Fish Nutrition And Its Relevance To Human Health

A. S. Ninawe

Former Advisor & Scientist
Department of Biotechnology, New Delhi

J. R. Dhanze

Former Dean
College of Fisheries, Central Agricultural University
Lembucherra, Agartala, Tripura

R. Dhanze

Ex. Prof. and Head
College of Fisheries, Central Agricultural University
Lembucherra, Agartala, Tripura

S. T. Indulkar

Professor & Head
College of Fisheries, Ratnagiri, Maharashtra

NARENDRA PUBLISHING HOUSE

First Edition 2020

ISBN : 978-93-89235-142

Published by :

NARENDRA PUBLISHING HOUSE
Publisher and Distributor
C-21, Varun Apartments, Sector-9, Rohini, Delhi- 110085 (INDIA)
Ph: +91-11-45025794, +919717874875
Email: info@nphindia.com, nphindia@gmail.com
Website: www.nphindia.com

5

Next Generation Fish Feeds for Sustainable Aquaculture

Dipesh Debnath[1], Sona Yengkokpam[1],
Basanta Kumar Das[2] and Bimal Prasanna Mohanty[2]
[1]ICAR-Central Inland Fisheries Research Institute, Regional Centre, Guwahati
[2]ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata

Introduction

Use of fish feeds in aquaculture can be considered as the most important factor contributing to increased fish production for the growing human population on planet earth. Growth of this sector can easily be distinguished into three generations – 1st generation was *extensive* in nature where cultured fish was dependent on the natural fish food organisms, 2nd generation was *semi-intensive* where fish was dependent on supplemental feeds in addition to the natural fish food organisms and 3rd generation was *intensive* where fish was fed with complete artificial feeds containing all nutrients necessary for normal growth and maintenance of fish. The present generation of aquaculture can be called 4th generation, where fish is reared with customized feeds (with precise/ accurate nutrient concentrations, pellet diameter, buoyancy/ sinking characteristics, water stability etc.) with use of demand feeders, automatic feeders, medicated feeds and feeds developed for special purposes such as early or repeated maturation. This can also be called *precision aquaculture*. The rapid growth of the sector was possible because of understanding of the science of feeding fish to ensure its optimal development, health and maintenance called *fish nutrition*. In recent years, fish nutritional practices have gained increasingly important role, not only for economic optimization, but also for sustainability of aquaculture. Supplying various nutrients to meet the requirements of the cultured fish is fundamental in achieving optimal growth and production efficiency and hence maximizing economic return. The natural feeding habits of a particular fish give us useful clues on its requirement for various nutrients. There is wide range of raw materials (ingredients) available to feed a particular species of fish, and hence an infinite number of combinations of those ingredients that correspond to the formulae of utilizable feeds. Knowledge on the nutrient requirements of the cultured fish along with the chemical composition of raw materials would help the nutritionist to formulate an optimally utilizable feed. However, the best formulated feed is the one that meets the requirements of the cultured fish at minimum cost without adversely affecting the environment.

Important Considerations While Feeding Fish

In aquaculture operations, fish feed accounts for more than one-half of variable cost. Therefore, practical know-how on nutrition and feeding of fish is most essential to successful aquaculture. Generally, nutrient requirements do not vary greatly among various fish species. Any exception to this generalization can often be identified with warm-water or cold-water, finfish or shellfish, carnivore or omnivore, and marine or freshwater fish. Therefore, when nutrient requirement is not available for a particular species, a cautious comparison can be made with other species while feeding the former. Feeding fish in the aqueous environment differs considerably from those of feeding terrestrial animals. Some of the confounding aspects of fish feeding include the nutrient contribution of natural aquatic organisms present in ponds, the effects of feeding and diet composition on dissolved oxygen of water and other water quality parameters, and the loss of nutrients if feed is not consumed by fish immediately. Fish feeds require processing methods that provide special physical properties to facilitate feeding in water, and variation in feeding behavior requires special feeding regimens for various species. Nutrient requirements are determined primarily with small fish and represent concentrations affecting maximum growth rate. Fish size, metabolic function, management, and environmental factors have slight to profound effects on nutrient requirements for optimum performance. The requirement data do not include additional allowances for processing and storage losses, bioavailability of nutrients in feed ingredients, or economic considerations.

Feeding Behavior of Fish

Amount of feed distributed and the amount of feed ingested by the fish determines whether feeding management in aquaculture is good or bad. The amount of feed to be distributed is in the hands of the aquaculturist. But whether the feed is going to be ingested by the fish depends on several factors, which can start, prolong or stop the ingestion of feed (Fig. 1).

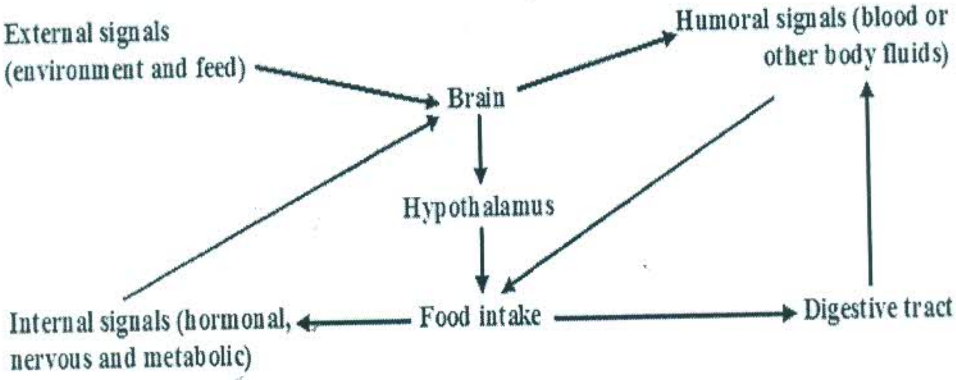


Fig. 1. Neuro-hormonal regulation of appetite and feed intake in fish

Appetite in fish appears to be regulated by the amount of stomach distension and the level of certain nutrients circulating in the blood. When fish has fed enough, stomach distension acts through the brain to reduce feeding response to the point of cessation. However, as food is digested and passed through the intestine, distension reduces and the brain triggers feeding behavior by means of a search for more food. Absorbed nutrients from food circulate in blood and changes in levels of these nutrients modify feeding behavior. In some species of fish, the time taken for the stomach to empty influences the return of appetite, which determines how many times fish will feed in a 24-h period. Fish metabolism also has an influence on feeding behaviour. Fish eat to meet their energy requirements. Thus, if a diet has a low-energy value fish will compensate by eating more (within the limits of how much can be packed into their stomachs); the case will be opposite when the diet is high-energy. The physical limits of a fish stomach means that diets with different energy values, but nutritionally balanced, will produce the same amount of growth weight-for-weight. Addition of feed attractants to high energy diets is a sensible feed management policy that can improve growth on a weight-for-weight basis.

Digestive Physiology of Fish

Digestion is the process by which the ingested food material is broken down into simple, small, absorbable molecules. This task is performed by the digestive fluids and enzymes (Table 1). Most digestion of the food is extracellular, taking place in the lumen of the alimentary canal. The digestive enzymes are hydrolases, compounds capable of catalysing hydrolytic reactions. They are water-soluble proteins. Based on the physiological function they are mainly divided into proteases, lipases, esterases and carbohydrases.

Table 1. Digestive fluids and enzymes secreted in teleosts

Site/ type	Fluid/ enzyme	Function
Stomach (gastric glands)	Zymogens	Proteolytic enzymes; pepsinogen
	HCl	Reduces gut pH and prepares pepsinogens to act. Cleaves peptide links and other structures such as cell walls by hydrolysis
	Pepsin	Attacks most proteins
	Amylase	Acts on carbohydrates
	Lipase	Acts on lipids
	Chitinase	Acts on chitin
Pancreas	HCO_3^-	Neutralizes HCl entering intestine, and prepares intestine for alkali digestion
	Trypsin	Cleaves peptide linkages at carboxy groups of lysine and arginine
	Chymotrypsin	Attacks peptide with carbonyls from aromatic side-chains
	Carboxypeptidases	Hydrolyse the terminal peptide bond of their substrates
	Elastase	Attacks peptide bonds on elastin
	Amylase	Carbohydrate digestion at non-acidic pH
	Lipases	Hydrolyse triglycerides, fats, phospholipids and wax esters
	Chitinase (& NAGase)	Splits chitin into dimers and trimers of N-acetyl-D-glucosamine (NAG), which is further broken down by NAGase
Liver/ anterior intestine	Bile (bile salts, organic anions, cholesterol, phospholipids, inorganic ions	Makes the intestinal medium alkaline; most bile salts are reabsorbed from the intestine and returned to the liver in entero-hepatic circulation
Brush border of the intestinal epithelium but could be partly pancreatic in origin	Aminopeptidases	Split nucleosides
	Polynucleotidases	Split nucleic acids
	Lecithinase	Split phospholipids into glycerol and fatty acids
	Various carbohydrate-digesting enzymes	Act on various carbohydrates

Protein digestion: Proteolytic enzymes arise from inactive precursors known as zymogens. Proteases break down peptide links of proteins. Different enzymes are capable of acting on peptide bonds either at the end of the protein (exopeptidases) or at a point within the protein (endopeptidases). Among the endopeptidases, pepsin hydrolyses the bond on the amino side of the aromatic radical while chymotrypsin hydrolyses the bond on the carboxyl side. Trypsin, on the other hand, acts on the peptide bonds between arginine and lysine. There are three groups of exopeptidases: carboxypeptidases, amino peptidases and dipeptidases. Carboxypeptidases remove terminal amino acids in which the carboxyl radical is free, while amino peptidases act on the other end of the polypeptide chain and remove the terminal amino acid possessing free amino group. Most of the digestion of protein occurs in the stomach as a result of action of pepsin. In stomachless fish, the role of pepsin is taken up by alkaline proteases.

