*Brit. J. Nutr.* (1963), **17**, 309, *with* 1 *plate*



# A growth assay with chicks for the lysine content of protein concentrates

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(*Received* 19 *November* 1962—*Revised* 28 *February* 1963)

The amino acids that can be determined in a food by chemical analysis are sometimes not all nutritionally available; it is known that the discrepancy between 'total' and 'available' lysine, in particular, can be large in processed foods and feeding-stuffs. Procedures have been suggested for obtaining by chemical means values more likely to approximate to the levels of lysine nutritionally available, but the usefulness of such values must be checked empirically against the direct results of biological tests.

In this paper we report the use of weight gains of young chicks as a measure of their response to dietary sources of lysine. Such an assay was first described by Tsien, Johnson & Liener (1957) in their study of *Torula* yeast and by Kratzer & Green (1957), who were interested in blood meals; both these groups used basal diets containing natural feeding-stuffs of low lysine content. More recently, Ousterhout, Grau & Lundholm (1959) have conducted assays for a range of amino acids, including lysine, using a mixture of synthetic amino acids as the only nitrogen source in the basal diets. From studies on the rat (Gupta, Dakroury, Harper & Elvehjem, 1958; Calhoun, Hepburn & Bradley, 1960) and on the chick (Fisher, Griminger, Leveille & Shapiro, 1960; Fisher & Shapiro, 1961), it appears that, in assays of this kind, the relationship between live-weight gain and lysine content of the diet can be distorted by the nature of the supplement, in part through an effect on the appetite of the test animal.

The intention of the work reported here was to find an assay procedure with chicks that was not liable to distortion. We also wished to design a growth assay that would provide results suitable for statistical analysis, as was done for the B vitamins by Coates, Kon & Shepheard (1950); one condition is that each test material should be given at more than one level. Owing to the high cost of synthetic diets, we took advantage of the primary deficiency in lysine that sesame seed has for the chick (Grau, 1948).

## EXPERIMENTAL

### *Management of the chicks*

For each of the four experiments 250 White Leghorn × Rhode Island Red day-old cockerels were purchased from a commercial hatchery. In Expts 3 and 4 only, they were 'debeaked' on arrival by cutting off the tips of the upper beak with sharp scissors,

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as a precaution against later cannibalism. They were reared to 4 days of age on a commercial diet and then transferred to the preliminary diet described below. They were weighed at 5, 7 and 9 days of age and the 192 showing the most even gains in weight were selected. They were further stratified into four blocks of forty-eight chicks each, according to their growth up till then, and each block was divided at random into twelve groups of four. Each group was allotted to a separate cage to receive one of the twelve experimental diets used in each experiment.

At 10 days of age the selected birds were transferred to an experimental battery of forty-eight suspended cages, as used by Carpenter & March (1961). The battery was designed especially for short-term assays of this kind and is illustrated in Pl. 1. Each block was allotted a group of twelve cages on either the two top or the two lower decks on one side of the battery. The battery was housed in a room kept at a controlled temperature with tubular electric heaters, at floor level, connected to a thermostat, the air being kept in motion with a swinging fan. The battery was turned daily to reduce the effect of any inequality of environment on the two sides.

The birds received their experimental diets *ad lib.* as finely ground dry powders. They were weighed individually after 3 and 6 days in the cages and also when the experiment ended after 9 or 10 days. The food consumption of each cage of birds was recorded after each weighing in Expts 3 and 4; only the total consumption of each experimental diet at the various times was recorded in Expts 1 and 2. The response used in the statistical analysis was the mean weight gain of the four chicks in a cage. Expts 1 and 2 were exact replicates, and so were Expts 3 and 4.

### *Basal diet*

The basal diet (A) had the percentage composition: extracted sesame-seed meal 50, coarse-ground whole wheat 20, hydrogenated vegetable fat (Trex; J. Bibby & Sons Ltd, Liverpool) 10, dried-grass meal 2, ground oat husks 2, steamed bone flour 2, limestone flour 1, L-leucine 0·06, oxytetracycline supplement (TM-5, containing 2·2% oxytetracycline; Pfizer Ltd, Folkestone, Kent) 0·07, salts and vitamins, and maize starch to 100.

The salts and vitamins were added so as to contribute (mg/kg diet): NaCl 5000, KI 13, $MnSO_4.4H_2O$ 32, choline chloride 1750, thiamine 10, riboflavin 10, pyridoxine 10, nicotinic acid 50, calcium pantothenate 30, biotin 1, pteroylglutamic acid 4, cyanocobalamin 0·01, menaphthone (vitamin K) 0·5, Rovimix A and D (Roche Products Ltd, Welwyn Garden City; containing $4 \times 10^6$ i.u. vitamin A and $1 \times 10^6$ i.u. vitamin $D_3$/lb) 1000, Rovimix E (Roche Products Ltd; containing 10% α-tocopheryl acetate) 200.

The preliminary diet consisted of the basic diet A with the addition, at the expense of maize starch, of 3·5% white-fish meal. In Expt 1 only, the L-leucine was omitted from the preliminary diet.

### *Supplements to the basal diet*

The supplements to the basal diet in the various experimental treatments were as follows.

*X.* 92; *herring meal*: an English commercial sample prepared by the cook-and-press procedure with no return of the solubles to the meal.

*X.* 118; *zein*: a commercial sample supplied by Corn Products Ltd, Paisley.

*X.* 119B; *anchovy meal*: a Peruvian sample selected for its high content of available lysine as judged by its reaction with fluorodinitrobenzene.

*X.* 120B; *anchovy meal*: another Peruvian sample selected for its low content of available lysine as judged by the same test.

