

Digestion in the pig between 7 and 35 d of age
5. The incorporation of amino acids absorbed in the small intestines
into the empty-body gain of pigs given milk or soya-bean proteins

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(Received 18 October 1979 – Accepted 29 August 1980)

1. In two separate experiments, forty-two pigs weaned at 4–5 d of age were given diets containing milk or soya-bean protein and slaughtered at 7, 28 or 35 d of age.
2. The amino acid composition of the empty body of the pigs did not vary with age or sources of protein fed.
3. The nitrogen and amino acid contents of the empty body gain were calculated between 7 and 28, and 7 and 35 d of age. The retention of absorbed N was greater in pigs given a soya-bean meal (SBM) diet than in pigs given milk or isolated soya-bean protein (ISP) diets.
4. The retention of total absorbed N was greater in pigs given a pelleted milk diet (0.72) than in pigs given a liquid milk diet (0.58).
5. Methionine and threonine appeared to be the first two limiting amino acids, as indicated by their high incorporation into the empty body, for pigs given soya-bean proteins in Expt 3, while methionine and lysine were the first two limiting amino acids for pigs given ISP in Expt 4.
6. The addition of methionine to diets in Expt 3 reduced the retention of absorbed methionine in the empty body by 43–76%. Lysine supplementation did not alter the retention of the absorbed lysine.
7. Retention of arginine, proline and glycine in the body was greater than the apparent absorption of each of these amino acids.

The quality of a protein source to a pig depends to a large extent on how its amino acid content meets the requirements of the pig. The over-all efficiency of protein use is a function of protein quality, which is usually measured by the retention of N in relation to total N intake or absorbed N. This over-all efficiency is the sum of the efficiency of use of individual amino acids by the tissues, and should be measured under carefully controlled conditions for specific rates of growth or milk production.

The levels of protein synthesis in the body tissues will influence to a great extent the utilization of dietary amino acids. The fractional retention of each amino acid absorbed into carcass gain should in theory be inversely related to its amount and availability in the diet. In rapidly-growing animals consuming diets containing sub-optimal amounts of a readily-digested protein, the most limiting amino acid would be expected to show the highest fractional retention.

During the turnover of protein there will be an inevitable loss of amino acids to the animal, especially as the protein turnover in muscle is more rapid than protein deposition (Edmunds *et al.* 1978). Losses of histidine, lysine and proline may limit the amino acid supply for the resynthesis of protein, as they are partially converted to other compounds and excreted in the urine (Munro, 1970; Harris *et al.* 1977).

The value for the amount of an amino acid recovered from the carcass gain: the amount consumed in the diet or absorbed from the small intestine should provide a valuable measure of the fractional retention of an amino acid by the tissues for protein synthesis.

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EXPERIMENTAL

Animals and diets

Expt 3. Thirty-six pigs (mean weight 2.05 kg, mean age 7 d) were allocated to six diets as a 3×2 factorial with three replicates of two pigs per replicate. Three protein sources were compared in isonitrogenous diets: milk, isolated soya-bean protein (ISP; Promine D; Central Soya Co., Chicago, Ill.) and soya-bean meal (SBM), each with and without methionine supplementation, and lysine supplementation of the ISP and SBM diets. The composition of the diets is given in Wilson & Leibholz (1981 *a*). After 17 d, one pig per pen was removed and the remaining eighteen pigs were given the experimental diets sprayed with indigestible markers for a further 5 d. The pigs were then slaughtered at 28 d of age as described in Wilson & Leibholz (1981 *b*).

