

# The Essential Nutrients

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# 2

This chapter discusses the essential food nutrients, their classification or types, chemical structures, general functions, and importance in the nutrition of aquatic animals. It is divided into six sections: proteins and amino acids, lipids and fatty acids, carbohydrates, energy, vitamins, and minerals. There are specific learning objectives for each section.

## PROTEINS AND AMINO ACIDS

### Introduction

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Proteins are macromolecules made up of carbon, hydrogen, oxygen, nitrogen, and may also contain sulfur. The nitrogen content of protein distinguishes it from fats and carbohydrates and other organic compounds. Proteins occur in every living cell as compounds of tissues and organs and are major components of fish tissues. They are needed for growth and tissue repair and maintenance. No other nutrient can take the place of protein in its major role of building and repairing worn out cells and tissues. In addition, proteins are also responsible for muscle contraction and are components of enzymes, hormones, and antibodies. Proteins may be complexed with heme, carbohydrate, lipid, or nucleic acids.

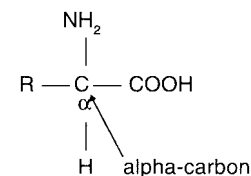
Aquatic animals must consume protein to provide a continuous supply necessary for replacing worn-out tissues (maintenance) and for the synthesis of new tissues (growth and reproduction). Inadequate dietary protein will result in retardation or cessation of growth or a loss of weight due to withdrawal of protein from less vital tissues in order to maintain the functions of more vital ones.

This section aims to teach the reader the ten essential amino acids required by fish and their chemical structures, distinguish between essential and non-essential amino acids; the fate of absorbed amino acids in fish; effects of deficiencies and excesses of dietary amino acids in fish diets; the procedure on how to determine the qualitative and quantitative amino acid requirements of fish; methods of evaluating protein quality; and how to determine protein requirements of some aquaculture species.

## Amino Acids

Proteins can be broken down or hydrolyzed into a number of basic units called amino acids. These amino acids are called the building blocks of proteins. The term amino comes from the  $-NH_2$  or an amino group which is “basic” in nature and the “acid” part comes from the  $-COOH$  or a carboxyl group, hence the term amino acid. In protein molecules, amino acids form peptide bonds (bonds between amino and carboxyl groups) in long strands called polypeptide chains. There are many amino acids in nature but only 20 are naturally occurring. These are also the 20 amino acids specified in the genetic code common to all life.

The components of the general structure of an amino acid are: 1) a carboxyl ( $-COOH$ ) group, 2) an amino group ( $-NH_2$ ) on the alpha ( $\alpha$ ) carbon and 3) an alkyl (R) group attached to the  $\alpha$  C atom. The amino acids differ in their R group. The R group gives an amino acid its



