

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

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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<sub>2</sub>O<sub>3</sub> 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 ( $N \times 6·25$ ) 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, 1970a–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

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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<sub>12</sub> 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*

Diet no ...	Low crude protein				Medium crude protein			High crude protein	
	1	2	3	4	5	6	7	8	9
<b>Ingredients (g/kg)</b>									
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
<b>Analysis (g/kg)</b>									
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)<sup>0.75</sup>, 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<sub>2</sub>O<sub>3</sub> 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<sub>2</sub>O<sub>3</sub> 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<sub>2</sub>O<sub>3</sub> 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<sub>2</sub>SO<sub>4</sub> (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<sub>2</sub>O<sub>3</sub>). 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<sub>2</sub>O<sub>3</sub>) in feed (g/kg),  $n_f$  is 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		Pooled SED
	1	2	3	4	5	6	7	8	9	
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 <sup>a</sup>	0.84 <sup>ab</sup>	0.84 <sup>ab</sup>	0.84 <sup>ab</sup>	0.86 <sup>bcd</sup>	0.87 <sup>c</sup>	0.87 <sup>c</sup>	0.88 <sup>c</sup>	0.87 <sup>c</sup>	0.014

<sup>a, b, c</sup> 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		Pooled SED
	1	2	3	4	5	6	7	8	9	
Digestible lysine	1.21	3.14†	4.69	6.56†	2.89†	4.84†	6.74†	4.74	6.34†	—
Lysine digestibility	0.58 <sup>a</sup>	0.78†	0.85 <sup>b</sup>	0.89†	0.65†	0.75†	0.81†	0.69 <sup>c</sup>	0.77†	0.021

<sup>a, b, c</sup> 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.* 1990b), 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:

$$y = 0.58 x + 0.96 \quad (r^2 0.87).$$

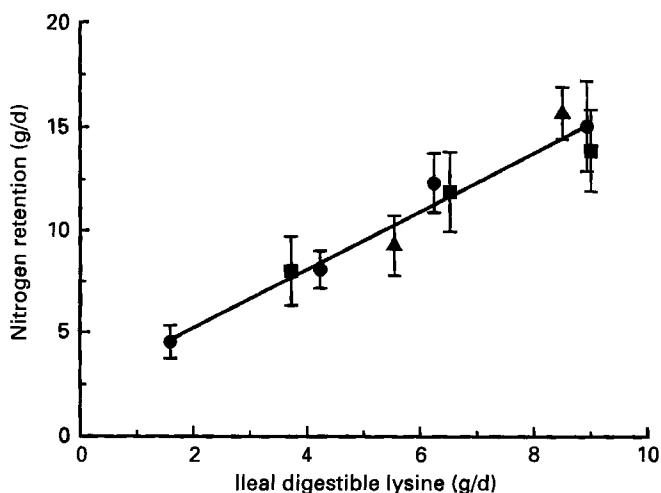


Fig. 1. Nitrogen retention in relation to ileal digestible lysine at three levels of protein: 100 g crude protein/kg (●); 200 g crude protein/kg (■); 300 g crude protein/kg (▲). Values are means for six pigs, with standard deviations indicated by vertical bars.

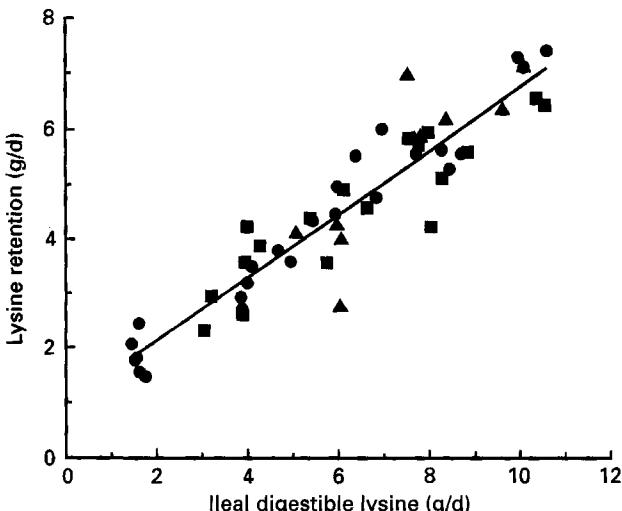


Fig. 2. Lysine retention as a function of ileal digestible lysine intake at different levels of protein intake, calculated from nitrogen retention assuming 65 mg lysine/g body protein ( $N \times 6.25$ ) gained. Diets contained 100 g crude protein/kg (●); 200 g crude protein/kg (■); 300 g crude protein/kg (▲). 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:

$$y = 0.63x + 1.04 \quad (r^2 0.87).$$

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 (Tanksley & 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.* (1990b) 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.* (1990b) 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.* (1990b)) gave the following equation:

$$y = 0.15x + 5.27 \quad (r^2 0.99).$$

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:

$$y = 0.65x + 0.41 \quad (r^2 0.90).$$

Batterham *et al.* (1990a) 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.* 1990b) 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.* (1990b) intact male pigs had a considerably higher efficiency than females. It may, therefore, be inappropriate to expect a constant value for all animals.

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