Expt 4. Four pigs were slaughtered at 7 d of age. A further twenty-four pigs (mean weight 2.43 kg, mean age 7 d) were allocated to three diets: an all-milk-protein diet fed either pelleted or liquid or a pelleted diet in which the protein source was ISP (Supro 610; Ralston Purina, St Louis, Mo.). There were eight replicates of one pig per replicate. The composition of the diets is given in Wilson & Leibholz (1981 *a*). Twelve of these pigs were fed the diets *ad lib.* from 4–5 d of age until 9 d of age and then at 2 g nitrogen/kg live weight^{0.75} per d for 5 d until slaughter at 14 d of age. The other twelve pigs were given the diets *ad lib.* until 30 d of age and then at 2 g N/kg live weight^{0.75} per d for 5 d until slaughter at 35 d of age. The pigs were given equal amounts of food every 2 h over the 5 d preceding slaughter. Indigestible markers were administered to the pigs immediately preceding each 2 h feed.

Indigestible markers

The indigestible markers used were the ⁵¹Cr complex of ethylenediaminetetra-acetic acid (EDTA) (⁵¹Cr EDTA) (Downes & McDonald, 1964) and ¹⁰³Ru-labelled Tris-(1,10-phenanthroline)-ruthenium (II) chloride (¹⁰³Ru-P) (Tan *et al.* 1971). Flow rates were calculated as described by Wilson & Leibholz (1981 *b*).

Analytical methods

Amino acid analysis was carried out on freeze-dried samples of whole empty body by the methods described for dietary amino acid analysis in Wilson & Leibholz (1981 *d*).

Statistical methods

The results were subjected to analysis of variance with treatment means being statistically compared using least significant difference comparisons (Steel & Torrie, 1960).

RESULTS

Empty-body amino acids

In Table 1, the mean values for the amino acid composition (g/16 g N) of the empty body of pigs at 14, 28 and 35 d of age are given. In Expt 3 there were no significant differences between the amino acid composition of pigs given the three protein sources. Amino acid supplementation of these diets did not influence empty-body amino acid contents. Also, the amino acid contents of the empty bodies at 14, 28 and 35 d of age were similar (Expts 3 and 4).

Utilization of dietary and absorbed N

There were differences in the efficiency of incorporation of absorbed N into empty-body gain of pigs given the protein sources in Expts 3 and 4. Pigs given the unsupplemented ISP diet in Expt 3 had a lower N utilization (when calculated from the apparent and total

Table 1. Expts 3 and 4. Amino acid composition (g/16 g nitrogen) of the empty body of pigs at 14, 28 and 35 d of age

Expt no. ...	3										4 (14 d of age)						4 (35 d of age)													
	Milk			ISPT			SBM			SEM			Milk			ISPT			SEM			Milk			ISPT			SEM		
	-	+	+	-	+	+	-	+	+	SEM	-	+	+	P	L	L	P	L	L	SEM	-	+	+	P	L	L	P	L	L	SEM
Protein source ...	6.76	6.28	6.03	6.52	6.09	6.27	0.387	5.88	5.70	5.49	0.260	5.81	6.46	6.83	0.188**															
Amino acid	2.67	2.69	2.80	2.23	2.51	2.65	0.168	2.89	2.76	3.02	0.049*	2.68	2.99	3.01	0.049***															
supplementation ...	4.25	4.37	4.16	3.84	3.61	4.38	0.225	4.05	3.82	3.64	0.189	3.65	4.13	4.28	0.195															
Arginine	7.30	7.21	6.62	7.12	7.23	7.06	0.346	7.33	7.27	7.79	0.186	6.61	6.81	6.79	0.168															
Histidine	6.20	6.55	5.61	5.49	5.58	6.27	0.339	6.87	7.01	7.22	0.205	6.52	6.87	6.77	0.153															
Isoleucine	2.33	2.38	1.97	2.43	2.42	2.25	0.151	2.37	2.05	2.40	0.038***	2.12	2.59	1.99	0.223															
Leucine	1.15	1.08	1.21	1.12	1.01	1.23	0.073	1.08	1.03	1.78	0.020***	1.09	1.18	0.96	0.036															
Lysine	4.40	4.04	3.80	4.08	4.37	3.88	0.297	4.52	4.23	4.72	0.083**	4.16	4.36	4.28	0.063															
Methionine	3.23	3.65	3.52	3.63	3.70	3.54	0.153	3.72	3.72	3.06	0.073***	3.79	3.46	3.76	0.113															
Cystine	5.10	5.47	5.55	5.75	4.73	5.04	0.368	3.86	4.00	5.02	0.068***	5.56	5.67	5.78	0.199															
Phenylalanine	7.18	6.59	6.60	6.30	6.75	7.05	0.304	7.02	6.62	5.84	0.110***	6.65	6.89	7.16	0.255															
Threonine	10.86	11.36	10.43	11.17	10.66	10.34	0.393	10.98	10.97	9.45	0.195***	10.50	11.48	10.95	0.340															
Valine	12.53	12.70	12.43	12.24	12.81	11.63	0.236	13.35	12.67	12.93	0.289	12.92	13.02	12.77	0.287															
Alanine	8.86	8.82	9.70	8.50	9.46	10.39	0.805	9.07	8.13	7.90	0.140***	6.95	8.89	8.65	0.452*															
Aspartic acid	5.87	6.23	5.99	6.21	5.95	5.85	0.396	6.17	5.80	5.61	0.245	5.93	5.34	5.66	0.147															
Glutamic acid	3.25	2.98	3.02	3.02	3.61	2.87	0.251	3.60	3.25	3.40	0.115	3.23	3.94	3.44	0.048															
Glycine	4.61	4.89	3.95	4.29	4.15	3.67	0.259	4.22	4.70	4.34	0.193	4.18	4.50	5.02	0.077***															
Proline																														
Tyrosine																														
Serine																														

