Oxidation of an indicator amino acid by young pigs receiving diets with varying levels of lysine or threonine, and an assessment of amino acid requirements

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1. The catabolism of [^{14}C]phenylalanine was used to indicate the effects of varying the dietary level of lysine and threonine on the retention of dietary amino acids by 2-week-old pigs receiving diets containing skim milk and a mixture of free amino acids.

2. Reducing the dietary level of lysine from 16 to 12 g/kg had no influence on phenylalanine oxidation, reducing the lysine level from 12 to 11 then to 10 g/kg caused an almost linear increase in phenylalanine oxidation whereas further reduction to 9 or 8 g/kg resulted in a less-marked increase in phenylalanine oxidation. This showed that 12 g lysine/kg was required to maximize amino acid retention and indicated that lysine was conserved more effectively at low dietary concentrations than at dietary concentrations approaching the requirement.

3. Reducing the dietary level of threonine from 8 to 6 g/kg had no influence on phenylalanine oxidation, whereas further reduction to 4 g/kg caused a linear increase in phenylalanine catabolism showing that 6 g threonine/kg was required to maximize amino acid retention.

4. Reduction of the levels of lysine, threonine and methionine from the generous levels characteristic of a diet containing 240 g protein from skim milk/kg, to the requirement levels determined separately in the presence of the generous levels of all the other amino acids, resulted in a twofold increase in phenylalanine catabolism. This shows that the pig seems able to conserve limiting intakes of a single amino acid, but not if the intakes of two or three amino acids are limiting.

Lysine, threonine and methionine are the essential amino acids most likely to be limiting in diets prepared for pigs. Many studies have been carried out to determine the dietary requirements using growth as the primary indicator of response to the adequacy of the amino acid levels in the diet. The recommended dietary requirements for growing pigs are well based on experimental evidence ((US) National Research Council, 1979). This is not the situation for young piglets because normal growth rates can only be obtained by feeding diets based on milk, and such diets supply generous amounts of essential amino acids leaving no opportunity to manipulate amino acid levels from deficient to excess. Kim *et al.* (1983) showed that the oxidation of [^{14}C]phenylalanine could be used to indicate the adequacy of the dietary levels of other essential amino acids in short-term studies which reduced the difficulties associated with using experimental diets in piglets of less than 2 weeks of age.

The dietary requirements for individual amino acids have been established by varying the level of a single amino acid in diets which contained an excess of the other essential amino acids. Such an approach assumes that the requirement determined in this way is unaffected by the excesses of the other essential amino acids in the diets. This assumption needs to be evaluated by examining protein utilization by animals receiving diets with two or more essential amino acids at levels considered to be their requirements on the basis of previous experiments.

The experiments to be described involved measurement of the release of labelled carbon dioxide from [^{14}C]phenylalanine by piglets which received diets containing varying levels

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of lysine and threonine to indicate the requirements for these two amino acids. The effects of feeding diets containing the required levels of any two, or all three, of the amino acids lysine, threonine and methionine on phenylalanine oxidation, were also measured.

EXPERIMENTAL

The experimental diets and the procedures used have been described by Kim *et al.* (1983). Dietary protein was supplied as a mixture of amino acids and skim-milk powder, with 600 g/kg dietary nitrogen being provided by the free amino acids. In the diet the skim milk provided 8 g lysine/kg and 4 g threonine/kg. The oxidation of the [1-^{14}C]phenylalanine was measured by collecting and counting the $^{14}CO_2$ expired in 1 h by the piglets after they had consumed two meals of the experimental diets containing 20 μCi [^{14}C]phenylalanine. Measurement of the specific activity of the liver free phenylalanine and the assumption that the liver is the major site of phenylalanine oxidation allowed calculation of the rate of phenylanine oxidation during the CO_2-collection period.

