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in

Montero D. (ed.), Basurco B. (ed.), Nengas I. (ed.), Alexis M. (ed.), Izquierdo M. (ed.). Mediterranean fish nutrition

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 63

2005
pages 73-90

Amino acid nutrition of salmonids: Dietary requirements and bioavailablity

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SUMMARY – The amino acid (AA) requirements of salmonid fishes and the shortcomings of methods used to estimate AA requirement are briefly reviewed. The reported essential AA requirements for certain AA of salmon (Atlantic, coho, chum, chinook) and rainbow trout do not always agree well. For a proper feed formulation, prudent analogy of the reported AA requirement values and the estimates of AA bioavailability data from plant and animal protein sources are necessary. The apparent and true AA (TAA) availability values have been published for only a few animal and protein sources. TAA values give a better indication of the biological value, specifically for those feed ingredients with relatively low protein content. Further research on a better estimate of feedstuffs amino acid bioavailability, as well as maintenance requirement and protein accretion of fish, will result in the more efficient use of alternate protein sources to fish meal in salmonid feeds and lower nitrogenous waste from commercial aquaculture operations.

Key words: Salmon, trout, amino acid, protein, bioavailability.

RESUME – *"Nutrition des salmonidés en acides aminés : Besoins nutritionnels et biodisponibilité". Cet article présente brièvement les besoins en acides aminés (AA) des salmonidés et les limitations des méthodes utilisées pour les estimer. Les besoins en AA indispensables rapportés pour certains salmonidés (saumon Atlantique, saumon coho, saumon chum, saumon du Pacifique) et pour la truite arc-en-ciel ne concordent pas toujours très bien. Pour une formulation alimentaire adéquate, il est nécessaire de mettre au point une analogie prudente des valeurs rapportées pour les besoins en AA, et des estimations de données de biodisponibilité des AA à partir de sources de protéines végétales et animales. Les valeurs de disponibilité apparente et réelle des AA ont été publiées uniquement pour quelques animaux et quelques sources de protéines. Les valeurs réelles des AA nous donnent une meilleure indication de la valeur biologique, spécialement pour les ingrédients alimentaires ayant une teneur relativement faible en protéines. D'autres recherches pour une meilleure estimation de la biodisponibilité des AA dans l'aliment, ainsi que des besoins pour l'entretien et de l'accrétion protéique des poissons, permettront une meilleure utilisation de sources de protéines alternatives pour l'aliment poisson destiné aux salmonidés, ainsi qu'une réduction des rejets azotés provenant de l'aquaculture commerciale.*

Mots-clés : *Saumon, truite, acides aminés, protéines, biodisponibilité.*

Introduction

Proteins are an essential component of aquatic animal diet, needed for growth, development, reproduction and survival of fish. In the fish body, proteins are the primary constituent of structural and protective tissues (e.g. bones, ligaments, scales, and skin), soft tissues (organs, muscle) and body fluids. If a diet contains an inadequate amount of protein, there is a reduction or cessation of growth and ultimately withdrawal from certain less vital tissues to maintain the essential function of the vital tissues. There are about 22 or more amino acids (AA) that forms the building blocks for all complex proteins. Therefore dietary requirement for protein is essentially a requirement of the amino acids contained in the protein. The composition of experimental and commercial diets is generally described in terms of protein concentration. However, the protein quality in terms of nutritional value of diet depends on its amino acid content and on physiological utilization of specific AA after digestion, absorption, and a minimal rate of oxidation. Availability of amino acids varies with protein source, processing treatment, and interaction with other components of the diet.

Dietary protein and AA supply are major factors influencing the productivity of farmed fish. The protein and amino acid requirements for several salmonids including salmon, trout and arctic char

have been investigated over the past three decades. Most of the protein requirement values have been obtained from a typical dose response curve of growth and they show large variations in the requirements (Table 1). Dietary protein required for maximum growth generally ranges from 40-50% crude protein. Some of the variations in the requirement values among various reports are probably due to the differences in experimental conditions and dietary factors including the energy concentration of the test diet, and AA composition and digestibility of the dietary protein source. Net retention of dietary nitrogen in fish is in the range of 30-40%; therefore much of the dietary protein is lost to the animal.

Table 1. Dietary protein requirement (%) for maximum growth of salmonids

Species	Protein source	Requirement
Arctic charr	Fish meal	39[1]
Atlantic salmon	Casein, gelatin	45[2]
	Fish meal	55[3]
Chinook salmon	Casein, gelatin, amino acids	40[4]
Coho salmon	Casein	40[5]
Sockeye salmon	Casein, gelatin, amino acids	45[6]
Rainbow trout	Casein, gelatin	40[7]

[1]350 g digestible protein/kg, Gurure *et al*. (1995); [2]Lall and Bishop (1977); [3]Grisdale-Helland and Helland (1997); [4]DeLong *et al*. (1958); [5]Zeitoun *et al*. (1974); [6]Halver *et al*. (1964); [7]Zeitoun *et al*. (1973).

Amino acid requirement

Amino acids provide essential nitrogen for the synthesis of protein and other biological molecules. It is widely recognized that the specific requirement for amino acids should be determined in terms of optimum amount of dietary protein necessary for most efficient animal production. The amino acids incorporated in fish protein are α-amino acids, with the exception of proline, which is an α-amino acids. The term indispensable (essential) and dispensable (non-essential) is widely used to classify the nutritional importance of amino acids in fish. The ten essential or indispensable amino acids (EAA), arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, cannot be synthesized by fish, and therefore must be provided in the diet. However, the nutritional classification of amino acids for terrestrial animals is divided into three categories based on their absolute or relative rates of protein synthesis *in vivo*: (i) essential or indispensable (EAA) – histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine; (ii) conditionally dispensable – arginine, cysteine, tyrosine; and (iii) dispensable (non-essential) – alanine, aspartic acid, asparagine, glutamic acid, glutamine, glycine, proline and serine. The metabolism and requirement of certain conditionally dispensable amino acids may vary among animals including fish (D'Mello, 2003). Species-specific requirement for arginine has been reported for salmon and trout (NRC, 1993; Lall *et al.*, 1994; Luzanna *et al.*, 1998) and taurine for flounder larvae (Park *et al.*, 2002). Therefore protein requirement has two components: (i) EAA needed by the fish because it cannot be synthesized or the synthesis is not rapid enough; (ii) protein required to supply dispensable amino acids (NEAA) or to supply amino nitrogen for the synthesis of NEAA.

