

## NUTRITION AND FEEDS

The forms of traditional animal aquaculture rely largely on the production of foods through natural processes, or by fertilization and water management in enclosed areas. To a certain extent, this practice is still followed

in extensive and semi-intensive pond farming, but supplementary feeding is resorted to for ensuring adequate availability of food to dense stocks and for enhanced growth and production.

In the case of mollusc culture, live foods continue to be the source of nutrition, even though experimental studies are under way to develop inert feeds. In nature, one can observe distinctly different feeding habits among fish and shellfish species, such as those that feed on zoo- and phytoplankters, filamentous algae, macrophytes, benthos, detritus, molluscs and other smaller animal species, etc. Many of them feed on more than one type of food or even on quite a number of them. Fish generally use one or more sensory systems for acquiring feed, such as visual detection, sound and water turbulence and chemical stimuli released by food. Of these, the visual stimuli are best understood and include the properties of size, movement, shape and colour contrast.

Digestion involves the conversion of the three major nutrients (proteins, carbohydrates and lipids) which occur as macromolecules in nature into sizes that pass through the walls of the alimentary canal and are absorbed into the bloodstream. Proteins are converted into amino acids or polypeptide chains of a few amino acids, carbohydrates into simple sugars and lipids into glycerols and fatty acids. This is made possible through the activity of enzymes. Digestibility ranges from 100 per cent for glucose to as little as 5 per cent for raw starch or 5–15 per cent for plant material containing cellulose. Digestibility of most natural proteins and lipids ranges over 80–90 per cent.

**Digestibility of a food component is indicative of its bioavailability. Digestibility capacity is species specific and varies with nutrient source and method of treatment of the samples as well as ambient conditions such as temperature**

(Pfeffer et al., 1991). Indigestible materials are eventually voided as faeces.

The enzyme amylase catalyses the digestion of starch and together with dextrinases produces maltose. Maltase hydrolyses maltose to give the final product of starch digestion, glucose. Most fish have amylase; in plant-eating fish such as tilapia it may be present in all parts of the digestive tract, whereas in carnivorous fish it may be found only in the pancreas, pyloric caeca and intestines.

Cellulase and cellobiase are the enzymes involved in digesting cellulose. Cellulase hydrolyses cellulose to disaccharide cellobiose, which is then acted upon by cellobiase, producing the final breakdown product, glucose.

Very few fish have cellulase activity, but the microflora in their intestines may serve as a source of cellulases.

Protein digestion in fish begins in the stomach and is catalysed by pepsin and acid pH ranging from 1 to 4. Pepsin is synthesized in the gastric gland in the inactive form called pepsinogen. Hydrochloric acid, produced by another enzyme-controlled reaction between sodium chloride and carbonic acid, activates

the pepsinogen. Pepsin attacks most proteins where the linkages are formed by aromatic and acidic amino acids, such as phenylalanine, tyrosine, tryptophan and aspartic and glutamic acids.

Trypsin and chymotrypsin are involved in the alkaline digestion of proteins. These enzymes are generally synthesized and stored in the pancreatic cells as inactive forms, viz. trypsinogen and chymotrypsinogen. These are then transported mainly to the intestines and pyloric caeca, or in some cases to the liver. In the intestines, trypsinogen is converted to the active form trypsin by the enzyme enterokinase.

Trypsin in turn activates chymotrypsinogen into chymotrypsin. Trypsin is specific for peptide linkages which come from basic amino acids: arginine and histidine. Chymotrypsin attacks linkages with aromatic amino acids: phenylalanine, tyrosine and tryptophan.

Carboxypeptidases (A and B) hydrolyse the C-terminal peptide of proteins. This is found in the pancreas, pyloric caeca and intestines of fish. Carboxypeptidase A is not active towards proteins with aromatic C-terminal amino acids: phenylalanine, tyrosine and tryptophan, while carboxy-peptidase B acts preferentially on these with lysine and arginine. Amino peptidase hydrolyses the amino terminal peptide of polypeptidia, releasing one amino acid at a time from the N-terminal end. Most fish also have lipase enzymes that hydrolyse ester linkages in triglyceride and produce glycerol and fatty acids.

