Effect of frequency of feeding of diets containing free or protein-bound lysine on the oxidation of [^{14}C]lysine or [^{14}C]phenylalanine by growing pigs

BY E. S. BATTERHAM* AND H. S. BAYLEY

Department of Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada

(*Received 23 March 1989 – Accepted 21 July 1989*)

The effect of frequency of feeding (once daily or six equal portions at intervals of 3 h) of diets containing lysine (6.4 g/kg) supplied as either protein-bound or free lysine on the metabolism of essential amino acids was investigated in pigs over the 23-34 kg weight range. The amounts of radioactivity from $^{14}CO_2$ produced by the pigs given oral doses of [^{14}C]lysine or [^{14}C]phenylalanine were determined. [^{14}C]Lysine was confirmed to be unsuitable presumably because the oral dose was diluted by the dietary free lysine or was absorbed ahead of the protein-bound lysine, or both. With [^{14}C]phenylalanine addition, there was a significantly ($P < 0.01$) greater production of $^{14}CO_2$ by pigs fed once daily on the diet containing free lysine. However, with frequent feeding, there was no significant difference ($P > 0.05$) in the amount of $^{14}CO_2$ produced by pigs from [^{14}C]phenylalanine for either diet. Growth responses of the pigs were lower when the diet containing free lysine was given once daily ($P < 0.01$) but were similar when the diets were given frequently. It is concluded that the more rapid absorption of free lysine by growing pigs fed once daily results in an imbalance of amino acids at the sites of metabolism. This leads to greater oxidation of essential amino acids than occurs when all the amino acids are supplied in the protein-bound form. With frequent feeding, a better balanced supply of amino acids is absorbed, leading to similar rates of oxidation of excess essential amino acids from diets containing either free or protein-bound lysine.

Feeding frequency: Lysine: Amino acid oxidation: Pig

Supplements of free lysine in diets for growing pigs fed once daily are used with an efficiency of half that with which free lysine is used when the pigs are fed frequently (Batterham & Murison, 1981). The low utilization appears due to more rapid absorption of the free lysine than of the other, protein-bound, amino acids, resulting in a temporary imbalance at the sites of metabolism. This leads to the catabolism of the temporary surplus of free lysine. Frequent feeding would lead to the absorption of the balanced supply of amino acids preformulated for the diets, allowing more efficient utilization of both the free lysine and the other, protein-bound, essential amino acids.

The free lysine utilization studies of Batterham & Murison (1981) are based on growth experiments. Studies have shown that free lysine is absorbed more rapidly than protein-bound lysine (Williams & Dunkin, 1980). However, the metabolism of free lysine (and of protein-bound amino acids) after absorption has not been studied.

The use of an indicator amino acid to monitor amino acid metabolism (Kim *et al.* 1983) would appear to have application. The oxidation of an indicator amino acid, such as [^{14}C]phenylalanine, monitors the metabolism of the test amino acid and it should be able to monitor the metabolism of essential amino acids following absorption of diets containing either free or protein-bound lysine.

The aim of these studies was to investigate the effects of frequency of feeding on the metabolism of essential amino acids, using [^{14}C]phenylalanine as an indicator amino acid.

* Present address: NSW Agriculture and Fisheries, North Coast Agricultural Institute, Wollongbar, New South Wales 2480, Australia.

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

	Wheat	Wheat gluten	Soya-bean meal
Crude protein (nitrogen × 6·25)	79	784	471
Dry matter	888	934	892
Light petroleum (b.p. 40–60°) extract	19	9	6
Crude fibre	26	4	37
Ash	17	10	63
Essential amino acids			
Threonine	2·6	18·7	18·7
Valine	4.2	38.9	24.9
Cystine	0·9	13·2	5·2*
Methionine	1·5	12·2	8·2*
Isoleucine	3·1	25·5	20·8
Leucine	5.3	64·6	34·8
Tyrosine	1·9	26·2	10·0
Phenylalanine	3·3	35·3	20·9
Histidine	1·4	12·4	9·5
Lysine	2·6	12·4	29·1
Tryptophan	0·6	3·7	5·8

* Cystine and methionine were estimated from values for similar meals.

The diets contained either free or protein-bound lysine and the overall level of lysine in the diets was suboptimal, to make protein metabolism sensitive to the lysine supply. In a preliminary experiment the effect of frequency of feeding on the catabolism of [^{14}C]lysine was examined for diets containing free and bound lysine.

