Protein quality of feeding-stuffs

5.* Collaborative studies on the biological assay of available methionine using chicks

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1. The reproducibility of an assay for methionine based on live-weight gains in young chicks has been tested in a collaborative study in six laboratories.

2. Results were analysed by the slope-ratio procedure and, in general, the assays were statistically valid. The variability between laboratories was similar to that found in previous studies of variability within a single assay in one laboratory.

3. With the combined estimates from five or six laboratories the standard error of the estimates was approximately 10% of the mean. Expressing response as 'g gain/g food eaten' gave no more precision than using 'weight gain' alone, but is nevertheless thought to be less open to error due to appetite effects.

4. The experiments have shown that materials can be ranked consistently, but that the absolute estimates of potency varied between assays and further improvements are desirable if potency estimates are to be used for the calibration of in vitro procedures of protein evaluation.

The following workers also collaborated in the work described in the present paper:

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The general objectives of this series of collaborative studies have already been described (Boyne, Carpenter & Woodham, 1961). The purpose of the investigation now described was to study the reproducibility in six laboratories of a procedure for the biological assay of methionine, which has already been published (Miller, Carpenter,

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Morgan & Boyne, 1965), and also of a modification of it, with a view to their possible use in providing reference values for the assessment of a microbiological assay developed by other participants (Ford, 1962; Boyne, Price, Rosen & Stott, 1967).

EXPERIMENTAL

Test materials

Six test materials were used. Each was obtained by the Rowett Research Institute, for the Agricultural Research Council's collaborative programme, re-mixed and stored under refrigeration until it was distributed to individual laboratories in polyethylene bags and stored there at room temperature for a maximum of 2 months before the experiments were completed. The analytical results obtained for the samples are set out in Table 1.

Table 1. *Percentage composition of the test materials*

	Moisture	Crude protein	Ether extract	Crude fibre	Ash	Ca	P
Fish meal							
FM 101	9·6	68·9	3·3	—	20·0	5·75	3·27
FM 102	17·2	45·5	6·4	—	29·6	5·81	2·28
Meat meal MM 101	8·6	58·6	3·1	—	22·5	7·40	3·22
Groundnut meal							
GN 101	10·1	50·2	0·7	7·9	5·6	0·18	0·55
GN 102	11·4	48·3	1·3	3·9	4·6	0·10	0·62
Sunflower-seed meal							
SF 101	8·4	30·7	1·9	24·1	4·5	0·40	0·71

Of the two fish meals, FM 101 was a locally produced white fish meal; the other FM 102 was an unusual type of meal, imported from Pakistan, which had given low values in preliminary screening tests. The meat meal MM 101 was a blend of four different commercial samples manufactured in Britain. The decorticated, extracted groundnut meals GN 101 and GN 102 were commercial samples manufactured in the UK and chosen for their low aflatoxin content, $< 0{\cdot}5$ μg/g. The semi-decorticated extracted sunflower-seed meal SF 101 was an imported sample.

L-Methionine was used as the standard, commercial samples having been obtained either from Koch–Light Laboratories Ltd, Colnbrook, Bucks, or from Cambrian Chemicals Ltd, Croydon, Surrey.

Basal diets

The compositions of the two diets used are set out in Table 2. Diet 1 was similar to that used by Miller *et al.* (1965); diet 2 was a modification of this that had been used successfully in one laboratory (Harwood & Shrimpton, 1969), in which part of the groundnut meal was replaced by gelatin and a methionine-free mixture of amino acids. The same sample of dried whey and of groundnut meal was used in each laboratory; other materials were those available at the different laboratories.

Table 2. *Composition of the basal diets*

	Diet 1 (%)	Diet 2 (%)
Groundnut meal (GN 101)	40	26·5
Gelatin	—	6·16
Dried whey	5	5·0
Maize oil	5	5·0
Choline chloride	0·15	0·15
Inositol	0·10	0·10
Vitamin mix*	0·5	0·5
Salt mix†	3·13	3·13
$CaCO_3$	2	2
$CaHPO_4.2H_2O$	2·5	2·5
Glycine	0·2	—
L-Lysine hydrochloride	0·4	—
L-Cystine	0·2	—
Special amino acid supplement‡	—	1·566
Maize starch	To 100	To 100

* To provide per 100 g basal diet: vitamin A 880 i.u., cholecalciferol 220 i.u., vitamin E 5 mg, menaphthone 0·2 mg, biotin 20 μg, folic acid 300 μg, thiamin 300 μg, pyridoxol 1 mg, riboflavin 1 mg, nicotinic acid 5 mg, calcium pantothenate 3 mg, cyanocobalamin 2 μg, glucose to 0·5 g.

