Aquaculture

Aquaculture 124 (1994) 1–11

Review

Amino acid requirements of fish: a critical appraisal of present values

C.B. Cowey*

Department of Nutritional Sciences, University of Guelph, Guelph, Ont., N1G 2W1 Canada

Abstract

There are large variations in the measured essential amino acid requirements of different species of fish when expressed as a proportion of the diet. The question of whether or not these are real differences is considered. Dietary amino acids are needed for growth and for maintenance, and the former is quantitatively much the more important in young, rapidly growing fish. It is noted that the amino acids laid down during growth are sensibly the same in different species. Maintenance is considered to consist of losses from the integument and intestine, from oxidation of amino acids, from conversion of amino acids to other N-molecules and from protein turnover. Losses from these causes are considered and are not thought likely to differ appreciably between species. When amino acid requirements are expressed as a proportion of the dietary protein, differences, while reduced somewhat, are still wide. The dilemma is illustrated by reference to the differences in amino acid requirement values for rainbow trout from different laboratories. Factors likely to affect the overall performance of fish in requirement studies (water quality, different sources of amino acids and so on) are enumerated, but are not thought likely to explain the observed discrepancies. Dietary energy density is an important factor affecting amino acid requirement, but there are uncertainties surrounding metabolisable energy contents of major dietary components and this tends to preclude expression of amino acid requirement in terms of metabolisable energy. Other methods of assessing amino acid requirement are regarded as subsidiary to, and confirmatory of, growth data. Where amino acid deficiencies lead to tissue pathologies it is important that the stated requirement level is such as to prevent such pathologies. A table of requirement values for channel catfish and trout is provided; it is based on all published values but with greater weight being given to studies characterised by high rates of growth. The relative proportions of essential amino acids in the requirement pattern of the two species bear a strong similarity.

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* Present address: Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB9 2TN, UK.

SSDI 0044-8486(94)00063-T

1. Introduction

The essential amino acid requirements for several species of fish have been measured over the past 20 years or so. The values obtained, when expressed as a proportion of the diet, indicate large variations in the requirements of different species. For many of the amino acids shown in Table 1, the variation in reported requirement values between species is at least two-fold and in some cases approaches three-fold.

The immediate question is whether or not these are real differences. Would such differences be expected, on the basis of what is known of fish growth and metabolism? Dietary amino acids are required for two purposes, firstly for growth, which mainly consists of protein deposition, and secondly for a number of processes that are conceptually described as maintenance. The relative requirements for protein deposition and maintenance clearly depend on the relative rate of growth: the amino acid requirements of fish virtually all apply to young, rapidly growing animals so that growth should be the major component of requirement. In this context the maintenance component of the protein requirement for growth in the rat accounts for about 22% of the total (Reeds, 1988). That of fish is likely to be much smaller as, among other things, they do not expend energy maintaining body temperature.

Growth

Much of the protein being laid down as tissue protein during growth consists of relatively large amounts of a small number of different proteins such as myosin, actin, collagens and so on. While important, interspecific differences undoubtedly exist between these proteins, they are, nevertheless, molecules in which amino acid sequences have been highly conserved during evolution and few differences in amino acid composition are evident between them. It is well established that whole-body protein of different species of fish differs little in amino acid com-

Table 1
Measured requirements of certain amino acids in channel catfish, chinook salmon and common carp

	Percent of dry diet		
	Channel catfish	Chinook salmon	Common carp
Dietary protein (%)	24	40	38.5
Arginine	1.0	2.4	1.7
Methionine + cystine	0.6	1.6	1.2
Tryptophan	0.12	0.2	0.3
Phenylalanine + tyrosine	1.2	2.1	2.5
Lysine	1.2	2.0	2.2
Threonine	0.48	0.9	1.5

Values are from Wilson (1989) which should be consulted for primary references.

position (Wilson and Cowey, 1985). Consequently amino acid requirements of different species for growth would not be expected to differ greatly.

