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PROTEIN AND AMINO ACID REQUIREMENTS OF FISHES

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INTRODUCTION

Fish are poikilothermic animals with metabolic rate determined by water temperature. They also require almost half their diet to be protein. Fish can be carnivores, omnivores, or herbivores. All hatchlings are carnivorous and this review concentrates on the apparent protein and amino acid requirements of the major carnivorous and omnivorous fishes on which nutritional requirement studies have been done.

Proteins are the major organic material in fish tissue, making up about 65–75% of the total on a dry-weight basis. Fish consume protein to obtain amino acids and use excess amino acids as an energy source. The protein is digested to release free amino acids, which are absorbed from the intestinal tract and used by various tissues to synthesize new protein. A consistent intake of amino acids is required because these are used continually by the fish to build new proteins. Inadequate protein in the diet results in a reduction of growth and loss of weight. When excess protein is supplied in the diet, only part is used for protein synthesis; the remainder is converted into energy.

The first definitive studies on the protein and amino acid nutrition of fish were made in the late 1950s and early 1960s with chinook salmon, *Oncorhynchus tshawytscha*. The initial amino acid test diets were formulated on the amino acid content of chicken whole-egg protein, chinook salmon egg protein, and chinook yolk-sac fry protein (23). The diet with an amino acid profile of chicken whole-egg protein gave the best growth and feed efficiency, and was therefore adopted. It was used to determine the qualitative amino acid requirements of the chinook salmon (26). The gross protein requirement was determined by feeding test diets containing a mixture of casein, gelatin, and crystalline amino acids to simulate the amino acid content of whole-egg protein (17). Subsequent experiments utilized test diets containing a mixture of casein, gelatin, and crystalline amino acids to form an amino acid pattern of 40% whole-egg protein to determine the quantitative amino acid requirements of the 10 indispensable amino acids for the chinook salmon (9, 18, 24, 27).

GROSS PROTEIN REQUIREMENTS

Fish do not have a true protein requirement, but need a well-balanced mixture of indispensable and dispensable amino acids. Many investigators have used semipurified and purified diets to estimate the protein requirement of fish (Table 1). Most of these values have been estimated from dose response curves yielding the minimum of dietary protein necessary for maximal growth. Some of these requirement values may have been overestimated.

The dietary protein requirement for fish is influenced by the dietary protein-to-energy balance, the amino acid composition and the digestibility of the test

Table 1 Estimated protein requirements of juvenile fish

Species	Protein source(s)	Estimated requirements (%)	References
Channel catfish (*Ictalurus punctatus*)	Whole-egg protein	32–36	21
Chinook salmon (*Oncorhynchus tshawytscha*)	Casein, gelatin, and amino acids	40	17
Coho salmon (*Oncorhynchus kisutch*)	Casein	40	108
Common carp (*Cyprinus carpio*)	Casein	38	64
		31	89
Estuary grouper (*Epinephelus salmoides*)	Tuna muscle meal	40–50	91
Gilthead bream (*Chrysophrys aurata*)	Casein, FPC[a], and amino acids	40	79
Grass carp (*Ctenopharyngodon idella*)	Casein	41–43	15
Japanese eel (*Anguilla japonica*)	Casein and amino acids	44–145	60
Largemouth bass (*Micropterus salmoides*)	Casein and FPC	40	3
Milkfish (fry) (*Chanos chanos*)	Casein	40	46
Plaice (*Pleuronectes platessa*)	Cod muscle	50	13
Puffer fish (*Fugu rubripes*)	Casein	50	35
Rainbow trout (*Salmo gairdneri*)	Fish meal	40	80
	Casein and gelatin	40	107
	Casein, gelatin, and amino acids	45	25
Red sea bream (*Chrysophrys major*)	Casein	55	104
Smallmouth bass (*Micropterus dolomieui*)	Casein and FPC	45	3
Snakehead (*Channa micropeltes*)	Fish meal	52	98
Sockeye salmon (*Oncorhynchus nerka*)	Casein, gelatin, and amino acids	45	25
Striped bass (*Morone saxatilis*)	Fish meal and soy proteinate	47	55
Tilapia			
(*Tilapia aurea*)	Casein and egg albumin	34	103
(*T. mossambica*)	White fish meal	40	34
(*T. nilotica*)	Casein	30	97
(*T. zillii*)	Casein	35	50
Yellowtail (*Seriola quinqueradiata*)	Sand eel and fish meal	55	87