+, Present; -, absent.
P, pelleted; L, liquid; ISP, isolated soya-bean protein; SBM, soya-bean meal.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Promine D; Central Soya Co., Chicago, Ill.
‡ Supro 610; Ralston Purina, St Louis, Mo.

Table 2. *Expt 3. The incorporation of dietary and absorbed nitrogen in the empty body of pigs between 7 and 28 d of age*

Protein source	Amino acid supplement	Efficiency of utilization of N		
		A	B	C
Milk	—	0.812	0.721	0.667
	+	0.854	0.745	0.662
Isolated soya-bean protein†	—	0.618	0.547	0.508
	+	0.892	0.736	0.446
Soya-bean meal	—	0.986	0.817	0.515
	+	0.927	0.760	0.453
SEM		0.104*	0.083*	0.067*

+, Present; —, absent.

A, gain in empty-body N (g/d): apparent N absorption to ileum (g/d); B, gain in empty-body N (g/d): true N absorption to ileum (g/d); C, gain in empty-body N (g/d): dietary N intake (g/d).

* $P < 0.05$.

† Promine D; Central Soya Co., Chicago, Ill.

Table 3. *Expt 4. The incorporation of dietary and absorbed nitrogen in the empty body of pigs between 7 and 35 d of age*

Protein source	Efficiency of utilization of N		
	A	B	C
Milk: pelleted liquid	0.808	0.717	0.672
	0.645	0.576	0.565
Isolated soya-bean protein†	0.691	0.615	0.610
SEM	0.036**	0.031**	0.027

A, gain in empty-body N (g/d): apparent N absorption to ileum (g/d); B, gain in empty-body N (g/d): true N absorption to ileum (g/d); C, gain in empty-body N (g/d): dietary N intake (g/d).

** $P < 0.01$.

† Supro 610; Ralston Purina, St Louis, Mo.

absorbed N) (Table 2) than for pigs given the milk or SBM diets. Amino acid supplementation improved the N utilization for pigs given ISP but not for pigs given the milk or SBM diets.

In Expt 4, the incorporation of absorbed N into body gain was greater for pigs given the pelleted milk diet than for pigs given the liquid milk and pelleted ISP diets (Table 3). There were no significant differences in this retention due to the protein sources fed when expressed on an N intake basis.

