Protein quality of feeding-stuffs

2.* The comparative assessment of protein quality in three fish meals by microbiological and other laboratory tests, and by biological evaluation with chicks and rats

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In a previous paper this Group has reported the results of biological, microbiological and other laboratory assessments of protein quality of a large range of protein concentrates (Boyne, Carpenter & Woodham, 1961). In particular, it was shown that for animal by-products including fish meals the available lysine values (ALV) correlated well with the results of a chick growth test, the estimation of gross protein value (GPV). Although all the concentrates tested are commonly used for the supplementation of cereal-based diets for non-ruminants in general, no information was available on the applicability of the assessments in evaluating the materials as constituents of diets for growing pigs. It was decided therefore that new samples of fish meal should be acquired sufficient in quantity for a large-scale pig experiment, and that these should be significantly different as judged by ALV and by a chick growth test. Eventually two Peruvian anchovy meals were found to satisfy the requirements, and a sample of good-quality British white-fish meal that had been collected previously was used for

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comparison. The results presented here are from the work carried out on these samples by the various collaborating laboratories, but the pig experiment is dealt with in the accompanying paper (Barber, Braude, Chamberlain, Hosking & Mitchell, 1964). Further studies of available amino acid contents by microbiological techniques will be the subject of a separate paper.

EXPERIMENTAL

Meals

Samples of a British white-fish meal and two Peruvian anchovy meals (10 cwt of each) were allocated code numbers FM 24, FM 25 and FM 26, respectively. FM 24 was a meal with the normal pale colour of a good white-fish meal; both of the anchovy meals were dark in colour and had apparently become overheated on board ship. FM 25 was designated a 'fertilizer grade meal' by the trade and FM 26 had been rejected by the consignee as not being of the 'fair average quality' stated in the contract.

Chemical procedures

Total nitrogen. Though there was some individual variation in technique, most collaborators in the Group followed fairly closely the method described by the Association of Official Agricultural Chemists (1960*a*). The crude protein figure (N × 6·25) used for each meal was the mean of the results obtained in five collaborating laboratories and was expressed on an 'as-received' basis. It should be noted that slightly different figures were used in the accompanying paper (Barber *et al.* 1964) reporting the assessments with pigs.

True protein. The method was that described by the Association of Official Agricultural Chemists (1960*b*).

Ash content. The method was that described by the Association of Official Agricultural Chemists (1960*c*), but with the sample heated at 520° for 5 h.

Available lysine value (ALV). The method described by Carpenter (1960) was used.

Total sulphur. The method of Miller & Donoso (1963) was used.

Orange G binding values. A modification (Bunyan, 1959) of the method of Fraenkel-Conrat & Cooper (1944) was used.

Total amino acids and tryptophan. In one laboratory the method of Spackman, Stein & Moore (1958) was employed using a Beckman–Spinco automatic ion-exchange chromatographic analyser. In another, the protein was oxidized with performic acid as described by Bidmead & Ley (1958); excess performic acid was reduced by HBr (Moore, 1963), and the oxidized protein then hydrolysed for 22 h at 110° under N_2 in a closed bottle. The quantities of amino acids in the hydrolysate were estimated by means of a Technicon amino acid analyser. Tryptophan was determined without preliminary hydrolysis by the method of Spies & Chambers (1949, procedure N).

Microbiological procedures

Evaluation of protein quality with Streptococcus zymogenes. The method of Ford (1960) was used. Results are expressed as relative nutritive values (RNV); casein = 100.

Evaluation of protein quality with Tetrahymena pyriformis *W.* The method of Stott, Smith & Rosen (1963) was used. Results are expressed as organism counts in units of 10^4/ml on the basis of 0·3 mg meal N/ml.

Total methionine. Samples containing precisely 100 mg N were sealed in Carius tubes with 40 ml 3N-HCl and autoclaved for 16 h at 121°. After cooling, the tubes were opened and their contents brought to pH 7·0 with aqueous 10N-NaOH and then diluted to 1 l. for assay by the method of Ford (1962).

Assay of the availability of methionine with Strep. zymogenes. The method of Ford (1962) was used.

Assay of the availability of lysine and methionine with T. pyriformis *W.* The basal medium and general technique were those of Stott *et al.* (1963). The assay medium contained an amino acid mixture based on the 2C medium of Kidder & Dewey (1951) with the omission of either lysine or methionine. A response curve was obtained by the addition of graded levels of lysine or methionine, and the organism was also grown in media containing meal + lysine or methionine-free amino acid mixture. Lysine or methionine values corresponding to growth on the meal were read from the response curve, and the available amino acid per 16 g N was calculated.