Fat digestion: Liver plays an important role in the digestion of fat. The bile that is produced in the liver and stored in the gall bladder is released when food arrives in the intestine. It contains gallic acids of high surface activity, and these emulsify the fats, breaking large fat droplets into very small droplets, thereby increasing the surface area and making them more accessible to fat-splitting enzymes. All fat digesting enzymes are classed as lipolytic enzymes or lipases. Lipases show relatively little substrate specificity, and many will catalyse hydrolysis of almost any organic ester. All fat digesting enzymes act in alkaline media.

Carbohydrate digestion: Carbohydrases have been demonstrated in pancreatic juice, stomach, intestine and in the bile. They have a broad temperature tolerance (20-40°C) and optimal activity occurs at pH 6-8. Carbohydrase activity responds to the level of dietary carbohydrate and appears to be related to their feeding habits. A higher level of dietary starch has been found to stimulate higher levels of amylase activity in some fish.

Feed Formulation

Artificial feed: Artificial feed includes a wide array of feeds ranging from simple, on-farm based mixtures of a few ingredients to microencapsulated diets. This may be either complete or supplemental in nature. Complete diets supply all the nutrients necessary for the optimal growth and health of fish, while supplemental diets are intended only to help support the natural food normally available to fish in ponds. Usually the nursery and grow-out feeds are artificially formulated to satisfy the known nutritional requirement of the cultured fish. The bulk of feeds used in aquaculture belong to this category. Now-a-days, artificial feeds having a particle size of 50-200 µm are formulated for larval rearing that are called microparticulate diets. These diets are fabricated by micro-binding (particles mixed with binding agent, dried, crushed and sieved), micro-coating (an external coating is added to graded microparticles) or micro-encapsulation (liquid nutrient phase is emulsified with non-miscible liquid phase forming an insoluble film at the interface). Fish fry are given feed in the form of flakes or crumbles. For fingerlings and grow-out fish, feed are given in various forms (e.g., mash, pellets).

Table 3. Desired characteristics of artificial feed for aquaculture

Property	Desired characteristics
Acceptability	Artificial feed must be attractive and readily ingested; particles must be suitable for ingestion and must elicit a feeding response.
Stability	Artificial diet particles must maintain integrity in water and nutrient leaching must be minimal.
Digestibility	Artificial feed must be digestible and its nutrients easily assimilated.
Nutrient composition	Artificial feed must have an appropriate nutritional composition to meet the nutritional requirements of the cultured animal.
Storage	Artificial feed must be suitable for long-term (6-12 months) storage with stable nutrient composition and particle integrity.

Fish feed Ingredients and Additives

Common ingredients of animal origin

They are widely used in aquaculture feed formulation because they are better in terms of digestibility, essential amino acids (EAA) profile, essential fatty acid (EFA) profile, palatability, richness of vitamin A, content of growth factors and also rarity of anti-nutritional factors. However their use in aquaculture is restricted at present due to high cost and non-availability of such ingredients.

Fishmeal: It is a source of high protein (may vary between 55-70% crude protein) in fish feeds. It is rich in EAAs and EFAs. Fishmeal is also a good source of minerals, vitamins (except vitamin C) and also often contains carotenoid pigments. All these characteristics make fishmeal an ideal feed ingredient. However, it is becoming increasingly scarce and expensive worldwide, and therefore lot of research is on for its partial or complete replacement with mostly plant-derived feed ingredients.

Crustacean meal: It is made from shrimp heads, small prawn, mantis shrimp, crabs and krills. Crude protein level varies from 30-50% depending on raw material. Crustacean meal is a good source of cholesterol, carotenoids, chitin, calcium, iron, manganese, choline, niacin, panthothenic acid and is powerful attractant.

Animal meal: It includes meat and bone meal, feather meal and blood meal. They are often not used in aquaculture feed, because their protein is of low biological value, lipids are mostly saturated or mono-unsaturated and often lack few essential amino acids.

Silkworm pupae: It can be used as ingredient in fish feeds at low level. It is prone to rancidity due to high level of lipid.

Common ingredients of plant origin

There are several ingredients of plant origin. They are less expensive than those of the animal origin. Some have certain binding properties and also contain B groups of vitamins. Most of these ingredients lack unsaturated fatty acids (HUFA) and have complex carbohydrates that are indigestible to fish. Most of these ingredients contain various anti-nutritional factors affecting digestion and assimilation of feeds.

Oilseed meals: They are by-products of oil extraction from various oilseeds such as soybean, cotton, rapeseed, sunflower, groundnut etc. They have less protein (30-50%) compared to the ingredients of animal origin and have lower mineral contents. Oilseed meals such as soybean meal, cotton meal, sunflower meal and groundnut meal are now sought after ingredients for aquafeed formulation because of non-availability of fishmeal and other meals of marine origin. The anti-nutritional factors of such ingredients are often taken care of by heat treatment, water soaking or by exogenous enzyme supplementation.

Cereals and by-products: Crude protein contents in these ingredients vary from 10-20%, hence mainly constitute energy sources. They have low mineral contents and EAAs. They are good source of group B and E vitamins. Heat treatment of these ingredients improves starch digestibility. Bulk of the farm-made aquafeeds in India is constituted by such ingredients, i.e., wheat bran/ flour, rice bran/ flour, maize flour, etc.

Pulses: These are seeds of leguminous plants like broad beans, lupins, peas. They contain 25-45% proteins. They are not widely used in fish feed.

Alfalfa and leaf meal: They are sought after as balanced source of protein, certain vitamins and carotenoids. But fish utilizes their nutrients poorly, mostly because they contain anti-nutritional factors and indigestible fibre. They are often processed to improve the protein digestibility and remove the anti-nutritional factors.

Commonly used purified ingredients

Fish oil: They are well digested, rich in HUFAs and lipid soluble vitamins (A and D). Their quality depends on the origin of raw materials and also freshness of the product used for oil extraction.

Plant oil: They constitute energy source but contain anti-nutritional factor. They contain lipid soluble vitamins, astaxanthin, linoleic and linolenic acid. They are costly to be used in aquafeeds.

Starches: They are used for their binding properties and also for their energy value.

Purified amino acids: They are used in diets prepared for experimental purposes, and also to supplement formulated feeds deficient of certain amino acids.

Some commonly used additives in fish feeds

Attractant: They are used to attract or orient fish towards the diet. They include L-amino acids, glycine, betaine, inosine etc.

Antioxidants: These are substances that are easily oxidised and hence protect other compounds which are sensitive to oxidation. E.g., ascorbic acid, citric acid, EDTA, BHA, BHT, etc.

Binding agents: These include agar-agar, carrageenan, pectins, arabic gum, chitosans, xanthan gums, carboxymethylcellulose (CMC), etc.

Preservatives: These are used to preserve moist feeds. E.g., potassium sorbate, organic or mineral acids.

Probiotics: Dietary supplementation with probiotics such as live micro-organisms (bacteria or yeast) to change composition of the gastrointestinal tract of fish was shown to enhance growth as well as reduce disease risk in various species of fish (Halver and Hardy, 2002).

Enzymes: Enzymes are added to the feed in order to improve the digestibility of the feed and hence improve growth of the fish. For example, phytase, amylase, protease, lipase, chitinase and cellulase. Phytases are phosphatase enzymes that sequentially cleave orthophosphate groups from the inositol ring of phytic acid to yield available free inorganic phosphorus. Enzymes that break down non-starch polysaccharides would be of specific interest in fish nutrition (especially to improve energy availability), because plant meals (which are gradually replacing fish meal) including soybean meal contains such carbohydrates.

Acidifiers: Acidifiers consisting of organic acids and their salts are promising alternative to antibiotic growth promoters in fish and shellfish. Acidifiers such as citric acid, benzoic acid, propionic acid, fumaric acid, lactic acid and formic acid are known to improving performance and health of livestocks and fish. They can exert their effects on performance via three different mechanisms: (1) in the feed, (2) in the gastro-intestinal tract of fish and (3) effects on fish' metabolism.

Nucleotides: Nucleotides are low molecular weight biological compounds that play a major role in almost all biological functions. An exogenous source of nucleotide may optimize the function of rapidly dividing tissue, such as those of the immune system, which lack the capacity to synthesize nucleotide and therefore must depend on the preformed nucleotides. Generally fish and prawns get stressed, especially at times of vaccination, grading and netting and these are all known to result in the release of cortisol causing immune suppression, reduction in growth rate and increased susceptibility to disease. These create additional demand for available nucleotides and an additional exogenous supply in the diet may alleviate the stress and hence health and growth of fish will improve. Nucleotides are now being used commercially as feed additives to improve growth and/ or disease resistance.