Utilization of dietary and absorbed amino acids

Differences in the efficiency of incorporation of absorbed amino acids into the empty-body gain of pigs given the six diets in Expt 3 are shown in Table 4. Methionine supplementation of the three protein sources resulted in a significant reduction in the efficiency with which methionine was incorporated into empty-body gain. The addition of lysine to the ISP and SBM diets did not result in any reduction in the utilization of lysine. Arginine and aspartic acid levels in milk protein are well below the levels in soya-bean proteins. These amino acids

Table 4. Expt 3. Incorporation of apparently absorbed amino acids into the empty body of pigs between 7 and 28 d of age

Amino acid supplement ...	Efficiency ratio‡						SEM
	Milk		Isolated soya-bean protein†		Soya-bean meal		
	-	+	-	+	-	+	
Essential							
Arginine	1.648	1.660	0.475	0.428	0.692	0.709	0.141***
Histidine	0.780	0.678	0.585	0.429	0.691	0.635	0.106
Isoleucine	0.564	0.591	0.453	0.461	0.542	0.706	0.085
Leucine	0.649	0.624	0.434	0.547	0.739	0.756	0.110
Lysine	0.595	0.605	0.465	0.420	0.585	0.594	0.092
Methionine	0.609	0.349	0.754	0.239	0.929	0.226	0.126*
Cystine	0.814	0.729	0.646	0.571	0.430	0.653	0.072*
Phenylalanine	0.645	0.548	0.319	0.348	0.604	0.494	0.081
Threonine	0.690	0.710	0.665	0.646	0.926	1.100	0.115
Valine	0.496	0.517	0.661	0.784	0.652	0.695	0.090
Non-essential							
Alanine	1.710	1.597	0.938	0.935	1.958	1.819	0.344
Aspartic acid	1.020	1.039	0.572	0.731	0.548	0.536	0.099**
Glutamic acid	0.542	0.587	0.419	0.616	0.554	0.500	0.084
Glycine	3.632	4.172	1.681	2.497	3.729	3.289	0.510*
Tyrosine	0.585	0.497	0.475	0.544	0.786	0.563	0.051*
Serine	0.844	0.674	0.783	0.749	1.161	0.922	0.099

+, Present; -, absent.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Promine D; Central Soya Co., Chicago, Ill.

‡ Gain in empty-body amino acid (g/d): apparent amino acid absorption to ileum (g/d).

showed a higher fractional retention into the empty-body gain of pigs given the milk-protein diets compared to pigs given the ISP and SBM diets.

Similar results were obtained in Expt 4 (Table 5). Methionine was more efficiently incorporated into empty-body gains of pigs given ISP compared to pigs given milk protein. The fractional retention of amino acids followed a similar trend to absorbed N in that the values were generally lower for pigs given the liquid milk diet than the two pelleted diets.

DISCUSSION

The amino acid composition of the empty body of pigs for both experiments (mean of twenty-four pigs) was similar to published values (Williams *et al.* 1954; Aumaitre & Duée, 1974). It would be difficult to determine whether the small differences would be due to the different methods of amino acid analysis, sample preparation, or whether there are any real differences in the amino acid composition of the empty bodies.

The results presented show that the amino acid composition of the empty body of pigs does not alter greatly during the growth of the pig, which agrees with the results of Williams *et al.* (1954) and Aumaitre & Duée (1974). It would also appear that the amino acid composition of the diet does not markedly affect the amino acid composition of the body. Similar results were obtained by Nielsen (1973) with pigs, and by Pellett & Kaba (1972) with rats.

