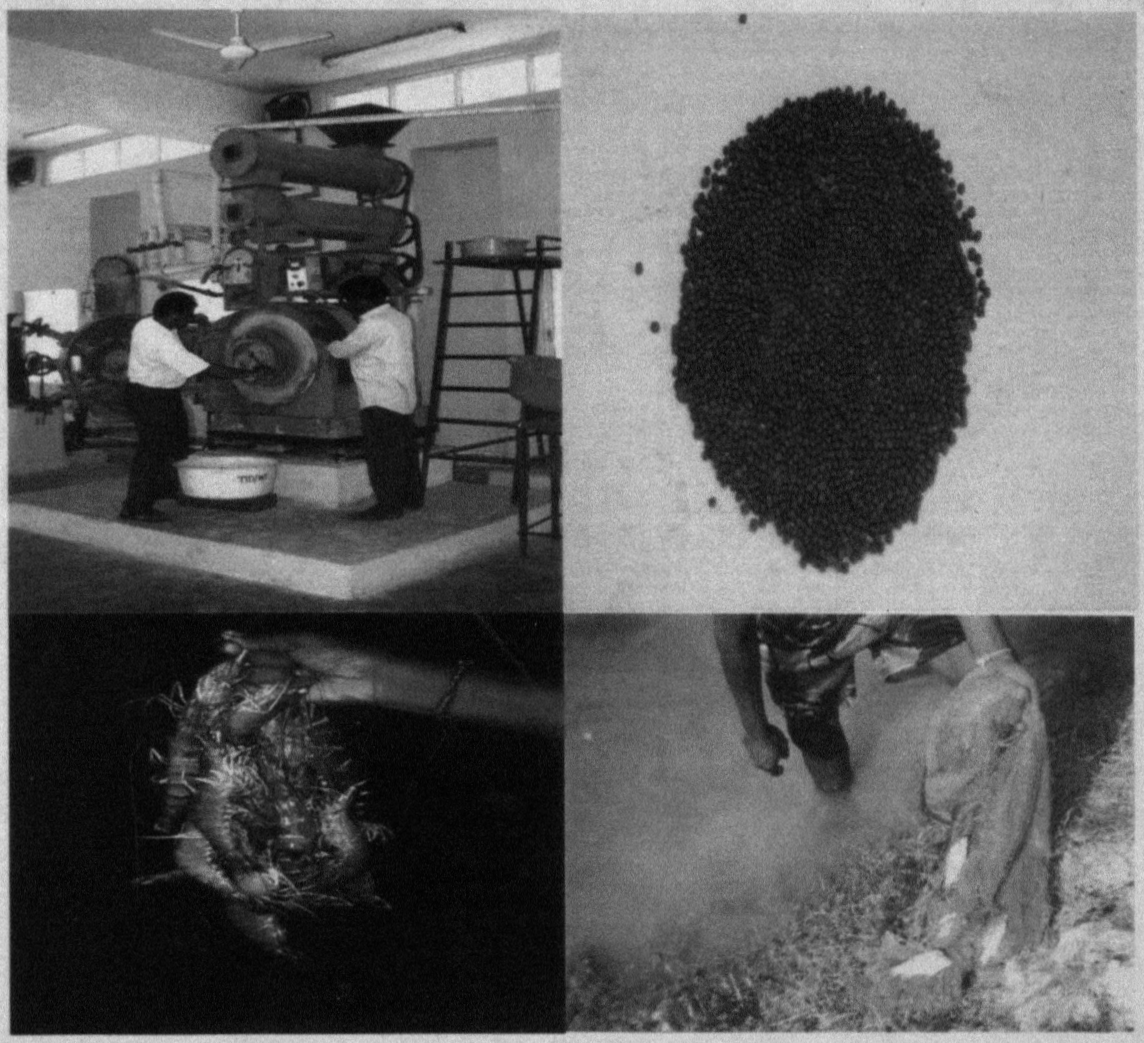
TRAINING MANUAL ON

SHRIMP AND FISH NUTRITION AND FEED MANAGEMENT

14-23 November 2006



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CENTRAL INSTITUE OF BRACKISHWATER AQUACULTURE
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Training programme on
"Shrimp and Fish Nutrition and Feed Management"
14-23rd November 2006

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CHAPTER -1

PHYSIOLOGY OF DIGESTION IN FISH AND SHRIMP

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INTRODUCTION

An understanding of the Physiology of digestion of any fish species is essential for development of suitable feeds for its Culture. The steps involved in obtaining nutrients from food are ingestion, digestion and absorption in digestive tract. The Physiology of digestion differs in fishes and crustaceans and the steps involved in the process are briefly dealt below.

PHYSIOLOGY OF DIGESTION IN FISHES

Food and feeding habits.

Many workers had investigated food and feeding habits of many species of fishes. Based on the presence of food items in the gut, fishes are broadly categorised into the following groups:

- herbivores - feeding on plant materials
- carnivores - feeding on fish and other invertibrates
- omnivores - feeding on mixed diets
- detritivores - feeding on detritus

While the above categorisation is broad based, majority of fishes feed on mixed diets. Fishes are also categorised on ecological basis viz., pelegic, plankton feeders, benthos etc.

Morphology of alimentary canal

The digestive tract in fishes may be a straight tube or divided in to many parts such as the mouth, buccal cavity, pharynx, oesophagus, stomach, intestine, rectum and pyloric caecae. The length of the gut varies in relation to body length of fish and the relationship is known as relative gut length (RGL). In herbivores the mean RGL is more than that of the

carnivores.The mouth, buccal cavity and pharynx are associated with the process of ingestion of food. Lips are used in Picking up the food and its shape indicates the feeding habits of the fish. Carps do not have teeth in their jaws, while carnivores have teeth. Buccal teeth help in capturing and holding the food. In *Mugil cephalus* gill rakers are useful in straining the food from the bottom mud. Oesophagus or gullet is essentially a lubricating tube for the smooth passage of food into the stomach or intestine. In *M. cephalus* the Oesophagus has well developed mucous cells.Not all fishes have stomach. The stomach when present is divided in to two regions namely an anterior and pyloric. The gastric glands are more common in the anterior region. *M. cephalus* has a gizzard like stomach, which is attributed to its bottom feeding habit and is thought to be a compensation for its poor dentition. The intestine consists of simple columnar epithelium lined with intestinal brush border or microvilli which increases the surface area for absorption of nutrients. The intestinal surface area in relation to the body size decreases with growth of fish. The distal portion of intestine with thicker muscular coat and goblet cells is known as rectum. Pyloric caecae are auxillary intestinal appendages in fishes. They are supposed to be useful in digestion of food as a site for absorption of carbohydrates and fats. The gross anatomy of four cultured fishes having diverse feeding habits are given in Fig.1.

Digestion

Digestion is the process by which food material is broken down in to simple molecules for absorption through the gut wall after which they enter in to the blood stream. This means that proteins are hydrolyzed to aminoacids or polypeptide chains of a few aminoacids, carbohydrates to simple sugars and lipids to fatty acids and glycerol. Those materials that are not digested and absorbed are voided as faeces. Digestion is a continuous process beginning in stomach and ending only when the food leaves the rectum as faeces. The entire process of digestion is brought about by the action of variety of digestive fluids and enzymes.

Digestive fluids and enzymes

The digestive fluids and enzymes are located in the stomach, pancreas, liver and intestine. HCl is generally produced in the stomach in most fishes

and is useful in reducing the pH of gut which is useful in activating pepsinogens. The enzymes produced by gastric glands are: pepsin (acts on protein) amylase (acts on carbohydrates) lipase and esterase (acts on lipids and esters of lipid) and chitinase (acts on chitin). Pancreatic enzymes are stored as zymogens. The enzymes produced by pancreas are proteases (trypsin, chymotrpsin, carboxypetidases and elastase), amylase (carbohydrate digesting enzyme) chitin (acts on chitin) and lipases (hydrolyses triglycerides, fats, phospholipids)

The liver secretes bile and bile salts which make the intestinal medium alkaline and is useful in emulsification of lipids. The intestinal enzymes secreted by the brush horder of the epethelium are aminopeptidases (splits nucleosides), polynuclueotidase (splits nucleic acids) and lecithinase (splits phospholipids in to glycerol and fatty acids). Digestive enzymes are water soluble proteins and are classified generally as proteases (acts on proteins) lipases and esterases (acts on lipids), carbohydrases (acts on carbohydrates). Digestion of food is extra cellular taking place in the lumen of the intestine.

Absorption of food

Digested food products are absrovbed either by diffusion or active transport mechanism. Almost all inorganic and organic nutrients are absorbed in the small intestine. The lining of intestine is highly absorptive which is further increased by formation of folds called rugae or villi. The folds are covered with epithelium within which are present capillaris and lymph vessels.The mechanism of absorption of food particles by active transport requires energy. Uptake of glucose is through active transpoft. Lipids are broken down into fatty acids and are absorbed in to the intestinal epithelium by simple diffusion which does not require energy. The lipids form droplets called chylomicrons and enter into the blood stream. Proteins are broken down to simple amino acids absorbed in to the intestinal capillaries and are carried in to the blood stream through liver. The amino acids are taken up by cells to build new tissues.

Digestive enzymes of Milkfish

Considerable progress had been made in understanding the digestive enzymes of milk fish (***Chanos chanos)***. Enzymes involved in digestion of

carbohydrate, lipid and protein had been detected in pyloric caecae, intestine and pancreas. Intestinal amylase activity reached the maximum during noontime which indicated that milk fish is a day time feeder. No cellulase activity was detected, though it is an algal feeder. *Chaetomorpha spp.*, which is a main constituent of lumut contains a trypsin inhibitor. Pancreatic lipases have two pH maxima indicating acidic and alkaline lipases. Enzyme activity was optimal in warmer temperatures than in colder temperatures. This suggests that a prolonged cold spell could have adverse effect on growth of milk fish.

Digestive enzymes of grey Mullets

Studies carried out at CIBA on grey mullets, ***Mugil cephalus*** indicated that α-amylase activity was at a higher level in the intestine than in the pancreas. In the case of L-glucosidase the activity level was more in Pancreas than in intestine. Highest activity level of trypsin was detected in Pancreas than in stomach and intestine. Carboxymethyl cellulase activity was detected in stomach and Pancreas. Chymotrypsin could not be detected. The gut microflora showed wide fluctuations indicating their role in digestion of protein, stomach and to a certain extent cellulos

PHYSIOLOGY OF DIGESTION IN SHRIMP

Our knowledge on the Physiology of digestion in crustacea is largely due to the studies on Decapoda. The general process involved in digestion are outlined below.

Mouth and Mouth parts

The mouth or anterior opening of the gut, lies between the large mandibles. The anterior wall of the mouth cavity is formed by the labrum, a muscular mobile structure suspended between the expistoma, the anterior medial edge of the mandibles, and the oesophagus. To the rear of the mouth lies the paragnatha. The mouth is connected to the gut by a short oesophagus.The mandibles posses both cutting and crushing processes and act to reduce food particles to a size suitable for ingestion. The function in co-ordination with accessory feeding appendages, especially the 2nd and 3rd maxillipeds, which hold and pull the food.The labrum may be analogous to the

vertebrate tongue and aids the passage of food particles into the oesophagus. Chemosensory and mechano sensory cells are located in the labrum.

Organs of alimentary canal

The gut in crustaceans is mostly a straight tube. Extensive winding is rare. The gut is divided in to three parts: The fore gut, the mid gut and the hind gut. The fore gut and the hind gut are of ectodermal origin while the mid gut is of endodermal origin.In the foregut storage,trituration and early digestion of food take place.In almost all crustaceans the midgut bears glandular appendages designated as hepatopancreas.Secretion of enzymes and absorption of digested food mostly occur in the midgut.Faecal formation takes place in the hind gut. If the food particles are larger,mixing of enzymes is achieved by triturition and gastric mill.Since both secretion and absorption of digested food take place in the midgut,the epithelial layer is differentiated into secretive and absortive cells.