Practical Feed Formulation

Semi-intensive and intensive aquaculture practices require artificial feeds. The major aim in developing commercial diets for aquaculture is to formulate a diet that will satisfy the nutritional requirements of the target species at minimum possible cost. This is known as least-cost formulation. Information that are required before feed formulation can begin are: (1) a list of available raw materials and information on their compositions and costs, (2) knowledge of the nutritional requirements of the target species, (3) the specifications of the diet to be made (i.e., desired levels of protein, lipid, amino acids, fatty acids, etc.), (4) knowledge on the suitability of available raw materials for the target species. Information on the proximate composition of feed ingredients is useful for the initial choice of potential raw materials for aquaculture feeds. Usually the information on the proximate composition of feed ingredients is given according to six major components.

- » Moisture is a measure of the water content of the feed or feed ingredient.
- » Crude protein (CP) is the measure of the protein content, estimated after nitrogen analysis.

- Ether extract (EE) is the measure of the lipid and fat-soluble vitamin content.
- Crude fibre (CF) is the measure of the insoluble polysaccharide content (e.g. cellulose).
- Nitrogen-free extract (NFE) is equivalent to the carbohydrate content.
- Ash is a measure of the inorganic content.

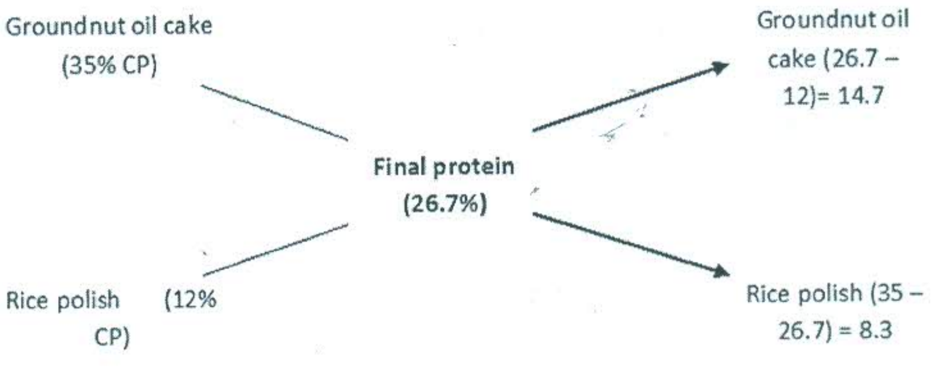
Various computer software packages are available now-a-days to formulate feed. For a diet that contains few ingredients, a simple algebra method called the Pearsons' square method can be used to formulate a feed.

i. **Pearsons' square method:** This method is used to formulate a diet with a few ingredients. For e.g., to formulate a diet containing 30% protein using the following feed ingredients with protein content given as:

Fish meal - 60% crude protein
Groundnut cake - 35% crude protein
Rice polish - 12% crude protein

Step 1. Fish meal, an animal source of protein which contain well balanced amino acids and palatable to the fish is generally included @ 10% of the diet since it is expensive and limited in availability. So, protein from fish meal is 60 x 10% = 6%. Therefore, the protein to be obtained from other sources is 30 – 6 =24% in remaining 90% of the diet.

Step 2. 24% in 90 parts of feed will be equivalent to 24 x 100/90 = 26.7 % in 100 parts.



Balancing the crude protein level in a feed using the Persons' Square method

Actual amount of ingredient needed (in 100 parts)

Groundnut oil cake	= 14.7 ÷ (14.7 + 8.3) x 100 = 63.9%
Rice polish	= 8.3 ÷ (14.7 + 8.3) x 100 =36.1%

For 90%, it will be 63.9 x 90% =57.5% for groundnut and 36.1 x 90% = 32.5% for rice polish.

Therefore, the diet formulated will contain:

10% Fish meal + 57.5% groundnut cake + 32.5% rice polish

ii. **Linear programming:** A process by which various feed ingredients are combined in proportion to get the desired amount of nutrients in the feed with a given cost is called linear programming. The idea is to prepare a least cost feed that meets the nutrient requirements of the cultured fish. For this one requires to know about the various nutrient values of each feed ingredient, nutrient requirement of the target species, cost of ingredients, number of ingredients to be used, etc. This is a tedious job to do manually where as a computer can perform various calculations within no time.

iii. **Diet Formulator:** Diet Formulator developed by Dr. Kevin C Williams, CSIRO Marine & Atmospheric Research is a simple Excel-based diet calculation program (www.enaca.org). It is *not* a least-cost diet formulation program, but simply a tool that allows the user to select ingredients and calculate the nutritional specification of the formulated diet. The strength of the program is the database of feed ingredients that are common to SE Asia. However, it must be recognized that the nutritional specifications for these ingredients are only the best average values that the author was able to source. The program will allow the user to insert other ingredients for which the user has his/ her own chemical analysis. Also, ingredient costs can be entered in the database and the program will calculate the feed cost.

Feed Manufacture

The manufacture of a compound feed consists of a series of operations, the end goal is to combine several raw materials in previously determined proportions for a precise nutritional objective. The various steps in feed manufacture are performed by specific instruments/ equipments, and lot of developments are happening with respect to the design/ efficiency of such machineries:

***Grinding*:** Grinding is done to reduce the raw material to finer particles and to increase the surface area so as to facilitate mixing, pelleting and improve digestibility of the ingredients. The most commonly used mill for grinding is hammer mills.

***Weighing*:** Weighing ensures the input of different ingredients of the formula in well defined proportions.

***Mixing*:** Mixing is an essential process so as to form a homogenous blend. Mixing can be done in batches or in continuous mixers. Types of mixers are horizontal ribbon mixers, vertical mixers or turbine mixers.

***Structuring of the feed*:** This refers to giving a final structure to the feed. The feed can be given as paste after addition of water or can be pelleted. Pelleting is the compacting of feeds formed by extruding the ingredients, which are durable and suitable for modern-day aquaculture. Different types of pellets are compressed pellets and extruded dry pellets.

a. ***Compressed pellets***: Compressed pelleting involves exposing the mixture to steam for 5-20 s at high temperature (85⁰C) and low moisture (16%), followed by forcing the mix through a metal die to form pellets.

b. ***Extruded dry pellets***: In this type of pelleting, the temperature is increased to about 125-150⁰C in a pressurized conditioning chamber (20s) and the moisture is brought to 20-24%, enhancing gelatinization of starch. This results in the mixture being made into a dough-like consistency, which is then forced through a die at high pressure. As the pellet leaves the die, fall in pressure causes the trapped water to evaporate and the gelatinized materials expand forming air pockets. When cooled, the density is generally about 0.25-0.3 g/cm^3, so the pellets float or sink slowly in water.

Most of the recent developments in fish feed manufacturing is taking place with respect to pelletization and extrusion technologies. Commercial fish feeds are manufactured as either extruded (floating or buoyant) or pressure-pelleted (sinking) feeds. Both floating and sinking feeds are getting increasingly used for feeding fish, which are producing satisfactory results in terms of growth. However, type of feed (floating or sinking) used should be based on species' preference (i.e., feeding biology, mouth structure etc.). For example, prawns/ shrimps/ flat fish will not accept a floating feed but most fish species can be trained to accept a floating pellet. It is a fact that producing extruded/ floating pellets is more expensive due to higher manufacturing costs. However, it is advantageous to feed a floating pellet, because the farmer can directly observe the feeding intensity of fish and adjust feeding rates accordingly.

An extruder is a pump that provides the pressure necessary to force the mash through a restrictive die. During the transport through barrel, large amount of heat is added to the mash through friction generated between the mash and stationary & rotating components of an extruder. The pressure and temperature profiles experienced by the mash can, within limits, be chosen and controlled by variations in screw design and operational conditions. The shape of the final product can be easily controlled through die selection and design. In most cases, a pre-conditioner is used in conjunction with the extruder to increase moisture and heat absorption into the mash, reduce mechanical power requirements and increase capacity. The conditioner normally operates at atmospheric pressure and provides a means in which either water or steam or both are uniformly incorporated into the process mash. In addition, additives such as vitamins, flavors, colors and even meat slurries may be incorporated. The conditioner provides retention time necessary for the mash to absorb the heat and moisture needed before entering the extrusion barrel.

Barrel of a single-screw extruder can be divided into three separate zones. In the 'feed zone', the conditioned mash is simply received from the conditioner and transported forward in the barrel to a point where the cross section of the barrel is completely full and an elastic plug is formed. The 'transition zone' of the barrel is identified by the fact that the mash changes, rheologically, from a powder to an elastic dough. In the 'metering zone' (or 'cooking zone'), extreme pressure is applied to the mash and high level of heat is induced by friction causing the temperature of the dough to increase to well above 100°C. As compression on the extrudate is increased, the mechanical energy created by the screw turning is dissipated as heat into the extrudate. In many designs, the surface of the barrel is grooved, either in a spiral or straight manner so that the barrel 'grips' the extrudate and the rotating screw can force the material forward toward the die.

Table 4. Difference between single-screw and twin-screw extruders used for aquafeed extrusion.

