Proc. Nutr. Soc. (1975), **34**, 57

Aspects of protein utilization by fish

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Most animals which have been domesticated as farm animals are either omnivorous or herbivorous. By contrast most fish are carnivorous and many which are farmed, or are considered potential species for farming, have a high dietary protein requirement. As food proteins are expensive, fish farming has tended to concentrate on esteemed species with a high market value.

In general, three types of feeding regimen have been adopted, namely: (1) a pelleted ration containing a sufficiency of known nutrients; (2) trash fish (i.e. fish of little commercial value used as food); (3) a natural food chain which includes invertebrate herbivores as a component of the system.

Natural food chains such as those employed in the farming of carp may proceed at different levels: (1) non-intensive with low stocking density, fertilization of the water with compounds containing nitrogen or phosphorus to stimulate a food chain, and no supplemental feeding; (2) semi-intensive with medium stocking density, supplemental feeding with maize, wheat or barley, and water fertilization only if the excretory products of the fish are inadequate to support a food chain; (3) intensive with high stocking density, feeding of a pelleted ration of relatively high protein content and no fertilization of pond water. At all levels of intensity, polyculture techniques (stocking together of fish utilizing different types of natural food) give superior results. Thus, for instance, carp, tilapia and mullet might be grown together.

Dietary protein and amino acid requirements

The dietary protein levels necessary for maximal growth of several species of young fish are shown in Table 1. The values given contrast markedly with protein levels in the rations of conventional terrestrial farm animals. In arriving at the values shown in Table 1 most investigators claim to have used isoenergetic diets to obtain dose-response curves. This claim is frequently based on the assumption that carbohydrate and protein are isoenergetic for fish. As ammonia, rather than either urea or uric acid, is the main end-product of N metabolism in fish, food protein may have a higher metabolizable energy content than carbohydrate in fish (Cowey, Pope, Adron & Blair, 1972). Until this matter is resolved and the metabolizable energy content of protein for fish is fixed with certainty, difficulties will remain in arriving at precise values for the energy content of diets and in the expression of protein requirement in relation to dietary energy level.

Table 1. *Estimated dietary protein requirement of certain species of juvenile fish*

Fish species	Crude protein level in diet for optimal growth (g/kg)	Reference
Rainbow trout (*Salmo gairdnerii*)	400	Satia (1974)
Carp (*Cyprinus carpio*)	380	Ogino & Saito (1970)
Chinook salmon (*Oncorhynchus tschawytscha*)	400	DeLong, Halver & Mertz (1958)
Eel (*Anguilla japonica*)	445	Nose & Arai (1972)
Plaice (*Pleuronectes platessa*)	500	Cowey, Pope, Adron & Blair (1972)

The high dietary protein requirement of fish suggests that their requirement for essential amino acids is also likely to be relatively high. This has proved to be so in the only species for which complete quantitative information on essential amino acid requirements is available, namely Chinook salmon *(Oncorhynchus tschawytscha)*. These results, from J. E. Halver and his colleagues, have been summarized by Mertz (1969) and appear in Table 2. The requirements of Chinook salmon for essential amino acids are indeed appreciably greater than those of omnivorous birds and mammals.

Table 2. *Requirement of Chinook salmon* (Oncorhynchus tschawytscha), *and certain omnivorous animals for essential amino acids (g/kg dry diet)**

Amino acid	Chinook salmon fingerling	Chick	Young pig	Rat
Arginine	2·4	1·1	0·2	0·2
Histidine	0·7	0·3	0·2	0·4
Isoleucine	0·9	0·8	0·6	0·5
Leucine	1·6	1·2	0·6	0·9
Lysine	2·0	1·1	0·65	1·0
Methionine†	1·6	0·8	0·6	0·6
Phenylalanine‡	2·1	1·3	0·46	0·9
Threonine	0·9	0·6	0·4	0·5
Tryptophan	0·2	0·2	0·2	0·2
Valine	1·3	0·8	0·4	0·4

*From Mertz (1969).
†In the absence of cystine.
‡In the absence of tyrosine.

