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

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1. The availability of lysine in nine vegetable-protein concentrates was assessed using the slope-ratio assay for growing pigs and rats. Diets were equalized for crude fibre using solka floc to minimize any possible effects of variation in fibre content on availability estimates.

2. The availability of lysine in the nine proteins for pigs, using food conversion efficiency (FCE) on a carcass basis as the criterion of response were (proportion of total): cottonseed meal 0·39, lupin (*Lupinus angustifolius*) seed meal no. 1 0·37, no. 2 0·65, no. 3 0·54, no. 4 0·54, field peas (*Pisum sativum*) 0·93, peanut (groundnut) meal 0·57, soya-bean meal no. 1 0·98, no. 2 0·89.

3. Estimates of available lysine for rats as assessed by the slope-ratio assay using FCE on a carcass basis were in close agreement with the pig estimates for cottonseed meal (0·35) and soya-bean meal no. 1 (0·91) and no. 2 (0·89), higher for lupin-seed meals (range 0·70–0·94 with a mean of 0·81) and peanut meal (0·76) and lower for field peas (0·76).

4. The differences in available lysine were not detected by the chemical Silcock available-lysine test (Roach *et al.* 1967) or by the direct 1-fluoro-2,4-dinitrobenzene procedure (Carpenter, 1960).

Previous work (Batterham *et al.* 1979) showed that, for growing pigs, the availability of lysine in cottonseed meal and meat-and-bone meal was approximately half that in fish 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).

Further studies (Batterham *et al.* 1981) on the availability of lysine in vegetable-protein concentrates confirmed that lysine availability varied widely and was not detected by chemical techniques. However, there was a wide variation in the crude fibre content of the diets (up to 50 g/kg), as a result of the large quantities of vegetable-protein concentrates needed to supply the desired concentrations of total lysine in the assays (up to 250 g/kg). Amino acid digestibility is negatively correlated with fibre content in cereals (Taverner & Farrell, 1981) and the addition of fibre to diets can depress the digestibility and utilization of lysine and other nutrients (Just, 1982). Thus the high crude fibre contents in some of the diets used by Batterham *et al.* (1981) could have depressed lysine digestibility and hence availability, yielding estimates which may not apply in diets of lower crude fibre content.

In the present paper the results of two assays conducted with pigs to determine the availability of lysine in vegetable-protein concentrates using diets equalized for crude fibre content are reported. Following a very low assay value for a sample of lupin (*Lupinus angustifolius*)-seed meal in Expt 1, additional samples of lupin-seed meal were assayed in Expt 2 to assess the range in lysine availability in this meal. Lysine availability was also estimated by a slope-ratio assay with rats, the Silcock available-lysine technique and by the direct 1-fluoro-2,4-dinitrobenzene (FDNB)-available-lysine assay (Carpenter, 1960).

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Table 1. *Composition (g/kg) of the nine protein concentrates*

| | Expt 1 | | | | Expt 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cotton-seed meal | Lupin (*Lupinus angustifolius* meal no. 1 (cv. Uniharvest) | Peanut (groundnut) meal | Soya-bean meal no. 1 | Lupin-seed meal no. 2 (cv. Unicrop) | Lupin-seed meal no. 3 (cv. Uniharvest) | Lupin-seed meal no. 4 (cv. Uniharvest) | Field peas (*Pisum sativum*) | Soya-bean meal no. 2 |
| Crude protein (nitrogen × 6·25) | 424 | 337 | 448 | 439 | 309 | 323 | 301 | 216 | 449 |
| Dry matter | 932 | 895 | 913 | 883 | 891 | 906 | 903 | 889 | 891 |
| Light petroleum (b.p. 40–60°) extract | 54 | 39 | 60 | 11 | 42 | 54 | 58 | 9 | 17 |
| Fibre | | | | | | | | | |
| Crude | 106 | 137 | 63 | 64 | 153 | 168 | 162 | 33 | 64 |
| Acid-detergent | 179 | 174 | 121 | 81 | 223 | 211 | 221 | 76 | 85 |
| Neutral-detergent | 187 | 201 | 141* | 112 | 247 | 231 | 237 | 103 | 110 |
| Hemicellulose | 8 | 27 | 20 | 31 | 24 | 20 | 16 | 27 | 25 |
| Ash | 71 | 28 | 46 | 65 | 29 | 30 | 26 | 25 | 59 |
| Gross energy (MJ/kg) | 19·8 | 18·5 | 19·2 | 18·0 | 18·2 | 18·4 | 18·8 | 18·7 | 18·0 |
| Alkaloid | — | ⩽ 0·14 | — | — | ⩽ 0·1 | ⩽ 0·1 | ⩽ 0·1 | — | — |
| Essential amino acids | | | | | | | | | |
| Threonine | 15 | 11 | 13 | 17 | 11 | 11 | 11 | 8 | 18 |
| Valine | 19 | 11 | 19 | 20 | 11 | 12 | 11 | 10 | 23 |
| Methionine + cystine | 12 | 3 | 9 | 8 | 6 | 8 | 9 | 5 | 9 |
| Isoleucine | 13 | 12 | 15 | 20 | 13 | 13 | 13 | 9 | 19 |
| Leucine | 27 | 23 | 30 | 35 | 24 | 25 | 25 | 16 | 35 |
| Phenylalanine + tyrosine | 36 | 26 | 40 | 37 | 22 | 23 | 23 | 17 | 38 |
| Histidine | 12 | 8 | 10 | 13 | 8 | 9 | 9 | 6 | 13 |
| Lysine | 18 | 15 | 15 | 28 | 14 | 15 | 14 | 16 | 26 |
| Arginine | 45 | 40 | 60 | 41 | 33 | 35 | 34 | 23 | 34 |