Item	Single-screw extruder	Twin-screw extruder
Conveying manner	Feed mash is conveyed forward by friction	Feed mash is forced to slid forward
Self-cleaning function	Unavailable	Excellent self-cleaning performance
Running	Stable and occasional blockage	Stable and reliable
Heat distribution of feed mash	Non-uniform	Uniform
Ingredient adaptability	Common	Wider (including ingredients rich in moisture and fat)
Moisture content of ingredients	10-30%	5-95%
Product variety	Limited	Various
Machine price	Relatively cheap	Expensive
Capacity	Common	Relatively high

Feeding Rate, Frequency and Methods

Feeding fish in aquaculture presents some difficulties such as (1) it is hard to ensure that all feed distributed in the pond is eaten up by fish, (2) if feed is not consumed fully, it can lead to deterioration of water quality, and (3) the requirements of fish vary in relation to the environmental factors viz. temperature, dissolved oxygen etc. In practice, feeding of fish involves the following three stages:

- determining how much feed should be given to fish (ration size or feeding rate)
- determining how many times the fish should be fed in a day (feeding frequency) and the optimum time of feeding, and
- selecting the method of feed distribution (feeding methods).

Feeding rate: Feeding rate or ration size defines the amount of feed made available to the cultured fish. Determining the optimum ration size is one of the most important tasks in aquaculture. The optimum ration will give the best growth and feed conversion, minimize wastage and reduce environmental problems. However, the ration size varies with the size of cultured organisms. A fry or fingerling requires more energy for metabolism per unit weight and has the potential to grow faster than an adult fish. Therefore, a fry or fingerling needs a higher feeding rate than a yearling or adult. The feeding rate is normally calculated as a percentage of the biomass being cultured. to be provided to fish stock will depend on the biomass present which can be calculated as follows: Biomass = average weight of fish x number. Ration size also depends on temperature of water and stages of life cycle of fish.

Fry –	7-10% of body wt
Small fingerlings –	6-7 % of body wt
Advanced fingerlings –	5% of body wt
Yearlings –	2-4% of body wt

Feeding frequency: In ideal condition feed @ 5% of biomass is to be fed daily in five times. But as it involves labour and cost, practically the same feed was fed twice daily preferably morning and afternoon at fixed time and place.

Feeding methods: Method is of two types as described below: Non-demand feeding involves predetermined feed at predetermined time interval. Suitable way of giving feed for initial life stages like spawn and fry is to broadcast feed powder over water surface whereas for grow-out fish to keep feed pellet or ball in earthen pot or tray 1-1.5 feet below water surface. Demand feeding provides *ad libitum* feeding which is activated by fish stock. Of late, developments are taking place in designing automatic (timed) feeders. Automatic and demand feeders can save time, labor and money. However, in Indian scenario, both these technologies are yet not popular due to various reasons. Tray feeding and bag feeding methods are getting popularised at present in India which reduce feed wastage to a great extent.

Innovative Fish Feeds For Future

Research and development in fish feeds has transformed this sector into a fast growing aquafeed industry. Rapid growth of finfish and shellfish aquaculture into global prominence has been mainly due to availability and provision of fish feeds. To sustain the present growth rate of the sector i.e., 8-10%, the supply of feeds and their ingredients will have to grow at similar rates. That is to say that output from a unit water area mainly depends on the inputs provided. Hence, optimizing the inputs for efficient utilization by fish should be given preference. Some of the ideas in this direction are presented below.

Digestive optimization

Digestive enzyme activities in the digestive tract of fish and the utilization of nutrients are of utmost importance for optimizing fish feeding procedures (Suarez *et al.*, 1995). Digestive enzyme pattern can reflect the feeding habit of fish (whether it is herbivore, detritivore, omnivore or carnivore) and also reflects the digestive capacity of fish (Smith, 1980). The type, source and amounts of nutrients can alter the enzyme profile/ concentration in the digestive tract of fish. This adaptive characteristic of the enzymes can be successfully used to take advantage of the nutrient content of diets (Moraes and Bidinotto, 2000).

Growth of fish is one of the most desirable qualities sought after by an aquaculturist, which depends on several factors like species, culture conditions, feeds and feeding regime. Growth rate of fish could partly be set by the digestive capacity, oxygen availability, or the metabolic capacity required to support tissue protein synthesis (Blier *et al.*, 1997). It has been observed that trypsin (a protein digesting enzyme) activity has an influence on growth rate and food conversion efficiency in Atlantic cod (Lemieux *et al.*, 1999) and Atlantic salmon (Torrisen and Shearer, 1992). Glycolytic enzyme activities have been reported to be directly correlated with growth rate in Atlantic cod (Pelletier *et al.*, 1994) and coho salmon (Blier *et al.*, 2002). Trypsin, chymotrypsin and alkaline phosphatase (ALP) activities were found to be positively correlated with growth in case of Atlantic cod (Lemieux *et al.*, 1999).

Breakdown of large nutrients into small absorbable subunits in the digestive tract (called digestion) of the animal depends largely on the available enzymes, which may lead to a loss of food energy upto 20 to 80% (Cho, 1987). Estimations on the digestive enzymes in fish could help recommend appropriate dietary protein (Twining *et al.*, 1983) and carbohydrates (Spannhof and Plantikow, 1983), i.e. matching an artificial diet to the nutritive capabilities of fish. Further, it is also possible to predict the ability of a species of fish/ shellfish to utilize different nutrients from the digestive enzyme profile (Hofer and Kock, 1989). It is strongly felt that studies on digestive enzymes in fish might clarify some aspects of their nutritive physiology and thus could help solve some nutritional problems in fish feeding and diet formulation.

Metabolic optimization

Utilization of the dietary nutrients can be reflected in the metabolic profile in various tissues, as metabolic efficiency determines the growth characteristics of fish. Metabolic profile may reflect the availability of nutrients which in turn is associated with digestion and absorption (Moraes and Bidinotto, 2000; Lundstedt *et al.*, 2004). However, in warm water fish, correlation between growth and metabolism is controversial (Vieira *et al.*, 2005). Debnath et al. (2007) studied the metabolic capacity of *Labeo rohita* fingerlings in relation to the dietary level of protein and carbohydrates. After 45 days of feeding, activity of digestive enzymes and metabolite concentrations were assayed. Amylase, lipase and alkaline phosphatase (ALP) activities were not influenced by the dietary protein, but proteolytic and acid phosphatase (ACP) activities varied ($P<0.05$) between the treatments. Proteolytic activity showed a second order polynomial relationship with dietary crude protein. A positive correlation was observed between dietary CP and amylase ($r^2 = 0.78$). All the metabolites except muscle glucose showed significant change corresponding to the dietary protein levels. Glucose and glycogen levels corresponded to the dietary carbohydrate levels. Muscle and plasma pyruvic acid increased as the crude protein in the diet increased, whereas liver pyruvic acid showed the opposite trend. Muscle protein content was not affected by dietary CP. Protein fractions in plasma (total protein, albumin and globulin) showed maximum values in 30% CP fed group. Considering the cost-effectiveness of the diet, and based on liver and plasma free amino acid levels and plasma protein fractions, 30% crude protein was recommended as the optimal dietary protein for *L. rohita* fingerlings.

A number of studies have reported that nutritional status leads to changes in the levels of enzyme activities involved in intermediary metabolism of fish (Sundby *et al.*, 1991; Baanante *et al.*, 1991; Moon and Foster, 1995; Meton *et al.*, 1999a; Panserat *et al.*, 2001). Gilthead seabream has been shown to respond rapidly to changes in dietary composition and ration size through regulating the expression of key enzymes in intermediary metabolism

(Meton *et al.*, 1999b; Caseras *et al.*, 2000). Liver plays a vital role in almost all the metabolic pathways, exhibiting sensitive control to govern the substrates and products in the glycolytic/gluconeogenic pathways. Muscle is an important tissue for metabolic studies because muscle growth represents the somatic growth in fish (Blier *et al.*, 1997) and lower aerobic capacity of muscle could limit growth.

Physiological optimization

Among the dietary nutrients, protein is the most important one affecting growth performance of fish. Dietary protein provides essential and non-essential amino acids to synthesize body protein and energy for maintenance. Protein requirement of fish ranges from 30-55% depending on the species of fish, size of fish, dietary protein sources and environmental conditions (NRC, 1993). With the change in diet composition, protein and carbohydrate contents also change, which may limit energy availability for absorption of amino acids as amino acids utilization is affected by diet composition, and metabolic utilization of absorbed glucose (Kaushik and Seiliez, 2010). The synchronization of amino acids and glucose availability following hydrolysis in the gut on given dietary nutrient levels are also critical determinants for performance (van den Borne et al., 2006). Thus, it is not just the dietary protein level in balanced feed but physiological ability to handle plasma amino acids and the liver's capacity to deaminate amino acids and produce ammonia for excretion determine optimal dietary protein level. The deviation from optimal levels can be reflected as excretion of ammonia, plasma amino acids profiles and enzyme kinetics.

Post-absorption physiological handling of amino acids in *Labeo rohita* fingerlings was assessed using attributes pertinent to intermediary metabolism through a feeding trial employing diets varying in protein content (Debnath et al., unpublished). Plasma free amino acids and NH_3 excretion, activities and kinetics of enzymes clearly indicated the physio-biochemical limitations posed on handling of amino acids beyond 30% dietary protein in a practical feed used in the above study.