Biological procedures

Protein efficiency ratio method (PER) (*chick*). Six hundred day-old Rhode Island Red × White Leghorn cockerels were fed on cracked wheat and maize for 4 days and then for the next 10 days on a standard high-protein chick starter diet containing 21–22% crude protein. The birds were then individually weighed and 192 were selected as being as near the upper end of the total weight range as was consistent with close weight similarity in the selected group. The selected birds were allocated at random, commencing with the lightest birds, to sixteen groups each of twelve birds. Group weights were recorded. Diets containing 18·6% crude protein, of which 6·6% was supplied by barley, weatings, oatfeed and dried yeast and 12·0% by the supplement under test, were offered *ad lib.* To improve discrimination between diets the cages were lit with 150 W red bulbs and every attempt was made to avoid unnecessary disturbance of the birds during the 2-week experimental period. At the conclusion of this period groups were weighed on 2 successive days and the food consumption of each group was recorded. The PER was taken to be the weight gain of the group per unit total protein eaten by the group. Four groups were given diets containing each of the three test fish meals and the remaining four were given a standard white-fish meal diet as control.

Gross protein value method (GPV) (*chick*). The method of Duckworth, Woodham & McDonald (1961) was used.

Soya protein efficiency ratio method (*soya* PER) (*rat*). For 21 days 3-week-old rats

were fed *ad lib.* on diets containing barley meal (40%), sucrose (40%), maize starch, salt, cellulose, arachis oil, maize oil, minerals and vitamins along with either soya-bean meal or a test protein which contributed 5% protein. The total protein level of the diets was 9% and there were between six and eight rats on each diet.

$$\text{Soya PER} = \frac{\dfrac{\text{Weight gain}}{\text{weight of test protein in diet consumed}} \times \dfrac{100}{\%\text{ crude protein in test protein diet}}}{\dfrac{\text{weight gain}}{\text{weight of soya diet consumed}} \times \dfrac{100}{\%\text{ crude protein in soya diet}}} \times 100.$$

Net protein utilization method (NPU) (*rat*). The method of Miller & Bender (1955), which depends on carcass analysis, was used. The number of animals was as stated in the method.

Net dietary protein calories % (*NDpCals* %) (*rat*). This measurement is the NPU of a practical diet multiplied by the percentage of protein calories of the diet (Platt & Miller, 1959). The tests were carried out with the diets used in the pig experiment described in the accompanying paper (Barber *et al.* 1964) and containing 3 or 7% of the fish meal under test. The total protein contents of the diets were 12·4 and 14·6% to which supplementary fish meal contributed approximately 1·9 and 4·5% respectively. The unsupplemented diet was also tested.

RESULTS

Chemical tests (*Table* 1)

The only test in this table that has been suggested as an indicator of protein quality for fish meals is the measurement of Orange G absorption, and this ranked the meals in the order FM 24 > FM 25 > FM 26.

Table 1. *Results of chemical tests on the fish meals*

	FM24	FM25	FM26
Orange G absorption (mg/g)	107	101	48
Total sulphur (%)	0·81	0·77	0·84
Ash (%)	19·5	15·7	15·5
True protein (%)	58·1	58·8	59·7
Crude protein* (%)	66·7	64·7	64·4

* Values are means of those determined in five laboratories and are on an 'as-received' basis.

Amino acid analysis

Sixteen amino acids in addition to ammonia were estimated in hydrolysates of the unoxidized fish meals in the laboratory with the Beckman–Spinco automatic ion-exchange chromatographic analyser. The presence of methionine sulphoxide and hydroxyproline was noted but the amounts were not estimated. The Technicon analyser was used in the examination of hydrolysates of the meals after oxidation with

performic acid, and the amounts of fifteen amino acids were estimated. Values for tyrosine, proline, hydroxyproline and ammonia were not obtained by this procedure, and the apparent values for serine, methionine, isoleucine and histidine may be corrected to allow for losses found in recovery tests with another fish meal. These results, together with the values for tryptophan in the unhydrolysed meals and methionine determined with *Strep. zymogenes*, are given in Table 2.

