Protein quality of feeding-stuffs

6.* Comparisons of the results of collaborative biological assays for amino acids with those of other methods

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1. Twelve commercial samples of high-protein feeding-stuffs, six of them fish meals, were used for a study of the reproducibility of chemical, microbiological and biological assay values for individual amino acids. Comparisons were also made of results obtained by different methods for the same amino acid.

2. For lysine there was quite good agreement between the results from the different laboratory tests for 'available' lysine. The values for 'total' lysine were, on average, 10% higher than those for 'available' lysine.

3. For methionine the correlations between the chick assay and each of the laboratory tests were similar.

4. The results from the 'total protein efficiency' test of over-all protein quality, using chicks, showed a close correlation with the results of lysine or methionine assays according to which of the two amino acids was most deficient.

5. The results with tryptophan were difficult to interpret and no recommendations could be made as to procedures suitable for general use.

6. Although most of the present samples proved to be of good quality, only screening of a much larger number of samples will reveal to what extent commercial protein concentrates of low availability are still liable to come onto the market.

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An earlier report of an Agricultural Research Council collaborative group (Boyne, Carpenter & Woodham, 1961) dealt with the use of laboratory procedures for predicting the over-all quality of high-protein concentrates as supplements in non-ruminant diets. It is clear that the quality is influenced by various factors but primarily by amino acid composition. With the development of methods of amino acid analysis and assay, it has become usual to balance non-ruminant diets for their content of each of the essential amino acids that are likely to be limiting in practice. The purpose of the present paper is to summarize the results from a further collaborative study of some of the procedures in use for the analysis and assay of these amino acids.

As with any attempts at the assessment of some aspect of nutritional value, one is always concerned with two problems: (i) the reproducibility of the results when the procedure is applied to the same sample both at different times in the same laboratory and at different laboratories; (ii) the relevance of the experimental results obtained to the actual values of the samples in practice.

Under the second heading for example, an analytical result may give a 'false' impression of the nutritional value of a sample either because the procedure is non-specific or the necessary preliminary manipulations cause partial loss of the nutrient, but even if it is correct in analytical terms, some of the amino acid in the particular food may be indigestible or unavailable to the species for which it is intended. This collaboration has been designed therefore to include comparisons of the results from chemical analyses with those from biological assays with animals (mostly chicks). In addition, studies have been carried out with laboratory procedures (chemical or microbiological) that are simpler than biological assays, but have shown promise of predicting the latter more closely than do ordinary chemical analyses.

The procedures used in this study are all discussed in more detail elsewhere. In the present paper they will be described only in sufficient detail to avoid confusion about procedures.

The bulk of analytical results obtained would only be confusing if reported here in full. Copies are, however, available for those who wish to have more detailed informa-

Table 1. *Test materials used for amino acid assays, their country of origin and crude protein content (g/kg)*

Material			Country of origin	Crude protein (nitrogen × 6·25) assuming 900 g dry matter/kg
Fish meal	FM 101	White fish meal	UK	708
	FM 108	Herring meal	Iceland	712
	FM 113	Anchovy meal (with added anti-oxidant)	Peru	676
	FM 122	Pilchard meal (with added ethoxyquin)	South Africa	665
	FM 123	Pilchard meal	South Africa	642
	FM 102	'Fish' meal (not a commercial sample)	Pakistan	509
Meat meal	MM 101		UK	596
Decorticated groundnut meal	GN 101		Solvent-extracted in UK	503
Soya bean	SB 101		China; extracted and tested in UK	474
Sunflower-seed meal	SF 102	Extracted sunflower-seed meal	Argentine	302
Yeast	HY 101	Grown on *n*-paraffins	UK	650
	HY 104	Grown on gas oil	UK	659

tion. The present paper is largely restricted to over-all mean values together with estimates of their precision based on inter-laboratory variability.

Originally a more comprehensive programme of work was planned than has, in fact, proved possible. However, it is hoped that the results which have been obtained will prove useful and, at the same time, their limitations will be understood.

