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Availability of lysine in protein concentrates as determined by the slope-ratio assay with growing pigs and rats and by chemical techniques

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1. A slope-ratio assay was developed with growing pigs to determine the availability of lysine in five protein concentrates. The basal diet contained 5·2 g lysine/kg and six levels of lysine, in 500 mg/kg increments, were used to determine the pig's response to standard lysine. The protein concentrates were incorporated into the basal diet to provide five levels of total lysine, again in 500 mg/kg increments, at the expense of wheat starch. A daily feeding scale based on live weight was used to ensure similar nutrient intakes. Pigs were fed at three-hourly intervals to ensure the utilization of free amino acids in the diets. Four pigs were allotted to each dose level and response was assessed over the 20–45 kg growth phase.

2. Potency estimates for available lysine in the five protein concentrates varied, depending on whether live-weight gain or carcass gain was used as the criterion of response. Carcass gain was considered more appropriate as it was not influenced by variation in gut fill. Availability of lysine in the five proteins, using carcass gain/d were (proportion of total) cottonseed meal 0·39, fish meal 0·89, meat-and-bone meal 0·50, skim-milk powder 0·88 and soya-bean meal 0·87.

3. Rat slope-ratio assay results for available lysine in the five protein concentrates were in general agreement with those from the pigs. In contrast, the differences in available lysine were not detected by the chemical Silcock available-lysine test (Roach *et al.* 1967) nor by the direct fluorodinitrobenzene procedure (Carpenter, 1960).

Previous work (Batterham *et al.* 1978) showed that for growing pigs, the content of available lysine in locally produced cottonseed meal, two meat meals and sunflower meal was approximately 60% less than that in rapeseed meal, skim-milk powder and soya-bean meal. Similar differences were detected with a slope-ratio assay with rats but not with the chemical Silcock available-lysine assay as developed by Roach *et al.* (1967).

In view of these differences, it seemed appropriate to attempt to quantify the available lysine content of the major protein concentrates for pigs and to evaluate the efficiency of some of the different techniques for estimating available lysine. This paper reports the results of a slope-ratio assay that was developed with growing pigs to determine the availability of lysine in five protein concentrates. Lysine availability in these proteins was also estimated by a slope-ratio assay with rats, the Silcock available-lysine technique and by the direct fluorodinitrobenzene (FDNB)-available-lysine assay (Carpenter, 1960).

EXPERIMENTAL

Pig slope-ratio assay

Diets. For the pig assay, five protein concentrates were assayed in the one experiment. This involved the use of thirty-two diets; the basal diet (blanks), six diets to determine the pig's response to standard lysine and twenty-five for the five protein concentrates (five/protein concentrate). The basal diet contained (g/kg): wheat 730, wheat gluten 50, L-lysine monohydrochloride (anhydrous) 1·03, DL-methionine 0·30, L-threonine 0·70, mineral and vitamin premix 5, bone flour 30, and starch 182·97 (Table 1). The wheat was a high-protein

Table 1. *Composition (g/kg) of the wheat, wheat gluten and basal diet used for the growth assay with pigs*

	Wheat	Wheat gluten	Basal* diet
Crude protein (nitrogen × 6·25)	192	754	178
Dry matter	890	909	891
Petroleum-ether extract	21	20	16
Crude fibre	31	1	23
Essential amino acids			
Threonine	5·4	17	4·8
Valine	7·7	25	6·8
Methionine + cystine	4·6	21	4·5
Isoleucine	5·8	26	5·5
Leucine	12·3	51	11·5
Phenylalanine + tyrosine	15·5	62	14·5
Histidine	4·0	16	3·7
Lysine	5·2	13	4·4
Arginine	8·4	29	7·7

* Supplements of L-threonine, DL-methionine and L-lysine-monohydrochloride (anhydrous) were added to the basal diet to bring the levels of these amino acids to 5·6, 4·8 and 5·2 g/kg respectively.