The response of the pigs to increasing concentrations of dietary lysine was measured using thirty-six piglets which weighed 1·5 kg on average when they were weaned at 3 d of age. The diets were supplemented with L-lysine hydrochloride and contained 8, 9, 10, 11, 12 and 16 g lysine/kg. The phenylalanine level in these diets was 7·5 g/kg as determined by Kim *et al.* (1983). The response of the pigs to increasing the concentration of dietary threonine was measured using twenty-seven piglets which weighed 1·5 kg on average when they were weaned at 3 d of age. The diets were supplemented with L-threonine and contained 4·0, 5·0, 5·5, 6·0, 6·5 and 8·0 g threonine/kg. Phenylalanine was added to these diets at 8·0 g/kg to ensure that this would not be limiting.

The effects of reducing the levels of lysine, threonine and methionine from those in a diet in which skim milk provided all the protein to the levels shown to minimize phenylalanine oxidation in the present and a previous study (Kim & Bayley, 1983) were investigated in a third experiment in which twenty-five piglets were used. Their average weight was 1·8 kg when they were weaned at 3 d of age. The control diet contained (g/kg): lysine 20, threonine 10, methionine 5·7. Three more diets contained pairs of these amino acids at the newly-determined requirement levels (g/kg; lysine 12, threonine 6, methionine 2·7) with the third amino acid at the control level. A fifth diet contained all three amino acids at their determined-requirement levels. These diets all contained 8 g phenylalanine/kg.

RESULTS

Estimation of lysine requirement

Increasing the lysine level in the diet from 8 to 12 g/kg reduced the phenylalanine oxidation rate, but an increase to 16 g/kg resulted in no further change in the release of $^{14}CO_2$ from phenylalanine (Fig. 1). The specific activity of the liver free phenylalanine was not influenced by dietary lysine level (Table 1) so that the release of $^{14}CO_2$ in response to increasing dietary lysine levels was equivalent to the rate of heptic phenylalanine oxidation. The first two increments of dietary lysine from 8 to 9 g/kg and from 9 to 10 g/kg appeared to have less effect on phenylalanine catabolism than the increments from 10 to 11 g/kg and from 11 to 12 g/kg suggesting that the piglets were conserving lysine more effectively at the lower dietary levels than at the dietary levels which approached adequacy. The apparent curvilinear form of the influence of dietary lysine level on both the recovery of radioactivity in CO_2 and on phenylalanine oxidation rate precluded analysis of the results using a linear regression model, and thus no confidence limits can be applied to the estimate that the lysine requirement was 12 g/kg diet. The piglets gained 106 g/d in the period preceding the measurement of phenylalanine oxidation.
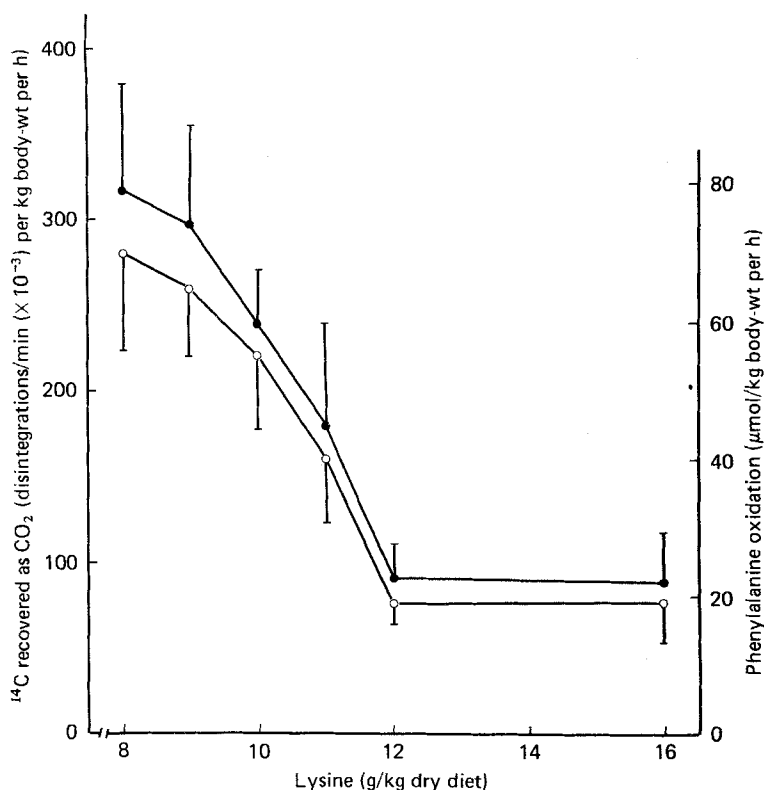
Fig 1. Influence of dietary lysine level on phenylalanine oxidation. Mean values with their standard errors represented by vertical bars for the radioactivity recovered as carbon dioxide (●—●) and for phenylalanine oxidized (○——○) in 1 h by pigs which had received 20 μCi L-[1-^{14}C]phenylalanine in diets containing graded levels of lysine.