EXPERIMENTAL

Test materials

A short description of the samples used in the work described in this paper is set out in Table 1. These were more-or-less random purchases in 1968 and they may or may not represent average-quality samples of the particular types of protein concentrate. All were of commercial origin except for the yeasts grown on hydrocarbons, which were obtained from large pilot plants, and for fish meal (FM) 102, which was made available to us by a research institute as an atypical sample (on the basis of its low protein and high ash content) and which would probably not have been used in commercial animal feeding. The groundnut meal (GN) 101 had a low aflatoxin content (0·35 mg/kg).

Some results have already been reported for five samples in the present series (Carpenter, McDonald & Miller, 1972). The crude protein values given in that paper differ slightly from those given now in Table 1, partly because of differences in moisture content and partly because only the results from the single laboratory that analysed all of the samples have been used in Table 1.

Analytical procedures for amino acids

Total lysine, methionine and cystine. Lysine was estimated after hydrolysis with 6 M-HCl, and for methionine and cystine the samples were first oxidized with performic acid (Moore, 1963). The hydrolysates were analysed essentially by the method of Spackman, Stein & Moore (1958).

Total tryptophan. The methods used were based on that of Spies & Chambers (1949). In two laboratories the modification of Miller (1967) was followed and, in another, that of Matheson (1974).

Fluorodinitrobenzene (FDNB) – reactive lysine. The determinations were carried out both by a 'direct' method (Carpenter, 1960) and by a 'difference' method (Roach, Sanderson & Williams, 1967).

Streptococcus zymogenes *assays.* Assays with this organism were first described by Ford (1962). Total methionine was assayed after a stronger acid-hydrolysis procedure (Henry & Ford, 1965). Available methionine and tryptophan were assayed after partial pre-digestion with papain using modified procedures (J. E. Ford & D. H. Shrimpton, unpublished results).

Tetrahymena pyriformis W. *assays.* Assays for available lysine were carried out using a modification (Shorrock & Ford, 1973) of the procedure described by Boyne, Price, Rosen & Stott (1967).

Biological assays for amino acids

The chick growth assay for lysine followed the procedure of Carpenter, March, Milner & Campbell (1963) except that the sesame meal used in the basal diet has been replaced with groundnut meal heat-damaged by autoclaving at 121° for 4 h to reduce its level of available lysine (Varnish & Carpenter, unpublished results). The rat growth procedure was similar, the basal diet was that described by Bjarnason & Carpenter (1969). The chick growth assays for methionine follow a procedure previously reported by the ARC Protein Evaluation Group (Carpenter *et al.* 1972) with calculations based on food conversion efficiency. For tryptophan the chick growth assay of Harwood & Shrimpton (1969) was used.

Procedures for over-all protein quality

Net protein utilization (NPU). The determinations were done using rats given diets containing 100 g crude protein/kg, and nitrogen retention was estimated either from analysis of the carcasses (Miller, 1963) or of urine and faeces (Henry & Toothill, 1962).

Gross protein value (GPV). The supplementary value of concentrates for chicks was estimated using a procedure reported by Duckworth, Woodham & McDonald (1961) except that Ross 1 broiler chicks were used. In this test the nutritive value is estimated from the growth produced by the addition to an 80 g protein/kg diet based on cereals of test protein equivalent to 30 g/kg diet.

Total protein efficiency (TPE). This is another growth test using Ross 1 broiler chicks for the evaluation of near-practical diets containing 180 g protein/kg, in which

Table 2. *Amino acid values (g/kg crude protein (nitrogen × 6·25)) obtained for the test materials, using different procedures, and estimates of their 'over-all' protein quality*

