

Some Principles and Requirements in Fish Nutrition

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Abstract: Fish, especially when reared in high densities, require a high-quality, nutritionally complete, balanced diet to grow rapidly and remain healthy. Fish gut is a tubular structure beginning from the mouth and ending at the anus. Many fish chew their food and have the ability to secrete mucus substances from the mucus gland. In fish, the enzymes are liberated into the lumen of the gut and the products of their action absorbed (extra cellular digestion). Chromium oxide (Cr₂O₃) mixed with prepared diets, measured in the faeces, provides a general comparison of the digestibility of all feed. A proper understanding of the fish digestive system, description of fish gut, physiology of fish gut, peristalsis and its control, gastric evacuation time, digestion and absorption, Specific Dynamic Action (SDA), characteristics of enzymes and other digestive secretions, digestion in the mouth and oesophagus, digestion in the stomach, digestion in the mid gut and pyloric caeca, the role of bile, gall-bladder and liver in digestion, measurement of digestibility and related factors, feed conversion and efficiency calculations, nutrient requirement, proteins, fats and lipids, carbohydrates, vitamins and minerals are vital for effective culture fisheries.

Key words: Digestibility, digestion, enzymes, feed conversion, feed efficiency, nutrient requirement

INTRODUCTION

Nutrition is the series of processes by which living organisms obtain food substances and use them to provide energy and materials for growth, activities and reproduction. Good nutrition in animal production systems is essential to economically produce a healthy, high quality product (Winfree, 1992). In fish farming, nutrition is critical because feed represents 40-50% of the production costs. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health (Tom and Van-Nostrand, 1989). The development of new species-specific diet formulations supports the aquaculture (fish farming) industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish and seafood products (Robinson *et al.*, 1998).

Prepared or artificial diets may be either complete or supplemental. Complete diets supply all the ingredients (protein, carbohydrates, fats, vitamins, and minerals) necessary for the optimal growth and health of the fish. Most fish farmers use complete diets, those containing all the required protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (<8.5%), phosphorus (<1.5%), water (<10%), and trace amounts of vitamins, and minerals. When fish are reared in high density indoor systems or confined in cages and cannot forage freely on

natural feeds, they must be provided a complete diet (Roberts, 1989).

In contrast, supplemental (incomplete, partial) diets are intended only to help support the natural food (insects, algae, small fish) normally available to fish in ponds or outdoor raceways. Supplemental diets do not contain a full complement of vitamins or minerals, but are used to help fortify the naturally available diet with extra protein, carbohydrate and/or lipid. Fish, especially when reared in high densities, require a high-quality, nutritionally complete, balanced diet to grow rapidly and remain healthy (Robert, 1979).

Fish cannot manufacture their food from simple substances. They either eat plants or the flesh of other aquatic animals, which feed on plants (Lovell, 1988).

Fish which feed entirely on plants are called herbivores and those which eat flesh are called carnivores. Some feed on both plants and flesh and are called omnivores. Others feed on either planktons (planktivores) or detritous (detritivores). The food for most fish is solid, but before the animal can use it, the food must be in solution (Houlihan *et al.*, 2001).

There are four main processes concerned with the use of food by fish:

- The taking in of solid food (Ingestion)
- The dissolving of the solid food (Digestion)

- The process by which the soluble parts of the food are taken into the animal's body fluids (Absorption)
- The process by which the insoluble parts of the food are discharged from the body as faeces (Egestion)

Dietary nutrients are essential for the construction of living tissues. They also are a source of stored energy for fish digestion, absorption, growth, reproduction and the other life processes. The nutritional value of a dietary ingredient is in part dependant on its ability to supply energy. Physiological fuel values are used to calculate and balance available energy values in prepared diets. They typically average 4, 4, and 9 kcal/g for protein, carbohydrate and lipid, respectively (Helfrich and Smith, 2001).

To create an optimum diet, the ratio of protein to energy must be determined separately for each fish species. Excess energy relative to protein content in the diet may result in high lipid deposition. Because fish feed to meet their energy requirements, diets with excessive energy levels may result in decreased feed intake and reduced weight gain. Similarly, a diet with inadequate energy content can result in reduced weight gain because the fish cannot eat enough feed to satisfy their energy requirements for growth. Properly formulated prepared feeds have a well-balanced energy to protein ratio (CAN, 1993).

Commercial fish diets are pressure-pelleted (sinking) feeds. Both floating or sinking feed can produce satisfactory growth, but some fish species prefer floating, others sinking. Shrimp, for example, will not accept a floating feed, but most fish species can be trained to accept a floating pellet (CAN, 1993).

Extruded feeds are more expensive due to the higher manufacturing costs. Usually, it is advantageous to feed a floating (extruded) feed, because the farmer can directly observe the feeding intensity of his fish and adjust feeding rates accordingly. Determining whether feeding rates are too low or too high is important in maximizing fish growth and feed use efficiency (Helfrich and Smith, 2001).

Feed is available in a variety of sizes ranging from fine crumbles for small fish to large (1/2 inch or larger) pellets. The pellet size should be approximately 20-30% of the size of the fish species mouth gape. Feeding too small a pellet results in inefficient feeding because more energy is used in finding and eating more pellets. Conversely, pellets that are too large will depress feeding and, in the extreme, cause choking. Select the largest sized feed the fish will actively eat (Houlihan *et al.*, 2001).

Feeding rates and frequencies are in part a function of fish size. Small larval fish and fry need to be fed a high protein diet frequently and usually in excess. Small fish have a high energy demand and must eat nearly

continuously and be fed almost hourly. Feeding small fish in excess is not as much of a problem as overfeeding larger fish because small fish require only a small amount of feed relative to the volume of water in the culture system (Lovell, 1988).

As fish grow, feeding rates and frequencies should be lowered, and protein content reduced. However, rather than switching to a lower protein diet, feeding less allows the grower to use the same feed (protein level) throughout the grow-out period, thereby simplifying feed inventory and storage (Robert, 1979).

Feeding fish is labor-intensive and expensive. Feeding frequency is dependent on labor availability, farm size, and the fish species and sizes grown. Large catfish farms with many ponds usually feed only once per day because of time and labor limitations, while smaller farms may feed twice per day. Generally, growth and feed conversion increase with feeding frequency. In indoor, intensive fish culture systems, fish may be fed as many as 5 times per day in order to maximize growth at optimum temperatures (Robert, 1979).

Many factors affect the feeding rates of fish. These include time of day, season, water temperature, dissolved oxygen levels, and other water quality variables. For example, feeding fish grown in ponds early in the morning when the lowest dissolved oxygen levels occur is not advisable. In contrast, in re-circulating aquaculture systems where oxygen is continuously supplied, fish can be fed at nearly any time. During the winter and at low water temperatures, feeding rates of warm water fishes in ponds decline and feeding rates should decrease proportionally (Roberts, 1989).

Feed acceptability, palatability and digestibility vary with the ingredients and feed quality. Fish farmers pay careful attention to feeding activity in order to help determine feed acceptance, calculate feed conversion ratios and feed efficiencies, monitor feed costs, and track feed demand throughout the year (Roberts, 1989).

Published feeding rate tables are available for most commonly cultured fish species. Farmers can calculate optimum feeding rates based on the average size in length or weight and the number of fish in the tank, raceway, or pond (Robinson *et al.*, 1998). Farmed fish typically are fed 1-4% of their body weight per day.

Fish can be fed by hand, by automatic feeders, and by demand feeders. Many fish farmers like to hand feed their fish each day to assure that the fish are healthy, feeding vigorously, and exhibiting no problems. Large catfish farms often drive feed trucks with compressed air blowers to distribute (toss) feed uniformly throughout the pond (Robinson *et al.*, 1998).