Maintenance

The processes involved in maintenance comprise replacement of protein lost via the body surface (integument) and gastrointestinal tract, together with losses due to (1) oxidation of amino acids, (2) synthesis of other N-compounds from amino acids, and (3) protein turnover.

Comparatively large amounts of ingested amino acids are deaminated and their carbon skeletons used as a source of energy. Net retention of dietary N in fish is in the range 30–40% so that much of the dietary protein is lost to the animal. Interestingly, retention of dietary protein is of a similar order in birds and mammals (Bowen, 1987). Whether or not there is any marked degree of specificity with respect to which amino acids are oxidised is by no means finally resolved. For example, kinetic mechanisms appear to exist at the substrate level which would conserve essential amino acids at the expense of non-essential amino acids (Cowey and Walton, 1989). It is unlikely, however, that marked differences would occur, between fish species, in the pathways or control mechanisms involved in amino acid oxidation, or that differences in amino acid requirement would arise from this source.

Many other N-compounds are formed from amino acids; these include purines (glycine, glutamine), polyamines and methylated compounds (methionine), catecholamines (phenylalanine), thyroid hormones (tyrosine), carnitine (lysine), creatine (arginine, glycine), histamine (histidine), taurine (cysteine) and serotonin (tryptophan). Although qualitatively and physiologically important, the flow of amino acids via these pathways is low compared to that involved in amino acid oxidation or protein synthesis. In addition, pool sizes and turnover rates of these compounds seem unlikely to differ in different fish species.

Protein turnover itself does not necessarily result in a net loss of amino acids from the body. Losses may occur during turnover as a result of the activity of catabolic enzymes present in the tissues and the loss of an essential amino acid may restrict the degree to which other amino acids can be re-incorporated. It should be noted, however, that rates of protein turnover in fish muscle are very low indeed (Smith, 1981; Houlihan et al., 1986) and as muscle accounts for about 60% of the weight of the fish whole-body protein turnover is not likely to be a big factor in amino acid losses. Nor is it likely to differ qualitatively between species.

2. Amino acid requirement and protein intake

Amino acid requirements have been determined by dose–response curves in which the response measured has usually been weight gain. Requirement levels have then been obtained from the data by broken line analysis, applying a range test or by fitting a growth curve.

In warm-blooded animals a constant relationship was shown between essential

Table 2
Measured requirements of certain amino acids in channel catfish, chinook salmon and common carp

	Percent of dietary protein		
	Channel catfish	Chinook salmon	Common carp
Arginine	4.3	6.0	4.3
Methionine + cystine	2.3	4.0	3.1
Tryptophan	0.5	0.5	0.8
Phenylalanine + tyrosine	5.0	5.1	6.5
Lysine	5.1	5.0	5.7
Threonine	2.0	2.2	3.9

Values are from Wilson (1989) which should be consulted for primary references.

Table 3
Values for arginine and tryptophan requirement of juvenile rainbow trout measured in different laboratories

Crude protein in diet (%)	Requirement (% dietary protein)	Requirement (% dry diet)	Reference
Arginine			
36	3.3	1.19	Kaushik (1979)
45	3.6	1.62	Walton et al. (1986)
35	4.0	1.41	Kim et al. (1992b)
33	4.7	1.55	Cho et al. (1992)
47	5.9	2.77	Ketola (1983)
Tryptophan			
55	0.5	0.28	Walton et al. (1984)
35	0.6	0.21	Kim et al. (1987)
42	1.4	0.59	Poston and Rumsey (1983)

amino acid requirement and protein intake up to the level of protein needed for maximum growth (Almquist, 1972). For several essential amino acids, intake and weight gain were apparently linearly related and this relationship was presumed to hold for all essential amino acids. On this basis amino acid requirements of fish have been expressed as a percentage of dietary protein as well as on a dry-matter basis.

