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The nutritive quality and available amino acid composition of some animal protein concentrates

By D. G. WATERWORTH

J. Bibby and Sons Ltd, Nutrition Research Department, 'Weatherstones', Neston, Wirral, Cheshire

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The nutritive quality of protein foods is conventionally assessed in feeding trials with higher animals. These tests are cumbersome and expensive, and quite unsuited to the practical needs of the feeding-stuffs compounder who requires a quick and simple grading test. The crude protein content (total nitrogen × 6·25) gives little guide to nutritive quality and there is not necessarily any correlation between protein quality and crude protein content.

In recent years various laboratory techniques for assessing protein quality have been recommended. A chemical test for available lysine (Carpenter & Ellinger, 1955*a*, *b*) has aroused much interest, especially since practical diets are largely comprised of cereals which, as a class, are deficient in lysine, making it important that the available lysine content of supplementary protein concentrates be known. The available lysine value of a food protein is a measure of the content of lysine that contains free ε-amino groups, as distinct from lysine that is bound in this grouping. Bound lysine is considered nutritionally unavailable because of the resistance of these bound groups to enzymes (Eldred & Rodney, 1946; Lea & Hannan, 1950; Henry & Kon, 1950). This test involves the combination of free ε-amino groups of lysine molecules in the protein with 2:4-dinitrofluorobenzene. After acid hydrolysis, the content of the dinitrophenyl-lysine complex is measured and the original available lysine can be calculated. Furthermore, the ε-amino group of lysine is susceptible during processing to chemical reactions of the Maillard type, which may bind the amino acid into unavailable complexes. The available lysine value of protein supplements is, therefore, of special importance, and it has been established (cf. Boyne, Carpenter & Woodham, 1961) that, for a wide variety of protein foods, these values correlate closely with gross protein values (GPV). This term was first introduced by Heiman, Carver & Cook (1939) to supply information to the poultry industry on the supplementary value of various protein concentrates when added to a basal cereal diet. Three-day-old chicks are first maintained on the basal diet up to 14 days of age and then either continued on it for 2 weeks to form a control, or given the basal diet supplemented with the test concentrate to supply 3 % supplementary protein. The increase in weight per head of birds on the control diet is subtracted from the increase in weight per head on the supplemented diet, and the difference so obtained divided by the weight of concentrate protein consumed, to find the increase in weight per unit of concentrate protein. This value is expressed as a percentage of the value for casein as a supplement to the basal diet, under the same conditions of test, to give the GPV of the concentrate, relative to casein = 100.

Several microbiological tests have been proposed. Ford (1960) used the proteolytic bacterium *Streptococcus zymogenes* to obtain a measure of the relative nutritive value (RNV) of a variety of food proteins, and demonstrated a close correlation between microbiological and biological estimates of protein quality. The RNV is defined as the amount of growth of the bacterium that occurs on a protein food, compared with the amount of growth on an equi-nitrogenous quantity of casein under the same conditions of test. The growth on the casein standard is given an arbitrary value of 100. This type of grading test, capable of distinguishing good- from bad-quality samples of the same protein, is potentially of great value to the feeding-stuffs compounder. The method described by Ford (1960) has been used in this laboratory to survey the quality of a wide selection of commercial meat meals, whale-meat meals and fish meals. For most of them it has been possible to compare the microbiological values with GPVs and available lysine values reported by Boyne *et al.* (1961) for the same samples. For the whale-meat and meat meals, the RNV results have also been compared with net protein utilization values (NPU), also quoted by Boyne *et al.* (1961), originally from Bunyan & Price (1960). The NPU value of a sample, given as the sole source of nitrogen for growing rats, is the percentage of the nitrogen consumed that is retained in the tissues.

Many of the meals tested were collected by the Agricultural Research Council 8 years ago and represented meals available on the commercial market at that time. These samples were stored under nitrogen in sealed tins and were chosen for this survey because they came from the commercial market and because biological criteria of protein quality were also available for them. Fifteen samples available on the commercial market in the last 2 years were also included.

The RNV gives no useful information about the value of a protein as a source of individual amino acids, which the feeding-stuffs compounder must have if he is to make efficient use of the materials at his disposal. Most of the published values for the amino acid composition of food proteins must be interpreted with caution, since, for most of the analyses, the test samples were subjected to drastic conditions of chemical hydrolysis. These total values give no indication of the biological availability of the amino acids as they exist in the intact protein, and evidence is growing that in processed food proteins the content of available amino acids may be appreciably less than that of the total (cf. Ford, 1962).

By adapting the *Strep. zymogenes* assay technique for RNV measurements, Ford (1962) was able to measure the availability to this organism of methionine, leucine, arginine and tryptophan in a wide range of proteins. He also determined, with the same organism, the total amounts of these amino acids, except tryptophan, present in acid hydrolysates of the proteins. It was evident from his findings that values for available amino acids reflected nutritive quality much more accurately than did those for total. This modified assay procedure has been used in this laboratory to measure total and available methionine, arginine, leucine, histidine, valine, isoleucine, and available tryptophan in all the fish, meat and whale-meat meals for which RNVs were determined.