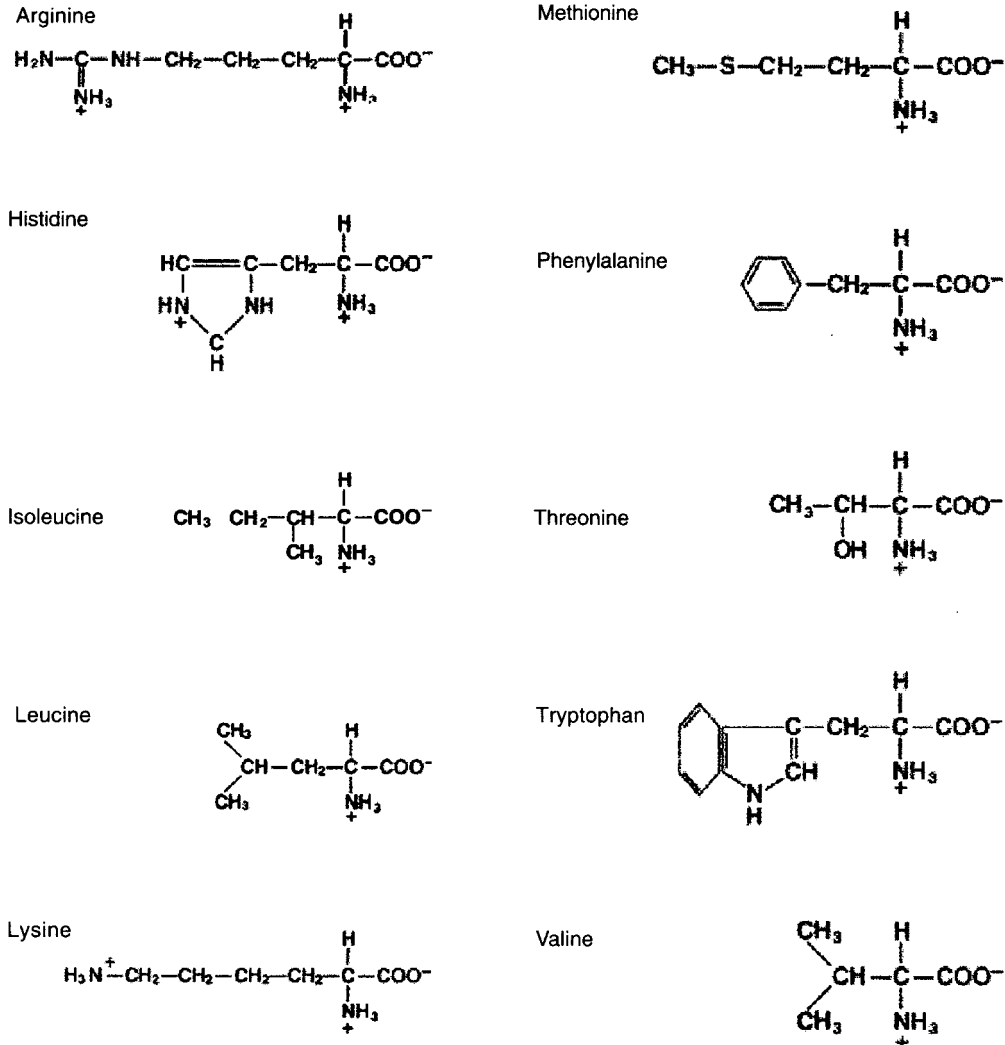
individual chemical characteristic. The formation of a peptide bond or amide linkage involves covalent bonding between an amino group of one amino acid and the carboxyl group of the adjoining amino acid. Proteins may consist of one or more polypeptide chains held together in the protein molecule.

Amino acids are commonly referred to by three- or one-letter abbreviations as listed in Table 2.1.

**Table 2.1** Names and abbreviations of the common amino acids

Essential Amino Acids	Abbreviation Three-letter	One-letter
Arginine	Arg	R
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Threonine	Thr	T
Tryptophan	Trp	W
Valine	Val	V
Non-essential Amino Acids		
Alanine	Ala	A
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Proline	Pro	P
Serine	Ser	S
Tyrosine	Tyr	Y

The chemical structures of each of these essential amino acids are shown in Figure 2.1.



**Figure 2.1**  
Chemical structures of the ten essential amino acids.

## Classification of Amino Acids

Amino acids are classified as essential or indispensable, and non-essential or dispensable. Essential or indispensable amino acids cannot be made or synthesized by the animal or which are synthesized in amounts not enough to support maximum growth and have to be present in their diet. The capacity of different feed proteins to meet the amino acid needs of fish differs considerably. The essentiality of an amino acid will also depend on the animal being fed. For example, glycine is required by chicken but is not essential for fish. Non-essential or dispensable amino

acids can be adequately synthesized by the animal or formed from other amino acids in sufficient amounts in tissues and does not have to be present in their diet. Amino acids can also be classified according to the chemical composition of their side chain as listed below:

1. Aliphatic amino acids
  - a. basic - arginine, lysine
  - b. acidic - aspartic acid, glutamic acid
  - c. neutral - leucine, isoleucine, valine, alanine, glycine  
methionine, cysteine  
threonine, serine
2. Aromatic amino acids  
phenylalanine, tyrosine
3. Heterocyclic amino acids  
histidine, tryptophan, proline

### **Essential Amino Acids**

There are ten essential amino acids (EAA) required by fish for growth and maintenance of life: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Aside from being the building blocks of proteins, some amino acids are precursors or supply part of the structure of other substances. Methionine is the precursor of cysteine and cystine. Methionine also supplies methyl (CH<sub>3</sub>) groups for creatine, choline, and many other substances. When a hydroxyl (OH) group is added to phenylalanine, tyrosine is formed. Tyrosine is needed to form the hormones thyroxine, epinephrine and norepinephrine, and melanin pigments. Arginine yields ornithine when urea is formed in the urea cycle. The removal of a carboxyl (COOH) group from histidine forms histamine. Tryptophan is the precursor of serotonin and the vitamin, nicotinic acid. All finfishes have a requirement for the same ten essential amino acids.