The effectiveness of digestive enzymes is influenced by temperature and pH. In general, the reaction rate increases with temperature until the enzymes begin to denature around 50–60°C. However the range of pH within which they function is very limited, often as little as 2 pH units. In the case of channel catfish, which is probably representative of many teleosts, **the pH in the stomach ranges between 2 and 4, becoming alkaline (pH 7–9) below the pylorus, decreasing to 8.6 in the upper intestine, and finally nearing neutrality in the hind gut**

Absorption of amino acids, peptides and simple carbohydrates in fish have not been studied much, but presumably they diffuse through or are transported across the gut epithelium into the bloodstream. Digested food, particularly protein, is not fully available to the fish even after it has been absorbed.

Amino acids may be used as absorbed for building new tissue. But if digested food has to be oxidized for energy, deamination (removal of the amino group) which requires an input of energy (a process known as specific dynamic action) would have to occur first. Fish that have not

grown due to low temperature or due to low levels of feeding would deaminate most or all of their amino acids. Those reared at high temperatures or having very high metabolic rates due to high activity levels would also do likewise. On the other hand, fish having rapid growth and high protein intake would deaminate a relatively small portion of the digested protein. The energy for deamination need not come from amino acids, but may be preferentially taken from carbohydrate or lipid, if available. This 'protein-sparing' action accounts for the addition of limited amounts of inexpensive carbohydrate in the diet of fish, which helps in reducing feed costs. The calorie-to-protein ratio (kcal : g) can be applied in diets containing adequate energy and protein. Optimal ratios for catfish diets are reported to be between 6.5 and 8.3 kcal of digestible energy per g protein.

## 7.2 Energy metabolism

From among the two types of energy, heat energy utilized for maintaining body temperature and the free energy available for biological activity and growth, the latter is more important for poikilothermal animals like fish. Free energy is needed for maintenance, growth and reproduction. Seaweeds and other plants can obtain it directly from the sun and water and synthesize the complex molecules that constitute its structural parts. Animal species have to depend on the oxidation of the complex molecules contained in the food that they eat for energy requirements. The complex molecules are broken down during digestive processes to simpler molecules and are absorbed into the body, where oxidation occurs and energy is released. The biological process of energy utilization is known as metabolism and the rate at which it is utilized is referred to as the metabolic rate. Energy metabolism in cold-blooded animals such as fish is different from that in mammals and birds in that they do not expend energy to maintain a body temperature different from their environment as warm-blooded animals do, and the excretion of waste nitrogen requires less energy than in homeothermic land animals. Fish are the most efficient converters of energy and protein among all farmed animals. While the energy and protein conversion efficiencies in farmed warm-blooded animals (sheep, cattle, pigs, chicken) **are in the ranges of 1.7–17 per cent and 3–12 per cent respectively, the corresponding ranges for salmonids are much higher at 30–40 per cent and 20–25 per cent respectively** (Rerat and Kaushik, 1995; De Silva, 1999). The metabolic rate in fish, which is probably the most studied aquaculture animal group, is influenced by temperature, age or size, activity and seasonal and diurnal fluctuations of body function. It is also affected by oxygen or carbon dioxide concentration, pH and salinity of the

water. The energy requirements necessary for all metabolic functions can be calculated for each species. For example, carp utilize 25 cal/dec<sup>2</sup>/h at 25°C. Approximately 70 per cent of this is used for maintenance and growth and the remaining 30 per cent is lost to the environment. As the body temperature of the fish is maintained at or near the environmental water temperature, the heat that is produced is lost to the environment. Most of the available information on nutrient requirements of aquaculture species is based on researches on a small number of these (trouts, salmon, channel catfish, common carp, grass carp, eel, plaice, gilthead bream, red sea-bream and yellowtail).