Foregut and its functions

Storage, trituration and early digestion take place in the foregut. This region consists of a short oesophagus opening into the stomach (cardiac sac) through the oesophageal-cardiac sac valve. The oesophagus exhibits rhythemic peristaltic movement which start after the commencement of mandibular movement (chewing). The various glands associated with the oesophagus appear to be lubricating rather than digestive in function. The stomach can be divided into three sections - the proventriculus (cardiac sac), the gastric mill and the pyloric filter (Fig.2a). The gastric mill divides the proventriculus into an anterior distendable part which serves as a crop for storage and a posterior part. The gastric mill has large calcified cuticular ossicles (one median dorsal ossicle and two lateral ossicles), which function as teeth. The posterior part of the proventriculus is in turn divided into dorsal and ventral chambers. The dorsal chamber (which bears lateral grooves) leads into the midgut. The ventral chamber contains the filter-press (compressed W-shaped in cross section) which leads into the digestive gland. The floor of the anterior proventriculus bears a median groove and two ventro-lateral grooves, with fringing dense setae. The ventrolateral grooves leads to the filter-press.All the regions of the foregut exhibit neurogenic rhythmic movements.

Powell's model of foregut function

As food enters the anterior chamber of the proventriculus it is penetrated by fluid from the digestive gland that flows forward dorsolaterally in grooves (PCG) in the posterior chamber (PC). Trituration and further mixing with fluid occurs at the gastric mill ossides (O). The food mass is continually being manipulated by the lateral plates of the anterior chamber and forced into the gastric mill. Eventually fluid passes anterior chamber. Dense setae exclude larger particles and the fluid passes backwards through the filter-press (FP) which excludes particles above 1 um and finally into the openings of the digestive gland (DG). Fluid from the digestive gland is pumped dorsally into the dorsolateral grooves, joined by fluid squeezed from the food mass in the posteriof chamber. Some fluid is also pumped in and out of the anterior diverticulum of the midgut. The combined fluid then pass forwards to the anterior chamber. The circulation is driven by the pumping action of the filter press and associated structures, probably aided by other pumps. The lateral grooves (LG) of the anterior chamber may also have a role in fluid circulation.In the digestive gland, dissolved nutrients are absorbed and the fluid, perhaps with the addition of more enzymes, is then returned to the general proventricular circulation. The midgut diverticulum, which is lined with secretory epithelium contributes essential components to the digestive fluid, such as activators of proteolytic enzymes or pH change.

Midgut and its functions

From the digestive gland; the midgut extends well back into the abdomen. The wall of the midgut may be defined histologically as a simple glandular epithelium lined with "light" and "dark" cells. Both cells have microvillous borders suggesting absorptive functions and granules which are secreted (Fig 3 & 4). Except for a very thin layer of connective tissue and muscle fiber, this single layer of cells is in direct contact internally with the blood, enabling absorbed nutrients to be rapidly translocated. The apical `E' cells in the midgut epithelium gives rise to two basic types of cells (i) the `R' cells, which absorb nutrients and also store and metabolise lipids and glycogen; (2) the `F' cells, which synthesise digestive enzymes which accumulate in vacuoles that enlarge and coalesce to transform finally into "B" cells. The vascular contents of "B" cells are liberated into the tubule lumen for digestion. Water and electrolyte regulation and secretion of a peritrophic

membrane around the faecal pellet are important functions of the midgut. Shrimps have a well defined anterior (lined with very tall columnar epithelium) and a posterior diverticulum, providing additional absorptive surfaces, digestive and osmoregulatory functions.

Hindgut

The function of hindgut, is defaecation. In penaeid shrimp, six smooth-surfaced longitudinal pads containing a spongy tissue, fill the lumen of the hindgut. These pads grasp the faecal pellet in its peritorphic membrane and rhythmically expel it.

Digestive enzymes of crustacea

In most of the crustaceans the presence of proteases, lipases and carbohydrases have been detected.Trypsin has been isolated and characterised from *Penaeus sp* Trypsin is secreted by the digestive gland. They are similar to mammalian trypsin in the pH optima (pH 7-9), molecular weight (about 25000) and sensitivity to soybean trypsin inhibitor. Carboxypeptidases A and B have been isolated and characterized from the digestive gland of *Penaeus setiferus*. Other peptidases such as arylamidase and dipeptidases have been reported for *Macrobrachium lamerrei*. No zymogens of proteolytic enzymes have been found.

Lipase and esterase activities have been demonstrted in many crustacea and a lipase has been purified from the lobster, *Homarus americanus*. α -amylase and α -1-4 glucosidase and cellulosase activity have been demonstrated in crustacea. Chitinase and Chitobiase has been established in crustacea. The foregut fluid is most crustaceans is reported to be slightly acidic (pH 5-7). *Penaeus setiferus* have microorganisms specially adapted for living in the gut. A possible function of microbial activity in the digestive tract is the supply of vitamins and essential amino acids.

Digestive enzymes of Tiger shrimp

Studies carried out at CIBA on tiger shrimp, ***Penaeus monodon*** indicated the activity levels of major digestive enzymes namely proteases (trypsin, chymotrypsin) lipase, carbohydrases (amylase and glucosidase) involved in the digestion of protein, lipid and carbohydrate in the hepatopancreas, stomach and intestine of all size groups. While carboxymethyl cellulase was observed in the digestive tract, pepsin was not

detected. The highest activity levels of trypsin, amylase, glucosidase and lipase were recorded in the hepatopancreas. These enzymes showed the highest activity around neutral pH. The activity levels of digestive enzymes were more in young shrimp than in larger sized ones. Hence different feed formulations are to be developed for different sizes.

For example young sized shrimp require high protein diet while larger shrimp require low protein diets as the protease activity is markedly reduced in large sized shrimp. Among the environmental factors, salinity and pH are observed to influence the activity of the digestive enzymes. For example the activity levels of amylase and protease were reduced in higher and lower salinities and pH levels. The optimum activity levels for salinity and pH were 25 put and 8.0 for amylase and protease. Hence at low or high salinities *P. monodon* should be feed with low protein diets. The enzyme activity of gut microflora of tiger shrimp indicated their ability to digest protein, fat, starch and chitin. The chief point in which digestion of crustacea differs from vertebrates is that enzymes are mostly secreted in hepato pancreas in shrimp while in vertebrates they are secreted by different glands.

Absorption of digested

Fully digested nutrients in solution is absorbed immediately by the `R' cells in the digestive gland tubules. It is not known whether small particles and partly digested substance are absorbed as such or whether digestion is completed in the lumen of the tubule after additional secretion of enzymes.Glycine and lysine transport in the midgut of *P.merguinensis* and glucose transport in the midgut of *Macrobrahium rosenbergii* has been shown to be an active carrier mediated process with a sodium requirement. *Penaeus japonicus* absorbed aminoacids primarily between the proventriculus and the fore intestine. Little fatty acid absorption occurrred in the lower intestinal tract. Dietary fat is absorbed mainly as amixture of free fatty acids and mono or di-acylglycerol.

Conclusion

From the foregoing account it is evident that for development of suitable formulated feeds for culture of any species, proper understanding of the Physiology of digestion is highly essential.

CHAPTER – 2

PROTEIN, LIPID AND ENERGY REQUIREMENTS OF SHRIMP AND FISH

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INTRODUCTION

Shrimp and finfish farming have shown phenomenal growth in the last decade in India producing protein rich health food and earning valuable foreign exchange. Feed is a major input in shrimp and fish farming. The development of nutritionally balanced feed involves understanding the dietary requirements of candidate species, selection of feed ingredients, formulation of feeds and appropriate processing technology for producing water stable pellet feeds. Depending upon the type of farming, a wide range of feeds are used for feeding stocked shrimp and fish. While no feed is used in traditional farming systems, supplementary and balanced feeds are used in extensive and semi-intensive aquaculture.

NUTRITIONAL REQUIREMENTS OF SHRIMPS

Shrimp diet should have adequate energy, not only to meet the needs of body maintenance called basal metabolism, but also for growth. In nature shrimp feeds on a variety of food items and derive their balanced nutrition for healthy growth. When shrimps are cultured in confined systems (ponds), they should be provided with a balanced diet as close to natural food as possible. It is for this reason understanding the nutritional requirements of candidate species is essential.

Protein requirement

Protein is the most important and essential nutrient in the diet of shrimp. It also contributes a major share to the cost of feed. The requirement of protein varies with size of shrimp and also with the source of protein used in diet. The dietary requirement of protein for tiger shrimp *Penaeus monodon* ranges from 35 to 45% and for *P. indicus* it ranges from 30 to 43%, which are the most sought after species for culture. It has been demonstrated that postlarvae and juveniles

require higher protein in diet and the requirement decreases, as the shrimp grows larger in size.

Amino acids

The growth of shrimp is directly related to the quality of protein in terms of amino acids. Out of the twenty-five odd amino acids that are generally found in proteins, ten are essential amino acids (EAA). These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine threonine, tyrosine, tryptophan and valine. Shrimps are not capable of synthesizing these amino acids and should be provided through diet and hence they are termed as essential. It is found that if the amino acid composition of the protein in the feed matches with the amino acid composition of shrimp body tissue, such feed promotes good growth. The quantitative requirement of EAA in the diet is related to protein level in diet and their recommended levels in shrimp feeds are given Table 1.

Table 1: Essential Amino Acid requirement in shrimp feed

Amino acid	As % of protein	% of feed at protein level in feed			
		36.0	38.0	40.0	45.(
Arginine	5.8	2.09	2.20	2.32	2.61
Histidine	2.1	0.76	0.80	0.84	0.95
Isoleucine	3.5	1.26	1.33	1.40	1.58
Leucine	5.4	1.94	2.05	2.16	2.43
Lysine	5.3	1.91	2.01	2.12	2.39
Methionine	2.4	0.86	0.91	0.96	1.08
Methionine+ Cystine	3.6	1.30	1.37	1.44	1.62
Phenylalanine	4.0	1.44	1.52	1.60	1.80
Threonine	3.6	1.30	1.37	1.44	1.62
Tryptophan	0.8	0.29	0.30	0.32	0.36
Valine	4.0	1.44	1.52	1.60	1.80

Source: Modified from Akiyama and Dominy, 1989

Lipid requirement

Lipid is a complex mixture of simple fat, phospholipids, steroids, fatty acids and other fat soluble substances such as pigments, vitamins, A, D, E and K. The quantitative requirement of fat in the diet of shrimp is in the range of 5 to 10%. However, the quality of fat in terms of fatty acids is more important.

Fatty acids

Fats are triesters of glycerol. Among the long chain fatty acids polyunsaturated fatty acids (PUFA) such as linoleic acid (18:2n6), linolenic acid (18:3n3), eicosapentaenoic acid (20:5n3) (EPA) and docosahexaenoic acid (22:6n3) (DHA) are essential for growth, survival and good feed conversion ratio (FCR) for *P. monodon* and other penaeid shrimps. The n3 fatty acids are more essential than the n6 acids (also known as ω fatty acids). The fatty acids, EPA and DHA, which are known as highly unsaturated fatty acids (HUFA) of n3 series, are particularly important. Quantitatively EPA and DHA are needed at 0.5% to 1.0% in the diet of larvae and juvenile shrimp. Studies in *P.indicus* have shown that oils rich in PUFA such as fish (sardine) oil, squid oil and prawn head oil produce superior growth when incorporated in its diet. These oils are rich in HUFA.

Phospholipids

The phospholipid, phosphatidylcholine (lecithin), is essentially required in the diet of shrimp for fast growth and good survival. Soya lecithin is a good source of phospholipid for shrimps. It is required at 2% level in the diet. The development and survival of larvae is significantly improved when the diet contained lecithin. It was established that phospholipids lipid having choline and ethanolamine are only effective. Those phopholipids having other groups such as serine are not as effective as these derivatives. Phospholipids are found to be involved in the transport of lipid, especially steroids in the haemolymph.

Steroids

Shrimps grow through the process called moulting in which they periodically shed body skin (shell). Steroid hormones called, ecdysones, are responsible for moulting. To synthesize these hormones, the steroid cholesterol is required in the diet. Shrimps are not capable of synthesizing cholesterol in their body and hence must be supplied through diet. The requirement of cholesterol in shrimp

diet was shown to vary from 0.5% to 1.0%. For *P. monodon* and *P. indicus* the dietary requirement of cholesterol is 0.5%. Plant sterols, such as phytosterol, ergosterol and β-sitosterol were also tested for shrimp *P. japonicus*. Though these sterols support growth and survival, the performance of cholesterol is superior to these sterols. 24-methylcholesta-5, 22-dienol was also found to be as effective as cholesterol. Phytosterols are converted to cholesterol and utilized by the pathway suggested by. Many natural feed ingredients, such as prawn head waste and squid are good sources of cholesterol which can be included in the feed formulations.