† To provide per 100 g basal diet: K_2HPO_4 1·61 g, $MgSO_4.7H_2O$ 0·51 g, NaCl 0·837 g, Fe citrate. $5H_2O$ 0·137 g, KI 0·004 g, $MnSO_4.4H_2O$ 0·025 g, $ZnCl_2$ 1·5 mg, $CuSO_4.5H_2O$ 1·5 mg.

‡ Containing: L-lysine hydrochloride 427·5 mg, L-cystine 273·1 mg, L-tryptophan 63·9 mg, L-valine 129·1 mg, L-leucine 372·9 mg, L-isoleucine 166·5 mg, L-threonine 133·2 mg.

Test diets

In Expt 1, basal diet 1 was used. Standard and test supplements were added to the basal diet at the levels given in Table 3. GN 101 was added at the expense of starch, the other materials, FM 101, FM 102 and MM 101, were added at the expense of $CaCO_3$, $CaHPO_4$ and starch so as to keep the levels of calcium and phosphorus approximately equal to those in the basal diet. The standard methionine was added at three levels and each test material at two levels, the higher of which was expected to give a response on the upper range of the standard.

In Expt 2 the two basal diets were compared. The standard and test supplements (GN 102 and SF 101) were added at the expense of starch to each basal diet at the levels given in Table 5. It is to be noted that in the test diets based on diet 2, in which groundnut (GN 102) was the test material, a further addition was made of the same amino acid mixture as used in the basal diet, in proportion to the level of groundnut meal present in the diet. The intention of this addition was to correct for the imbalance of groundnut in amino acids other than methionine. In Expt 2 the standard methionine and test supplements were each added at two levels to each of the two basal diets.

Chicks

The chicks were all males of rapidly growing 'broiler strains', except that for Expt 2 one laboratory (E) used surplus male chicks from a laying strain. The mean weights of those selected at 10 d of age in each laboratory are given in Tables 3 and 5.

For Expt 1 the chicks were reared from hatching to 10 d of age on the standard diet in use in each laboratory. For Expt 2 a single batch of one high-energy diet was

distributed for use in the different laboratories. In each instance, at least 50% more chicks were reared than would be subsequently used and those used were selected for uniformity of weight at 10 d of age.

Allocation to treatments

Three laboratories, A, B and C (group 1), were equipped with caging suitable for holding ten chicks each; the remaining three laboratories (group 2) used smaller cages, holding only three chicks each, but these were available in larger numbers. Therefore different experimental designs had to be adopted for the two groups.

For Expt 1, each laboratory in the first group allocated two cages of ten chicks to each of twelve experimental treatments, the chicks having been first divided according to weight, and then allotted at random to the cages. In group 2, Laboratories D and E used four cages of three chicks each, allocating the chicks in the same way. Laboratory F did not participate.

In Expt 2, involving fourteen treatments, there was the same degree of replication except that Laboratory D used only three cages (of three chicks each) per treatment.

Conduct of the experiments

The chicks were weighed on being put into the cages and offered the test diets *ad lib.* for the following 8 d.

They were then weighed again and the food consumption in each cage was measured. Any death that occurred was recorded and food consumption up to that point was measured, so that an estimate could be made of the proportion eaten by the animal that had died; this amount was deducted from the total food consumption in the cage over the whole period, so as to give an estimate of the food eaten by the survivors.

Statistical analysis

Weight gain and food conversion efficiency (FCE), i.e. 'g gain/g food eaten' were used as two alternative responses by which to compare the effect of test supplements with that of the standard methionine supplement. The apparent potencies of the supplements as sources of methionine were estimated for each laboratory by the slope-ratio technique described by Finney (1964).