EXPERIMENTAL

Diets

The composition of the dietary ingredients is given in Table 1. The diets were formulated to be lysine deficient (6.4 g lysine/kg) using wheat, wheat gluten, and L-lysine monohydrochloride or wheat and soya-bean meal and to contain an estimated 14.3 MJ digestible energy/kg (Table 2).

Pigs

Large White × Yorkshire female pigs with an initial weight of 16–24 kg were used. Dietary treatments were allocated to pairs of pigs on a weight basis. The pigs were housed in individual pens and water was supplied by 'nipple' drinkers. They were fed at a daily rate of 1 kg at 20 kg live weight, incremented by 0·1 kg/d for each additional 2·5 kg live weight. The diets were offered wet (feed–water, 1:1 w/w). For once-daily feeding, the pigs were trained to consume their daily ration in 10 min. With frequent feeding the pigs were given their daily ration in six equal portions at three-hourly intervals, commencing at 07.00 hours. Collections were conducted with the pigs over the 23-34 kg live weight range.

$^{14}CO_2$ collection

Pigs were placed in wooden chambers with a Perspex window. The chambers measured 0·4 × 1·0 × 0·6 m. The pigs were placed in the chambers on two occasions before the collections to ensure their familiarity with the procedure. Air (35 litres/min) was drawn through the chamber, passed through a cold water condenser to remove water vapour and then through three gas-washing bottles, each containing 200 ml CO_2-absorber (etha-

Table 2. *Components and composition (g/kg, air-dry basis) of the diets*

	Diet 1 Protein-bound plus free lysine	Diet 2 Protein-bound lysine
Ingredients		
Wheat	845	795
Wheat gluten	100	—
Soya-bean meal	—	150
L-Lysine hydrochloride	3·85	—
Mineral and vitamin premix*	6	6
Dicalcium phosphate	30	30
Maize oil	15·15	19
Composition		
Crude protein (nitrogen × 6·25)	145	133
Dry matter	896	893
Light petroleum (b.p. 40–60°) extract	32	35
Crude fibre	22	26
Essential amino acids		
Threonine	4·1	4·9
Valine	7·4	7·1
Cystine	2·1	1·5
Methionine	2·5	2·4
Isoleucine	5·2	5·6
Leucine	10·9	9·4
Tyrosine	4·2	3·0
Phenylalanine	6·3	5·8
Histidine	2·4	2·5
Tryptophan	0·9	1·4
Total lysine	6·40	6·40
Protein-bound lysine	3·40	6·40
Free lysine	3·00	—
Digestible energy (estimated) MJ/kg	14·2	14·4

* Contributed the following (mg/kg diet): iron 60, zinc 100, manganese 30, copper 5, iodine 2, selenium 0·15, sodium chloride 2·8 g, retinol equivalent 0·96 μg, cholecalciferol 12 μg, α-tocopherol 20, thiamin 1·5, riboflavin 3, nicotinic acid 14, pantothenic acid 10, pyridoxine 2·5, cyanocobalamin 15 μg, pteroylmonoglutamic acid 2, choline 500, ascorbic acid 10, biotin 0·1.

nolamine and ethylene glycol monomethyl ether (1:2, v/v)). The CO_2-absorbing mixture was changed at 30 min intervals. Radioactivity of the absorbed CO_2 was measured by mixing a 1 ml portion of the CO_2-absorbing mixture with 15 ml ACS (Amersham Corp., IL, USA) and counting in a liquid-scintillation spectrometer (model Delta 300; Searle Analytical, IL, USA).

[^{14}C]*lysine studies*

Single 25 μCi tracer doses of L-[U-^{14}C]lysine (New England Nuclear, Boston, MA, USA) were diluted with water and mixed with the daily ration. Respired $^{14}CO_2$ was collected for 6 h following consumption of the daily meal. Collections were completed for six pigs per diet for the first 3 h, but for only four pigs per diet between 3 and 6 h.

[^{14}C]*phenylalanine studies*

Single 25 μCi tracer does of L-[1-^{14}C]phenylalanine (New England Nuclear) were diluted with water and mixed with the meal before feeding. With once-daily feeding, $^{14}CO_2$

collections were made every 30 min for the first 3 h after feeding, and then for a 30 min period for the latter portion of each hour for the subsequent 5 h, using six pigs per diet.