Estimation of threonine requirement

Increasing the dietary threonine level from 4 to 6 g/kg resulted in a linear decrease in the release of carbon dioxide from the labelled phenylalanine (Fig. 2), but increasing the threonine level to either 6·5 or 8·0 g/kg caused no further reduction in $^{14}CO_2$ release by the pigs. The specific activity of the liver free phenylalanine was not affected by dietary threonine level (Table 1) so that again the release of the $^{14}CO_2$ was equivalent to hepatic phenylalanine oxidation.

Analysis of the values in Fig. 2 using a 'broken-line' regression model (Seber, 1977) showed that the change-over point of the two components of the line for the $^{14}CO_2$ recovery occurred at a dietary threonine level of 5·99 g/kg, (95% confidence limits, 5·85–6·14 g/kg). The change-over point for the phenylalanine oxidation occurred with a dietary threonine concentration of 6·03 g/kg (95% confidence limits, 5·54–6·53 g/kg), showing that inclusion of the measurement of the specific activity of the liver free phenylalanine in the calculation considerably reduced the precision of the estimate. The piglets gained 121 g/d in the period preceding the measurement of phenylalanine oxidation and the results of the present study show that their dietary threonine requirement was 6 g/kg.

Table 1. *Influence of dietary amino acid concentration on the specific activity of liver free phenylalanine in young pigs 3·25 h after receiving meals containing 20 μCi L-[1-^{14}C]phenylalanine providing a specific activity of 15 ($\times 10^3$) disintegrations/min per mol for the dietary phenylalanine source*

Dietary variable			No. of pigs	Specific activity of liver free phenylalanine (disintegrations ($\times 10^{-3}$) per min per μmol)	
				Mean	Range
Lysine (g/kg)					
8			6	4·8	3·8–6·9
9			6	4·4	2·9–5·2
10			6	5·6	2·1–11·6
11			6	4·0	2·2–6·5
12			6	4·8	2·4–7·7
16			6	3·7	2·3–4·7
Threonine (g/kg)					
4·0			5	3·0	2·0–4·9
5·0			6	2·7	1·3–3·2
5·5			5	3·8	2·2–5·2
6·0			3	2·4	2·3–2·4
6·5			5	3·4	2·0–4·7
8·0			3	2·4	1·5–2·6
Lysine (g/kg)	Threonine (g/kg)	Methionine (g/kg)			
20	10	5·7	5	3·3	2·0–5·6
12	6	5·7	5	3·0	2·5–3·3
12	10	2·7	5	3·1	1·9–5·0
20	6	2·7	5	3·1	2·0–5·0
12	6	2·7	5	3·5	2·1–5·3

Interactions of lysine, threonine and methionine levels on phenylalanine oxidation