### **Non-essential Amino Acids**

The non-essential amino acids for fish are: alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine. These amino acids have nutritional significance because their presence in the diet conserves energy that is required for protein synthesis. Some dispensable amino acids can partially replace or spare indispensable amino acids. Phenylalanine can be partially replaced by tyrosine and methionine by cystine. Thus, requirements for phenylalanine and methionine decrease when tyrosine and cystine, respectively, are present in the diet.

## Classification of Proteins

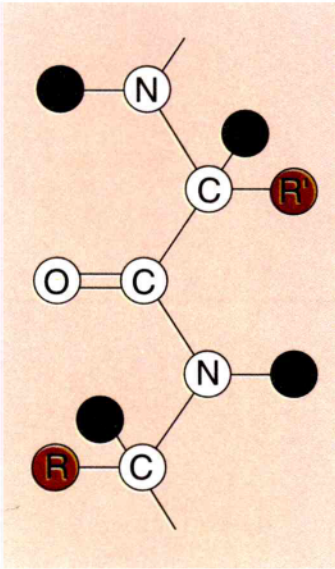
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Proteins are broadly classified according to their form, solubility, and physical properties.

- ❑ **Globular proteins** have compact spherical or globular shape. They are mostly soluble in aqueous systems. They include: enzymes, protein hormones, proteins of the serum fraction of the blood and antibodies.
- ❑ **Fibrous proteins** consist of a long chain of polypeptides. They are highly insoluble and resistant to action of the digestive enzymes. They include collagen, found in cartilage or soft bones, blood vessels, bone matrix, tendon, fins and skin; elastins, that are a component of arteries and ligaments; and keratins, in protective coverings such as skin and scales.

Based on physical properties, proteins are grouped into simple, conjugated, and derived proteins:

- ❑ **Simple proteins** yield only amino acids or their derivatives when hydrolysed. They include albumins (egg albumin, serum albumin from blood, lactoalbumin from milk, leucosin from wheat); albuminoids (keratin from hair, fingernails, feathers, wool, silk fibroin, elastin from connective tissue, collagen from cartilage and bones); globulins (edestin from hemp seed, serum globulin from blood, lactoglobulin from milk, legumin from peas); histones (globin from hemoglobin, scombrone from spermatozoa of mackerel); and protamins (salmine from salmon, scombrine from mackerel). These groups are differentiated by solubility in various solvents such as water, salt solution, alcohol, and by other characteristics.
- ❑ **Conjugated proteins** yield amino acids and non-protein components when broken down by hydrolysis. They include the nucleoproteins, glycoproteins, phosphoproteins, hemoglobins, and lecithoproteins. Nucleoproteins are compounds of one or more protein molecules with nucleic acid present in all cell nuclei. Glycoproteins are compounds of the protein molecule and substances containing a carbohydrate group other than nucleic acid (e.g., mucin). Phosphoproteins are compounds of the protein molecule with a phosphorus-containing substance other than nucleic acid or lecithin (e.g., casein). Hemoglobins are compounds of the protein molecule with hematin or a similar substance (e.g., hemoglobin). Lecithoproteins are compounds of the protein molecule with lecithin (e.g., tissue fibrinogen).
- ❑ **Derived proteins** consist of compounds representing altered and degraded products of naturally occurring proteins, produced by the action of heat, enzymes, or chemical agents.

**Figure 2.2A**

Primary structure consists of amino acids joined in sequence by peptide bonds.

## Protein Structure

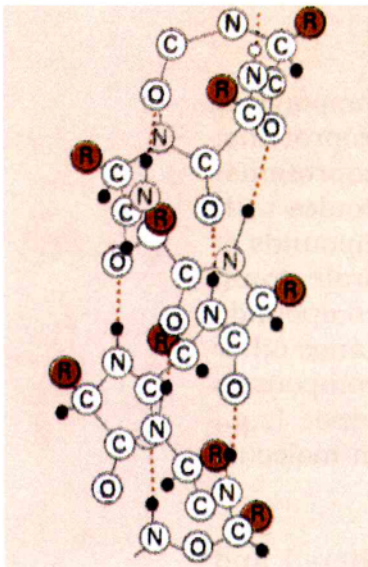
The composition, organization and shape of proteins are directly related to their function. Because of the complicated structure of proteins, a new terminology has developed as an aid in describing the structural organization of a protein molecule. These are designated as primary, secondary, tertiary, and quaternary structure of proteins.

- **Primary structure** describes the specific sequence in which the amino acids are linked together by peptide bonds in a polypeptide chain (Figure 2.2A). For example, the peptide Leu-Gly-Thr-His-Arg-Asp-Val has a different primary structure from the peptide Val-Asp-His-Leu-Gly-Arg-Thr, even though both have the same number and kinds of amino acids.