	Lysine						Methionine				Cystine	Tryptophan			'Over-all protein'		
	Total	'FDNB-reactive'		'Available'			Total		'Available'		Total	'Available'			NPU	GPV	TPE
Procedure*... Material†	Chemical	Direct method	Difference method	Tetra-hymena	Rat assay	Chick assay	Chemical	Micro-biological	Micro-biological	Chick assay	Chemical	Chemical	Micro-biological	Chick assay	Rats	Chicks	Chicks
FM 101	62 (3)	63	58	54	71	60	23 (2)	26	23	25	8·5 (2)	11	5·2	6·8	0·56	0·73	0·028
FM 108	66 (3)	64	61	58	—	—	26 (2)	28	24	20	8·2 (2)	14	5·7	9·9	0·58	0·72	0·030
FM 113	80	67	69	64	—	73	30	29	26	22	12	13	7·7	11	0·72	0·87	0·031
FM 122	74 (3)	72	70	60	—	75	26 (2)	28	22	21	11 (2)	13	7·8	11	0·66	0·84	0·031
FM 123	78 (3)	71	74	65	—	80	26 (2)	31	23	22	10 (2)	14	9·0	12	0·71	0·84	0·031
FM 102	53 (3)	46	52	39	48	47	18 (2)	23	12	18	8·0 (2)	9·1	3·4	4·6	0·36	0·58	0·025
MM 101	48 (3)	40	39	36	41	35	12 (2)	14	7·4	11	14 (2)	9·9	2·0	4·5	0·30	0·35	0·023
GN 101	34	26	31	—	39	25	9·9	10	7·8	7·6	12	13	8·2	8·0	0·48	0·24	0·022
SB 101	59	56	58	—	—	54	14	17	13	—	16	15	10·8	14	0·58	0·76	0·024
SF 102	28	21	27	—	—	24	18	17	16	19	16	14	10·5	—	0·51	0·49	0·020
HY 101	71	56	62	60	—	65	17	16	14	9·9	11	13	—	12	0·57	0·78	0·023
HY 104	75	61	71	71	—	73	14	15	13	9·9	10	13	—	12	0·52	0·76	0·023
Approximate SE‡	1·8 (3) 1·6	2·6	—	—	3·0	3·4	1·3 (2) 1·0	1·0	1·0	0·9	1·0 (2) 0·8	0·8	—	0·5	—	—	—
No. of laboratories (except where otherwise stated (in parentheses))	4	2	1	1	5	4	3	2	6	5	3	3	1	3	1	1	1

FDNB, fluoridinitrobenzene; NPU, net protein utilization; GPV, gross protein value; TPE, total protein efficiency.

* For details see p. 650. † For details see Table 1.

‡ The standard errors are approximate and are based on laboratory *v.* material interactions, indicating within-laboratory variability only.

§ Determined by carcass analysis.

Table 3. *Correlation coefficients for variation between chick assays and other methods for estimation of individual amino acid content or protein quality*

(No. of degrees of freedom in parentheses)

Variable*	Chick assay: Lysine	Chick assay: Methionine	Chick assay: Tryptophan
Lysine			
Total, chemical	0·98 (9)	—	—
'FDNB-reactive'			
Direct	0·97 (9)	—	—
Indirect	0·99 (9)	—	—
Available, microbiological	0·94 (6)	—	—
Methionine			
Total			
Chemical	—	0·86 (9)	—
Microbiological	—	0·89 (9)	—
Available, microbiological	—	0·86 (9)	—
Tryptophan			
Total, chemical	—	—	0·91 (9)
Available, microbiological	—	—	0·92 (7)
Growth tests			
NPU	0·70 (9)	0·53 (9)	0·76 (9)
GPV	0·92 (9)	0·56 (9)	0·67 (9)
TPE	0·74 (9)	0·73 (9)	0·19 (9)

FDNB, fluorodinitrobenzene: NPU, net protein utilization, GPV, gross protein value; TPE, total protein efficiency.

* For details of procedures for estimating lysine, methionine, tryptophan, NPU, GPV and TPE, see p. 650.

the test material was the only variable (Woodham, 1968). In this method the test material provides two-thirds of the total protein and a basal cereal diet, one-third.

RESULTS

Evaluation of materials as sources of lysine. Amino acid values for the test materials and estimates of their over-all protein quality are summarized in Table 2. It was considered that there were too few laboratories carrying out any one test to provide a meaningful estimate of between-laboratory variability. Consequently the standard errors in Table 2 are based on sample *v.* laboratory interactions. The correlations between the results of chick assays and those of other tests are set out in Table 3.

Eleven of the twelve samples were assayed with chicks in four or five laboratories (the sample omitted being FM 108) and four samples, FM 101, FM 102, meat meal (MM) 101, and GN 101, were assayed in five laboratories with rats. Each laboratory performed one assay on each sample. No special problems were encountered with these assays and the coefficients of variation were as close as can usually be obtained with growth procedures and small in relation to the range of mean values for the different materials. There was a tendency for the rat values to be higher.