Reducing the level of any two of the three amino acids from the level equivalent to that in a diet containing 240 g protein from skim milk/kg, to the requirements determined by feeding graded levels of single amino acids significantly increased both $^{14}CO_2$ release from the labelled phenylalanine and the calculated oxidation rate of phenylalanine (Table 2). The increases in both measures of phenylalanine catabolism were almost twofold, but there were no significant differences between the results for the piglets receiving diets with the levels of any two, or all three, of the amino acids reduced to the determined requirement. These findings show that the requirement for an amino acid determined in the presence of generous levels of the other essential amino acids may underestimate the requirement for diets in which the levels of more than one amino acid are just adequate.
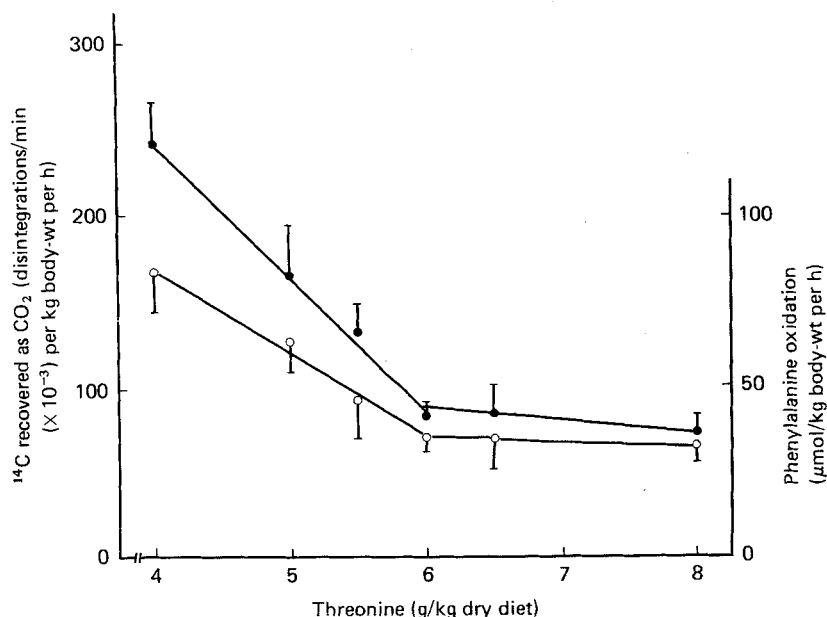
Fig. 2. Influence of dietary threonine level on phenylalanine oxidation. Mean values with their standard errors represented by vertical bars for the radioactivity recovered as carbon dioxide ($\bullet$———$\bullet$) and for phenylalanine oxidized ($\bigcirc$———$\bigcirc$) in 1 h by pigs which had received 20 μCi L-[1-^{14}C]phenylalanine in diets containing graded levels of threonine. 'Broken-lines' were fitted by regression analysis; for the CO_2 recovery the values for dietary threonine levels of 4, 5, 5·5 and 6·0 g/kg diet were ascribed to the first part of the regression line, and for the phenylalanine oxidized the values for dietary threonine levels of 4, 5 and 5·5 g/kg diet were ascribed to the first part of the regression line.

Table 2. *Interactions of lysine, threonine and methionine levels on phenylalanine oxidation by the young pig*

(The mean (with SE) growth rate of the pigs was 116 (4) g/d in the period preceding the oxidation determination)

Dietary level of amino acid (g/kg)			$^{14}CO_2$ recovered in 1 h (disintegrations ($\times 10^{-3}$)/min per kg body-wt per h)	Phenylalanine oxidation (μmol/kg body-wt per h)
Lysine	Threonine	Methionine		
20	10	5·7	110	33·7
12	6	5·7	183	63·2
12	10	2·7	218	71·4
20	6	2·7	180	59·7
12	6	2·7	205	59·6
SEM*			34	7·8
Least significant difference ($P < 0.05$)			70	16·3

* Based on error mean square from analysis of variance (20 df).

DISCUSSION

Lysine requirement

A requirement for 12 g lysine/kg in a diet containing 240 g protein/kg for the 2-week-old pig is very close to the requirement of 12·8 g/kg in diets containing 270 g protein/kg ((US) National Research Council, 1979). The coincidence of these two values may be misleading as their is little evidence to support the estimate of 12·8 g/kg if, as seems likely, this value (for the 1–5 kg pig) is based on a 30% increment of the protein and amino acid requirements listed for 5–10 kg pigs by the (US) National Research Council (1979). In a preliminary experiment Kim et al. (1983) showed that $^{14}CO_2$ release from $[^{14}C]$phenylalanine was minimized by diets containing 12 g lysine/kg or more, confirming the results of the present study. However, this only shows the consistency of the technique and does not relate the result to those of other studies using different indices of response to varying lysine levels in the diets.