Work on penaeid shrimps and the giant freshwater prawn has shown considerable similarities with the fish species studied, although there are some differences. Very little work has been done on the nutritional needs of molluscs, as culture has been based on filter feeding of naturally occurring phytoplankton and similar organisms. However, efforts are presently underway to develop encapsulated or fineparticulate feeds, which should lead to a better understanding of their feed requirements. Energy requirements have in most cases been derived primarily from experimentation, in which fish were fed rations varying in calorific value. The ration yielding the best growth was assumed to be the most satisfactory calorific value for the species concerned. Carbohydrates are the most abundant and relatively least expensive source of energy in animal aquaculture. These may range from easily digested sugars to complex cellulose which is difficult to digest. Based on results of research on carnivorous species, doubts have been expressed on the value of carbohydrates in fish feeds, but practical experience in fish culture shows that digestible carbohydrate can be an energy source if kept in proper balance with other nutrients. The ability to assimilate starches depends on enzymatic activity (production of amylase). In herbivores, amylase occurs through the entire digestive tract. Up to levels of 25 per cent in the diet, it can be as effective an energy source as fat for several species of fish, such as channel catfish, rainbow trout and plaice (Cowey and Sargent, 1972). Stickney and Shumway (1974) have shown the presence of cellulase activity associated with cellulolytic microflora in several species of brackish-water fish and fresh-water catfish. Metabolizable energy values of carbohydrates may range up to 3.8 kcal/g for easily digestible sugars, whereas for indigestible cellulose it may be near zero. Values for raw starch range from 1.2–2.0 kcal/g. When processed in high moist temperatures, for making pelleted feed, starch gelatinizes and its digestibility therefore improves. When

digested, the products of hydrolysis are assimilated into the blood-stream, where their known function is to provide energy. Therefore they have a protein sparing action (see the end of Section 7.1) and any excess is partially stored in the liver as glycogen and partially converted into visceral and muscular fat.

Successful fish feeds contain a certain amount of carbohydrates, as for example 20 per cent for cold-water fish feeds and 30 per cent for warm-water fish feeds. Besides providing energy, they have the physical function of texturizing manufactured feeds and acting as a binder in the formulation of pellets. Cereal grain products are generally used as 'fillers' to complete feed formulae. Formulae for expanded pellets often contain up to 50 per cent of whole cereal grains, to achieve the floating properties.

Fish and shrimp vary in their ability to digest carbohydrate (New, 1989). The utilization of dietary carbohydrate has also been found to vary with the complexity of the carbohydrate source used.

#### **Proteins**

Dietary protein is the main source of nitrogen and essential amino acids in animals. It is also the most expensive source of energy in artificial diets. In nature, carnivorous fish consume foods which are about 50 per cent protein. They have a very efficient system for excretion of waste nitrogen from protein, which is catabolized for energy. Therefore high-protein diets are not harmful but, being expensive, it is necessary to keep the proportion of protein down to optimum levels necessary for good growth and feed conversion. Protein has a metabolizable energy value of about 4.5 kcal/g in fish, which is higher than that of mammals and birds.

The requirements are highest in the initial feeding of fry and decrease as fish size increases. For maximum growth, young fish require between 40 and 60 per cent of their diet as proteins, which is much higher than the requirements of terrestrial animals. However, most of the wet weight gain in lean fish is in the form of muscle tissue, unlike in terrestrial animals where there is considerable deposition of both fat and protein. Salmonids continue to need, from young to adult stages, higher levels of 40–60 per cent protein in their diets. But other species like the milkfish (*Chanos chanos*) appear to make a rapid transition to a diet of algae, containing 10–20 per cent protein in its natural environment. The protein component of this material is digested and the amino acids absorbed, while most of the undigestible cellulose is excreted. The dietary protein level resulting in the highest growth rate in various farmed shrimp species ranges from 28–57 per cent, the highest requirement

being 40–58 per cent and 40–51 per cent for *Penaeus japonicus* and *P. aztecus* respectively and the lowest 28–32 per cent for *P. setiferus*. The optimal growth ranges for *P. indicus*, *P. merguensis* and *P. monodon* were found to be with 30–40 per cent, 32–42 per cent and 34–46 per cent diets respectively. The corresponding range for the freshwater prawn, *Macrobrachium rosenbergii*, is 35–40 per cent (Tacon, 1990).