Weather optimization

Requirement for maximal growth is the most commonly used parameter for determining requirement of micro- or macronutrients. This is defined as the minimum amount of nutrient required to obtain maximum growth, which may be higher than the requirement for least cost production. As feed constitutes the lion's share of operating cost in any aquaculture enterprise, feed should be used effectively and economically, which is considerably influenced by weather conditions. It is to be remembered that the requirements determined for certain nutrients under optimal culture conditions may increase when fish are exposed to unfavorable weather conditions such as higher or lower than optimal water temperature. Normally, fish grows faster in summer months consuming/ digesting/ metabolizing higher quantities of feed compared to winter months. Hence, reducing ration size in winter will help in saving cost on feeds in addition to avoiding water quality issues.

Environmental optimization

Dietary protein intake is the most important factor, which affects ammonia excretion in animals. Higher rates of ammonia excretion are directly related to protein intake in fish (Beamish and Thomas, 1984; Alexis and Paparaskeva-Papatsoglou, 1986). Most bony fish excrete predominantly ammonia. However, there are several examples of increased excretion of urea in fish subjected to stressful conditions, such as high concentrations of environmental ammonia, high pH, air exposure, or crowding (Wright and Land, 1998).

A proportional increase in urea-N excretion compared to ammonia-N could decrease specific growth rate because excretion of ammonia is energetically less expensive than converting ammonia to urea (Mommsen and Walsh, 1991; Korsgaard *et al.*, 1995). A greater energy expenditure on excretion could influence energy balance and therefore growth efficiency.

The environmental impact of fish farming depends on species, rearing method, stocking density, feed type, hydrography of the site and husbandry practices (Wu, 1995). Excessive nutrient discharges at fish farms, especially nitrogen and phosphorus, are of increasing concernwhich can lead to eutrophication of receiving water. Since the major source of nitrogen and phosphorus is fish feed, manipulating feed formulation to meet the nutrient requirements of fish is an efficient way to reduce environmental pollution (Talbot and Hole, 1994; Cho and Bureau, 1997). With increasing concern about pollution in the aquatic environment and production costs in aquaculture, researchers are focused on identifying ways of reducing water pollution and aquaculture production costs. Fishmeal accounts for 30-50% by weight in most carnivorous fish feeds. Fishmeal is used in fish feeds as the primary protein source. It contains 51.1-72.0% crude protein (CP) and 1.67-4.21% phosphorus. On the other hand, plant protein meals, such as soybean meal, contains 44.8-50.0% CP, but only 0.6-0.7% phosphorus (NRC, 1993). Therefore, formulating feed with plant protein meals to replace a portion of fishmeal will reduce dietary CP and phosphorus, thereby reducing the levels of total ammonia nitrogen (TAN) and phosphorus discharged from fish farms. However, many plant protein meals are low in lysine and thus, supplementing lysine may be necessary to maintain fish growth.

The main end-product of protein metabolism in teleosts is ammonia, which accounts for at least 80% of total nitrogen excretion (Elliott, 1976). A direct relation between protein intake and ammonia excretion has been found in fish (Rychly, 1980; Beamish and Thomas, 1984; Ballestrazzi *et al.*, 1994), and ammonia excretion has been proposed as an index for comparing the efficiency of dietary protein utilization among rainbow trout of different strains (Ming, 1985). In addition to ammonia, a significant proportion of nitrogenous waste is also excreted as urea (Wood, 1993). Consequently, measurements of ammonia and urea excretion have been used as indicators of the effects of various environmental and nutritional factors on protein metabolism and can give an insight into the nitrogen balance of fish (Rychly and Marina, 1977; Jobling, 1981; Beamish and Thomas, 1984; Perera *et al.*, 1995). Therefore, quantification of ammonia and urea-nitrogen excretion for fish in relation to nutrition is important for intensive fish culture operations because protein metabolism partly defines the success of a particular nutritional regimen (Dosdat *et al.*, 1995; Gelineau *et al.*, 1998). The rate of ammonia excretion increases rapidly in response to feed intake (Savitz, 1971; Brett and Zala, 1975; Jobling, 1981; Ballestrazzi *et al.*, 1994) and the majority of the nitrogen excreted is derived from deamination of amino acids from dietary proteins (Wood, 1993; Brunty *et al.*, 1997). Excretion peaks some hours after feed intake and is mainly dependent upon nitrogen intake, temperature and fish species (Lied and Braaten, 1984; Ramnarine *et al.*, 1987; Kaushik and Cowey, 1990). Although in early studies, urea-nitrogen excretion was not found to correlate with nitrogen intake in the same way as ammonia-nitrogen excretion (Brett and. Zala, 1975), several authors have now demonstrated a linear relationship in flatfish (Kikuchi *et al.*, 1991; Carter *et al.*, 1998; Verbeeten *et al.*, 1999) and eel (Knights, 1985). The mechanism behind this is not clear but the adaptive significance of urea synthesis in some teleosts appears to be ammonia detoxification during times when ammonia cannot be freely excreted into the environment, because of high environmental ammonia concentration (Walsh, 1998).

Species optimization

The number of species cultured has increased dramatically and search for newer species still continues for diversification of aquaculture. Nutrient requirements for most of the cultured species are not yet defined. Therefore, the current recommendations are mostly based on the data obtained with few selected fish species under intensive cultivation. For this reason, research on the basic fish nutrition is needed for any candidate species of interest to aquaculture. Optimizing feed (that is nutritional requirements) for the species of interest is most important to grow that fish economically. Recognizing the importance of fish nutrition research in aquaculture development and the differences found in the literature for not only different but the same species, it is necessary to standardize the applied methodology to determine the nutrient requirements of fish. The requirement for a particular nutrient could be defined from a physiological or a practical point of view.

The dietary protein requirement of several species of fish has been measured and data for newer species is getting generated at a faster rate. In almost all these studies, the method used has been measurement of weight gain in response to graded levels of dietary protein when all other essential nutrients in the diet are supplied at or in excess of requirement (Cowey, 1995). The values obtained in protein requirement studies may be affected by several factors including rate of growth, feeding rate, balance of energy source and the method used to analyze the data. Requirement experiments are usually been carried out using young rapidly growing fish. This is particularly important in protein or amino acid requirement studies wherein the maintenance constitutes a relatively small proportion of total requirement. On the other hand, at low rates of growth and protein deposition, maintenance would become an increasing proportion of requirement and may distort the value obtained (Cowey, 1995).

Epigenome optimization

It is believed that the impact of epigenetics and epigenetic inheritance on evolutionary theory and the philosophy of biology will be profound (Jablonka and Raz, 2009). As different aspects of heritable changes are being linked to epigenetic control mechanisms in this century, there is need to redefine the concept of evolution and heredity. Jablonka and Lamb (2007a,b) suggested that *evolution* be redefined as the "set of processes that lead to changes in the nature and frequency of heritable types in a population" and *heredity* as "the developmental reconstruction processes that link ancestors and descendants & lead to similarity between them". This is in recognition of the fact that there are processes other than natural selection and genetics in the fray which are responsible for intra- and transgenerational inheritance of the acquired phenotypes.

The importance of improved growth performance and increased disease resistance of the aquaculture organisms need no emphasis, because the industry is looked upon by millions as a source of high quality, nutritive and affordable animal protein. Enhanced feed conversion efficiency is another important consideration for aquaculture, because feed accounts for more than half of the total operational cost. It is believed that feed can prime the epigenome of cultured fish for the benefit of the present generation or the future generations. For example, utilization of plant protein sources for cultured fish could be improved by early (at spawn, fry or fingerling stage) exposure to such feeds having higher percentage of plant ingredients or feeds which are exclusively based on plant proteins. It is foreseen that production of *omega fish* on a sustainable basis may be possible if the genes responsible for desaturation/ elongation in the target fish are tailored by some dietary or non-dietary manipulations. Naturally occurring polyphenolic compounds (basically having antioxidant/ prooxidant properties) may be used for immersion treatments at a dose sufficiently high to induce optimal stress response towards single or multiple stressors. Hence, there is ample scope in each and every stage of the aquaculture cycle where one can manipulate to get a desired outcome in the present generation or future. In all or most of the cases, an epigenetic approach may be proved to be associated. However, the type of treatment (low or high dose, short or long-term, dietary or immersion) will be a determinant factor. For instance, to induce a protective immune response, the shock needs to be sufficiently detrimental to majority of the organisms undergoing the treatment, and only then the survivors may be displaying (cross) protection or improved growth response which could be explained by epigenetics.

Epidemiological studies suggest that the environment is one of the major factors causing diseases (Jirtle and Skinner, 2007; Szyf, 2007), which is evident from the fact that each geographic region around the world normally has a distinct disease frequency. The ability of the environmental factors to promote a phenotype or disease state not only in the individual in the present case but also in the subsequent progenies is termed transgenerational inheritance. It is a fact that majority of the environmental factors including nutrition or toxicants do not promote genetic mutations in the DNA sequence, however, these factors do have the capacity to alter the epigenome (Skinner *et al.*, 2010). For example, endocrine disruptors are the largest group of environmental contaminant affecting human health in recent years. These compounds can have profound consequences because of the fact that hormones play crucial role in the development. Endocrine disruptors have the ability to alter the DNA methylation patterns of key genes that produce related transcriptional changes (Li *et al.*, 2003; Edwards and Myers, 2007; Jirtle and Skinner, 2007; Guerrero-Bosagna and Valladares, 2007). The concept of transgenerational inheritance has received utmost importance in understanding diseased conditions or formulating mitigation strategies.