Later studies bear on the finding of Almquist (1972) in that they show the relationship to be not a linear but an exponential function (Finke et al., 1987). The response of an animal to a given nutrient does not break at a particular point. An accurate representation of the so-called "diminishing returns" area of the response curve is claimed (Finke et al., 1989) to be critical in assessing the efficiency of incremental increases of dietary amino acid concentration as the response approaches the maximum. The use of a logistic model supports a more accurate assessment, than is provided by broken line analysis, of the diminishing returns area of the response curve and of the maximum response (Finke et al., 1989).

The implication of these later studies is that essential amino acid requirements are not best expressed as a percentage of dietary protein. Nevertheless, because the dose–response relationship is sensibly linear for much of its length (e.g. Fig. 3 of Gahl et al. 1991 shows it to be linear for up to about 85% of the asymptotic value for N-gain), amino acid requirements can reasonably be described as a percentage of dietary protein. In this mode, variations are less than when expressed as a proportion of the dry diet; even so, there are obvious discrepancies between the requirements of different species (Table 2).

The extent of the dilemma is further illustrated by reference to Table 3. These show reported values from different laboratories for arginine and tryptophan requirement of rainbow trout. Whether stated in terms of dry diet or of dietary protein, wide variations are evident, variations that exceed credibility.

3. Causes of apparent species differences

Some, at least, of the discrepancies observed may be attributed to different overall levels of performance by the fish. In some experiments growth rates have been very low—much lower than would be the case with a good practical diet; this would militate against accurate measurement of requirements. In general,low rates of growth give rise to high requirement levels.

These different overall levels of performance might arise for a number of reasons (see also Kim et al., 1992b who refer to "laboratory variances"), from differences in water quality, water flow rate and biomass density. The use of different "reference" proteins as sources of amino acids may affect their availability. In some experiments diets with large amounts of crystalline amino acids have given lower growth rates than diets containing whole protein (Robinson et al., 1981; Walton et al., 1982), although more recently both Cho et al. (1992) and Kim et al. (1992a,b) have obtained good growth rates with diets containing high levels of crystalline amino acids.

When all these factors are considered, it remains highly questionable as to whether they provide a satisfactory explanation of the discrepancies observed in published amino acid requirements.

A factor of particular importance in amino acid requirement studies is energy density. Requirements (expressed in terms of dietary concentration) would be expected to increase as energy density of the diet increases. Quantitative information concerning the effects of energy density of the diet on the metabolisable energy content of feedstuffs at high energy densities, however, is inadequate and precludes listing of amino acid requirements on the basis of dietary energy. Available data suggest that at higher or lower energy densities amino acid requirements (as percent diet) will need to be adjusted upward or downward, respectively.

4. Methods other than growth

In several studies growth data have been supplemented by other measurements considered useful in confirming the values obtained. These measurements have basically centred around changes in the concentration of the amino acid under examination in plasma, liver or muscle. At sub-requirement intakes of the amino acid under study its concentration in free amino acid pools should be consistently very low; as dietary concentration increased beyond the requirement level, then tissue concentrations of the amino acid would increase. A sharp change (increase) in concentration of the amino acid in blood or tissues as dietary level of the amino acid increased would be seen as indicating the requirement level.

Direct and indirect oxidation studies are also based on tissue free amino acid concentrations. At low intakes, rates of oxidation of the amino acid under study would be low (because concentrations in the free amino acid pool would be small) and would remain low until the requirement level had been reached when they would increase sharply. Indirect oxidation studies measure the oxidation of an essential amino acid other than the one under study. In this instance incorporation of this other amino acid into tissue protein is limited at low (sub-requirement) intakes of the amino acid under study; consequently high rates of oxidation of this other amino acid will occur. As dietary concentration of the amino acid under study increases, tissue protein synthesis will increase progressively, and the amounts of other amino acids being oxidised will decrease as proportionately larger amounts are used for protein synthesis.

These methods have always been subsidiary to growth studies and a recent paper on amino acid catabolism (Kim et al., 1992c) concluded that "the use of amino acid oxidation techniques for the determination of amino acid requirements of fish is probably inappropriate".

