Nutrition* **58**, 277–285.

Fuller, M. F., Livingstone, R. M., Baird, B. A. & Atkinson, T. (1979). The optimal amino acid supplementation of barley for the growing pig. 1. Response of nitrogen metabolism to progressive supplementation. *British Journal of Nutrition* **41**, 321–331.

Fuller, M. F., Reeds, P. J., Cadenhead, A., Sève, B. & Preston, T. (1987*b*). Effects of the amount and quality of dietary protein on nitrogen metabolism and protein turnover of pigs. *British Journal of Nutrition* **58**, 287–300.

Furuya, S. & Kaji, Y. (1989). Estimation of the true ileal digestibility of amino acids and nitrogen from their apparent values for growing pigs. *Animal Feed Science and Technology* **26**, 271–285.

Genstat 5 Committee (1993). *Genstat 5.2*. Oxford: Clarendon Press.

Harper, A. E., Benevenga, N. J. & Wohlhueter, R. M. (1970). Effects of ingestion of disproportionate amounts of amino acids. *Physiological Reviews* **50**, 428–558.

Henry, Y., Colléaux, Y. & Sève, B. (1992). Effects of dietary level of lysine and of level and source of protein on feed intake, growth performance, and plasma amino acid pattern in the finishing pig. *Journal of Animal Science* **70**, 188–195.

Just, A., Jørgensen, H. & Fernández, J. A. (1981). The digestive capacity of the caecum-colon and the value of the nitrogen absorbed from the hind gut for protein synthesis in pigs. *British Journal of Nutrition* **46**, 209–219.

Just, A., Sauer, W. C. & Jørgensen, H. (1980). The influence of diet on the apparent ileal and faecal digestibility of protein and amino acids in pigs. In *Proceedings of the 3rd EAAP-Symposium on Protein Metabolism and Nutrition*, vol. I. *EAAP Publication* no. 27, pp. 215–219 [H. J. Oslage and K. Rohr, editors]. Braunschweig: European Association for Animal Production.

Klay, R. F. (1964*a*). The lysine requirement for growth of pigs at four protein levels. *Journal of Animal Science* **23**, 881 (Abstr).

Klay, R. F. (1964*b*). Lysine and nitrogen utilization by pigs at four protein levels. *Journal of Animal Science* **23**, 881 (Abstr).

Langer, S. & Fuller, M. F. (1995)., Lysine utilization in growing pigs at three different levels of protein. *Proceedings of the Nutrition Society* **54**, 64A.

Leung, P. M.-B. & Rogers, Q. R. (1969). Food intake: regulation by plasma amino acid pattern. *Life Sciences* **8** (2), 1–9.

Li, S., Sauer, W. C. & Fan, M. Z. (1993). The effect of dietary crude protein levels on ileal and fecal amino acid digestibility in early-weaned pigs. *Journal of Animal Physiology and Animal Nutrition* **70**, 117–128.

Menke, K. H., Gruber, F. & Gaus, G. (1983). The product of amino acids functions as a measure of protein quality for pigs. In *IVth International Symposium on Protein Metabolism and Nutrition*, pp. 431–434 [R. Pion, M. Arnal and D. Bonin, editors]. Paris: INRA.

Mitchell, H. H. & Block, R. J. (1946). Some relationships between the amino acid contents of proteins and their nutritive values for the rat. *Journal of Biological Chemistry* **163**, 599–620.

Morris, T. R., Al-Azzawi, K., Gous, R. M. & Simpson, G. L. (1987). Effects of protein concentration on responses to dietary lysine by chicks. *British Poultry Science* **28**, 185–195.

Mørup, I. L. K. & Olesen, E. S. (1976). New method for prediction of protein value from essential amino acid pattern. *Nutrition Reports International* **13**, 355–365.

Sauer, W. C. & Ozimek, L. (1986). Digestibility of amino acids in swine: results and their practical applications. A review. *Livestock Production Science* **15**, 367–388.

Stevenson, A. E. & Clare, N. T. (1963). Measurement of feed intake by grazing cattle and sheep. IX. Determination of chromic oxide in faeces using an auto analyzer. *New Zealand Journal of Agricultural Research* **6**, 121–126.

Susenbeth, A. (1995). Factors affecting lysine utilization in growing pigs. An analysis of literature data. *Livestock Production Science* **43**, 193–204.

Tanksley, T. D. & Knabe, D. A. (1984). Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. In *Recent Advances in Animal Nutrition*, pp. 75–95 [W. Haresign and D. J. A. Cole, editors]. London: Butterworths.

Tews, J. K., Lee Kim, Y.-W. & Harper, A. E. (1979). Induction of threonine imbalance by dispensable amino acids: relation to competition for amino acid transport into the brain. *Journal of Nutrition* **109**, 304–315.

Udén, P., Colucci, P. E. & Van Soest, P. J. (1980). Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *Journal of the Science of Food and Agriculture* **31**, 625–632.

Wang, T.C. & Fuller, M. F. (1989). The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *British Journal of Nutrition* **62**, 77–89.

Wünsche, J., Hennig, U., Meinl, M., Kreienbring, F. & Bock, H. D. (1982). Investigations of the absorption and utilization of amino acids infused into the caecum of growing pigs. 1. N-balance measuring with regard to the

utilization of lysine and isoleucine and the isoleucine requirement of growing pigs. *Archiv für Tierernährung* **32**, 337–348.
Yen, H. T., Cole, D. J. A. & Lewis, D. (1986). Amino acid requirements of growing pigs. 7. The response of pigs from 25–55 kg live weight to dietary ideal protein. *Animal Production* **43**, 141–154.
Zebrowska, T. (1973). Digestion and absorption of nitrogenous compounds in the large intestine of pigs. *Roczniki Nauk Rolniczych* **95B**, 85–90.

Printed in Great Britain