Fig. 1 is in the form of a scatter diagram showing the relationship between the results from laboratory tests and those from the chick growth assays. The four laboratory tests each show a similarly high degree of correlation with the results from the

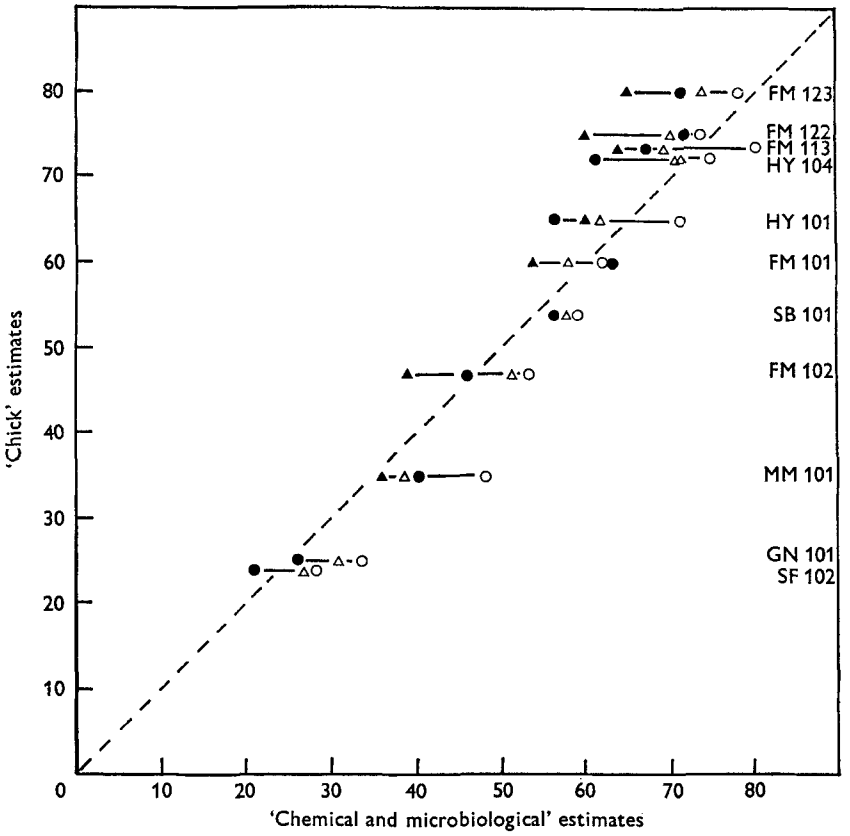

Fig. 1. 'Available' lysine (g/kg crude protein (nitrogen × 6·25)) in protein samples (see Table 1 for details) determined biologically with chicks compared with chemical (total, ○; fluorodinitrobenzene, direct, ●; indirect, △) and microbiological (Tetrahymena, ▲) estimates. The 45° broken line has been included to give a visual indication of the correlations. For details of estimation procedures see p. 650.

chicks (Table 3). Over-all, the absolute values obtained in the laboratory tests are also similar to those obtained with chicks, though there is a tendency for the samples of lower potency to give relatively higher values by chemical tests than with chicks. On average the 'FDNB' values are approximately 10% below the total lysine values; the 'Tetrahymena' values are a further 10% lower for the fish meals.

Evaluations for methionine. The values for total methionine are generally similar whether determined chemically or microbiologically, though in general the latter were somewhat higher (Fig. 2).

Evaluations for tryptophan. The chick assay procedure for this amino acid was applied successfully to the full series of samples, i.e. the results were judged valid by statistical analysis and they gave reasonable standard errors for the estimates. For all but one of the samples assayed microbiologically for available tryptophan, the values were considerably lower than for chicks, though the ranking was similar.