* Difficult to filter.

## EXPERIMENTAL

### *Protein concentrates*

The chemical composition of the nine protein concentrates is presented in Table 1. The cottonseed meal and peanut (groundnut) meal were expeller processed and the soya-bean meals 'prepress' solvent extracted. The field peas (*Pisum sativum* cultivar Early Dun) and lupin (cultivars Unicrop and Uniharvest)-seed meal were coarsely crushed through a hammer mill. The lupin-seed meal samples were analysed for alkaloids, aflatoxins and for the presence of *Phomopsis leptostromiformis*, a fungus which produces a mycotoxin on the leaves of lupin plants. As an additional precaution against *P. leptostromiformis* infection, the lupin-seed meals for Expt 2 (nos 2, 3 and 4) were stored at $15 \pm 1°$ and $55 \pm 5\%$ relative humidity before incorporation into diets.

### *Pig slope-ratio assay*

A slope-ratio assay was used to determine the availability of lysine in proteins. Linear regression coefficients of response (say to food conversion efficiency) to increasing dose level of test protein and standard lysine are calculated and the ratio of the test protein's linear regression coefficient to the standard lysine's linear regression coefficient provides the potency of the lysine in the test protein. In our assays the dose levels for the test proteins were formulated to contain the same total lysine as that of the standard lysine doses so that the potency estimate for lysine in the test protein was an expression of lysine availability as a proportion of total lysine. The statistical analysis of the slope-ratio assay was as outlined in Chapter 7 of Finney (1964).

There are a number of criteria that can be used to assess response. Food conversion efficiency is preferred to weight gain as it takes into account any differences in food intake (Carpenter, 1973). Previous work (Batterham *et al.* 1979, 1981) had shown that results expressed on a carcass basis were preferred to those expressed on a live-weight basis as the former eliminates any effect of variation in gut content on availability estimates. This is important, as in the formulation of diets the test protein was included at the expense of highly digestible wheat starch.

*Expt 1.* For this assay, four protein concentrates were assayed in the one experiment. This involved the use of twenty-six diets: the basal diet (blanks), five diets to determine the pig's response to standard lysine and twenty for the four protein concentrates (five for each protein concentrate). The basal diet (Tables 2 and 3) was formulated using a medium-protein wheat (Timgalen cultivar) which, 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 five 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, feed grade, supplied by Miwon Co. Ltd, Korea. 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 cottonseed meal contained 15100 and 940 mg total and free gossypol/kg respectively and ferrous sulphate was added to inactivate any effects it may have had (Husby & Kroening, 1971).

Solka floc was included in the basal diet and the level reduced to make allowance for the crude fibre content of the vegetable-protein concentrates so that all diets were fed on an equal crude fibre basis. Maize oil was included in the basal diet to improve the texture of the diet. The digestible energy content of the components was calculated using results of previous determinations at this Agricultural Research Centre or literature values. Dietary

Table 2. *Composition (g/kg) of the basal diets used for the slope-ratio assays with pigs*

| | Expt 1 | Expt 2 |
|---|---|---|
| Wheat | 670 | 650 |
| Wheat gluten | 100 | 90 |
| L-Lysine monohydrochloride | 1·54 | 1·79 |
| DL-Methionine | 0·70 | 0·30 |
| L-Threonine | 0·60 | 0·70 |
| Mineral and vitamin premix* | 5·5 | 5·5 |
| Bone meal | 30 | 30 |
| Solka floc | 32 | 39 |
| Maize oil | 20 | 25 |
| Wheat starch | 139·66 | 157·71 |

* Contributed the following (per kg diet): iron 60 mg, zinc 100 mg, manganese 30 mg, copper 5 mg, iodine 2 mg, selenium 150 $\mu$g, sodium chloride 2·5 g, retinol equivalent 960 $\mu$g, cholecalciferol 12 $\mu$g, $\alpha$-tocopherol 20 mg, thiamin 1 mg, riboflavin 3 mg, nicotinic acid 12 mg, pantothenic acid 10 mg, pyridoxine 1·5 mg, cyanocobalamin 15 $\mu$g, pteroylmonoglutamic acid 2 mg, choline 500 mg, ascorbic acid 10 mg, biotin 100 $\mu$g, arsanilic acid 9 mg.