Table 2. *Composition (g/kg) of the five protein concentrates*

	Cottonseed meal	Fish meal	Meat-and-bone meal	Skim-milk powder	Soya-bean meal
Crude protein (nitrogen × 6·25)	392	649	527	345	475
Dry matter	936	867	935	956	890
Petroleum-ether extract	15	68	114	8	30
Crude fibre	138	—	—	—	40
Ash	71	141	305	82	62
Gross energy (MJ/kg)	17·8	18·3	15·4	17·1	17·3
Essential amino acids					
Threonine	13	27	16	17	19
Valine	16	31	19	20	23
Methionine + cystine	10	16	11	13	10
Isoleucine	12	27	12	17	21
Leucine	21	46	28	33	38
Phenylalanine + tyrosine	31	45	27	35	46
Histidine	11	17	12	11	14
Lysine	17	51	28	29	30
Arginine	40	36	—	16	39

Timgalen cultivar and, in combination with the wheat gluten, supplied adequate quantities of all the amino acids except lysine, which was added to bring the basal level up to 5·2 g/kg, and methionine and threonine, which were added to ensure adequacy according to estimates of Lewis & Cole (1976). The six levels of lysine used to determine the pig's response to standard lysine were in 500 mg increments of L-lysine/kg and were obtained by the addition to the basal diet of L-lysine monohydrochloride, anhydrous, 98% pure, supplied by Ajinomoto Co., Inc., Japan. The protein concentrates were incorporated into the basal diets to provide five levels of total lysine, again in 500 mg/kg increments, at the expense of wheat starch. The level of bone flour was reduced to make allowance for the calcium and phosphorus in the diets containing meat-and-bone meal. The mineral and vitamin premix contributed (/kg diet): iron 60 mg, zinc 100 mg, manganese 30 mg, copper 5 mg, iodine 2 mg, selenium 150 μg, sodium chloride 2·5 g, retinol equivalent 960 μg, cholecalciferol 12 μg, α-tocopherol 20 mg, thiamin 1 mg, riboflavin 3 mg, nicotinic acid 12 mg, pantothenic acid 10 mg, pyridoxine 1·5 mg, cyanocobalamin 15 μg, pteroylmonoglutamic acid 2 mg, choline 500 mg, ascorbic acid 10 mg, biotin 100 μg. Dietary energy was maintained at 14·5 MJ digestible energy/kg diet using wheat starch and tallow as non-protein energy sources.

The chemical composition of the five protein concentrates is given in Table 2. The cottonseed and soya-bean meals were 'prepress' solvent-extracted, the fish meal and meat-and-bone meal were dry-rendered, and the skim-milk powder was spray-dried. All meals were locally produced, except fish meal which was imported from South Africa. The cottonseed meal contained 8300 and 260 mg total and free gossypol/kg respectively and ferrous sulphate was added to inactivate any effects it may have had (Husby & Kroening, 1971).

Animals and procedure. The thirty-two diets were arranged in a randomized block design with four pigs allocated to each diet. Two pigs were females and two were males, all of the Large White breed. The pigs were penned individually and water supplied by 'nipple' drinkers. Dietary treatments were introduced when the pigs reached 20 kg live weight.

The diets were offered at a daily rate of 1000 g at 20 kg live weight, with 100 g increments/2·5 kg live-weight gain. The pigs were fed eight times daily, at intervals of 3 h, to ensure the utilization of the added free lysine (Batterham & O'Neill, 1978). The food was offered dry. Rations were adjusted after the weekly weighings of the pigs.

The pigs were slaughtered after reaching a minimum weight of 45 kg and hot eviscerated carcass weights recorded. The ham was dissected and the lean content used as an indicator of carcass leanness. Pig response was assessed in terms of daily live-weight gain, food conversion efficiency (FCE; kg live-weight gain/kg food eaten), dressing percentage (hot carcass weight as a percentage of live weight), lean content of the ham, carcass gain/d (kg hot carcass weight – kg initial live weight ÷ d on experiment) and FCE on a carcass basis (kg hot carcass weight – kg initial live weight ÷ kg food intake).