The accurate determination of amino acid (AA) requirement for each of the dietary essential amino acids (EAA) and the contribution of NEAA is considered the fundamental basis of protein and amino acid nutrition of fish. Studies on the quantitative essential amino acid requirements of salmonids have relied largely on dose-response curves of growth in juvenile fish. In purified, semi-purified, and practical diets graded increments of the amino acid have been tested. The nitrogen component of the test diets has consisted of either amino acids or a mixture of amino acids, casein, and gelatin that provides an amino acid composition similar to a reference protein such as fish body protein or whole hen's egg protein less the amino acid under test. Earlier studies on rainbow trout showed that growth rates obtained on diets with large amounts of free amino acids were inferior to diets of similar amino acid composition in which the nitrogen component was protein (Wilson *et al.*, 1978; Walton *et al.*, 1982, 1986). However, more recently relatively good growth rates with diets containing high levels of

crystalline amino acids have been reported (Cho *et al.*, 1992; Rodehutscord *et al.*, 1995). Amino acid requirement of several salmonid species is summarized in Table 2. Where several estimates are available for one amino acid in a single species, as in the case of rainbow trout, marked discrepancies occur. Some of these may be due to differences in growth rate, amino acid sources, feed intake, and other aspects of methodology. There are not any experiments reported that simultaneously estimate all amino acid requirements of fish for maintenance and for tissue accretion.

Table 2. Essential amino acid requirement of salmonids†,††

EAA	Chum salmon	Chinook salmon	Coho salmon	Atlantic salmon	Rainbow trout
Lys	4.8[1], 5.0[2]	5.0[3]	3.8[4]	4[5], 3.2-3.6[6], 6.1[7]	3.7[8], 4.2[9,10], 5.3[11]*, 6.1[12]
Met (Cys)	3.0 (1.2)[2]	4.0 (1)[13]	2.7 (0)[4]	3.1[7§]	2.2 (0)[14], 2.3 (0)[15], 1.9 (0.2)[16], 2.4 (0.2)[16‡], 3.0 (0.3)[17], 1.8 (0.9)[11]*
Trp	0.7[18]	0.5[19]	0.5[4, 19]	-	0.5[11]*,[20], 0.6[21], 1.4[22]
Thr	3.0[1]	2.2[23]	2.0[4]	3.2[7§]	3.4[11]*, 3.2-3.7[24]
Leu	3.8[2]	3.9[25]	3.4[4]	5.2[7§]	4.4[11]*
Val	3.0[2]	3.2[25]	2.2[4]	3.9[7§]	3.1[11]*
Ileu	2.4[2]	2.2[25]	1.2[4]	3.2[7§]	2.4[11]*
Phe + Tyr	6.3[2]	5.1[25]	4.5[4]	5.8[7§]	4.3[26], 5.2[11]*
His	1.6[1,2]	1.8[27]	1.8[27], 0.9[4]	1.8[7§]2.0[37]	1.6[11]*
Arg	6.5[2]	6.0[27]	5.8[27], 3.2[4], 4.9-5.5[28]	4.1[29], 5.0-5.1[30], 4.6[7§]	3.3[31], 3.5[1]*[1,24], 3.5-4.2[33], 3.8[33], 3.6-4.0[34], 4.0[8], 4.1[35], 4.7[36], 5.4-5.9[12]

†Requirement listed as % of protein, results based upon growth – no superscript, *protein accretion, §ideal protein, ‡lens pathology.

††The following references cited above are available from the senior author: [1]Akiyama *et al.* (1985a); [2]Akiyama and Arai (1993); [3]Halver *et al*. (1958); [4]Arai and Ogata (1993); [5]Anderson *et al*. (1993); [6]Berge *et al.* (1998); [7]Rollin *et al*. (1994); [8]Kim *et al.* (1992); [9]Walton *et al*. (1984a,b); [10]Pfeffer *et al*. (1992); [11]Ogino (1980); [12]Ketola (1983); [13]Halver *et al*. (1959); [14]Walton *et al*. (1982); [15]Kim *et al*. (1992); [16]Cowey *et al*. (1992); [17]Rumsey *et al*. (1983); [18]Akiyama *et al*. (1985b); [19]Halver (1965); [20]Walton *et al.* (1984); [21]Kim *et al*. (1987); [22]Poston and Rumsey (1983); [23]DeLong *et al.* (1962); [24]Rodehutscord *et al.* (1995); [25]Chance *et al*. (1964); [26]Kim, (1993); [27]Klein and Halver (1970); [28]Luzzana *et al*. (1998); [29]Lall *et al*. (1994); [30]Berge *et al*. (1997); [31]Kaushik, S. (1979); [32]Chiu *et al*. (1988); [33]Forster (1993); [34]Walton *et al*. (1986); [35]Pack *et al.* (1995); [36]Cho *et al*. (1992);[37]Scott (1998).

The measurement of amino acid requirement by growth experiments shows large differences between species and even for single species. Some of these requirements obtained were based on growth rates below the optimum. These variations in the EAA requirement values reported for salmonids as well as between other finfish species may also be due to the use of different test proteins as sources of AA, a large amount of crystalline AA supplement used in test diets, and the variation in the protein energy: total energy ratio in the diet. Other sources of variations among fish species could be due to the differences in genetic strain, age, feed utilization and environmental conditions for rearing of fish. However, marked differences may not occur between fish species in the pathway or control of mechanisms involved in amino acid metabolism and protein utilization.