Finally, the first limiting amino acid for *Strep. zymogenes* has been determined in these meals by supplementation with each, in turn, of the essential amino acids.

EXPERIMENTAL

For the measurement of RNV and available amino acid content of the test samples, the methods used were those of Ford (1960, 1962), modified as follows.

Preparation of samples for assay. All the meals were milled to pass a 40-mesh sieve. Samples were weighed into 100 ml beakers and mixed with 20 ml buffer containing 0·5 % (w/v) sodium citrate and 0·003 % (w/v) sodium cyanide (Ford, 1962), and the pH was adjusted to 7·0 with N-H_3PO_4. Two ml of 1 % papain were added to the beakers, which were then incubated in a water-bath at 56° for 2 h, with stirring every 10 min.

For total amino acid assays, samples providing 100 mg nitrogen were sealed in Carius tubes with 40 ml 3N-HCl and autoclaved for 9 h at 121°. These conditions of hydrolysis gave the highest values for the amino acids assayed. Hydrolysis with 40 ml 2N-HCl for 5 h, as suggested by Ford, did not liberate maximum amounts of several amino acids. Results were generally in good agreement with those obtained with *Leuconostoc mesenteroides* P. 60, which is commonly used for amino acid assays. Longer periods of hydrolysis or stronger acid gave dark-coloured hydrolysates and lower values for methionine.

Estimation of growth. Growth was assessed turbidimetrically by measuring the density of the bacterial suspensions in a Hilger absorptiometer, modified to indicate extinction directly on a galvanometer. The culture tubes were shaken vigorously, left to stand for 2 min to allow any undigested particles to settle, and the supernatant bacterial cultures poured into optically matched cells.

First limiting amino acid. The extracts from the RNV test were set up in eight duplicate pairs of tubes, all at the 4 ml level. To one pair, the control tubes, 1 ml water was added. To each of the other seven pairs of tubes, in turn, 0·5 mg was added of a different essential L-amino acid dissolved in 1 ml water. The fluid content of all the tubes was made up to 8 ml with water, and 2 ml of 5 × strength basal medium were added to each. The tubes were steamed, inoculated and incubated in the usual manner, and the growth in the tubes with supplement was compared with that in the control tubes.

Sample code. The samples preceded by the code letters FM (fish meal), WM (whale-meat meal) and MM (meat meal) were collected by the Agricultural Research Council to cover a wide range of sources and qualities found on the commercial market. They formed the subject of a detailed collaborative investigation on measurements of protein quality, reported by Boyne *et al.* (1961). Samples without code letters were bought through normal commercial channels.

RESULTS

Comparison of RNVs with biological indices of protein quality

Whale-meat meals. Fourteen samples (Table 1) were tested microbiologically. RNVs ranged from 22 (WM7) to 89 (WM13). The mean RNV was 56 (SD ± 15). For

Table 1. *Total and available amino acid composition (g/16 g N) and protein quality ratings of whale-meat meals*

Whale-meat meal code no. (see p. 505)	Arginine Total	Arginine Available	Histidine Total	Histidine Available	Isoleucine Total	Isoleucine Available	Leucine Total	Leucine Available	Methionine Total	Methionine Available	Valine Total	Valine Available	Tryptophan, available	GPV*	NPU†	RNV	ALV‡
WM2	6·0	3·1	2·8	1·1	4·9	2·1	8·8	3·7	3·0	1·3	5·8	3·1	0·34	86	37	50	5·8
WM3	5·2	3·4	2·9	1·1	5·1	2·3	8·9	4·2	3·1	1·5	5·3	3·0	0·35	94	46	53	5·9
WM4	5·4	4·2	2·8	1·6	4·7	2·5	9·3	5·5	3·0	1·5	6·3	4·1	0·48	99	57	63	6·3
WM5	5·2	3·6	3·2	1·2	4·7	2·2	9·2	5·3	2·4	1·4	5·5	3·3	0·37	96	38	53	6·2
WM6	5·9	4·2	2·6	1·3	4·7	2·9	8·9	5·5	3·0	1·5	5·7	3·9	0·43	101	52	65	6·6
WM7	5·4	1·9	2·0	0·34	4·0	1·0	7·3	1·2	2·5	0·40	4·8	1·2	0·13	52	17	22	3·5
WM9	5·5	4·5	2·4	1·6	5·0	2·9	8·1	4·2	2·7	1·7	5·7	3·7	0·60	114	61	72	6·7
WM10	5·7	3·1	2·2	0·91	4·4	2·1	8·3	4·0	3·0	1·3	6·0	3·1	0·32	87	35	49	5·0
WM11	5·8	3·7	2·4	1·2	4·3	2·4	8·5	5·5	2·8	1·4	5·5	3·5	0·37	104	41	57	5·6
WM12	5·8	3·6	2·9	1·1	4·2	2·3	9·2	4·3	2·8	1·4	6·1	3·2	0·34	86	31	54	5·3
WM13	6·4	4·8	2·8	2·0	4·3	3·2	8·8	7·2	3·0	2·3	5·5	5·5	0·58	98	62	89	6·8
WM14	5·5	2·7	2·6	0·79	4·3	1·8	7·9	3·6	1·9	1·0	6·2	3·0	0·22	72	—	47	4·8
1320	5·8	2·8	2·5	0·73	4·2	1·6	8·5	2·7	2·9	—	5·8	2·7	0·29	—	—	46	—
1321	5·6	4·0	2·7	1·7	—	3·0	8·6	5·3	2·9	—	6·2	3·7	0·53	—	—	67	—