Chavez & Bayley (1976) showed that the oxidation of $[^{14}C]$lysine by 6 kg pigs receiving diets containing 191 g/kg protein did not increase in response to supplementation with lysine until the diets contained more than 13–15 g lysine/kg. Sunflower-seed meal and maize-gluten meal were the protein sources in these diets and they contributed 4·7 g lysine/kg: fractional availability of this lysine would lead to a greater estimate of lysine requirement than that determined in the present study using skim milk and free amino acids, which are well absorbed, as lysine sources.

There are several earlier estimates of the lysine requirement of young pigs based on growth studies. Hutchinson et al. (1957) found that growth between 2 and 6 weeks was maximized by a lysine level of 9·35 g/kg in a diet containing 142 g protein/kg. However, in a later study Mitchell et al. (1965), using a diet containing 220 g protein/kg, found that 12·4 g lysine/kg were needed to maximize growth of pigs between 2 and 8 weeks of age. More recently it has been shown that the lysine requirement increases with the total protein level in the diet. Baker et al. (1975) found that the growth of pigs from 18 kg was maximized by 6·9 g lysine/kg in a diet containing 120 g protein/kg, but that increasing the protein level to 160 g/kg resulted in a need for 7·7 g lysine/kg to maximize growth. This observation helps to explain some of the discrepancies in the estimates of lysine requirements of growing pigs, but there are still such inconsistencies between values reporting the response of pigs to dietary lysine level that the (UK) Agricultural Research Council (1981) made only a tentative recommendation for dietary lysine requirements of pigs of 3–8 weeks of age.

Changing the lysine level in the low-lysine diets had less effect on phenylalanine oxidation than changing the levels of other amino acids in this and in the previous studies (Kim et al. 1983; Kim & Bayley, 1983). The curvilinear response of phenylalanine oxidation to dietary lysine level indicates that at low lysine levels changing the lysine concentration in the diet had little effect on phenylalanine oxidation, suggesting conservation of lysine under these conditions. Lysine conservation at low lysine intakes by rats was reported by Said & Hegsted (1970). A satisfactory explanation of this phenomenon has been provided for the rat by Chu & Hegsted (1976) who measured the activities of lysine ketoglutarate reductase (EC 1.5.1.8) and threonine dehydratase (EC 4.2.1.16) activities in the livers of rats which had been receiving various levels of lysine and threonine. The activity of the enzyme initiating lysine catabolism responded to lysine intake, and also to the intake of other amino acids, whereas the activity of the threonine dehydratase was not influenced by the intake of either threonine or the other amino acids. The observations in the present report extend the occurrence of this lysine-conserving phenomenon to the young pig; the rapidity of this response in these short-term studies is particularly noteworthy.

Threonine requirement

The (US) National Research Council (1979) lists the threonine requirement for pigs of 1–5 kg as 7·6 g/kg which is approximately 30% greater than the requirement of 5·6 g/kg listed for pigs of 5–10 kg. The requirement determined in the present study of 6 g/kg diet is much closer to that listed for the 5–10 kg pigs by the (US) National Research Council (1979). There may be less justification for increasing the threonine requirement for these smaller pigs than there was for increasing the lysine requirement, particularly as the enzymic studies of Chu & Hegsted (1976) showed that threonine degradation did not increase in response to a greater protein flux. The observation of Mitchell *et al.* (1968) that a dietary threonine level of 6 g/kg diet maximized N retention levels lends support to the currently determined requirement; however, pigs weighing 10 kg were used in their study.

Since lysine and threonine can be the first- and second-limiting amino acids in cereal-based diets for pigs, it is interesting to note that the requirements for the two amino acids of 12 and 6 g/kg respectively determined in the present study represents a ratio of 2:1 which is similar to that of the two amino acids in the pig's body protein. Fuller *et al.* (1979*a*) found that for 33 kg pigs receiving a barley diet, urinary N excretion was minimized by supplementing with lysine and threonine to provide levels of 8·15 and 4·7 g/kg respectively giving a relative value of 1·73:1 for the two amino acids. The corresponding value relating the dietary levels of these two amino acids in different diets has been of interest to nutritionists since Rose (1937) first discussed it, and Rosenberg *et al.* (1960) utilized the values for the ratio as a guide for the amino acid supplementation of rice and wheat.