[a] Fish protein concentrate.

protein(s), and the amount of nonprotein energy sources in the test diet. Excess energy in the test diet may limit consumption, since fish, like other animals, eat to meet their energy requirement (45, 65). Most investigators state they used isoenergetic diets to determine the protein requirements; however, the metabolizable energy of the various ingredients have not been determined for most fishes and various physiological fuel values have been used. Values of 4, 8, and 1.6 kcal/g of protein, fat, and carbohydrate have been used for salmonids (67). Other values are used for different fish, for example 3.5, 8.1, 2.5 (57) and 4, 9, 4 (21) for channel catfish, *Ictalurus punctatus;* 4, 8, 2 (63) and 4, 9, 4 (107) for rainbow trout, *Salmo gairdneri;* 4.5, 9.0, 4.0 (50) and 4.5, 8.5, 3.5 (34) for *Tilapia zillii* and *T. mossambica*, respectively; 5, 9, 2 (98) for snakehead, *Channa micropeltes;* and 5.7, 9.5, 4 (13) for plaice, *Pleuronectes platessa*. The influence of changes in dietary protein-to-energy ratios on growth and protein utilization has been demonstrated in several species of fish: rainbow trout (45, 90); yellowtail, *Seriola quinqueradiata* (83, 87); common carp, *Cyprinus carpio* (89); channel catfish (21, 65); brook trout, *Salvelinus fontinalis* (72); *Tilapia aurea, T. mossambica* (34, 103); and the estuary grouper, *Epinephelus salmoides* (91).

Many investigators have demonstrated the important sparing effect of non-protein energy sources on the utilization of dietary protein. The utilization of dietary carbohydrate is known to vary among species, and it has been shown to spare protein in salmonids (7, 8, 45, 68, 72); plaice (11); turbot, *Scophtalmus maximus* (1); channel catfish (21, 22); sea bass, *Dicentrarchus labrax* (2); common carp (63, 81, 89); and red sea bream, *Chrysophrys major* (20). Lipids have also been shown to spare protein and enhance protein utilization in salmonids (45, 71, 72, 88, 90, 93, 105, 106); common carp (84, 85, 93); channel catfish (21, 22, 56, 65, 86); turbot (1); striped bass, *Morone saxatilis* (55); and *Tilapia aurea* (103). The quality of the dietary protein used in the test diets to determine the protein requirements also affects the estimated requirement value.

FACTORS AFFECTING REQUIREMENTS

Generally, the protein requirements of fish decrease with increasing size and age. For example, the optimal dietary protein level for very young salmonids is 45–50% of the diet, while juveniles require 40% and yearlings require about 35% dietary protein (31, 58). Similarly, channel catfish fry require about 40% protein, whereas fingerlings require 30–35% protein, and larger fish require 25–35% protein (57, 65). Dietary protein levels have also been recommended (57) for common carp of various sizes: 43–47% for fry, 37–42% for fingerlings and subadults, and 28–32% for adult and brood fish. Balarin & Haller (6) have reviewed various studies on tilapia and concluded that fish of less than 1 g require 35–50% protein, 1–5-g fish require 30–40% protein, 5–25-g fish require 25–30% protein; tilapia weighing more than 25 g require 20–25% protein.

Changes in water temperature have been shown to alter the protein requirement in some fish. Chinook salmon require 40% protein at 8°C and 55% protein at 15°C (17). Similarly, striped bass were found to require 47% protein at 20°C and about 55% at 24°C (54, 55). Rainbow trout were fed practical diets containing 35, 40, and 45% crude protein at temperatures ranging from 9 to 18°C and no apparent differences in protein requirement were reported (58).

MAINTENANCE REQUIREMENTS

The methodology used to evaluate the excretion of endogenous nitrogen by fish was recently reviewed by Luquet & Kaushik (48). Two types of methods have generally been used: direct methods that involve measuring the combined fecal, urinary, and branchial losses; and an indirect method based on carcass analysis. The fish are maintained without food, fed on protein-free diets, or fed diets containing low levels of protein.