Edmunds *et al.* (1978) found that 57% of N absorbed from the gastro-intestinal tract

Table 5. *Expt 4. Incorporation of apparently absorbed amino acids into the empty body of pigs between 7 and 35 d of age*

Protein source ...	Efficiency ratio†			SEM
	Milk		Isolated soya-bean protein†	
	Pelleted	Liquid		
Essential				
Arginine	1.198	0.975	0.585	0.071***
Histidine	0.704	0.580	0.662	0.038
Isoleucine	0.476	0.415	0.526	0.022*
Leucine	0.562	0.484	0.528	0.047
Lysine	0.641	0.511	0.807	0.050*
Methionine	0.538	0.513	1.035	0.092**
Cystine	0.866	0.689	0.512	0.042***
Phenylalanine	0.613	0.451	0.479	0.032*
Threonine	0.786	0.521	0.672	0.063*
Valine	0.611	0.440	0.799	0.041***
Non-essential				
Alanine	1.732	1.317	1.381	1.113
Aspartic acid	0.899	0.699	0.724	0.042*
Glutamic Acid	0.598	0.411	0.454	0.029**
Glycine	3.044	2.995	1.737	0.115***
Tyrosine	0.625	0.572	0.591	0.038
Serine	0.934	0.730	1.402	0.077***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Supro 610, Ralston Purina, St Louis, Mo.

‡ Gain in empty body amino acid (g/d): apparent amino acid absorption to ileum (g/d).

of 25 kg pigs was deposited as body protein. Hodge (1974) found that the retention of digested N in the empty body of 10–30-d-old pigs given liquid milk diets was 0.502 when fed *ad lib.* and 0.572 when feeding was restricted. The efficiency with which the digested N intake was retained in the empty body declined with age of pigs to 0.302 when fed *ad lib.* and 0.392 when feeding was restricted for pigs at 30–50 d of age (Hodge, 1974). Higher values of 0.78–0.82 were determined by Jordan & Weatherup (1976) using 30-d-old pigs given reconstituted dried whole milk for the amount of N retained in the carcass as a percentage of the N intake.

In the present experiments, 0.72–0.75 of the total absorbed N was retained in the empty body of pigs given the pelleted milk diets, which is higher than the values reported by Hodge (1974) and Edmunds *et al.* (1978), but similar to the values obtained by Jordan & Weatherup (1976) with reconstituted milk. However, in the present experiment with the liquid milk diet only 0.58 of the total absorbed N was retained in the empty body, but the pigs were not fed *ad lib.* It was shown earlier (Wilson & Leibholz, 1981a) that the performance of pigs given the liquid milk diet was below that of pigs given the pelleted milk diet. The retention time of the liquid milk in the anterior gastro-intestinal tract was less than that for the pelleted milk diet (Wilson & Leibholz, 1981b). This may lead to a lower utilization of the absorbed N.

The incorporation of absorbed N into the empty body of pigs given ISP varied from 0.55–0.62. The lower values for the ISP diets may be due to the poorer amino acid balance of the diets as suggested by Lewis *et al.* (1976).

Both the retention of N and of individual amino acids in the empty body could be affected by the balance of amino acids in the diet in relation to the amino acid requirement of the

animal for maintenance and growth (Solberg *et al.* 1971). This means that a high efficiency of incorporation of an amino acid into the carcass would indicate that the amino acid may be limiting. This is illustrated by the results of Expt 3. For the pigs given the ISP and SBM diets the incorporation of the apparently absorbed methionine into the empty body was 0.754 and 0.929 respectively. These values were greater than for the other essential amino acids. Methionine supplementation of ISP and SBM diets reduced these values to 0.239 and 0.226 respectively. This would indicate an excess of methionine. With the milk protein diets in Expt 3, the fractional retention of methionine was similar to that of several other essential amino acids, but dietary supplementation of the milk diet with methionine also reduced methionine incorporation. Methionine supplementation of milk protein diets has been shown to improve the N retention of rats (Schiller & Ocio, 1963) and preruminant lambs (Walker, 1975) in balance experiments, but this was not observed in the N-balance studies in the present experiments with pigs (Wilson & Leibholz, 1981*a*).

The soya-bean protein diets in Expt 3 were also supplemented with lysine, but it would appear that in this instance lysine was not limiting the growth of pigs given these diets. Zebrowska & Kotarbinska (1972) found that in the 30 kg pig approximately 0.50 of the lysine intake was incorporated into the carcass of pigs given a barley and skim-milk diet. This would be approximately 0.60 of the absorbed lysine, which is similar to the values obtained in the present experiments for the milk-fed pig. The higher value observed for the pigs given ISP in Expt 4 (0.807) would be due to the lower lysine content of the ISP diet.