Table 2. *Total amino acid composition (g/16 g N) of the fish meals*

	FM24		FM25		FM26	
	Moore & Stein* (*a*)	Moore & Stein* (*b*)	Moore & Stein* (*a*)	Moore & Stein* (*b*)	Moore & Stein* (*a*)	Moore & Stein* (*b*)
Lysine	6·7	6·7	7·5	7·3	6·4	6·8
Histidine	2·0	1·8	2·4	1·6	2·0	1·7
Arginine	6·1	6·8	5·5	5·5	5·0	5·4
Aspartic acid	8·6	8·9	9·3	9·7	8·8	9·7
Threonine	3·8	4·3	4·1	4·4	3·8	4·3
Serine	4·1	4·7	3·5	3·9	3·2	3·8
Glutamic acid	13·4	13·6	13·0	13·8	12·9	14·4
Proline	5·5	—	3·9	—	3·6	—
Glycine	9·3	9·0	5·7	5·3	5·4	5·1
Alanine	6·3	6·5	6·1	6·4	5·7	6·3
Valine	4·5	4·3	5·3	4·9	4·9	4·9
Cystine	—	0·91	—	0·87	—	0·8
Methionine†	2·4	2·7	2·5	2·6	2·2	2·4
Isoleucine	3·8	3·7	4·6	4·2	4·3	4·3
Leucine	6·4	6·6	7·5	7·4	7·0	7·4
Tyrosine	2·8	—	3·3	—	3·1	—
Phenylalanine	3·4	3·4	4·1	3·9	3·7	4·1
Methionine sulphoxide	‡	—	‡	—	‡	—
Hydroxyproline	Trace	—	Trace	—	Trace	—
Ammonia	1·5	—	1·5	—	1·6	—
Tryptophan§	—	0·88	—	1·05	—	0·82

* (*a*) Results of single estimations carried out on direct hydrolysates of the fish meals; cystine was not measured. (*b*) Means of duplicate tests on duplicated hydrolysates of the oxidized fish meals. Cystine was measured as cysteic acid. All tyrosine is destroyed in this procedure. Corrections for loss of other amino acids have not been applied, but experiments on other fish meals suggest that the values given for serine, methionine, isoleucine and histidine should be increased by 9, 16, 10 and 30 % respectively.

† The *Strep. zymogenes* method gave values for FM24, FM25 and FM26 of 2·6, 3·0 and 2·8 respectively.

‡ Presence noted but quantity not measured.

§ By the method of Spies & Chambers (1949) on the unhydrolysed meal.

AI.VS determined by the method of Carpenter (1960), available lysine and methionine determined microbiologically with *T. pyriformis* W, and available methionine with *Strep. zymogenes* are given in Table 3.

The total levels of arginine, glycine and proline were higher for FM 24 than for FM 25 and FM 26, and FM 25 had the highest content of total lysine. As regards available lysine values, it is clear that those for FM 24 and 25 were higher than those for FM 26, but the difference between the values for FM 24 and 25 was of doubtful significance. Similarly, for available methionine content all the collaborating labora-

tories found FM 26 inferior and concluded that the difference between the values for FM 24 and 25 was not significant.

Table 3. *Amounts (g/16 g N) of available lysine and methionine in the fish meals*

	FM24		FM25		FM26	
Method	Lysine	Methionine	Lysine	Methionine	Lysine	Methionine
Fluoro-2:4-dinitrobenzene*	6·60	—	6·52	—	4·76	—
Strep. zymogenes	—	2·4	—	2·5	—	1·6
T. pyriformis W	6·4	3·0	5·7	3·0	3·7	1·6

* Values obtained by this chemical procedure were obtained in three laboratories and the ranges were: FM24, 6·30–6·79; FM25, 6·40–6·64; FM26, 4·45–5·03. Results are means of six values each calculated on the assumption that 92 % of ε-dinitrophenyl-lysine is recovered. One laboratory obtained recoveries of 97 % with resulting available lysine values of 6·38, 6·27 and 4·75 for the three meals.

Biological tests (Table 4)

PER measured by the chick technique showed significant differences between the three meals and ranked them in the order FM 24 > FM 25 > FM 26. In this test the standard error of differences between the means was ±0·02 (9 df) and the PER of FM 24 was significantly higher than that of FM 25 ($P < 0·001$), which was in turn significantly higher than that of FM 26 ($P < 0·001$).

Table 4. *Results of biological evaluations of the fish meals*

	FM24	FM25	FM26
(*a*) Tests in which the fish meal was given as a supplement in a cereal-based ration			
Protein efficiency ratio (chick)	2·62	2·45	2·01
Gross protein value (chick)	100	103	74
Soya protein efficiency ratio (rat)	106	110	51
(*b*) Tests in which the fish meal was given as a sole source of protein			
Net protein utilization (rat)*	60, 66	51, 66	31, 27
Relative nutritive value (*Strep. zymogenes*)†	62, 65	76, 80	38, 50
Nutritive value for *T. pyriformis* W	44	53	35
(*c*) Tests on pig diets containing 3 or 7 % of the fish meals			
NDpCals % (3 %) (rat)	8·4	8·6	7·7
NDpCals % (7 %) (rat)	10·0	10·0	8·3

* First values of each pair reported by one laboratory and second by another.
† Replicate values obtained in same laboratory.