The calculations for the slope-ratio assay assume that, when mean responses are plotted against levels of supplementation, the points lie approximately on a series of straight lines, one for the standard and one for each test supplement, and that when the lines are extrapolated down to zero level of supplementation they all intersect at a common response. If statistical tests depart significantly from 'linearity' or from 'intersection', the assay is said to be invalid. If the point of intersection is found to differ significantly from the measured response to zero supplementation ('blanks'), the assay is not considered to be invalid, but the statistical analysis must be repeated with the exclusion of the results from the blanks, for in such an event the response is evidently not linearly related to dose all the way down to zero level.

As a means of comparing the precision of assays between laboratories we have used

λ, the ratio of the standard deviation in response between replicate measurements to the change in response per increase of 0·01 g/100 g in the concentration of L-methionine in the diet. Lower values of λ indicate higher precision, but since λ includes an arbitrary dose unit, it cannot be compared directly with corresponding values in assays of other nutrients.

The estimates of potency were compared between test supplements and between laboratories by analysis of variance, using a logarithmic transformation. Estimates of variation between laboratories and of residual variation, from this analysis, were used in calculating 95% fiducial limits for the final combined estimates of potency, taken as unweighted means of the individual laboratory estimates. The arithmetical calculations of the slope-ratio assay procedure were carried out on the Rowett Institute's I.B.M. 1130 computer, instructed by a Fortran programme 'KEN' written for the purpose.

RESULTS

Expt 1

The mean weight gains and FCE of chicks receiving each treatment at the different laboratories are set out in Table 3. The chicks appeared to remain generally healthy on the test diets, and it is seen that, in every instance, the addition of standard methionine produced a graded response in the chicks receiving it. On the other hand, the actual response to a particular diet (e.g. the basal diet alone) differed considerably between laboratories. It is also seen from Table 3 that the initial weights of the chicks differed markedly from one laboratory to another.

Table 3. *Expt* 1. *Mean weight gain and food conversion efficiency* (FCE) of chicks in five laboratories, A–E, given a basal diet supplemented with either* L-*methionine, fish meal (FM), meat meal (MM) or groundnut meal (GN)*

	Laboratory†									
	A (111)		B (151)		C (115)		D (171)		E (115)	
Supplement	Gain (g)	FCE	Gain (g)	FCE	Gain (g)	FCE	Gain (g)	FCE	Gain (g)	FCE
None	33	0·31	65	0·34	50	0·36	79	0·36	54	0·42
0·02 % L-methionine	53	0·38	76	0·36	74	0·46	104	0·42	61	0·43
0·04 % L-methionine	68	0·46	87	0·41	76	0·48	112	0·44	94	0·54
0·06 % L-methionine	92	0·52	118	0·47	92	0·54	131	0·50	108	0·57
2·0 % FM 101	59	0·44	95	0·44	69	0·45	104	0·44	72	0·49
4·0 % FM 101	77	0·51	128	0·51	107	0·59	128	0·50	104	0·59
2·0 % FM 102	45	0·37	77	0·37	64	0·46	93	0·41	57	0·43
4·0 % FM 102	53	0·41	98	0·45	70	0·46	101	0·42	72	0·49
4·0 % MM 101	51	0·39	84	0·41	71	0·45	106	0·43	67	0·48
8·0 % MM 101	69	0·47	101	0·48	87	0·51	119	0·47	89	0·54
5·0 % GN 101	50	0·39	70	0·33	52	0·42	97	0·41	52	0·39
10·0 % GN 101	56	0·43	91	0·39	77	0·48	112	0·46	81	0·56
Standard error of a treatment mean	1·7	0·011	4·7	0·018	5·8	0·025	6·3	0·018	3·7	0.016

* g weight gain/g food eaten.
† Figures in parentheses are the initial weights (g) of the chicks in each laboratory.