The results for dressing percentage and lean content of the hams were analysed by analysis of variance; (6 concentrates × 5 levels) + 2 factorial. The results for daily live-weight gain, FCE, carcass gain/d, and FCE on a carcass basis were analysed by the slope-ratio technique of Finney (1964) for multiple assays. The potencies and their standard deviations were calculated.

Rat slope-ratio assay

For the rat assays, single separate assays were conducted for each protein concentrate. A total of seven diets were used for each assay: the basal diet (blanks), three diets to determine the rat's response to standard lysine and three diets to determine the rat's response to the protein concentrate. The basal diet contained (g/kg): wheat 650, wheat gluten 100, DL-methionine 1·5, L-threonine 0·7, maize oil 20, bone flour 25, mineral and vitamin premix 5 (composition described previously) and wheat starch 197·8. The combination of wheat and gluten supplied adequate levels of all amino acids except lysine (4·7 mg/kg) methionine and threonine. The latter two were added to ensure adequacy according to estimates of the (US) National Research Council (1972). The three levels of L-lysine used to determine the rat's response to standard lysine were 0·75, 1·5 and 2·25 g/kg (same batch of lysine as used for the pig assay). The protein concentrates were incorporated into the diets to supply the same three levels of total lysine as used to determine the standard lysine response. This was done at the expense of wheat starch. Additional maize oil was used with some protein concentrates to maintain the estimated digestible energy content of the diets.

For the rat assays, two female and two male albino rats, approximately 24–26 d old, were used per dose and were blocked on the basis of litter and sex (block size seven). The rats were individually caged in a room where the temperature and relative humidity were maintained at 21° ± 1 and 50 ± 5% respectively. Lighting was provided for 12 h daily.

Table 3. *Live-weight gain (g/d), food conversion efficiency and lean content of hams of pigs during the 20–45 kg growth phase when fed on the diets for the slope-ratio assay for lysine**

Lysine dose level (g/kg)	Form of lysine addition: Free lysine	Cottonseed meal	Fish meal	Meat-and-bone meal	Skim-milk powder	Soya-bean meal
			Live-wt gain (g/d)			
0	384					
0·5	429	431	470	439	432	449
1·0	505	464	468	463	469	486
1·5	499	471	531	478	496	506
2·0	514	496	540	512	538	519
2·5	593	509	578	505	552	544
3·0	610					
			SEM 17			
			Food conversion efficiency†			
0	0·300					
0·5	0·329	0·336	0·354	0·335	0·327	0·344
1·0	0·373	0·352	0·362	0·359	0·368	0·366
1·5	0·372	0·361	0·392	0·364	0·368	0·377
2·0	0·389	0·378	0·401	0·383	0·396	0·403
2·5	0·437	0·393	0·433	0·379	0·408	0·405
3·0	0·450					
			SEM 0·010			
			Lean in ham (g/kg)			
0	611					
0·5	609	590	606	597	602	602
1·0	602	604	618	617	623	603
1·5	620	616	621	605	621	628
2·0	629	620	618	619	595	628
2·5	642	610	605	601	634	632
3·0	626					
			SEM 11			

* For details, see p. 383.
† kg live-weight gain ÷ kg food intake.

Food was supplied in 'self-feeders'. The rats were assessed for weight gain and FCE (g gain/g food eaten) over a 14 d test.

The results were analysed by the slope-ratio technique of Finney (1964) for single assays. The mean potencies and standard deviations were calculated.

Chemical analyses

The dietary components were analysed for crude protein (nitrogen × 6·25) using a macro-Kjeldahl method with selenium as the catalyst; total amino acids using reflux hydrolysis under N_2 in 6 M-hydrochloric acid and separation of the amino acids by ion-exchange chromatography with a Technicon NC-2P amino acid analyser (Lewis & Lowe, 1977); Silcock available lysine according to Roach *et al.* (1967); available lysine using the direct FDNB dye-binding procedure (Carpenter, 1960); crude fibre adapted from the closed-tube 'Filtrex' technique of Moir (1971); and ash, dry matter and petroleum ether extract by the methods of the Association of Official Analytical Chemists (1975). Digestible energy in the diets was calculated using literature values and results of previous determinations at this Agricultural Research Centre.

