

Studies on the nutrition of marine flatfish. Utilization of various dietary proteins by plaice (*Pleuronectes platessa*)

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1. Five proteins, freeze-dried cod muscle, a white fish meal, a yeast-protein concentrate, a soya protein and a fish-protein concentrate (a solvent-extracted fish meal) were given separately, as single sources of protein in diets containing 500 g crude protein/kg, to small plaice (*Pleuronectes platessa*) of mean initial weight 12–15 g. Weight gain and net protein utilization (NPU) were examined.
2. Even at these high protein intakes, significant differences in growth rate between fish given the different proteins were evident. These findings were reflected in different NPU values between the proteins. This position contrasts with that in the rat where proteins which differ markedly in NPU at low protein intakes have similar NPU values when given at high dietary levels.
3. Coefficients of apparent digestibility measured on larger plaice (150–200 g) were high for all the proteins used except soya-bean meal, which had a digestibility coefficient of 0.68.
4. At high protein intakes there was no clear relationship between NPU values and essential amino acid content (as measured chemically) of the proteins tested.
5. The preparation of protein concentrates from fatty fish by the use of detergents is described. One of the preparations obtained gave weight gains and NPU values similar to those obtained with commercially available feedstuff proteins.

Net protein utilization (NPU) by mammals decreases with increasing dietary protein level, progressively more of the protein being used as an energy source as protein intake increases. NPU values for proteins of high and low nutritional quality differ widely at low protein intakes when the amino acid composition of the protein under test is the limiting feature, but are very similar at high protein intakes (Miller & Payne, 1961), presumably because the essential amino acid requirements of the animal are met even by proteins of low nutritional quality, provided a sufficient amount of the protein is ingested. Thus, studies on mammals indicate that the quantities of essential amino acids required for optimal growth remain constant provided measurements are made at, or above, the minimum dietary level for optimal growth (Bressani & Mertz, 1958). Conversely, when expressed as a percentage of protein in the diet, calculated essential amino acid requirements fall as the dietary protein level is increased above the minimum level for optimal growth.

The minimum protein requirement for optimal growth of many fish is high and much of the ingested protein is used as a source of energy, the relationship between NPU and protein intake being similar to that in mammals (Cowey, Pope, Adron & Blair, 1972). Consequently, unless the quantitative essential amino acid requirements of fish are considerably in excess of those of mammals, it might be expected that at

these high protein intakes different proteins would not differ greatly in their ability to support growth (i.e. NPU values at this dietary protein level would be similar). In order to examine this possibility, plaice were given several single sources of protein at a high dietary level (500 g crude protein/kg diet) and growth rate and NPU were measured. The results obtained indicate that, even at high protein intakes, feedstuff proteins differ in their ability to support the growth of plaice.

In addition, protein concentrates were prepared from two species of fatty fish by the use of surface-active agents. These materials were prepared in an attempt to obtain a low-lipid protein concentrate from fatty fish without the use of organic solvents. They were also tested as sources of dietary protein for plaice at high protein intakes. The results obtained suggest that this type of process could be a useful means of obtaining protein concentrate from trash fatty fish.

EXPERIMENTAL

Diets

The composition of the experimental diets is shown in Table 1. White fish meal was purchased from Caledonian Fish Meal Co., Aberdeen; promine-D (extracted soya protein) was from Oppenheimer Casing Co. (UK) Ltd, London. Sources of other materials were as described previously (Cowe, Pope, Adron & Blair, 1971). As BP protein concentrate and promine-D are relatively deficient in methionine, they were supplemented with 10.0 g L-methionine/kg and 19.0 g L-methionine/kg respectively before use. Thus the quantity of L-methionine in these two proteins was raised to 30 g/kg crude protein. Promine-D was used in diet 4 rather than the more appropriate commercial feed, soya-bean meal, to permit use of a soya protein alone at a dietary level of 500 g protein/kg diet. All diets contained 500 g crude protein/kg dry diet; the estimated metabolizable energy content per kg dry diet varied slightly from 15480 kJ (3700 kcal) in diet 5, to 17570 kJ (4200 kcal) in diet 3. The ratio protein energy:total energy in the different diets varied between 0.68 and 0.77.