Table 3. *Composition (g/kg) of the wheat, wheat gluten and basal diets used for the growth assays with pigs*

| | Expt 1 | | | Expt 2 | | |
|---|---|---|---|---|---|---|
| | Wheat | Wheat gluten | Basal diet | Wheat | Wheat gluten | Basal diet |
| Crude protein (nitrogen × 6·25) | 147 | 814 | 179 | 156 | 841 | 177 |
| Dry matter | 905 | 923 | 905 | 904 | 925 | 903 |
| Light petroleum (b.p. 40–60°) extract | 17 | 7 | 34 | 14 | 6 | 41 |
| Fibre | | | | | | |
| Crude | 23 | 0 | 38 | 25 | 0 | 44 |
| Acid-detergent | 42 | —* | 55 | 45 | —* | 64 |
| Neutral-detergent | 96 | 3 | 94 | 105 | 5 | 105 |
| Hemicellulose | 54 | — | 39 | 60 | — | 41 |
| Essential amino acids | | | | | | |
| Threonine | 4·3 | 21 | 5·6 | 4·4 | 22 | 5·6 |
| Valine | 6·0 | 28 | 6·8 | 6·3 | 32 | 7·0 |
| Methionine + cystine | 4·4 | 30 | 6·7 | 4·6 | 39 | 6·8 |
| Isoleucine | 5·0 | 28 | 6·2 | 5·2 | 29 | 6·0 |
| Leucine | 9·7 | 57 | 12·2 | 10·2 | 62 | 12·2 |
| Phenylalanine + tyrosine | 10·6 | 63 | 13·4 | 10·9 | 65 | 12·9 |
| Histidine | 3·2 | 17 | 3·8 | 3·1 | 19 | 3·7 |
| Lysine | 4·0 | 13 | 5·2 | 3·9 | 14 | 5·2 |
| Arginine | 7·5 | 39 | 8·9 | 7·5 | 27 | 7·3 |

* Assay appeared inapplicable as abnormally high values recorded (198 and 161 g/kg), possibly due to high glutamic acid content in wheat gluten.

energy was maintained at 14·5 MJ digestible energy/kg diet using wheat starch and maize oil as non-protein energy sources.

The twenty-six diets were arranged in a randomized block design. The pigs were blocked on 7-week weight, sex and position in the experimental facilities. There were four blocks, two containing males and two females, 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.

Table 4. *Expt 1. Analysis of variance*

| Source of variation | Degrees of freedom | Source of variation | Degrees of freedom |
|---|---|---|---|
| Lysine (linear) | 1 | Curvature | |
| Test 1 (linear) | 1 | Lysine | 3 |
| Test 2 (linear) | 1 | Test 1 | 3 |
| Test 3 (linear) | 1 | Test 2 | 3 |
| Test 4 (linear) | 1 | Test 3 | 3 |
| Blanks | 1 | Test 4 | 3 |
| Intersection | | Error | 78 |
| Test 1–lysine | 1 | Total | 103 |
| Test 2–lysine | 1 | | |
| Test 3–lysine | 1 | | |
| Test 4–lysine | 1 | | |

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, with a solenoid-controlled automatic frequent feeder to ensure the utilization of added free amino acids (Batterham & Murison, 1981). 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 proportion (hot carcass weight as a proportion of live weight), lean content of the ham, carcass gain/d (kg hot carcass weight – (kg initial live weight × 0·69) ÷ period (d) on experiment) and FCE on a carcass basis (kg hot carcass weight – (kg initial live weight × 0·69) ÷ kg food intake). The factor of 0·69 for estimated carcass weight was previously determined with ten piglets (five males and five females) slaughtered at 20 kg live weight.

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 analysis of variance was as shown in Table 4. The error degrees of freedom were reduced to seventy-six as there were two missing plots (see Results section).

The results for dressing proportion and lean content of the hams were regressed *v.* lysine for each protein concentrate. These analyses were conducted to determine if there was any effect of inclusion level of protein concentrate on dressing proportion or dietary lysine concentration on lean deposition.

*Expt 2.* This was conducted in a similar manner to Expt 1 except that five protein concentrates were assayed. This involved the use of thirty-one diets: the basal diet (blanks), five diets to determine the pig's response to standard lysine and twenty-five for the five protein concentrates (five/protein concentrate). The basal diet (Tables 2 and 3) was formulated using a medium-protein wheat (Condor cultivar) which, 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).

Inclusion levels of lysine, equalization of dietary crude fibre and digestible energy content were as in Expt 1 except that the digestible energy content of the field peas and soya-bean meal no. 2 (13·9 and 14·8 MJ/kg, air-dry basis) were determined by the method outlined by Batterham (1979).

The design of the experiment and allocation of animals was as for Expt 1 with four pigs allotted to each diet except for diets 1 (blanks) and 6 (2500 mg standard lysine/kg) where six pigs were allotted (three males and three females). Feeding rates, method of procedure, assessment of performance and statistical analyses of the results were as for Expt 1 except that with the extra protein concentrate and additional pigs on diets 1 and 6 there were ninety-seven degrees of freedom in the analysis of variance.