There are a variety of automatic (timed) feeders ranging in design from belt feeders that work on wind-up springs, to electric vibrating feeders, to timed feeders that can be programmed to feed hourly and for extended

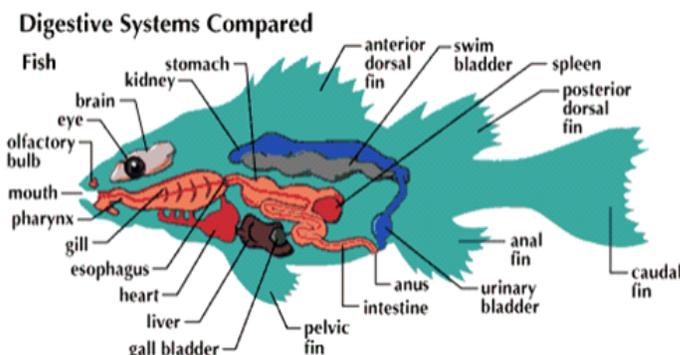


Fig.1: Anatomy of fish showing the digestive system (<http://cache.eb.com/eb/image?id=63663&rendTypeI=4>)

periods. Demand feeders do not require electricity or batteries. They usually are suspended above fish tanks and raceways and work by allowing the fish to trigger feed release by striking a moving rod that extends into the water. Whenever a fish strikes the trigger, a small amount of feed is released into the tank. Automatic and demand feeders save time, labor and money, but at the expense of the vigilance that comes with hand feeding. Some growers use night lights and bug zappers to attract and kill flying insects and bugs to provide a supplemental source of natural food for their fish (Tom and Van-Nostrand, 1989). The most important rule in fish nutrition is to avoid overfeeding. Overfeeding is a waste of expensive feed. It also results in water pollution, low dissolved oxygen e manufactured as either extruded (floating or buoyant) or levels, increased biological oxygen demand, and increased bacterial loads. Usually, fish should be fed only the amount of feed that they can consume quickly (less than 25 min). Many growers use floating (extruded) feeds in order to observe feeding activity and to help judge if more or less feed should be fed (Tom and Van-Nostrand, 1989)

Even with careful management, some feed ends up as waste. For example, out of 100 units of feed fed to fish, typically about 10 units of feed are uneaten (wasted) and 10 units of solid and 30 units of liquid waste (50% total wastes) are produced by fish. Of the remaining feed, about 25% is used for growth and another 25% is used for metabolism (heat energy for life processes). These numbers may vary greatly with species, sizes, activity, water temperature, and other environmental conditions (Tom and Van-Nostrand, 1989). This article reviews the fish digestive system, description of fish gut, physiology of fish gut, peristalsis and its control, gastric evacuation time, digestion and absorption, Specific Dynamic Action (SDA), characteristics of enzymes and other digestive secretions, digestion in the mouth and oesophagus, digestion in the stomach, digestion in the mid gut and pyloric caeca, the role of bile, gall-bladder and liver in

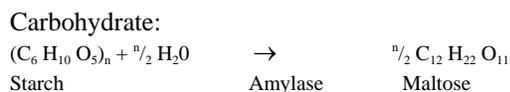
digestion, measurement of digestibility and related factors, feed conversion and efficiency calculations, nutrient requirement, proteins, fats and lipids, carbohydrates, vitamins and minerals to provide vital information for effective culture fisheries management.

Fish digestive system (Fig. 1): Digestion is the means whereby the various items of the diet become broken up into a form in which they can be assimilated into the blood or lymph. The breaking down of large molecules that takes place in digestion is based on the chemical reaction of hydrolysis, whereby organic and inorganic compounds are split into fragments by the addition of water (Winfree, 1992).

When food enters the fish's body it goes down a short and expandable esophagus that is lined with muscle. This allows it to swallow large objects. The food then enters a stomach that is a bent muscular tube. Here, gastric glands release substances to break down the food to prepare it to be digested. After the stomach the food moves into the intestine where enzymes that have been secreted by the pancreas digest the food. Here most of the absorption takes place (Winfree, 1992).

The length of the intestine varies between types of fish. In herbivore fish it is usually long and coiled and in carnivorous fish it is short. This is due to the amount of time that it takes for the intestine to break down the food. After this the waste then leaves the body through the anus. This shows the digestive system of the fish. Unlike many of the organisms that we dissected, the fish only has one intestine as opposed to a large and a small intestine (Tom and Van-Nostrand, 1989).

Typical examples of hydrolysis, as it occurs in digestion, are as shown:



from infolded body wall is absent in teleost fish, but present in sharks and rays (Robinson *et al.*, 1998).

Physiology of fish gut: Many fish chew their food and have the ability to secrete mucus substances from the mucus gland. This facilitates the ingestion of abrasive food. These secretions are similar to saliva but lacks enzymatic activity. The mucus substance is only partly comparable to saliva. Pylorus is developed variously in different fish species. Some species lack pylorus. This is the case with fish without stomach. The oesophageal sphincter serves to prevent regress of food from the intestine. This is because the mid gut is attached directly to the oesophagus (Roberts, 1989).

The digestive process of the mid gut resembles the higher vertebrates' histo-chemically. It is mildly alkaline and contains enzymes from the pancreas and intestinal wall, as well as bile from the liver. These enzymes act on all classes of food. Pyloric caeca have the same structure and enzyme contents as the upper mid gut. The pyloric caeca contain bacteria, which does not produce vitamin B. Functionally, it only increases the surface area of the mid gut and contains anatomical demarcation of the mid gut. There is a comparable blood supply between the hindgut and the posterior mid gut, so that a continuous absorption pattern is discernible as the mid gut (Roberts, 1989).

Peristalsis and its control: This process involves a traveling wave of contraction of the circular and longitudinal layers of muscles in the gut wall, such that, materials made inside the gut is moved along. The existence of an intrinsic nerve network in the intestine controls the process (Roberts, 1989).

Gastric evacuation time: The development of optimum feeding schedule and gastric evacuation time for culture fish include variables like temperature, season, active body size, gut capacity, safety and metabolic rate. Gastric emptying rate declines exponentially with time. Large meals are not digested as fast as small meals. The amount of pepsin and acid produced, are proportional to the degree of distention of the stomach. The appetite, digestion rate and amount of secretion produced decrease with decreased temperature. These secretions also decreased at temperatures in excess of the acclimation temperature (CAN, 1993).

The quantity of food eaten voluntarily at a time, determine the reversed stomach fullness. This does not explain the eutric phenomenon of appetite. Appetite continues to increase for a number of days when the stomach is empty. This indicates that additional metabolic or neutral mechanisms are operating (CAN, 1993).

Food passage time becomes important when one wishes to analyze faeces resulting from ingestion of a specific meal. If sufficient time is allowed for feeding, a

test meal when the gut is completely empty. The digestion processes observed would only be typical for starved fish. When this is given as part of a regular feeding programme, the major problem marks the food for appropriate faecal analysis (CAN, 1993).

Digestion and absorption: Digestion is the process by which ingested food particles are reduced to smaller molecules. In this process, proteins are hydrolyzed to amino acids, carbohydrates to glycogen and lipids to glycerol. Indigestible food materials are voided as faeces. The digestibility of most natural proteins and lipids ranges from 80 to 90%. Digestion is a progressive process starting from the stomach and ends when food leaves the rectum as faeces. For the channel catfish, digestion is continuous through each part of the gut (Lovell, 1988).

Temperature and pH play major roles in determining the effectiveness of digestive enzymes. In general, enzyme reaction rate continues to increase at high temperatures, even when the temperature increase is beyond its lethal level. Enzymes have limited ranges of pH they function. The pH for the channel catfish, *Ictalurus punctatus* range from 2 to 4. When the pH becomes alkaline, below the pylorus decreases slightly to a maximum pH of 8.6 in the upper intestine and finally approached neutrality in the hindgut (Lovell, 1988).

Fish with no stomach have no acid phase in digestion. The physical state of feed passing through the gut varies with species and types of food. Fish feeding on relatively large prey, reduces the prey in size. Digestion in the gastric entericus is aided by, mucus substances, acid and enzymes when the stomach wall contacts food. Food liquefies in the mid gut and solidifies again during the formation of faeces. Pellets of commercial feed are treated similarly (Lovell, 1988).

Absorption of soluble food takes place predominantly in the mid gut but also in the hindgut. Fat droplets are present in the intestinal epithelial cells on consumption of a lipid rich meal. Villi and lacteal are absent in fish. However, the folding and ridging of the gut wall increases its surface area. Lacteals serve as primary uptake routes of droplets of emulsified lipids. Absorbed amino acids, peptides and glycogen are transported across the gut epithelium into the blood stream. Microvilli are present on the surface of the epithelial cells facing the gut lumen. These are sub cellular finger like projections of the cell membrane. They increase the surface are and are involved in absorption (Lovell, 1988).