The potencies of the test materials estimated (*a*) from weight gain and (*b*) from FCE are set out in Table 4, together with their fiducial limits and values of λ. The value of λ is useful in comparing precision between different assays of the same factor, as it is based on the two most important quantities affecting precision other than degree of replication and choice of dose levels. When the fiducial limits are symmetrical their breadth is directly proportional to λ, so it is desirable that λ should be as small as

Table 4. *Expt* 1. *Estimates from five laboratories* (*A–E*) *of the potencies* (*g methionine/16 g N*) *of fish meal* (*FM*), *meat meal* (*MM*) *and groundnut meal* (*GN*), *together with their* 95% *fiducial limits as derived from* (*a*) *weight gain or* (*b*) *food conversion efficiency* (*FCE*), *together with* λ *values calculated as indicators of the precision of the assay in each laboratory* (*see this page*)

Test material	Group 1 laboratories A	B	C	Group 2 laboratories D	E*	(Combined estimates)
	(*a*) Estimates from weight gain					
FM 101	1·7 (1·5–1·8)	2·8 (2·3–3·5)	2·6 (1·9–3·7)	2·0 (1·4–2·8)	1·7 (1·5–2·1)	2·2 (1·8–2·6)
FM 102	1·1 (0·84–1·3)	2·3 (1·6–3·0)	1·6 (0·55–2·6)	1·2 (0·25–2·2)	1·4 (1·0–1·7)	1·5 (1·2–1·8)
MM 101	0·76 (0·67–0·85)	1·0 (0·76–1·3)	1·1 (0·71–1·6)	1·0 (0·64–1·4)	0·81 (0·67–0·98)	0·95 (0·78–1·2)
GN 101	0·51 (0·42–0·59)	0·67 (0·42–0·91)	0·62 (0·23–1·0)	0·72 (0·37–1·1)	0·64† (0·50–0·81)	0·64 (0·53–0·79)
λ	0·26	0·73	1·12	1·58	0·58	
	(*b*) Estimates from FCE					
FM 101	2·1 (1·8–2·4)	3·1 (2·3–4·4)	2·5 (1·7–3·9)	2·4 (1·6–3·5)	2·2 (1·6–3·1)	2·5 (2·0–3·1)
FM 102	1·5 (1·1–1·8)	2·7 (1·7–4·0)	1·8 (0·66–3·1)	1·5 (0·34–2·5)	1·5 (0·89–2·3)	1·8 (1·4–2·2)
MM 101	0·93 (0·77–1·1)	1·4 (1·0–2·1)	1·0 (0·56–1·6)	1·1 (0·69–1·6)	1·0 (0·70–1·4)	1·1 (0·88–1·4)
GN 101	0·72 (0·58–0·87)	0·50 (0·10–0·88)	0·73 (0·29–1·2)	0·81 (0·42–1·3)	1·1† (0·79–1·6)	0·76 (0·61–0·96)
λ	0·44	1·10	1·29	1·81	1·07	

* The results for the highest level of the standard were excluded from the analysis for this laboratory, because of a significant deviation from linearity. In the weight gain analysis the blanks were also excluded on the basis of a significant test.

† These estimates are based on the response to the higher level of GN 101. The results from the lower level were inconsistent and their inclusion led to an invalid assay.

possible. The 95% limits for the combined estimates of potency in Table 4 are based on estimates of variability within and between laboratories obtained by combining evidence from assays 1 and 2.

The conventional statistical tests of validity for slope-ratio assays, i.e. for intersections, blanks and linearity, were carried out on the results from each laboratory and were generally satisfactory, with the following exceptions. Results from one replicate of the blank treatment at Laboratory A were excluded because they appeared out of line and their inclusion resulted in a significant 'blanks' test. Results from one

replicate of the highest standard at Laboratory B were also excluded, because they too seemed out of line and their inclusion resulted in a significant 'linearity' test and hence an invalid assay. At Laboratory E it appeared that the highest standard and the lower level of GN 101 both gave responses outside the linear range, and these treatments had to be excluded in order to obtain a valid assay. Indications of invalidity were similar, although not identical, for the two response criteria and the same conclusions were applied to each. The net effect of this exclusion of results was negligible, changing each of the combined potency estimates by less than 0·05; the fact that it was necessary is relevant to any assessment of the biological method.