Other methods used to determine the AA requirement include oxidation of amino acids or the measurement of the concentration of free amino acids in blood and tissues (Wilson, 2003). The

shortcomings of methods used to measure the AA requirements of fish have been reviewed by Cowey (1995) and are beyond the scope of this paper.

Whole body amino acid profile

Early studies to develop the formulation of test diets for dietary AA requirements were based on the composition of chicken egg protein. It was later discovered that test diets for AA requirements of fish could be improved by simulating the AA profile of the whole body tissue of the species under investigation (Ketola, 1982; Wilson and Cowey, 1985). There are several reports now to confirm that amino acid profiles of whole body tissue of a given species of fish resemble those of the dietary requirements of the fish (Arai, 1981; Ogata *et al.*, 1983; Wilson and Poe, 1985; Mambrini and Kaushik, 1995). Limited differences in the AA profile of salmonids, chickens and pigs have been observed. Lysine is the most abundant essential amino acid in the whole body of salmonids and its concentration is higher compared with chicken and pigs. However the ratio of AA to lysine is similar in trout and pigs. The ratio of sulfur amino acids and branched chain AA to lysine is higher in chicken than trout.

Generally, the amino acid profile of fish and terrestrial animals are affected by the changes in contribution of different protein tissues to whole body protein. As the dietary concentration of an AA increases beyond the requirement level, the tissue concentration also increases indicating where the requirement may lie on a dose-response curve (Cowey, 1995). Metabolism of amino acids is estimated by the proportion of amino acids used for protein synthesis. Generally, rates of oxidation of amino acids are low until the amount consumed exceeds the amount needed for protein synthesis; oxidation then increases rapidly. The concentration of AA in the whole body may be lower than muscle (Arzel *et al.*, 1995). Whole body AA composition of several salmonid fish species is summarized in Table 3. There are some clear differences among various species and definitely there is a need to determine the AA requirements of individual fish species.

Table 3. Whole body amino acid composition (g/100 g) of salmon and trout

Amino acid	Atlantic salmon[1]	Coho salmon[2]	Cherry salmon[3]	Rainbow trout[4]	Arctic charr[5]
Ala	6.52	6.08	6.35	6.57	7.02
Arg	6.61	5.99	6.23	6.41	6.28
Asp	9.92	9.96	9.93	9.94	11.18
Cys	0.95	1.23	1.34	0.80	–
Glu	14.31	15.25	15.39	14.22	15.74
Gly	7.41	7.31	7.62	7.76	7.14
His	3.02	2.99	2.39	2.96	2.48
Ile	4.41	3.70	3.96	4.34	3.10
Leu	7.72	7.49	7.54	7.59	6.95
Lys	9.28	8.64	8.81	8.49	8.94
Met	1.83	3.53	3.14	2.88	2.85
Phe	4.36	4.14	4.63	4.38	4.82
Pro	4.64	4.76	4.33	4.89	6.10
Ser	4.61	4.67	4.48	4.66	5.17
Thr	4.95	5.11	4.63	4.76	5.00
Try	0.93	1.40	0.83	0.93	–
Tyr	3.50	3.44	3.58	3.38	3.12
Val	5.09	4.32	4.85	5.09	4.10

[1]Wilson and Cowey, 1985; [2]Arai, 1981; [3]Ogata *et al.*, 1983; [4]Wilson and Cowey, 1985; [5]Gurure, 1997.

The AA composition and the A/E ratios [(each essential amino acid content/total essential amino acid content including cystine and tyrosine) x 1000] of whole body tissue have been widely used to develop AA test diets for AA requirement studies in fish (Table 4). Wilson (1991) calculated the amino

acid requirements of channel catfish based on the determined lysine requirement and the A/E ratios of the amino acids of whole body and found a close similarity between the determined requirement values and the calculated ones. Other investigators have predicted the requirements of the essential amino acids in a similar manner after determining the lysine requirement through dose-response trials for red drum (Moon and Gatlin, 1991), striped bass (Brown, 1995) and Japanese flounder (Foster and Ogata, 1998). More recently, Rodehutscord *et al.* (1997) indicated that estimates of dietary amino acid requirements based upon the amino acid composition of the whole body might not agree with the values obtained from a controlled growth study.

Table 4. Determined and predicted EAA requirement of chum salmon and Japanese eel (Akiyama *et al*., 1997)

EAA	Chum salmon			Japanese eel		
	A/E Ratio	Determined	Predicted	A/E Ratio	Determined	Predicted
Arg	115	6.5	3.44	133	4.5	4.32
His	67	1.6	2.01	76	2.1	2.47
Ileu	77	2.4	2.31	82	4.0	2.67
Leu	140	3.8	4.19	145	5.3	4.71
Lys	167	5.0	–	163	5.3	–
Met + Cys	80	3.0	2.40	77	3.2	2.50
Phe + Tyr	147	6.3	4.40	137	5.8	4.45
Thr	90	3.0	2.69	77	4.0	2.50
Trp	29	0.7	0.87	13	1.1	0.42
Val	88	3.0	2.63	96	4.0	3.12
Total		35.3	24.9		39.3	27.2

Ideal protein concept and amino acid nutrition

A concept of ideal protein in which AA needs could be proportioned one to another was first introduced in practical diet formulation of pigs (Cole, 1978; ARC, 1981). Ideal AA ratios, with lysine as the reference amino acid, are used widely for diet formulation of chicken and pigs. Generally, the ideal AA ratios do not change whether diets contain high or low levels of energy or protein. Certain physiological and environmental factors such as stress, temperature, disease and rearing density that affect the voluntary feed consumption may affect lysine requirement but not ideal ratios. Some of the essential prerequisites to estimate the ideal AA ratios include: (i) the same basal diet, sex and strain of animals, and the same assay period in all requirement studies; (ii) true digestibility values of AA in the basal diet; (iii) clear-cut graded response of the limiting AA under investigation; and (iv) proper statistical methods with consistent and appropriate curve fitting procedures.