The chemical values for total tryptophan were in every instance higher than the estimates from the chick assays and some were considerably so. However, for the

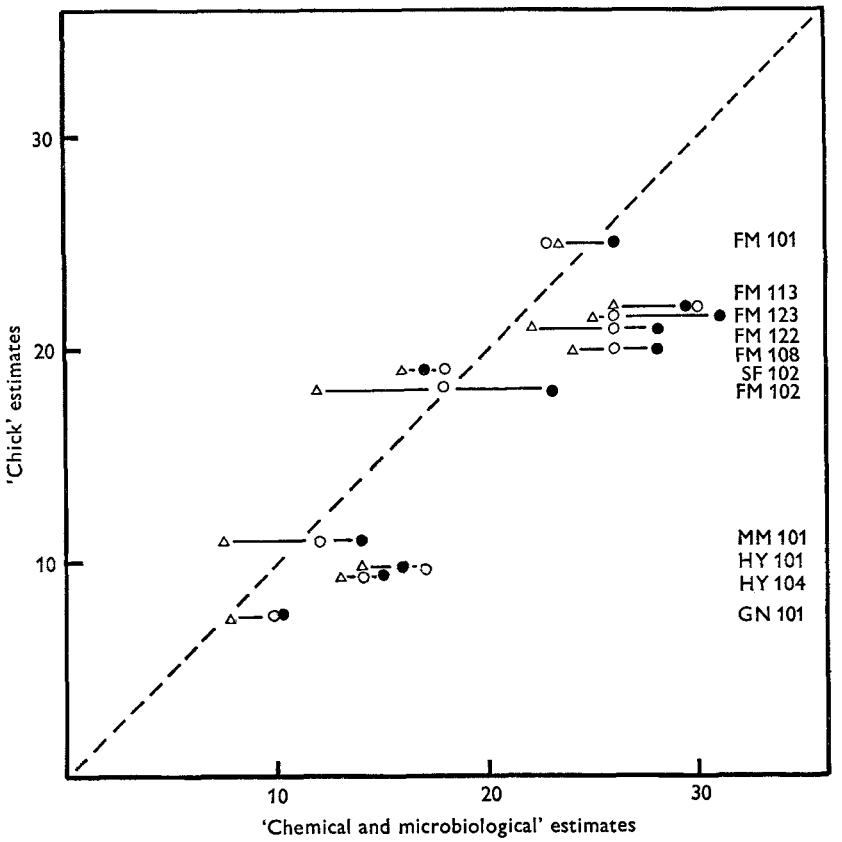

Fig. 2. Available methionine (g/kg crude protein (nitrogen × 6·25)) for protein samples (see Table 1 for details) determined biologically with chicks compared with total methionine estimated chemically (○) and microbiologically (total, ●; 'available', △). The 45° broken line has been included to give a visual indication of the correlations. For details of estimation procedures see p. 650.

Table 4. *Net protein utilization values for four protein samples assayed in six laboratories with young rats using either the carcass analysis or a nitrogen-balance procedure*

(No. of degrees of freedom in parentheses)

Procedure	Laboratory	Level of test protein (g/kg diet)	Test materials* FM 101	FM 102	MM 101	GN 101	Over-all mean	SE of means†
Carcass analysis	A	100	0·56	0·36	0·30	0·48	—	—
	D	100	0·64	0·28	0·31	0·46	—	—
	H	100	0·59	0·35	0·30	0·55	—	—
	J	100	0·65	0·39	0·41	0·25	—	—
	Mean		0·61	0·34	0·33	0·43	0·43	0·044 (9)
N-balance	C	50	0·74	0·42	0·51	0·58	—	—
	K	80	0·70	0·58	0·43	0·56	—	—
	Mean		0·72	0·50	0·47	0·57	0·57	—

* For details see Table 1.

† Based on laboratory *v.* material interactions, indicating within-laboratory variability only.

soya-bean meal and the two yeasts they were similar. The spread of total values is very much smaller (9·9–15 g/kg crude protein (N × 6·25)) than that from the chick assays (4·5–14).

Over-all quality tests. The same three fish meal samples FM 113, 122 and 123 gave the highest values in each of this group of tests but the ranking differed for the remaining samples, with yeasts (HY) 101 and 104 giving relatively higher values in the GPV test than in the NPU and TPE series. The GPV test showed the highest correlation with lysine values and the TPE test with methionine values.

Table 2 lists the NPU results from the only laboratory that assayed the full series of samples, using the carcass analysis procedure. Four of the samples (FM 101, FM 102, MM 101 and GN 101) were also assayed in five further laboratories with the results shown in Table 4. The over-all mean value from the balance experiments was fourteen units higher than that from the carcass analysis determinations. Such differences have been reported before (Henry & Toothill, 1962; Miller & Carpenter, 1964). It does not appear to be justified to mix results obtained by two different methods, and a standard error has been quoted only for the carcass analysis results.