Specific Dynamic Action (SDA): Digested food, particularly protein is scarcely available to fish, even when absorbed into the blood stream. Amino acid used for building new tissues could be absorbed. When amino acids are oxidized for energy, deamination occurs. The reaction requires input of energy. This process is known

as specific dynamic action. It could be measured externally in fish as an increase in oxygen consumption due to ingestion of food. The process is accompanied by an increase in ammonia excretion (Lovell, 1988).

Characteristics of enzymes and other digestive secretions: The characteristics of enzymes critical to the functioning of living systems, is their specificity. Most enzymes are highly specific with regard to the reactants they affect and the type of reaction they catalyze. The ability of any organism to digest a given substance depends on the type of enzyme present and conditions for the operation of that enzyme. Digestion for convenience is subdivided into sections (Houlihan *et al.*, 2001).

Digestion in the mouth and oesophagus: Many fish do not produce any secretion because of the hard surface in the mouth. Others chew with pharyngeal teeth or similar structures. These produce mucus while chewing. Oesophageal mucus cells show no sign of enzymatic activity. However, gastric-like secretory cells are present (Helfrich and Smith, 2001).

Digestion in the stomach: Pepsin is the predominant gastric enzyme in fish. The optimal pH for maximum proteolytic activity obtained for some fish species are:

- pH of 2 for pike and plaice
- pH range of 3-4 for Ictalurus

The stomach wall of fish produces the hormone, gastrin that stimulates gastric secretion. Lipase may be present in the stomach of fish (Helfrich and Smith, 2001).

Digestion in the mid gut and pyloric caeca: Two sources of enzymes are known in the mid gut. These are pancreas and the secretory cells in the gut wall. The pancreas secretes greater variety and quantities of enzymes in fish. Because of the variety of enzymes present in different fish species, fish diet correlate with enzyme activity. Since trypsin and the predominant proteases are easily isolated, the proteolytic activity of pH range, 7-11 is known for trypsin (Helfrich and Smith, 2001).

One major limitation of obtaining relatively crude extracts from mixed tissues for the localization of the enzymes is the diffuse nature of the pancreas. When extract of pancreas are mixed with extracts from the intestine, the tryptic activity increased tenfold or more. pancreatic origin. Cod's lipase is essential to fish because fatty acids are essential dietary component for fish (Helfrich and Smith, 2001).

Amylase activity is also present in some fishes. For example, gold fish, bluegill sunfish in extracts of mixed liver and pancreas, oesophagus and intestine. However, it



Fig. 4: The liver

is absent in large-mouth bass. Similar activity is present in rainbow trout, perch, tilapia, cod, common carp and eel. Enzymes responsible for the digestion of carbohydrate are glycosidase, maltase, sucrase, lactase, melibiase and cellobiase (Helfrich and Smith, 2001). This confirms the presence of the enzyme, enterokinase in the intestinal wall of the fish. This activates the pancreatic trypsin as it reaches the intestine in mammals (Helfrich and Smith, 2001).

The use of radioisotope labeled lipids in cod explains that cod's lipase acted in the same manner as mammalian pancreatic lipase. It implies that fish lipase is of

The role of bile, gall-bladder and liver in digestion: The function of bile in fish resembles those in higher vertebrates. Bile is composed mainly of bilirubin and biliverdin. These are products of the breakdown of hemoglobin. These salts act like detergents and emulsify lipids, making them more accessible to enzymes. Because of the increased surface area, some lipids are absorbed as micro droplets. About 80% of the bile is recycled through the liver and gall bladder (CAN, 1993).

The basic functions of the liver (Fig. 4) in processing digested and absorbed food are entirely cellular and molecular in scope. There is no functional requirement for shape at any level above the cellular level. Liver can be of any shape. However, some restrictions occur due to its position in the circulatory system between the gut and the heart. Changes in normal size and shape indicate dietary problems. For example, a large yellowish liver with white blotches indicates fatty degeneration of the liver caused by excess starch or saturated fats in the diet (CAN, 1993).

Measurement of digestibility and related factors:

Chromium oxide (Cr₂O₃) mixed with prepared diets, measured in the faeces, provides a general comparison of the digestibility of all feed and is expressed as:

Percentage digestibility (%) = 100

% Cr₂O₃ in faeces

% Cr₂O₃ in feed

The caloric value of ingested food and faeces produced replaces the use of Cr₂O₃ as an indicator. This provides information for estimating the energy balance for a fish (CAN, 1993). Alternatively, the protein and lipid contents of food and faeces can be measured. In both cases the equation can be expressed as:

Digestibility (%) = Nutrient intake in faeces × 100

The most acceptable equation for the determination of the percentage digestibility is:

$$\begin{aligned} \text{Digestibility (\%)} &= 100\% \frac{\text{Cr}_2\text{O}_3 \text{ in feed}}{\text{Cr}_2\text{O}_3 \text{ in faeces (\%)}} \times \text{Protein in feed (\%)} \\ &= \frac{\text{Cr}_2\text{O}_3 \text{ in faeces (\%)}}{\text{Cr}_2\text{O}_3 \text{ in feed (\%)}} \times \text{Protein in feed (\%)} \end{aligned}$$

Feed conversion and efficiency calculations: Because feed is expensive, Feed Conversion Ratio (FCR) or Feed Efficiency (FE) are important calculations for the grower. They can be used to determine if feed is being used as efficiently as possible. FCR is calculated as the weight of the feed fed to the fish divided by the weight of fish growth. For example, if fish are fed 10 pounds of feed and then exhibit a 5 pound weight gain, the FCR is 10/5 = 2.0. FCRs of 1.5-2.0 are considered, good ¾ growth for most species (CAN, 1993). FE is simply the reciprocal of FCRs (1/FCR). In the example above, the FE is 5/10 = 50%. Or if fish are fed 12 pounds of feed and exhibit a 4 pound weight gain, the FE = 4/12 = 30%. FEs greater than 50% are considered, good¾ growth (CAN, 1993).

Fish are not completely efficient (FEs of 100 %, FCRs of 1.0). When fed 5 pounds of feed, fish cannot exhibit 5 pounds of growth because they must use some of the energy in feed for metabolic heat, digestive processing, respiration, nerve impulses, salt balance, swimming, and other living activities. Feed conversion ratios will vary among species, sizes and activity levels of fish, environmental parameters and the culture system used (Lovell, 1988).

Commercial fish feed is usually purchased by large farms as bulk feed in truckloads and stored in outside bins. Smaller farms often buy prepared feed in 50-pound bags. Bag feed should be kept out of direct sunlight and as cool as possible. Vitamins, proteins, and lipids are especially heat sensitive, and can be readily denatured by

high storage temperatures. High moisture stimulates mold growth and feed decomposition. Avoid unnecessary handling and damage to the feed bags which may break the pellets and create fines ¾ which may not be consumed by fish (Lovell, 1988).

Feed should not be stored longer than 90 to 100 days, and should be inventoried regularly. Bags should not be stacked higher than 10 at a time. Older feed should be used first, and all feed should be regularly inspected for mold prior to feeding. All moldy feed should be discarded immediately. Mice, rats, roaches and other pests should be strictly controlled in the feed storage area, because they consume and contaminate feed and transmit diseases (Lovell, 1988).

When fish reduce or stop feeding, it is a signal to look for problems. Off-feed behavior is the first signal of trouble such as disease or water quality deterioration in the fish growing system. Relatively few therapeutic drugs are approved for fish by FDA (Helfrich and Smith, 2001), but some medicated feeds for sick fish are available. Although using medicated feeds is one of the easiest ways to treat fish, they must be used early and quickly because sick fish frequently will stop feeding.

Nutrient requirement: The major nutrients required for fish growth and maintenance are protein, lipids, carbohydrate, mineral salts and vitamins. An outline of these major nutrients can give a better understanding for fish nutrient requirements (CAN, 1993).

Proteins: Because protein is the most expensive part of fish feed, it is important to accurately determine the protein requirements for each species and size of cultured fish. Proteins are formed by linkages of individual amino acids. Although over 200 amino acids occur in nature, only about 20 amino acids are common. Of these, 10 are essential (indispensable) amino acids that cannot be synthesized by fish (CAN, 1993).

The 10 essential amino acids that must be supplied by the diet are: methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine. Of these, lysine and methionine are often the first limiting amino acids. Fish feeds prepared with plant (soybean meal) protein typically are low in methionine; therefore, extra methionine must be added to soybean-meal based diets in order to promote optimal growth and health. It is important to know and match the protein requirements and the amino acid requirements of each fish species reared (CAN, 1993).