Protein requirements are influenced by water temperature, body size, stocking density, oxygen levels and the presence of toxins. As water temperature declines, the body temperature of fish also declines and consequently the metabolic rate is reduced. The most favourable temperature for a given species is the one at which the difference between maintenance requirement and voluntary food intake is greatest and at which optimum efficiency of growth occurs (Smith, 1980). Chinook salmon (*Oncorhynchus tshawytscha*) need food containing 40 per cent protein in water temperatures of about 8°C for optimum growth, whereas in temperatures of about 14°C the same fish will need food containing 55 per cent protein (De Long et al., 1958). At lower temperatures, foods containing more than 40 per cent protein produce stress due to an excess of ammonia released from gills. Channel catfish (*Ictalurus punctatus*) show optimum growth at 20°C on a 35 per cent protein diet, whereas at 25°C they need a 40 per cent protein diet to achieve optimum growth (Dupree and Sneed, 1966). However, work by Slinger et al. (1977) and Cho and Slinger (1978), does not confirm the results relating to temperature effects. The greater absolute need for protein at higher temperatures might be satisfied through increased consumption of the lower protein diets.

Readily digested high-protein materials have higher metabolizable energy (ME) values for fish than other mono-gastric animals. Similarly, protein has more net energy for fish than it has for mammals or birds. Smith et al. (1978) showed that less than 5 per cent of the ME is lost as heat increment in fish. Fish are among the most efficient of all animals in converting feed energy into high quality protein. Available information does not seem to support the general view that omnivorous and herbivorous aquaculture species require less protein in their diets. For example, the juveniles of herbivorous grass carp (*Ctenopharyngodon idella*) require levels of protein similar to salmon and trout. Phytoplankton and zooplankton contain high percentages of protein (40–60 per cent) and there is reason to believe that the protein requirements of plankton feeding species are also similarly high. The real difference between species of different feeding

habits would appear to be in the ability to digest carbohydrates. Most of the carnivores, like trout and yellowtail, have a limited ability to digest complex carbohydrates.

Juveniles and adults of most cultured crustaceans have protein requirements in the range of 30–50 per cent of their dry diet weight. Like fish, they also require much higher levels of protein than terrestrial animals. However, there are differences in their nitrogen metabolism. One major difference is based on the habit of moulting. The crustacean exoskeleton consists of a mineral-organic matrix. Chitin, one of the primary compounds, is composed of glucosamine units (an amine group and glucose).

Growth occurs when the old exoskeleton is partly resorbed, then shed and a new one grown in its place. Prior to moulting, they produce high levels of ammonia, indicating the resorption of the old exoskeleton. Even when the exuvia is eaten, substantial losses of nitrogen occur.

Nitrogen balance can be used to evaluate the amino acid and nitrogen requirements. Moulting (ecdysis) affects the animal's nitrogen balance.

Unlike terrestrial vertebrates, crustaceans appear to have a limited ability to store protein (Maynard and Loosi, 1969). Recent studies show that both carbohydrates and lipids can be used to spare dietary protein in crustaceans.

There are wide variations in the protein requirements of shrimp species.

Little work has been done on defining the nutritional requirements of molluscs, probably because of the successful use of algae as food and the lack of successful microencapsulated diets. However, as pointed out earlier, the protein content of phytoplankton species is generally high (above 40 per cent), although it varies with environmental conditions.

#### **Lipids and essential fatty acids**

Lipids are a group of fat-soluble compounds occurring in the tissues of plants and animals and broadly consist of fats, phospholipids, sphingomyelins, waxes and sterols. Fats are the fatty acid esters of glycerol and are the principal form of energy storage. They contain more energy per unit weight than any other biological product – it is estimated that they provide 8.5kcal metabolizable energy (ME) per gram. Natural diets may contain as much as 50 per cent fat. Phospholipids are the esters of fatty acids and phosphatic acid. These are the main constituent lipids of cellular membranes, determining the hydrophobic or hydrophylic properties of the membrane surfaces.