The extracted herring muscle and extracted sprats used in diets 6 and 7 were prepared by extracting these fish with non-ionic, surface-active agents. The procedure applied to herring muscle was: 600 g herring muscle (previously deep-frozen) were homogenized with 1.2 l water and the homogenate was brought to a temperature of 80°; 36 g tergitol 15-S-7 (Union Carbide Ltd) were added to the homogenate, which was held at 80° with constant stirring for 1 h. The homogenate was then centrifuged (5000 g, 20 min) in polypropylene buckets while still hot. The supernatant fraction was discarded and the precipitate resuspended in 1 l water; this suspension was heated to 80°, 30 g tergitol were added and the suspension was again held at 80° for 1 h with constant stirring. It was then centrifuged, the supernatant fraction discarded and the precipitate suspended in water. The suspended precipitate was transferred to a large Buchner funnel and washed well with water to remove any remaining tergitol. Finally the precipitate was dried in a forced-draught oven at 40°; the yield was 98 g and the composition of the product is shown in Table 2. Recovery of protein was about 75%, and the lipid content of the material was reduced from 43 g at the start to 4 g.

Table 1. *Composition of experimental diets (g/kg dry diet)*

Ingredient	Diet						
	1	2	3	4	5	6	7
Freeze-dried cod muscle	575	—	—	—	—	—	—
White fish meal	—	715	—	—	—	—	—
BP protein concentrate*	—	—	830	—	—	—	—
Promine-D*	—	—	—	545	—	—	—
A fish-protein concentrate	—	—	—	—	620	—	—
Extracted herring muscle	—	—	—	—	—	563	—
Extracted sprats	—	—	—	—	—	—	602
α -Cellulose	265	125	10	295	220	227	238
Additional components†	160	160	160	160	160	160	160

* Supplemented with methionine (see p. 298).

† Vitamin premix (Cowey *et al.* 1972) 25 g, mineral mixture (Cowey *et al.* 1972) 5 g, maize oil 60 g, cod-liver oil 30 g, binder 40 g.

Table 2. *Extraction of 600 g of herring muscle and of sprats with surface-active agents*

Material	Composition of starting material (g)			Composition of product (g)		
	Water	Protein	Lipid	Total weight	Protein	Lipid
Herring muscle	420	116	43	98	87	4
Sprats	410	102	58	79	65	3.5

A similar procedure was applied to sprats, except that Tween 80 (polyoxyethylene sorbitan mono-oleate; Sigma Chemical Co. Ltd, London) was used at a concentration of 20 g/l instead of tergitol. The composition of the product is shown in Table 2.

In addition to the diets listed in Table 1, a low-protein diet was given to a further group of fish, thus permitting evaluation of NPU of the proteins under examination as described previously (Cowey *et al.* 1972). Values for growth obtained with this diet were not included in the analysis of weight gains.

The digestibility of the different proteins was determined by a ratio method using an inert indicator (Furukawa & Tsukahara, 1966; Knapka, Barth, Brown & Cragle, 1967). Fish of 150–200 g live weight were given diets containing 5 g chromic oxide per kg of the protein under test for 2–3 weeks; they were then lightly anaesthetized by immersion in sea-water containing 1 part of M.S. 222 (*m*-ethoxycarbonylanilinium methanesulphonate; Sandoz Products Ltd, London) in 10000. After removal from the sea-water, gentle finger pressure was applied to the surfaces of the fish in the rectal region and the faecal material so expelled was collected in a petri dish. This material was dried at 100° before its nitrogen and chromic oxide contents were measured.

Animals and tank system

Young plaice (*Pleuronectes platessa*), about 9 months old, were obtained from the White Fish Authority Marine Fish Cultivation Unit, Hunterston, Ayrshire, where they had been fed on a diet of minced, trash fish. The plaice were distributed