Table 4. *Dressing percentage, carcass gain and food conversion efficiency on a carcass basis of pigs during the 20–45 kg growth phase when fed on the diets for a slope-ratio assay for lysine**

Lysine dose level (g/kg)	Form of lysine addition					
	Free lysine	Cottonseed meal	Fish meal	Meat-and-bone meal	Skim-milk powder	Soya-bean meal
			Dressing percentage (g/kg)†			
0	766					
0·5	756	747	765	747	754	761
1·0	749	741	757	763	758	763
1·5	772	756	753	759	757	748
2·0	759	737	740	743	754	747
2·5	748	728	739	738	757	750
3·0	756					
			SEM 8			
			Carcass gain (g/d)‡			
0	219					
0·5	241	234	270	241	243	259
1·0	278	254	268	264	265	284
1·5	295	267	302	271	285	281
2·0	294	265	292	284	302	304
2·5	329	267	311	266	318	306
3·0	351					
			SEM 11			
			Food conversion efficiency§			
0	0·172					
0·5	0·185	0·183	0·203	0·184	0·183	0·198
1·0	0·206	0·193	0·207	0·205	0·207	0·214
1·5	0·220	0·204	0·223	0·206	0·212	0·210
2·0	0·223	0·202	0·217	0·212	0·222	0·224
2·5	0·243	0·206	0·233	0·199	0·235	0·227
3·0	0·259					
			SEM 0·007			

* For details, see p. 385.
† Hot carcass weight as a percentage of live weight before slaughter.
‡ Hot carcass weight – initial live weight ÷ period (d) on experiment.
§ Hot carcass weight – initial live weight ÷ food intake.

RESULTS

Performance results for the pigs are presented in Tables 3 and 4. The lean content of the ham increased slightly as the level of dietary lysine increased (lean = 605+6·2 lysine dose, g/kg; SE of slope = 0·89) and there was no significant difference between the slopes of each protein concentrate. There were also slight but significant ($P < 0·01$) effects of the level of inclusion of the protein concentrates on dressing percentage, but only with cottonseed meal was the dressing percentage significantly lower relative to the standard lysine diets ($P < 0·05$).

The availability of lysine in the five protein concentrates, as determined by four production criteria, are presented in Table 5. Lysine availability was considerably lower in the cottonseed meal and meat-and-bone meal compared to fish meal, skim-milk powder and soya-bean meal, by all criteria. Availabilities based on carcass content were generally lower than those based on live weight. There were small and inconsistent effects on the availability estimates of including food intake in the criterion of response.

Rat slope-ratio assay estimates for lysine availability ranged from 0·95 for soya-bean meal to 0·53 for cottonseed meal when gain was the criterion (Table 6). With FCE as the criterion, lysine availability ranged from 1·04 for fish meal to 0·58 for cottonseed meal.

Table 5. *Availability of lysine (proportion of total) in the protein concentrates as assessed using daily live-weight gain, food conversion efficiency, daily carcass gain and food conversion efficiency on a carcass basis as the criteria for availability*

(Mean values and standard deviations)

Protein concentrate	Daily live-wt gain		Food* conversion efficiency		Daily carcass† gain		Food‡ conversion efficiency	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cottonseed meal	0·62	0·082	0·69	0·078	0·39	0·107	0·43	0·093
Fish meal	1·04	0·090	1·06	0·086	0·89	0·104	0·89	0·098
Meat-and-bone meal	0·66	0·082	0·67	0·078	0·50	0·098	0·49	0·093
Skim-milk powder	0·88	0·086	0·86	0·081	0·88	0·103	0·85	0·097
Soya-bean meal	0·93	0·087	0·91	0·082	0·87	0·103	0·84	0·097

* kg live-weight gain ÷ food intake.
† Hot carcass weight – initial live weight ÷ period (d) on experiment.
‡ Hot carcass weight – initial live weight ÷ food intake.