#### **7.3.4 Vitamins**

Vitamins are a chemically diverse group of organic substances that are either not synthesized by organisms or are synthesized at rates insufficient to meet the organisms' needs. They

constitute only a minute fraction of the diet and are more catalytic in their function, but are critical for the maintenance of normal metabolic and physiological functions. They can be classified into two groups: water-soluble and fat-soluble vitamins. Water-soluble vitamins include eight members of the vitamin B complex: thiamin, riboflavin, pyridoxine, pantothenic acid, niacin, biotin, folic acid and vitamin B<sub>12</sub>. They include the essential nutritional factors choline, inositol and ascorbic acid, and vitamins with less-defined activities for fish: paminobenzoic acid, lipoic acid and citrin. The fat-soluble group comprises vitamins A, D, E and K. The information available on vitamin nutrition of aquatic animals is limited. The leaching of water-soluble vitamins from test diets, before the animals feed on them has been a major problem in determining their requirements. This is more so in the case of crustaceans because of their slow feeding habits, and in the case of molluscs because they feed on algae and other natural food. Much of the available information is based on the work done on salmonids, but some data are also available for a few other species.

If natural food organisms are available to cultured animals, as in extensive pond culture, prepared feed may not need any vitamin supplements. On the contrary, in intensive farming, where natural food items do not contribute much to dietary intake, the addition of adequate quantities of vitamins will be essential.

Insufficient information often makes it difficult to decide with precision the quantities which should be added. Hypervitaminosis is rare in fish, although it is possible at very high levels (for example excess vitamin A causes enlargement of liver and spleen, abnormal growth and bone formation and epithelial keratinization)

### **Minerals**

Minerals are required by all animals, either in their elemental form or incorporated into specific compounds, for various biological functions such as the formation of skeletal tissue, respiration, digestion and osmoregulation. Of the 26 naturally occurring essential elements described for animals, only nine have been shown to be required by finfish. Very little is known of the mineral requirements of crustaceans and molluscs. One of the main difficulties in determining the quantitative mineral requirements of aquatic animals is their ability to absorb inorganic elements from their external environment in addition to their diets. Calcium and phosphorus are closely related in metabolism, especially in bone formation and the maintenance of acid-base equilibrium. While fish can obtain calcium from food and also from the environment through gills and fins in fresh water, phosphorus has to come mainly from food, as both fresh and salt waters



are generally deficient in phosphates. Almost the entire store of calcium (99 per cent) and most of the phosphorus (80 per cent) in the body of fish are in the bones, teeth and scales. The remaining small portions are widely distributed throughout the organs and tissues.

Calcium is present in body fluids in nondiffusible form bound to protein and in a diffusible fraction largely as phosphate and bicarbonate compounds. It is this diffusible fraction that is of significance in calcium phosphorus nutrition (Chow and Schell, 1980).

Ionized calcium in the extracellular fluids and in the circulatory system participates in muscle activity and osmoregulation. Phosphorus combinations with proteins, lipids, sugars, nucleic acids and other compounds are vital exchange currencies in life processes, and are distributed throughout the organs and tissues.

Among the feed ingredients in common use, fish meal is rich in both calcium and phosphorus. However, feedstuffs of plant origin usually lack calcium, and phosphorus, though abundant, occurs predominantly in the form of phytin or phytic acid, which are generally not readily absorbed.

Magnesium is closely associated with calcium and phosphorus in distribution and metabolic activities. While the bulk of the magnesium is stored in the skeleton, the rest (40 per cent) is distributed throughout the organs and muscle tissues and extracellular fluids. This fraction plays a vital role in enzyme co-factors and as an important structural component of cell membranes.

Among the trace elements of importance in fish nutrition, mention has to be made of cobalt.