Studies on animal models have established that a wide variety of stressors experienced by breeding females can exert profound impacts on progeny characteristics (Huizink *et al.*, 2004). It is also widely accepted that chronic toxicological, psychological, physical or physiological stress adversely affects reproductive success in vertebrates, but there is no simple explanation about the manner in which these stressors elicit the response. For example, in mammals and birds, prenatally stressed offspring typically suffer from increased mortality, lowered birth weight, congenital abnormalities and immunological problems (Braastad, 1998; Eriksen *et al.*, 2003; Hayward and Wingfield, 2004; Huizink *et al.*, 2004; Janczak *et al.*, 2006; Merlot *et al.*, 2008). Prenatal stress has most prominent impact on behaviour of the offspring, in the form of reduced stress coping, increased fearfulness in newer situations, impaired competitive capability, cognitive deficits, modified sexual and maternal behaviour (Braastad, 1998; Huizink *et al.*, 2004). Increased exposure to maternally derived glucocorticoids during the prenatal (stress) period is suggested to be the main mediator of such behavioural changes in offspring (Rhees and Fleming, 1981; Williams *et al.*, 1995; Huizink *et al.*, 2004).

Prenatal stress is normally thought to have adverse impacts on the progeny phenotype, but it may not be the case always. From an evolutionary point of view, the transfer of information from the maternal environment to the embryo can benefit offspring by calibrating their biological systems to the environmental conditions experienced by the mother (Bakken, 1995; Braastad, 1998). This transfer of 'inside information' from the mother to her offspring could induce adaptive behavioural changes that might optimize survival in risky environments (Eriksen *et al.*, 2011).

Maternally derived substances in the fish yolk may influence or modulate offspring development (Leatherland, 1999; Schreck *et al.*, 2001; Leatherland *et al.*, 2010). In intensive aquaculture operations, broodstock may face stressful situations. Such stress experienced by the matured female fish might produce increased levels of various hormones, which might get deposited in the eggs. This is one way through which mothers may affect offspring phenotype in aquaculture. It is well known that stress exerted upon matured female fish would show a general reproductive suppression, which might be reflected by reduced levels of sex steroids and vitellogenin in plasma, decreased fecundity, reduced oocyte size and altered timing of ovulation (Carragher *et al.*, 1989; Campbell *et al.*, 1992; Schreck *et al.*, 2001). Maternal stress may be detrimental to various characters of the progeny, such as offspring survival, embryo size at hatching, incidence of morphological malformations (Campbell *et al.*, 1994; Eriksen *et al.*, 2006, 2008; Gagliano and McCormick, 2009).

Adult growth in Atlantic salmon (*Salmo salar*) was found to be affected when they were exposed to different temperatures as embryos from fertilization until the completion of eye pigmentation (Macqueen *et al.*, 2008). Briefly, the embryonic eye was completely pigmented (the 'eyed stage') after 94 days at 2°C, 53 days at 5°C, 33 days at 8°C and 25 days at 10°C, and the subsequent growth and muscle phenotypes were also significantly different.

Zebrafish has a short generation time, and its genome is fully sequenced and annotated, which makes it a unique model organism. Stewart et al. (2009) investigated the regeneration of caudal fin in zebrafish and demonstrated that histone modifications silenced the promoters of some genes involved in the regeneration process. These authors suggested that zebrafish maintains a normal, non-regenerating gene expression in the caudal fin under normal circumstances. But the gene expression program is switched-on when the caudal fin is removed through the actions of plastic epigenetic mechanisms (i.e,, demethylation of me^{3}K27 H3 histone).

Eriksen *et al.* (2011) studied the effect of simulated maternal stress (intraperitoneal cortisol implants 1 week prior to stripping) in matured female Atlantic salmon on behavioral responses in the offspring one and half years after hatching. The cortisol treated offspring were more aggressive than the control, but were easily affected by environmental challenges such as acute confinement that usually occur during intensive aquaculture. They hypothesised that environmental challenges (such as crowding) would generate more stress and fearfulness in offspring from stressed mothers, which might be detrimental to farmed fish, because competition for food and space are regular phenomena in intensive aquaculture.

Dietary polyphenols were attributed abilities to prevent cancer by reversing epigenetic marks in cancer cells (Berghe, 2012). Since food is a conditioning environment that shapes the activity of the epi(genome) and determines stress adaptive responses, metabolism, immunity and the overall physiology of the body (vel Szic *et al.*, 2010), there is immense scope for dietary manipulations including polyphenols (also via dip treatment or bath) for aquaculture. Brief exposure of an aquaculture model organism (i.e., *Artemia franciscana*) to pure polyphenols was shown to modulate disease resistance and growth potential of the animals (Debnath *et al.*, unpublished data). Relative expression of immune genes such as heat shock proteins 60/70/90 and prophenol oxidase was also modulated by such exposures (Debnath *et al.*, unpublished data). There is a huge possibility that such responses will not only be observed in the parent generation, but will also be carried forward to the next generation. Recently, Li and Leatherland (2012) have underscored the implications of epigenetic programming of endocrine system of fish embryos for aquaculture practice. For example, environmental factors acting at the maternal epigenome could elicit epigenetic programming of the embryo genome which might determine the phenotype of the F1 generation. Since both the somatic and germ cell lines of the F1 generation could be affected by the epigenetic programming, these characteristics could be inherited by the future generations (Li and Leatherland, 2012).

Consumptive-value optimization

Fish production through aquaculture has increased markedly over the last two decades. Bulk of the inland fish production comprises the three Indian Major Carps – catla, rohu and mrigal and the three exotic carps – silver, grass and common carp. Efforts are now on to diversify the aquaculture species for culture taking into consideration the regional preference and market values of fish. There is no doubt that fish is the best animal protein for human consumption because of inherent nutritional characteristics of fish, such as highly digestible protein (2nd highest digestibility only after eggs) with highly balanced amino acids (all essential amino acids are present in fish that we need for our tissue building and maintenance), fish lipids being mostly unsaturated in nature providing us essential fatty acids, vitamins and minerals as well. Further, it is possible to make the cultured fish contain certain nutrients at higher concentrations so that it becomes healthier for human beings, mainly through dietary manipulations. For example, nutritional intervention such as changing the source of lipid in the diet could influence tissue fatty acid profile in fish. Incorporation of vegetable oils (coconut, olive, sunflower, linseed) in fish diets was shown to demonstrate a different fatty acid profile of flesh compared to fish oil fed fish. Hence, it is possible to obtain a desirable tissue fatty acid composition by changing the dietary fatty acid composition in a short period of time. Due to the importance of n-3 fatty acids for human health, fatty acid profile of fish fed plant oils (that are poor in HUFA) is considered of inferior quality. After using regular feeds containing vegetable oils for most part of the culture period, switching over to a fish oil diet in the last one or two months of culture is a way of increasing human cardio-protective n-3 fatty acids in fish flesh.

Innovative Feeding Methods: Need-Of-The-Hour

Dispensing or distributing fish feeds to the target fish is one of the most important factors that affects proper utilization of fish feeds and hence feed efficiencies. The best of fish feeds may not lead to best growth and feed efficiencies if the feeding method is faulty. For example, a sinking pellet, completely balanced in nutrients for the target fish, if dispensed by broadcasting method may lead to non-availability of a portion of the feed to fish. This may happen due to many reasons – presence of mud in the bottom of the fish ponds, uneven bottom, target fish not being a good bottom feeder, improper timing and frequency of feeding, etc.

The feeding regime adopted should permit maximal growth rates. Schemes involving restricted feeding generally prevent this objective being achieved. Such schemes negate one of the two components involved in the growth response to an essential nutrient, i.e. the increase in feed intake (Cowey, 1992). As a consequence, under conditions of restricted feeding, differences in the growth rate between treatments would result only from differences in metabolic efficiency. The most acceptable approach to maximal feed intake is therefore to feed the fish to satiation several times a day. In one experiment (Ogino, 1980), it was found that the dietary protein levels necessary for maximal protein retention increased from 35% to 50% (of the dry matter) when feeding rate was lowered from 3.5% to 2.5% of the body weight.

Concluding Remarks

Of late, research and development in the area of fish nutrition is underway in greater intensity than anticipated, and hence new data on requirements are getting generated for a variety of fish and shellfish species. Days are not far away when precision aquaculture will become the norm in farming aquatic organisms. Aquafeeds will be individualized for different farmed species as well as for each life-history stage of each species, which will be called 'special feeds', as foreseen by Pigott and Tucker (2002). The optimization concept in aquaculture presented here is thought of from various angles. The cultured aquatic organism should be able to optimally utilize the feed. Feeds should be environment-friendly, low polluting. The consumer should get a fish which is safe and healthy. Finally, the farmer has to get a market price for his produce which is remunerative. When these four major issues are addressed, mainly through specialized (next generation) feeds and innovative feeding methods, only then aquaculture is going to be sustainable.