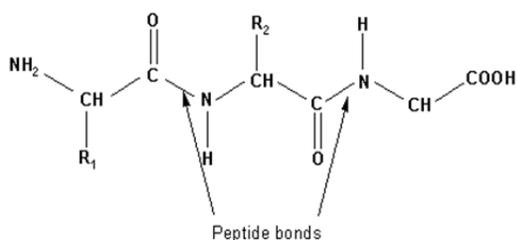
Protein levels in aquaculture feeds generally average 18-20% for marine shrimp, 28-32% for catfish, 32-38% for tilapia, 38-42% for hybrid striped bass. Protein requirements usually are lower for herbivorous fish (plant eating) and omnivorous fish (plant-animal eaters) than they are for carnivorous (flesh-eating) fish, and are higher

for fish reared in high density (re-circulating aquaculture) than low density (pond aquaculture) systems (CAN, 1993).

Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Protein requirements also vary with rearing environment, water temperature and water quality, as well as the genetic composition and feeding rates of the fish. Protein is used for fish growth if adequate levels of fats and carbohydrates are present in the diet. If not, protein may be used for energy and life support rather than growth (CAN, 1993).

Proteins are composed of carbon (50%), nitrogen (16%), oxygen (21.5%), and hydrogen (6.5%). Fish are capable of using a high protein diet, but as much as 65% of the protein may be lost to the environment. Most nitrogen is excreted as ammonia (NH₃) by the gills of fish, and only 10% is lost as solid wastes. Accelerated eutrophication (nutrient enrichment) of surface waters due to excess nitrogen from fish farm effluents is a major water quality concern of fish farmers. Effective feeding and waste management practices are essential to protect downstream water quality (CAN, 1993).

Proteins are complex, organic compounds composed of many amino acids linked together by peptide bonds, cross linked by hydrogen bonds and van der Waals forces by attraction. There is a greater diversity of chemical composition in proteins than in any other group of biologically active compounds. Proteins can be classified as:



Simple proteins: On hydrolysis they yield amino acids and occasionally small carbohydrate compounds. Examples include albumens, globulins, glutelins, albuminoids, histones and protamines.

Conjugated Proteins: These are simple proteins combined with some non-protein materials in the body. Examples include nucleoproteins, glyco proteins, phosphoproteins, and hemoglobin and lecithin proteins.

Derived proteins: These are proteins derived from simple or conjugated proteins by physical or chemical means. Examples include denatured proteins and peptides.

The configuration of protein molecules, are so complex that some molecules can be constructed. These are present in biological materials with different physical

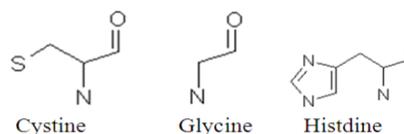
characteristics. Globular proteins are present in blood and tissue fluids in amorphous globular forms with very thin membranes. Other types of protein are present in definitive structures such as collagenous protein in connective tissue. For example, cell membranes fibrous protein in brain muscle and connective tissue. Crystalline proteins are typical of eye lens and similar tissues.

Enzymes are also proteins that catalyses most physiological process in life. Polypeptides act as hormones in tissue systems controlling water alcohol, dilute base or various concentrations of salt solutions. Proteins have characteristic coiled structure determined by the sequence of amino acids. This determinant is the primary polypeptide chain. They are heat labile. The liability depends on the type, solution and temperature profile. They can be reversible or irreversible but are denatured by heating, salting, freezing or ultrasonic stress. They undergo a characteristic bonding with other proteins in plastein reaction and combines with free aldehyde and hydroxyl groups of carbohydrates to form mallard type compounds (CAN, 1993)

Ingested proteins are split into smaller fragments by pepsin in the stomach, trypsin or chymotrypsin in the pancreas. The peptides are further reduced by the action of carboxyl peptidase. This hydrolyses one amino acid at a time. It starts from the free carboxyl end of the molecule. Amino peptidase also splits one amino acid at a time starting from the free amino end of the peptide chain. Protein requirements are known for a few fish species. Simulated whole egg protein contains an excess of indispensable amino acids (CAN, 1993).

Protein requirements are higher in fry, fingerlings and yearling fish. These requirements decrease as the fish increases in size. Maximum growth rate can be achieved when nearly half of the digestible ingredients consist of balanced protein. This requirement decreases to 40% of the diet at 6-8 weeks for salmon and trout. In salmonids, the decrease is 35% when cultured at standard environmental temperature.

Diets lacking arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine retard fish growth. Dispensable amino acids are alanine, aspartic acid, cystine, glutamic acids, glycine, proline, serine and tyrosine. These amino acids are known for fish growth. Quantitative study of the requirement of this indispensable amino acid can be carried out using a caeseingelatin mixture supplemented with crystalline amino acid in test diet with an amino acid of 40% whole egg protein for the nitrogen component. Carp and eel showed retarded growth when indispensable amino acids were absent in a diet (CAN, 1993).



Lipids: Lipids (fats) are high-energy nutrients that can be utilized to partially spare (substitute for) protein in aquaculture feeds. Lipids supply about twice the energy as proteins and carbohydrates. Lipids typically comprise about 15% of fish diets, supply Essential Fatty Acids (EFA) and serve as transporters for fat-soluble vitamins (Robert, 1979).

A recent trend in fish feeds is to use higher levels of lipids in the diet. Although increasing dietary lipids can help reduce the high costs of diets by partially sparing protein in the feed, problems such as excessive fat deposition in the liver can decrease the health and market quality of fish (Robert, 1979).

Simple lipids include fatty acids and triacylglycerols. Fish typically require fatty acids of the omega 3 and 6 (n-3 and n-6) families. Fatty acids can be: a) saturated fatty acids (SFA, no double bonds), b) polyunsaturated fatty acids (PUFA, >2 double bonds), or c) highly unsaturated fatty acids (HUFA; > 4 double bonds). Marine fish oils are naturally high (>30%) in omega 3 HUFA, and are excellent sources of lipids for the manufacture of fish diets. Lipids from these marine oils also can have beneficial effects on human cardiovascular health (Robert, 1979).

Marine fish typically require n-3 HUFA for optimal growth and health, usually in quantities ranging from 0.5-2.0% of dry diet. The two major EFA of this group are eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA:22:6n-3). Freshwater fish do not require the long chain HUFA, but often require an 18 carbon n-3 fatty acid, linolenic acid (18:3-n-3), in quantities ranging from 0.5 to 1.5% of dry diet. This fatty acid cannot be produced by freshwater fish and must be supplied in the diet (Robert, 1979).

Many freshwater fish can take this fatty acid, and through enzyme systems elongate (add carbon atoms) to the hydrocarbon chain, and then further de-saturate (add double bonds) to this longer hydrocarbon chain. Through these enzyme systems, freshwater fish can manufacture the longer chain n-3 HUFA, EPA and DHA, which are necessary for other metabolic functions and as cellular membrane components. Marine fish typically do not possess these elongations and de-saturation enzyme systems, and require long chain n-3 HUFA in their diets. Other fish species, such as tilapia, require fatty acids of the n-6 family, while still others, such as carp or eels, require a combination of n-3 and n-6 fatty acids (Robert, 1979).

Lipids are fat-soluble compounds present in the tissues of plants and animals. They are broadly classified as fats, phospholipids, sphingomyelins, waxes and sterols. Fat are fatty acid esters of glycerol. In fish, energy is stored as glycogen. The energy is being utilized during periods of extensive exercise, inadequate food and energy intake. Phospholipids are esters of fatty and phosphatidic

acids. These are main constituent lipids of cellular membrane surfaces that are hydrophilic. This depends on the orientation of the lipid compounds into intra or extra cellular spaces (Robert, 1979).

Sphingomyelins are fatty acid esters of sphingosine present in brain and nerve tissue compounds. Waxes are fatty acid esters of long-chain alcohols. They are metabolized for energy and impart physical and chemical characteristics through the stored lipids of some plant and animal compounds. Sterols are polycyclic, long chain alcohols. These function as components of several hormone systems especially in sexual maturation and sex related physiological functions (Robert, 1979).