With frequent feeding, the [^{14}C]phenylalanine was administered with the 13.00 hours feed, and $^{14}CO_2$ was collected every 30 min for 6 h. These collections were interrupted for the 16.00 hours meal, which took 3 min. Six pigs per treatment were used for these studies. Three of the six pigs per treatment had previously been used in the $^{14}CO_2$ collections for the once-daily feeding regimen. These pigs were changed to frequent feeding for approximately 7 d and then the production of $^{14}CO_2$ was measured for 60 min to determine background levels of $^{14}CO_2$ production. On the following day they were used for $^{14}CO_2$ collection as described for the frequent feeding regimen. The background of $^{14}CO_2$ production of the pigs, which was negligible, was subtracted from the $^{14}CO_2$ frequent feeding measurements.

Chemical analysis

The feed ingredients were analysed for nitrogen, dry matter, diethyl ether extract, crude fibre and ash using the methods of the Association of Official Analytical Chemists (1975). Total amino acids (except tryptophan) were determined using a Technicon TSM amino acid AutoAnalyzer following hydrolysis in 6 M-hydrochloric acid at 110° for 24 h. Tryptophan was determined by the method of Hugli & Moore (1972).

Statistical analysis

The values for $^{14}CO_2$ production by pigs receiving the two diets in the two feeding regimens were treated by analysis of variance.

RESULTS

[^{14}C]*lysine*

Pigs given diet 2, containing protein-bound lysine, produced more $^{14}CO_2$ than pigs given diet 1, containing free and protein-bound lysine (Fig. 1). The differences in rates of production were significant ($P < 0.05$) for the first hour after the meal (Fig. 1*a*) while the differences in cumulative production (Fig. 1*b*) were significant ($P < 0.05$) for the first 1.5 h after the meal.

There was a marked increase in the variability of the $^{14}CO_2$ production rates for the pigs receiving the protein-bound-lysine diet (diet 2) after 3 h.

[^{14}C]*phenylalanine*

With once-daily feeding, production of $^{14}CO_2$ increased to a maximum at 90 min after feeding for pigs given both diets and then declined gradually. Production of $^{14}CO_2$ was significantly greater ($P < 0.05$) throughout the 8 h collection period for the pigs receiving the diet with added free lysine (Fig. 2*a*).

Production of $^{14}CO_2$ by the pigs fed frequently increased rapidly for 1·5–2 h after feeding, and then increased again 1-1·5 h after the second meal (Fig. 2*b*). There was no difference in the production of $^{14}CO_2$ for the pigs given the two diets for the first 4·5 h after ingestion of the tracer dose of [^{14}C]phenylalanine. From 4·5 h to 6 h it was slightly greater for pigs given the diet with the free lysine ($P < 0.05$). The variability (SE) of the $^{14}CO_2$ production was greater for the frequent than for the once-daily-fed pigs.

With once-daily feeding, cumulative $^{14}CO_2$ production for the pigs given the diet containing free lysine was 1·33 times greater than for the pigs given the diet containing protein-bound lysine ($P < 0.01$) (Fig. 3*a*). With frequent feeding, there were no significant differences ($P > 0.05$) in the total production of $^{14}CO_2$ by pigs given the two diets (Fig. 3*b*).