Although the ideal AA ratio concept has been tested in some fish species, there has been limited research effort in this area. The amino acid requirement of a growing fish generally includes two components: (i) requirement for maintenance; and (ii) a requirement for tissue protein accretion. There is evidence from studies with other species that the pattern of amino acids required for each of these two components may be different, and the total requirement of fish, must therefore depend on the relative contribution of maintenance and tissue protein accretion to its total needs. The requirement for maintenance may include losses due to oxidation of amino acids, synthesis of other nitrogenous compounds from amino acids, protein turnover and the metabolic cost to replace protein lost from the body surface (mucus and integument) and sloughing off of mucosa from the gastrointestinal tract.

Protein synthesis in fish has been measured using the constant infusion technique of Garlick and Marshall (1972) and the use of a single high-dose injection of [^{3}H] phenylalanine to flood the intracellular pools and stabilize precursor specific activity over the duration of measurement (Garlick *et al.*, 1980). The rate of protein synthesis varies in different tissues in the following order: liver>gills>digestive tract>red muscle>white muscle. Protein synthesis in white muscle is low in fish

(4-5 g/kg$^{0.75}$/day) as compared with mammals (16 g/kg$^{0.75}$/day) (Smith, 1981; Houlihan *et al.*, 1986). Most of the protein synthesized is retained in white muscle of fish (50-70%). Protein synthesis in the liver contributes to less than 2% of protein accretion in fish, in contrast to 60% for white muscle and 65% of total muscle. At these protein turnover rates in fish muscle whole body protein turnover is not likely to be a major factor in amino acid losses. Loss may occur during turnover as a result of the activity of catabolic enzymes present in tissues.

Estimate of amino acid maintenance requirements

Certain amounts of amino acids ingested by animals are not deposited and this loss is often referred o as "maintenance" or "non-productive" requirement of amino acids. When a growing aquatic animal is neither gaining nor losing net body protein, metabolic processes continue to occur due to the loss of proteinaceous material from the body. An estimate of these losses provides the maintenance amino acid requirement of fish. They reflect the continuous loss of amino acids in urine due to inefficient turnover of body protein, a loss of amino acid nitrogen via gills and integument, gut endogenous amino acid losses, and the use of amino acids by cells to synthesize essential non-amino acid and non-protein nitrogen metabolites. Major nitrogenous compounds formed from amino acids includes purines (glycine, glutamine), polyamines and methylated compounds (methionine), catecholamines (phenylalanine), histamine (histidine), carnitine (lysine), creatine (arginine, glycine), taurine (cysteine), thyroid hormones (tyrosine), and serotonin (tryptophan).

Estimates of the maintenance requirement for EAA are available for rainbow trout only and there is a need for additional studies to determine the factors that influence the maintenance requirement. Rodehutscord *et al.* (1997) has reported the maintenance requirement for certain essential amino acids. The estimated requirements from exponential functions for protein deposition for amino acids were [in mg/(100 g BW.d)]: lysine, 1.93; tryptophan, 1.05; histidine, 1.07; valine, 2.92; leucine, 8.26 and isoleucine, 0.91. This indicates approximately 4% of the requirement for protein deposition for lysine and isoleucine and 32% for leucine and the remaining amino acids between the range of these two values. In young rapidly growing fish, the average maintenance requirement of first liming AA may be only a small proportion (<10%) of the total daily amino acid requirement. Information on the maintenance requirement is necessary for modelling amino acid metabolism and for the estimation of EAA requirement. To a limited extent these non-productive requirements of amino acids could be minimized, however, there is a greater need to study maintenance requirement to effectively use protein and amino acids in salmonid feeds.

Lysine and methionine utilization in salmonids

Unlike terrestrial animals, lysine is not the first-limiting amino acid in either natural food or a formulated diet based on fish meal for carnivorous fish (Table 5). The lysine requirement for fish is between 3.8 and 6.6 g/16 g N. In swine and poultry a possible inhibitory effect of arginine on lysine absorption has been demonstrated, and therefore the dietary lysine concentration must be increased when there is an excess amount of arginine present. This information is not available for salmonids. Lysine is also a precursor for carnitine, but conversion occurs from peptides derived from protein catabolism and not from free lysine.

Lysine is the most abundant essential amino acid in the body of salmon and trout. Since lysine is normally the first limiting amino acid in most feedstuffs, the requirement for other EAA are expressed in relation to the lysine requirement based on the ideal protein concept (Cole, 1980). Several investigators have determined the lysine requirement and the A/E ratios of the amino acids of whole body and predicted the requirement of EAA after determining the lysine requirement through dose-response trials for chum salmon (Akiyama *et al.*, 1997) catfish (Wilson, 1991), red drum (Moon and Gatlin, 1991), striped bass (Brown, 1995) and Japanese flounder (Foster and Ogata, 1998). Recently, Akiyama *et al.* (1997) reported that the variations in the essential amino acid requirements of different species possibly reflect the true differences between phylogenetically distinct families or species.

Table 5. Requirement and concentrations of lysine (as % protein) of selected feed ingredients for fish

	Requirement†
Fish species	
Atlantic salmon / rainbow trout	3.7 – 6.1
Carp	5.3 – 5.7
Japanese eel	5.3
Channel catfish	5
Striped bass	4 – 6.1
Coho salmon	3.8 – 5.0
Milkfish	5
Sea bass	4.8
Red drum	4.4 – 5.7
Gilthead bream	5
Japanese flounder	4.6
Protein source	
Fish meal (72% CP)	7.5
Fish protein concentrate (72% CP)	7.5
Soybean protein concentrate (70% CP)	8.0
Soybean meal (44% CP)	6.0
Casein (92% CP)	8.6
Gelatin (92% CP)	4.2
Corn gluten meal (60% CP)	1.7

†NRC (1993).