*Rat slope-ratio assay*

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 in Table 2) 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. Solka floc was incorporated into the diets to equalize dietary crude fibre content to that supplied by the highest level of protein concentrate. 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 $21 \pm 1°$ and $50 \pm 5\%$ respectively. Lighting was provided for 12 h daily. Food was supplied in 'self-feeders'.

At the completion of a 14 d test, the rats were weighed, killed with chloroform and the alimentary tract, heart and lungs removed. The weight of the eviscerated carcass was recorded. Performance was assessed in terms of weight gain, FCE (g weight gain/g food eaten), carcass gain (g eviscerated carcass weight − (g initial live weight × 0·79)) and FCE on a carcass basis (g eviscerated carcass weight − (g initial live weight × 0·79) ÷ g food eaten). The factor of 0·79 for estimated eviscerated carcass weight was previously determined with eight rats (four male and four female) of similar live weight and age to those used for the assays.

The results were analysed by the slope-ratio technique of Finney (1964) for single assays. The analysis of variance for each protein was as follows:

| Source of variation | Degrees of freedom |
|---|---|
| Lysine (linear) | 1 |
| Test (linear) | 1 |
| Blanks | 1 |
| Intersection | 1 |
| Curvature of lysine | 1 |
| Curvature of test | 1 |
| Error | 21 |
| Total | 27 |

Table 5. *Expt 1.* Live-weight gain, 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 | Lupin (*Lupinus angustifolius*) seed meal no. 1 | Peanut (groundnut) meal | Soya-bean meal no. 1 |
| | | | Live-wt gain (g/d) | | |
| 0 | 478 | — | — | — | — |
| 0·5 | 447 | 494 | 495 | 465 | 495 |
| 1·0 | 558 | 532 | 514 | 543 | 549 |
| 1·5 | 599 | 486 | 534 | 575 | 608 |
| 2·0 | 597 | 527 | 542 | 555 | 609 |
| 2·5 | 631 | 579 | 548 | 591 | 643 |
| | | | SEM 24 | | |
| | | | Food conversion efficiency† | | |
| 0 | 0·381 | — | — | — | — |
| 0·5 | 0·370 | 0·375 | 0·393 | 0·367 | 0·388 |
| 1·0 | 0·426 | 0·413 | 0·405 | 0·427 | 0·418 |
| 1·5 | 0·449 | 0·402 | 0·420 | 0·443 | 0·457 |
| 2·0 | 0·453 | 0·412 | 0·418 | 0·431 | 0·471 |
| 2·5 | 0·479 | 0·445 | 0·439 | 0·458 | 0·485 |
| | | | SEM 0·012 | | |
| | | | Lean in ham (g/kg) | | |
| 0 | 594 | — | — | — | — |
| 0·5 | 606 | 593 | 587 | 590 | 568 |
| 1·0 | 609 | 592 | 606 | 583 | 603 |
| 1·5 | 610 | 590 | 610 | 600 | 580 |
| 2·0 | 627 | 599 | 623 | 649 | 626 |
| 2·5 | 633 | 614 | 617 | 609 | 619 |
| | | | SEM 12 | | |

* For details, see p. 87.
† Live-weight gain (kg) ÷ food intake (kg).

### *Chemical analyses*

The techniques used were as reported by Batterham *et al.* (1979) except for acid-detergent fibre (Van Soest, 1963), neutral-detergent fibre (Van Soest & Wine, 1967, as modified by King & Taverner, 1975), alkaloids (Ruiz, 1976) and aflatoxins (Romer, 1975).

## RESULTS

Performance results of the pigs for Expt 1 are presented in Tables 5 and 6 and for Expt 2 in Tables 7 and 8. Two pigs died in Expt 1 (diets nos 2 and 5) with post-mortem symptoms of *Escherichia coli* infections. There was a smaller amount of feed rejection by most pigs in Expt 1 and lesser amounts by pigs in Expt 2.

Lean in the ham of pigs increased slightly as the level of dietary lysine increased. In Expt 1 the slope of the increase was greater ($P < 0.05$) in pigs given soya-bean meal than with those given cottonseed meal but neither was significantly different ($P > 0.05$) from the standard lysine response. In Expt 2 the slope of the increase in pigs given field peas was