Carcass analysis is the most convenient method to use for fish. Nitrogen retention is revealed by the difference between nitrogen consumed and nitrogen retained by the fish at the end of the experimental period. These data can also be combined with growth data obtained by feeding increasing ration size and obtaining the nitrogen or protein intake that results in zero growth.

Luquet & Kaushik (48) summarized the various estimated endogenous nitrogen excretion values for several fish species. These workers also discussed several factors that appear to influence these measurements. The protein requirement for maintenance can be calculated from the endogenous nitrogen excretion data by taking into account the digestibility and biological value of the test protein. Ogino & Chen (62) obtained a maintenance requirement of 0.95 g protein/kg body weight/day for carp fed casein as the sole protein source. Kaushik et al (38) estimated the maintenance requirement for rainbow trout to be 1.6 g protein/kg body weight/day based on data obtained by feeding fish meal as the sole protein source. The maintenance requirement for channel catfish was found to be 1.3 g protein/kg body weight/day based on growth rates of fish fed at 0 to 5% of body weight/day of diets containing either 25 or 35% crude protein diets made from a casein-gelatin mixture (R. P. Wilson, unpublished data). The requirement was found to be about 1.0 g protein/kg body weight/day based on protein retention data from the above growth studies.

QUALITATIVE AMINO ACID REQUIREMENTS

The first successful amino acid test diet for fish was reported in 1957 (23). It was based on previous amino acid test diets used by Rose and co-workers in determining the amino acid requirements of the young albino rat (52). Halver

(23) compared test diets containing 70% crystalline L-amino acids formulated on the amino acid patterns of whole chicken egg protein, chinook salmon egg protein, and chinook yolk-sac fry protein. The test diet based on whole chicken egg protein gave the best growth and feed efficiency for chinook salmon over a 12-week period. Therefore, this test diet was used to determine the qualitative amino acid needs of chinook salmon (26). These workers determined the essentiality of the 18 common protein amino acids by comparing the relative growth rates of fish fed the basal and specific amino acid--deficient diet over a 10-week period. For each of the 10 indispensable amino acids, groups of the deficient fish were split at 6 weeks, with one lot being continued on the deficient diet and the other lot fed the basal diet. In each of the lots shifted to the basal diet, the fish showed an immediate and substantial growth response to the complete diet.

Several other workers have utilized amino acid test diets similar to those developed by Halver (23) to study the essentiality of various amino acids in other species. Such studies in common carp were initially unsuccessful. The young carp would consume the test diets but a marked reduction in growth rate was observed corresponding to the relative amount of free amino acids in the test diets (4). Dupree & Halver (19) and Nose et al (61) found that the amino acid test diets must be neutralized before they can be utilized by channel catfish and common carp, respectively.

Cowey et al (10) used radioactive labelled glucose to determine the qualitative amino acid requirements of the plaice and sole, *Solea solea*. Small (2–3 g) fish were injected intraperitoneally with [U-^{14}C] glucose and fed a natural diet for 6 days. The fish were then killed, homogenized, and the protein isolated. A sample of the protein was hydrolyzed and the constituent amino acids were separated by chromatography and counted for radioactivity. Significant radioactivity was incorporated into the dispensable amino acids and not into the indispensable amino acids. Similar studies have been done with the sea bass, *Dicentrarchus labrax* (53). All finfish studied to date have required the same 10 amino acids considered indispensable for most animals (Table 2). These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

QUANTITATIVE AMINO ACID REQUIREMENTS

Most investigators have used the basic method developed by Halver and co-workers (52) to determine the quantitative amino acid requirements for fish. This procedure involves feeding graded levels of one amino acid at a time in a test diet containing either all crystalline amino acids or a mixture of casein, gelatin, and crystalline amino acids formulated so that the amino acid profile is identical to whole chicken egg protein (except, of course, for the amino acid