Expt 2

The mean weight gains and FCE of chicks receiving each treatment at each laboratory are set out in Table 5. In one laboratory (C) the chicks failed to grow at the normal

Table 5. *Expt 2. Mean weight gain and food conversion efficiency* (FCE) of chicks in six laboratories, A–F, given basal diet 1 or basal diet 2 (see Table 2) alone or supplemented with* L-*methionine, groundnut meal (GN) or sunflower-seed meal (SF)*

	Laboratory†											
	A (155)		B (187)		C (97)		D (169)		E (86)		F (155)	
Diet ...	1	2	1	2	1	2	1	2	1	2	1	2
Supplement	(*a*) Weight gain per chick (g)											
None	56	36	69	57	73	47	74	123	50	25	98	78
0·03 % L-methionine	78	59	109	93	95	71	106	129	60	48	135	118
0·06 % L-methionine	114	81	132	114	133	99	131	134	71	67	168	140
5·0 % GN 102†	67	47	101	67	86	58	90	136	55	46	130	105
10·0 % GN 102†	88	59	110	84	99	67	98	144	67	60	150	116
3·5 % SF 101	77	48	101	77	84	69	80	133	57	51	116	101
7·0 % SF 101	97	62	117	90	94	76	102	143	65	58	150	119
Standard error of a treatment mean	±3·6		±8·2		±4·4		±5·6		±2·7		±3·3	
	(*b*) FCE											
None	0·36	0·26	0·30	0·26	0·43	0·36	0·38	0·48	0·40	0·28	0·27	0·21
0·03 % L-methionine	0·43	0·38	0·43	0·38	0·54	0·44	0·45	0·50	0·45	0·40	0·36	0·32
0·06 % L-methionine	0·54	0·45	0·47	0·45	0·58	0·52	0·51	0·51	0·49	0·48	0·44	0·37
5·0 % GN 102‡	0·39	0·33	0·42	0·37	0·48	0·40	0·42	0·54	0·43	0·40	0·36	0·29
10·0 % GN 102‡	0·45	0·39	0·44	0·40	0·54	0·46	0·45	0·58	0·49	0·45	0·41	0·31
3·5 % SF 101	0·45	0·33	0·41	0·36	0·48	0·46	0·39	0·50	0·44	0·41	0·31	0·28
7·0 % SF 101	0·43	0·39	0·44	0·39	0·49	0·47	0·46	0·52	0·49	0·46	0·41	0·34
Standard error of a treatment mean	±0·014		±0·018		±0·017		±0·018		±0·009		±0·013	

* g weight gain/g food eaten.

† Figures in parentheses are the initial weights (g) of the chicks in each laboratory.

‡ When 10 % GN 102 was added to diet 2, the test diet was further supplemented with L-lysine hydrochloride 0·155, L-cystine 0·099, L-tryptophan 0·023, L-valine 0·047, L-leucine 0·135, L-isoleucine 0·06 and L-threonine 0·048 %; with 5 % GN 102, one-half of these quantities of amino acids was added.

Table 6. *Expt 2. Estimates from six laboratories of the potencies (g methionine/16 g N) of groundnut meal (GN) and sunflower-seed meal (SF) derived from (a) weight gain and (b) food conversion efficiency (FCE) in assays with each of two basal diets, together with λ values calculated as indicators of the precision of each assay* (see p. 12)

Basal diet	Test material	Group 1 laboratories A	Group 1 laboratories B	Group 1 laboratories C	Group 2 laboratories D	Group 2 laboratories E	Group 2 laboratories F	Mean values (excluding Laboratory D)	Mean of single-laboratory '95 % fiducial limits', as % of estimates (excluding Laboratory D) Lower %	Upper %
					(*a*) Estimates from weight gain					
1	GN 102	0·69	0·80	0·60	0·54	0·94	0·95	0·80	57	146
	SF 101	2·1	2·1	1·1	1·2	2·0	1·9*	1·8	59	146
	(λ)	(0·71)	(1·47)	(0·65)	(0·95)	(0·92)	(0·64)	(0·88)		
2	GN 102	0·63	0·50	0·46	—†	1·0	0·63	0·64	59	139
	SF 101	1·6	1·6	1·7	—†	2·3	1·5*	1·7	70	131
	(λ)	(0·35)	(0·70)	(0·76)	(8·15)	(0·84)	(0·58)	(0·65)		
					(*b*) Estimates from FCE					
1	GN 102	0·57	1·0	0·77	0·63	1·1	1·0	0·89	60	144
	SF 101	1·4	2·2	1·1	1·4	2·5	2·1*	1·9	54	148
	(λ)	(0·93)	(1·11)	(0·73)	(1·27)	(0·83)	(1·05)	(0·93)		
2	GN 102	0·79	0·90	0·68	—†	0·95	0·71	0·81	64	137
	SF 101	1·8	1·9	2·2	—†	2·4‡	1·9*	2·0	71	134
	(λ)	(0·34)	(0·82)	(1·16)	(10·16)	(0·74)	(0·75)	(0·76)		