Table 6. *Expt 1.* Dressing proportion, 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 | Lupin (*Lupinus angustifolius*) seed meal no. 1 | Peanut (groundnut) meal | Soya-bean meal no. 1 |
|---|---|---|---|---|---|
| | Dressing proportion (g/kg)† | | | | |
| 0 | 752 | — | — | — | — |
| 0·5 | 770 | 754 | 748 | 769 | 755 |
| 1·0 | 758 | 756 | 742 | 733 | 755 |
| 1·5 | 751 | 746 | 718 | 735 | 748 |
| 2·0 | 762 | 738 | 744 | 738 | 751 |
| 2·5 | 758 | 734 | 736 | 736 | 741 |
| | SEM 8 | | | | |
| | Carcass gain (g/d)‡ | | | | |
| 0 | 381 | — | — | — | — |
| 0·5 | 373 | 397 | 391 | 386 | 398 |
| 1·0 | 450 | 428 | 401 | 415 | 441 |
| 1·5 | 478 | 384 | 394 | 442 | 481 |
| 2·0 | 488 | 408 | 426 | 428 | 487 |
| 2·5 | 509 | 444 | 423 | 454 | 500 |
| | SEM 18 | | | | |
| | Food conversion efficiency (carcass basis)§ | | | | |
| 0 | 0·304 | — | — | — | — |
| 0·5 | 0·309 | 0·301 | 0·311 | 0·305 | 0·311 |
| 1·0 | 0·344 | 0·332 | 0·316 | 0·327 | 0·336 |
| 1·5 | 0·358 | 0·318 | 0·310 | 0·340 | 0·362 |
| 2·0 | 0·370 | 0·320 | 0·328 | 0·332 | 0·377 |
| 2·5 | 0·386 | 0·341 | 0·339 | 0·353 | 0·378 |
| | SEM 0·010 | | | | |

* For details, see p. 87.
† Hot carcass weight as a proportion of live weight before slaughter.
‡ Hot carcass weight (kg) – (initial live weight (kg) × 0·69) ÷ period (d) on experiment.
§ Hot carcass weight (kg) – (initial live weight (kg) × 0·69) ÷ food intake (kg).

greater and those given soya-bean meal smaller ($P < 0.05$) compared with pigs given the standard lysine or other protein concentrates.

The level of inclusion of protein concentrate depressed dressing proportion in Expt 1, but this was only significant ($P < 0.05$) for cottonseed and peanut meals. In Expt 2 the addition of standard lysine increased dressing proportion ($P < 0.05$), field peas had no effect and the lupin-seed and soya-bean meals had small but non-significant ($P > 0.05$) depressing effects.

The availabilities of lysine in the nine protein concentrates, as determined by the four production criteria, are presented in Table 9. Availability estimates were lower on a carcass compared to live-weight basis and in the majority of cases marginally higher on a FCE compared to weight-gain basis. Using FCE on a carcass basis as the criterion of response, availability estimates were very low in cottonseed meal, low and variable in lupin-seed meal and high in field peas and soya-bean meal.

Table 7. *Expt 2.* Live-weight gain, 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 | Lupin (*Lupinus angustifolius*) seed meal no. 2 | Lupin-seed meal no. 3 | Lupin-seed meal no. 4 | Field peas (*Pisum sativum*) | Soya-bean meal no. 2 |
|---|---|---|---|---|---|---|
| | | | Live-wt gain (g/d) | | | |
| 0 | 493 | — | — | — | — | — |
| 0·5 | 535 | 541 | 502 | 515 | 523 | 502 |
| 1·0 | 543 | 550 | 510 | 536 | 563 | 541 |
| 1·5 | 587 | 574 | 562 | 572 | 589 | 576 |
| 2·0 | 587 | 555 | 585 | 544 | 564 | 601 |
| 2·5 | 590 | 598 | 566 | 567 | 614 | 631 |
| | | | SEM 15 | | | |
| | | | Food conversion efficiency† | | | |
| 0 | 0·371 | — | — | — | — | — |
| 0·5 | 0·397 | 0·406 | 0·388 | 0·383 | 0·391 | 0·382 |
| 1·0 | 0·413 | 0·406 | 0·391 | 0·399 | 0·418 | 0·406 |
| 1·5 | 0·441 | 0·429 | 0·423 | 0·425 | 0·441 | 0·443 |
| 2·0 | 0·440 | 0·417 | 0·440 | 0·424 | 0·430 | 0·453 |
| 2·5 | 0·444 | 0·444 | 0·428 | 0·435 | 0·463 | 0·475 |
| | | | SEM 0·015 | | | |
| | | | Lean in ham (g/kg) | | | |
| 0 | 590 | — | — | — | — | — |
| 0·5 | 585 | 602 | 599 | 601 | 589 | 631 |
| 1·0 | 604 | 608 | 617 | 618 | 606 | 614 |
| 1·5 | 631 | 625 | 632 | 635 | 634 | 638 |
| 2·0 | 640 | 652 | 651 | 655 | 620 | 627 |
| 2·5 | 628 | 636 | 653 | 645 | 671 | 649 |
| | | | SEM 9 | | | |

* For details, see p. 89.
† Live-weight gain (kg) ÷ food intake (kg).

Rat slope-ratio estimates on the nine protein concentrates are presented in Table 10. There was no consistent pattern of gain *v.* FCE but estimates were generally lower on a carcass basis. Using FCE on a carcass basis as the criterion of response, there was close agreement with the pig estimates for cottonseed and soya-bean meals, higher values for lupin-seed meal and peanut meal and a lower value for field peas.