*X.* 135; L-*lysine monohydrochloride* (*Light & Co., Colnbrook, Bucks.*): tests for the purity of this sample are described below; our estimate from the results of these tests is that it contained the equivalent of 78% L-lysine.

*X.* 136; *cod fillets*: a sample of freeze-dried material (Ministry of Agriculture, Fisheries and Food Experimental Factory, Aberdeen) whose preparation was similar to that described in an earlier paper (Carpenter, Ellinger, Munro & Rolfe, 1957); it had 3% moisture.

*X.* 137; *heated cod fillets*: prepared by the addition of sufficient water to a sample of X. 136 to bring the moisture content to 11%, sealing in nitrogen with a small head-space and heating in an oven at 105° for 36 h.

### *Chemical analyses for lysine*

The protein supplements were analysed for their total lysine content: 0·25 g of material was refluxed with 900 ml constant boiling-point aqueous HCl for 24 h; the hydrolysate was filtered and made up to 1 l. with water and a measured portion was concentrated almost to dryness at 35–40° in a rotary evaporator. The residue was made up to 50 ml with a citrate buffer of pH 5·28, and 2 ml were then applied to a 15 cm resin column and eluted, as specified by Moore, Spackman & Stein (1958). We followed the further stages of this procedure except for collecting fractions of 1·68 ml instead of 2 ml and using the ninhydrin reagent of Jacobs (1956). Under our conditions, the lysine was usually eluted in the twenty-fifth to thirtieth fractions. We calculated the results from reference to a standard curve of ninhydrin colour developed with graded levels of lysine hydrochloride (X. 135).

The same materials were also analysed for lysine free to react with fluorodinitrobenzene by the Carpenter (1960) procedure, with application of the '$\times$ 1·09' correction factor for the loss of approximately 8% of the dinitrophenyl lysine during the hydrolysis stage that has been found to occur with animal protein concentrates of the type used here. Some of the materials were re-analysed after a preliminary extraction of the lipids.

### *Expts* 1 *and* 2

They were replicates and their purpose was to provide a test of the relative merits of the addition of the test material for assay at the expense of (*a*) a non-nitrogenous constituent such as starch, or (*b*) nitrogenous material of little or no lysine content

such as zein, so as to keep the total level of crude protein constant in the experimental treatments. It was also hoped to obtain a measure of the relative precision of assays conducted over 3, 6 or 9 days.

Table 1. *Supplements (%) added to the basal diet, at the expense of maize starch, to make the test diets used in Expts 1 and 2*

| Diet code | L-lysine hydrochloride (X. 135) | Zein (X. 118) | Herring meal (X. 92) | Protein (%) contributed by zein and herring meal* |
|---|---|---|---|---|
| A | — | — | — | — |
| B | 0·15 | — | — | — |
| C | 0·30 | — | — | — |
| D | 0·75 | — | — | — |
| E | — | 3·6 | — | 3·24 |
| F | 0·15 | 3·6 | — | 3·24 |
| G | 0·30 | 3·6 | — | 3·24 |
| H | 0·75 | 3·6 | — | 3·24 |
| J | — | — | 2·4 | 1·62 |
| K | — | — | 4·8 | 3·24 |
| L | — | 1·8 | 2·4 | 3·24 |
| M | — | 3·6 | 4·8 | 6·48 |

* Calculated as N × 6·25 from zein (90·0% crude protein) and herring meal (67·6% crude protein). If the lysine hydrochloride contributions at 0·15, 0·30 and 0·75% had been included they would have added 0·14, 0·29 and 0·72% crude protein respectively.

The composition of the test diets is set out in Table 1. Diets A–D consisted of the basal diet with graded additions of lysine; diets E–H replicated these diets with the addition throughout of 3·6% zein. Diets J and K contained 2·4 and 4·8%, respectively, of the herring meal X. 92; 4·8% of it contributed 3·24% of crude protein (N × 6·25), as did the 3·6% of zein in diets E–H. Diet L contained 2·4% of X. 92 and also 1·8% of zein, which together contributed 3·24% of crude protein. Finally, diet M contained the top level of both X. 92 and zein. (The letter I was omitted from the diet series in order to avoid possible confusion with the number 1.)

### *Expts 3 and 4*

They were again replicates. In each, four test materials were assayed, each at the two levels of protein addition, 1·62 and 3·24%, used in the previous experiments. For comparison, a negative control and diets supplemented with L-lysine hydrochloride at three levels, 0·15, 0·30 and 0·60%, were also included. All the diets were adjusted when necessary with zein, so as to bring the level of supplementary crude protein in each to 3·24%. The composition of each diet is set out in Table 4.

### *Statistical analysis*

The relations of weight gain and food conversion to the dietary level of supplementary lysine were of the standard slope-ratio type; the results were analysed by the methods of Finney (1952). The relation of weight gain to weight of lysine eaten was also of the slope-ratio type, but with the complication that the response lines for the

test substances would not be expected to meet at zero dose, because the extra lysine eaten by the test groups came in part from the extra quantity of basal diet as well as from the test supplement eaten. An account of the modifications made to the standard analysis is being prepared for publication elsewhere.

## RESULTS

### *Expts* 1 *and* 2

The only difficulty encountered in carrying out these experiments was sporadic pecking amongst the birds. Two individuals were taken out of Expt 1, and a whole group, which had been receiving diet B, was taken out of Expt 2. An adjusted mean weight gain has been calculated for this treatment, based on the growth of the birds in the remaining three cages, and a 'missing plot' value calculated in the usual way; it was not possible to make a similarly adjusted estimate of food consumption, which was not measured separately for each cage in the first two experiments.