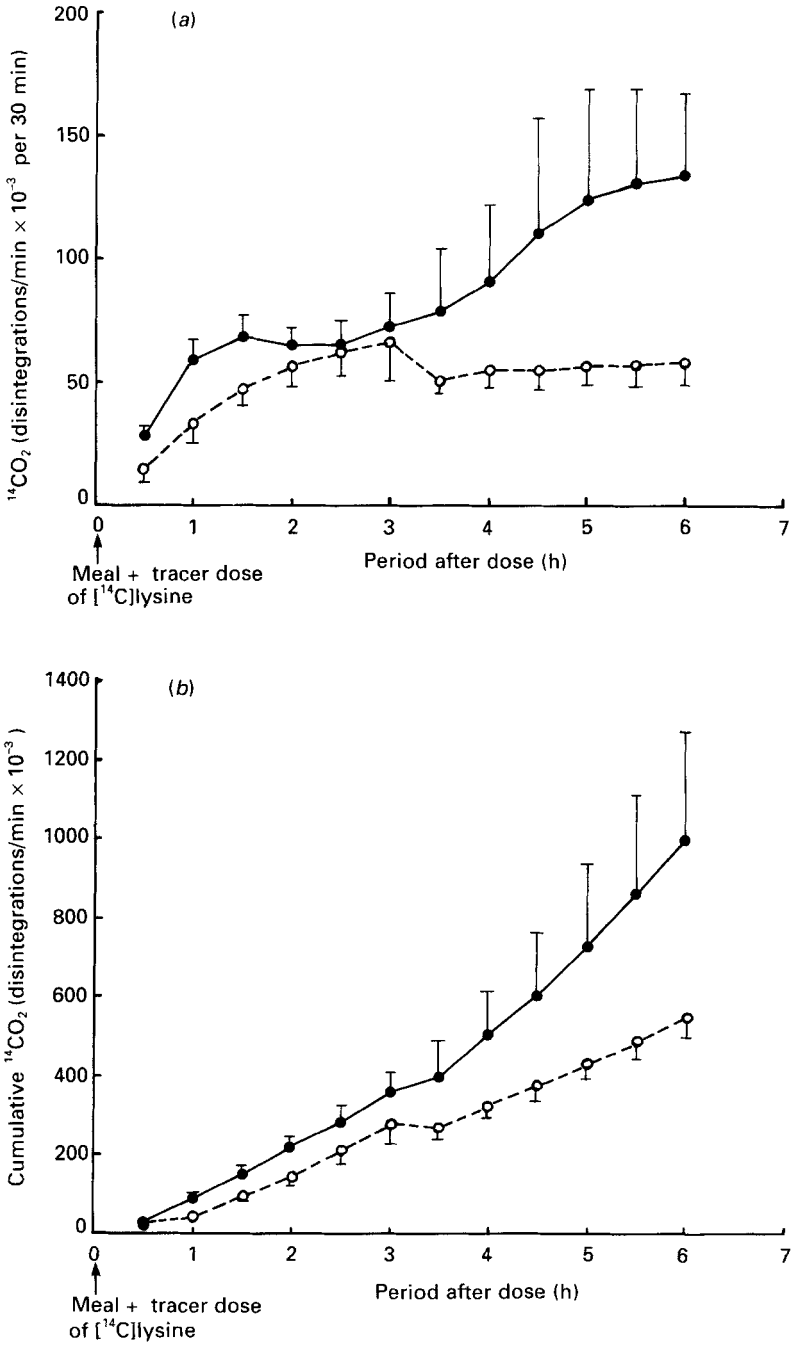

Fig. 1. Radioactivity released as $^{14}CO_2$ from L-[U-^{14}C]lysine by pigs given a single meal containing free (○----○) or protein-bound (●——●) lysine. (*a*) Rate per 30 min, (*b*) cumulative production of $^{14}CO_2$ for the 6 h period following the tracer dose of L-[U-^{14}C]lysine. Values are means with their standard errors represented by vertical bars for six pigs per diet for 0–3 h and four pigs from 3–6 h.