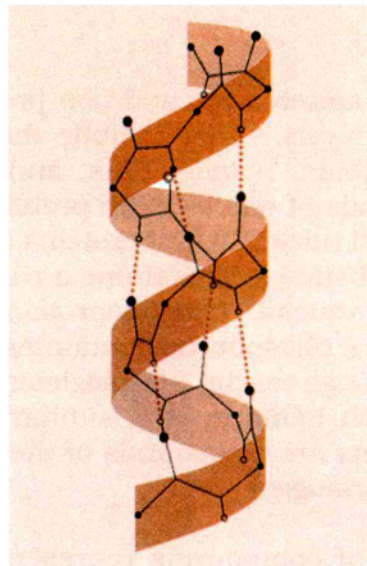
The primary structure determines the three-dimensional conformation of the protein molecule and its cellular role. It also shows the sequence of nucleotides in DNA or RNA and thus provides information on the genetic input to protein synthesis and cellular potential. Thus, a change in any one amino acid in the primary structure of a protein may produce a drastic effect in the animal.

- **Secondary structure** describes the manner in which the amino acids are arranged in the polypeptide chain. This develops through the interactions between adjacent amino acid residues in a polypeptide chain (Figure 2.2B).

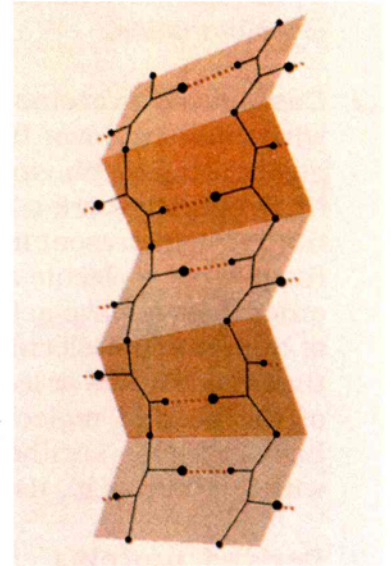
The secondary structures may exist in the form of coiled helices (alpha-helix) (Figure 2.2C) or in an extended form (beta-sheet) (Figure 2.2D).

**Figure 2.2B**

Secondary structure develops through interactions between neighboring residues, as by hydrogen bonds between adjacent monomer units in an alpha-helix region.

**Figure 2.2C**

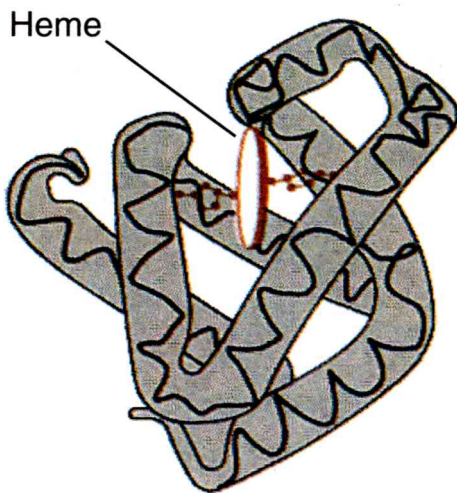
The alpha-helix structure of a polypeptide chain, or part of the chain, is stabilized by hydrogen bonds (dotted lines) between the oxygen (color) of one peptide bond and the hydrogen of another.

**Figure 2.2D**

The beta-sheet structure of all or part of a polypeptide chain is characterized by hydrogen bonds (dotted lines) between every peptide bond and its neighbor.

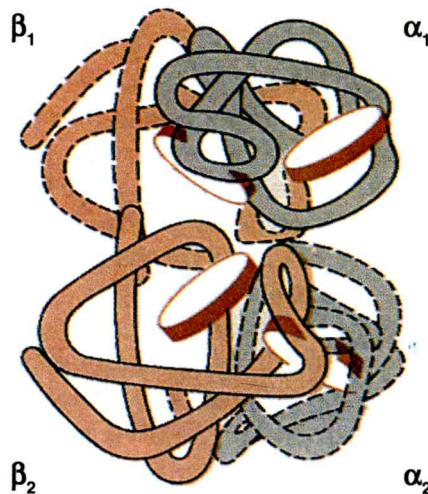
The alpha-helix structure is stabilized by hydrogen bonds between the oxygen of one and the hydrogen of another peptide bond while the beta-sheet structure is characterized by hydrogen bonds between adjacent peptide bonds. The hydrogen bonding between the peptide chains in the beta-sheet gives rise to a repeated zigzag structure hence, the name pleated sheet. Fibrous proteins exhibit a high degree of secondary structure forming sheets of molecules that are involved in cellular construction.