DISCUSSION

Lysine. Although it is known that lysine can be particularly sensitive to damage under some conditions of processing, the present series of samples show general agreement between estimates for 'total' and 'available' lysine, whatever method is used. The greatest proportional differences were seen with the proteins of lowest lysine content (i.e. GN 101 with 34 g/kg crude protein (N × 6·25) and sunflower-seed meal (SF) 101 with 28). It is possible that these differences reflect a short-coming of the chick assay, and that with such protein sources there is a significant growth-depressant effect from the imbalanced amino acid pattern, which results in artificially low assay values being obtained. Further work will be required to determine whether or not this is the situation.

It is unfortunate that, of the two low-lysine protein sources one, SF 101, was not also assayed with rats. The other example, GN 101, gave a 'rat' value of 39 g/kg crude protein (N × 6·25), which was above even the total chemical value of 34 g/kg crude protein (N × 6·25).

Methionine. Although there are highly significant correlations between the chick values and each of the laboratory values for methionine, there are still some individual discrepancies. For example, with two materials, FM 102 and MM 101, the '*Strep. zymogenes* – available' values are considerably lower than the corresponding chick values. Although the difference was about 30% in each instance it can still be the result of random variation in the determinations. Taking the present results for methionine as a whole the correlation between 'total chemical' values and 'chick-available' values is approximately equal to that between '*Strep. zymogenes*–available' and chick values. For four of the samples 'total chemical' and 'chick-available' values are quantitatively undistinguishable; only in the instances of four fish meals, the two yeasts and the groundnut meal were 'total' values considerably higher than 'available' values.

Tryptophan. There is clearly need for more work in the instance of this amino acid. For four of the samples (FM 101, 102, MM 101 and GN 101) the chick growth assay value is only 50–70% of the chemical values for total tryptophan in the same samples. The corresponding results with methionine and with lysine are all over 70%. Are we to accept this as evidence that the percentage availability of tryptophan in protein concentrates is commonly lower than that of other amino acids?

Atkinson & Carpenter (1970), who first assayed MM 101 (then coded X 804), obtained values of 64 and 66 g/kg crude protein (N × 6·25) by chick growth assay and chemical analysis respectively; this corresponds to almost complete availability in contrast to approximate availability of 0·50 found in the present study. On the other hand, Pongpaew & Guggenheim (1968) also obtained some very low availability ratios for tryptophan from a growth assay with rats, including one of 0·35 (44 g/kg crude protein (N × 6·25)) for a sample of fish meal. This must surely be an abnormal value since fish meals in the present study and in previous studies (Miller, 1970) have usually given NPU values for rats of 0·60–0·75, or more with supplementary methionine. If it is accepted that the rat's requirement for tryptophan in its dietary protein is approximately 10 g/kg crude protein (N × 6·25) (Bender, 1965) then a NPU value of 0·75 would presumably correspond to at least 7·5 g/kg crude protein (N × 6·25) of available tryptophan.

The microbiologically determined values that we have for 'available' tryptophan of animal protein in a trial in the present series are only 20–75% of the corresponding total values. It seems agreed that although the '*Strep. zymogenes*' procedure gives values for available methionine that are in generally good agreement with those from chick assays, with tryptophan it does not (Atkinson & Carpenter, 1970; J. E. Ford, unpublished results). This difference would be reduced if a new correction to the method of adjusting results for 'enzyme blanks' (J. E. Ford & D. H. Shrimpton, unpublished results) had been applied, as it would with other very low reported values for meat meals and other protein concentrates (Ford, 1962; Waterworth, 1964). Pongpaew & Guggenheim (1968) found that even though most samples gave very low values, i.e. availability of 0·40 or less, when assayed by Ford's (1962) procedure starting with pre-digestion in papain, very much higher values, 0·90 or more, were obtained when 'pepsin + trypsin' were used.

With tryptophan we can only advise caution in drawing conclusions from any assay or analytical results at present. There are apparent contradictions both within our own results and between the published results of other workers.

'*Over-all*' *protein tests.* The estimates of over-all protein quality have served as a link with the earlier collaborative work with a different set of samples (Boyne *et al.* 1961).