Table 2. *Mean weight gain and food consumption (g/chick) over 9 days, with each of the test diets used in Expts 1 and 2*

| Diet code | Dietary supplements, other than zein | Expt 1 | | Expt 2 | |
|---|---|---|---|---|---|
| | | Wt gain (0); (3·6) | Food eaten (0); (3·6) | Wt gain (0); (3·6) | Food eaten (0); (3·6) |
| A; E | None | 60; 39 | 151; 116 | 52; 40 | 144; 115 |
| B; F | 0·15% lysine hydrochloride, X. 135 | 73; 65 | 167; 152 | 66*; 58 | —; 138 |
| C; G | 0·30% lysine hydrochloride, X. 135 | 94; 95 | 188; 175 | 97; 86 | 192; 166 |
| D; H | 0·75% lysine hydrochloride, X. 135 | 128; 134 | 195; 196 | 136; 140 | 207; 209 |
| J; L | 2·4% herring meal, X. 92 | 70; 66† | 158; 149† | 69; 67† | 154; 148† |
| K; M | 4·8% herring meal, X. 92 | 96; 88 | 186; 167 | 96; 90 | 174; 168 |

Figures in parentheses are the amounts (%) of zein in the diet.
* Adjusted mean for three cages only.
† Only 1·8% of zein was included in diet L.

The mean gains in weight and food consumption on each treatment and in each experiment are set out in Table 2; there was a progressive growth response to increasing levels of the lysine supplement, X. 135, both with the basal diet alone (diets A–D) and with the basal diet plus zein (diets E–H). Fig. 1 *A*, in which the means of the results of the two experiments are plotted, shows that, at the zero and 0·15% levels of X. 135 addition, the daily gain was less with the diets containing zein, but that at the highest level of lysine addition the response was almost the same whether zein was included or not.

If the response metameter is taken to be g gain/g food eaten (Fig. 1 *B*), there were again increases with successive additions of lysine and an apparent depression (though not a significant one; $0{\cdot}05 < P < 0{\cdot}10$) with addition of zein at the zero level of addition of X. 135.

Gupta *et al.* (1958) found with rats that the disturbing influence of a change in carbohydrate source could be removed by plotting the growth response not against the percentage of lysine added to the diet but against the weight of available lysine eaten. This finding was confirmed in further rat experiments by Calhoun *et al.* (1960). To study similarly our results, an estimate of the available lysine in the basal diet is

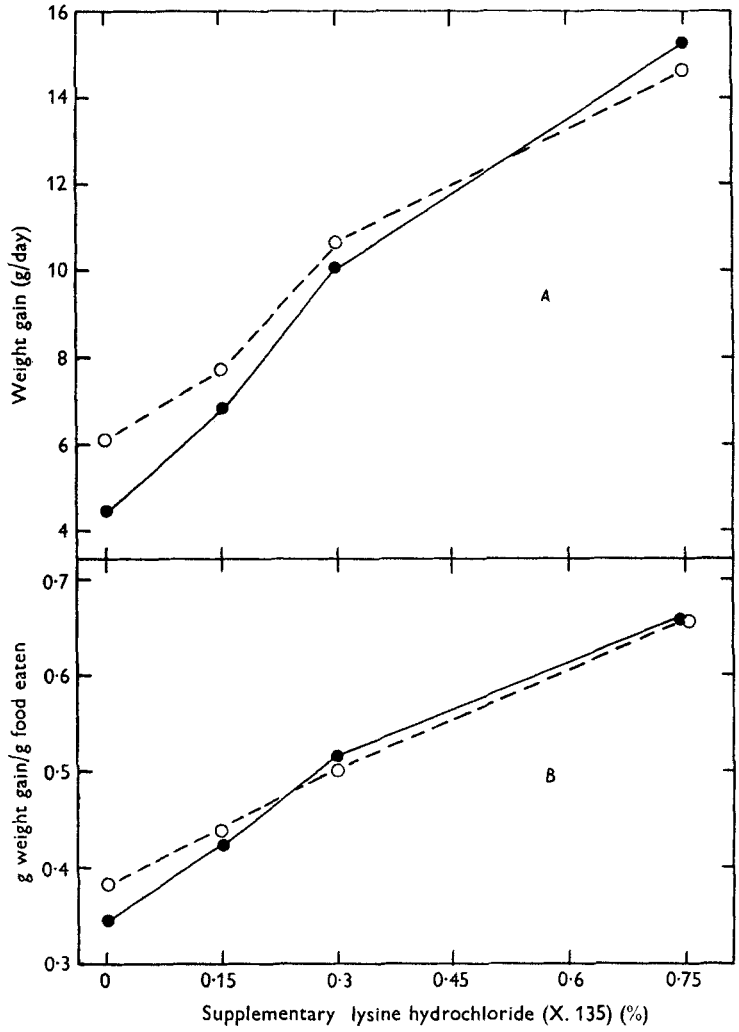



Fig. 1. Effect of level of dietary supplementation with L-lysine hydrochloride (X. 135) on (*A*) weight gain/chick and (*B*) weight gain/g food eaten. Mean results for Expts 1 and 2 over 9 days. o - - - o, basic diet; ● — ●, basal diet plus 3·6% zein.

required. Taking this to be 0·637%, we plotted in Fig. 2 the growth response against g available lysine consumed/day. Thus the effect of added zein on the lysine-deficient diets that had been apparent in Fig. 1 was eliminated; the interpretation of these results and the validity of the estimate used for the basal diet is considered on p. 321.

The gain in weight and food consumption of the chicks receiving diets J, K, L

and M, which contained herring meal, are set out in Table 2; the apparent potency of the fish meal as a lysine source has been calculated in several ways, and the results are summarized in Table 3. The methods of calculation depend upon the choice of standards and of dose and response metameters. The metameters used were:

(1) g weight gain/day and percentage added lysine in diet;
(2) g weight gain/g food eaten and percentage added lysine in diet;
(3) g weight gain/day and g available lysine eaten.