Fatty acids can exist as straight or branch chain components. Most fish fats contain numerous unsaturated double bonds in the fatty acid structures. A short bond designation for fatty acids is used. The number indicates the position of the first double bond from the methyl end. Linolenic acid can therefore be written as 18:3. The first number indicates the number of carbon atoms; the second number, the number of double bonds and the last number, the position of the double bond.

The main emphasis on lipid requirements, are on its Essential Fatty Acid (EFA) and energy value. Widely accepted theories explaining the presence of high levels of 20:5W3 and 22:6W3 fatty acids in fish oils is related to the effect of unsaturation on the melting point of a lipid. Higher degree of unsaturation of fatty acids in the fish phospholipids allows for flexibility of cell membrane at lower temperatures. The W3 structures allow more unsaturation than the W6 or W9. This is consistent with the fact that coldwater fishes have more nutritional requirement for W3 fatty acids. The EFA requirement of some warm water fish can be achieved by mixing W6 and W3 (Robert, 1979).

Fatty acid metabolism in fish: Fish are capable of synthesizing fatty acids from acetate and pufa. Fish can convert 16.0 to the W7 monoene and 18.0 to the W9 monene. The W5 and W13 monoenes are proposed based on the identification of these fatty acids (Robert, 1979).

Eskimo study suggests fish oils curb diseases. A study of Alaska's Yup'ik Eskimos, who consume 20 times more omega-3 fats from fish than most Americans, suggests these oils can prevent obesity-related illness such as diabetes and heart disease. The researchers analyzed data from 330 people living in the Yukon Kuskokwim Delta region of southwest Alaska.

Although 70% of the population was overweight or obese, they did not show the same risk factors for heart disease and had a lower prevalence of diabetes than the overall US population. The fats the researchers were interested in measuring were those found in salmon, sardines and other fatty fish - docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

In participants with low blood levels of these fats, obesity strongly increased heart disease risk measured by triglycerides and C-reactive protein, a measure of overall body inflammation. Additionally, the researchers said that although the Yup'ik Eskimos have overweight/obesity levels similar to those in the US overall, their prevalence of type 2 diabetes is significantly lower-3.3% versus 7.7%.

"Because Yup'ik Eskimos have a traditional diet that includes large amounts of fatty fish and have a prevalence of overweight or obesity that is similar to that of the general US population, this offered a unique opportunity to study whether omega-3 fats change the association between obesity and chronic disease risk", "Interestingly, we found that obese persons with high blood levels of omega-3 fats had triglyceride and CRP concentrations that did not differ from those of normal-weight persons."

"It appeared that high intakes of omega-3-rich seafood protected Yup'ik Eskimos from some of the harmful effects of obesity." The study was led by researchers at Fred Hutchinson Cancer Research Center and conducted in collaboration with the Center for Alaska Native Health Research at the University of Alaska-Fairbanks. It published online March 23 in the European Journal of Clinical Nutrition.

For the study, the participants provided blood samples and health information via in-person interviews and questionnaires. Diet was assessed by asking participants what they ate in the past 24 h and asking them to keep a food log. The researchers said however that further studies would be needed to make specific recommendations on diets or supplements. "If the results of such a trial were positive, it would strongly suggest that omega-3 fats could help prevent obesity-related diseases such as heart disease and diabetes,".

Carbohydrates: Carbohydrates are compounds that contain carbon, hydrogen and oxygen. Hydrogen and oxygen are present in the same proportion. Some carbohydrates such as starch and cellulose are very large and complex molecules. Other examples are sugars and gums. The basic carbohydrate molecules are simple sugars or monosaccharide. All sugars, when in straight chain form, contain a C=group. If the double bonded O is attached to the terminal C of a chain, the combination is called a ketone group. The carbon chain that forms the backbone of the sugar can be of different lengths. Some sugars contain as few as three carbons (trioses). Others contain five carbons (pentose), six carbons (hexoses) or more. Though, both trioses and pentoses play an important biological roles, hexoses are the important building block compounds for more complex carbohydrates (Robert, 1979).

Carbohydrates (starches and sugars) are the most economical and inexpensive sources of energy for fish diets. Although not essential, carbohydrates are included in aquaculture diets to reduce feed costs and for their

binding activity during feed manufacturing. Dietary starches are useful in the extrusion manufacture of floating feeds. Cooking starch during the extrusion process makes it more biologically available to fish (Robert, 1979).

In fish, carbohydrates are stored as glycogen that can be mobilized to satisfy energy demands. They are a major energy source for mammals, but are not used efficiently by fish. For example, mammals can extract about 4 kcal of energy from 1 of carbohydrate, whereas fish can only extract about 1.6 kcal from the same amount of carbohydrate. Up to about 20% of dietary carbohydrates can be used by fish.

There are many six-carbon sugars of which glucose and fructose are two of the most important. Since these two sugars contain the same number of carbon atoms, the proportions of oxygen and hydrogen atoms are constant in carbohydrates. It follows that glucose and fructose are isomers of each other because they have the same molecular formula, $C_6H_{12}O_6$. In addition to this kind of structural isomerism, there is another more-subtle one called stereo-isomerism (Robert, 1979) (Fig. 5).

When a five-membered ring is formed it is called a *furanose* (Fig. 6).

In stereoisomers, identical groups are attached to the carbon atoms, but the spatial arrangements in the attached groups are different. Stereo isomerism can be geometric or optical. In geometric, all the carbon-to-carbon bonds in the two molecules are single bonds. Free rotation is possible around a single bond. Therefore, the two compounds could be the same. The carbon valences in optical stereoisomers are arranged around the carbon like the apexes of a tetrahedron. When the groups attached to each of the four valences are different, there would be some alternative arrangement of these groups that would not be super-impossible (Robert, 1979) (Fig. 7).

Glucose is an aldehyde sugar. Its double bonded oxygen is attached to a terminal carbon. Glucose seldom exists as straight chain aldehyde compound but with a ring composed of five carbons with one oxygen atom. It is said to resonate between forms because its internal

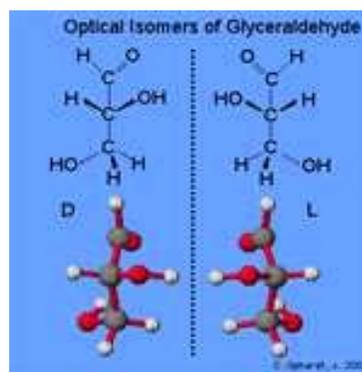


Fig. 5: Optical Isomers of Glyceraldehyde

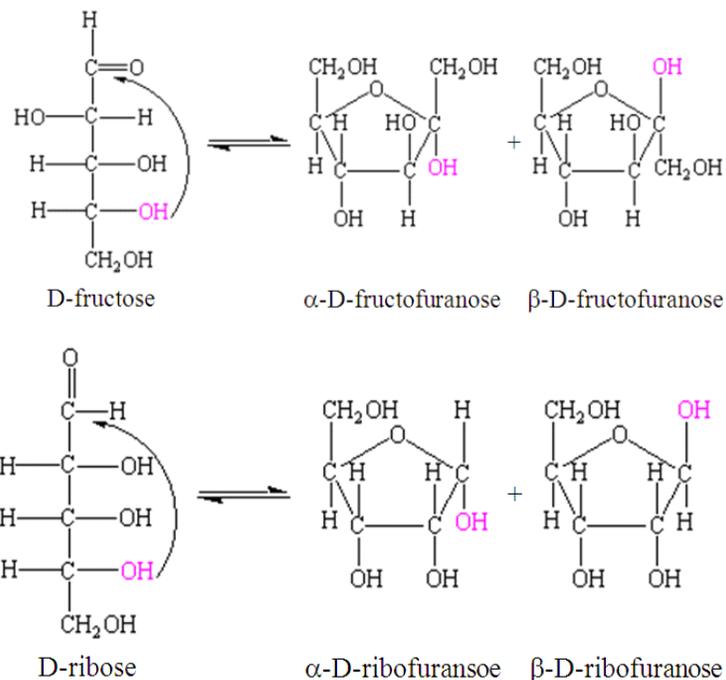


Fig. 6: Five member ring formation

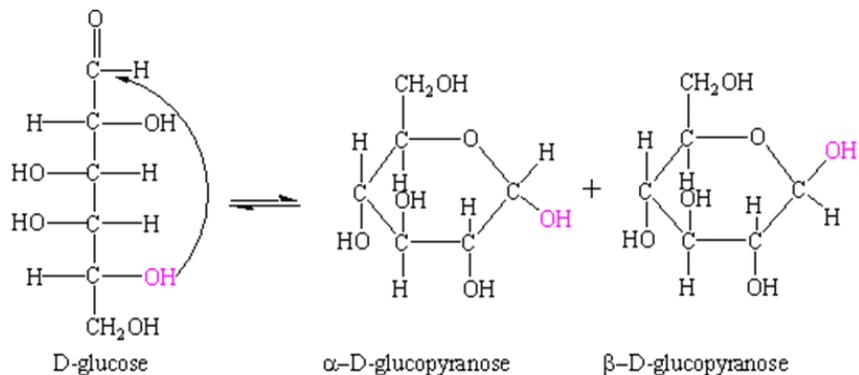


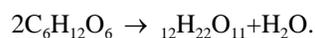
Fig. 7: Six member ring formation

configuration is constantly in a state of flux, and no single structural formula fully depicts it. If the carbon chain is long enough, the alcohol at one end of a monosaccharide can attack the carbonyl group at the other end to form a cyclic compound. When a six-membered ring is formed, the product of this reaction is called a *pyranose*.