Table 2 Finfish known to require the same 10 indispensable amino acids

Species	Kind of studies	Reference
Channel catfish (*Ictalurus punctatus*)	Growth	19
Chinook salmon (*Oncorhynchus tshawytscha*)	Growth	26
Common carp (*Cyprinus carpio*)	Growth	61
European eel (*Anguilla anguilla*)	Growth	5
Japanese eel (*Anguilla japonica*)	Growth	5
Plaice (*Pleuronectes platessa*)	^{14}C-labeling	10
Rainbow trout (*Salmo gairdneri*)	Growth	82
Red sea bream (*Chrysophrys major*)	Growth	104
Sea bass (*Dicentrarchus labrax*)	^{14}C-labeling	53
Sockeye salmon (*Oncorhynchus nerka*)	Growth	29
Sole (*Solea solea*)	^{14}C-labeling	10
Tilapia (*Tilapia zillii*)	Growth	51

being tested). Diets are designed to contain protein levels at or slightly below the optimal protein requirement for that species to assure maximal utilization of the limiting amino acid. This procedure has been used successfully in chinook, coho, and sockeye salmon, Japanese eel, and rainbow trout. However, the amino acid test diets must be neutralized with a base before they can be utilized by common carp (61), and channel catfish (101).

Other investigators have used semipurified and practical-type test diets to estimate amino acid requirements in fish. The semipurified diet includes an imbalanced protein as the major source of dietary amino acids, for example zein (16, 36) or corn gluten (27, 39), which are deficient in certain amino acids. The practical-type diet involves normal feedstuffs to furnish the bulk of the amino acids in the test diets. These may be formulated with a fixed amount of the desired protein level and the remaining amount of the protein equivalent is made up of crystalline amino acids (32, 33, 49, 92, 94, 96). Wilson (99) has pointed out some of the possible problems inherent in using these types of diets in assessing the amino acid requirements of fish.

Most of the reported amino acid requirement values are estimates based on the conventional growth response curves or Almquist plots. Replicate groups of fish are fed diets containing graded levels of the test amino acid until measurable differences appear in their growth. A linear increase in growth rate is normally observed with increasing amino acid intake up to a plateau corresponding to the requirement for the specific amino acid.

Cowey & Tacon (14) and Cowey & Luquet (12) have reviewed the various problems involved in the accurate determination of the amino acid requirements of fish based on growth studies. Some of these problems are (*a*) imprecise interpretation of the growth response curves, i.e. the breakpoint of the curve is often determined subjectively; (*b*) the growth rates commonly observed with amino acid test diets being generally lower than those observed with intact protein diets; and (*c*) some of the crystalline amino acids in the test diets being leached during the feeding studies.

Some investigators have found a high degree of correlation of either serum or blood and muscle free amino acid levels to dietary amino acid intake in fish. The basic hypothesis suggests serum or tissue content of the amino acid will remain low until the requirement for that amino acid is met, and then increase to high levels when excessive amino acid is consumed. This technique has been useful in determining the amino acid requirement in only a few cases. The studies in the channel catfish of the 10 indispensable amino acids showed that serum lysine (101), threonine (100), histidine (102), and methionine (30) were required.

Amino acid oxidation studies have been used to estimate amino acid requirements for fish. Rainbow trout were fed test diets containing increasing levels of dietary lysine for 12 weeks. Three fish from each dietary treatment were injected intraperitoneally with a tracer dose of [U-^{14}C] lysine and the respired carbon dioxide was collected over a 20-hr period. The level of $^{14}C\text{-}CO_2$ produced was used as a direct measurement of the rate of oxidation of lysine in the fish. The level of oxidation observed was very low in those fish fed low dietary levels of lysine, somewhat higher for the intermediate dietary levels, and much higher for the higher levels of dietary lysine (96). The break-point of the dose response curve indicated a dietary requirement of 20 g lysine/kg diet, which was in close agreement with a value of 19 g lysine/kg diet obtained from growth data.

These same investigators have also used this method to assess the tryptophan requirement of rainbow trout (94). In this case, the requirement value was lower, 2.0 vs 2.5 g/kg diet, when determined by tryptophan oxidation than by growth data. Using one or more of the above techniques, researchers determined the indispensable amino acid requirements for several species of fish.