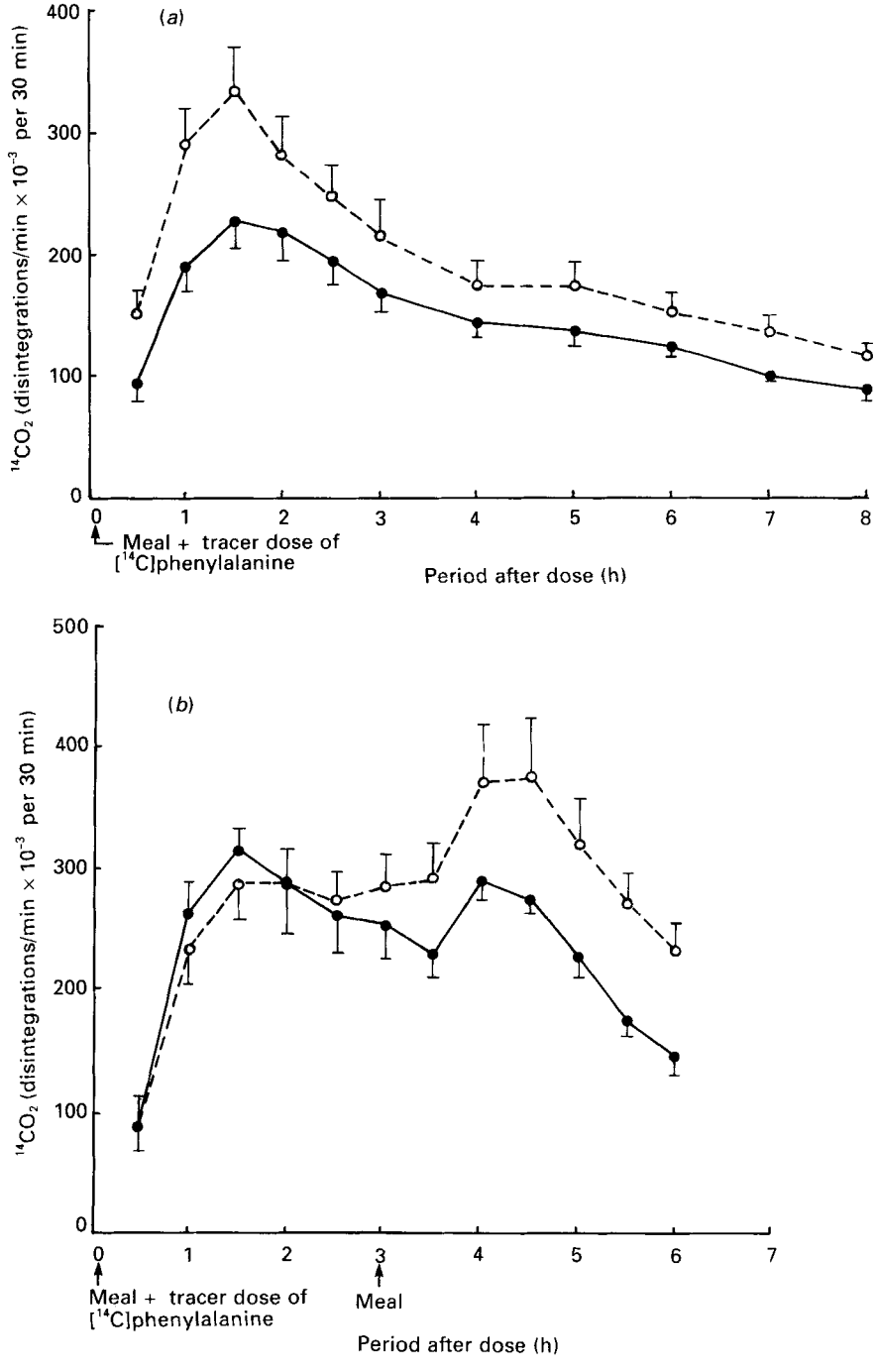

Fig. 2. Radioactivity released as $^{14}CO_2$ per 30 min from L-[1-^{14}C]phenylalanine by pigs given diets containing free (○----○) or protein-bound (●——●) lysine once daily (*a*) or in six equal portions at intervals of 3 h (*b*). Values are means with their standard errors represented by vertical bars for six pigs.