It should be emphasized that the essential amino acid requirements of Chinook salmon may differ appreciably from those of other species and these values cannot be safely extrapolated to the formulation of rations for other species of fish. In fact requirements of Japanese eel *(Anguilla japonica)* for at least two essential amino acids are substantially greater than those of Chinook salmon (T. Nose & Y. Hashimoto, personal communication).

Nutritional quality of food proteins

Since fish are more demanding than are birds and mammals in their requirement for essential amino acids, greater amounts of high-quality protein must be supplied in their food. This demand has generally been met by fish meal and virtually all

successful published diets (Halver, 1972) contain substantial amounts (100 g/kg or more) of crude protein derived from this commodity. Moreover, attempts to replace all or part of the fish meal in piscean rations have not so far proved successful. Thus, when soya-bean meal was substituted on an isonitrogenous basis for menhaden meal in catfish (*Ictalurus punctatus*) diets, growth and food conversion efficiency fell significantly (Andrews & Page, 1974). Addition of methionine, cystine and lysine (the most limiting amino acids) to these soya-bean-substituted diets did not enhance growth or food conversion. This finding raises doubt as to whether, as in the poultry industry, plant protein sources can replace fish meal extensively in piscean foods.

Comparatively little information exists on the relative nutritional quality of food proteins for fish, and few of the available methods have yet been rigorously applied to fish. There is a considerable need for such information as well as for knowledge of the first limiting amino acids in food proteins given to fish. Such knowledge appears mandatory for the rational formulation of the protein component of practical fish diets.

Values obtained for the nutritional quality of food proteins by two separate groups of workers using different species of fish have given different values. Ogino & Chen (1973) measured the biological value (BV) of a number of proteins for carp at a dietary protein level of 100 g crude protein/kg. They obtained high values comparable with those of Miller & Bender (1955) using rats (Table 3). By contrast Cowey *et al.* (1972), using plaice *(Pleuronectes platessa)*, obtained a markedly lower value for net protein utilization (NPU) of freeze-dried cod muscle than Miller & Bender (1955) obtained with a similar material using rats (0·63 *v.* 0·83).

Table 3. *The relative nutritional quality of certain dietary proteins* for carp* (Cyprinus carpio) *and rats*

Protein	Biological value for carp†	Net protein utilization for rats‡
Casein	0·80	0·60
Whole egg	—	0·91
Dried egg yolk	0·89	—
Soya-bean meal	0·74	0·56
Wheat germ	0·78	0·67

*Dietary protein level 100 g/kg.
†Results of Ogino & Chen (1973).
‡Results of Miller & Bender (1955).

One point that the results of Ogino & Chen (1973) and Cowey, Adron, Blair & Shanks (1974) have in common is that differences in nutritional value of food proteins for fish are evident even at high protein intakes. By contrast, NPU values for proteins of high and low nutritional quality obtained using rats differ widely at low protein intakes but are very similar at high protein intakes (Miller & Payne, 1961). Presumably, at high protein intakes, essential amino acid requirements of the rat are met even by proteins of low nutritional quality provided the protein intake is sufficiently high. The persistence of differences in nutritional quality of different proteins for fish even at high protein intakes may be an expression of the high essential amino acid requirement of the fish.

A feature of the results of Ogino & Chen (1973) is the high BV obtained for various proteins given at high levels in the diet. At a dietary protein level of 400 g/kg, the BV obtained for dried egg yolk, casein, white fish meal and maize gluten meal were approximately 0·70, 0·65, 0·65 and 0·40 respectively. This is unexpected as it implies that, even at high protein intake, the synthesis of new tissue protein from assimilated amino acids exceeds the oxidation of amino acids. In fact high protein intakes would tend to elevate the tissue levels of (1) enzymes which catabolize amino acids and (2) the amino acids themselves. Both factors would accelerate amino acid oxidation (Krebs, 1972) and it is somewhat surprising that, at high dietary protein levels, more ingested food is retained as tissue protein than is burned as an energy source.