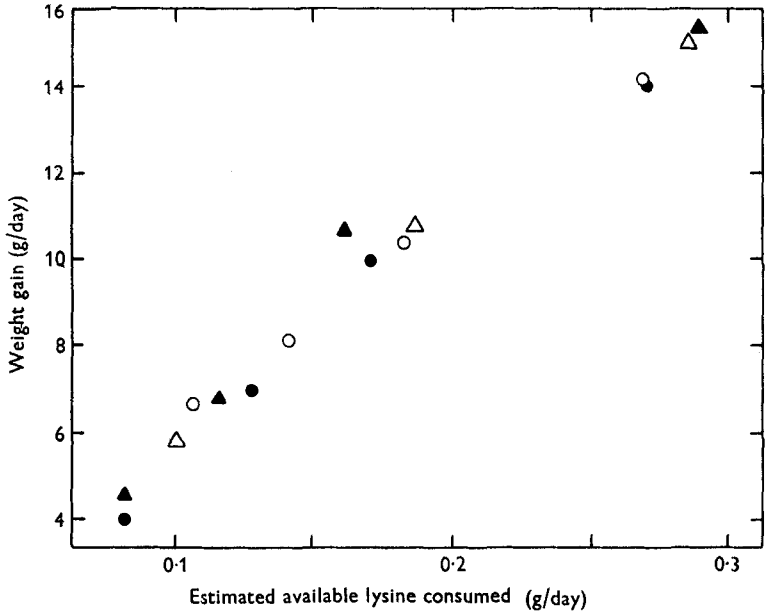



Fig. 2. Relationship between weight gain and weight of available lysine consumed (on the assumption that the basal diet contained 0·637% lysine available to the chick) for chicks receiving the basal diet with or without zein and with supplementary lysine at various levels. Expt 1: ○, basal diet; ●, basal diet+zein. Expt 2: △, basic diet; ▲, basal diet+zein.

Table 3. *Estimates of the potency (g lysine/16 g N) of herring meal X. 92 as a source of lysine, from the mean results of Expts 1 and 2 (see p. 313)*

| Basis of computation | | | | Potency according to method of computation | | |
|---|---|---|---|---|---|---|
| | | | | Readings from graphs | | Statistical analysis (with 95% fiducial limits)† |
| Relationship used | Standard | Unknown | Principle of comparison | (*a*)* | (*b*)* | |
| g wt gain/day—% added lysine in diet | A–D | J, K | Straight addition | 7·2 | 7·3 | Not valid |
| | E–H | M | Straight addition | — | 7·1 | — |
| | E–H | L, K | Constant protein | 8·4 | 8·6 | 8·5 (7·5–9·7) |
| g wt gain/g food eaten —% added lysine in diet | A–D | J, K | Straight addition | 8·2 | 9·8 | 9·0 (6·7–12·6) |
| | E–H | M | Straight addition | — | 8·8 | — |
| | E–H | L, K | Constant protein | 9·2 | 8·9 | 8·7 (7·0–10·9) |
| g wt gain/day—g available lysine eaten | A–H | J, K, L, M | All values pooled | 9·0 | 9·0 | — |
| | | | | 9·3 | 8·6 | — |

* Values obtained with (*a*) 2·4% and (*b*) 4·8% level of addition of herring meal (X. 92).
† The responses to diets D and H were not used in these calculations.

The standards were results with either diets A–D or diets E–H. The use of standards A–D implies the assumption that simple addition of lysine to the basal diet leads to a correct measure, whereas the use of standards E–H implies the assumption that comparisons have to be made at constant protein content. Graphical estimates were always obtained, and numerical methods also were used when the response relationship made possible a valid assay. Three examples of these methods will now be described.

(*a*) Reference to the curve of weight gain/day in Fig. 1*A* for diets A–D shows that virtually the same responses were obtained from the addition of 0·15% and of 0·30% X. 135 to the basal diet as were in fact obtained by the addition of 2·4 and of 4·8% of fish meal X. 92 in diets J and K, respectively. On the assumption that simple addition to the basal diet in this way gives a correct result and that X. 135 contains 78% L-lysine, the estimates of potency obtained for X. 92 would be 7·2 and 7·3 g lysine/16 g N respectively.

(*b*) Comparing the responses to diets L and K with those to diets E–H in Fig. 1*A*, we obtain values of 8·4 and 8·6 g/16 g N for diets L and K, respectively. Statistical analysis of the same results with the fitting of one straight-line response for diets E, F and G and another for diets E, L and K gives an overall estimate of 8·5 g/16 g N, with 95% fiducial limits of 7·5 and 9·7 g/16 g N.

(*c*) With the response relationship shown in Fig. 2, the mean gain of 96 g on diet K corresponds to an intake of 1·655 g available lysine, which, with a mean food intake of 180 g, corresponds to 0·925% of the diet. Fig. 2 is based on an assumed 0·637% content of available lysine in the basal diet, so that by difference 0·283% appears to come from the 3·24% protein contributed by the fish meal, and its apparent potency is 8·7 g/16 g N.

### *Expts 3 and 4*

These experiments were carried through without mishap, except that one chick, receiving diet L in Expt 4, caught its leg in the wire mesh of a cage floor and was removed from the experiment. A missing value has been fitted for calculating the mean response to this treatment. The mean results for each treatment, after 3, 6 and 10 days, and in each experiment are set out in Table 4. The pattern of the response to lysine and the four test materials is illustrated in Fig. 3 in which the mean gain in weight for the two experiments on each treatment over the full period is plotted against the level of supplement used.