Energy requirements

The major components of shrimp diet are protein, fat and carbohydrate, which are the main sources of energy to animals. One gram of protein is approximately equal to 5.5 kcal of energy for shrimp, while fat is the highest energy source equal to 9.5 kcal/g. The energy equivalent of carbohydrate is 4.5 kcal/g. The total digestible energy content of a diet varies with the proportion of protein, fat and carbohydrate. While keeping minimum essential levels of these nutrients, the energy requirement in the diet of penaeid shrimp was found to be 2800 kcal to 4300 kcal/kg for tiger shrimp, *Penaeus monodon* and 3500 to 4000 kcal/kg for *P. indicus*.

Carbohydrate

Carbohydrate is an inexpensive source of energy in shrimp diet. Among the different types of carbohydrates available, shrimp are found to utilize disaccharides and polysaccharides better than monosaccharides. The Indian white shrimp *P. indicus* showed superior growth on diets containing maltose and starch than those containing glucose, fructose, galactose and glycogen. Tiger shrimp *P. monodon* showed preference for trehalose, sucrose and glucose for growth. However, diets containing maltose and molasses gave inferior results.

The quantitative requirement of carbohydrate in the diet of shrimp is related to dietary protein and lipid levels. Depending upon the total energy content required in the diet, carbohydrate can be used from 10 to 40% level. Carbohydrate has protein sparing effect in. Using starch as source of carbohydrate in diet has dual advantage. Besides being energy source, it can act as binder if gelatinized by cooking with moisture and improves water stability of diet. Corn flour, wheat flour, tapioca flour and other grain flours are good sources of starch in shrimp feeds.

Another polysaccharide, cellulose (also known as crude fiber) is also found to be required in shrimp diet. The enzyme cellulase is detected in digestive tract of penaeid shrimp. But the digestibility of cellulose in shrimp is negligible. However, it is needed in the diet as roughage for improving the feed efficiency. Cellulose levels in shrimp diet should be in the range of 1 to 3% for best results and should not exceed 6%. However, in good quality feeds the crude fiber levels are maintained below 3% level.

The dietary requirements of major nutrients for tiger shrimp and Indian white shrimp are summarized in Table 2.

Table 2: Requirement of major nutrients of shrimps cultured in India

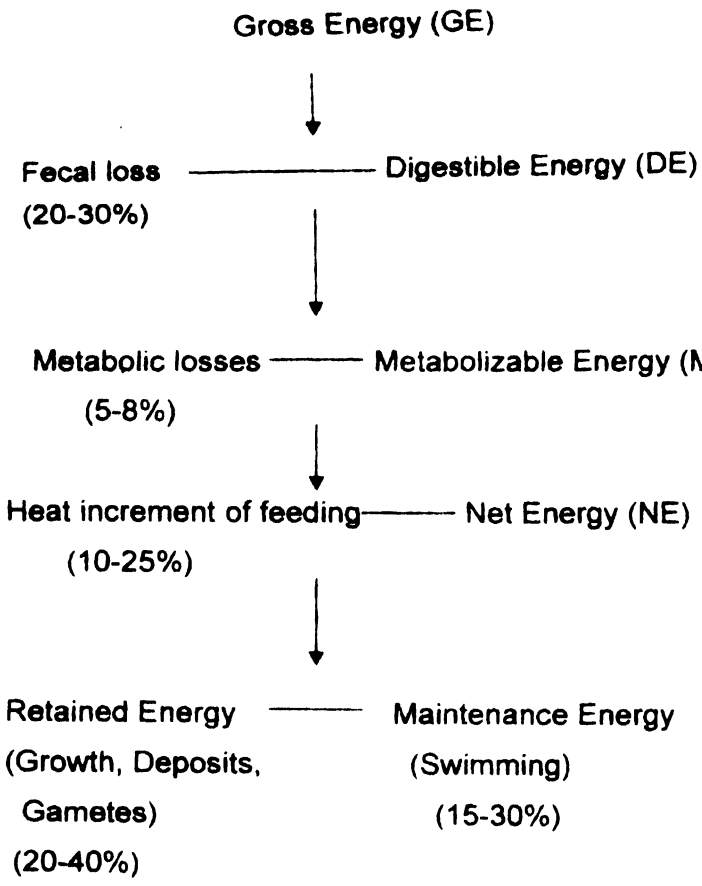
Nutrient	Dietary requirement	
	P. monodon	*P. indicus*
1. Energy (Kcal/kg)	2800 - 4300	3500 – 4000
2. Protein %	35.0 - 45.0	30 – 43
3. Lipid ,,	5 - 15	6 – 10
4. Carbohydrate ,,	20 - 25	25- 30
5. Phospholipids ,,	0.1 - 2.0	0.1 - 2.0
6. Cholesterol ,,	0.5	0.5

NUTRITIIONAL REQUIREMENTS OF FINFISH

Feeding nutrients more than needed leads wastage and economic loss. Similarly under feeding nutrients leads to poor performance. Hence balanced feeding essential for optimum performance with economic viability. To evolve balanced feed, study of nutrition of candidate species is imperative, which helps to understand what a fish requires in its diet for optimum and healthy growth and cost-effective conversion of feed into biomass. Dietary requirements are influenced by digestive physiology and environmental conditions.

Gross Energy Metabolism in Fish

Fish need energy for cellular function of maintenance and production. Energy in diet is stored as chemical energy and it is liberated by oxidation of organic compounds, protein, fat and CHO.



Fish require 10 – 30 folds lower maintenance energy than land animals, because they are poikilotherms and hence there is no regulation of body temperature. Their aquatic mode of life also does not require much energy. Secondly fish are ammoniotelic and excrete nitrogen as ammonia expending less energy than the other animals that excrete nitrogen as urea and uric acid. The maintenance energy needs of different animals are compared with that of fish are as given below

Animal	**Basal metabolic energy** (MJ/kg0.75)
Cow	0.32
Pig	0.31
Sheep	0.29
Foul	0.36

Rat	0.30
Chick	0.36
Man	0.29
Average	0.27
Fish	0.01 – 0.07

The estimated metabolic rates in fish are maintenance energy requirement 85-110 J/kg/day and heat increment 16-24KJ/kg/d. The digestible energy levels for maximum growth of fish is 14-17 MJ/kg dry diet, while the gross energy of the diet is17-20 MJ/kg dry diet. A 300-400 g fish needs 270-320 KJ/kg/d for maximum growth. Young fish need less energy (protein deposition) than old fish (fat deposition). Gonadal maturation depletes 60% body energy reserves. The energy content of egg in fish is estimated to be 27 KJ/g dry and the total energy stored in eggs is 8-15% of gross body energy. This is found equal to gonado-somatic index.

PROTEIN NUTRITION

Protein requirement of all teleosts is higher than that of land animals because fish utilizes significant portion of protein for energy needs. But fish appears to be efficient in converting protein into growth - PER is around 2.2 to. 2.7, which is higher than land in animals. Therefore production of fish by aquaculture is far more efficient than any other animal production. It is estimated that protein required for maintenance is 1.5-2.0 g/kg/day and for maximum growth it is 7 – 11 g/kg/day. Fish retains about 40% of the protein fed. The protein requirement of some selected fishes is given below

Freshwater fishes	**% Protein in diet**
Atlantic salmon	45.0
Rainbow trout	40.0
Common carp	31-38
Grass carp	41-43
Tilapia Nile	30.0
T. zilli	35.0
T. mossambica	40.0
Eels	45.0
Siberian sturgeon	40.0
Snake head	52.0

Marine fishes

Gilthead sea bream	40.0
Red sea bream	55.0
European sea bass	50.0
Asian sea bass	40-45
Sole	55-60
Turbot	65.0
Plaice	57.0
Yellow tail	55.0
Estuarine grouper	40-50
Puffer fish	50.0
Red drum	40-50
Milkfish	40.0
Mullet	35-40

Amino acids

The same ten EAAs, which essential for other animals are also found essential for fish

EAAs are required for protein synthesis (growth), maintenance- protein lost from body surface, gastro-intestinal tract, and oxidation of AAs, synthesis of other N compounds such as purines (gly, glu), polyamines (met), catechol amines (phe), thyroxine (tyr), carnitine (lys), creatine (arg, gly) histamine (his), taurine (cys) and serotonin (try). EAAs constitute 1/3 of total protein and 2/3 are non EAAs. Estimate show that 30-40% of protein N is retained in body. EAA requirement determined by dose response studies of some of the fishes is compared with that other animals as given below

EAA requirement of animals (as % of P)

EAA	Rat	Chick	Pig	Salmon	Cat fish	Carp
Arginine	5.0	6.3	1.3	5.0	4 .3	4.3
Histidine	2.5	1.5	1.2	1.8	-	-
Ileucine	4.2	3.5	3.2	2.3	-	-
Leucine	6.3	6.8	3.8	4.0	-	-
Lysine	5.8	5.2	4.8	5.0	5.1	5.7
Methionine + Cystine	5.0	4.0	2.8	4.0	2.3	3.1
Phenyalanine + Tyrosine	6.7	6.7	4.4	5.3	5.0	6.5
Threonine	4.2	3.3	2.8	2.3	2.0	3.9
Tryptophan	1.3	1.0	0.8	0.5	0.5	0.8
Valine	5.0	3.6	3.2	3.3	-	-
Protein in Diet%	12.0	23.0	20.0	>35.0	24.0	38.5

LIPID NUTRITION

Fat levels of 6 – 8% are adequate in most of the fish diets. However, higher fat levels can help sparing protein in the diet. The quality of fat in terms of fatty acid composition is more important from nutrition point of view. Polyunsaturated fatty acids (PUFA) are essential in fish diets. The following fatty acids are found to be essential for fish

Oleic acid	-	18 : 1w9
Linoleic acid	-	18 : 2w6
Arachidonic acid	-	20 : 4w6
Linolenic acid	-	18 : 3w3
Eicosapentaenoic acid	-	20 : 5w3
Docosahexaenoic acid	-	22 : 6w3

PUFA are involved in synthesis of important physiological compounds, such as steroid hormones and prostaglandins. Deficiency of EFA affects maturation and spawning. Rainbow trout fed EFA deficient diets produced eggs with poor hatching rate. Similar observations were also made in red sea bream – defective oil globule formation in the eggs. Higher levels of EFA seems to result in depressed growth and FCR and also alters the fatty acid composition of phospholipid. The essential fatty acid requirement of some of the fish species is given below

Fish	EFA	% required in diet
Rain bow trout	18:3w3	0.8 - 1.0
	W3 HUFA	10% of lipid
Carp	18:2w6	1.0
	18:3w3	1.0
Eel	18:2w6	0.5
	18:3w3	0.5
Salmon	18:3w3	1.0
	18:2w6	1.0
	W3 HUFA	0.5
Tilapia zilli	18:2w6	1.0
	Or 20:4w6	1.0
T.nilotica	18:2w6	0.5
Red sea bream	w3 HUFA	0.5

	Or 20:5w3	0.5
Turbot	w3 HUFA	0.8
Yellow tail	w3 HUFA	2.0
Coho salmon	18:3w3	1.0 – 2.5

Fresh water fish show requirement for w6 & w3 EFA, where as marine fish show requirement for w3 and also HUFA. Phospholipid, lecithin is essential in lipid transport, cell membrane structure and brain function. Tocopherol plays an important role in fish

It is essential to prevent oxidation of PUFA in the body of fish. Carotenoids are required for pigmentation of fish. Vitamin K is involved in prothrombin factor of blood, which helps in quick clotting of blood

CARBOHYDRATE NUTRITION

Omnivorous fishes have enzymes to digest carbohydrates while carnivorous fishes have poor digestibility of CHO. Polysaccharides are better utilized than monosaccharides. Monosaccharides are rapidly absorbed poorly utilized. Polysaccharides are slowly absorbed and better utilized. Insulin secretion seems to be poor in fishes. Generally carbohydrate utilization by fish is found to be lower than that of higher animals. Carps can utilize dietary carbohydrate as energy source up to 30 – 40%. For carnivorous fishes the CHO levels in the diet are generally in the range of 10 – 20%. Adequate levels of CHO and lipid in diet as energy source can spare dietary protein. Cooked starch is better digested and utilized by fish α – starch is the best source of CHO for salmon. On the other hand sea bream, yellow tail, carp and other warm water fishes better utilize β-starch. Cellulose (roughage) in diet up to 10% helped to improve FCR and PER .