ALV, available lysine value.
* Quoted from Boyne *et al.* (1961), originally from Duckworth, Woodham & McDonald (1961).
† Quoted from Boyne *et al.* (1961), originally from Bunyan & Price (1960).
‡ Quoted from Boyne *et al.* (1961).

twelve of the meals, Boyne *et al.* (1961) quote GPV and NPU values. The GPVs ranged from 52 to 114 and were closely correlated with the RNVS ($r = 0.81$; $P < 0.01$). The NPU values, which ranged from 17 to 62, were also highly significantly correlated with the RNVS ($r = 0.91$; $P < 0.001$).

Fish meals. Twenty-six samples (Table 2) were tested. They included ten herring and eight white-fish meals of British manufacture, three Peruvian fish meals from anchovy, and five South African fish meals which were probably manufactured from pilchards. The RNVs ranged from 46 to 80, with a mean of 64 (SD ± 9). Peruvian and South African meals gave RNVs in the middle and lower range, white-fish meal values were scattered throughout the range, and values for herring meals were mainly concentrated in the upper half of the range. Since the original samples were tested, samples of fish meal have been regularly tested for RNV. None of the results has fallen outside the limits shown by this survey. Peruvian and South African samples from several factories, tested over the last 15 months, August 1962 to November 1963, proved to be generally better and more consistent in quality than those included in this survey, although they did not reach the highest values given by white-fish and herring meals.

For fifteen of the meals, GPVs were known (Boyne *et al.* 1961). They ranged from 92 to 121 and were significantly correlated ($r = 0.59$; $P < 0.05$) with the RNVs.

Meat meals. Fourteen samples (Table 3) were tested. RNVs ranged from 20 to 42, with a mean of 31 (SD ± 7). For eleven of the samples, Boyne *et al.* (1961) gave GPVs ranging from 39 to 91 and NPU values from 9 to 34. These GPVs were significantly correlated with the RNVs ($r = 0.76$; $P < 0.01$), as were the NPU values ($r = 0.66$; $P < 0.05$).

Comparison of total and available amino acids with nutritive quality ratings

For the whale-meat meals all three criteria of protein quality correlated with each of the available amino acids. As seen in Table 4, the RNVs formed highly significant correlations with all the seven available amino acids, determined microbiologically, from $r = 0.89$ for leucine to $r = 0.97$ for methionine. The RNVs also correlated significantly ($r = 0.90$) with the chemically determined available lysine values.

The concentrations of each available amino acid in these whale-meat meals correlated with those of every other available amino acid, only one correlation coefficient (available arginine–available histidine) being less than 0.77. *Strep. zymogenes* has no absolute requirement for lysine, which, therefore, could not be measured. Chemically determined published values (Boyne *et al.* 1961) for available lysine, determined by the dinitrofluorobenzene procedure of Carpenter (1960), have been reported for the Agricultural Research Council samples. These available lysine values also correlated very closely with all the values for available amino acids determined with *Strep. zymogenes*.

Among the fish meals (Table 5), GPVs correlated significantly ($r = 0.88$; $P < 0.001$) with values for available histidine, and for tryptophan and valine (r values about 0.6). RNVs also correlated with values for several available amino acids, the highest correlation coefficient being with available isoleucine ($r = 0.81$). There were several

Table 2. *Total and available amino acid composition (g/16 g N) and protein quality ratings of fish meals*