- **Tertiary structure** describes the three-dimensional arrangement or the actual conformation of all the atoms in the protein molecule. The interactions between distant amino acid residues of a polypeptide chain leads to the folding and a more globular conformation of the polypeptide chain assuming a three dimensional shape, for example, myoglobin (Figure 2.2E). Globular proteins usually have a considerable amount of unordered random coil regions, along with regions of ordered alpha-helix and other types of secondary structure. The secondary and tertiary structure of a protein can be determined simultaneously.



**Figure 2.2E**

Tertiary structure depends on the interaction between more distant residues, leading to folding and to a more globular conformation of the polypeptide chain.



**Figure 2.2F**

Quaternary structure is the result of interactions between two or more polypeptide chains in a protein molecule (four chains in hemoglobin).

- **Quaternary structure** refers to the spatial organization when two or more polypeptide chains are part of a single protein molecule. Commonly occurring examples are dimers, trimers, tetramers, consisting of two, three, and four polypeptide chains, respectively. The polypeptides are held together by weak chemical bonds (Figure 2.2F). The absolute number of polypeptides and the number of different kinds of polypeptides in a protein varies. For example, hemoglobin molecules consist of two alpha- chains and two beta- chains. Each globin chain in hemoglobin is bonded to a heme group, which functions

in oxygen transport to body tissues. Disruption of any level of molecular organization, including quaternary structure, leads to malfunction of a protein.

### **Fate of Absorbed Amino Acids**

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Proteins are absorbed from the intestine, mainly as amino acids. The absorbed amino acids are used in one of the following ways: a) incorporated into the metabolic pool, mixed with free amino acids originating from various tissues and synthesized into tissue proteins; b) synthesized into nitrogen-containing tissue constituents such as nucleic acids, hormones and enzymes; c) deaminated (removal of nitrogen), resulting in a carbon chain (-C-C-) and an amino group (-NH<sub>2</sub>).

The carbon chains are oxidized to produce energy or synthesized into sugars and fats, or again react with amino groups, forming amino acids. Amino groups separated from amino acids are excreted in the form of nitrogen compounds such as urea and ammonia in the urine or excreted through the gills.

### **Importance of Amino Acid Profiles in Fish Nutrition**

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Fish tissues contain about 65-75% protein on a dry weight basis. Dietary protein often constitutes the principal and most expensive item in fish diets. The free amino acids released from proteins by the action of digestive enzymes are absorbed in the intestinal tract and used by various cells to build and repair worn out tissues. Excess amino acids are used as energy source or converted to fat.

Information on gross protein requirement of fish is of limited value without data on essential amino acid requirements since protein quality depends largely on its amino acid composition and digestibility. The determination of essential amino acid profiles is helpful in the design of amino acid test diets used for research studies to determine the amino acid requirement of fish. It is also an important parameter in the evaluation of the protein quality of feedstuffs.

The nutritive value of a dietary protein is dependent on the extent to which the composition of its essential amino acids fulfills the requirement of the organism. The closer the profile to the requirement, the higher is the nutritional value. The amino acid lacking in the protein is known as the limiting amino acid. For example, lysine is limiting in corn while methionine is limiting in soybeans.

### **Qualitative Amino Acid Requirements**

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The two methods used to determine whether an amino acid is essential or non-essential are: 1) growth response method, and 2) the radioisotope method.

The growth response method as used by Halver (1957) uses a series of amino acid test diets containing crystalline L-amino acids as source of nitrogen. The diets are formulated based on the amino acid pattern of a reference protein such as whole chicken egg protein, chinook salmon egg protein, or chinook yolk-sac fry protein.