Glucose plays a unique role in the chemistry of life. It is the cross roads of chemical pathways in the bodies of plants and animals. Other six-carbon monosaccharide such as fructose and galactose are constantly being converted to glucose or synthesized from glucose. The more complex carbohydrates such as disaccharides and polysaccharides are composed of monosaccharide bonded together in sequence. And even such classes of

compounds as fats and proteins could be converted into simple sugars in the living body (Robert, 1979).

Disaccharides (double bonded sugars) are compound sugars composed of two simple sugars bonded together through a reaction that involve the removal of a molecule of water. This kind of reaction is called condensation or dehydration reaction. Maltose (malt sugar) is synthesized by a condensation reaction between two molecules of glucose:

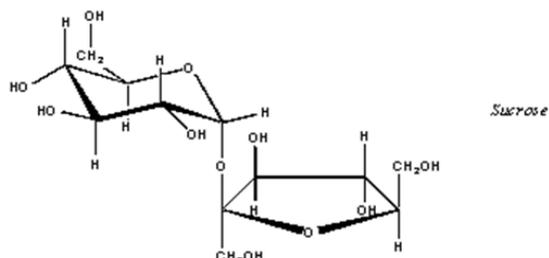


Disaccharides are formed by condensing a pair of monosaccharides. The structures of three important

disaccharides with the formula $C_{12}H_{22}O_{11}$. Maltose, or malt sugar, which forms when starch breaks down, is an important component of the barley malt used to brew beer. Lactose, or milk sugar, is a disaccharide found in milk. Very young children have a special enzyme known as lactase that helps digest lactose. As they grow older, many people lose the ability to digest lactose and cannot tolerate milk or milk products. Because human milk has twice as much lactose as milk from cows, young children who develop lactose intolerance while they are being breast-fed are switched to cows' milk or a synthetic formula based on sucrose.

The substance most people refer to as "sugar" is the disaccharide sucrose, which is extracted from either sugar cane or beets. Sucrose is the sweetest of the disaccharides. It is roughly three times as sweet as maltose and six times as sweet as lactose. In recent years, sucrose has been replaced in many commercial products by corn syrup, which is obtained when the polysaccharides in cornstarch are broken down. Corn syrup is primarily glucose, which is only about 70% as sweet as sucrose. Fructose, however, is about two and a half times as sweet as glucose. A commercial process has therefore been developed that uses an isomerase enzyme to convert about half of the glucose in corn syrup into fructose. This high-fructose corn sweetener is just as sweet as sucrose and has found extensive use in soft drinks.

constituent sugars on hydrolysis. A number of complex polysaccharides are of great importance in fish nutrition. Starches for example, are storage products of plants. They are composed of many glucose units bonded together.



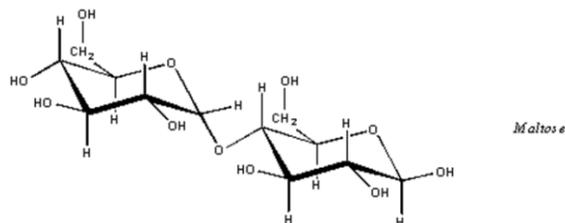
The monosaccharides and disaccharides represent only a small fraction of the total amount of carbohydrates in the natural world. The great bulk of the carbohydrates in nature are present as polysaccharides which have relatively large molecular weights. The polysaccharides serve two principal functions. They are used by both plants and animals to store glucose as a source of future food energy and they provide some of the mechanical structure of cells.

Very few forms of life receive a constant supply of energy from their environment. In order to survive, plant and animal cells have had to develop a way of storing energy during times of plenty in order to survive the times of shortage that follow. Plants store food energy as polysaccharides known as starch. There are two basic kinds of starch: amylose and amylopectin. Amylose is found in algae and other lower forms of plants. It is a linear polymer of approximately 600 glucose residues whose structure can be predicted by adding α -D-glucopyranose rings to the structure of maltose. Amylopectin is the dominant form of starch in the higher plants. It is a branched polymer of about 6000 glucose residues with branches on 1 in every 24 glucose rings. A small portion of the structure of amylopectin.

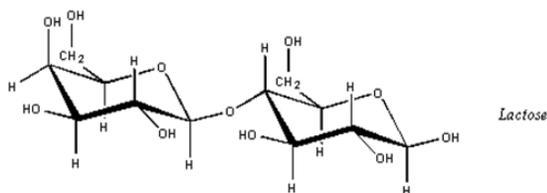
The polysaccharide that animals use for the short-term storage of food energy is known as glycogen. Glycogen has almost the same structure as amylopectin, with two minor differences. The glycogen molecule is roughly twice as large as amylopectin, and it has roughly twice as many branches.

There is an advantage to branched polysaccharides such as amylopectin and glycogen. During times of shortage, enzymes attack one end of the polymer chain and cut off glucose molecules, one at a time. The more branches, the more points at which the enzyme attacks the polysaccharide. Thus, a highly branched polysaccharide is better suited for the rapid release of glucose than a linear polymer.

Polysaccharides are also used to form the walls of plant and bacterial cells. Cells that do not have a cell wall often break open in solutions whose salt concentrations are



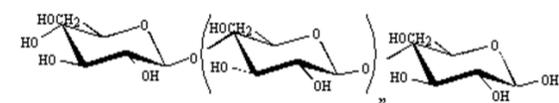
Lactose (milk sugar) is a double sugar composed of glucose and galactose. Double sugars are broken into its constituent simple sugars. This reaction involves addition of a water molecule and is called hydrolysis:



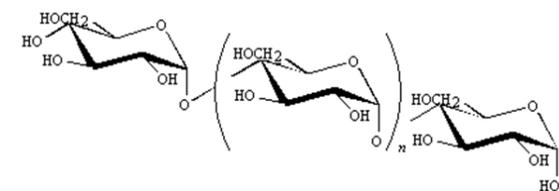
Polysaccharides are complex carbohydrates composed of many simple sugar building blocks bonded together in long chains. They are synthesized by exactly the same kind of condensation reactions as the disaccharides. Like the disaccharides, they can be broken down to their

either too low (hypotonic) or too high (hypertonic). If the ionic strength of the solution is much smaller than the cell, osmotic pressure forces water into the cell to bring the system into balance, which causes the cell to burst. If the ionic strength of the solution is too high, osmotic pressure forces water out of the cell, and the cell breaks open as it shrinks. The cell wall provides the mechanical strength that helps protect plant cells that live in freshwater ponds (too little salt) or seawater (too much salt) from osmotic shock. The cell wall also provides the mechanical strength that allows plant cells to support the weight of other cells.

The most abundant structural polysaccharide is cellulose. There is so much cellulose in the cell walls of plants that it is the most abundant of all biological molecules. Cellulose is a linear polymer of glucose residues, with a structure that resembles amylose more closely than amylopectin, as shown in the Fig. The difference between cellulose and amylose can be seen by comparing the figures of amylose and cellulose. Cellulose is formed by linking β -glucopyranose rings, instead of the α -glucopyranose rings in starch and glycogen.