The Silcock available-lysine values ranged from 0·98 for lupin-seed meal no. 1 and field peas to 0·87 for cottonseed meal (Table 11). Values for the direct-FDNB procedure were lower and more variable and ranged from 0·57 for lupin-seed meal no. 1 to 0·94 for soya-bean meal no. 2. For comparative purposes, the slope-ratio estimates for pigs and rats (based on FCE on a carcass basis) are also presented in Table 11. There was little relationship between these values and the chemical techniques.

All samples of lupin-seed meal had low levels of aflatoxins ($\leqslant 30\ \mu g/kg$), alkaloids ($\leqslant 0{\cdot}10$–$0{\cdot}14$ g/kg) and presence of *P. leptostromiformis* (nos 1 and 4 not detectable, nos 2 and 3 one seed per 200 seeds examined).

Table 8. *Expt 2.* Dressing proportion, 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 | Lupin (*Lupinus angustifolius*) seed meal no. 2 | Lupin-seed meal no. 3 | Lupin-seed meal no. 4 | Field peas (*Pisum sativum*) | Soya-bean meal no. 2 |
| | | | Dressing proportion (g/kg)† | | | |
| 0 | 749 | — | — | — | — | — |
| 0·5 | 746 | 743 | 751 | 749 | 749 | 755 |
| 1·0 | 745 | 742 | 737 | 737 | 740 | 736 |
| 1·5 | 760 | 736 | 738 | 746 | 751 | 739 |
| 2·0 | 759 | 751 | 714 | 731 | 769 | 731 |
| 2·5 | 768 | 729 | 745 | 729 | 736 | 741 |
| | | | SEM 10 | | | |
| | | | Carcass gain (g/d)‡ | | | |
| 0 | 392 | — | — | — | — | — |
| 0·5 | 421 | 422 | 400 | 407 | 413 | 404 |
| 1·0 | 428 | 428 | 395 | 414 | 436 | 417 |
| 1·5 | 475 | 441 | 434 | 450 | 468 | 447 |
| 2·0 | 475 | 442 | 428 | 413 | 467 | 457 |
| 2·5 | 487 | 452 | 444 | 431 | 471 | 490 |
| | | | SEM 12 | | | |
| | | | Food conversion efficiency (carcass basis)§ | | | |
| 0 | 0·295 | — | — | — | — | — |
| 0·5 | 0·313 | 0·317 | 0·310 | 0·303 | 0·309 | 0·307 |
| 1·0 | 0·325 | 0·317 | 0·302 | 0·308 | 0·324 | 0·314 |
| 1·5 | 0·357 | 0·330 | 0·326 | 0·335 | 0·350 | 0·344 |
| 2·0 | 0·356 | 0·332 | 0·322 | 0·322 | 0·356 | 0·344 |
| 2·5 | 0·366 | 0·336 | 0·336 | 0·330 | 0·356 | 0·368 |
| | | | SEM 0·017 | | | |

* For details, see p. 89.
† Hot carcass weight as a proportion of live weight before slaughter.
‡ Hot carcass weight (kg) – (initial live weight (kg) × 0·69) ÷ period (d) on experiment.
§ Hot carcass weight (kg) – (initial live weight (kg) × 0·69) ÷ food intake (kg).

## DISCUSSION

The results confirm earlier findings (Batterham *et al.* 1979) that the availability of lysine in cottonseed meal is low for pigs, and that of soya-bean meal high. There appears to be little effect of the method of processing on cottonseed meal in that the low availability in the cottonseed meal in Expt 1 (0·39, expeller processed) was similar to that of a 'prepress' solvent-extracted meal (0·43, Batterham *et al.* 1979). The results also indicate that the availability of lysine in peanut meal is low to medium (0·57) and in field peas high (0·93).

The low and variable results for lupin-seed meal were unexpected as no heat processing was used in their preparation. Consequently, an availability similar to field peas (also given raw) was anticipated. The low availabilities were unlikely to be due to the presence of aflatoxins, mycotoxins or alkaloid content. The production history of meal no. 1 (availability 0·37) was traced and there was no evidence of pesticides or chemicals used during

Table 9. *Expts 1 and 2. Availability of lysine (proportion of total) in the protein concentrates as assessed with pigs using daily live-weight gain, food conversion efficiency (FCE), daily carcass gain and FCE on a carcass basis as the criteria for availability*

(Mean values and standard deviations)