between seven tanks ($1.9 \times 0.84 \times 0.61$ m deep) standing along one wall of a rectangular room; each tank contained sea-water to a depth of 0.35 m. Light was provided only from fluorescent tubes which gave an intensity of 60 lx at the water surface in each tank. Sea-water flowed by gravity through the fish tanks at a rate of 60 l/h per tank from a 10000 l reservoir situated in the rectangular room adjacent to that containing the fish tanks. From the fish tanks, water flowed through a biological filter (where oxidation of ammonia, excreted by the fish, to nitrate occurred) to a sump from where it was pumped back to the reservoir through an ultraviolet sterilizer (Hanovia Model 6, Nafloc Ltd, Northwich, Cheshire). The biological filter, sump, u.v. light and pumps were housed in a separate rectangular room adjacent to the fish tank room and distant from the reservoir room. Sea-water was discarded from the reservoir at weekly intervals and replaced by fresh sea-water. Ammonia levels in the water were monitored regularly (not less than once per week) and never exceeded 0.2 mg/l. All three rooms were thermally insulated and both air and water temperatures controlled at $15 \pm 2^\circ$.

Fish were first accustomed to an artificial diet (diet 4 of Cowey *et al.* 1972). Some fish were then discarded from each tank and small number of others re-allocated between the seven tanks in such a way as to provide in each tank approximately equal numbers of individuals, a similar total biomass, and a similar size distribution. The fish in each tank were then marked by subdermal injection of differently coloured rubber latex dyes (Riley, 1965) on the ventral surface so that they were individually identifiable.

After 2-3 weeks on this diet, fish were given the seven experimental diets (Table 1) for 3 d, all the fish within the same tank receiving the same diet. All the diets were readily accepted by the fish, so initial weight measurements were made and the experiment was begun. All the fish were removed from each experimental tank and transferred to separate, small, portable tanks, one for each treatment. From each of the portable tanks in turn fish were removed singly with a hand net, drained for 10 s during which time the colour code was identified, and placed on a piece of rigidly-backed, water-absorbent sponge. The weight of sponge + fish was rapidly determined on a Mettler P 1200 top-pan balance and the fish was put into another small, portable tank. The weight of the sponge (plus any water it had absorbed) was then determined. When all the individuals on any treatment had been weighed they were returned to one of the experimental tanks. This method of weighing proved satisfactory and reproducible. Fish were not fed for 18 h before weighing. Feeding procedure was as described previously (Cowey *et al.* 1972).

With the resources available it was not possible to replicate the tanks allotted to each diet. Experience with this tank system has shown, however, that groups of fish of a similar size kept in separate, identical tanks and given the same diet do not differ in growth rate. Moreover, at each bi-weekly weighing, fish were returned to a different experimental tank from that in which they had spent the previous fortnight.

At the start of the experiment there were twenty to twenty-two individuals in each tank. No mortalities occurred during the experiment. A small number of fish given some of the diets showed abnormally large or small growth, and values for them were excluded from the analysis of weight gains, but were included in the measurement of

Table 3. *Initial weights (g) of experimental plaice*

Diet no.	No. of fish	Distribution of initial wt			Mean initial wt
		< 10	10- < 20	≥ 20	
1	16	4	11	1	12.65
2	22	7	12	3	13.52
3	21	5	10	6	14.98
4	17	5	9	3	13.48
5	21	9	9	3	13.36
6	21	7	12	2	13.39
7	19	8	8	3	14.11

Table 4. *Mean weights (g) of plaice at successive fortnightly intervals*

Diet no.	Wt after weeks						Average wt gain after 10 weeks
	0	2	4	6	8	10	
1	12.65	14.62	17.52	19.79	24.73	29.47	16.82
2	13.52	15.32	17.48	19.14	22.32	25.80	12.28
3	14.98	16.75	19.58	20.92	23.44	25.13	10.16
4	13.48	14.38	16.55	17.63	19.72	20.99	7.52
5	13.36	14.73	16.98	17.23	19.87	20.21	6.85
6	13.39	15.45	17.21	18.75	20.41	20.79	7.40
7	14.11	15.70	17.52	19.21	21.48	23.36	9.26

NPU. Table 3 gives the number of fish used in the analysis of growth, the distribution of their initial weights, and the mean initial weight for each diet.