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CHAPTER – 3

VITAMIN AND MINERAL REQUIREMENTS OF FINFISH AND SHRIMP

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INTRODUCTION

Micro- nutrient such as vitamins and minerals significantly influence the growth and survival of fish and shrimp and these cannot be synthesized by these organisms. Even though, some vitamins such as niacin can be synthesized by number of animal's but are typically insufficient to meet physiological demand. Hence, supplementation of vitamins in feed becomes necessary for most aquatic organisms.

VITAAMINS

Vitamins are complex organic compounds with distinct role in various metabolic processes. They are indispensable for normal growth, maintenance and reproduction in animals. Since most central metabolic pathways are common to all multicellular organisms, there are similar interactions between vitamin - dependent coenzyme system and cellular functions. Vitamin deficiency symptoms in fish and shellfish are non- specific unlike in mammals. There are four fat-soluble (A, D, E, K) and 11 water soluble (B, C) vitamins required by various organisms. Most of the water-soluble vitamins function directly or in a modified form as coenzyme for virtually all-amino transferases. In certain instances this functional role of vitamins has been used as a means of assessing the nutritional status of an animal with respect to vitamins.

Requirement of vitamins in fish and shellfish has been widely studied using deletion or dose response technique. The requirement depends on various factors such as size, age, growth rate, water temperature and composition of diets. Information available in literature on vitamin requirements widely varies in shrimp which is not so in case of fishes. Thus, presently well-defined vitamin formulations are in practice in shrimp than in fish feeds. Usually, vitamins are used at higher dosage as a safety margin in crustaceans compared to that of fishes (Conklin, 1980). Unlike domestic higher animals, the recommended

dosages of vitamins for aquatic animals are higher, as many vitamins are lost during the process of feed manufacture and also due to leaching. Destruction of vitamin C due to oxidation is one of the biggest problems during feed manufacture.

Comparing the vitamin requirement in bird, fish and shrimp (Table 1) it is evident that there is quite a large variation between bird/fish and that of shrimps. This variation is related to metabolic demand amongst the groups. These metabolic demands are affected by host of other factors that are well defined in vertebrates compared to crustaceans. In shrimps and prawns individual normal metabolic demand varies in response to various nutrient levels, so variation amongst different groups of animals is quite evident.

Water Soluble Vitamins Requirements

Amongst water soluble vitamins B group and vitamin C are most important for both finfish and shellfish. The requirements were estimated based on growth response or tissue levels of the vitamin. Many of the recent works have shown that B vitamins supplemented in feeds are near optimum. However, levels of some vitamins which were higher than recommended levels resulted in decreased growth in prawns (Deshimaru and Kuroki, 1979) and others when deleted from vitamin mixture (riboflavin) increased growth rates were observed (Heinen, 1984). Thus a balance of vitamin requirement has to be evolved for species grown under different environmental conditions. In fishes, the characteristic nervous disorders and behavioral changes are good signs for observation for deficiency syndromes unlike in crustaceans (Table II). For this reason in crustaceans there is wide fluctuation in vitamin requirement studies and hence no standard vitamin premix has been evolved like in fishes and other vertebrates.

The individual vitamin requirement and their deficiency syndromes in finfish and shellfish are as follows:

Thiamin (B_1)

Chemically, thiamin is a substituted pyrimidine and thiazole moiety. It exists in enzyme form as thiamin pyrophosphate (TPP). This compound (TPP) has primary role in pyruvic acid cycle as it participates in oxidative decarboxylation of α-keto acids especially pyruvic acid to aldehyde eventually releasing CO_2.

Another role of this vitamin is in transketolase system of glucose metabolism. The vitamin is highly labile to heat and is destroyed when moisture content is high. Studies have shown that in shrimp feeds 60-70% of thiamin loss occurs within 1 hour of immersion in water. The vitamin has important role in growth, reproduction and normal digestion. The requirement in finfish ranges from 1 – 15 mg/kg as against 15-30-mg/kg feed for shellfishes. Deficiency syndromes are associated with nervous disorders, poor appetite, growth and sensitivity to shock.

Riboflavin (B_2)

The main function of riboflavin is as coenzyme form of flavinadenine mononucleotide (FMN) and flavin adenine dinucleotide (FAD). It is also involved in many oxidative enzyme systems (dehydrogenases, oxidase and reductase) and also in retinal pigment reactions. Many plant and animal products are rich in this vitamin. The feeds can be protected from this vitamin loss by keeping a check on the pH, moisture and exposure to light. The requirement in fishes ranges from 7–30 mg /kg as against 15 –60 mg/kg diet in crustaceans. Deficiency syndromes include poor appetite, growth, photophobia and hemorrhagic eyes.

Pantothenic acid (B_5)

This vitamin is one of the most readily absorbed compounds in the intestine and phosphorylated to 4 – phosphopantothenic acid that is converted to coenzyme A (CoA). This CoA is a high-energy bond participating in condensation and addition reactions in energy metabolism. The vitamin is destroyed by heat at either alkaline or acid pH. Fish requirement ranges from 25-50 mg/kg dry diet as against 60-120 mg/kg in crustacean diet. Main deficiency syndromes are loss of appetite, poor growth, cellular atrophy and clubbed gills. In prawns it is observed that the problem of partial molting can be overcome by addition of pantothenic acid in diets (Gopal, 1987).

Niacin (B_3)

This vitamin can be synthesized in the body through aminoacid- tryptophan. But in most fish species the synthesis is slow, hence it should be added in feed. Basically niacin has major role in metabolism of fat, carbohydrate and aminoacid in transferring hydrogen electron to another coenzyme in the hydrogen transport

series. In fish the requirement ranges from 120 – 200 mg/kg dry diets and in crustaceans it ranges from 60 – 120 mg/kg diets. Deficiency syndromes observed in fishes are lesions and edema of colon, fin erosion and skin lesions and hemorrhages.

Pyridoxine (B_6)

It has important role in the protein metabolism. Carnivorous fishes exhaust the body stores of pyridoxine rapidly. This vitamin is available in grains, legumes, egg yolk and yeast. It is destroyed by light and pH changes. The requirement in fish ranges from 3 – 20 mg/kg diets where as for crustaceans it is 60 – 120 mg/kg diets. Pyridoxine deficiency in fish and shellfishes results in nervous disorders, loss of appetite, edema of the peritoneal cavity, anemia, and hyperirritability.

Biotin

Microorganism, algae and certain plant species biosynthesize biotin and are good sources for others which are unable to do so. Yeast, liver, peanuts, rice bran, egg yolk are rich sources of biotin Biotin is bound to egg white, which can be inactivated by avidin – a heat labile protein. It is bounded with lysine residues that can be released by heating or use of the enzyme biotinase. This vitamin serves as a cofactor for CO_2 transfer in biotin dependant reactions in glucose metabolism and Acetyl-CoA carboxylase. Biotin requirement in fish ranges from 1-1.5 mg/kg but in crustaceans, even though no detailed work has been done, it is incorporated at 0.05-0.5 mg/kg diet. In fishes other than general disorders, spastic convulsions are observed. In crustaceans no significant disorders have been reported.

Cyanocobalamine (B12)

This vitamin is a complex molecule containing corrin nucleus attached with 5,6-dimethyl benzimidazole ring by D-1-amino-2-propanol to cobalt. The main function of B_{12} is alkyl transfer group during de novo synthesis of labile methyl groups. The requirement in fishes and shellfishes ranges from 0.01-0.02 mg/kg diet. In presence of adequate folic acid, the action of the vitamin (B_{12}) is more effective in the metabolic processes as both are necessary in synthesis of DNA

especially in erythrocyte producing tissues. The deficiency symptoms observed in fishes are highly fragmented erythrocytes and lower levels of hemoglobin.

Folic acid

The main function of folic acid is in the transfer of 1-C units (methyl, formyl). As coenzyme, it is involved in purine and pyrimidine synthesis of DNA/RNA bases, aminoacid inter- conversions and metabolism of methyl groups as C-1 units or oxidation to CO_2. The requirement in fishes and shellfishes ranges from 6-10 mg/kg diet. Deficiency syndromes are usually anorexia, dark skin pigmentation, fragile fins, and infraction of spleen.

Lipoic acid

A dual soluble (water and fat) vitamin with major function in α - keto acids decarboxylation. As a part of pyruvate dehydrogenases complex along with TPP, CoA, FAD helps in pyruvate oxidation to Acetyl-CoA. No specific requirement and deficiency syndromes have been listed in fishes and shellfishes.

Para Aminobenzoic acid (PABA)

Little is known about the mode of action of p- Aminobenzoic acid. The only information available on its requirement to crustaceans was given in the results of an experiment with *Oniscus ascellus* where the presence of 1% of the antagonist sulpha pyridine in the diet produced no inhibitory effect.

Choline

Choline is found substantially in animal tissues. Choline does not function as cofactor of an enzyme (Zeisel, 1981) but this trimethylated compound is a source of labile methyl group for many enzymatic reactions. It also forms an important structural component of biological membranes. In finfishes the requirement ranges from 2500 to 3000 mg/kg feed and in crustaceans from 600 – 800 mg/kg (Kanazawa *et al.*, 1976, Gopal, 1987). The commonly observed dietary deficiency symptoms in fish and shellfish are poor appetite, nervous disorders, poor growth and survival.

Inositol

Basically inositol is a constituent of phospholipid and has similar function as that of Choline. It is an important constituent of cell membrane and also has lipotropic activity. It has significant role in prevention of cholesterol accumulation in one type of fatty liver disease in fishes. The requirement of inositol varies from 1000 to 2000 mg/kg diets. Though plant seeds are rich sources of inositol, it forms complexes with phytic acid, hence it is not available. Deficiency causes increased gastric time, edema, dark color, distended stomach in fishes.

Ascorbic acid (ASC)

An important water soluble vitamin for fish and shellfishes. It is essential in maintenance of cell membrane. Its involvement in collagen formation is well known in vertebrates. In crustaceans, collagen is not a predominant structural element, yet ASC deficiencies are related to insufficient synthesis of this protein. In shrimps the well-documented deficiency syndrome is the Black Death caused due to melanization lesions observed in the underlying collagen tissues of the endoskeleton. ASC is essential for synthesis of carnitine needed for utilization of lipid stores for energy and also to combat various stresses including infection and reproduction (Sander *et al.*, 1984). Most of the vertebrates and some invertebrates are capable of synthesizing ASC in presence of gulonolactone oxidase (GLO). However, in many finfishes and shellfishes the presence of two enzymes has not been established and this could vary among species within a genus (Soliman *et al.*, 1985). Even in the presence of GLO, many species require ASC supplementation in the feeds for normal physiological functions. In many cases tissue levels of ASC is in the form of ascorbate -2– SO_4. In salmonoids this compound is cleaved to SO_4 and ASC enzymatically (Tsujimura *et al.*, 1981).

Dietary requirement of ASC in shrimp feeds is high compared to other animals. Optimum amount of ASC in diet of fish and shellfish depends on species, size, growth rate and culture conditions. In fishes the requirement ranges from 50 – 70 mg/kg diet as compared to 8000 – 10000 mg/Kg diet in shrimps (Kanazawa, 1985; Gopal, 1987). Dietary ASC is highly soluble in water and easily oxidizable to dehydro ascorbic acid. Therefore, derivatives like SO_4, PO_4 are used to improve the ASC quality in feeds. In recent times Mg-L-ascorbyl-

2-PO_4 is most widely used in feeds. The prominent ASC deficiency syndromes in finfish and shellfish are scoliosis, poor growth and survival.

Fat-soluble vitamins

Vitamin (A)

Vitamin A is also known as retinol. It exists as an aldehyde (retinal- rhodopsin), as an alcohol (retinol- hormone), acid (retinoic acid- carrier) and also as an esters (retinyl ester- storage form). Vit A exists in two forms - Vit A_1 (saltwater fishes) and vit A_2 (Freshwater fishes) and both are interconvertible. Cod liver oil is rich in Retinol or retinyl esters. Synthetic vit A (retinyl palmitate) is generally used in feeds of fishes and shellfishes. β- carotene present in plants is broken down by β-carotene dioxygenase to retinol and retinoic acid in intestine and acts as provitamin A. Retinol is absorbed, re-esterfied and stored in liver as retinyl palmitate. Vit A is essential for maintenance of epithelial cells and generation of light sensitive rhodopsin in retina. In fishes the requirement ranges from 2000-5000 IU/kg diet and shellfishes from 3000 - 5000 IU/100g diet. Poor growth and vision, keratinization of epithelial cell characterizes deficiency syndromes. Hypervitaminosis of vitamin A causes enlargement of liver and spleen, abnormal growth and skin lesions in fishes.