A statistical analysis was carried out for the two experiments combined, and the results are summarized in Table 5. With weight gain as a function of percentage added lysine, the fiducial limits were widest for the estimates based on the results for the first 3 days of the trial, but there was little difference in precision between the estimates calculated from the 6- and 10-day figures. There was an apparent tendency for the calculated potencies of the materials to decrease as the assay period increased, but the differences were well within the calculated limits of error of the estimates.

The corresponding estimates of potency based on g gain/g food eaten over 6 and 10 days ranked the four materials in the same order as did the estimates based on

Table 4. *Mean weight gain and food consumption (g/chick) over experimental periods of 3, 6 and 10 days, with each of the test diets used in Expts 3 and 4*

(All diets were adjusted to contain 3·24% supplementary crude protein)

| Diet code | Dietary supplements | Expt 3 | | | | | | Expt 4 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0–3 days | | 0–6 days | | 0–10 days | | 0–3 days | | 0–6 days | | 0–10 days | |
| | | W | F | W | F | W | F | W | F | W | F | W | F |
| A | 3·6% zein | 7·8 | 29·4 | 17·9 | 61·8 | 32·2 | 106·7 | 4·2 | — | 13·4 | 51·7 | 21·3 | 90·8 |
| B | 0·15% L-lysine hydrochloride + 3·6% zein | 14·2 | 35·3 | 31·5 | 76·8 | 57·1 | 140·5 | 11·7 | — | 26·5 | 66·2 | 40·8 | 117·5 |
| C | 0·30% L-lysine hydrochloride + 3·6% zein | 20·2 | 39·3 | 45·0 | 87·5 | 82·1 | 163·4 | 17·1 | — | 37·5 | 78·1 | 62·6 | 144·0 |
| D | 0·60% L-lysine hydrochloride + 3·6% zein | 31·5 | 47·7 | 69·6 | 105·7 | 127·2 | 200·0 | 25·4 | — | 58·4 | 95·2 | 102·4 | 180·3 |
| E | 2·47% X. 119B + 1·8% zein | 13·4 | 33·3 | 29·6 | 71·6 | 53·2 | 127·3 | 13·0 | — | 29·2 | 70·2 | 47·5 | 126·7 |
| F | 4·95% X. 119B | 21·8 | 42·0 | 48·9 | 91·0 | 86·8 | 171·0 | 18·4 | — | 43·0 | 82·4 | 70·8 | 153·5 |
| G | 2·54% X. 120B + 1·8% zein | 13·0 | 34·3 | 26·4 | 71·2 | 47·1 | 125·4 | 8·9 | — | 22·3 | 64·0 | 35·6 | 113·2 |
| H | 5·09% X. 120B | 16·6 | 38·3 | 35·6 | 81·8 | 62·4 | 150·3 | 10·1 | — | 28·5 | 67·8 | 46·7 | 124·5 |
| J | 1·92% X. 136 + 1·8% zein | 13·5 | 34·5 | 29·9 | 72·9 | 55·0 | 131·0 | 14·4 | — | 31·1 | 72·0 | 49·7 | 129·0 |
| K | 3·85% X. 136 | 25·3 | 43·4 | 53·8 | 93·7 | 96·4 | 176·7 | 21·3 | — | 47·7 | 83·7 | 79·2 | 158·9 |
| L | 2·10% X. 137 + 1·8% zein | 13·9 | 35·0 | 30·1 | 73·7 | 53·3 | 131·0 | 11·1 | — | 26·8 | 67·8 | 42·8 | 121·3 |
| M | 4·20% X. 137 | 21·4 | 40·7 | 44·1 | 86·4 | 78·2 | 160·4 | 15·9 | — | 36·4 | 73·0 | 58·7 | 131·7 |

W, weight gain; F, food eaten.

weight gain, but they were on the average 6% higher; the fiducial limits were less widely spaced.

Lastly, estimates were obtained, as for Expts 1 and 2, by considering weight gain as a function of the total weight of available lysine eaten and making the same assumption that the basal diet contained 0·637% available lysine. Again the four materials showed the same ranking, and the overall mean was the same as for the 10-day estimates by the first procedure.