RESULTS

The average bi-weekly weights and the average total growth of fish given each diet are shown in Table 4; they show that the highest average weight gain was produced by diet 1. Within treatments, it appeared that gain in weight over the experimental period was linearly related to initial weight. To take account of this association, linear regressions of weight gain on initial weight were calculated for each diet. Although there was evidence that the relationship between weight gain and initial weight was more marked for some diets than others, a common slope was calculated for all diets. That is, it was assumed that the difference in total weight gain corresponding to any given difference in initial weight was the same for all diets. The relationships for each diet were:

$$\text{diet 1, } Y = 12.744 + 0.3221X;$$

$$\text{diet 2, } Y = 7.926 + 0.3221X;$$

$$\text{diet 3, } Y = 5.333 + 0.3221X;$$

$$\text{diet 4, } Y = 3.177 + 0.3221X;$$

$$\text{diet 5, } Y = 2.550 + 0.3221X;$$

$$\text{diet 6, } Y = 3.093 + 0.3221X;$$

$$\text{diet 7, } Y = 4.715 + 0.3221X;$$

Y is the gain in weight (g) and X is the initial weight (g).

Table 5. *Estimated average weight gains (g) after 10 weeks, with their standard errors, of plaice of 13.7 g initial weight*

Diet	Estimated gain in wt	SE
1	17.2	0.80
2	12.3	0.68
3	9.7	0.70
4	7.6	0.77
5	7.0	0.70
6	7.5	0.70
7	9.1	0.73

From these equations, the expected weight gains of fish given different diets were calculated using a value for initial weight of 13.7 g (the mean initial weight of all the experimental fish) and these are given in Table 5.

The values in Table 5 show that diet 1 (freeze-dried cod muscle) clearly promoted much greater growth than any of the other diets. The next-highest average weight was obtained with diet 2 (white fish meal), which was significantly ($P < 0.01$) better than diets 3 (BP protein concentrate) and 7 (extracted sprats), the difference in expected weight gains between diets 2 and 3 being 2.6 ± 0.98 g and between diets 2 and 7, 3.2 ± 1.00 g. Diets 3 and 7 in turn produced higher expected weight gains than diets 4 (promine-D), 5 (fish-protein concentrate) and 6 (extracted herring muscle), which produced the poorest gains of all the diets and were not themselves significantly different.

The interesting feature of these results is the poor growth rate of fish given diet 5 containing, as sole source of protein, a fish-protein concentrate obtained by solvent extraction of fish meal. The growth rate of fish given this diet is significantly less than, and contrasts markedly with, that of animals given other fish proteins (diets 1 and 2). It is noteworthy (Table 6) that the fish given diet 5 consumed more protein than did either of the groups given diets 1 or 2. It must be deduced, therefore, that there are real differences in nutritional value between this fish-protein concentrate and the other two proteins derived from fish (freeze-dried cod muscle and white fish meal).

Results of measurements of protein quality of the diets appear in Table 6. NPU values for diets 1, 2, 3 and 4 were somewhat similar, indicating that at the dietary protein level used there was little difference in their relative nutritional value. Diet 5, on the other hand, gave a much lower NPU value than most other diets, especially diets 1-4. The absence of a facility to replicate treatments precluded any assessment of the significance of differences in NPU but, taken in conjunction with the findings on weight gain, real differences seem to exist in relative nutritional value of the fish-protein concentrate and the other proteins derived from fish (diets 1 and 2). The detergent-extracted fatty fish (diets 6 and 7) were not markedly inferior to the best of the commercial proteins (diets 2, 3 and 4), and to this extent the method used for their extraction is promising.

With the exception of soya-bean meal, the proteins under examination were of

Table 6. Total live-weight gains of the plaice and biological evaluations of the proteins

Diet no.	Total biomass		Live-wt gain (g)	Protein consumed (g)	PER	NPU
	Initial (g)	Final (g)				
1	295.3	678.4	383.1	215	1.78	0.42
2	297.5	567.7	270.2	210	1.29	0.35
3	297.9	506.9	209.0	171	1.23	0.38
4	298.2	420.6	122.4	138	0.89	0.34
5	290.6	431.2	140.6	240	0.60	0.23
6	288.5	442.5	154.0	184	0.84	0.30
7	279.1	455.2	176.1	180	0.98	0.33

PER, g wt gain per g protein eaten; NPU, $[B - (B_k - I_k)]/I$, where B and B_k are the total body nitrogen and I and I_k the nitrogen intakes of the fish on the test and low-protein diets respectively.