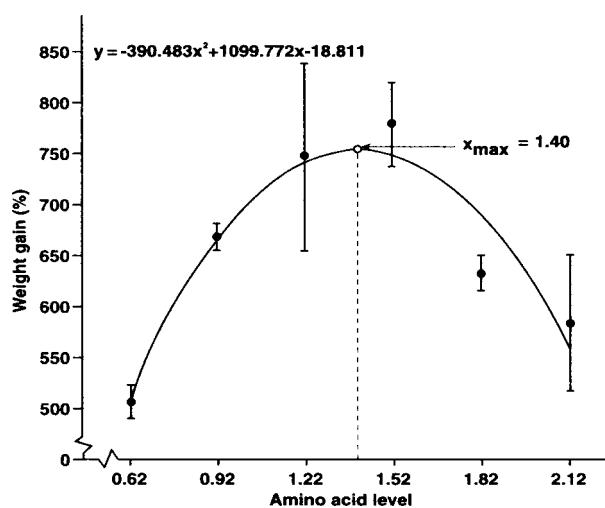


For each of the ten essential amino acids, a 10-week feeding trial is conducted using a basal diet containing all the amino acids and a diet deficient in the amino acid being tested. Fish are weighed every two weeks to measure growth response to the test diet. Fish fed the amino acid-deficient diet show poor growth but a substantial growth response is observed when fish is fed the complete amino acid diet. Subsequently, other investigators used a similar test diet for determining the essentiality of some amino acids in other fishes.

In the radioisotope method used by Cowey et al. (1970), fish are injected intraperitoneally with radioactively labeled  $^{14}\text{C}$  glucose and fed on a natural diet for 7 days. Fish are then killed, homogenized, and protein was isolated. A sample of the isolated protein is then hydrolyzed and the constituent amino acids are separated by chromatography and counted for radioactivity. Significant radioactivity is incorporated into non-essential amino acids while the essential amino acids have very little or no radioactivity. This method was also used by Coloso and Cruz (1980) to determine the qualitative amino acid requirements of tiger shrimps.

### Quantitative Amino Acid Requirements

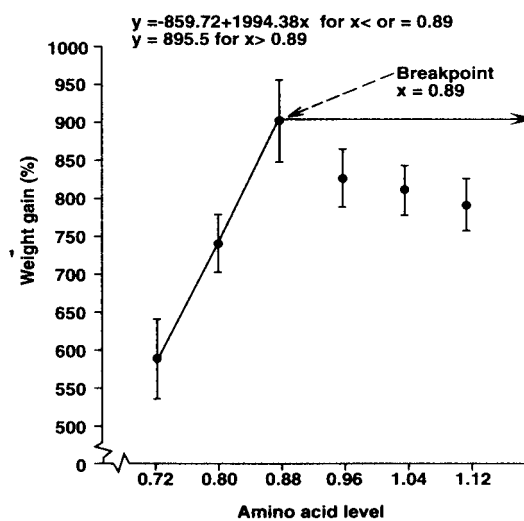
The quantitative amino acid requirements of fish is determined using either purified or semi-purified test diets. A purified diet is made up of pure substances (casein, gelatin, crystalline amino acids) while a semi-purified diet may contain other ingredients such as fish meal or soybean meal. The test diet is formulated so that the amino acid profile is identical to that of a reference protein usually fish muscle protein. A series of experimental diets is then prepared containing graded levels of one amino acid for which the requirement is to be determined. Diets are fed to fish and the gain in weight are measured at weekly or biweekly intervals. The required dietary level is determined using the dose response curve (Figures 2.3A, B).



**Figure 2.3A**

Growth response of tiger shrimp fed graded levels of phenylalanine for 8 weeks as described by the quadratic regression model.

Source: Millamena et al. 1999



**Figure 2.3B**

Growth response of tiger shrimp fed graded levels of methionine for 8 weeks as described by the broken-line regression model.

Source: Millamena et al. 1996

A dose response curve indicates the growth response of the fish to graded levels of an essential nutrient in their diet. This method assumes that weight gain is linearly related to increasing dietary levels of the essential amino acid at or below the requirement level. When the requirement is met, weight gains abruptly plateau and then decline if the dietary concentration of the nutrient exceeds the animal's tolerance. Figure 2.3 (A) shows a quadratic curve with the requirement of phenylalanine at 1.4 percent of diet or 3.7 percent of dietary protein.

The broken line analysis has also been used to estimate the essential amino acid requirement. This method also assumes a linear relation between growth and dietary level of the essential amino acid at or below the requirement. At the requirement level, the linearly ascending line instantly breaks to horizontal. The point is known as breakpoint. Figure 2.3 (B) shows a linear curve with the requirement for methionine at 0.89 percent of diet or 2.4 percent of dietary protein.

In young growing animals, the greatest proportion of body weight is in the form of muscle. It is reasonable to infer that the dietary amino acid requirement will be closely related to the amino acid profile of muscle or whole body protein. Thus, when this hypothesis was examined for pigs, chicken, fish and shrimp, the amino acid requirement was closely correlated to the amino acid profile of the muscle protein.