Vitamin D

The D vitamins are prohormone of sterol type generated from provitamin-ergosterol and 7-dehydrocholesterol. UV radiation converts ergosterol to ergocalciferol ($VitD_2$) in plants and 7-dehydrocholesterol to Cholecalciferol in the skin of animals. Fish liver oil is rich sources of vitamin D3. Vit D_3 is the most potent forms of Vit D and stimulates the absorption of Ca in intestine. It has important role in homeostasis of Ca and P from the water through gill membranes. The requirement in fishes ranges from 1000-2400 units/100g diet and in crustaceans from 2000-2400 units/100g diet. Prominent deficiency symptoms are lethargy, dark pigmentation, and impaired growth. Hypervitaminosis results mobilization of Ca and PO_4 leading to fragile bones.

Vitamin (E)

The chemical name of vitamin E is α- tocopherol. Among the many tocopherols, α- tocopherol has highest biological activity. They have specific role in acting as

intracellular antioxidants, maintaining homeostasis of labile metabolism in the cells and tissue plasma. It has a role in selenium metabolism too. In combination with ascorbic acid and selenium it helps in stopping chain reaction of lipid oxidation with the help of glutathione peroxidase enzyme. Wheat germ oil, soybean oil and corn oil are rich sources of vitamin E and synthetic form of alpha - tocopherol acetate/phosphate is used in feeds. Vit E prevents oxidation of oils. Generally for fishes 30-100 mg/kg diet is recommended and for shrimps it is used at 30 – 40 mg/kg diet. Deficiency of Vit E in fishes causes immaturity, variable size, muscular dystrophy and fragmentation and fragility of erythrocytes. Hypervitaminosis in fishes results in mortality due to toxicity.

Vitamin K

The chemical name of vitamin K is menadione. It is found in green leaves and vegetables. Highly susceptible to oxidation and UV radiation. Menadione added in feeds should be protected from oxidation and UV radiation. It is an important cofactor for carboxylation of glutamyl residues of blood clotting proteins (prothrombin). The requirement in fishes ranges from 10-1000 mg/kg diet and in shellfishes 4 - 6 mg/kg diet. In fishes deficiency leads to prolonged bleeding due to slow clotting of blood, anemia, and hemorrhagic areas in gills, eyes and vascular tissues.

MINERALS

Mineral requirement studies in fish and shellfish had been in vogue for quite some time. The investigations are carried out primarily in relation to osmoregulation, heavy metal toxicity and other physiologically related functions in finfish and shellfish. Dietary studies had been restricted because the organisms have the tendency to absorb some of the mineral elements from the surroundings. Yet in dietary formulations mineral mixtures are added. In case of shrimps the periodic molting necessitates incorporation of mineral mix so as to compensate for the mineral loss during ecdysis. Thus, for shrimps the availability of minerals from the dietary source is important and essential.

About 20 inorganic elements (macro and micro) are required to meet the metabolic and structural functions in the body of animals. These minerals differ from other nutrient as they are neither produced nor consumed by the organisms. The aquatic organisms regulate the mineral needs through dietary source and

also through internal regulatory mechanisms in the kidneys and gills. The requirement studies are therefore based on dietary deficiency symptoms using purified diets (Table III). Use of practical diets with fishmeal often interfered with requirement studies, as fishmeal is a rich source of minerals. Among the minerals, 7 are required comparatively in large quantities and 15 are required in trace amounts. Non-supplementation of trace minerals in fish feeds results in reduced growth and low feed efficiency. The minerals such as Ca, P, Mg, K, Na, Cl, and S are classified as macro-nutrients while Fe, Zn, Cu, Mn, Ni, Co, Mo, Se, Cr, I, Fl, Sn, Si, Va and As are known as micro-nutrients.

Calcium (Ca)

In saline waters Ca is abundant, which is absorbed by most aquatic animals. Also diet is an alternative source for meeting the requirements. The ratio of Ca: P has significant role in meeting the dietary needs of these minerals. Since the availability of P through water medium is poor, P should be made available through diet. Usually the preferred Ca: P ratio is 1: 1 in feeds of aquatic species. Higher levels of incorporation of Ca results in abnormalities such as Ca deposition in kidneys in fishes (Kloppel and Post, 1973; Smith *et al.*, 1974).

Phosphorus (P)

Ca and P requirements are generally considered together because the metabolism of the two elements is intimately connected. Phosphorus is the most important because of its essential requirement for growth and bone mineralization and also for lipid and carbohydrate metabolism. However, the requirement of P is unaffected by dietary Ca. The availability of inorganic P depends on its solubility . Mono and dicalcium phosphate ($CaPO_4$) contain more available P than tri calciumphosphate. Phosphorus in fishmeal exists in the form of insoluble hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_6$) originating from bones and scales. Incorporation of P should be very discrete in fish and shellfish feeds, as most of it gets excreted leading to eutrophication. The dietary requirement of P ranges from 0.5-0.9 % in fishes and 1-2% in shellfishes.

Magnesium (Mg)

Magnesium occurs not only as a component of bone but also in many metaloenzymes. Its deficiency affects many metabolic functions. One of the best

source of Mg is fishmeal and bone meal. Important Mg deficiency symptoms is the flexibility of muscle, which is the result of increased extracellular fluid accumulation. The requirement of Mg in shrimp and fish ranges between 0.04 - 0.3% .

Zinc (Zn)

Many of the metaloenzymes (superoxides, dismutase, and carboxypeptidase) have Zn as one of the inorganic element. Several metabolic function are affected due to Zn deficiency. The requirement of Zn ranges from 15-30 mg/Kg diet for fishes and 80-120 mg\Kg for shellfishes. In fishes the prominent Zn deficiency symptoms are eye lens cataract and short body dwarfism and depressed growth.

Iron (Fe)

Dietary Fe is essential to maintain normal hemoglobin content, haematocrit value and mean corpuscular diameter. The requirement of Fe ranges from 150 - 200 mg/Kg for fishes from 60-100 mg/Kg for shrimps.

Manganese (Mn)

Major deficiency symptoms of manganese in fishes are cataracts and abnormal curvature of the backbone, decreased levels of manganese and malformation of tail. A dietary supplementation of 11-13 mg/Kg restores normal growth in fishes. In shrimps and prawns, the requirement goes up to 40-60 mg/Kg which may be due to periodic ecdysis.

Trace minerals

Other trace elements like copper (Cu), cobalt (Co), selenium (Se), iodine (I) and chromium (Cr) have some role in general upkeep of the organism. Their dietary incorporation enhances growth and survival (Dadd, 1983). Copper is needed by crustaceans because of hemocyanin. Optimum dietary level of Cu ranges from 40 – 60 ppm and it was also observed that omission of Cu from the diet was not detrimental as, crustaceans are able to meet their demands from seawater.

Suggested Reading

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Table I: Comparative vitamin requirement in poultry, fish and shrimp.

Vitamin (m g/Kg)	Poultry	Fish	Shrimp
Thiamin	15	10	120
Riboflavin	15	20	40
Pyridoxine	6	10	120
Pantothenic acid	20	40	100
Niacin	50	150	150
Folic acid	6	5	5
Vitamin B12	<0.1	0.1	<0.1
Choline	1000	3000	600
Inositol	100	400	2000
Vitamin C	150	100	10000
Vitamin E	50	30	200
Vitamin A (IU)	4500	2500	5000
Vit D (IU)	400	2400	1000
Vitamin K	1.5	10	40

Table II: Vitamin deficiencies syndromes

Vitamin	Deficiencies
Thiamin	Poor appetite & growth, muscle atrophy, convulsions, moribund
Riboflavin	Hemorrhagic eyes, incordination dark coloration, poor appetite & growth
Pyridoxine	Nervous disorders, hyperirratability, loss of appetite, gasping breathing
Pantothenic acid	Clubbed gills, prostration, loss of appetite, gill exudate, sluggishness, partial molting
Inositol	Poor growth, increased gastric emptying time, skin lesions.
Biotin	Loss of appetite, skin lesions, poor growth, spastic convulsions.
Folic acid	Poor growth, lethargy, fragility of caudal fin, macrocytic anemia.
Choline	Poor growth & food conversion, hemorrahgic kidney.
Nicotinic acid	Loss of appetite, lesions in colon, weakness, muscle spasms while resting.
Vitamin B12	Poor appetite, low hemoglobin, macrocytic anemia.
Ascorbic acid	Scoliosis, lordosis, impaired collagen formation, hemorrahgic skin, intestine, muscle.
PABA	No specific syndromes
Vit A	Impaired growth, edema, depigmentation, degeneration of retina.
Vit D	Poor growth, impaired calcium homeostasis.
Vit E	Reduced survival, poor growth, anemia, fragile erythrocytes, elevated body water.
Vit K	Prolonged blood clotting, lipid peroxidation, reduced haematocrit.

Table III: Dietary mineral requirement and deficiencies in finfishes and shellfishes

Element	Requirement %	Deficiencies	Requirement %	Deficiencies
	Fish		Shrimp/Prawn	
Calcium	0.2-0.4		1-2	
Phosphorus	0.6-0.8	Skeletal abnormalities, high lipid content, low ash and feed efficiency	1-2	Poor growth, low ash
Ca:P	*P. monodon* /*P. japonicus*			
			1:2	
			1.2:1	
Magnesium	0.04-0.07	Poor growth, high mortality, sluggishness, high Ca content in bone, poor growth	0.2-0.3	Poor growth and high mortality
Sodium				
Potassium			0.9-1.0	
Chloride				
Iron mg/Kg	150-200		60-100	
Copper mg/Kg	3-4		8-12	
	Trace elements			
Zinc mg/kg	15-30	Poor growth, high mortality, erosion of fins and skin, low Zn content in bone, dwarfism	80-120	Poor growth
Manganese	11-13	Dwarfism, poor growth, low Ca, P, Mg, Mn and Zn content	40-60	Poor growth
Cobalt			0.8-12	
Iodine	0.6-1.1		4.0-0.6	
Chromium	0		0.6-1.0	
Selenium	0.03-0.04		0.17-0.25	

CHAPTER-4

FEED FORMULATION FOR SHRIMPAND FISH AQUACULTURE

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INTRODUCTION

Feed formulation is the method of combining available raw materials to satisfy the pre-established nutrient requirement of the species intended for aquaculture. It is essentially the application of nutrition studies to a practical situation. The main objective or aim of feed formulation is to utilise the knowledge of nutrient requirements, locally available feed ingredients and digestive capacity of the organism for the development of a nutritionally balanced mixture of feedstuffs which will be eaten in adequate amounts to provide optimum production of the cultured fish or shrimp at an acceptable cost. Diet formulation is not easy, it usually follows a certain sequence of 'trial and error' steps, involving lot of educative guesses and skill. The nutritionist who attempts to formulate a feed must have in depth knowledge of the biology of the target species and its environment.

The performance and success of a formulated diet for fish or shrimp depends on many factors, the most important being

a) Feed formulation and nutrient content.
b) Feed manufacture and physical characters of the feed.
c) Feed handling and storage.
d) On – farm feed management- feed application methods, feeding regime.
e) Aquatic environment and natural food availability.

Further, formulation of practical diet is also based on several technical and economical factors, which can be summarized as follows:

I. The market value of the species – the value of the species cultured using the feed will set the upper limit which can be spent on the formulation and

Selection of Ingredients

Before proceeding with formulating a feed, the ingredients are to be selected from many available sources. The common shrimp/fish feed ingredients generally used for feed preparations are dealt in another lecture. No single ingredient can be expected to provide all the nutrient requirement. Any ingredient considered for inclusion in the feed must be present for a specific reason i.e. either to supply a specific nutrient or physical property to the diet. By selecting various ingredients in the correct proportions, a compounded ration, which is nutritionally balanced, pelletable, palatable and easy to store and use, can be formulated. Good quality feeds can be made only from good quality ingredients.

The basic technique used in ingredient selection is through "Least-Cost" or "Best-buy" calculations. This will enable the nutritionist to compare the cost of supplying a particular nutrient from different feedstuffs.

Least-cost or Best-buy technique

When several feedstuffs are available to supply a particular nutrient then it is useful to calculate the cost per unit of nutrient from each of the ingredient and compare. Cottonseed meal and groundnut meals are typical examples of materials available as dietary protein sources.