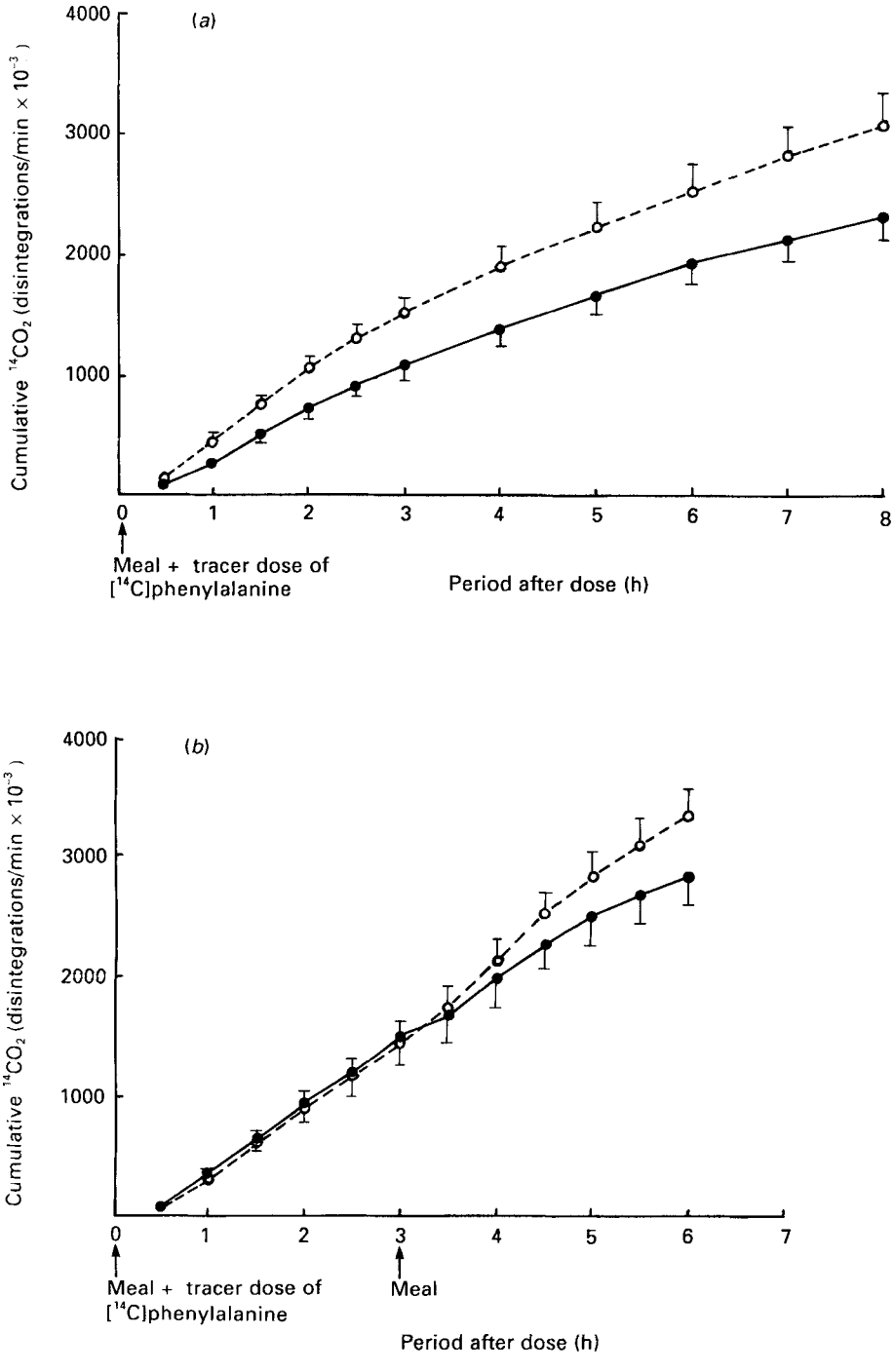

Fig. 3. The cumulative release of $^{14}CO_2$ from L-[1-^{14}C]phenylalanine by pigs fed on diets containing either free (○----○) or protein-bound (●——●) lysine, for pigs fed once daily (*a*) or in six equal portions at intervals of 3 h (*b*). Values are means with their standard errors represented by vertical bars for six pigs.

Growth performance

Pigs given diet 2 (containing protein-bound lysine) grew significantly ($P < 0.01$) faster (536 *v.* 409 (SEM 14) g/d) and had lower ($P < 0.01$) feed:gain ratios (2·09 *v.* 2·61 (SEM 0·06)) than pigs given diet 1 (containing free lysine). When the pigs were fed frequently there were no significant differences ($P > 0.05$) in weight gains (528 *v.* 514 (SEM 17·3) g/d) or feed:gain ratios (2·34 *v.* 2.35 (SEM 0·09)) for diets 1 and 2 respectively.

DISCUSSION

The initial more rapid oxidation of [^{14}C]lysine by pigs given the diet containing protein-bound lysine in a single daily meal confirms that this compound is unsuitable for monitoring the metabolism of protein-bound lysine. The initial rapid oxidation could be explained by the tracer dose of free [^{14}C]lysine being more rapidly absorbed and entering the circulation before the other protein-bound amino acids. With the diet containing free lysine, the tracer dose would have been diluted with the dietary free lysine, reducing the specific activity of the lysine being oxidized and hence reducing the amount of $^{14}CO_2$ produced. To use [^{14}C]lysine for these studies it would be necessary to label the protein-bound lysine as well as the free lysine.