There are several problems that occur in the accurate determination of amino acid requirements of fish when based on growth studies: a) growth rates that are commonly observed in the amino acid test diets are frequently inferior or lower than those observed with natural or intact proteins. b) some of the amino acids in test diets may leach out during feeding. c) the interpretation of the breakpoint in the growth response curve could be subjective if the appropriate statistical tool is not used. The requirements of some fishes and shrimp for essential amino acids are shown in Table 2.2.

**Table 2.2** Amino acid requirements of some fishes and shrimp in percent of protein

	Eel	Carp	Rainbow trout	Chinook salmon	Milkfish	Nile tilapia	Sea bass	Tiger shrimp
Arginine	4.5	4.4	4.0	6.0	5.2	4.2	3.6	5.3
Histidine	2.1	1.5	1.8	1.8	2.0	1.7		2.2
Isoleucine	4.0	2.6	2.8	2.2	4.0	3.1		2.7
Leucine	5.3	4.8	5.0	3.9	5.1	3.4		4.3
Lysine	5.3	6.0	6.0	5.0	4.0	5.1	4.5	5.2
Methionine + Cys/2	5.0	2.7	3.3	4.0	2.5	3.2	2.9	2.4
Phenylalanine+ Tyr	5.8	5.7	6.0	5.1	4.2	5.5		3.7
Threonine	4.0	3.8	4.1	2.2	4.5	3.8		3.5
Tryptophan	1.1	0.8	0.6	0.5	0.6	1.0	0.5	0.5
Valine	4.0	3.4	3.6	3.2	3.6	2.8		3.4

Source: NRC 1993; FDS Manual 1994; Santiago and Lovell 1993; Borlongan and Coloso 1998; Coloso et al. 1999; Millamena et al. 1996-99

## Deficiencies and Excesses of Dietary Amino Acids

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Essential amino acid deficiency may be caused by the use of feed ingredients usually protein sources that are slightly or grossly deficient in at least one essential amino acid. Amino acid deficiency may also arise from the presence of chemicals that may affect some feed ingredients, excessive heat treatment during feed manufacture, and leaching out of nutrients.

Amino acid imbalance due to amino acid antagonism or amino acid toxicity may also cause reduced growth in animals. Amino acid antagonism occurs when some amino acids are fed in excess of the required levels, causing an increase in the requirement for another amino acid of similar structure. Some examples are the leucine-isoleucine antagonism and arginine-lysine antagonism observed in some fish species. Amino acid toxicity occurs when excess amounts of certain amino acids are fed to the animal and the negative effects cannot be improved by adding amino acids in the diet.

In practical diet formulation, the recommended dietary levels of essential amino acids can be met by carefully selecting and properly combining two or more protein sources. The limiting or deficient amino acid in one protein source can be supplemented by another protein source abundant in the same amino acid and hence make a better feed. Another way of meeting the EAA needs of an animal is by supplementing the practical diet with crystalline L-amino acids. Nutrient leaching can be minimized by using water stable diets through the use of efficient binders and employing appropriate feeding practices.

## Evaluation of Protein Quality

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Proteins are said to be of high quality when their amino acid composition closely resembles the amino acid pattern or approximates the essential amino acid requirements of the animal under consideration and when they are highly digested by the animal. The quality of proteins is usually evaluated by biological and chemical methods. The chemical method determines the quantity of protein or amino acid in a feedstuff whereas the biological method determines how the fish reacts to the protein in terms of growth and survival. In the biological method, body weight gain and nitrogen retention are used as criteria for protein quality which is considered as more accurate than the chemical method. Protein efficiency ratio, biological value, and net protein utilization are used to measure the biological value of a protein and can be calculated, by using the following formula:

### 1. Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Live weight gain (grams)}}{\text{Amount of protein fed (grams)}}$$

**2. Biological value (BV)\***

$$BV = \frac{\text{True nitrogen retained}}{\text{Nitrogen absorbed}} \times 100$$

where, R = true nitrogen retained

A = nitrogen absorbed

and A = I - (F - Fo),

R = A - (U - Uo)

where, I = nitrogen intake

F = nitrogen excreted in the feces

Fo = metabolic faecal nitrogen

U = nitrogen excreted in the urine

Uo = endogenous nitrogen

$$\text{Thus, } BV = \frac{R}{A} \times 100 = \frac{I - (F - Fo) - (U - Uo)}{I - (F - Fo)} \times 100$$

- \* Insufficient data on the biological value are available for fish due to difficulties in determining the metabolic faecal and endogenous nitrogen separately

**3. Net protein utilization (NPU)**

$$NPU = \frac{\text{True nitrogen retained}}{\text{Nitrogen intake}} \times 100$$

where, NPU is determined by the following formula:

$$NPU = \frac{\text{Nitrogen increase in fish fed the test protein diet} + \text{Nitrogen decrease in fish fed the protein free diet}}{\text{Nitrogen intake from the test protein diet}} \times 100$$

A detailed procedure for protein evaluation is discussed in Chapter 6.