Meal		Arginine		Histidine		Isoleucine		Leucine		Methionine		Valine		Trypto-phan, avail-able	GPV*	RNV	ALV†
Code no. (see p. 505)	Description	Total	Avail-able	Total	Avail-able	Total	Avail-able	Total	Avail-able	Total	Avail-able	Total	Avail-able				
FM1	Herring	6·8	4·7	2·3	1·4	4·2	3·2	8·2	5·2	3·0	1·9	5·4	3·8	0·61	112	70	6·4
FM8		6·0	4·5	2·0	1·6	4·4	3·4	8·3	4·9	3·2	2·3	6·4	3·5	0·51	117	76	7·1
FM14		6·8	5·4	2·0	1·3	4·3	3·6	7·4	5·5	2·6	1·8	6·5	4·3	0·66	115	71	6·9
FM5		6·8	5·2	2·1	1·5	4·3	3·0	8·0	5·2	2·7	1·9	6·3	3·7	0·57	119	67	6·5
FM2		5·9	4·9	1·8	1·2	4·7	3·0	7·1	5·7	2·4	1·8	4·6	3·1	0·40	106	55	5·8
FM6		6·8	5·1	1·9	1·4	4·8	3·5	7·7	5·6	3·1	2·3	6·0	4·5	0·66	116	68	6·6
FM11		6·8	4·9	1·9	1·4	4·7	3·1	6·2	5·2	2·8	2·2	5·4	3·7	0·56	113	60	6·2
FM10		6·0	4·8	1·8	1·4	4·1	3·8	7·4	5·8	2·6	2·5	5·8	4·5	0·75	112	80	6·3
2380		7·3	4·8	2·2	1·3	4·4	3·0	6·4	4·5	3·5	2·3	5·9	3·6	0·55	—	72	—
2092		7·1	4·7	2·3	1·2	4·3	2·7	7·9	4·7	3·1	1·9	5·7	3·5	0·53	—	56	—
FM21	White-fish	6·5	5·3	2·0	1·2	4·0	3·2	7·5	4·8	3·0	2·2	5·4	3·1	0·57	98	63	—
FM19		7·1	4·7	2·0	1·2	4·3	3·2	7·9	5·2	3·1	2·2	5·4	3·1	0·55	100	67	—
FM20		7·2	4·7	2·1	1·0	3·9	2·6	6·8	4·7	3·2	2·1	5·4	3·1	0·46	93	55	—
62		6·8	4·7	2·2	1·3	4·2	3·0	7·4	5·0	3·3	2·1	5·8	3·5	0·64	—	49	—
1808		7·3	3·9	1·9	0·85	4·4	2·4	7·2	5·0	3·2	1·7	5·5	3·3	0·22	—	49	—
FM16		6·8	4·8	1·5	1·4	4·0	3·1	5·7	4·9	2·8	2·5	4·4	3·3	0·53	106	69	5·6
FM23		6·3	4·9	1·9	1·6	4·3	3·8	8·7	6·1	3·0	3·0	5·7	4·2	0·77	115	77	—
FM22		6·4	4·8	1·8	1·1	4·3	2·9	6·7	4·8	2·8	2·2	4·4	3·5	0·40	92	54	—
FM7	South African	6·4	4·3	2·5	1·5	4·9	2·9	7·5	4·4	2·9	1·9	6·6	3·6	0·66	121	63	6·7
1124		6·2	4·6	2·5	1·8	4·8	3·5	8·0	5·5	2·9	2·3	6·4	4·3	0·72	—	65	—
2091		6·8	4·3	2·5	1·1	4·8	2·5	7·6	4·2	3·0	1·6	6·6	3·0	0·52	—	55	—
1809		6·2	4·2	2·5	2·2	4·7	3·5	8·4	6·0	3·1	2·0	6·1	3·2	0·62	—	69	—
1170		6·6	5·0	2·1	1·3	4·1	3·0	7·6	4·3	3·2	2·1	6·1	3·8	0·63	—	57	—
231	Peruvian	5·5	4·9	2·4	2·0	4·6	3·6	7·8	4·5	3·8	2·0	6·0	3·2	0·68	—	70	—
2882		7·2	4·4	2·6	2·0	4·8	3·8	8·7	5·6	3·1	2·2	5·8	4·3	0·54	—	67	—
1221		4·9	3·5	2·2	1·4	4·6	2·7	7·2	5·3	2·7	2·0	5·5	3·5	0·42	—	46	—

ALV, available lysine value.

* Quoted from Boyne *et al.* (1961), originally from Duckworth *et al.* (1961).

† Quoted from Boyne *et al.* (1961).

Table 3. *Total and available amino acid composition (g/16 g N) and protein quality ratings of meat meals*