If cottonseed meal costs Rs. 14 and contains 54% protein –

$$\text{Cost/Kg protein} = \frac{14}{0.54} = \text{Rs. } 25.93 \text{ per kg protein.}$$

Groundnut meal costs Rs. 11 and contains 44% protein –

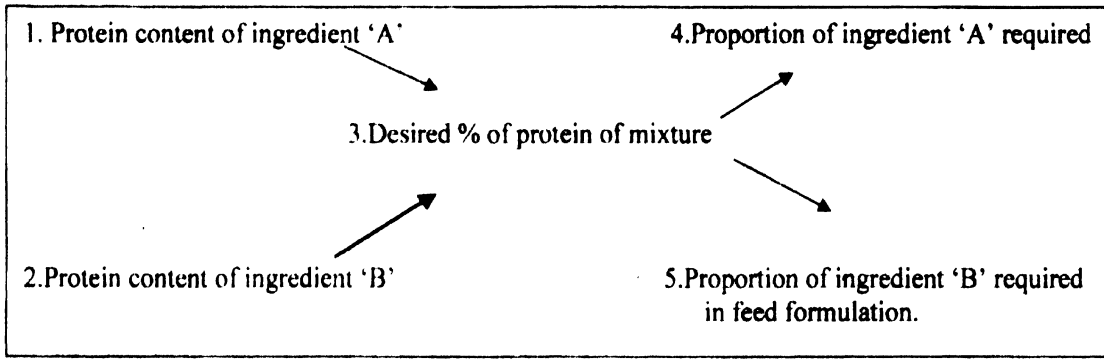
$$\text{Cost/Kg protein} = \frac{11}{0.44} = \text{Rs. } 25.00 \text{ per kg protein}$$

thus, although cottonseed meal contains higher levels of protein, the cost of per kg protein from groundnut meal is less. Therefore groundnut meal is a better buy.

Similar calculations are performed to find the Least-cost ingredient for supplying any nutrient.

Balancing nutrient levels

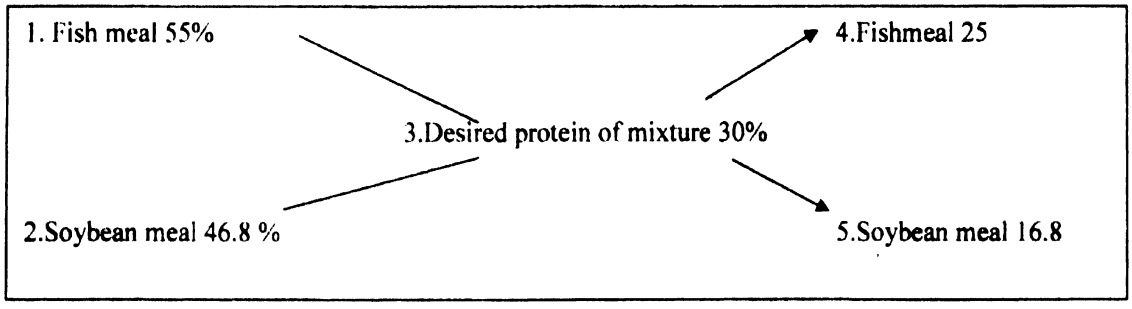
The mathematical techniques used for balancing the nutrient levels during feed formulations are simple. This can be achieved easily by using the Pearson's Square method. Using this method, the proportions of each ingredient that would provide the desired nutrient level in the feed mix can be worked out.



Since protein is the most expensive component in a feed, it is the first nutrient considered in

Protein sources are first selected from those available on the basis of their cost per unit of protein supplied and their amino acid profile (protein source with high chemical scores or essential amino acid indices are selected initially).

Supposing it is desired to formulate a 30% protein feed from two protein sources, say, fishmeal (55 % protein) and soybean meal (46.8 % protein). By substituting the values of fishmeal and soybean meal in the above square we have,



The protein level of the mixture is subtracted from that of each of the ingredient in turn and the answer is placed at the opposite corner to the ingredient ignoring positive or negative signs. The two figures on the right hand side of the square are then added together (25 +16.8 =41.8). To obtain the 30% protein feed mix we then need:-

Fish meal 25 + 41.8 x 100 = 59.81 %

Soybean meal 16.8 + 41.8 x 100 = 40.19 %

Thus to make 100 kg of 30% protein feed we need 59.81kg of fishmeal and 40.19 kg of soybean meal.

Assuming that a nutritionist has to formulate a diet with 26% protein and 5.0% lipid using locally available ingredients given below.

	Lipid (%)	Protein (%)
Fish meal	6.0	55
Groundnut cake	13.7	34.5
Soybean meal (fat extracted)	1.3	46.8
Rice bran	2.4	13.3
Maize meal	4.5	9.8

The nutritionist decides that 10% fishmeal needs to be incorporated and hence the task is primarily to determine the proportion of other four ingredients that will make up the diet. Fishmeal (10%) will contribute 5.5% protein to the diet. Therefore the other 90% of the ingredients will have to make up to 20.5% of protein. The non-fish meal portion of the diet must actually contain 20.5 x 100/90 = 22.8% protein. In order to provide 22.8% protein the amounts of each of the four possible pairs of ingredient combinations which will supply this level of protein can be calculated by Pearson's square, as detailed above.

The four combinations containing 10% fishmeal that will provide a overall protein level of 26.0% are –

40.3% groundnut cake + 49.7% rice bran (diet a)

47.3% groundnut cake + 42.6% maize (diet b)

25.5% soybean cake + 64.5% rice bran (diet c)

31.6% soybean cake + 58.4% maize (diet d)

Now if the lipid levels for the different combinations are calculated –

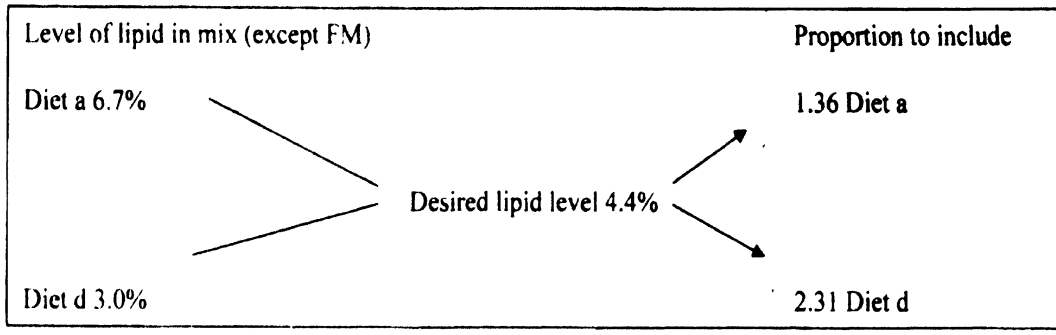
Fishmeal + rice bran + groundnut cake = 8.06% (diet a)

Fishmeal + maize meal + groundnut cake = 9.0% (diet b)

Fishmeal + rice bran + soybean meal = 2.48% (diet c)

Fishmeal + maize meal + soybean meal = 3.64% (diet d)

The required lipid level of 5% cannot be provided by any of the above combinations. Therefore, reformulation has to be done by using the above ingredient combinations (except 10% fishmeal, fixed) but this time by considering the each combination as a single ingredient.



Thus the proportion of the ingredients in 'Diet a' to be used in the final formula would be

(1.36 /(1.36+2.31)) x 100 = 37.1%

Similarly the proportion of ingredients in 'Diet d' to be used would be

(2.31/(1.36+2.31)) x 100 = 62.9%.

Accordingly, the final formulation would appear as

Fishmeal 10% x 100 % = 10.0 %

Rice bran 49.7% (from Diet a) x 37.1% = 18.4

Groundnut cake 40.3% (from Diet a) x 37.1% = 15.0

Maize meal 58.4% 9 (from Diet d) x 62.9% = 36.7

Soybean meal 31.6% (from Diet d) x 62.9% = 19.9

Finally the contribution of each of the ingredients to a diet of 26% and 5% lipid will be

	Inclusion level (%)	Protein Contribution (%)	Lipid Contribution (%)
Fishmeal	10.0	5.50	0.60
Rice bran	18.4	2.45	0.44
Groundnut cake	15.0	5.18	2.06
Maize meal	36.7	3.60	1.65
Soybean meal	19.9	9.31	0.26

Spreadsheet software such as Lotus 1-2-3 or Microsoft Excel can also be utilised for formulating feeds by arranging the variables in tabular form as given in the Worksheet (Table 1 & 2).

Another mathematical technique available for selecting the best combination of feed ingredients at the least possible cost is Linear Programming. Additional informations necessary for formulation using LP are -

- Nutrient content and DE or ME of ingredients;

- Unit prices of feedstuffs including vitamin and mineral mixtures;
- Information on other additives; and
- Minimum and maximum restrictions on the amounts of each ingredient in the feed.

A point of caution - feeds formulated without due consideration to nutritional aspects, purely by the computer will not be useful. The knowledge gathered by nutritionists through experience cannot always be programmed into a computer.

General procedure for feed formulation:

1. Use least cost analysis to select protein and energy sources.
2. Ensure that these sources will provide the desired levels of EAA and EFA.
3. Remember that a better nutrient balance is likely to be achieved by using several feedstuffs in combination.
4. Balance crude protein level.
5. Balance digestible energy level.
6. Calculate the levels of EAA and EFA in the finishes feed and if these do not satisfy the requirements of the species, repeat steps 4 and 5. Return to step 4 readjusting the protein until EAA requirements are satisfied and return to step 5 to adjust the lipid sources until the EFA requirements are satisfied.

WORKSHEETS FOR DIET FORMULATION

Table 1. Major nutrients and cost.

INGREDIENT	% FEED	IN	COST/100 KG		% PROTEIN		% LIPID		% CHO		%
			ING	FEED	ING	FEED	ING	FEED	ING	FEED	IN
TOTAL	100		-		-		-		-		-
REG	100		-		-		-		-		-

Table 2. Essential amino acids.

Ingredient	% in feed	% ARG		% HIS		% ISO		% LEU		% LYS		% ME
		ING	FEED	ING	FEED	ING	FEED	ING	FEED	ING	FEED	ING
TOTAL	100	-		-		-		-		-		-
REQ	100	-		-		-		-		-		-

CHAPTER-5

NUTRITION AND FEEDING OF ASIAN SEABASS IN HATCHERY, NURSERY AND GROW-OUT PONDS

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INTRODUCTION

Asian sea bass (*Lates calcarifer*) has emerged as an important candidate finfish species for aquaculture in the Brackishwater sector. Availability of seed and appropriate feed are two important prerequisites for the development and propagation of aquaculture of any fish species. After considerable efforts and extensive research the Central Institute of Brackishwater Aquaculture succeeded in developing captive brood stock and seed production technology for Asian seabass. Researches on nutritional requirements and development of suitable formulated feeds have been in progress simultaneously at CIBA.

NUTRITIONAL REQUIREMENTS

Investigations on Asian sea bass (Barramundi) (also known as Bhetki in Bengal)) have been mainly concentrated on energy nutrient requirement in the diet.

Protein

Seabass being highly carnivorous showed a dietary requirement of 45 – 55% protein as determined by different workers (Cuzon and Fuchs, 1988; Tucker et al, 1988; Wong and Chou, 1989). Subsequently Catacutan and Coloso (1995) suggested 42.5% in the diet of the fish. Experiments conducted in CIBA with different level protein feeds on the young-ones of seabass showed a protein requirement of 43 % for this fish. The protein quality in the feed diet influences the requirement. Most of the finfish show the requirement of the same ten amino acids (arginine, histidine, isoleucine, leucine, lysine, methionin, phenylalanine, threonine tryptophan, tyrosine or valine) as essential. However, determination of quantitative essential amino acid requirement would help in assessing the protein requirement more accurately.

Lipid

The quantitative lipid requirement in the diet of seabass is estimated to be in the range of 6-18% (Cuzon and Fuchs, 1988; Tucker et al, 1988; Wong and Chou, 1989). Catacutan and Coloso (1995) suggested 10% lipid (in combination of 42.5% protein) in the diet for the juveniles *L. calcarifer* for good growth and FCR. When the lipid level was raised to 15% in the diet the protein sparing effect was not observed in this fish. The highly unsaturated fatty acids (HUFA) of n-3 series are reported as essential for sea bass (Buranapanidgit et al., 1988) suggesting its marine species characteristics. The requirement in the diet is suggested to be 1.72% for this fish. Deficiency of HUFA in the diet caused red colouration of the fins in the fish fed such diets.