**Protein Requirement**

The optimum protein requirement is the level of high quality dietary protein needed for maximum growth. To determine the protein requirement of a fish species, feeding trials have to be conducted using test diets containing graded levels of protein from sources of high biological value. Growth response, usually weight gain, is measured for each diet. The diet that gives the highest weight gain and survival is considered as the best diet. Maximum tissue protein retention may also be used as the criterion for determining protein requirement instead of weight gain. This is done by analyzing the amount of nitrogen in the tissues at certain intervals e.g. every two weeks until there is no more decrease in nitrogen retained in the tissues.

The minimum amount of dietary protein needed for optimum growth of most cultured aquatic species ranges from 27% to 60%. A summary of optimal protein levels and the protein sources used to determine these values are presented in Table 2.3.

**Table 2.3** Optimal dietary protein levels (% of dry diet) for some aquaculture species

Species	Protein sources	Optimal protein level	References
Asian sea bass	fish meal, soybean meal	43	Catacutan & Coloso 1994
Common carp	fish meal, casein	31-38	Takeuchi 1979
Grouper	tuna, muscle meal	40-50	Teng et al. 1978
	fish meal, meat meal, shrimp meal	43	Shiau 1972
Japanese eel	casein + amino acids.	44	Nose & Arai 1972
Kuruma shrimp	squid meal	60	Deshimaru & Shigeno 1972
	casein + egg albumin	>55	Teshima & Kanazawa 1984
Milkfish	fish meal, casein	40	Lim et al. 1979
	casein, gelatin	30-40	Pascual 1989
	fish meal, soybean meal, cassava meal	24*	Sumagaysay & Borlongan 1995
Red sea bream	casein	55	Yone 1976
Snakehead	fish meal	52	Wee & Tacon 1982
Red Snapper	fish meal, soybean meal, squid meal	44	Catacutan & Pagador 2001
Tiger shrimp	casein	40	Alava & Lim 1983
	fish meal, soybean meal, shrimp meal	40	Millamena & Triño 1994
Nile tilapia	fish meal, casein	30	Wang et al. 1985
	fish meal	28	Santiago et al. 1986
White shrimp	mussel meal, fish meal, collagen,	34-42	Andrew & Sick 1972
	squid meal	28-32	Sedgwick 1979
Yellow tail	fish meal, casein	55	Takeda et al. 1975
Abalone	soybean meal, rice bran	27	Bautista-Teruel & Millamena 1999
	fish meal, squid meal		

\* tested under pond conditions

Protein of high biological value such as whole egg protein, casein, combination of casein and gelatin, and fish meal are often used in determining protein requirements. Several factors influence protein requirements for maximum fish growth: species, fish size or age, water temperature, protein quality as reflected by the amino acid profile, dietary level of non protein energy and daily food allowance. Smaller or younger fish have higher protein requirements than older fish of the same species. Fish fed at feeding rates below satiation will require a higher protein level. Fish also respond better to a higher level of dietary protein when fed poor quality protein.

**Guide Questions**

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1. What is a protein? What is the most important function of a protein?
2. How are proteins classified? Distinguish between simple, conjugated, and derived proteins.
3. What is the general structure of amino acids?
4. Why are amino acids called the building blocks of proteins?
5. Name the ten essential amino acids required by fish.
6. Differentiate between essential and non-essential amino acids.
7. Describe the fate of absorbed amino acids.
8. Why is the amino acid profile of a feed a valuable index in the assessment of the nutritive value of dietary proteins?
9. What is the difference between qualitative and quantitative amino acid requirement? Describe each briefly.
10. What are the two methods used to determine qualitative amino acid requirements?
11. Describe a dose-response curve used to determine the quantitative amino acid requirement.
12. What are the causes of dietary essential amino acid deficiency?
13. What are some of the problems that occur in determining essential amino acid requirements?
14. Explain briefly how the essential amino acid requirements can be met in a practical diet formulation.
15. In evaluating protein quality, why is the biological method considered more accurate than the chemical method?