Cellulose
n = 5000-10,000



Amylose
n = 1000-6000

The -OH substituent that serves as the primary link between β -glucopyranose rings in starch and glycogen is perpendicular to the plane of the six-membered ring. As a result, the glucopyranose rings in these carbohydrates form a structure that resembles the stairs of a staircase. The -OH substituent that links the β -glucopyranose rings in cellulose lies in the plane of the six-membered ring. This molecule therefore stretches out in a linear fashion. This makes it easier for strong hydrogen bonds to form between the -OH groups of adjacent molecules. This, in turn gives cellulose the rigidity required for it to serve as a source of the mechanical structure of plant cells.

Cellulose and starch provide an excellent example of the link between the structure and function of biomolecules. At the turn of the century, Emil Fischer suggested that the structure of an enzyme is matched to

the substance on which it acts, in much the same way that a lock and key are matched. Thus, the amylase enzymes in saliva that break down the α -linkages between glucose molecules in starch cannot act on the β -linkages in cellulose.

Most animals cannot digest cellulose because they don't have an enzyme that can cleave β -linkages between glucose molecules. Cellulose in their diet therefore serves only as fiber, or roughage. The digestive tracts of some animals, such as cows, horses, sheep, and goats contain bacteria that have enzymes that cleave these β -linkages, so these animals can digest cellulose.

There are many slightly different types of starch, varying in number of constituent sugar molecules and degree of branching. Glycogen is a storage product in animals and is sometimes called "animal starch". Its molecules are more branched than those of starch. Cellulose is a highly insoluble polysaccharide occurring frequently in plants. It is a major supporting material. The bonds between its sugars are different from those of starch and glycogen, and seem to be more resistant to hydrolysis. The reactions that form polysaccharides are called polymers.

Raffinose, stachyose and verbasose are major oligosaccharides present in significant quantity in seeds. Raffinose, which spreads mostly among the three, consist of one molecule of glucose, linked to a molecule of sucrose at the 6 positions. Its abbreviated chemical name is - D - Gal (1,5) - - D-Glu - (1,2) - BD - Fru. Further chain elongation at the galactose end with another galactose molecule can yield stachyose.

Starch is a high molecular weight polymer of D-glucose and is the principal reserve carbohydrate in plants. Most starch consist of mixture of two types of polymers namely, anuglose and anuglopectin. Glycogen is the only complex carbohydrate of animal origin and exists in limited quantities in liver and muscle tissues, and acts as a readily available energy source. Dextrins are intermediate compounds resulting from incomplete hydrolysis or digestion of starch. The presence of 1-D (1, 6) linkage in amyl pectin and the inability of α -amylase to cleave these bonds can result to low molecular weight carbohydrate. Termites provide an example of the symbiotic relationship between bacteria and higher organisms. Termites cannot digest the cellulose in the wood they eat, but their digestive tracts are infested with bacteria that can. Propose a simple way of ridding a house from termites, without killing other insects that might be beneficial.

For many years, biochemists considered carbohydrates to be dull, inert compounds that filled the space between the exciting molecules in the cell - the proteins. Carbohydrates were impurities to be removed when "purifying" a protein. Biochemists now recognize that most proteins are actually glycoprotein in which

carbohydrates are covalently linked to the protein chain. Glycoproteins play a particularly important role in the formation of the rigid cell walls that surround bacterial cells.

Vitamins: Vitamins are organic compounds necessary in the diet for normal fish growth and health. They often are not synthesized by fish, and must be supplied in the diet.

The two groups of vitamins are water-soluble and fat-soluble. Water-soluble vitamins include: the B vitamins, choline, inositol, folic acid, pantothenic acid, biotin and ascorbic acid (vitamin C). Of these, vitamin C probably is the most important because it is a powerful antioxidant and helps the immune system in fish.

The fat-soluble vitamins include A vitamins, retinols (responsible for vision); the D vitamins, cholecalciferols (bone integrity); E vitamins, the tocopherols (antioxidants); and K vitamins such as menadione (blood clotting, skin integrity). Of these, vitamin E receives the most attention for its important role as an antioxidant. Deficiency of each vitamin has certain specific symptoms, but reduced growth is the most common symptom of any vitamin deficiency. Scoliosis (bent backbone symptom) and dark coloration may result from deficiencies of ascorbic acid and folic acid vitamins, respectively.

These are organic substance present in certain food and are essential to the health and growth of fish. Vitamins are divided into water-soluble and fat-soluble ones. Water-soluble vitamins include eight recognized members of the vitamin B complex: Thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, biotin, folic acid and cyanocobalamin. The water-soluble essential nutritional factors are choline, inositol, ascorbic acid and vitamins with less defined function for fish such as P-amino benzoic acid, lipoid acid and citrin. The first eight, though required in small quantities in the diet, play major roles in growth, physiology and metabolism. Choline, inositol and ascorbic acid are required in appreciable quantities in the diet and are referred to as major dietary nutrients rather than vitamins.

Thiamine (B₁): This is found in plant seeds, in particular the germ, in yeast and in certain green vegetables. It has a complex structure with formula, C₁₂H₁₈O₂N₄S and is not readily affected by boiling. A deficiency of the vitamin causes beriberi in man and a similar disease called polyneuritis in birds. The diseases are by an accumulation of pyruvic acid in the cells of the body and by interference in nervous conduction. The muscles become weakened and atrophied, though the tissues themselves may become distended with fluid. Thiamine is concerned with respiration. It acts on pyruvic acid formed from anaerobic glycolysis in the tissues. Normally the pyruvic acid is passed on to an oxidative cycle but this does not take place in the absence of

thiamine. Hence, this vitamin is a principal part of the coenzyme that catalyses the oxidation of pyruvic acid.

Thiamine is a water-soluble colorless, monoclinic crystalline compound. It is comparatively stable of dry heat but rapidly broken down in neutral or alkaline solutions. Sulphites can split thiamine into pyrimidine and thiazole moieties. Pyrimidine ring is relatively stable but the thiazole ring is easily opened by hydrolysis. It functions in all cells as the co enzymes; co-carboxylase and thiamine pyrophosphate are involved in all oxidative decarboxylation of pyruvic acid to acetate for entry into the tricarboxylic acid cycle. It is also a co enzyme of the transketolase system by which direct oxidation of glucose occur in the cytoplasm of cells via the pentose phosphate pathway. Vitamin B₁ also acts as a growth factor.

Riboflavin (B₂): This occurs in the free form only in the eye, whey and wine. It is a yellowish brown crystalline pigment. The vitamin is slightly soluble in water but soluble in alkali. It is insoluble in most organic solvents and stable to oxidizing agents in strong mineral acids and neutral aqueous solution. It functions as enzymes of tissue respiration and is also involved in hydrogen transport to catalyze the oxidation of reduced pyridine nucleotide (NADH) and NADPH). Thus, it functions as coenzyme for many oxidase and reductase such as cytochrome C reductase, D and L amino acid oxidase, Xanthine and aldehyde oxidase, nicotinic dehydrogenase, glucose oxidase and fumaric dehydrogenase.

Niacin or Nicotinic acid (B₃): This is found in milk, yeast and eggs. It is necessary to man but can be synthesized by other mammals, such as dogs, provided they are supplied with tryptophan. It is a water-soluble vitamin stable to boiling. The deficiency disease caused by absence of the vitamin is pellagra. Nicotinic acid is concerned in the formation of coenzymes that are important in the activity of dehydrogenase (an enzymes that can store or transfer hydrogen from one substance to another) enzymes responsible for removal or transfer of hydrogen during respiration. Again, the disease is caused by accumulation of intermediate metabolic products of respiration. Pellagra affects the epithelia and nervous system. This white crystalline solid, soluble in alcohol and water is stable in the dry state and can be autoclaved without distinction. Its main function in NAD NADP are involved in the synthesis of high energy phosphate bonds which furnish certain steps in glycolysis, pyruvate metabolism, amino acid, protein metabolism and photosynthesis.

Pantothenic acid (B₅): This is found in yeast, eggs, and rice bran. It is used as calcium salt in fish diet formulation. Pantothenic acid is yellow and viscous and is responsible for the formation of coenzymes involved in cellular respiration. Disorder of nervous system and gut

are disease resulting from deficiency of the vitamin. Animals therefore need pantothenic acid or its reduced alcohol form. Since the free acid is labile to heat, acid and alkali, some losses do occur during most diet preparation and during storage.