Table 7. Digestibility, for plaice, of the proteins used

Protein source	Concentration in dry diet (g/kg)	Digestibility ratio
Freeze-dried cod muscle	500	0.91
Soya-bean meal	400	0.68
BP protein concentrate	500	0.90
White fish meal	500	0.86
A fish-protein concentrate	500	0.89

uniformly high digestibility (Table 7). The digestibility of soya-bean meal was examined because this material, rather than promine-D, might feature in commercial diets. The low apparent digestibility of the protein in soya-bean meal may be due to the relatively high levels of carbohydrate in this material. Promine-D has a high digestibility. We have found that marine flatfish have very limited amylolytic activity in their gastrointestinal tract (unpublished), and it has been observed by Kitamikado, Morishita & Tachino (1964), using rainbow trout (*Salmo gairdnerii*), that high dietary starch levels have a detrimental effect on the digestibility of protein.

The measurements of apparent digestibility were made on fish weighing 150–200 g, that is, considerably larger animals than those on which the weight-gain measurements were made (mean initial weights 12–15 g, Table 3). Kitamikado *et al.* (1964) examined the relationship between digestibility and weight of rainbow trout; they found no significant differences in the digestibility of protein (casein, white fish meal) in fish of body-weight 'from about 10 g to 100 g' but in smaller fish ('less than about 6 g body-weight') apparent digestibility of these proteins showed considerably lower values. Nevertheless, the possibility that the feedstuffs we used differ in digestibility for small plaice (12–15 g) cannot be ruled out. It is not easy to apply our method for collection of faeces to small flatfish because the gastrointestinal tract is short and is concentrated in a small peritoneal cavity. There is a likelihood of damage to the fish and the expulsion of material from the mid-gut region, producing spurious results.

The gross composition of fish given the different diets was similar at the end of the experiment, mean values (\pm SE) being (g/kg): water 762.1 \pm 3.03, protein

Table 8. *Chemically measured essential amino acids* in diets containing the proteins under test at levels of 500 g crude protein/kg dry diet*

Amino acid	Amount in test protein (g/kg dry diet)					Essential amino acid requirement of Chinook salmon† (g/kg dry diet)
	Freeze-dried cod muscle	White fish meal	BP protein concentrate	Promine D	A fish protein concentrate	
Lysine	46.0	37.9	36.0	30.3	46.5	20
Histidine	11.0	9.9	10.0	12.3	14.0	7
Arginine	30.5	33.9	25.0	39.0	33.0	24
Threonine	22.0	18.9	26.0	18.5	26.5	9
Valine	24.0	23.3	28.5	24.1	29.5	13
Methionine	14.5	14.6	15.0‡	15.0‡	14.4	16
Isoleucine	23.5	19.4	23.5	24.2	24.5	9
Leucine	37.5	32.4	36.5	38.7	41.5	16
Phenylalanine	20.5	17.4	22.0	26.8	23.5	21 §
Tryptophan	6.0	4.7	7.0	6.7	5.0	2
Cystine	5.5	3.4	5.0	5.2	5.0	
Tyrosine	17.5	14.8	18.5	18.5	20.5	

* From Cowey *et al.* (1971).

† Results of Halver and co-workers summarized by Mertz (1969).

‡ Includes methionine used to supplement these proteins.

§ Phenylalanine and tyrosine together (with 4 g tyrosine/kg diet, 17 g phenylalanine/kg were required for maximal growth rate).

155.8 ± 0.74, lipid 51.0 ± 2.52 and ash 28.7 ± 0.47. Lipid was the most variable component, ranging from 43 g/kg live weight (diet 6) to 62 g/kg live weight (diet 3), values considerably higher than those of wild fish (Cowey *et al.* 1972).

DISCUSSION

Proteins differ in their ability to support the growth of plaice even when given at high dietary concentrations. Moreover, there is no evidence that these differences are due to differences in digestibility of the proteins concerned. A possible explanation may lie in a high requirement by the fish for one or more essential amino acids coupled with either a relative deficiency or a low biological availability of some essential amino acids in some of the proteins.