Standard errors have not been quoted in Table 2 for these tests. In general it has been shown that the SE for a difference between the means of four replicates within a TPE trial is 0·046 (Woodham, 1968), and for a GPV the standard deviation of an individual result has been calculated to be 8 (Duckworth *et al.* 1961).

The estimate of TPE in which protein concentrates are tested at a higher ratio to cereals than in the GPV test would be expected to be intermediate between that of

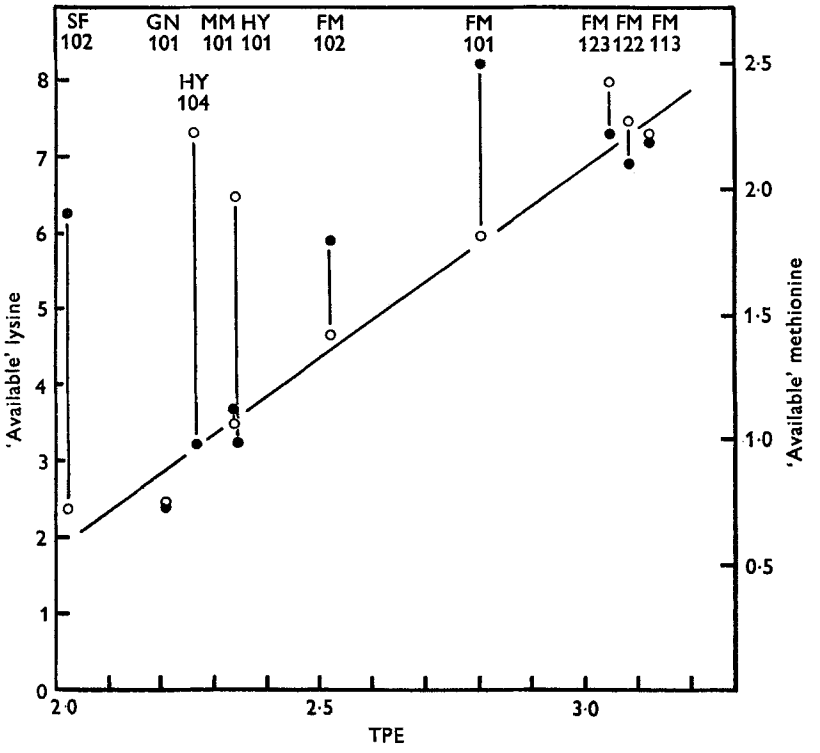

Fig. 3. Total protein efficiency (TPE) compared with 'available' lysine (○) and 'available' methionine (●) (g/kg crude protein (nitrogen × 6·25)) for protein samples (see Table 1 for details) both estimated with chicks. The line has been fitted by eye to indicate the correlation between TPE and the lower of the lysine or methionine values in each instance.

the GPV test in which lysine is usually limiting, and of the 'sole protein' tests (such as NPU) in which the sulphur-containing amino acids are usually limiting. In Fig. 3 the TPE values are plotted against both 'chick-available' lysine and methionine values, on scales roughly proportional to the chick's requirements for these amino acids. It is seen that there is a close correlation between TPE and the lower of the two amino acid values for each material when plotted in this way. This is an encouragement to believe that the values obtained under the necessarily abnormal artificial conditions of assay for a single amino acid have relevance for the construction of practical diets, for which the conditions of the TPE test are a much closer model.

Choice of laboratory evaluation methods. One basic difficulty in drawing conclusions from the present results is the element of error in the biological assays, i.e. the chick 'weight-gain' assays, which have to serve as standards by which to judge the other procedures of analysis or microbiological assay. Further, for both lysine and methionine, there is a close correlation between the 'available' and 'total' laboratory values, with the mean value for the former being only approximately 10% below that of the latter in each instance. As would be expected under such circumstances, we did not find that one type of test was a significantly better predictor of nutritional value than another.

The availability problem in practice. As explained above, the experiment was not designed to study the range of quality of different types of protein concentrates in the UK, but rather the applicability of different evaluation procedures to them. However, it is useful to consider what the present results add to the over-all body of information, and whether the generally high availability of lysine and methionine indicated by the results is in line with what could have been expected.