Soya PER determinations with rats indicated a possible slight superiority of FM 25, and the meals were ranked in the order FM 25 > FM 24 > FM 26.

Chick GPVs failed to discriminate between FM 24 and 25 but ranked both significantly superior to FM 26.

NPU determinations with rats carried out in one laboratory failed to discriminate between FM 24 and 25, although in a second laboratory the same test suggested that

FM 24 might be the better. In both laboratories FM 26 was shown to be decidedly inferior to the other two meals.

Determinations of NDpCals % of the mixed diets used in the pig experiment in which the test meal contained 3 or 7 % fish meal failed to discriminate between FM 24 and 25, though this test also confirmed the inferiority of FM 26.

RNV determined with *Strep. zymogenes* and with *T. pyriformis* W ranked the three meals in the order FM 25 > FM 24 > FM 26.

DISCUSSION

All the tests, microbiological, biological and chemical, agreed as to the inferiority of FM 26 compared with the other meals tested. The first objective, namely the choice of two meals of widely differing quality for chicks, was therefore attained and we were able to conclude that the two Peruvian meals, FM 25 and 26, were suitable for use in the trial with pigs described in the accompanying paper (Barber *et al.* 1964).

Although there was complete agreement between the tests regarding the quality of FM 26, there was no such clear-cut verdict on FM 24 and 25. From the results of the biological tests it will be seen that usually the difference between these two meals was small and for all practical purposes they were rated equivalent in nutritive value. This conclusion applies to GPV (chick), soya PER (rat), and NDpCals % measured at both the 3 and 7 % levels. As regards NPU, although one laboratory found what might appear to be a significant difference between the meals, another found them to be identical. In the remaining two tests, one laboratory found FM 24 to be significantly superior to FM 25 (PER, chick) and one found FM 25 to be significantly superior to FM 24, (RNV, *Strep. zymogenes*).

This apparent discrepancy may have been due to the possibility that FM 24 was slightly higher in available lysine value than FM 25. We do not have enough evidence yet to be able to say that this particular measurement of PER can detect such small differences, particularly in view of the fact that in the diet a total protein level of 18·6 % was used. On the other hand, we do know that for *Strep. zymogenes* the limiting amino acid is probably isoleucine or methionine, and certainly not lysine.

In chemical tests, in addition to the higher ALV mentioned, the higher Orange G absorption value was possibly also related to the higher available lysine content of FM 24, though it must be realized that acid dye absorption should indicate the sum of lysine, histidine and arginine. This sum was indeed higher for FM 24 considering total amino acid values obtained for the oxidized meals, though not for the unoxidized meals.

In a number of tests the 'total' levels of individual amino acids, including lysine, were not greatly different for FM 24 and 26. The explanation for the relative total lysine values for the three meals probably lies in the fact that the two Peruvian meals, FM 25 and 26, were made from whole anchovies, whereas there was evidence that the white-fish meal FM 24 contained filleter's waste. This can be inferred from the similarity of the results of the amino acid analysis to those of previous analyses of samples known to consist solely of offal meal (G. M. Ellinger, private communication). In particular, the high levels of arginine, glycine and proline in FM 24 strongly suggest

that it contained a high proportion of filleter's waste. Such a meal would be expected to contain less total lysine than a good-quality whole meal such as FM 25. FM 26, on the other hand, though a whole meal, had been damaged as evidenced by its content of available lysine.

We may conclude that though a wide variety of tests may agree on the relative nutritional values of two fish meals which are widely different in quality, such as FM 25 and 26, they may differ in the way in which they discriminate between two meals of rather similar quality such as FM 24 and 25.

The relative placings given to the meals by these various tests are of particular interest in the light of the results obtained in the pig trial of Barber *et al.* (1964) and by the microbiological results that will be published later.

SUMMARY

1. A sample of British white-fish meal and two samples of Peruvian anchovy meal were subjected to a wide variety of tests including total amino acid analysis, biological evaluation with chicks and rats, microbiological assessment, and other miscellaneous laboratory tests.

2. All of the tests agreed as to the inferiority of one of the anchovy meals compared with the other anchovy meal and the white-fish meal, though the differentiation between these two good meals was not so clear-cut.

3. The significant difference in quality for chicks between the two anchovy meals fitted them for the large-scale pig trial described in the accompanying paper (Barber *et al.* 1964).

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