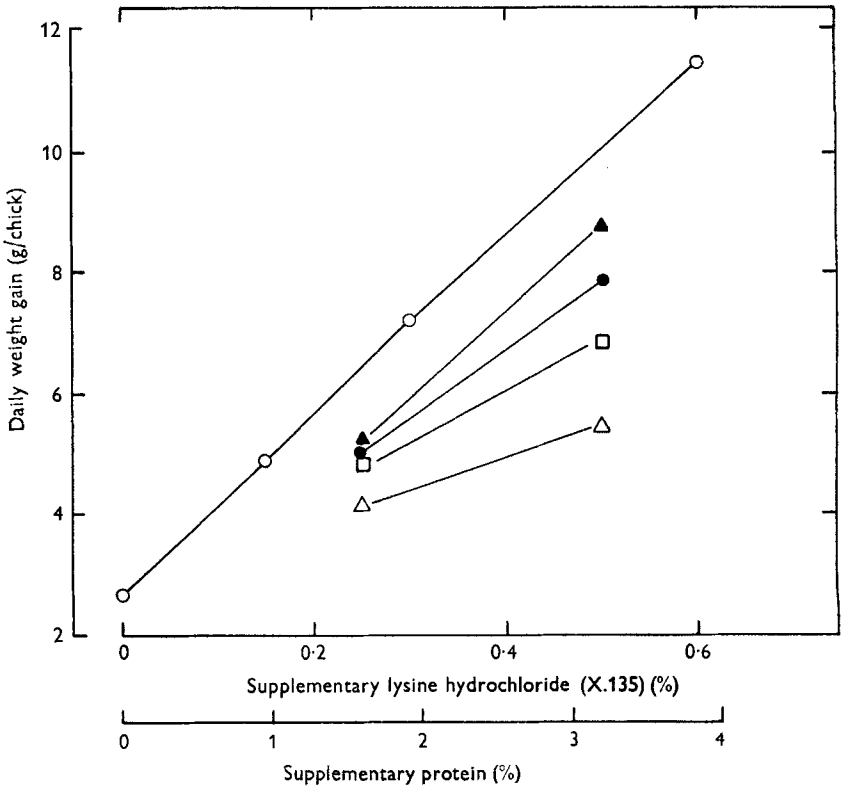



Fig. 3. Effect of levels of dietary supplementation with L-lysine hydrochloride (X. 135) or fish meal on the mean weight gains of chicks over 10 days in Expts 3 and 4. Supplements used: ○—○, L-lysine hydrochloride; ●—●, meal 119B; △—△, meal 120B; ▲—▲, meal 136; □—□, meal 137.

### *Chemical analysis*

Six replicate runs for lysine free to react with fluorodinitrobenzene were carried through on each of the four samples used in Expts 3 and 4, so as to make possible an estimate of the reproducibility of the results. Both the mean values, ranging from 3·9 to 8·7 g/16 g N, and their 95% fiducial limits are set out in Table 5. The same procedure applied to the zein used gave a value of only 0·02 g/16 g N.

Although samples X. 119B and X. 120B, after drying, contained only 2·3 and 2·9% respectively of ether-extractable materials, extraction with chloroform–methanol (2:1, v/v) removed 8·6 and 13·0% of material respectively; 7·6 and 6·9%, respectively,

were soluble in chloroform alone. In case the apparently 'bound' lipid was affecting the values obtained with the fluorodinitrobenzene procedure, the analyses were repeated on samples extracted with chloroform–methanol, but the values obtained were unchanged.

Table 5. *Estimated potency (g lysine/16 g N) of four protein concentrates calculated in different ways from the results of Expts* 3 *and* 4 *over* 3, 6 *or* 10 *days, together with the approximate fiducial limits of these estimates, and also the results of the chemical analyses of the same protein concentrates and of the concentrate used in Expts* 1 *and* 2

| Relationship used | Period | Dietary supplement (X. 92)* | X. 119B | X. 120B | X. 136 | X. 137 | Approximate distance of the 95% fiducial limits from the estimate† |
|---|---|---|---|---|---|---|---|
| | | Chick assays | | | | | |
| g wt gain/day—% lysine added to diet | 0–3 days | — | 8·7 | 4·8 | 10·5 | 7·8 | ±1·24 |
| | 0–6 days | — | 8·8 | 4·9 | 10·1 | 7·3 | ±0·94 |
| | 0–10 days | (8·5) | 8·4 | 4·7 | 9·7 | 6·9 | ±0·81 |
| g wt gain/g food eaten—% lysine added to diet | 0–6 days | — | 9·3 | 4·9 | 10·9 | 8·3 | ±0·75 |
| | 0–10 days | (8·7) | 8·7 | 4·3 | 10·2 | 7·6 | ±0·70 |
| g wt gain/day—g available lysine eaten | 0–10 days | — | 8·5 | 4·3 | 9·7 | 7·3 | ±0·54 |
| | | Chemical analyses‡ | | | | | |
| Available lysine (i.e. ε-$NH_2$ groups reactive with fluorodinitrobenzene) | | (7·45) | 6·85 | 3·9 | 8·7 | 7·25 | ±0·17 |
| Total lysine, after acid hydrolysis | | (7·7) | 7·6 | 6·7 | 8·85 | 8·35 | ±0·42 |

* Assayed in Expts 1 and 2.

† For each material the distances of the upper and lower 95% fiducial limits from the estimate differed slightly, and there were also small differences between the distances for different materials in the same statistical analysis, but only in the third significant figure.

‡ The results are all expressed to the nearest 0·05 g/16 g N.

Duplicate analyses for total lysine were also carried out on each material, with the results shown in Table 5. When the lysine standard was added to samples of test material before acid hydrolysis, 100% recovery was obtained, so that no correction was made to the values found. The 95% fiducial limits of the mean values are again shown. The lysine hydrochloride (X. 135) used in the assays was found to be chromatographically pure on the column, with 100% recovery; $(\alpha)_D = +19{\cdot}8°$ in 5 N-HCl compared with the expected $+20{\cdot}7°$ for the hydrochloride in view of the reported value of $+25{\cdot}9°$ for pure L-lysine (Dawson, Elliott, Elliott & Jones, 1959); nitrogen found was 14·67% (15·32% expected), m.p. 263–4° (as expected); weight loss on heating at 70° for 5 h over $P_2O_5$ in a vacuum oven was 2·0%. Our estimate is that the material contained the equivalent of 78% L-lysine instead of the theoretical 80·0%.

## DISCUSSION

As judged by the usual statistical criteria, the assay procedure chosen for Expts 3 and 4, after the preliminary study of the first two experiments, has been successful. The range of response to the substance to be assayed was wide, and the pattern of response to the standard and the unknowns tested has allowed a valid analysis by the slope-ratio technique, whether weight gain or food conversion efficiency was taken as the response metameter.

Statistical analysis can give us an estimate of the reproducibility of the results. It is more difficult with an assay of this kind to be certain that results are not affected by systematic errors, which do not distort the linearity of the response but reflect the influence in the test material of factors other than the one directly under study. These factors may be classified as protein as such, specific amino acids and, lastly, other non-protein factors in the food.