Meat meal code no. (see p. 505)	Arginine		Histidine		Isoleucine		Leucine		Methionine		Valine		Tryptophan, available	GVP*	NPU†	RNV	ALV‡
	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available					
MM1	6·9	1·9	1·9	1·0	3·2	1·1	8·4	2·7	1·8	0·64	5·2	2·4	0·15	71	25	25	3·9
MM2	7·3	2·2	2·0	1·1	3·2	1·5	7·2	2·9	1·9	0·64	5·2	2·6	0·18	70	18	28	4·0
MM3	7·4	2·1	1·2	0·86	2·1	1·2	4·8	2·6	1·2	0·57	3·5	2·0	0·11	64	9	24	3·9
MM5	7·2	1·5	1·3	0·76	2·0	1·0	4·7	2·5	1·2	0·48	3·3	1·7	0·10	68	11	21	3·9
MM6	7·3	3·1	2·2	1·7	3·0	1·8	7·4	3·1	1·7	0·67	5·2	2·6	0·24	73	22	34	4·1
MM10	7·3	2·0	1·7	0·80	3·0	1·4	7·0	2·1	1·7	0·48	4·8	1·9	0·15	64	16	28	3·6
MM12	8·0	1·8	1·8	0·67	3·5	1·6	8·2	2·8	1·6	0·54	5·5	2·0	0·16	61	30	33	2·9
MM16	7·7	1·4	1·4	0·58	4·4	1·5	9·0	2·1	1·5	0·32	6·6	2·1	0·13	39	26	20	2·7
MM22	8·0	2·2	1·9	1·1	2·8	1·3	8·0	2·1	1·6	0·48	4·6	1·8	0·16	57	15	26	3·3
MM23	8·0	2·2	2·3	1·4	3·7	2·1	10·5	3·0	2·9	0·77	5·5	2·4	0·19	91	34	41	4·4
MM25	8·1	2·2	1·9	1·1	3·2	1·5	9·9	2·5	—	—	4·9	2·1	0·19	—	—	34	4·0
1246	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	34	—
687	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	37	—
362	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	42	—

ALV, available lysine value.
* Quoted from Boyne *et al.* (1961), originally from Duckworth *et al.* (1961).
† Quoted from Boyne *et al.* (1961), originally from Bunyan & Price (1960).
‡ Quoted from Boyne *et al.* (1961).

Table 4. *Whale-meat meals: table of significant correlations*

	ALV	GPV	RNV	NPU	Available arginine	Available histidine	Available isoleucine	Available leucine	Available methionine	Available tryptophan	Available valine
ALV	+1	—	—	—	—	—	—	—	—	—	—
GPV	—	+1	—	—	—	—	—	—	—	—	—
RNV	+0·90***	+0·81**	+1	—	—	—	—	—	—	—	—
NPU	—	+0·86***	+0·91***	+1	—	—	—	—	—	—	—
Available arginine	+0·93***	+0·88***	+0·96***	+0·93***	+1	—	—	—	—	—	—
Available histidine	+0·72**	+0·82**	+0·97***	+0·93***	+0·66*	+1	—	—	—	—	—
Available isoleucine	+0·94***	+0·64*	+0·95***	+0·82**	+0·98***	+0·92***	+1	—	—	—	—
Available leucine	+0·83***	+0·76**	+0·89***	+0·77**	+0·87***	+0·88***	+0·86***	+1	—	—	—
Available methionine	+0·89***	+0·78**	+0·97***	+0·86***	+0·93***	+0·95***	+0·96***	+0·89***	+1	—	—
Available tryptophan	+0·91***	+0·88***	+0·94***	+0·96***	+0·95***	+0·96***	+0·85***	+0·77**	+0·89***	+1	—
Available valine	+0·85***	+0·73**	+0·97***	+0·85***	+0·91***	+0·94***	+0·94***	+0·95***	+0·95***	+0·86***	+1

ALV, available lysine value.
*** $P < 0·001$ (highly significant). ** $P < 0·01$ (very significant). * $P < 0·05$ (significant).

Table 5. *Fish meals: table of correlations*

	ALV	GPV	RNV	Available arginine	Available histidine	Available isoleucine	Available leucine	Available methionine	Available tryptophan	Available valine
ALV	+1	—	—	—	—	—	—	—	—	—
GPV	—	+1	—	—	—	—	—	—	—	—
RNV	NS	+0·59*	+1	—	—	—	—	—	—	—
Available arginine	NS	NS	+0·45*	+1	—	—	—	—	—	—
Available histidine	NS	+0·88***	+0·45**	NS	+1	—	—	—	—	—
Available isoleucine	NS	NS	+0·81***	+0·39*	+0·69***	+1	—	—	—	—
Available leucine	NS	NS	NS	NS	+0·39*	+0·63***	+1	—	—	—
Available methionine	NS	NS	+0·54**	NS	NS	+0·58**	+0·40*	+1	—	—
Available tryptophan	NS	+0·62*	+0·72***	+0·44*	+0·55**	+0·72***	NS	+0·48*	+1	—
Available valine	NS	+0·60*	+0·52**	NS	NS	+0·68***	+0·49*	+0·49*	+0·61***	+1

ALV, available lysine value.
*** $P < 0·001$ (highly significant). ** $P < 0·01$ (very significant). * $P < 0·05$ (significant). NS (not significant).

correlations of available amino acid values with each other and noticeably a pattern of the values for available isoleucine forming correlations with each of those for the other available amino acids, which were determined with *Strep. zymogenes*, but not with the available lysine values.