Energy

Carbohydrate levels of 10 –16 % were suggested in the diet of seabass (Cuzon and Fuchs, 1988; Tucker et al, 1988; Wong and Chou, 1989). Subsequently Catacutan and Coloso (1995) suggested 42.5% and 10% lipid with a protein – energy ratio of 128mg protein/kcal as optimum for the juveniles *L. calcarifer* for growth and good FCR and PER.

Experimenting with carbohydrate and lipid for juveniles of Asian seabass, Catacutan and Coloso (1997) reported that best growth and FCR were observed in fish fed with 20% carbohydrate (bread flour as source) and 12% or 18% lipid (cod liver oil and soybean oil in 1:1ratio).

Summary of energy nutrient requirements for seabass

Nutrient	Requirement in diet
Protein	45 – 55%
Lipid	6 - 18%
Fatty acids	
(n-3 HUFA essential)	1.72%
Carbohydrate	10 – 20%
Protein : Energy ratio	128mg protein/kcal

Thus the information on the dietary nutrition of seabass started coming. The dietary requirements of individual vitamins and minerals are still not known.

FEEDS AND FEEDING OF SEABASS

Feeds and feeding of larvae in hatchery and nursery

Larvae of finfish and shellfish are generally fed with live food organisms (Phyto or Zooplanktons or both) in the initial phase, Investigations revealed that the developing larvae do not have the full complement of digestive system developed. The larvae of seabass are no exception to this. Being carnivorous, seabass larvae are fed with zooplankton such as rotifers for the first two weeks post hatch (PH) and then switched over to brine shrimp (*Artemia*) nauplii. The size of the rotifers plays an important role in successful rearing of the larvae. Super small size rotifers are preferred for feeding seabass larvae. Since Artemia is an expensive live-food, its replacement by prepared diets has assumed significance in the hatchery and nursery rearing of fish larvae. In this context formulated micro particulate and microencapsulated diets have been successfully used for feeding the growing fish larvae.

In case of seabass larvae it is all the more important to wean the larvae to prepared diet so as to continue them in grow-out system where formulated feed has to be used for their culture. Seabass is very conservative in its feeding habit. The larvae

of seabass are successfully weaned to prepared diet in CIBA by adopting co-feeding technique. The larvae after 18 to 19 days PH are first introduced to prepared diet in semi-moist form by co-feeding with boiled fish meat. The fish meat is gradually replaced with prepared diet and in about seven days the larvae are totally on prepared diet (Ahamad Ali et al., 2000). The larval weaning diet is prepared using marine fish and soybean meal containing 45-50% of crude protein. This diet is successfully used for rearing the larvae in hatchery and nursery. Use of formulated micro diet for feeding larvae improved the survival rate and reduced the incidence of cannibalism. Frequent feeding of larvae in hatchery and nursery is essential for achieving good survival rates. The larvae, trained on prepared diet are given to farmers for further grow-out culture. Dry particulate feeds in the size range of 200 to 300 micron size containing 50-55% protein are being evolved at CIBA for the larvae of seabass. Such feeds are used in Australia, Thailand Taiwan and other East Asian countries.

Feeds and feeding of seabass in grow-out culture

In some of the East Asian countries and also in India seabass is cultured in grow-out ponds using low value fish (trash fish) and tilapias in fresh condition. Since procurement and storage of these feed-fish is not only laborious but also quite expensive. Hence formulated feeds are essential for the propagation large-scale farming of seabass.

Asian sea bass is cultured in Australia and Thailand using formulated feeds (Mackinnon, 1989; Boonyaratpalin, 1991). As in the case of other carnivorous species, feed formulations for seabass utilize marine fish resources (for meeting protein requirement) and fish oils along with plant protein sources. The animal ingredients are kept above 60% of the formulation to get protein levels in the range of 45-52%. Experiments conducted at Muttukadu field laboratory of CIBA had shown that feeds with substantial fishmeal component (30-40%) only have good acceptability for seabass. Higher the proportions of fishmeal better the acceptability. The texture and size of the feed effects acceptability of the feed. If the flavour and texture of the feed are not to the liking of the fish, it spits out such feed even after taking it into its mouth. For keeping higher protein levels in the feed, use of animal protein sources such as fishmeal is inevitable. However, plant ingredients such soybean meal and other oil seed residues

may be utilized in the feed formulations. For providing the polyunsaturated fatty acids (PUFA) use of marine fish oils should be included in the feed formulations. Studies conducted at CIBA on the feed attractants for seabass revealed that the amino acid - glutamic acid and trimethyl amine are useful feed attractants for this fish (Syama Dayal et al., 2002).

A formulated feed was prepared into three types in an extruder. These are floating, slow sinking and quick sinking pellets. These three types of feeds were tested for the fry of Asian seabass (*Lates calcarifer*) for acceptability. The results showed that slow sinking and the quick sinking pellet types are more acceptable to the fry.

Pellet feeds with 38-44% and 8-15% fat developed at CIBA, were tested in three grow-out ponds in Tamil Nadu and Andhra Pradesh. In a culture period of 5 to 6 months the fish had grown to 300-500g with production of 1250 to 1600 kg per hectare.

Seabass feeds on moving prey; hence the physical design of the feed plays a very important role. The fish readily accepts soft semi-moist feeds with appropriate size to swallow vis-à-vis the size of the fish. The lower lip of the fish is curved slightly upward, which is disadvantageous for biting the feed. Floating and slow sinking pellet feeds are more suited for feeding seabass. Such feeds are generally processed in extruders.

Extruder technology

The basic components in an extruder are a barrel fitted with a die plate and a screw shaft conveyer, which is connected to a high-speed motor. The feed mixture is fed into an extruder by proper arrangement of water/steam injection facility. The extruder operates at high pressure (14-98 kg/cm^2) and steam (Pressure 5 - 7 kg/cm^2) injection. Depending upon the characteristics of the feed mixture and moisture content, the pressure develops before the material passes through the die. Because of this the temperature rises and the material is forced through the die and the pressure suddenly drops. The temperature of the material rises to 110 - 130°C for a short spell of time and cooks the food, gelatinizing the starch present in the feed mixture. This imparts good binding and water stability to the resultant pellets. However, the pellets expand as they

come out of the die due to sudden drop of pressure and air gaps develop inside the pellet, which makes them float or sink very slowly. This is an excellent process for producing floating pellets for finfish culture. By adjusting the pressure in the barrel and moisture in the feed, it is possible to prepare sinking pellets by extruder. The new generation extruders are made with twin screw-barrel arrangement, which are more versatile for feed manufacture. The size of the pellet diameter ranges from 0.5 mm to 8.0 mm.

The characteristics of extruder pellets are

1. Reduction in pellet disintegration and loss in water.
2. Increases starch digestibility due to good cooking
3. Can be worked with higher moisture and oil (fish oil) levels in the feed.
4. Extruder pellets float or sink slowly.
5. Making charges for extruder pellets are higher due to high cost of extruders

At CIBA formulated feeds developed as floating and sinking pellets were successfully tested in grow-out ponds and grown the fish to 500 g in six months.

The fish should be fed at the rate of 10% of their body weight to start with. After four to six weeks the feeding rate may be reduced to 8%. As the fish grow in size the feeding rate should be gradually reduced to 5%, 3% and even 2% finally. The total bio bass in the pond should be periodically estimated by a suitable means (by caste netting) for adjusting the feed. The entire quantity of feed in a day should not be given at one time. It should be divided and fed 3-4 times a day.

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CHAPTER - 6

METHODOLOGIES IN SHRIMP AND FISH NUTRITION

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INTRODUCTION

The success of aquaculture operations depends much upon feeding the stock with nutritionally balanced feeds which can be formulated by understanding the dietary requirements of the candidate species of shrimp and fish. Hence the importance of methodologies in shrimp and fish nutrition assumes greater significance in aquaculture research. Research diets and practical feeds are formulated and evaluated for its nutritional attributes and development of feeds for aquaculture. The following parameters are generally measured for evaluation of research diets and practical feeds.

A. GROWTH EVALUATION

1. Growth

The increment in length / weight during the period of test trial is an index of growth. It is expressed as

$$\text{Percent gain in length/ weight} = \frac{\text{(Final length/weight)} - \text{(Initial length/weight)}}{\text{Initial length / weight}} \times 100$$

2. Specific Growth Rate

Specific growth rate indicates the growth per unit time (per day) and is expressed as

$$SGR = \frac{(\ln Wt_2 - \ln Wt_1)}{\text{No of days}} \times 100$$

Where Wt_2 = Final body weight (g)

Wt_1 = Initial body weight (g)

3. Daily growth coefficient

Daily growth coefficient (DGC) (Kaushik 1998) can be calculated using the following equation:

$$DGC = \frac{[(\text{final weight})^{0.33} - (\text{initial weight})^{0.33}]}{\text{Number of days}} \times 100$$

This is commonly followed in larval stages.

4.Cannibalism

Cannibalism ids most common in the early life stages of aquatic animal and it can be calculated as follows. Cannibalism was calculated as a percentage of the initial number of animals that could not be accounted for other than mortalities as a result of handling at initiation of the trial and daily mortalities removed during the trial and were calculated according to the following equation:

$$\text{Cannibalism} = \frac{I - S - F - M}{I} \times 100$$

where, *I* is the initial larvae numbers stocked, *S* the number of larvae sampled for observation during the trial, *F* the final number of larvae in each tank and *M* the number of mortalities removed during the trial.

5. Feed Conversion Ratio (FCR)

In nutritional studies the quality of feed can be best evaluated by estimation of FCR, which tells how many kilograms of feed is required to produce one kilogram of shrimp or fish. FCR of feed has direct bearing on the cost of production of fish or shrimp. It is expressed as

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

It is also expressed as feed conversion efficiency and calculated as

$$\text{Feed conversion efficiency} = \frac{\text{Live weight gain}}{\text{Feed intake}}$$

Feed intake means it is feed eaten.

For the fish farmer for comparing the monetary value of feed FCR is the best tool.

B. PROTEIN EVALUATION

1. Chemical score (CS)

The level of each essential amino acid in the test protein is calculated as percentage of corresponding level of each amino acid in the reference material. The chemical score is the percentage of amino acid in the test protein that is in maximum deficiency. The reference material can be of whole chicken egg protein composition, the composition of fish meal or the requirement of fish/ prawn, if they have been determined

$$\text{Chemical score} = \frac{\text{EAA content in the test protein}}{\text{EAA content in the reference protein Or EAA requirement of animal}} \times 100$$

2. Essential amino acid index (EAAI)

The relative proportion of the essential amino acids in the test protein is compared to the requirement of the animal or the reference protein. A feedstuff is rated as good quality protein material when its EAAI is 0.90; useful when its EAAI is around 0.80; and inadequate when its EAAI is below 0.70

$$EAAI = n \frac{100a}{a_e} \times \frac{100b}{b_e} \times \frac{100c}{c_e} \ldots \frac{100j}{j_e}$$

Where 'n' is the number of EAA included in the formula

a, b, c, ... j are the percentages of the ten essential amino acid in the test protein and

a_e, b_e, c_e ...., j_e are the corresponding percentages of the tern essential aminoacids in the reference protein.

3. Protein Efficiency Ratio (PER)

The nutritive value of dietary protein is determined by the rate at which the animal grows. It is defined as the weight gained per unit intake of protein and may be calculated as

PER = Gain in body weight (g) / protein intake (g)

PER will vary with the percentage of protein the diet, with a maximum efficiency at an optimum protein level. The determination of PER demands feeding over a longer period, and in fishes and shrimp it is strongly related to the water temperature

Constraints: Gain in body weight is assumed as gain in protein (muscle tissue)

4. Apparent Net Protein Utilization (Productive Protein Value)

This function is identical with the term productive protein value (PPV) and expressed as

$$\text{App NPU} = \frac{Ni - Nf - Nu - Nb}{Ni}$$

$$= \frac{\text{Nitrogen retained}}{\text{Nitrogen consumed}}$$

The advantage of the method is that it does not require a control group, and the nitrogen retention can be conveniently and very precisely determined. This method is well suited for assay of different proteins, as the endogenous nitrogen may be considered equal in all groups.