| | Daily live-wt gain (kg) | | FCE* | | Daily† carcass gain | | FCE‡ (carcass basis) | |
|---|---|---|---|---|---|---|---|---|
| Protein concentrate | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Expt 1 | | | | | | | | |
| Cottonseed meal | 0·51 | 0·12 | 0·57 | 0·09 | 0·36 | 0·11 | 0·39 | 0·09 |
| Lupin (*Lupinus angustifolius*) seed meal no. 1 | 0·51 | 0·12 | 0·62 | 0·09 | 0·31 | 0·11 | 0·37 | 0·09 |
| Peanut (groundnut) meal | 0·74 | 0·12 | —§ | 0·10 | 0·54 | 0·11 | 0·57 | 0·09 |
| Soya-bean meal no. 1 | 1·08 | 0·14 | 1·10 | 0·12 | 0·97 | 0·12 | 0·98 | 0·11 |
| Expt 2 | | | | | | | | |
| Lupin-seed meal no. 2 | 0·94 | 0·11 | 0·89 | 0·10 | 0·68 | 0·09 | 0·65 | 0·08 |
| Lupin-seed meal no. 3 | —§ | — | 0·82 | 0·10 | 0·49 | 0·09 | 0·54 | 0·08 |
| Lupin-seed meal no. 4 | 0·74 | 0·10 | 0·81 | 0·10 | 0·48 | 0·09 | 0·54 | 0·08 |
| Field peas (*Pisum sativum*) | 1·06 | 0·12 | 1·08 | 0·11 | 0·91 | 0·10 | 0·93 | 0·09 |
| Soya-bean meal no. 2 | —§ | — | —§ | — | 0·85 | 0·10 | 0·89 | 0·09 |

* Live-weight gain (kg) ÷ food intake (kg).
† Hot carcass weight (kg) − (initial live weight (kg) × 0·69) ÷ period (d) on experiment.
‡ Hot carcass weight (kg) − (initial live weight (kg) × 0·69) ÷ food intake (kg).
§ Slope-ratio values not calculated as curvature (peanut meal) and intersection (lupin-seed meal no. 3 and soya-bean meal no. 2) significant ($P \leqslant 0.05$).

Table 10. *Availability of lysine (proportion of total) in the protein concentrates as assessed with rats using live-weight gain, food conversion efficiency (FCE), carcass gain and FCE on a carcass basis as the criteria for availability*

(Mean values and standard deviations)

| | Live-wt gain | | FCE* | | Carcass gain† | | FCE‡ (carcass basis) | |
|---|---|---|---|---|---|---|---|---|
| Protein concentrate | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Cottonseed meal | 0·35 | 0·11 | 0·47 | 0·10 | 0·26 | 0·10 | 0·35 | 0·09 |
| Lupin (*Lupinus angustifolius*) seed meal no. 1 | 0·96 | 0·12 | 0·93 | 0·14 | 0·96 | 0·29 | 0·81 | 0·08 |
| Peanut (groundnut) meal | 0·87 | 0·15 | 0·92 | 0·12 | 0·74 | 0·10 | 0·76 | 0·09 |
| Soya-bean meal no. 1 | 0·80 | 0·14 | 1·00 | 0·15 | 0·78 | 0·13 | 0·91 | 0·10 |
| Lupin-seed meal no. 2 | 1·02 | 0·05 | 1·05 | 0·22 | 0·93 | 0·07 | 0·94 | 0·13 |
| Lupin-seed meal no. 3 | 1·07 | 0·15 | 0·80 | 0·13 | 0·96 | 0·15 | 0·70 | 0·13 |
| Lupin-seed meal no. 4 | 1·13 | 0·22 | 0·97 | 0·17 | 0·90 | 0·15 | 0·80 | 0·19 |
| Field peas (*Pisum sativum*) | 0·76 | 0·07 | —§ | — | 0·74 | 0·08 | 0·76 | 0·08 |
| Soya-bean meal no. 2 | 0·85 | 0·13 | 0·90 | 0·10 | 0·91 | 0·12 | 0·89 | 0·11 |

* Live-weight gain (g) ÷ food intake (g).
† Hot eviscerated weight (g) – (initial live weight (g) × 0·79)
‡ Hot eviscerated weight (g) – (initial live weight (g) × 0·79) ÷ food intake (g).
§ Slope-ratio values not calculated as responses were not linear ($P > 0.05$).

Table 11. *Total lysine (g/kg) and the availability of lysine (proportion of total) in the protein concentrates as assessed by the chemical Silcock technique (Roach* et al. *1967), the direct 1-fluoro-2,4-dinitrobenzene (FDNB) assay (Carpenter, 1960) and by the slope-ratio assay with pigs and rats using food conversion efficiency on a carcass basis as the criterion for availability*

(Mean values and standard deviations)

| | Total lysine | | | | Slope-ratio assay | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Pigs | | Rats | |
| Protein concentrate | Mean | SD | Silcock assay | Direct FDNB assay | Mean | SD | Mean | SD |
| Cottonseed meal | 18 | 0·03 | 0·87 | 0·83 | 0·39 | 0·09 | 0·35 | 0·09 |
| Lupin (*Lupinus angustifolius*) seed meal | | | | | | | | |
| No. 1 | 15 | 0·07 | 0·96 | 0·57 | 0·37 | 0·09 | 0·81 | 0·08 |
| No. 2 | 14 | 0·03 | 0·98 | 0·68 | 0·65 | 0·08 | 0·94 | 0·13 |
| No. 3 | 15 | 0·03 | 0·97 | 0·76 | 0·54 | 0·08 | 0·70 | 0·13 |
| No. 4 | 14 | 0·02 | 0·97 | 0·90 | 0·54 | 0·08 | 0·80 | 0·19 |
| | — | — | — | — | 0·53 | 0·04 | 0·81 | 0·06 |
| Peanut (groundnut) meal | 15 | 0·03 | 0·91 | 0·80 | 0·57 | 0·09 | 0·76 | 0·09 |
| Field peas (*Pisum sativum*) | 16 | 0·01 | 0·98 | 0·83 | 0·93 | 0·09 | 0·76 | 0·08 |
| Soya-bean meal no. 1 | 28 | 0·04 | 0·94 | 0·82 | 0·98 | 0·11 | 0·91 | 0·10 |
| Soya-bean meal no. 2 | 26 | 0·09 | 0·93 | 0·94 | 0·89 | 0·09 | 0·89 | 0·11 |