For both the fish and whale-meat meals there was a general lack of correlation between values for total amino acids and overall values of protein quality. The exceptions were the correlations between GPV and the values for isoleucine ($r = 0{\cdot}58$) and leucine ($r = 0{\cdot}59$) for the whale-meat meals, and between GPV and the values for isoleucine ($r = 0{\cdot}56$) and valine ($r = 0{\cdot}72$) for the fish meals. Both the whale-meat meals and the fish meals were obviously very uniform in composition, and correlations with the wide ranges of protein quality values were not expected; however, these were calculated, and the results confirmed that, on the whole, total amino acid estimations could not be used as indices of protein quality.

Among the meat meals (Table 6), there was no obvious division on the basis of the correlation of protein quality values with those for total and for available amino acids. Unlike whale-meat meals, the meat meals varied widely in total amino acid composition and the results for several total amino acids correlated with the biological assessments of protein quality. The GPVs gave a highly significant correlation with the values for methionine ($r = 0{\cdot}94$) and significant correlations, all at approximately $r = 0{\cdot}70$, with the values for available histidine and leucine, and for total histidine and methionine. The RNVs correlated with the values for several available amino acids (r about 0·6) and also correlated very highly with those for total histidine ($r = 0{\cdot}85$) and total methionine ($r = 0{\cdot}82$). In contrast, the NPU values correlated only with that for isoleucine among the available amino acids, but correlated highly with the values for all the total amino acids except arginine. The values for the available amino acids did not show the pattern of correlations with each other shown by those in the whale and fish meals. However, among the meat meals, the availability of amino acids was generally low, as were the biological values, and the correlations are probably meaningless.

Percentage availabilities of amino acids (Table 7)

Table 7 shows the average percentage availabilities, i.e. (available amino acid :total amino acid) × 100, of the amino acids of the meals. With only one exception, the order of decreasing percentage availability of any amino acid in meals was fish–whale-meat–meat. The exception was the greater percentage availability of histidine in the meat meals than in the whale-meat meals. This order of the meals, fish–whale-meat–meat, also corresponds to the order of average RNV, average GPV and average available amino acids. The meat meals were particularly poor sources of all the available amino acids and poor in overall protein quality compared with the fish and whale-meat meals.

Supplementation with amino acids

No single amino acid improved the RNV in any of the whale-meat meals. With some of the meat meals supplemental methionine produced a very slight and not significant increase in growth. Only when all the seven essential amino acids were added, how-

Table 6. *Meat meals: table of correlations*

	ALV	GPV	RNV	NPU	Available arginine	Available histidine	Available isoleucine	Available leucine	Available methionine	Available tryptophan	Available valine
ALV	+1	—	—	—	—	—	—	—	—	—	—
GPV	—	+1	—	—	—	—	—	—	—	—	—
RNV	NS	+0·76**	+1	—	—	—	—	—	—	—	—
NPU	—	NS	+0·66*	+1	—	—	—	—	—	—	—
Available arginine	NS	NS	NS	NS	+1	—	—	—	—	—	—
Available histidine	+0·70*	+0·72*	+0·70*	NS	+0·89***	+1	—	—	—	—	—
Available isoleucine	NS	NS	+0·79**	+0·72*	NS	NS	+1	—	—	—	—
Available leucine	NS	+0·70*	NS	NS	NS	+0·65*	NS	+1	—	—	—
Available methionine	+0·84**	+0·94***	+0·79**	NS	+0·62*	+0·80**	NS	+0·82**	+1	—	—
Available tryptophan	+0·63*	NS	+0·77**	NS	+0·82**	+0·85***	+0·71*	NS	+0·64*	+1	—
Available valine	NS	NS	NS	NS	NS	+0·65*	NS	+0·77**	+0·63*	+0·71*	+1

ALV, available lysine value.
*** $P < 0.001$ (highly significant). ** $P < 0.01$ (very significant). * $P < 0.05$ (significant). NS (not significant).

Table 7. *Mean values and mean percentage availability of amino acids*

	Fish meal			Whale-meat meal			Meat meal		
NPU	—			42			21		
GVP	109			91			67		
RNV	64			56			31		
ALV	6·4			5·7			3·7		
	Total	Available	Availability* (%)	Total	Available	Availability* (%)	Total	Available	Availability* (%)
Arginine	6·6	4·7	71	5·7	3·6	63	7·6	2·1	28
Histidine	2·1	1·4	67	2·6	1·2	46	1·8	1·0	56
Isoleucine	4·4	3·2	73	4·5	2·4	53	3·1	1·5	48
Leucine	7·5	5·1	68	8·6	4·4	51	7·9	2·6	33
Methionine	3·0	2·1	70	2·8	1·4	50	1·7	0·57	33
Valine	5·7	3·6	63	5·8	3·3	57	5·0	2·1	42
Tryptophan	—	0·67	—	—	0·38	—	—	0·16	—

ALV, available lysine value. * $\frac{\text{Available amino acid}}{\text{Total amino acid}} \times 100$.

ever, did increases of RNV result, of the order of from 20 to 100% for both whale-meat and meat meals. A similar finding was reported by Ford (1960). In contrast, all the fish meals showed increased RNV, of the order of from 20 to 50%, with added isoleucine (0·5 mg/0·4 mg test nitrogen). Eight fish meals, representing the full range of protein quality, were supplemented with isoleucine (0·5 mg/0·4 mg test nitrogen) plus each, in turn, of the remaining essential amino acids in an attempt to find the second limiting amino acid for *Strep. zymogenes*. The results were inconclusive but they did confirm isoleucine as being clearly the first limiting amino acid.