Thus the growth response of lysine-deficient rats or chicks may be increased by the addition of dextrin to the diet in place of sucrose (Gupta *et al.* 1958; Chang, 1962), but we would not accept as a meaningful result the value, calculable by comparison with the response to lysine, for g available lysine/g dextrin. The effect was no longer seen when weight gain was related to the weight of lysine eaten.

Again, in our Expts 1 and 2, the response to 0·12 g lysine + 3·6 g zein per 100 g of diet added at the expense of carbohydrate has been equivalent, judged from Fig. 1 *A*, to the response to an addition of about 0·05 g lysine alone. One possible explanation is, not that the lysine has become partially unavailable, but that the amino acids released on digestion of the zein have had a counteracting depressant effect on the appetite of the chick.

We tried to design our basal diet so that the addition of test materials should have the minimum effect on its balance of other nutrients. Grass meal and wheat were included as sources of possible unidentified growth factors, and fish meal was also included in the preliminary diet to allow the accumulation of reserves of any such factors it may contain. Oat husks and fat were also thought of as being suitable for partial substitution, to maintain fibre and energy levels, if low-protein test materials had to be included at a high level in the diets. Adjustments could also be made in mineral content. The protein level of the basal diet was high, over 20%, so that the change in amino acid proportions was minimized with the addition of the test material. However, even with the total protein content of the diets kept constant at the expense of zein, there is certainly some change in the levels of amino acids other than lysine.

Gupta *et al.* (1958) studied the effect of changing from sucrose to dextrin as the carbohydrate source in a series of diets containing graded levels of lysine. At any particular percentage level of lysine in a diet, rats gained weight faster on dextrin than on sucrose; however, 'if the weight gains are plotted against the total amino acid intake, the points fall almost on a straight line... irrespective of the nature of the dietary carbohydrate'. The authors conclude: 'This indicates that comparisons based on total amino acid intake are relatively little influenced by the type of carbohydrate and probably by other factors that affect food consumption primarily'. Calhoun

*et al.* (1960) reported similar observations with two series of lysine-deficient diets, one based on wheat gluten and the other on an amino acid mixture, but with the same carbohydrate.

We have applied this procedure to the results of our own feeding experiments. Since the basal diet was not free of the test amino acid, a value had to be assumed for its content of available lysine. Our own estimate of 0·637% available lysine was calculated from published figures (De Man & Zwiep, 1955) for the lysine content of the ingredients in the basal diet, corrected by a factor of 80% availability—the approximate figure found by Calhoun *et al.* (1960) for three cereal products. It has no special merit, except that it did suffice to bring the diets with and without zein into agreement in Fig. 2; further, calculation with figures assumed 20% above or below this estimate had little effect on the estimated potencies of the supplements.

One unusual aspect of this method of using the weight of lysine eaten as the dose metameter is that one cannot express the dose metameter without knowing the response of the chicks in terms of their intake of the experimental diet. As is seen from the increased intakes of birds receiving high levels of added pure lysine, a portion of the increased intake of a diet containing a test supplement may be due to the lysine that it contributes. However, as the procedure of the assay is solely to determine the slopes of the test and standard response curves, there appears to be no reason why the presence of some treatment effects in the dose metameter should affect the validity of the assay. Moreover, for the experiments reported here, there was no significant difference between the results obtained by this procedure and by the more conventional method of analysing the response as weight gain/g food eaten against the dose as percentage of lysine in the supplement; the standard error of the estimates from this second analysis was slightly the greater.

### *Correlation with chemical analysis*

Table 5 sets out both the chemical and the biological results for the four samples studied in Expts 3 and 4. For the two poorest samples (X. 120B and X. 137), the biological values, by the preferred methods of calculation (i.e. (2) and (3), p. 315) were close to those obtained for the chemical procedure with fluorodinitrobenzene, and the chemical values for total lysine were significantly higher. However, for the samples of highest activity (X. 136 and X. 119B) the biological values were higher than the chemical values. The relationships were therefore similar to those reported earlier for rat assays (Carpenter, 1957).

It is hoped to report later on the relationships between chemical and biological values for a larger series of materials. The finding reported here of a biological response for two materials higher than expected from their total lysine contents, as determined with all the controls that we could establish, does underline the need for caution in interpreting the results of biological assays for protein foods as sources of single amino acids. It is possible that added synthetic lysine is absorbed in a portion of the gut different from the site of absorption of lysine released from even an efficiently digested protein, and so is metabolized with different effect. Tsien *et al.* (1957) found that, when they supplied a high level of test protein as a supplement to a diet based

on free amino acids, the nitrogen content of the chicks' live-weight gains differed from that obtained with standard lysine additions.

If part of the response is due to a change in the balance of amino acids, and if the other amino acids in a particular protein will always have the same effect on its value as a supplement for lysine-deficient diets, then these factors should perhaps be valued as much as the lysine present as such.

## SUMMARY

1. Chicks fed from 10 to 20 days of age on a basal diet containing 50% sesame-seed meal, wheat, grass meal, fat, oat husks, leucine, oxytetracycline, vitamins, minerals and maize starch, showed a linear relationship of response by live-weight gain to dietary supplements of up to approximately 0·5% lysine.

2. The addition of 3·6% zein depressed the rate of weight gain of chicks receiving only a low level of supplementary lysine. In the selected assay procedure, test materials were added at the expense of zein, so as to keep the total protein content of the diets constant. Each material was added at two levels (1·6 and 3·2% protein), and the response was compared with that to pure lysine by the slope-ratio method of statistical analysis.

3. With eight groups, each of four chicks, per treatment, the mean estimated potencies (based on weight gain in 10 days related to percentage of lysine added) for the four test materials ranged from 4·8 to 9·9 g/16 g nitrogen with 95% fiducial limits of ±0·8 g/16 g nitrogen approximately for each estimate. The precision of estimates based on 6 days' growth was slightly less.