The published requirement values for methionine ranges between 1.3 and 3.6 g/16 g N. Adequate amounts of both sulfur amino acids (methionine and cystine) are necessary for protein synthesis and various physiological functions in the body. Cystine can be synthesized from methionine, however the conversion methionine to cystine in the body is not the reversible. Cystine is a diamer of cysteine with strong disulphide bond. In AA analysis of protein, only cystine is measured and widely reported as cystine in feed composition tables. Physiological methionine requirement can only be met by methionine supplied in the diet whereas cysteine requirement can be met by either of these two sulfur amino acids. If cystine is included in the diet, it reduces the amount of methionine needed.

According to some authors the methionine requirement in the absence of cytine must be considered. Approximately 70% of the total sulphur amino acids in rainbow trout are deposited as methionine (Rodehutscord *et al.*, 1997). Therefore total sulphur amino acid requirements that include both cystine and methionine will not represent the true methionine requirement of fish. Diets based on fish meal may not be deficient in methionine where the methionine to cystine ratio is 70:30. However in formulation-based diets based on alternate plant protein sources with estimates of AA requirement based on total sulphur amino acid, methionine could be a limiting AA.

In terrestrial animals, synthetically manufactured DL-methionine is as effective as the L-isomer, which is not industrially produced. In experimental diets L-methionine, DL-methionine and N-acetyl-DL-methionine have been shown to be efficiently utilized by fish, whereas glutathione and DL-methionine hydroxy analogue (a non-nitrogenous synthetic compound) appear to be significantly less efficient in fish than vertebrates. Inorganic sulphur or taurine are considered an ineffective source of sulphur amino acids for fish.

Protein accretion response to amino acid intake

The total amino acid requirement of fish depends on the relative contribution of maintenance and tissue protein accretion to its total needs. The protein accretion method has been used to estimate AA

requirement of some fish species. Ogino (1980) measured the retention of indispensable AA in whole body protein of rainbow trout fed a high quality protein diet and used the increase in indispensable AA content to estimate requirements. This method assumes that the maintenance requirements in young growing fish are low, so that the pattern of AA deposited in body weight gain is the main determinant of patterns of AA required. Amino acid requirements estimated by this method are generally lower than that based on growth studies probably due to the relatively low level (only 30 to 40%) of dietary N retention in growing fish. Although the efficiency of AA utilization for accretion is relatively constant, the efficiency may not be optimum when protein accretion (growth rate) is high and it is continuously decreasing with an increase in feed intake. Protein accretion may not vary between the species depending on diet composition (crude protein, digestible energy and body amino acid profile).

Amino acids and alternate protein sources

Formulation of low protein diets and the use of alternative feedstuffs to fish meal require a thorough understanding of amino acid requirements and their availability in feedstuffs. Utilization of alternative sources and supplements can be significantly improved by appropriate combination of alternative sources and supplements of insufficient EAA as the free form to adjust the dietary EAA balance. Recent research on salmonid feeds containing a major proportion of plant protein and supplemented with synthetic EAA show marked improvement in growth and protein utilization, however, the growth performance of fish remains below that of fish meal-based diets. Apart from the adverse effects of antinutritional factors and indigestible carbohydrates present in plant protein supplements, amino acid absorption utilization for growth is relatively less efficient than fishery by products. This decrease is reflected in the postprandial plasma and tissue free amino acid concentrations of fish fed diets containing either plant proteins or their combination with fish meal. The slow hydrolysis of AA from plant proteins in gut compared with amino acids released from fish protein earlier may delay their appearance in blood, which may cause inefficient use of AA for tissue protein synthesis and growth. A low palatability and an imbalance AA level in a diet also causes reduction in feed intake thus affecting nitrogen utilization from the diet. Close attention to excess amounts of certain amino acids as well as EAA deficiency are also necessary to improve the utilization of plant protein sources.

Amino acid availability

The nutritional value or quality of various animal and plant protein sources for fish varies considerably. It is affected by the amino acid composition, ratios of essential amino acids, extent of hydrolysis during digestion, source, and the effects of processing. The protein quality of a specific protein is higher when the ratio of indispensable to dispensable amino acids is high. The distribution of a specific amino acid within this ratio is also of paramount importance. Proteins that are deficient in one or more amino acids are of poor quality. For example, tryptophan and lysine are limiting in corn, lysine in wheat and other cereal protein, and methionine in soybean and other legumes. Three commonly purified proteins or their combinations are widely used in experimental diets of fish and other experimental animals, namely casein, gelatin and zein. Casein is sufficiently complete to permit growth but often methionine or cystine supplementation is necessary. Both gelatin and zein lack several indispensable amino acids that are necessary in the diet of salmonids.

Most high quality fish meals are considered to be a complete source of amino acids for salmonids on the basis of published analytical data (NRC, 1993). Animal meat by-products have surplus lysine and arginine but are deficient in sulphur amino acids and tryptophan. Milk protein available as dried whey is a good source of lysine and tryptophan but is deficient in arginine. Oilseed proteins show characteristically different patterns and they are generally deficient in lysine with the exception of soybean meal. Blood meal contains appreciable amounts of other amino acids, however, it is deficient in isoleucine. Corn gluten meal contains high amounts of isoleucine but has low levels of lysine and tryptophan. The ratio of amino acids is useful in differentiating closely related proteins and highlighting decreases in specific amino acids such as lysine following chemical modifications of proteins e.g. browning reaction during food processing.

In order to supply amino acids from protein sources, it must be digested. The nutritional value of a protein depends on the digestibility and availability of amino acids. Digestibility and availability are not

synonymous terms. Digestibility refers to the susceptibility of peptide bonds to hydrolysis, while availability refers to the chemical integrity of amino acids, i.e. its resistance to processing by heat, high pH, oxidation etc. These treatments can limit both digestibility and availability, particularly for lysine, methionine, threonine and tryptophan. Both parameters must be considered to define the nutritional value of proteins.