DISCUSSION

The correlation of each available amino acid with every other available amino acid in the whale-meat meals and many of those in the fish meals suggests that a portion of the entire protein may be unavailable. It was evident that the binding of individual amino acids was not responsible for the wide range of qualities found among these meals. Whether this parallel decrease of all available amino acids with decreased protein quality is caused by binding of the amino acids or by impairment of the digestibility of the particles of meal is unknown. The patterns of correlations would suggest that the latter is more probable, unless this pattern has been created by some influence of bound upon unbound amino acids. The inactivation of lysine by reactions with reducing sugars during processing has been long known, but the mechanism whereby other essential amino acids are bound is not so obvious. It is difficult to imagine how other amino acids, structurally dissimilar apart from the peptide bond, could be bound equally to produce the pattern of available amino acids found for whale-meat and fish meals. The specificity of proteolytic enzymes to split a given peptide bond is known to depend, to a large extent, on the nature of the amino acid residues on either side of the bond. Possibly, the presence of bound amino acids in a protein could be responsible for the unavailability of several unbound amino acids in the vicinity of the bound ones.

Harris & Mattill (1940) and others (for a fuller discussion see Ford, 1964) have suggested that heating might result in the cross linking of the ϵ-amino groups of lysine with carboxyl groups, forming enzyme-resistant bonds that decrease digestibility of the protein. This alternative view could account equally well for the pattern of available amino acids that I found for the fish and whale-meat meals. It is likely, in my opinion, that both processes are responsible.

The limiting amino acid in a food will, for a given organism, depend on the quantitative requirements of the organism and its ability to extract that amino acid from the food. The amino acid present in least amount relative to the demands of the organism will be the one limiting growth, unless there is some subtle interference from synergism or imbalance. For *Strep. zymogenes* isoleucine has been demonstrated as the first limiting amino acid in all the fish meals in the RNV test. The fish meals constituted, on average, a better source than the whale-meat meals of all the available amino acids, and by comparing their content of available amino acids with that of the whale-meat meals, an attempt was made to predict the combination of amino acids most likely to be limiting in the whale-meat meals, which had not exhibited a single limiting amino acid. The average content of each of the available amino acids of the whale-meat meals was expressed as a percentage of the content of the same amino acid in the fish meals. These percentage values were: valine 92 %, leucine 86 %, histidine 86 %, arginine 77 %, isoleucine 76 %, tryptophan 71 % and methionine 67 %. It was supposed that the amino acids most likely to be limiting in the whale-meat meals would be those that showed percentage deficiencies greater than, or about the same as, the percentage deficiency of isoleucine (i.e. 24 %). These amino acids are: methionine, tryptophan and arginine.

WM 6 and WM 13, at limiting levels of protein, were supplemented with every permutation and combination of two, three or four of the amino acids isoleucine, arginine, methionine and tryptophan at the levels used for single amino acid supplementation. The results showed that no combination of these amino acids improved the growth of *Strep. zymogenes* on the whale-meat meals. Again, only when all seven amino acids were added did a substantial increase of growth result. It would appear that more than these four available amino acids are present in whale-meat meals at a limiting level for *Strep. zymogenes*, and that possibly all the amino acids are present in proportions very similar to those required by *Strep. zymogenes.*

None of the fourteen whale-meat meals was limited by a single amino acid and none of the twenty-five fish meals was limited by an amino acid other than isoleucine. A possible explanation is suggested by the patterns of correlations among the available amino acids. For the whale-meat meals, the high correlations of every available amino acid with every other available amino acid indicate a uniform pattern of the amino acids for all the meals. Similarly, among the fish meals, the correlations of available isoleucine with all the other available amino acids suggest a constant ratio between isoleucine and these others, and probably explain why all the meals within the groups show the same supplementation effects.

The wide ranges of available amino acid values compared with the narrow ranges of values for total amino acids of the whale-meat meals suggest that raw whale-meat is uniform in composition and that the wide ranges of available amino acids reflect differing processing conditions at the factories. Similarly, the fish meals were fairly uniform in total amino acid composition but varied in available amino acid composition, depending probably on the type of fish and the processing. The meat meals, on the other hand, were found to be more variable in total amino acid composition, probably because they originated from a variety of raw materials, depending on the type of animal slaughtered.