Practical significance of amino acid requirements

Although the theoretical concept of specific requirements for each of the essential amino acids has provided a basis for the formulation of diets from feeds of differing composition, a review of the estimates of the requirements for each amino acid shows that definition of the requirements has been something of an unattainable goal. From the time of the classical work on response to amino acid supplementation by Rosenberg *et al.* (1959) to the present there have been indications that the simple concept of first-, second- and third-limiting amino acids in diets was inadequate to explain all the observations regarding an animal's response to supplementation of diets deficient in more than one amino acid. Rosenberg *et al.* (1959) found that for a diet with a marginal deficiency of a single amino acid, a small increment in the level of another amino acid above its requirement level would enhance protein accretion. A similar conclusion was drawn from an experiment in which the effects on the growth of chicks of reducing the dietary levels of the essential amino acids either individually or altogether were examined (Sugahara *et al.* 1969). Reducing the level of most of the individual amino acids had less effect on growth than reducing the levels of all the essential amino acids together.

Using phenylalanine oxidation as an indicator of amino acid catabolism has provided a sensitive assay of the effects of varying the levels of two or three amino acids in the diet which has not been previously available. This investigation has shown that when two amino acids are present at limiting concentrations in the diet, i.e. at what has traditionally been considered as the requirement, amino acid retention is reduced, with a greater surplus for oxidation than when there are generous levels of all but one of the amino acids in the diet. Few experiments have been reported which permit a critical evaluation of the interactions between two or more amino acids in the diet. However, Fuller *et al.* (1979*b*) did this in a study with pigs of between 25 and 60 kg weight which received barley diets supplemented with lysine and threonine. They found that 5 g threonine and 7·5 g lysine/kg diet maximized growth rates with higher levels of either amino acid reducing growth. However the lean

content of the carcass (as measured by specific gravity) continued to increase in response to the highest level of threonine employed, showing that this measure of protein metabolism responded differently than did total growth. Consequently their experiment left unanswered the question of what dietary threonine level would maximize protein deposition. The present study using amino acid oxidation as a sensitive index of protein metabolism suggests that such a question may, indeed, be unanswerable in terms of defining the level of any specific amino acid which would maximize protein deposition in the presence of various levels of the other essential amino acids.

Precise definition of the requirements for individual nutrients may be impossible if there are interactions between such nutrients as amino acids which depend on their relative abundance in the diet. The results of the present study show that the retention of a single amino acid is enhanced when this one amino acid is the only one present in the diet at the determined level. Lowering the dietary levels of two or more amino acids to the levels estimated as being 'required' on the basis of varying the amino acid levels singly showed those 'requirements' to be too low. Such a conclusion could be inferred from the work of Rosenberg et al. (1959) who suggested a 'mass action' effect increasing the incorporation of the 'limiting' amino acid into protein in diets leading to greater rates of protein deposition. A more rational explanation may arise from studies of protein turnover. Reeds et al. (1980) measured protein synthesis and degradation in growing pigs. They found that 30 kg pigs which were digesting 178 g protein/d were synthesizing 406 g protein/d, but degrading 312 g/d. Thus the endogenous contribution of amino acids to the body pool was almost twice that of the dietary contribution. The partition of amino acids between protein synthesis and amino acid catabolism must depend on many factors, the combined effects of which may be too complex to permit the definition of a single requirement for each nutrient. The effects of this uncertainty are embodied in such expressions as 'individual variability', and the 'margins of safety' used in the formulation of complete diets. In practice these uncertainties may be unimportant if the magnitude of the changes in requirement due to amino acid interactions are small compared to the margins of safety used in formulating diets. The magnitude of the uncertainty associated with the definition of the requirements for individual amino acids should be determined quantitatively and use of the indictor amino acid oxidation technique appears to be a suitable procedure to adopt.

There may be a biological 'uncertainty principle' which becomes important as procedures are refined to examine specific metabolic functions in detail. Precise estimates of the dietary requirements for individual amino acids whose utilization is influenced by other dietary factors, particularly energy intake, and whose net incorportion into protein depends on the complex of factors influencing protein turnover, need careful assessment using newly-available techniques for measuring protein metabolism.

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