Availability of any nutrient is the result of two processes: digestion and utilization. Thus, amino acid availability will be the result of protein hydrolysis and absorption of the products and the retention of absorbed amino acids.

Processing of feed ingredients and feeds can improve the nutritional value of protein and other nutrients as well as the food safety of products. However, uncontrolled heat and pH applied during the processing of feeds and feed ingredients may cause both enzymatic and Maillard type reactions of primary amino acid groups with reducing sugars and other enzymatic and non-enzymatic browning reactions with non-reducing carbohydrates. High pH induces racemization of L-amino acid residues to D-isomers and the formation of cross-linked amino acids such as lysinoalanine (Friedman and Pearce, 1989; Friedman and Gumbmann, 1984, 1989). A better understanding of the chemical changes that occur during processing is needed to optimize the protein quality while minimizing the formation of deleterious compounds. Limited information is available on the availability of amino acids from plant and animal protein sources. The following section describes our work on availability of AA in fish meal.

Fish meal is still an important source of protein in the diets of many intensively reared aquaculture species. Fish meals have widely varying nutrient compositions and amino acid profiles (Table 6) and also greatly vary in their qualities (Anderson *et al.*, 1993; 1997). Quality generally equates to the bioavailability in fish meal.

Table 6. Amino acid levels (g/16 g N) in various fish meals (for further details see Anderson *et al.*, 1997)

Amino acids	Groundfish meal	Mixed meal	Menhaden meal	Silver hake	Chilean - good quality	Chilean - poor quality	Norse-LT94®
Ala	6.70	7.16	6.68	6.60	6.34	7.21	6.73
Arg	6.70	7.23	5.93	6.98	6.14	6.85	6.48
Asp	8.46	8.89	8.43	10.29	9.00	10.19	9.54
Cys	1.07	1.07	0.97	1.09	0.98	1.00	1.06
Glu	12.65	12.85	12.13	15.06	12.95	14.63	13.80
Gly	9.46	9.15	7.55	6.50	6.22	7.15	6.81
His	1.79	1.99	2.12	2.28	3.59	3.52	2.40
Ile	3.79	4.16	3.99	5.10	4.75	5.10	4.73
Leu	7.15	7.81	7.53	8.42	7.76	8.40	7.92
Lys	7.01	7.58	7.34	8.59	7.89	8.87	8.32
Met	2.50	2.62	2.56	2.98	2.50	2.45	2.80
Phe	3.35	3.82	3.70	4.59	4.10	4.45	4.26
Pro	4.81	4.96	5.24	4.76	4.48	4.57	4.70
Ser	4.68	4.69	4.09	4.87	4.04	4.39	4.35
Thr	4.12	4.43	4.22	4.86	4.44	4.87	4.56
Trp	1.28	0.94	1.18	0.65	1.03	0.81	0.74
Tyr	2.91	3.25	3.01	3.61	3.18	3.50	3.33
Val	4.39	4.90	4.57	5.52	5.22	5.63	5.28

In the current circumstance of relatively high cost of raw materials, there is pressure to reduce the extent of over formulation and, more so, to increase the substitution of different protein sources besides fish meal. It is therefore increasingly recognized that unless the amount of each dietary amino acid becoming available to the animal can be determined for individual feedstuffs, little improvement can be made to current formulation practices.

Methods for determining amino acid availability

Methods of assessing quality fall into three categories: (i) gross inspection; (ii) chemical analyses; and (iii) biological evaluation. Gross inspection includes color, texture, odor, and microscopic examinations. These will not be discussed further. Numerous *in vitro* chemical tests have been used to assess the protein quality of fish meals (Table 7) including tests for: acid corrected pepsin digestible protein (Lovern, 1964; Lovern *et al.*, 1964; Olley and Pirie, 1966), multienzyme digestible proteins (Hsu *et al.*, 1977; Pedersen and Eggum, 1983), total volatile basic-nitrogen (Conway, 1933; Woyewoda *et al.*, 1986), available lysine (Booth, 1971; Carpenter, 1960; Nordheim and Coon, 1984), and sulphydryl groups and disulphide bonds (Opstvedt *et al.*, 1984).

One of the most used *in vitro* methods is enzymatic digestion with pepsin (Anderson *et al.*, 1993, 1997; March and Hickling, 1982). The primary limitation of this assay is that a 0.2% concentration (Williams, 1984) is excessive and can almost completely digest proteins of poor quality (Table 7). The use of dilute-pepsin concentrations has shown promise as a rapid method that correlates with *in vivo* assessments (Anderson *et al.*, 1993; 1997). Even so, a limitation of any *in vitro* chemical assay is not being sensitive enough to assess differences in quality among similar samples (Table 8) whereas biological assays can determine a difference (Anderson *et al.*, 1997).

Table 7. In vitro measures of protein quality of various fish meals

Fish meal	Dilute-pepsin digestibility (%)	Soluble protein (%)†	TVBN†† mg N/100 g fish meal (as-is)	Multi-enzyme pH-stat digestibility (%)
Herring meal 1	85.1	21.5	27.6	87.5
Herring meal 2	72.8	21.4	40.4	89.8
Herring meal 3	88.3	35.6	32.6	95.5
Herring meal 4	90.9	17.3	59.6	93.2
HM-FF-Lt	81.8	35.9	48.2	89.2
HM-FF-Std	83.4	30.4	49.2	89.5
HM-FM-Lt	68.7	38.4	30.1	89.1
HM-FM-Std	70.4	35.8	46.3	91.1
HM-SM-Std	70.5	35.8	67.2	89.7
Chilean - Good quality	95.2	35.2	59.6	92.9
Chilean - Poor quality	76.5	35.8	70.1	87.5
Groundfish meal	74.7	24.7	25.1	90.7
Menhaden meal	82.7	29.8	77.5	89.1
Mixed meal	80.1	36.7	49.8	95.9
Norse-LT94®	93.2	45.0	50.2	90.8
Silver hake	84.2	17.7	14.0	90.7

†The percentage of protein soluble in a weak acid solution (0.075 N HCL).
††Total volatile basic-nitrogen. Values for the raw fish used to make the HM-FF (fresh female) and HM-FM (fresh male) meals were 20.0 and 21.6 mg N/100 g raw fish, respectively. The value for the spoiled male herring used to make HM-SM-Std was 112.7 mg N/100 g raw fish. (Anderson *et al.*, 1997).