Table 6. *Availability of lysine (proportion of total) in the protein concentrates as assessed by the chemical Silcock technique, the direct fluorodinitrobenzene (FDNB) assay and by the rat slope-ratio technique using weight gain and food conversion efficiency (FCE) as the criteria for availability*

(Mean values and standard deviations)

Protein concentrate	Silcock assay	Direct FDNB assay	Rat slope-ratio assay: Gain		Rat slope-ratio assay: FCE	
			Mean	SD	Mean	SD
Cottonseed meal	0·93	0·65	0·53	0·13	0·58	0·11
Fish meal	0·89	0·90	0·86	0·09	1·04	0·26
Meat-and-bone meal	0·84	0·79	0·70	0·11	0·64	0·20
Skim-milk powder	0·96	0·79	0·92	0·12	0·94	0·10
Soya-bean meal	0·93	0·77	0·95	0·13	0·89	0·09

Silcock-available-lysine estimates ranged from 0·96 for skim-milk powder to 0·84 for meat-and-bone meal. In contrast, the values for the direct FDNB test ranged from 0·90 for fish meal to 0·65 for cottonseed meal.

DISCUSSION

There were considerable differences in the availability of lysine in the five protein concentrates as assessed by the slope-ratio technique with pigs. The values also varied depending on the criterion of response. Availabilities based on carcass gain appear more appropriate than those based on live-weight gain as the latter is subjected to variation in gut fill. This variation was greater than normally experienced in pig experiments due to the replacement in the diets of large quantities of highly-digestible wheat starch with less-digestible protein concentrates. There were only small and inconsistent differences in the availabilities derived from carcass gain or food conversion efficiency on a carcass basis. Little difference would be expected as the pigs were restrictively fed according to live weight. With *ad lib.* feeding systems, the slope-ratio technique can give considerable differences in availability estimates because of differences in food intake. In such instances potencies based on criteria involving food intake are normally preferred (Carpenter *et al.* 1972). Thus there are advantages in

Table 7. *Dietary crude protein contents (nitrogen × 6·25) (g/kg) of the diets used for the slope-ratio assay for pigs*

		Protein concentrates				
Lysine increment (g/kg)	Standard lysine diets	Cottonseed meal	Fish meal	Meat-and-bone meal	Skim-milk powder	Soya-bean meal
0·0	178					
0·5	179	189	184	187	184	186
1·0	179	201	191	197	190	194
1·5	180	212	197	206	196	202
2·0	180	224	203	215	202	210
2·5	181	236	210	225	208	217
3·0	181					

conducting the slope-ratio assay under a limited feeding system, where similar intakes of nutrients can be achieved. Problems of unpalatability of a protein concentrate affecting food intake, and thus the potency estimate, are also minimized. However, it is essential to feed frequently to ensure full utilization of the free amino acids in the diet (Batterham & O'Neill, 1978).