Since the first publication of the ARC Protein Quality Group (Boyne *et al.* 1961), there has been considerable work in this field. Combs, Bossard & Childs (1968) reported the results of a large number of growth assays with chicks for the available lysine and methionine values of commercial samples of protein concentrates used in the USA. They found values which corresponded to 90% or more of total chemical values for fish meals and soya-bean meals and to 80% or more for meat meals. We knew that chick assays of the kind used may give 'over-estimates' to some extent but these are much higher values than those reported from British work in which '*Strep. zymogens*-available' methionine values were compared with 'total' values obtained for acid digests assayed with the same organism (e.g. Ford, 1962; Boyne *et al.* 1967). In these studies the mean availability ratios were approximately 0·56 for fish meals and 0·36 for meat meals. The 'available' values by this procedure do seem to agree reasonably well with those from chick assays, the results in the present paper confirming the earlier findings of Miller, Carpenter, Morgan & Boyne (1965). However, as argued in detail elsewhere (Atkinson & Carpenter, 1970) it does appear that the very mild acid digestion used in the original procedure of Ford (1962) resulted in digests with growth-stimulating activity that seriously exaggerated their total methionine value. With stronger conditions of acid digestion (Henry & Ford, 1965) the values obtained are lower and close, in most instances, to chemical values. The effect of this correction is, of course, to raise values for 'availability ratios' considerably.

For lysine also, there was originally a misunderstanding of the degree of its unavailability in meat meals in particular. This arose from many of the reported values for the total lysine content of this material having come from microbiological assays which gave higher values than were obtained in later work by chemical analysis. The results are reviewed elsewhere (Carpenter, 1971). The discrepancy is probably explained by a synergistic action in the assays of the hydroxylysine present in these test samples. Typically, microbiological values have been in the range 70–90 g lysine/kg crude protein (N × 6·25), whereas chemical values for meat meals have usually been in the range 45–55.

It does appear therefore that the generally high 'availability ratios' found for the samples used in the present study are probably typical of materials entering into commercial use. Further, for samples of average quality, a 'total' amino acid value with a correction factor appropriate to the type of material as suggested by Combs *et al.* (1968) gives a reasonable estimate of its available amino acid content. However, for such material (if one can assume that it is of near-average quality) there is really no need for analysis at all and reference to tables of average composition would be sufficient.

The further question arises, however, as to whether or not an atypical, inferior

sample would be singled out by the system of quality control that was in force. If the inferiority were due to a poorer balance of ingredients in the raw material (e.g. addition of feathers to poultry offal), this would be shown up by analysis for total amino acids. However, if it were due to heat-damage during processing or storage or both, total amino acid values might fail to reveal the damage, and would certainly not reflect its extent. This would be so both for lysine and for methionine (cf. review by Carpenter, 1973). Although most of the present samples proved to be of good quality, only the screening of a much larger range would reveal the extent to which protein concentrates of low availability are liable to come onto the market. Thus, apart from the general impracticability of amino acid analysis by column chromatography for quality control, it would be wrong to conclude from the limited results of the present experiments that results of such 'total' analysis would be as useful as tests of 'available' amino acids in detecting inferior samples.

Of the methods used as indicators of available amino acids, the ones employed in the present study were chosen because they were in use in a number of laboratories and there was some background evidence of their worth. The microbiological procedure for 'available' tryptophan determination cannot be recommended in its present form. With the methods used for 'available' lysine and methionine none of the results appears to have been grossly in error. But for the procedures of determining 'FDNB-reactive' lysine, whilst it has been demonstrated repeatedly that controlled heat damage results in significant falls, there have been a number of other reports that commercial fish-meal samples may show variations in 'FDNB' values which do not correspond to differences in nutritional value (cf. review by Carpenter, 1973). This may be due to a variable degree of autolysis of the raw material with production of free (or N-terminal) lysine that is nutritionally available but not determined chemically.

So far the present '*Tetrahymena*' procedure for available lysine has only been used in one laboratory. It seems promising but, in the past, some laboratories have found difficulties in establishing assays with this organism. *Strep. zymogenes* has been more widely used and fewer problems have been encountered. There is however a general reluctance among quality-control laboratories to introduce a microbiological assay procedure where the rest of the work is confined to chemical procedures for which the staff have been trained.

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