A further refinement to the pepsin digestion has been the use of multienzyme systems (Hsu *et al.*, 1977; Pedersen and Eggum, 1983) or the use of fish enzymes (Dimes and Haard, 1994; Dimes et al 1994a; 1994b; Haard *et al.*, 1996). Although these methods show an improvement over the AOAC pepsin digestion method, their acceptance is limited due to the paucity of corresponding *in vivo* data or because the procedures are too complicated and time consuming for routine use.

A number of *in vivo* methods exist for the evaluation of amino acid availabilities. These methods include: growth assays, free amino acid levels in blood, and balance studies (amino acid availabilities).

Table 8. In vitro measures of protein quality of various fish meals (for further details see Anderson *et al*., 1993)

Fish meal	Acid-corrected pepsin-digestible protein		Multienzyme pH-stat digestibility
	AOAC (%)	Torry (%)	(%)
Herring meal 1	91.3	81.9	80.3
Herring meal 2	96.5	93.6	88.1
Herring meal 3	96.4	93.0	88.0
Herring meal 4	96.7	89.2	81.9
Herring meal 5	98.1	93.5	86.0
Herring meal 6	96.7	91.9	86.0
Herring	94.1	95.9	87.9
Menhaden meal	97.7	84.0	81.7
Anchovy meal	96.8	87.4	84.0
Norse-LT94®	98.5	96.8	85.2

Growth assays are the simplest to perform, but only provide limited direct information on amino acid availabilities. Growth assays need to be combined with whole body or tissue analysis to have a more detailed assessment of individual amino acid retention. Measuring the free amino acid levels in blood also has a similar drawback in that it provides limited direct information unless coupled with more sophisticated methods (Carter *et al*., 1995; Kaushik *et al*., 1994; Kaushik and Luquet, 1979; Lyndon *et al*., 1993; Nose, 1973; Plakas *et al*., 1980; Prieto *et al*., 1994; Tantikitti and March, 1995).

Amino acid availabilities are generally derived from the results of balance experiments that measure the difference between their input and output. Three pieces of information are required to determine the availability of amino acids: (i) the amount of the amino acids consumed; (ii) the amounts excreted; and (iii) a measure of the endogenous amino acid losses. The below term (Equation 1) should be referred to as the apparent amino acid availability because of the amino acids present in the feces, only some have arisen from undigested food residues. Part has come from the animal itself and consists of unabsorbed digestive secretions, sloughed-off gut tissue and bacteria.

$$\text{Amino acid availability} = \frac{\text{Amino acid consumed} - \text{amino acid in feces}}{\text{Amino acid consumed}} \quad [1]$$

These may be referred to as endogenous fecal material. Its measurement allows true amino acid availability to be calculated thus (Equation 2):

$$\text{True amino acid availability} = \frac{\text{Amino acid consumed} - \text{amino acid in feces} + \text{endogenous amino acid in feces}}{\text{amino acid consumed}} \quad [2]$$

The relationship between apparent and true availabilities can be seen in Fig. 1. As more amino acids are supplied in the diet, the amount of endogenous amino acids becomes proportionally less. Thus apparent amino acid availabilities values approximate more closely to the true amino acid availability values.

Part of the methodological problem with measuring amino acid availability in fishes is the method of fecal collection. A number of methods including intestinal dissection, fecal stripping, sedimentation, and automatic collection have been developed and evaluated, each method has its own unique drawback that may over- or underestimate amino acid availability (Anderson *et al.*, 1995; Austreng, 1978; Choubert *et al.*, 1982; Choubert *et al.*, 1979; Hajen *et al.*, 1993; Percival *et al.*, 2001; Satoh *et al.*, 1992; Smith *et al.*, 1980; Spyridakis *et al.*, 1989; Storebakken *et al.*, 1998; Windell *et al.*, 1978). These same problems also exist for the estimation of the amount of endogenous amino acids, where diet composition, food intake, and collection methods influence the results.

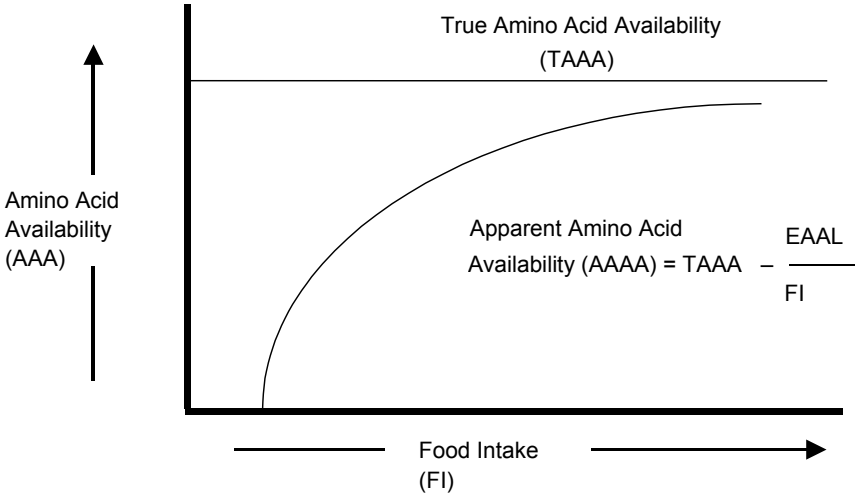

Fig. 1. The relationship between amino acid intake and apparent and true amino acid availabilities. Taken from McNab (1994).