production or storage that was likely to affect pig performance. There does not appear to be a toxic factor in the meal as a high inclusion level of meal no. 2 (430 g/kg) was given to weaner pigs with no adverse effect (Barnett & Batterham, 1981) and the response to inclusion level of the meals in the slope-ratio assay was linear. Nor is there evidence in the literature of anti-nutritional factors associated with cultivars of *L. angustifolius* (Hove *et al.* 1978; Hove & King, 1979; Hudson, 1979).

The true digestibility of lysine at the terminal ileum of pigs was determined for lupin-seed meal no. 1 (availability 0·37) by M. R. Taverner (personal communication) and was high (0·86). This indicates that the low availability is not associated with low digestibility. Nor was the low availability detected by the slope-ratio assays with rats or by the two chemical techniques. Whilst the values for the direct-FDNB procedure were lower than the Silcock values for lupin-seed meal, this may only be a reflection of the instability of dinitrophenyl-lysine in the presence of carbohydrates. Over-all, the lower availability of lysine in the lupin-seed meal for pigs may reflect either the presence of an unidentified growth depressant (which has a linear effect on performance with increasing inclusion level) or to the lysine being in a form that is digested but inefficiently utilized. Furthermore, the causal factor (or factors) appears specific to pigs. These aspects are currently being investigated.

Whilst crude fibre does not measure any definable chemical fraction of a feed (Van Soest & Wine, 1967) it does appear to be a suitable basis on which to equalize diets for fibre content using solka floc, as variation in dietary acid-detergent and neutral-detergent fibre levels was also much reduced (levels of 55–63 and 94–104 g/kg in Expt 1 and 64–70 and 105–115 g/kg in Expt 2 respectively). Nor did equalizing diets for fibre content affect the lowering of availability estimates based on carcass relative to live-weight performance. A limited number of viscera (including the trachea but excluding the kidneys) were examined and this indicated that whilst there was a slight increase in the weight of viscera minus gut contents in some pigs given the protein concentrates, the main effect was due to an increase in gut contents. In Expt 2 there was also an increase in the dressing proportion in pigs given standard lysine and this contributed to the lowering of availability estimates when based on a carcass basis. The reason for such an effect is unclear; it did not occur in Expt 1 or in previous slope-ratio assays (Batterham *et al.* 1979, 1981). It is desirable to express availability estimates on a carcass basis as it avoids the effect of variation in the weight of viscera and gut contents. It is also desirable to examine the effect of expressing availability estimates on a protein deposition basis to determine if the small differences in the slopes for lean in the ham of pigs given the different protein concentrates reflected differences in availability.

The slope-ratio estimate of 0·98 for lysine availability in soya-bean meal no. 1 (Table 9) was higher than the estimate for soya-bean meal no. 2 (0·89, Table 9) and that of a previously reported soya-bean meal (0·84; Batterham *et al.* 1979). The high value for soya-bean meal no. 1 may reflect variation in the availability estimate as the standard deviation was 0·11; it may also reflect variation in the total lysine determination. Whilst the standard deviation of the total lysine estimate was small (0·04), this reflects only within-laboratory variation, and between-laboratory variation may be greater (Porter *et al.* 1968; Williams *et al.* 1980). However, the estimates of lysine availability that were greater than 1·0 for a number of meals when gain and FCE were assessed on a live-weight basis (Tables 9 and 10) do not appear to be due to underestimation of total lysine but rather to overestimation of the availability estimates (due to the effect of variation in dressing proportion, as discussed earlier). The estimates on a carcass basis were considerably lower for these meals and less than 1·0.

The slope-ratio estimates with rats indicated that for some meals (cottonseed and soya-bean meal) there is a close agreement with the pig estimates. This also applies for sunflower meal (Batterham *et al.* 1981). For others, such as lupin-seed meal, the rat assay is inapplicable for predicting pig response. Additional assays need to be made with peanut meal and field peas before firm conclusions for these meals can be made. As in previous work (Batterham *et al.* 1981), there appeared to be little relationship between the values from the two chemical procedures and the slope-ratio values. Until the cause (or causes) of the variation in available lysine in the different vegetable proteins for pigs and rats is elucidated it is difficult to suggest suitable in vitro or in vivo techniques for predicting lysine availability in these meals for pigs.

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