With once-daily feeding, the greater oxidation of [^{14}C]phenylalanine by pigs given the diet containing free lysine indicates that the essential amino acids were used less efficiently for protein deposition than by the pigs given diet 2, containing protein-bound lysine. This indicates that the supplementary free lysine was absorbed ahead of the protein-bound lysine because of either separation in the stomach or because of the need for hydrolysis of the protein-bound lysine before absorption. Decreased efficiency of utilizing the absorbed free lysine would result in decreased utilization of the other essential amino acids, as lysine was the first limiting amino acid in the diet. In contrast, the similar amounts of $^{14}CO_2$ produced by the frequently fed pigs indicated that the free and bound forms of lysine were being utilized equally, allowing more efficient utilization of the other essential amino acids.

Although these studies were conducted over short time-periods of 7–14 d, the growth rates and feed:gain ratios of the pigs were affected by the feeding systems. With once-daily feeding, growth rates were lower and feed:gain ratios higher for pigs given the diet containing free lysine. However, with frequent feeding there were no differences in these variables. Thus, the growth responses support the conclusion from the oxidation studies and illustrate the sensitivity of growth to change in amino acid metabolism.

The diets were formulated to be lysine-deficient according to the estimates of the Agricultural Research Council (1981). However, recent estimates by Fuller & Wang (1987) indicate that tryptophan and threonine may have been more limiting than lysine in diet 1, for frequent feeding. With once-daily feeding, where only a 0·5 utilization of the added free lysine was anticipated, lysine was first limiting according to both estimates. The lower growth of pigs given diet 1 with once-daily feeding, and the similar response with frequent feeding, indicate that lysine was limiting in the diets.

With once-daily feeding, the pattern of production of $^{14}CO_2$ by pigs after administration of the tracer dose of [^{14}C]phenylalanine was relatively consistent between collections, reaching a peak 1–1·5 h after the meal. In contrast, with frequent feeding there was no consistent pattern of oxidation of [^{14}C]phenylalanine: in some collections, maximum production occurred within 1–2 h of ingestion of the tracer dose, whereas in other collections little $^{14}CO_2$ was produced initially but production peaked 4–5 h after the tracer dose. This suggests that there is greater variation in the flow patterns of amino acids with the ingestion of small individual meals than occurs when one large daily meal is consumed.

In general, these results support previous findings (Batterham & Murison, 1981) that supplements of free lysine are inefficiently utilized with once-daily feeding. As a consequence, there is more efficient utilization of other essential amino acids by pigs when diets containing free lysine are given at frequent intervals. These results extend the use of an indicator amino acid, such as [^{14}C]phenylalanine, from measurements of requirements (Kim *et al.* 1983) to other studies of amino acid metabolism. The indicator amino acids have application in situations such as these, where it was not possible to label the protein-bound lysine.

This work was supported by the Natural Science and Engineering Council of Canada and the Australian Pig Industry Research Committee.

REFERENCES

Agricultural Research Council (1981). *The Nutrient Requirement of Pigs*, 2nd ed. Slough: Commonwealth Agricultural Bureaux.

Association of Official Analytical Chemists (1975). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 12th ed. Washington, DC: Association of Official Analytical Chemists.

Batterham, E. S. & Murison, R. D. (1981). Utilization of free lysine by growing pigs. *British Journal of Nutrition* **46**, 87–92.

Fuller, M. F. & Wang, T. C. (1987). Amino acid requirements of the growing pig. In *Manipulating Pig Production*, pp. 97–111 [J. L. Barnett, E. S. Batterham, G. M. Cronin, C. Hansen, P. H. Hemsworth, D. P. Hennessy, P. E. Hughes, N. E. Johnston and R. H. King, editors]. Ferntree Gully: JFL Promotions.

Hugli, T. E. & Moore, S. (1972). Determination of the tryptophan content of proteins by ion-exchange chromatography of alkaline hydrolysates. *Journal of Biological Chemistry* **247**, 2828–2834.

Kim, K., McMillan, I. & Bayley, H. S. (1983). Determination of amino acid requirements of young pigs using an indicator amino acid. *British Journal of Nutrition* **50**, 369–382.

Williams, K. C. & Dunkin, A. C. (1980). Utilization of synthetic lysine by the growing pig. *Proceedings of the Australian Society of Animal Production* **13**, 149–152.

Printed in Great Britain