Effect of dietary protein level on amino acid metabolism

Recent studies on mammals have indicated that during dietary protein restriction metabolic mechanisms operate to conserve essential amino acids (McFarlane & von Holt, 1969). The oxidation of non-essential amino acids was not, however, appreciably altered by the level of protein in the diet. Sketcher, Fern & James (1974) confirmed this effect in rats with experiments on the metabolism of ^{14}C-labelled leucine. However, other experiments did not provide any evidence for the conservation of essential amino acids under conditions of protein restriction (Neale, 1971, 1972).

The interpretation of experiments in which isotopically labelled amino acids are administered either orally or by injection to animals is complicated by factors such as pool size, compartmentation and the position of the ^{14}C label in the amino acid (see, for example, Reeds, 1974).

Nevertheless it was considered of interest to ascertain the effects, if any, of dietary protein level on the metabolism of ^{14}C-labelled amino acids in fish. To this end plaice, which had been given diets containing either 60 or 500 g crude protein/kg, for not less than 3 weeks, were injected intraperitoneally with [1-^{14}C]leucine, [1-^{14}C]phenylalanine or [U-^{14}C]glutamic acid. Expiration of $^{14}CO_2$ and incorporation of radioactivity into liver and carcass protein during the ensuing 24 h period was then measured. The fish were not fed during this period.

The results are shown in Table 4. Glutamate was oxidized more rapidly than either of the essential amino acids, an effect to be expected since the deamination product of glutamate, α-ketoglutarate, is a tricarboxylic acid cycle intermediate. There was no significant difference in the rates of glutamate oxidation in fish given either high or low levels of dietary protein. Of the two essential amino acids, the metabolism of [1-^{14}C]phenylalanine was apparently unaffected by the dietary protein level. The conversion of [1-^{14}C]leucine to CO_2 was not significantly changed by the level of protein in the food, although there was significantly greater incorporation of [1-^{14}C]leucine into carcass and liver protein in plaice given the low-protein diet.

Variation in dietary protein intake is likely to affect the size of amino acid pools in tissues so that direct comparison of the metabolism of tracer amounts of amino

Table 4. *Incorporation of radioactivity from* L-[1-^{14}C]*leucine,* L-[1-^{14}C]*phenylalanine and* L-[*U*-^{14}C]*glutamic acid in vivo into liver protein, carcass protein and carbon dioxide of plaice* (Pleuronectes platessa) *given either a high- or low-protein diet*

(Mean values with their standard errors)

Amino acid	Protein level in diet (g/kg)	Oxidation as $^{14}CO_2$ (% dose given)	Incorporation into liver protein (% dose given)	Incorporation into carcass protein (% dose given)
Leucine	60	21·58±2·37	2·00±0·27[a]	25·58±4·67[b]
	500	14·58±3·33	0·84±0·20[a]	12·20±2·04[b]
Phenylalanine	60	11·23±4·76	1·37±0·15	21·30±2·84
	500	9·18±4·21	1·28±0·25	23·04±3·84
Glutamic acid	60	33·63±2·77	0·67±0·22	5·13±0·84
	500	40·23±1·45	0·27±0·03	2·93±0·58

[a] Values significantly different ($P<0·01$).

[b] Values significantly different ($P<0·02$).

acids in animals given different levels of dietary protein is hazardous. However, it would appear reasonable to assume that, in any given tissue, the same amino acid pool basically serves both catabolic (oxidative) and anabolic (protein synthesis) processes. The effect of dietary protein intake on amino acid metabolism may then be examined by comparing the ratio of amino acid oxidized on a low-protein diet : amino acid oxidized on a high-protein diet with the ratio of amino acid incorporated into protein on a low-protein diet : amino acid incorporated into protein on a high-protein diet.