Arginine

The arginine requirement values are summarized in Table 3. Salmon have the highest requirement at about 6% of dietary protein; the other species require about 4–5%. The arginine requirement in rainbow trout has been reported to be influenced by salinity (36). Kaushik found the requirement to be 3.3% of protein in freshwater and to decrease to 2.8% in 20 ppt salinity and to 2.2% of protein in full-strength seawater. These data indicate that the protein requirement should decrease as salinity increases. However, Zeitoun et al (107) reported that rainbow trout fingerlings require 40% protein for optimum growth at 10 ppt salinity and 45% protein at 20 ppt salinity. Additional studies are needed to confirm these observations before final conclusions can be drawn.

Histidine

The histidine requirements of fish exhibit excellent agreement among the species studied, with a range of 1.5–2.1% of protein. Wilson et al (102) found that the serum free-histidine levels in channel catfish responded positively to graded dietary histidine intake. In serum, free histidine concentration increased significantly up to the dietary requirement (as determined by growth data) and then remained constant at higher dietary intake.

Muscle carnosine concentration has been shown to be altered by dietary histidine in the chinook salmon (47). It was depleted when a histidine-deficient diet was fed. Carnosine could not be detected in muscle tissue of the channel catfish regardless of the dietary level of histidine (102). This may, however, serve as another potential indicator of histidine status in other fishes.

Isoleucine

The isoleucine requirements of fish appear to be about 2.0–2.6% of protein for those species studied except for the Japanese eel, which has a much higher value. The value originally reported for lake trout, *Salvelinus namaycush,* was lower (1.54–2.06% of protein) than those observed in other fishes (32). Hughes et al (32) state that the test diets were formulated to meet the required levels of amino acids for a 35% protein diet; however, when the total protein level is calculated based on the apparent nitrogen content of the test diets, a much lower dietary protein level is obtained. When the requirement value is recalculated based on the calculated nitrogen content of the diet, the requirement value is within the range reported for other species. Wilson et al (102) determined the effects of dietary isoleucine on serum free-isoleucine, leucine, and valine in the channel catfish. Even though the serum isoleucine increased somewhat with increasing isoleucine intake, these data did not confirm the requirement determined by growth data. The serum free-leucine and valine concentrations

Table 3 Indispensable amino acid requirements[a]

Fish	Arginine	Histidine	Isoleucine	Leucine	Valine
Chinook salmon	6.0(2.4/40)(43)	1.8(0.7/40)(43)	2.2(0.9/41)(9)	3.9(1.6/41)(9)	3.2(1.3/40)(9)
Coho salmon	5.8(2.3/40)(43)	1.8(0.7/40)(43)			
Common carp	4.3(1.6/38.5)(59)	2.1(0.8/38.5)(59)	2.5(0.9/38.5)(59)	3.3(1.3/38.5)(59)	3.6(1.4/38.5)(59)
Japanese eel	4.5(1.7/37.7)(59)	2.1(0.8/37.7)(59)	4.0(1.5/37.7)(59)	5.3(2.0/37.7)(59)	4.0(1.5/37.7)(59)
Channel catfish	4.3(1.03/24)(77)	1.5(0.37/24)(102)	2.6(0.62/24)(102)	3.5(0.84/24)(102)	3.0(0.71/24)(102)
Lake trout			2.6(0.72/27.6)(32)	3.5(0.96/27.6)(32)	3.3(0.78/23.7)(32)
Rainbow trout	3.3(1.2/36)(36)				
	4.0(1.4/35)(41)				
	5.9(2.8/47)(39)				
Gilthead bream	5.0(1.7/34)(49)				
Tilapia	4.01(1.59/40)(33)				

[a]Requirements are expressed as percentage of protein. In parentheses, the numerators are requirements as percentage of diet and the denominators are percentage of total protein in diet. References, in parentheses, follow each notation.

appeared to parallel the serum free-isoleucine concentrations. These workers also observed a much higher than expected mortality in their isoleucine-deficient fish.

Leucine

The leucine requirements of fish are between 3.3 and 4.0% of protein for those species studied except for the eel, which has a much higher requirement. The value reported for lake trout (32) was lower (2.74–3.66% of protein); however, after the data were recalculated, they appear to agree with those previously reported for the other species.