* Because of significant 'intersection', results from the lower level of SF 101 were disregarded.
† The responses to standard and test supplements were not significant and no valid estimate can be made.
‡ 'Blanks' were significant in these assays and were excluded from the potency estimation.

rate during the preliminary period on the standard diet which had been supplied to the laboratory, and, of the 280 put on experiment, six chicks died during the test period. In the other laboratories the chicks appeared to do well in the pre-experimental period and there were few deaths.

In most laboratories the growth rates were higher on basal diet 1 than on basal diet 2 but the responses in growth rate produced by adding methionine or one of the test supplements did not differ significantly between the two basal diets. The results from Laboratory D were anomalous in that growth on basal diet 2 without supplement was very good and there was little further response to supplementary methionine.

Separate assays were made for each laboratory and for each basal diet. As no supplement was added at more than two levels, there was no statistical test of linearity of response, except in so far as it is covered by the blanks test. The latter was statistically significant ($P < 0.025$) on two occasions with basal diet 2, at Laboratory E for the FCE assay and at Laboratory F for the weight-gain assay. The intersection test was not significant for any of the separate assays. The assays at Laboratory D involving diet 2 were of no value, as there was no statistically significant response to the addition of methionine.

The potencies estimated from the separate assays are set out in Table 6, together with λ values to indicate the relative precision of each assay. It may be noted that the anomalous assays with diet 2 at Laboratory D were accompanied by very large values of λ. To aid comparisons between basal diets and response criteria, a column of mean values over Laboratories A, B, C, E and F has been provided. Individual fiducial limits are not given, but each fiducial limit has been calculated as a percentage of the potency estimate to which it refers, and mean values are given for the upper and lower limits so expressed, again excluding Laboratory D.

As the results from the two basal diets showed, in general, no evidence of significant difference in response to added methionine, it appeared justifiable to carry out combined assays for each laboratory in order to obtain improved potency estimates for the test materials. These assays were again statistically valid except that the intersection test was significant at Laboratory F, both for gain and FCE. Investigation suggested that the results for the lower level of addition of SF 101 were responsible, and they were therefore excluded. The 'blanks' test was also significant for these two assays, and also for the FCE assays at Laboratories B and E.

Potency estimates for the six laboratories combined, with 95% fiducial limits, for GN 102 were 0·74 (0·63–0·87) from weight gain and 0·87 (0·75–1·0) from FCE. Corresponding estimates for SF 101 were 1·8 (1·6–2·2) and 1·9 (1·6–2·2).

DISCUSSION

It is obvious from the results of our experiment that there can be considerable variability between the estimates from different laboratories for the potency of particular samples even when the same procedure is used. For example, in Expt 1 the values calculated from weight-gain results from FM 101 ranged from 1·7 to 2·8 g/16 g N. It would clearly be dangerous, therefore, to compare values for different

materials when they have each been derived from single assays carried out in different laboratories. Whether or not one could expect better agreement between independent assays carried out in the same laboratory, it is not possible to say as a result of the present work. On the other hand, it was satisfactory that the ranking of the four samples was the same in all five laboratories, whichever response was measured.

Comparisons between different materials assayed at the same time would be free from these doubts. An assay with the same degree of replication and the same precision as the average in this series would be able to discriminate between diets differing in available methionine content by more than about 0·015–0·02 g per 100 g diet.