These ratios are shown in Table 5. They indicate that there are differences in the pattern of essential amino acid conservation, under conditions of dietary protein restriction, for the two essential amino acids. With phenylalanine, dietary protein level appeared to alter neither the rate of catabolism of the amino acid nor the rate at which it is incorporated into protein. With leucine, low-protein diets did lead to greater enhancement of incorporation into protein than of expiration as CO_2. However, a somewhat similar picture emerges for the metabolism of the non-essential glutamic acid.

Table 5. *Effect of dietary protein level on incorporation of radioactivity into carbon dioxide, liver protein and carcass protein from intraperitoneally injected* L-[1-^{14}C]*leucine,* L-[1-^{14}-*C*]*phenylalanine and* L-[*U*-^{14}C]*glutamic acid in plaice* (Pleuronectes platessa)

(Results are given as the ratio of the radioactivity incorporated by plaice given a low-protein diet (60 g crude protein/kg) to that of plaice given a high-protein diet (500 g crude protein/kg))

Amino acid	Oxidation as $^{14}CO_2$	Incorporation into liver protein	Incorporation into carcass protein
Leucine	1·5	2·4	2·1
Phenylalanine	1·2	1·1	0·9
Glutamic acid	0·8	2·5	1·8

https://doi.org/10.1079/PNS19750011 Published online by Cambridge University Press

The results suggest that plaice have no general mechanism for conserving essential amino acids as opposed to non-essential amino acids under conditions of dietary protein restriction.

Effect of dietary energy level on protein utilization

Several studies have been made of the sparing action of both carbohydrate and fat on protein requirement. An issue has been the extent to which fish can utilize dietary carbohydrate and whether elevated carbohydrate levels are deleterious. Buhler & Halver (1961) fed high levels (480 g/kg diet) of both monosaccharides and of dextrin to Chinook salmon and obtained protein efficiency ratios (PER) of about 2·0 without serious elevation of either liver glycogen or blood sugar levels. Palmer & Ryman (1972), on the other hand, showed that trout (*Salmo gairdnerii*) suffered from pronounced and persistent hyperglycaemia (with concomitant increases in liver glycogen) following oral administration of heavy loads of glucose. This implies that glucose tolerance in the fish is low and that metabolism of glucose is not rapid; it was inferred that fish suffer some degree of insulin deficiency.

When plaice were given diets containing up to 200 g carbohydrate/kg, as a mixture of equal amounts of glucose and dextrin, liver glycogen levels were only moderately elevated to 56 mg/g fresh liver compared with 25 mg/g fresh liver in plaice given diets free of carbohydrate (Cowey, Adron, Brown & Shanks, 1975). Moreover, diets containing these levels of carbohydrate gave higher PER and NPU values than iso-energetic diets which were free of carbohydrate.

The oxidation of intraperitoneally injected [U-^{14}C]glucose by plaice proceeded slowly and Cowey *et al.* (1975) were not able to induce any glucokinase (*EC* 2.7.1.2) in the livers of the fish. The latter observations suggest that plaice have only a moderate capacity to metabolize carbohydrate, but at the dietary levels used no pathological consequences were evident.

From a practical point of view there may be limited scope for achieving protein sparing via carbohydrate in at least some species of fish. In production diets carbohydrate would generally be present as starch; however, when the amount of starch in trout diets is increased, progressively less of it is absorbed (Singh & Nose, 1967). Thus with 200 g starch/kg the apparent digestibility was 0·69 but with 600 g starch/kg diet the apparent digestibility was only 0·26.

In line with this finding, two recent studies on the interaction of dietary protein and energy have used starch levels up to approximately 250 g/kg. Page & Andrews (1973) found that maize starch (up to 250 g/kg diet) and fat (up to 120 g/kg diet) were equally effective as energy sources for channel catfish. The estimated digestible energy derived from maize starch was 11·3 kJ (2·7 kcal)/g. In these experiments increasing energy levels at constant dietary protein level always resulted in improved food efficiency. At the same time an increase in the ratio digestible energy: protein in the diet led to an increased deposition of lipid in the fish although this effect was said to be less marked in channel catfish than in chickens.