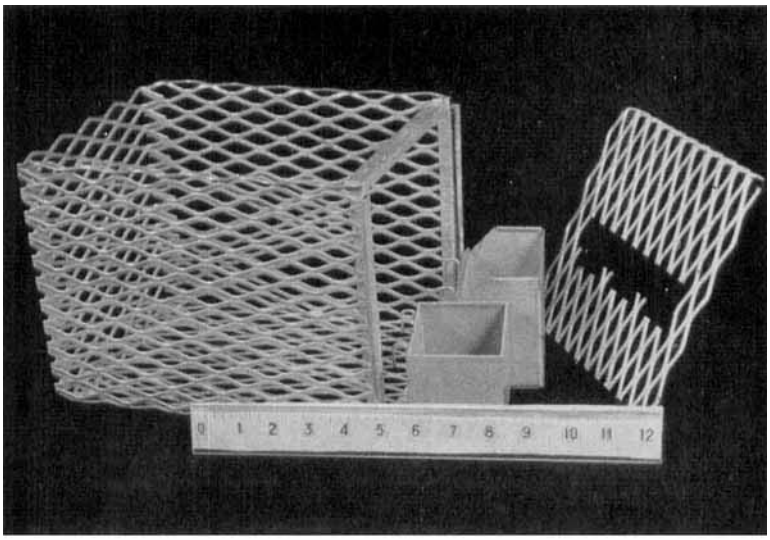
4. Estimates that took differences in food consumption into account were more precise and may also have been less affected by systematic errors due to substances other than lysine in the test materials.

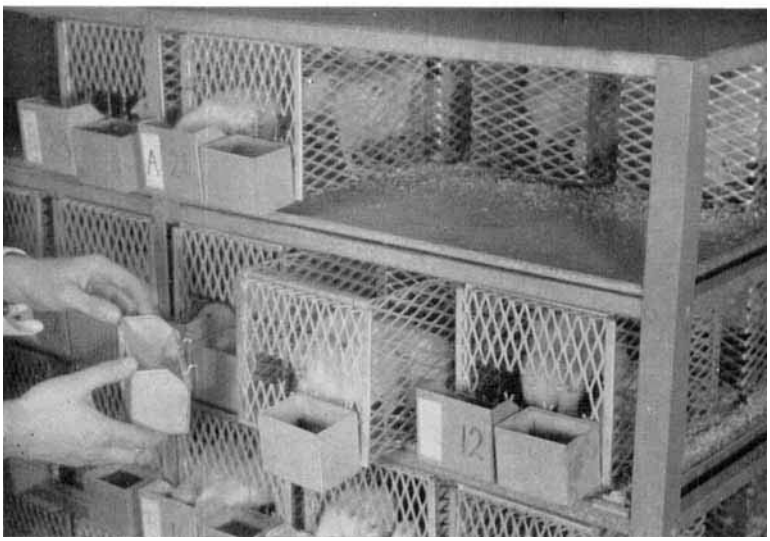
5. The assay results have been compared with chemical analyses for fluorodinitrobenzene-available lysine and for total lysine in acid hydrolysates of the test materials. For the two samples of highest quality the assay gave values slightly higher than those given by either chemical procedure.

One of us (B.E.M.) was in receipt of a Commonwealth Fellowship from the Royal Society and Nuffield Foundation during this work.

## REFERENCES

Calhoun, W. K., Hepburn, F. N. & Bradley, W. B. (1960). *J. Nutr.* **70**, 337.
Carpenter, K. J. (1957). *Proc. int. Congr. Nutr.* IV. *Paris*, p. 154.
Carpenter, K. J. (1960). *Biochem. J.* **77**, 604.
Carpenter, K. J., Ellinger, G. M., Munro, M. I. & Rolfe, E. J. (1957). *Brit. J. Nutr.* **11**, 162.
Carpenter, K. J. & March, B. E. (1961). *Brit. J. Nutr.* **15**, 403.
Chang, V. (1962). *J. Nutr.* **78**, 21.
Coates, M. E., Kon, S. K. & Shepheard, E. E. (1950). *Brit. J. Nutr.* **4**, 203.
Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (editors) (1959). *Data for Biochemical Research*. Oxford: Oxford University Press.
De Man, T. J. & Zwiep, N. (1955). *Voeding*, **16**, 147.
Finney, D. J. (1952). *Statistical Method in Biological Assay*. London: Griffin.



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Fisher, H., Griminger, P., Leveille, G. A. & Shapiro, R. (1960). *J. Nutr.* **71**, 213.
Fisher, H. & Shapiro, R. (1961). *J. Nutr.* **75**, 395.
Grau, C. R. (1948). *J. Nutr.* **36**, 99.
Gupta, J. D., Dakroury, A. M., Harper, A. E. & Elvehjem, C. A. (1958). *J. Nutr.* **64**, 259.
Jacobs, S. (1956). *Analyst*, **81**, 502.
Kratzer, F. H. & Green, N. (1957). *Poult. Sci.* **36**, 562.
Moore, S., Spackman, D. H. & Stein, W. H. (1958). *Analyt. Chem.* **30**, 1185.
Ousterhout, L. E., Grau, C. R. & Lundholm, B. D. (1959). *J. Nutr.* **69**, 65.
Tsien, W. S., Johnson, E. L. & Liener, I. E. (1957). *Arch. Biochem. Biophys.* **71**, 414.

## EXPLANATION OF PLATE

The upper picture shows an individual cage, made of cadmium-plated expanded metal, with the slotted front and food and water tins behind a 12 in. ruler. The lower picture shows the arrangement by which forty-eight cages are suspended in a battery frame.

*Printed in Great Britain*