Wilson et al (102) reported that the serum free-leucine level in channel catfish remained constant regardless of dietary leucine intake. There was, however, a marked effect of dietary leucine on the serum free-isoleucine and valine levels: about a six-fold increase in both concentrations at the 0.7% dietary leucine level, as compared to the 0.6% leucine level. These elevated levels of isoleucine and valine did not return to the base-line values until a dietary level of 1.2% or above was fed. This observation was interpreted to indicate that leucine may facilitate the tissue uptake of branched-chain amino acids and/or their intracellular metabolism.

Valine

Reasonable agreement exists among the valine requirements reported for the species studied. The requirement ranges from about 3 to 4% of protein. A much lower value (1.77–2.23% of protein) was originally reported for lake trout (32); however, upon recalculation of the data the revised value fits within the range of those reported for other species.

The effect of valine intake on serum valine levels in the channel catfish was similar to that described for isoleucine (102).

Isoleucine-Leucine-Valine Interactions

There are differences in the apparent isoleucine-leucine-valine interactions among different fishes. Chance et al (9) reported that the isoleucine requirement in chinook salmon was increased slightly with increasing levels of dietary leucine. This effect was not observed in either the common carp (59) or channel catfish (74). Nose (59) did, however, observe reduced growth rates in carp fed high dietary isoleucine levels during his study of leucine requirement. This reduced growth was not observed when the study was repeated at lower isoleucine levels. Hughes et al (32) observed a different pattern in plasma free branched-chain amino acids in lake trout fed increasing levels of valine than was previously observed in the channel catfish (102). No significant change in the plasma free-valine concentrations was observed until after the dietary requirement was met and then it increased about 2.5-fold. Plasma free-isoleucine and leucine were both elevated in the valine-deficient fish, and then decreased as dietary valine increased.

Robinson et al (74) concluded that a nutritional interrelationship does exist among the branched-chain amino acids in the channel catfish, but the interaction does not appear to be as severe as has been observed in certain other animals. These workers also suggest from their data that leucine may control either the tissue uptake or catabolism of valine and isoleucine in the channel catfish.

Lysine

The lysine requirement values are listed in Table 4. Similar values have been reported for the chinook salmon, Japanese eel, channel catfish, and gilthead bream. A slightly higher value was found for common carp. The lower value for tilapia may be due to low growth rates observed and/or the type of test diet used. The requirement for rainbow trout appears to be lower than that observed for other species. Walton et al (96) observed excellent agreement between requirement values determined by growth studies and amino acid oxidation studies in rainbow trout. The much higher value reported by Ketola (39) appears to be out of line with the other values. Ketola (39) observed very high mortality and incidence of caudal fin erosion in fish fed the lysine-deficient diets. Since the caudal fin erosion was not observed in fish fed diets deficient in arginine, the author attributed the fin erosion specifically to the lysine deficiency. A recent report from the same laboratory has also indicated a high incidence of caudal fin erosion in tryptophan-deficient rainbow trout (70).

Arginine-Lysine Interactions

A dietary interrelationship between arginine and lysine has been well documented in certain animals and is commonly known as the lysine-arginine antagonism. Robinson et al (77) could not demonstrate this antagonism in channel catfish when either excess lysine was fed in diets adequate or marginal in arginine, or when excess arginine was fed in diets adequate or marginal in lysine. Feeding excess lysine did not affect growth rates of rainbow trout fed low levels of arginine (41). Kaushik & Fauconneau (37) reported some biochemical evidence indicating that metabolic antagonism may exist between lysine and arginine in the rainbow trout. Increasing dietary lysine intake affected plasma arginine and urea levels and ammonia excretion. These changes were due to a decrease in the relative rate of arginine degradation as the level of dietary lysine increased (37).