In our assays, the dietary crude protein content varied as a result of differences in the concentration of lysine in the protein concentrates. The effects of increasing the protein level in slope-ratio assays on the utilization of the limiting amino acid have been investigated by a number of workers (e.g. Fisher *et al.* 1960; Guttridge & Lewis, 1964; Miller *et al.* 1965; De Muelenaere *et al.* 1967). These reports indicate that the effect of the addition of crude protein depends on the level of the addition, the degree of imbalance of the supplementary protein and the criteria used to assess response. The addition of a balanced protein appears to have little or no effect whilst the major effect of imbalanced protein is to reduce food intake. Provided food intake is taken into account when assessing availability, the effect of the addition of crude protein, even completely free of the limiting amino acid, is much reduced. It is therefore unlikely that the slightly higher crude protein contents of the diets containing meat-and-bone meal or cottonseed meal contributed to the very low estimates of lysine availability. The total levels of additional crude protein (Table 7) were within the range reported by Guttridge & Lewis (1964) to have no significant effect. In addition, the pig assay was conducted under limited feeding conditions and therefore the major effect of the addition of crude protein, that of depressing food intake, was eliminated. It is also possible that the digestibility of the crude protein in the meat-and-bone meal and cottonseed meal was lower than that for the fish meal, skim-milk-powder and soya-bean meal and thus differences in the digestible crude protein intakes may have been even less than those indicated by the crude protein analyses. The low estimates of available lysine content in the cottonseed meal and meat-and-bone meal by the slope-ratio assay are similar to estimates derived by Batterham *et al.* (1978) when using diets of conventional crude protein contents for growing pigs. This latter point also indicates that the estimates given by the slope-ratio technique are unlikely to have been influenced by differences in the dietary crude protein levels.

The low availability of lysine in the cottonseed meal and meat-and-bone meal appears to be due to processing conditions. With the cottonseed meal, heat and pressure are used not only to aid the extraction of oil, but also to reduce the level of free gossypol. Considering the extent of binding of the gossypol in the meal, it is not surprising that lysine availability is also reduced (Frampton, 1965). The meat-and-bone meal was typical of that produced in Australia, as indicated by its Silcock value and chemical composition (Fox, 1971;

Batterham, Murison & Lewis, unpublished results). It appears that the dry-rendering process used to produce meat-and-bone meal does considerable heat-damage to the meal. A similar potency for available lysine of 0·52 in meat meal for chicks using a slope-ratio assay was reported by Guo *et al.* (1971).

There was reasonable agreement between the slope-ratio assay results with rats and for those with pigs. The rat results were based on live weight and agreed best with the live-weight estimates with pigs. Subsequent work in our laboratories has indicated that gut fill may also affect availability estimates with rats and that it is preferable to use live-weight minus intestinal content, rather than live weight, when assessing response.

The Silcock assay results confirm previous observations (Batterham *et al.* 1978) that this technique gives only a narrow range of values for available lysine in protein concentrates. It appears unable to detect the extent of heat-damage in the cottonseed and meat-and-bone meal, as indicated by the slope-ratio assay with pigs and rats. Similar observations that the FDNB principle has been unsuitable for detecting heat-damage in severely-damaged protein has been made by Boctor & Harper (1968). In view of the discrepancies, it may be only coincidental that the Silcock values for fish meal, skim-milk powder and soya-bean meal were similar to the slope-ratio estimates. Available lysine estimates by the direct FDNB procedure showed a greater range in values than the Silcock assay, but also had little or no relationship to the slope-ratio values. The greater range in the direct FDNB values may have been a reflection of the instability of DNP-lysine in the presence of carbohydrates in the vegetable proteins. This interference is avoided with the Silcock assay.

Carpenter (1973) suggested that with slope-ratio assays at least two dose levels for the test protein and two for the lysine response, together with the basal diet alone (i.e. a five-point assay) are required. With pigs we chose a twelve-point assay, using seven points to define the lysine response and five to test the protein concentrate. As the response to lysine was linear, it may have been statistically slightly more efficient to reduce the number of dose levels and to concentrate the pigs at each end of the linear response (Batterham & O'Neill, 1978). This would also reduce the number of diets and hence the work associated with conducting the experiment. However, linearity of response with the test proteins cannot be assumed and a twelve-point assay is a better test of this. With the rat assay, in our colony, litter effects are very strong, and it has proven more efficient to block on litter with a seven-point assay than to use a greater number of dose levels with litter effects randomized.

The results of the slope-ratio assays with pigs and rats indicate considerable differences in the availability of lysine in protein concentrates. These differences were not detected by the Silcock assay or the direct FDNB procedure. Slope-ratio experiments however are time-consuming and expensive and accordingly are unsuitable for routine use. There is a need for the development of quicker, less expensive techniques for quantifying the available lysine content of protein concentrates for pigs.

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