Table 7. *Comparison of the mean biological estimates from all the collaborating laboratories for the potencies of the fish meal (FM), meat meal (MM), groundnut meal (GN) and sunflower-seed meal (SF) as sources of available methionine (g/16 g N) with microbiological estimates for the same materials*

Test material	Trial no.	Chick assay (diet 1) (*a*) With wt gain as criterion	Chick assay (diet 1) (*b*) With FCE as criterion	Microbiological assay using *Streptococcus zymogenes**
FM 101	1	2·2	2·5	2·29
FM 102	1	1·5	1·8	1·70
MM 101	1	0·95	1·1	0·90
GN 101	1	0·64	0·76	0·97
GN 102	2	0·80	0·89	0·93
SF 101	2	1·8	1·9	1·79

* J. E. Ford, private communication.

The absolute estimates, even when they are based on the combined replications from five laboratories, still have the upper fiducial limit approximately 50% above the lower one. This corresponds to a standard error approximately ± 10% of the mean (though it is not quite symmetrical), which compares quite favourably with the precision of other biological assays as reported by Bliss & Cattell (1943) when appropriate allowance is made for the latter being parallel line assays; nevertheless, for results of this type to be used for assessing absolute estimates obtained by other procedures (such as microbiological assays) a higher precision would be desirable.

As regards the difference in allocation of the chicks to groups in the various laboratories, there was no clear advantage one way or the other between three laboratories (group 1) having two cages of ten chicks each per treatment and those (group 2) having four cages of three chicks each. The 95% fiducial limits, for the potencies estimated from the individual assays and given in Tables 4 and 6, were on average from 63% to 140% of the estimated potencies. The values for group 1 were 59–144 and for group 2 68–137, but the difference was due entirely to the results of assay 2 (excluding Laboratory D), and there is no statistically sound basis for attributing it to the caging arrangements rather than to other factors, such as the uniformity of the chicks, which may have varied from occasion to occasion. The best individual assay had 95% fiducial limits as narrow as 85–115% of the potency estimate, and a number of the poorer assays had limits of about 50–160%.

There was very little difference between the average width of limits based on weight gain as criterion and of those based on FCE. The potencies estimated from weight gain were consistently higher from some laboratories than from others, both in assay 1 ($P < 0.01$) and in assay 2 ($P < 0.05$), but the laboratories were not ranked in the same order in the two assays so this variation would appear to be 'between-assay' rather than specifically 'between-laboratory'. Between-assay variability was not statistically significant in the results for the FCE assays.

The values derived from FCE tended to be higher on average by 13%, which is in accordance with the experience of Miller *et al.* (1965). The existence of these small but consistent differences between the results for the two calculations of response suggests that food consumption or growth, or both, of chicks was influenced by characteristics of the test materials other than their available methionine content. For example, it might be suggested that extra indigestible protein in the food alters its rate of passage in the gut. In our experiments the test materials were added simply at the expense of starch. Other workers using amino acid assays of this general type have been at pains to keep all their diets isonitrogenous, or even to attempt a constant balance in individual amino acids (Uwaegbute & Lewis, 1966). However, these precautions did not eliminate the tendency for values based on FCE to be slightly higher.

Our tentative conclusion is that it remains worth while to measure food intake in these experiments and to place most reliance on FCE results, since any appetite-depressing or appetite-stimulating effect of a particular test material might be expected to have a smaller effect on apparent potency determined in this way. However, errors in measurements of food consumption due to incomplete collection of spilled feed will reduce the precision of the estimates.

The possibility that the differences in composition of the basal diets being used routinely for methionine assays in two of the participating laboratories might affect the estimates of potency of test materials was investigated in Expt 2. No consistent difference either in estimate of potency or in precision of estimate was observed. The addition of an amino acid mixture to compensate for a possible amino acid imbalance of groundnut meal, when this was added as a test supplement, had no apparent advantage.

Finally, Table 7 compares our combined estimates for the potency of the test materials with the combined estimates obtained so far for the potency of the same materials in microbiological assays with *Streptococcus zymogenes*. The reproducibility of the latter values, as studied in collaborative experiments, will be discussed in a later paper.

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