It is not yet possible to assess the value of availability of amino acids determined with *Strep. zymogenes* as prediction of the availability to animals because insufficient work has been done with the latter. For this reason, the findings reported here have been assessed in terms of correlations and relationships among values rather than as absolute values. It is of interest, however, that Miller, Carpenter & Morgan (1963) found good agreement between chick and microbiological estimates of available methionine in a series of meat meals and fish meals. *Strep. zymogenes* was used for the microbiological assay but the samples were predigested with a larger amount of papain than was used in my study. Recent work has shown that very fine grinding of the test materials (J. E. Ford, private communication) and greater concentrations of papain (Miller *et al.* 1963) sometimes yielded somewhat higher values for available amino acids with *Strep. zymogenes*. This is unlikely to affect the relative values, and results have shown that groups of similar meals are still ranked in the same order for available methionine. These higher values agreed more closely with the results of animal experiments (cf. Miller *et al.* 1963).

The close correlations that can exist between available amino acids within groups of similar meals suggest a pitfall when a biological method of measuring protein quality correlates highly with a laboratory method of measuring an available amino acid. Within groups of similar meals the values for the different available amino acids may be closely correlated, and so it may be difficult to interpret differences in nutritive quality as being determined by any specific amino acid, unless that amino acid has first been shown to be limiting in the diet. Boyne *et al.* (1961) showed a high degree of correlation between GPV and chemically determined available lysine values for a number of different supplements, which might suggest that available lysine is limiting in the GPV test. Ellinger & Boyne (1963), using a basal diet slightly different from that of Boyne *et al.* (1961), demonstrated that lysine was limiting for the basal diet in the GPV test, but also demonstrated that, for some concentrates, methionine and lysine might be limiting, depending on the level of supplementation of the concentrate. Similarly, Boyne *et al.* (1961) demonstrated very close correlations between available lysine value and NPU for fish and whale-meat meals, although this biological test of protein quality usually lays greater emphasis on the sulphur amino acids (Boyne *et al.* 1961).

My experience of the *Strep. zymogenes* technique used in this work has convinced me of its value for grading samples of animal protein concentrates for overall protein quality, especially since it has been shown that a high RNV usually indicates the high availability of several amino acids. Until results of collaborative work, using both animal and microbiological techniques, are published there will inevitably be uncertainty of the accuracy of the microbiological values as indicators of availability to animals. Nevertheless, the determinations of available amino acids with *Strep. zymogenes*, though they may be imprecise, have emphasized some of the limitations of total amino acid analyses. They have indicated also the potential value of microbiological techniques for studying problems of nutrition. Species widely differing in structure and function, such as bacteria and mammals, are often remarkably similar biochemically, and microbiological techniques may reveal many reasons for the unavailability to higher animals of amino acids in protein foods.

SUMMARY

1. A survey was made of the protein quality (RNV) of typical commercial fish, whale-meat and meat meals measured by the *Streptococcus zymogenes* microbiological method of Ford (1960). Microbiological ratings of protein quality correlated closely with chick and rat assay values for the same meals quoted by Boyne *et al.* (1961).

2. Total and available arginine, histidine, leucine, isoleucine, methionine and valine and available tryptophan were measured in these meals by the method of Ford (1962), and the results compared with the microbiological and biological indices of overall protein quality.

3. For the whale-meat meals all three criteria of overall protein quality correlated with the values for each of the available amino acids which, in turn, all correlated very closely with each other and with available lysine values determined by the dinitrofluorobenzene procedure.

4. Among the fish meals, chick assay values correlated ($r = 0.88$) with the values for available histidine and for several other available amino acids (r about 0·6). RNVs measured microbiologically correlated with the values for several available amino acids, the highest correlation being with available isoleucine ($r = 0.81$). Both the fish and whale-meat meals were fairly uniform in total amino acid composition, which could not be used as an index of protein quality.

5. The meat meals varied widely in composition as regards several total amino acids. The GPVs found by chick assay correlated with the values for available methionine ($r = 0.94$), for available histidine and leucine, and for total histidine and methionine (r about 0·7). The RNVs correlated with the values for several available amino acids (r about 0·6) and for total histidine ($r = 0.85$) and total methionine ($r = 0.82$). In contrast, the NPU values found by rat assay correlated only with the value for isoleucine among the available amino acids, but correlated with all the total amino acid values except arginine.

6. The correlations of the values for several available amino acids with protein quality ratings, particularly for the whale-meat meals, indicated that in the poorer-quality meals a portion of the entire protein may be unavailable and the loss of protein quality may be largely the result of impairment of overall digestibility.

7. All the meals were supplemented singly with the essential amino acids to find the first limiting amino acid for *Strep. zymogenes* in the RNV test. The fish meals were clearly limited by isoleucine. No single amino acid was found to be limiting in whale-meat or meat meals.

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