Since this paper was submitted, Ogino & Chen (1973) have reported results somewhat similar to ours but obtained from experiments with carp (*Cyprinus carpio*). They examined the biological value of four proteins (casein, white fish meal, dried egg yolk and maize-gluten meal) at dietary protein concentrations of between about 100 and 400 g/kg. The point of interest is that the relationships between biological value and dietary protein level which they obtained for all four proteins were virtually parallel to each other. Thus, differences in biological value were as evident at high protein intakes as at low protein intakes.

The only values available for quantitative requirements of fish for essential amino acids are those for Chinook salmon (*Oncorhynchus tshawytscha*) determined by Halver and his colleagues (summarized by Mertz, 1969) using diets containing

400 g protein/kg. These results are shown in Table 8. Two points are worthy of note; first, the value for phenylalanine is really the requirement for phenylalanine and tyrosine together, it having been shown that with 4 g tyrosine/kg diet the phenylalanine requirement was 17 g/kg diet. Secondly, the position regarding methionine requirement is complicated by the sparing action of cystine. With a cystine level of 0.5 g/kg diet, Chinook salmon failed to show maximum growth even with 16 g methionine/kg diet. When 10 g cystine/kg diet were present, the methionine requirement appeared to be 5-6 g/kg diet.

Also shown in Table 8 are the quantities of essential amino acids in the proteins given to plaice in the present experiments. When expressed as a proportion of the diet, the requirements of the fish for most of the essential amino acids are greater than the corresponding requirements of mammals. Nevertheless, when these proteins are given at the high dietary levels required by fish, essential amino acids are apparently supplied considerably in excess of requirement, with the possible exceptions of methionine and phenylalanine, for which there is some difficulty in fixing an exact requirement.

Thus the differences in growth rate of plaice observed here cannot be explained on the basis of the essential amino acid content of the proteins used. This is made especially manifest by a comparison of the growth of fish given diets 1 and 5; chemically measurable quantities of essential amino acids in the two proteins involved are similar, but growth rates and NPU are markedly different at comparable protein intakes. It remains possible that differences may exist in the biological availability of some essential amino acids in the proteins, but this has not yet been shown to be so. Consequently the problem of relating amino acid composition to nutritional value at high protein intakes remains unresolved.

Treatment of fatty fish with surface-active agents gave products which were both acceptable to the test fish (no pathological signs appeared as a consequence of feeding) and more or less similar to commercial feedstuffs in nutritional value. Surface-active agents have been used to obtain a fish-protein concentrate by Caiozzi, Arrieta, Villarroch & Rauch (1968) and by Connell (1969). The former group used the ionic detergent sodium dodecyl sulphate to extract fat from a full-fat fish meal and were able to reduce the diethyl ether-extractable fat from 46 g/kg to 2 g/kg by their best procedure. Connell (1969), on the other hand, used sodium dodecyl sulphate at a concentration of 50 g/l to solubilize protein from comminuted whole fatty fish; the solubilized protein was then easily separated from the denser skin and bones and the less dense oil. Recovery of the solubilized protein was achieved by the use of either organic solvents (giving a product containing sodium dodecyl sulphate up to 40 g/kg) or by precipitation at pH 3-4 (giving a product containing up to 400 g sodium dodecyl sulphate/kg). Connell took the view that the latter product was unacceptable because of its detergent content, and the use of organic solvents to recover solubilized protein offered no advantage in cost over the procedures at present used.

The process used in the present experiment was designed to emulsify fat rather than solubilize protein; even so, considerable amounts of protein were lost and appreciable lipid remained in the product. No attempt was made to measure residual

detergent in the products but, as the fish ate diets containing high concentrations of them for 10 weeks without obvious signs of toxicity, this does not appear to be a particular problem. Since this work was carried out Hikota & Meguro (1969) have examined the properties of another group of surface-active agents, alkylsulphoacetates. These are said to be hydrolysed by dilute acid at 40°. Provided their emulsifying power is sufficiently great, these reagents may be of considerable use in the type of process envisaged because their removal from the product ought to be relatively easy. In fact, a readily hydrolyzable, surface-active agent of high emulsifying power could give rise to a useful process for the production of low-fat, high-protein meals from trash fatty fish.

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