Phenylalanine

Phenylalanine and tyrosine are classified as aromatic amino acids, and adequate amounts of both are needed for proper protein synthesis and other physiological

Table 4 Indispensable amino acid requirements[a]

Fish	Lysine	Phenylalanine	Methionine	Threonine	Tryptophan
Chinook salmon	5.0(2.0/40)(27)	5.1(2.1/41)(9)	4.0(1.6/40)(28)	2.2(0.9/40)(18)	0.5(0.2/40)(24)
		Tyr = 0.4%	Cys = 1%		
Coho salmon					0.5(0.2/40)(24)
Sockeye salmon					0.5(0.2/40)(24)
Common carp	5.7(2.2/38.5)(59)	6.5(2.5/38.5)(59)	3.1(1.2/38.5)(59)	3.9(1.5/38.5)(59)	0.8(0.3/38.5)(59)
		Tyr = 0%	Cys = 0%		
					0.3(0.13/42.5)(16)
Japanese eel	5.3(2.0/37.7(59)	5.8(2.2/37.7)(59)	3.2(1.2/37.7)(59)	4.0(1.5/37.7)(59)	1.1(0.4/37.7)(59)
		Tyr = 0%	Cys = 0%		
Channel catfish	5.1(1.23/24)(101)	5.0(1.20/24)(75)	2.3(0.56/24)(30)	2.0(0.53/24)(100)	0.5(0.12/24)(100)
		Tyr = 0.3%	Cys = 0%		
	5.0(1.5/30)(76)				
Rainbow trout	3.7(1.3/35)(40)		2.2(1.0/46.4)(95)		0.5(0.25/55)(94)
			Cys = 0%		
	4.2(1.9/45)(96)				1.4(0.58/42)(70)
	6.1(2.9/47)(39)		3.0(1.1/35)(78)		
			Cys = 0.3%		
			2.9(1.0/35)(42)		
			Cys = 0.5%		
Gilthead bream	5.0(1.7/34)(49)		4.0(1.4/34)(49)		0.6(0.2/34)(49)
			Cys = not stated		
Tilapia	4.1(1.62/40)(33)		3.2(1.27/40)(33)		
			Cys = 0.7%		
Sea bass			2.0(1.0/50)(92)		
			Cys = not stated		

[a]Requirements are expressed as percentage of protein. In parentheses, the numerators are requirements as percentage of diet and the denominators are percentage of total protein in diet. References, in parentheses, follow each notation. Cys and Tyr = percentage in the diet.

functions in fish. Fish can readily convert phenylalanine to tyrosine or utilize dietary tyrosine to meet their metabolic needs for this amino acid. Therefore, in order to determine the total aromatic amino acid requirement (phenylalanine plus tyrosine), the dietary requirement for phenylalanine is determined either in the absence of tyrosine or with test diets containing very low levels of tyrosine.

The phenylalanine or total aromatic amino acid requirements are listed in Table 4. Similar values have been reported for chinook salmon and channel catfish, with slightly higher values being required by the Japanese eel and common carp.

Methionine

A relationship similar to phenylalanine and tyrosine exists for methionine and cystine. Cystine is considered dispensable because it can be synthesized by the fish from the indispensable amino acid methionine. When methionine is fed without cystine, a portion of the methionine is used for protein synthesis, and a portion is converted to cysteine for incorporation into protein. If cystine is included in the diet, it reduces the amount of dietary methionine needed. Fish have a total sulfur amino acid requirement rather than a specific methionine requirement.

The methionine or total sulfur amino acid requirement values (Table 4) reveal some differences among species. Chinook salmon and gilthead bream require the highest level at about 4% of protein, channel catfish require the least at 2.3% of protein, and the other species studied require about 3.0% of protein.

Rainbow trout appear to be unique in that methionine deficiency results in bilateral cataracts (69). Poston et al (69) observed cataracts in rainbow trout fed diets containing isolated soybean protein. The cataracts were prevented by supplementing the diet with methionine. Cataracts have also been observed in methionine-deficient rainbow trout by Walton et al (95) and Rumsey et al (78). This deficiency sign has not been reported in any other species of fish.

Several studies have shown that the presence of dietary cystine reduces the amount of dietary methionine necessary for maximum growth. The cystine replacement value for methionine on a sulfur basis has been determined to be about 60% for channel catfish (30) and 40% for rainbow trout (42).