Because apparent availabilities of amino acids derived from balance experiments depend on the amount of amino acid eaten (Fig. 1), care must be taken to ensure that, when comparisons are made across foodstuffs, near constant food intakes are maintained. Otherwise, a systematic bias may inadvertently be incurred. For this reason, it is probably preferable to express values in terms of true availability coefficients, which are independent of food intake.

Compared with the literature for terrestrial animals, there is a lack of literature for fishes on amino acid utilization. A limited number of studies are available that have evaluated the true amino acid availabilities of various feedstuffs in different species: Atlantic salmon (*Salmo salar*) (Table 9) (Anderson *et al.*, 1992; 1995), common carp (*Cyprinus carpio*) (Hossain and Jauncey, 1989), channel catfish (*Ictalurus punctatus*) (Wilson *et al.*, 1981), yellowtail (*Seriola quinqueradiata*) (Masumoto *et al.*, 1996), and rainbow trout (*Oncorhynchus mykiss*) (Hudon and de la Noüe, 1985; Skrede *et al.*, 1980).

Table 9. Apparent (AAAA) and true (TAAA) amino acid availabilities values (%) calculated from feces collect by manual stripping of fish [see Anderson *et al.* (1995) for full details]

Amino acids	Herring meal		Menhaden meal		Anchovy meal		Norse-LT94®	
	AAAA	TAAA	AAAA	TAAA	AAAA	TAAA	AAAA	TAAA
Ala	86.1	91.1	87.9	93.8	81.1	87.4	94.2	99.5
Arg	90.8	94.4	91.3	96.4	81.5	87.0	95.9	99.9
Asp	83.2	90.3	69.9	83.7	78.7	89.6	86.3	94.4
Glu	86.3	92.4	85.5	94.5	81.5	90.3	94.4	99.9
Gly	81.1	88.8	72.1	84.8	82.3	92.5	85.1	92.9
His	74.6	76.5	85.4	86.5	75.9	77.2	96.4	97.9
Ile	84.9	91.3	90.6	97.4	81.6	89.7	94.9	99.9
Leu	86.3	92.8	89.3	97.3	82.3	91.2	95.3	99.9
Lys	86.2	89.3	84.1	88.8	80.6	85.2	94.6	98.0
Phe	84	90.4	89.2	95.7	80.2	87.9	94.4	99.9
Pro	83	98.0	83.8	98.1	73.6	92.8	89.3	99.9
Ser	81.9	87.2	85.6	91.8	81.1	87.8	94.2	99.9
Thr	80.8	96.6	85.1	99.9	80.7	99.9	93.2	99.9
Trp	83.5	86.4	72.7	79.0	81.3	91.2	85.9	89.1
Tyr	88.5	95.0	86.2	99.9	86.6	95.3	97.1	99.9
Val	85.8	92.5	89.7	97.5	81.5	90.9	94.8	99.9

Factors affecting amino acid availability

Unfortunately, nearly all sources of plant proteins possess antinutritional factors, which must be eliminated by special processing techniques to make them of maximum nutritional value. Of the many and various factors, which may be present in foods, particularly in foods of plant origin, a number of biochemical groups capable of interacting with proteins and amino acids exist. Of these diverse groups, phenolics (tannins), lectins, phytic acid, and the proteolytic enzyme inhibitors, are the most important in protein nutrition (Davídek *et al.*, 1990; Finley and Hopkins, 1985). Depending on the protein source and how it has been processed, these antinutrients may exert a significant affect on amino acid utilization and may need to be taken into consideration.

Summary and conclusions

Although the essential amino acid (EAA) requirement of several salmonid fishes has been investigated for the past 25 years, there are large variations in measured requirement values not only among species but also within a given species probably due to the differences in test diets, feed utilization and experimental conditions for rearing of fish. Essential AA requirement studies are laborious and expensive due to the need to use synthetic amino acids, in a purified diet. Many researchers have introduced the concept of an ideal amino acid pattern to estimate their dietary AA requirements. Proper formulation of a diet requires a prudent analogy of the reported AA requirement values. A brief discussion on the AA requirements of salmonids in relation to other finfish and the shortcomings of estimating the requirement of AA on the basis of the ideal protein concept has been given. Dose-response experiments are still considered a more reliable method to measure the amino acid requirements of fish. However, estimates of the AA requirement based on growth of salmonids reared on practical diets at various stages of development, preferably under experimental farm conditions, may be more reliable to estimate AA requirements than data obtained on fingerlings on purified diets.

The quality of protein is based not only on its AA composition of the protein but also on how well the concentrations and balance of available amino acids conforms to the essential AA requirements of fish. Protein digestibility values of fish meal and plant protein sources have been reported, however, limited information exists on the apparent and true AA availability values from animal and plant protein sources. Thus, efforts to completely replace fish meal with alternate protein sources in salmonid feeds have the certain risk of creating a deficiency or imbalance of dietary AA. True availability values of AA give a better indication of the biological value, specifically for those feed ingredients with relatively low protein contents, such as plant sources. Use of true availability allows for a more accurate and economical feed formulation.

In developing models for AA metabolism and estimation of AA requirements, fish growth data and body AA composition values obtained at various stage of life cycle of farmed salmonids may provide more realistic data for this purpose than values extrapolated from published values on fry or young fish. Moreover, test diets for AA requirement studies must show growth and protein deposition close to the practical diets successfully used for salmonid culture. Obviously, there is a need for a better understanding of AA requirements and reliable AA availability data from commonly used feed ingredients in commercial feeds as well. In this respect, some caution is necessary when using AA requirements values published for salmonids by NRC (1993) nearly a decade ago. There is a need to better define the requirements of tryptophan, histidine, and branch chain amino acids. Further work underway in several laboratories, including a better estimate of maintenance requirement and protein accretion, will result in the more efficient use of dietary protein in salmonids feeds and lower nitrogenous waste from commercial aquaculture operations.

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