5. Tissue pathology and amino acid requirement

Few amino acid deficiencies lead to well-defined pathologies; deficiency is generally manifest as a reduction in weight gain. In certain fish species, however, a deficiency of tryptophan or methionine does lead to pathologies and the relationship between amino acid intake and the incidence of pathology may provide useful information with respect to requirement.

Tryptophan deficiency led to scoliosis of the trunk in chum salmon fry (Akiyama et al., 1985); this occurred in 53–84% of the fish given a diet containing less than 0.16% supplemental tryptophan (the requirement being estimated as 0.29% of the diet). With 0.32% tryptophan in the diet scoliosis still apparently occurred with an incidence of 1.5%.

Were (1989) observed the occurrence of scoliosis, cataract and caudal fin erosion in rainbow trout deficient in tryptophan. A significant incidence of cataract (11%)—but not of scoliosis or caudal fin erosion—was observed in fish given a diet containing 0.26% tryptophan (requirement by broken line analysis being

0.27%). Some cataract (2%) was evident at higher levels of tryptophan intake. Were (1989) comments that if "deficiency symptoms still exist at that level (a level of tryptophan intake that gives maximal growth) then the criteria used for setting the requirement should be re-evaluated".

Methionine deficiency in rainbow trout led to the presence of cataracts (evident by simple visual inspection) in virtually all (95%) fish. No cataract was evident by visual inspection in fish fed a diet with 0.76% methionine (the requirement level from weight gain data). However, analysis of focal length variability of the lenses with a scanning lens monitor demonstrated that if abnormality of the lens was to be avoided, a higher concentration of dietary methionine (0.96%) is needed than that necessary to maximize growth (Cowey et al., 1992).

6. Amino acid requirements

The amino acid requirements of channel catfish and of rainbow trout are shown in Table 4. These values, from the National Research Council (1993), were arrived at by considering all the available data. While all experiments were considered, not all were given equal weight. Thus, recent experiments with more rapidly growing fish were given more emphasis than older experiments in which overall growth rate of fish was slower.

As a proportion of the diet, wide differences are again apparent in the requirements of the two species. However, the diets used were of different energy densities; consequently the relative proportions of amino acids have been considered—giving a requirement pattern. This pattern shows a close similarity, between the two species, for almost all amino acids; there are few disparities—none of them marked.

Table 4
Amino acid requirements of rainbow trout and channel catfish compiled from an assessment of published data

	Percent of diet		Pattern relative to leucine	
	Channel catfish	Rainbow trout	Channel catfish	Rainbow trout
Arginine	1.20	1.45	1.22	1.07
Histidine	0.42	0.65	0.43	0.48
Isoleucine	0.73	0.85	0.75	0.63
Leucine	0.98	1.35	1.00	1.00
Lysine	1.43	1.75	1.46	1.30
Methione + cystine	0.64	0.95	0.65	0.70
Phenylalanine + tyrosine	1.40	1.75	1.43	1.30
Threonine	0.56	0.75	0.57	0.56
Tryptophan	0.14	0.20	0.14	0.15
Valine	0.84	1.15	0.86	0.85

7. An additional method

An optimal dietary amino acid pattern for growing pigs was recently obtained from experiments on N-retention (Fuller et al., 1989; Wang and Fuller, 1989). Their experiments were based on the concept that when a single amino acid is limiting in the diet the rate of body protein accretion is directly related to that one amino acid. Conversely, removal of a non-limiting amino acid has no effect on N-retention. By measuring the changes in N-retention when a proportion of each amino acid in turn was removed from a standard diet the authors were able to arrive at a dietary amino acid pattern in which all amino acids were equally limiting.

As noted above, recent experiments have shown that high growth rates can be obtained when rainbow trout are given diets containing large amounts of crystalline amino acids (Kim et al., 1992a). Earlier, March et al. (1985) had shown the feasibility of using small rainbow trout, selected for uniformity of body weight, in 3-week experiments to assay differences in protein quality of feedstuffs. In doing this they could readily measure differences in protein retention between treatments.