References

Alexis, M. N. and Paparaskeva-Papatsoglou, E., 1986. Aminotransferase activity in the liver and white muscle of *Mugil capita* fed diets containing different levels of protein and carbohydrate. Comp. Biochem. Physiol., 83B: 245-249.

Baanante, I.V., Garcia de Frutos, P., Bonamusa, L., Fernandez, F., 1991. Regulation of fish glycolysis–gluconeogenesis: role of fructose 2,6 P2 and PFK-2. Comp. Biochem. Physiol. 100B, 11-17.

Bakken, M., 1995. Sex-ratio variation and maternal investment in relation to social environment among farmed silver-fox vixens (*V. vulpes*) of high competition capacity. J Anim Breed Genet 112, 463-468.

Ballestrazzi, R., Lanari, D., D'Agaro, E. and Mion, A., 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*). Aquaculture, 127: 197-206.

Beamish, F. W. H. and Thomas, E., 1984. Effects of dietary protein and lipid on nitrogen losses in rainbow trout, *Salmo gairdneri*. Aquaculture, 41: 359-371.

Berghe, W. V., 2012. Epigenetic impact of dietary polyphenols in cancer chemoprevention: lifelong remodelling of our epigenomes. Pharmol. Res. 65: 565-576.

Blier, P., Pelletier, D., Dutil, J.-D., 1997. Does aerobic capacity set a limit on fish growth rate? Rev. Fish. Sci. 5, 323-340.

Blier, P.U., Lemieux, H., Devlin, R.H., 2002. Is the growth rate of fish set by digestive enzymes or metabolic capacity of the tissues? Insight from transgenic coho salmon. Aquaculture 209, 379-384.

Braastad, B.O., 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. App Anim Behav Sci 61, 159-180.

Brett, J. R. and Zala, C. A., 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye (*Oncorhynchus nerka*) under controlled conditions. J. Fish. Res. Board Can., 32: 2479-2486.

Brunty, J. L., Bucklin, R. A., Davis, J., Baird, C. D. and Nordstedt, R. A., 1997. The influence of feed protein intake on tilapia ammonia production. Aquacult. Eng., 16: 161-166.

Campbell, P.M., Pottinger, T.G., Sumpter, J.P., 1992. Stress reduces the quality of gametes produced by rainbow trout. Biol Repro 47, 1140-1150.

Campbell, P.M., Pottinger, T.G., Sumpter, J.P., 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120, 151-169.

Carragher, J.F., Sumpter, J.P., Pottinger, T.G., Pickering, A.D., 1989. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. Gen Comp Endocrinol 76, 310-321.

Carter, C. G., Houlihan, D. F. and Owen, S. F., 1998. Protein synthesis, nitrogen excretion and long-term growth of juvenite *Pleuronectes flessus*. J. Fish Biol., 52: 272-284.

Caseras, A., Meton, I., Fernandez, F., Baanante, I.V., 2000. Glucokinase gene expression is nutritionally regulated in the liver of gilthead seabream (*Sparus aurata*). Biochim. Biophys. Acta 1493, 135-141.

Cho, C. Y. and Bureau, D. P., 1997. Reduction of waste output from salmonid aquaculture through feeds and feeding. Prog. Fish-Cult., 59: 155-160.

Cho, C.Y., 1987. La energía en la nutrición de los peces. In: Espinosa de los Monteros, J., Labarta, U. (Eds.), Nutrición en Acuicultura, vol. II. CAICYT, Madrid, pp. 197-244.

Cowey, C. B., 1992. Nutrition: estimating requirements of rainbow trout. Aquaculture, 100: 177-189.

Cowey, C. B., 1995. Protein and amino acid requirements: A critique of methods. J. Appl. Ichthyol., 11: 199-204.

Debnath, D., Pal, A. K., Sahu, N. P., Yengkokpam, S., Baruah, K., Choudhury, D. and Venkateshwarlu, G., 2007. Digestive enzymes and metabolic profile of Labeo rohita fingerlings fed diets with different crude protein levels. Comp. Biochem. Physiol. B, 146:107-114.

Dosdat, A., Metailler, R., Tetu, N., Servais, F., Chartois, H., Huelvan, C. and Desbruyeres, E., 1995. Nitrogenous excretion in juvenile turbot, *Scophthalmus maximus* (L.), under controlled conditions. Aquacult. Res., 26: 639-650.

Edwards, T.M., Myers, J.P., 2007. Environmental exposures and gene regulation in disease etiology. Environ. Health Perspect. 115, 1264-1270.

Elliott, J. M., 1976. Energy losses in the waste products of brown trout (Salmo trutta L.). J. Anim. Ecol., 45: 561-580.

Eriksen, M.S., Bakken, M., Espmark, A.M., Braastad, B.O., Salte, R., 2006. Pre-spawning stress in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. J Fish Biol 69, 114-129.

Eriksen, M.S., Espmark, A.M., Poppe, T., Braastad, B.O., Salte, R., Bakken, M., 2008. Fluctuating asymmetry in farmed Atlantic salmon (*Salmo salar*) juveniles: also a maternal matter? Env Biol Fish 81, 87-99.

Eriksen, M.S., Faerevik, G., Kittilsen, S., McCormick, M.I., Damsgard, B., Braithwaite, V.A., Braastad, B.O., Bakken, M., 2011. Stressed mothers – troubled offspring: a study of behavioural maternal effects in farmed *Salmo salar*. J Fish Biol 79, 575-586.

Gagliano, M., McCormick, M.I., 2009. Hormonally mediated effects shape offspring survival potential in stressful environments. Oecologia 160, 657-665.

Gelineau, A., Medale, F. and Boujard, T., 1998. Effect of feeding time on postprandial nitrogen excretion and energy expenditure in rainbow trout. J. Fish Biol., 52: 655-664.

Guerrero-Bosagna, C., Valladares, L., 2007. Endocrine disruptors, epigenetically induced changes, and transgenerational transmission of characters and epigenetic states. In: Endocrine disrupting chemicals, from basic research to clinical practice (Ed. Gore, A.C.), Humana Press Inc., Totowa, NJ, pp. 175-189.

Halver, J.E. and Hardy, R.W., 2002. Fish nutrition. 3rd Edition, Academic Press, California, USA.

Hofer, R. and Kock, G., 1989. Method for quantitative determination of digestive enzymes in fish larvae. Pol. Arch. Hydrobiol., 36:439-441.

Huizink, A.C., Muilder, E.J.H., Buitelaar, J.K., 2004. Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? Psychol Bull 130, 115-142.

Jablonka, E., Lamb, M.J., 2007a. Precis of evolution in four dimensions. Behav Brain Sci 30, 353-365.

Jablonka, E., Lamb, M.J., 2007b. Bridging the gap: the developmental aspects of evolution. Behav Brain Sci 30, 378-392.

Jablonka, E., Raz, G., 2009. Transgenerational epigenetic inheritance: prevalence mechanisms, and implications for the study of heredity and evolution. Quar Rev Biol 84, 131-176.

Janczak, A.J., Braastad, B.O., Bakken, M., 2006. Behavioural effects of embryonic exposure to corticosterone in chickens. App Anim Behav Sci 96, 69-82.

Jirtle, R.L., Skinner, M.K., 2007. Environmental epigenomics and disease susceptibility. Nat Rev Genet 8, 253-262.

Jobling, M., 1981. Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice (*Pleuronectes platessa* L.). J. Fish Biol., 18: 87-96.

Kaushik, S. J. and Cowey, C. B., 1990. Dietary factors affecting nitrogen excretion by fish. In: Nutritional strategies and aquaculture waste (eds., Cowey, C. B. and Cho, C. Y.), Proceedings of the first International symposium on nutritional strategies in management of aquaculture waste, Ontario, 5-8 June, University of Guelph, Canada, pp. 3-19.

Kaushik, S.J., Seiliez, I., 2010. Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. Aquac Res. 41, 322-332.

Kikuchi, K., Takeda, S., Honda, H., Kiyono, M., 1991. Effects of feeding on nitrogen excretion of Japanese flounder, *Paralichthys olivaceus*. Bull. Jpn. Soc. Sci. Fish., 57: 2059-2064.

Knights, B., 1985. Energetics and fish farming. In: Fish Energetics: New Perspectives (eds. Tytler, P. and Calow, P.), John Hopkins Univ. Press, Baltimore, pp. 309-341.

Korsgaard, B., Mommsen, T. P. and Wright, P. A., 1995. Adaptive relationships to environment, ontogenesis and viviparity. In: Nitrogen Metabolism and Excretion (eds., Walsh, P. J. and Wright, P. A.), CRC Press, Boca Raton, pp. 259-288.

Leatherland, J.F., 1999. Stress, cortisol and reproductive dysfunction in salmonids: fact or fallacy? Bull Euro Asso Fish Pathol 19, 254-257.

Leatherland, J.F., Li, M., Barkataki, S., 2010. Stressor, glucorticoids and ovarian function in teleosts. J Fish Biol 76, 86-111.

Lemieux, H., Blier, P., Dutil, J.-D., 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*)? Fish Physiol. Biochem. 20, 293-303.