Buttery & Annison (1976) recalculated the results of Cooke (1969) and showed that the maximum efficiency of lysine incorporation into the carcass was 0.38 at the lowest dietary lysine intake in 91 kg pigs. This is lower than the values determined in the present experiments and may indicate that the lysine levels used by Cooke (1969) were in excess of the pigs' requirements, or that the rapidly-growing young pig is more efficient in the utilization of dietary amino acids.

It would appear from the results of Expt 3 that threonine was the second limiting amino acid for the pigs given SBM protein, as the incorporation of the apparently-absorbed threonine was 0.926, as compared to a mean of 0.678 obtained with pigs given the milk and ISP diets. This result is rather surprising as the threonine content of the ISP and SBM diets was similar (3.3 g/16 g N), but the threonine content of the milk diet was higher (3.8 g/16 g N). In Expt 4 the order of limiting amino acids of the pigs given the ISP diet would be methionine, lysine, valine and threonine. Part of the reason for the difference from Expt 3 is due to the higher threonine and lower valine content of the ISP (Supro 610) used in Expt 4 compared to the ISP (Promine D) used in Expt 3. Recalculation of the results of Hardy (1974) and Taylor (1975) by Buttery & Annison (1976) showed an efficiency of threonine deposition into the total lean of the pig of approximately 0.42–0.45. Maximum retention of dietary threonine into the carcass of rats given a casein diet was 0.62 and 0.70 (Bunce & King, 1969*b*; Pellet & Kaba, 1972). Threonine is also a gluconeogenic amino acid and is involved in many amino acid reactions, although the extent of gluconeogenesis from amino acids in the diets fed would be relatively small.

In both experiments the arginine content of the milk diet was approximately half that of the soya-bean-protein diets. The fractional retention of arginine was greater than one for the pigs given the milk diets which must indicate net arginine synthesis by the pig. The efficiency of incorporation of proline into the empty body of pigs was zero for all diets fed. Proline is the major precursor for arginine and the net disappearance of proline exceeds the net increase in arginine. Similar results were obtained with the rat by Bunce & King (1969*a, b*).

In addition to the net synthesis of protein in the body, there is a continuous turnover of tissue proteins in the animal. The process of protein turnover constitutes some loss to

the animal of both energy-yielding nutrients and N. Lewis *et al.* (1976) showed that in a 100 g rat 713 mg muscle protein was synthesized daily, while 564 mg was degraded, and a rapidly-growing broiler chick (900 g) synthesizes 9.1 g muscle protein/d and degrades 5.2 g/d (Buttery *et al.* 1973). A 76 kg pig will synthesize 9 g protein/kg per d or 266 g/kg live weight^{0.75} per d (Garlick *et al.* 1976) while a 25 kg pig was found to synthesize 291 g protein/d but the actual amount of protein deposition was only 101 g/d (Edmunds *et al.* 1978). In the present experiments, protein deposition was approximately 50 g/d over the whole experimental period, hence on this basis the total protein synthesis may have been approximately 150 g/d.

From these results it is clear that the maximum efficiency of protein utilization can only be achieved when the effective supply of amino acids approaches physiological requirements. An understanding of the way in which protein turnover affects the efficiency of utilization of dietary or absorbed N for carcass protein synthesis is required as the comparatively low body retention values of certain essential amino acids may be due to losses associated with incomplete reutilization.

This study was made possible by the support of the Rural Credits Development Fund and the Australian Pig Industry Research Committee. The authors are indebted to Mayfair Farms for financially supporting R.H.W. and supplying the pigs and to Pfizer Agricare Pty Ltd for the vitamins and antibiotics. The authors also wish to thank Ms A. C. Kirby of the Department of Agricultural Genetics and Biometry, University of Sydney, for assistance with statistical analysis.

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