It therefore seems feasible that the procedures applied by Fuller and his colleagues to pigs could be adapted for use with fish. Such an approach, if successful, might do much to increase confidence in measured amino acid requirements of fish.

8. Non-essential amino acids as an energy source

Kim et al. (1991) recently showed that the dietary protein requirement of rainbow trout is not more than 30% on a dry matter basis—the lowest protein level used in their experiment. Protein requirement is probably best expressed in terms of dietary energy because, at dietary protein levels necessary for optimal growth, much of it is, in fact, utilized as an energy source. The 30% protein diet of Kim et al. actually supplied 16.1 MJ digestible energy/kg diet with 17.6 g digestible protein/MJ digestible energy (DP/DE ratio), a value in line with currently accepted values.

In a second experiment Kim et al. (1991) gave a diet containing 25% crude protein (and supplying all essential amino acids in quantities sufficient to meet requirement) together with a non-essential amino acid mixture equivalent to 10% protein (in conventional terms a 35% protein diet) to rainbow trout. It led to growth equal to that obtained with a diet containing 35% crude protein. The authors made the surprising conclusion that the protein requirement of trout is 25% "when appropriate energy sources that have ME values equivalent to protein are used to substitute for protein". This is a curious inference in that a mixture of non-essential amino acids is not seen as part of the protein component. It begs the question of why fat is not an "appropriate energy source". It has never been the case that non-protein energy sources, used to spare dietary protein, must have

ME values equivalent to protein, but this is implicit in the dictum of Kim et al. (1991).

The 25% protein diet of Kim et al. (1991) actually contained 16.6 MJ DE/kg and had a DP/DE ratio of 20.1 g/MJ (the non-essential amino acid mixture being part of the protein component). This value is appreciably higher than that of the 30% protein diet referred to above. The 35% protein diet of Kim et al. (1991) contained 16.5 MJ DE/kg and had a DP/DE ratio of 20.0 g/MJ—sensibly the same as the so-called 25% protein diet. Had a 25% protein diet (with fat substituting on a digestible energy basis for the non-essential amino acid mixture) been used, it would have provided 16.9 MJ DE/kg but with a DP/DE ratio of only 14.0 g DP/MJ DE. It would almost certainly have given lower rates of growth.

The experiments of Kim et al. (1991) also raise the question of amino acid balance in a diet. Is the proportion of essential to non-essential amino acids physiologically important? The effects of variation in this ratio, at a constant dietary protein concentration, on N-retention in growing pigs, were examined by Wang and Fuller (1989). From their data the optimal essential/non-essential amino acid ratio was 50:50 or 57:43; higher proportions of non-essential amino acids decreased N-retention.

The capacity of an animal to conserve essential amino acids at the expense of non-essential amino acids implies considerable metabolic adaptability to changes in dietary intake. As stated earlier, some control of amino acid catabolism appears to occur at the substrate level (Cowey and Walton, 1989), but there was little evidence that activities of amino acid de-aminating enzymes in the livers of fish show adaptive responses to gross changes in dietary protein level.

From recent experiments, Kim et al. (1992c) reached the contrary conclusion that fasting or feeding high-protein diets leads to increased amino acid catabolic activity in rainbow trout by increasing the activities of liver amino acid-degrading enzymes. In these experiments there were significant differences in relative liver weight (hepatosomatic index) between treatments. Consequently, comparisons between treatments should properly have been based on the total enzyme activity in the livers of fish of standard weight (e.g. fish of say 100 g). When this was done, there were no differences between treatments for glutamate dehydrogenase or alanine aminotransferase activities; for the third enzyme examined, histidase, there were no differences between trout given diets containing either 10% protein or 35% protein although some differences were seen between other treatments (commercial diet, 12-day fast). The conclusions of Kim et al. (1992c) are thus not supported by their data and are clearly not unequivocal.

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