Much higher dietary fat levels (up to 240 g/kg in some treatments) were used in a similar study on trout (Lee & Putnam, 1973). Food conversion was markedly

influenced by dietary energy level, PER being negatively correlated with the ratio dietary protein : energy. The best PER (3·0) was obtained with a diet containing 360 g protein, 240 g fat and 267 g carbohydrate/kg. On this latter regimen the body lipid increased from an initial value of 63 g/kg to 129 g/kg after 18 weeks.

Elevation of body fat as dietary energy levels were increased by the use of fat has also been observed in marine flatfish (Cowey *et al.* 1975). Any gross alteration in the composition of a food fish farmed in captivity seems undesirable, and some control in the levels of dietary fat used in fish cultivation may be advisable. Different species of fish from the natural environment contain very different amounts of fat in their muscle. Many marine fish have very little fat in their muscle (e.g. turbot, sole) and the production of relatively fatty flesh in farmed fish of these species may well prove unacceptable. Other fish such as trout and salmon contain substantial amounts of fat in their muscles and a moderate increase in this muscle fat level in farmed fish may not render them any less acceptable to the consumer than are their wild counterparts.

REFERENCES

Andrews, J. W. & Page, J. W. (1974). *J. Nutr.* **104**, 1091.
Buhler, D. R. & Halver, J. E. (1961). *J. Nutr.* **74**, 307.
Cowey, C. B., Adron, J., Blair, A. & Shanks, A. M. (1974). *Br. J. Nutr.* **31**, 297.
Cowey, C. B., Adron, J. W., Brown, D. A. & Shanks, A. M. (1975). *Br. J. Nutr.* **33**, 219.
Cowey, C. B., Pope, J. A., Adron, J. W. & Blair, A. (1972). *Br. J. Nutr.* **28**, 447.
DeLong, D. C., Halver, J. E. & Mertz, E. T. (1958). *J. Nutr.* **65**, 589.
Halver, J. E. (1972). In *Fish Nutrition*, p. 651 [J. E. Halver, editor]. New York: Academic Press.
Krebs, H. A. (1972). In *Advances in Enzyme Regulation*, vol. 10, p. 387 [G. Weber, editor]. New York: M. Dekker Inc.
Lee, D. J. & Putnam, G. B. (1973). *J. Nutr.* **103**, 916.
McFarlane, I. G. & von Holt, C. (1969). *Biochem. J.* **111**, 557.
Mertz, E. T. (1969). In *Fish in Research*, p. 233 [O. W. Neuhaus and J. E. Halver, editors]. New York: Academic Press.
Miller, D. S. & Bender, A. E. (1955). *Br. J. Nutr.* **9**, 382.
Miller, D. S. & Payne, P. R. (1961). *Br. J. Nutr.* **15**, 11.
Neale, R. J. (1971). *Nature New Biol.* **231**, 117.
Neale, R. J. (1972). *Biochim. biophys. Acta* **273**, 80.
Nose, T. & Arai, S. (1972). *Bull. Freshwat. Fish. Res. Lab., Tokyo* **22**, 145.
Ogino, C. & Chen, M. (1973). *Bull. Jap. Soc. scient. Fish.* **39**, 797.
Ogino, C. & Saito, K. (1970). *Bull. Jap. Soc. scient. Fish.* **36**, 250.
Page, J. W. & Andrews, J. W. (1973). *J. Nutr.* **103**, 1339.
Palmer, T. N. & Ryman, B. E. (1972). *J. Fish Biol.* **4**, 311.
Reeds, P. J. (1974). *Br. J. Nutr.* **31**, 259.
Satia, B. P. (1974). *Progve Fish Cult.* **36**, 80.
Singh, R. P. & Nose, T. (1967). *Bull. Freshwat. Fish. Res. Lab., Tokyo* **17**, 21.
Sketcher, R. D., Fern, E. B. & James, W. P. T. (1974). *Br. J. Nutr.* **31**, 333.

Printed in Great Britain