Robinson et al (73) studied the utilization of several dietary sulfur compounds in channel catfish. Growth and feed efficiency data indicated that DL-methionine was utilized as effectively as L-methionine. Methionine hydroxy analogue was only about 25% as effective in promoting growth as L-methionine. No significant growth response was observed when taurine or inorganic sulfate was added to the basal diet. Page et al (66) were also unable to detect the utilization of taurine and inorganic sulfate as sulfur sources in rainbow trout. D-methionine has been shown to replace L-methionine on an equal basis in rainbow trout (42).

Threonine

The Japanese eel and common carp appear to have a higher threonine requirement than the chinook salmon and channel catfish. DeLong et al (18) found the threonine requirement of young chinook salmon to be the same when determined at rearing temperatures of 8° and 15°C. These findings were not expected, since these workers have previously reported the protein requirement to increase from 40% at 8° to 55% at 15°C (17). The data on the threonine requirement would tend to indicate that the actual protein requirement for optimal protein synthesis does not change with increasing temperature.

Tryptophan

A tryptophan level of 0.5% of protein appears to be adequate for most fishes, with the exception of the Japanese eel and possibly the common carp. The value of 0.3% of protein reported by Dabrowski (16) for common carp appears to be low, and may have resulted from the experimental conditions. The value obtained by Poston & Rumsey (70) for rainbow trout appears to be too high when compared to the other data reported for this species.

Tryptophan deficiency results in several anatomical deformities in certain salmonid species, but not in other fishes. Halver & Shanks (29) observed scoliosis and lordosis in sockeye salmon, but not in chinook salmon fed tryptophan-deficient diets. Scoliosis and lordosis has also been observed in tryptophan-deficient rainbow trout (82). These deformities were found to be reversible when the fish were fed adequate dietary tryptophan (44, 82). Other tryptophan deficiency signs in rainbow trout include renal calcinosis (44), caudal fin erosion, cataracts, and short gill opercula (70), and increased liver and kidney levels of calcium, magnesium, sodium, and potassium (94).

SUMMARY

Tentative qualitative and quantitative amino acid requirements have been reported for the major species of fish reared for market or as replacement stocks for natural waters. Most work has concentrated upon juvenile fish or upon rapidly growing young market fish; these have high protein dietary requirements (30–50%) that are in direct contrast to the homothermic terrestial animals. Net protein utilization from the diet is similar or slightly better than that found in avian species, but energy needs are much lower in fish and as a result the body protein deposition in fish is larger (about 5 g protein/MJ for the chick versus about 10 g protein/MJ for young fish).

Qualitative amino acid requirements appear identical for all fish species examined; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are all required for normal growth

and metabolism. Quantitative requirements differ only slightly among species that have been tested. Salmon have higher arginine requirements than other fish examined (33, 36, 43, 59, 77). Catfish appear to have a lower requirement for histidine and threonine (100, 102), and the Japanese eel seems to need more tryptophan in the diet (59). However, when the quantitative requirements for indispensable amino acids are expressed as a percentage of the protein fed, then a remarkable harmony appears between values needed for maximal growth for most species examined. A review of Tables 3 and 4 will disclose the paucity of information available considering the large number of fish species reared commercially over the world. Most commercial diet formulations have relied upon the work done on salmon, catfish, and carp, and their amino acid and protein requirement values have been used. Remarkably, these diets have produced other species of fish economically.

Sparing effects of one amino acid on another have only been studied with cystine-methionine and tyrosine-phenylalanine. Arginine and analogues of methionine have been used as good nitrogen sources for salmon. Isoleucine-leucine ratios have been measured and experiments indicate some growth inhibition when the isoleucine-leucine ratio was greater than 2/1 (9). Valine at abnormally high levels also inhibited growth (9). Much more work needs to be done on the effects of subtle differences in amino acid ratios in the diet, and major emphasis should be placed on the important role of the dispensable amino acids in fish nutrition.

Fish production is expanding rapidly in northern Europe, in the southern US, and in the developing nations of the world. Protein and indispensable amino acid dietary intake are growth determinants, and the dietary requirement is higher for fish than for terrestrial animals. In the future, more emphasis can be expected on quantitative amino acid requirements and economical sources for protein for many new fish species reared under a variety of environmental conditions.

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