Li, M., Leatherland, J.F., 2012. The implications for aquaculture practice of epigenomic programming of components of the endocrine system of teleostean embryos: lessons learned from mammalian studies. Fish and Fisheries 12 (doi: 10.1111/j.1467-2979.2012.00486.x).

Li, S., Hursting, S.D., Davis, B.J., McLachlan, J.A., Barrett, J.C., 2003. Environmental exposure, DNA methylation, and gene regulation, lessons from diethylstilbesterol-induced cancers. Ann New York Acad Sci 983, 161-169.

Lied, E. and Braaten, B., 1984. The effects of feeding and starving and different ratios of protein energy to total energy in the feed on the excretion of ammonia in Atlantic cod (*Gadus morhua*). Comp. Biochem. Physiol., 78A: 49-52.

Lundstedt, L.M., Bibiano Melo, J.F., Moraes, G., 2004. Digestive enzymes and metabolic profile of *Pseudoplatystoma corruscans* (Teleostei: Siluriformes) in response to diet composition. Comp. Biochem. Physiol. 137 B, 331-339.

Macqueen, D.J., Robb, D.H.F., Olsen, T., Melstveit, L., Paxton, C.G.M., Johnston, I.A., 2008. Temperature until the 'eyed stage' of embryogenesis programmes the growth trajectory and muscle phenotype of adult Atlantic salmon. Biol Letters 4, 294-298.

Merlot, E., Couret, D., Otten, W., 2008. Prenatal stress, fetal imprinting and immunity. Brain Behav Immunity 22, 42-51.

Meton, I., Caseras, A., Mediavilla, D., Fernandez, F., Baanante, I.V., 1999b. Molecular cloning of a cDNA encoding 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase from liver of *Sparus aurata*: nutritional regulation of enzyme expression. Biochim. Biophys. Acta 1444, 153-165.

Meton, I., Mediavilla, D., Caseras, A., Canto, E., Fernandez, F., Baanante, I.V., 1999a. Effect of diet composition and ration size on key enzyme activities of glycolysis-gluconeogenesis, pentose phosphate pathway and amino acid metabolism in liver of gilthead sea bream (*Sparus aurata*). Br. J. Nutr. 82, 223-232.

Mommsen, T. P. and Walsh, P. J., 1991. Urea synthesis in fishes: evolutionary and biochemical prespectives. In: Biochemistry and Molecular Biology of Fishes (eds., Hochachka, P. W. and Mommsen, T.P.), Vol. 1. New York, Elsevier, pp. 137-163.

Moon, T.W., Foster, G.D., 1995. Tissue carbohydrate metabolism, gluconeogenesis and hormonal and environmental influences. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Metabolic Biochemistry, Elsevier, Amsterdam. pp. 65-100.

Moraes, G., Bidinotto, P.M., 2000. Induced changes in the amylohydrolytic profile of the gut of *Piaractus mesopotamicus* (Holmberg, 1885) fed different levels of soluble carbohydrate: its correlation with metabolic aspects. Revista de Ictiologia 8, 47-51.

NRC (National Research Council), 1993. Nutrient requirements of fish. National Academy Press, Washington, DC. pp. 114.

Ogino, C., 1980. Requirements of carp and rainbow trout for essential amino acid. Bull. Japan Sci. Soc. Fish, 46: 171-174

Palou, A., Remesar, X., Arola, M. and Alemany, M., 1980. Changes in alanine amino transferase activity in several organs in the rat induced by a 24 hour fast. Horm. Metab. Res., 12:505-508.

Panserat, S., Plagnes-Juan, E., Kaushik, S., 2001. Nutritional regulation and tissue specificity of gene expression for proteins involved in hepatic glucose metabolism in rainbow trout (*Oncorhynchus mykiss*). J. Expt. Biol. 204, 2351-2360.

Pelletier, D., Dutil, J.-D., Blier, P., Guderley, H., 1994. Relation between growth rate and metabolic organisation of white muscle, liver and digestive tract in cod, *Gadus morhua*. J. Comp. Physiol. 164 B, 179-190.

Perera, W. M. K., Carter, C. G. and Houlihan, D. F., 1995. Feed consumption, growth and growth efficiency of rainbow trout, *Oncorhynchus mykiss* (Walbaum) fed diets containing bacterial single cell protein. Br. J. Nutr., 73: 591-603.

Pigott, G.M. and Tucker, B.W., 2002. Special feeds. In: Halver, J.E. and Hardy, R.W. (eds.), Fish nutrition, 3rd Edition, Academic Press, California, USA. pp. 652-668.

Ramnarine, I. W., Pirie, J. M., Johnstone, A, D. F. and Smith, G. W., 1987. The influence of ration size and feeding frequency on ammonia excretion by juvenile Atlantic cod, *Gadus morhua* L. J. Fish Biol., 31: 545-559.

Rhees, R.W., Fleming, D.E., 1981. Effects of malnutrition, maternal stress or ACTH injections during pregnancy on sexual behavior of male offspring. Physiol Behav 27, 978-882.

Rychly, J. and Marina, A. B., 1977. The ammonia excretion of trout during a 24-hour period. Aquaculture, 11: 173-178.

Rychly, J., 1980. Nitrogen balance in trout: II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. Aquaculture, 20: 343-350.

Savitz, J., 1971. Nitrogen excretion and protein consumption of the bluegill sunfish (*Lepomis macrochirus*). J. Fish. Res. Board Can., 28: 449-451.

Schreck, C.B., Contreras-Sanchez, W., Fitzpatrick, M.S., 2001. Effects of stress on fish reproduction, gamete quality and progeny. Aquaculture 197, 3-24.

Skinner, M.K., Manikkam, M., Guerrero-Bosagna, C., 2010. Epigenetic transgenerational actions of environmental factors in disease etiology. Trends Endocrinol Metab 21, 214-222.

Smith, L.S., 1980. Digestion in teleost fishes. In: Lectures presented at the FAO/UNDP Training Course in fish feeding technology, ACDP/REP/80/11. pp. 3-17.

Spannhof, L. and Plantikow, H., 1983. Studies on carbohydrate digestion in rainbow trout. Aquaculture, 30: 95-108.

Stewart, S., Tsun, Z.-Y., Belmonte, J.C.I., 2009. A histone demethylase is necessary for regeneration in zebrafish. Proc Nat Acad Sci U S Ame 106, 19889-19894.

Suarez, M.D., Hidalgo, M.C., Garcia Galego, M., Sanz, A., De la Higuera, M., 1995. Influence of the relative proportions of the energy yielding nutrients on the liver intermediary metabolism of the European eel. Comp. Biochem. Physiol. A 111, 421-428.

Sundby, A., Hemre, G.I., Borrebaek, B., Christophersen, B., Blom, A.K., 1991. Insulin and glucagon family peptides in relation to activities of hepatic hexokinase and other enzymes in fed and starved Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). Comp. Biochem. Physiol. 100B, 467-470.

Szyf, M., 2007. The dynamic epigenome and its implications in toxicology. Toxicol Sci 100, 7-23.

Talbot, C. and Hole, R., 1994. Fish diets and the control of eutrophication resulting from aquaculture. J. Appl. Ichthyol., 10: 258-270.

Torrisen, K.R., Shearer, K.D., 1992. Protein digestion, growth and food conversion in Atlantic salmon and Arctic charr with different trypsine-like isozyme patterns. J. Fish Biol. 41, 409-415.

Twining, S.S., Alexander, P.A., Huibregste, K. and Glick, D.M., 1983. A pepsinogen from rainbow trout. Comp. Biochem. Physiol., 75B: 109-112.

van den Borne JJ, Verstegen MW, Alferink SJ, van Ass FH, Gerrits WJ., 2006. Synchronizing the availability of amino acids and glucose decreases fat retention in heavy preruminant calves. J Nutr. 136(8):2181-7.

Vel Szic, K. S., Ndlovu, M. N., Haegeman, G., Berghe, W. V., 2010. Nature or nurture: let food be your epigenetic medicine in chronic inflammatory disorders. Biochem. Pharmacol., 80: 1816-1832.

Verbeeten, B. E., Carter, C. G. and Purser, G. J., 1999. The combine effect of feeding time and ration on growth performance and nitrogen metabolism of greenback flounder. J. Fish Biol., 55: 1328-1344.

Vieira, V.P., Inoue, L.A.K., Moraes, G., 2005. Metabolic responses of matrinxa (*Brycon cephalus*) to dietary protein level. Comp. Biochem. Physiol. A 140, 337-342.

Walsh, P. J., 1998. Nitrogen excretion and metabolism. In: The physiology of fishes (ed. Evans, D. H.), CRC Press, Boca Raton, pp. 199-214.

Williams, M.T., Hennessy, M.B., Davis, H.N., 1995. CRF administered to pregnant rats alters offspring behaviour and morphology. Pharmacol Biochem Behav 52, 161-167.

Wood, C. M., 1993. Ammonia and urea metabolism and excretion. In: The Physiology of fishes (ed. Evans, D. H.), CRC press, Boca Rouge, pp. 379-425.

Wright, P. A. and Land, M. D., 1998. Urea production and transport in teleost fishes. Comp. Biochem.and Physiol., 119 A: 47-54.

Wu, R. S. S., 1995. The environmental impact of marine fish culture: towards a sustainable future. Mar. Pollut. Bull., 31: 59-166.