Pyridoxine (B₆): This is present in yeast, eggs and cereals. It is responsible for the formation of enzymes involved in the synthesis of amino acids. Anaemia and diarrhea are disease resulting from deficiency of the vitamin. Pyridoxine acts as a coenzyme in a number of enzyme systems. It is sensitive to ultraviolet light in neutral or alkaline solutions. Pyridoxamine and pyridoxine in dilute solutions are labile compounds, which are rapidly destroyed on exposure to air, heat or light. Pyridoxal phosphate as the coenzyme, co-de carboxylase is involved in the decarboxylation of amino acids. It is also the co-factor of the 22 different transaminases present in animal tissues. Supplementing commercial fish diets with pyridoxine is important because of the role it plays in protein metabolism.

Cyanocobalamin (B₁₂): The sources of this vitamin are kidney, liver, fish and milk. It is responsible for the formation of red blood cells. Pernicious anaemia is a disease for its deficiency. Cyanocobalamin is the anti-pernicious factor contained in liver. It is stable to mild heat in neutral solution, but is rapidly destroyed by heating in dilute acid or alkali. It is similar to the porphyrins in its spatial configuration with a central cobalt atom linked to form reduced pyrole rings in the haeme series. It is involved in the methylation of homocystine to methionine, in several other one-carbon reactions and in the synthesis of labile methyl compounds.

Biotin: This is a mono-carboxylic acid slightly soluble in water and alcohol but insoluble in organic solvent. It is destroyed by acids and alkaloids by oxidizing agents such as peroxides or permanganate. Avidin, a protein found in raw egg white, binds biotin and makes it unavailable to fish and other animals. Heating to denature the protein makes the bound biotin available again to fish. It is involved in the conversion of acetyl coenzyme A to malonyl coenzyme. It is involved in the synthesis of citrulline purines and pyrimidines. It can be present in many food including liver, yeast and fresh vegetable.

Folic acid: This is present in yeast and cures metaloblastic anaemia. It crystallizes into yellow spear shaped leaflets that are soluble in water and dilute alcohol. It can be precipitated with heavy metals. This vitamin is stable to heat in neutral and alkaline solution but unstable in acid solution. It is required for normal blood cell formation and acts as co-enzyme in one carbon transfer mechanism. Folic acid also functions in the conversion of metaloblastic bone marrow to a normoblastic one. It plays a major role in blood glucose

regulation. This improves all membrane function and hatchability of fish eggs.

Ascorbic Acid (vitamin C): This is an acid hexose sugar, which is found primarily in citrus fruits and fresh vegetables. The vitamin is water soluble and easily destroyed on heating. A deficiency of vitamin C causes scurvy, which is characterized by hemorrhages, an escape of blood into the tissues. It is thought that the vitamin is concerned with the synthesis of the mucopolysaccharides that cement individual cells together. Mucopolysaccharides are organic class of compound that has the general structure of cellulose or starch, made up of individual glucose molecules, but also contains nitrogen. Lack of this cement causes the cells to fall apart and the symptoms described. Vitamin C is also involved in the building of connective tissues important in wound healing, in antibody formation and for the health of the teeth.

Retinol (vitamin A): This is a fat-soluble vitamin present in green and yellow vegetables and fruits, dairy products, egg yolk and fish liver. Some deficiency symptoms of the vitamin are dry, bristle epithelia of skin, respiratory system and urogenital tract; night blindness and malformed rods.

Tocopherol (vitamin E): This vitamin is widely distributed in both plant and animal food, such as meat, egg, yolk, green vegetables and seed oils. It is a fat-soluble disease. The vitamin protects fatty acids and cell membranes from oxidation. Male sterility in rats (and perhaps other animals), muscular dystrophy in some animals, abnormal red blood cells in infants, abnormal eyes in embryo; death of rats and chicken embryos.

Phylloquinone (vitamin K): This is a fat-soluble vitamin present in liver and green vegetables. Vitamin K regulates the amount of prothrombin in the blood and affects its clotting.

Calciferol (vitamin D): This includes a number of fat-soluble substances related to sterol and anti-rachitic (against rickets) in action. In man the vitamin is synthesized by the action of ultraviolet light on the skin and otherwise found in such sources as egg and liver. Vitamin D provides a means for the assimilation of Ca²⁺ and PO₄³⁻ ions from the gut and possibly into the cells of the body as well. Without the vitamin the structures requiring these ions are weakened. This particularly applies to the bones and teeth. Such a condition is known as rickets and is particularly prevalent in temperate climates and slum conditions where sunlight cannot penetrate.

Minerals: Minerals are inorganic elements necessary in the diet for normal body functions. They can be divided into two groups (macro-minerals and micro-minerals) based on the quantity required in the diet and the amount

present in fish. Common macro-minerals are sodium, chloride, potassium and phosphorous. These minerals regulate osmotic balance and aid in bone formation and integrity.

Micro-minerals (trace minerals) are required in small amounts as components in enzyme and hormone systems. Common trace minerals are copper, chromium, iodine, zinc and selenium. Fish can absorb many minerals directly from the water through their gills and skin, allowing them to compensate to some extent for mineral deficiencies in their diet.

These are substances that occur naturally and not formed from animal or vegetative. A large number of different minerals are required to fulfill structural and other functions in an animal. These minerals may either be used as ions as the case with Na and K, or they may be built up into complex organic substances as with iodine and cobalt. Many salts are present as ions in the cytoplasm and extra cellular fluids and their presence is essential for cell function and operation of nervous and muscular tissues. Mineral salts are taken with the diet. A deficiency of certain salts sometime lead to a crave for a food rich in the mineral. Under certain conditions, it is possible to suffer from a deficiency disease due to lack of specific salts.

Though each mineral assimilated from the food may have a specific role in metabolism. It is the concentration of salts that largely determines the osmotic pressure of the body fluids. For normal functioning of the body tissue, the osmotic pressure must not vary more than the equivalent 0.1g NaCl in 100cc water. Body mechanisms dealing with correction of osmotic pressure are sensitive to much smaller changes than this. Ionic regulation, depending on both intake via the gut and excretion through the kidneys, ensures, as far as possible, that the salt concentration remains consistently within these limits. A list of important minerals and some of their uses in the body are:

- **Calcium:** Bone and cartilage formation, blood clotting, muscles contraction
- **Phosphorus:** Bone formation, high-energy phosphorus esters, other organic phosphorus compound
- **Magnesium:** Enzyme cofactor extensively involved in the metabolism of fats, carbohydrates and proteins
- **Sodium:** Primary monovalent action of intracellular fluid; involved in acid base balance and osmoregulation
- **Potassium:** Primary monovalent action of intracellular fluid; involved in osmoregulation
- **Sulphur:** Integral part of sulphur amino acids and collagen; involved in detoxification of aromatic compounds
- **Chlorine:** Primary monovalent anion in cellular fluid; component of digestive juice (HCL) acid base balance

- **Iron:** Essential constituent of haemoe in hemoglobin, cytochromes peroxidase
- **Copper:** Component of haeme in haemocyanin, cofactor in tryosinase and ascorbic acid oxidase
- **Manganese:** Cofactor for arginase and certain other metabolic enzymes involved in bone formation and erythrocyte regeneration
- **Cobalt:** Metal component of cyanocobalamin (B₁₂); prevent anaemia, involved in C₁ and C₃ metabolism.
- **Zinc:** Essential for insulin structure and function, cofactor of a carbonic anhydrase
- **Iodine:** Constituent of thyroxin regulates oxygen use.
- **Molybdenum:** Cofactor of hydrogenase and reduction
- **Fluorine:** Hardness of bones and teeth

Certain salts such as magnesium sulphate, which are very slowly assimilated, tend to hold backwater in the gut and, by stimulating peristalsis, act as laxatives.

CONCLUSION

The fish digestive system, description of fish gut, physiology of fish gut, peristalsis and its control, gastric evacuation time, digestion and absorption, Specific Dynamic Action (SDA), characteristics of enzymes and other digestive secretions, digestion in the mouth and oesophagus, digestion in the stomach, digestion in the mid gut and pyloric caeca, the role of bile, gall – bladder and liver in digestion, measurement of digestibility and related factors, feed conversion and efficiency calculations, nutrient requirement, proteins, fats and lipids, carbohydrates, vitamins and minerals are some vital principles in fish nutrition the fish culturist need to know for effective culture fisheries management.

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