5. True Net Protein Utilization (NPU)

This is a method of evaluating protein quality by comparing the amount animals retained to the amount they ingested. Evaluation parameters are digestibility and essential amino acid content. Net Protein Utilization (NPU) proposed by Bender and Miller (1953) is the most used in relation to protein evaluation. The original formula was modified for fishes by Castel and Tiews (1980) It is expressed as

$$NPU = \frac{Ni - (Nf - Nm) - (NU - Nen) - (Nb - Neb)}{Ni}$$

$$= \frac{Nct - Nco}{Ni}$$

Where Ni- nitrogen intake

Nf- Faecal nitrogen

Nm metabolic faecal nitrogen

Nu –Urinary nitrogen

Nen- endogenous urinary nitrogen

Nb – branchial nitrogen

Neb- endogenous branchial nitrogen

Nct – Carcass nitrogen of test group

Nco- Carcass nitrogen of group receiving nitrogen free diet

The first fraction of this equation requires determination of faecal and urinary nitrogen in groups on test diet as well as non protein diet. One of the major problem encountered is the solubility of the faecal nitrogen and collection of this part of the nitrogen.

The second fraction can be more easily applied to fishes. Body nitrogen can be determined by analysis of each single fish in the case of fingerlings, Whereas, careful homogenisation would give values based on analysis of samples from larger fish. The method requires adjustment of test and control diet to equal caloric (isocaloric diets) contents.

6. Biological Value of Protein (BV)

It is defined as percentage of absorbed protein which is utilised by the body. In this case the losses in digestion and metabolisim are taken in to account. It is expressed as

$$BV = \frac{Ni - (Nf - Nen) - (Nb - Neb)}{Ni - (Nf - Nm)}$$

From net protein utilisation and protein digestibility, we can derive this value

$$BV = \frac{\text{Net protein utilization}}{\text{True digestibility of protein}}$$

CHAPTER- 7

DIGESTIBILITY OF FEED AND NUTRIENTS IN SHRIMP/ FISH

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Introduction

Shrimp or fish feed, which is easily digested and assimilated gives good performance in terms of growth and FCR. By chemical analysis of the feed, the proximate composition *viz.*, protein, lipid, carbohydrate etc. can be quantitatively determined. However, the proportion of nutrients actually digested and assimilated by the fish or prawn can only be determined by conducting digestibility studies. The digestibility can be measured by conducting *in vivo* or *in vitro* methods.

Digestibility by *in vivo* method

In this method, the digestibility of diet is determined by quantitatively collecting the faeces. It can also be determined by using an inert marker, which is measured in diet and faeces. Biological evaluation of experimental diets in nutritional studies is generally done by conducting group-feeding experiments on candidate species. In digestibility, studies rearing tanks with white background are preferred for easy collection of faeces for analysis.

$$\text{Digestibility (\%)} = \frac{\text{Feed intake} - \text{Faeces excreted}}{\text{Feed intake}} \times 100$$

The digestibility of feed involves determination of dry matter or nutrient digestibility. In the case of nutrient digestibility, it is expressed as apparent digestibility of nutrient. If correction for metabolic loses of the nutrient are made it is expressed as the true digestibility of the nutrient.

$$\text{Apparent Digestibility of Nutrient (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient excreted}}{\text{Nutrient intake}} \times 100$$

The true digestibility takes the metabolic losses also into consideration while calculating the digestibility. It is expressed as:

$$\text{True Digestibility of Nutrient (\%)} = \frac{\text{Nutrient intake} - (\text{Nutrient excreted} - \text{Metabolic Nutrient})}{\text{Nutrient intake}} \times 100$$

The digestibility can be measured by **DIRECT** or **INDIRECT** methods.

DIRECT METHOD:

This is done directly from total water analysis involving the quantitative analysis of nutrients in uneaten solid and dissolved faeces from a feeding aquarium and solid and dissolved faeces in a balance aquarium (control). Digestibility is calculated as:

$$\textbf{Digestibility (\%)} = \frac{\textbf{Food nutrient intake in faecal} - \textbf{Nutrient in solid faeces} + \textbf{Nutrient dissolved aquarium water}}{\text{Food nutrient intake} - \text{Nutrient in solid feed} + \text{Nutrient dissolved in faecal aquarium water}} \times 100$$

INDIRECT METHOD:

In this method an inert marker, such as chromic sequioxide or chromium oxide (Cr_2O_3) is used at 0.5 to 1.0 % distributed in the feed. Use of other inert markers namely, titanium oxide is advantageous in protein digestibility studies where the indicator may be determined directly by Kjeldahl method. The test feed is fed to a group of animals, and after allowing an initial period of at least three days, the faeces are carefully collected daily over a period of time. These are dried at low temperature (freeze drying is advocated). Collection of faeces quantitatively is not needed. The

chromium oxide in feed and faeces is estimated spectrophotometrically at 540 nm using diphenyl Carbazide. The digestibility is calculated using the formula

$$\% \ Cr_2O_3 = \frac{Cr_2O_3 \text{ Concentration in sample x Dilution of sample}}{\text{ml. of sample used for colour development x Wt. Sample}} \times 100$$

$$\text{Dry matter digestibility of diet \%} = 100 - \frac{\% \ Cr_2O_3 \text{ in diet}}{\% \ Cr_2O_3 \text{ in faeces}} \times 100$$

$$\text{Nutrient digestibility of diet \%} = 100 - \frac{\% \ Cr_2O_3 \text{ in diet}}{\% \ Cr_2O_3 \text{ in faeces}} \times \frac{\% \text{ Nutrient in faeces}}{\% \text{ Nutrient in diet}} \times 100$$

Advantages of Indirect method

1. No need for measuring quantitatively either the amount of feed ingested or the amount of faeces excreted.
2. Restriction of short feeding time eliminated.
3. No necessity of handling large volumes of water for analysis of faecal excretion.
4. Digestion trial can be carried out in running water system, thus maintaining natural condition.

***In vitro* Digestibility methods**

The *in vitro*, method of protein and carbohydrate digestibility is quicker and provide valuable information on the biological quality of the prepared feed.

Protein digestibility

The protein digestibility by *in vitro* method is determined by incubating the test sample in phosphate buffer (pH 7.6) by adding homogenate prepared from shrimp/fish hepatopancreas/ liver. The digestive proteases in the tissue hydrolyses protein in to amino acids. The amino acid tyrosine released in the process is qualitatively estimated. After incubation for a given time intervals (30, 60 min). The amount of tyrosine released

due to hydrolysis of the substrate by the enzyme can be obtained from the tyrosine standard curve that is directly correlated with the digestibility of the substrate.

Materials

1. Phosphate buffer: Dissolve 0.157 g KH_2PO_4 and 1.575 g $NaHPO_4 2H_2O$ in about 90 ml double distilled water, adjust to p^H 7.6 and dilute to 100 ml with distilled water.
2. Substrates- Casein, feed samples
3. Folin Ciocalteu phenol reagent
4. Trichloro acetic acid -5%
5. Hydrochloric acid –0.2 N
6. Sodium hydroxide solution –0.5 N
7. Tyrosine standard: Dissolve 18.119 mg L (-) tyrosine in 0.2 N HCl and make up to 100 ml.
8. Enzyme solution homogenate: take the hepatopancreas/ liver tissue of shrimp/fish (pre weighed) in 10 ml distilled water. Centrifuge the homogenate at 10000 rpm for 15 min at 5^0 C in a refrigerated centrifuge. The supernatant is the enzyme solution used for incubation.

Method

To 1 g feed, add 49 ml of phosphate buffer and 1 ml of enzyme solution. Incubate for 30,60... minutes. At the end of each time interval transfer 2 ml of aliquots in to a centrifuge tube containing 3 ml TCA solution to stop hydrolysis.

After centrifugation at 3000 rpm, transfer 2.5 ml of the supernatant into a test tube. Add 5 ml of 0.5 N NaOH and 1.5 ml of Folin-phenol solution. After 10 minutes, measure the absorbance at 691 nm.

Prepare a standard curve taking different concentrations of tyrosine and developing the colour with Folin-phenol reagent.

Carbohydrate digestibility

Materials

1. Phosphate buffer: Dissolve 1.23 g KH_2PO_4 and 1.97 g $NaHPO_4 2H_2O$ in about 800 ml double distilled water. Add 65 ml 0.9 % NaCl solution, adjust to pH 6.9 with 0.1 N NaOH and dilute to 1000 ml with distilled water.
2. 0.9 % NaCl solution
3. 0.1N NaOH, 1 N NaOH solutions
4. Dinitro salicylic acid (DNS) reagent: Dissolve with warming 10 g of 3, 5 dinitro salicylic acid in about 300 ml double distilled water and 400 ml 1 N NaOH, add 300 g Potassium tartarate and dilute to 1000 ml with double distilled water
5. Maltose standard: Dissolve 100 mg maltose in double distilled water and make up to 100 ml.

6. nzyme solution: As described under protein digestibility.

Method

To 1 g feed, add 49 ml of phosphate buffer and 1 ml of enzyme solution. Incubate for 15, 30,45, 60... minutes and then transfer 2 ml of aliquots in to a centrifuge tube containing 2 ml
DNS reagent. Heat for 5 minutes in a boiling water bath. Cool in cold water and after 30 minutes, measure the absorbance at 546 nm.

Lipid digestibility

Materials

1. Standard 0.1 N NaOH, 0.01 N NaOH solutions
2. Diethyl ether- ethanol mixture: 1:1 v/v
3. Phenophthalein indicator
4. Gum arabic solution – 10% w/v gum arabic in distilled water
5. Standard oxalic acid solution 0.01 N- take 1,26 g of bi oxalic acid in 1000 l of distill water.
6. Thymalphthalein indicator
7. Enzyme solution: homogenize hepatopancreatic or liver tissue in 10 times its weight of chilled distill water in a tissue homogenizer. Centrifuge the homogenate at 10000 rpm for 30 minutes at 5^0 C. Freeze the supernatant, thaw and recentrifuge to obtain a clear, fat- free supernatant, which is used undiluted as the source of lipase enzyme.
8. Deoxycholate/ NaCl solution – Dissolve 1.60 g sodium deoxycholate and 187 mg NaCl in distilled water and make up to 100 ml.
9. Lipid substrates- Oil emulsions- determine the alkali required to neutralize 20 ml oil. Take 5 ml oil in a 100 ml conical flask, add 40 ml diethyl ether-ethanol mixture, titrate against 0.1 N NaOH using phenolphthalein indicator to end point (permanent pink colour). Note down the volume of NaOH required for 5 ml oil, multiply by 4 to obtain the volume required for 20 ml oil. Neutralize 20 ml oil in a separate flask with the required volume of NaOH solution. Blend 165 ml gum Arabic solution, 15 g broken ice (minus volume of 0.1 N NaOH required for neutralization) and 20 ml neutralized oil for 15 minutes in a mixer at medium speed.

Method

Take 10 ml substrate emulsion solution add 10 ml Deoxycholate/ NaCl solution, 9 ml distilled water and 1 ml enzyme solution and incubate in a shaking water bath set at 37^0 C for 6 hours. After the incubation period, add 10 ml rectified spirit. Titrate against 0.01N NaOH using Thymalphthalein indicator until the blue end point is reached. The volume of 0.01N NaOH required for titration is directly proportional to the quantity of fatty acids released from the substrates.

CHAPTER- 8

FEED PROCESSING AND PRODUCTION TECHNOLOGY FOR AQUACULTURE

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INTRODUCTION

Feed is one of the major inputs in aquaculture production. Consequently, there is a growing demand for good quality feeds, which can give good feed conversion ratio. Besides, the feed should have good water stability and acceptable physical appearance. One of the important factors that determine the final quality of feed is the adoption of appropriate processing technology. With the best of machinery at the disposal, working out right combination of various factors in processing and standardizing them would only lead to production of feed of desired water stability.

FEED PROCESSING

The following are the steps involved in processing of aquaculture feeds

Processing of feed ingredients

The quality of feed ingredients has an important bearing on the quality of final feed. Feed ingredients should be fresh and confirm to the nutrient quality. Contamination with foreign matter, especially, sand, stones and earthen materials will affect the quality of the materials. Old stock of oil cakes may contain aflatoxin, while PUFA rich fish oils are oxidized leading to rancidity. Quality control of the raw materials should be done at the time of their procurement itself, to ensure the quality of final feed. All the solid ingredients are procured in dry form with moisture levels preferably below 10%; otherwise the materials may be subjected to drying before they are processed.

Grinding

Pre-grinding of solid ingredients to uniform particle size is essential for making homogenous mixture of a compounded feed. Fine powdering of materials increases the surface area and improves the digestibility besides helping in making compact pellets. Materials such as dry fish, prawn head waste, squilla and squid are subjected to two stage grinding process. First, size reduction, by passing through a hammer mill. In this

the materials are roughly powdered so that they can be further powdered to finer particles. Subsequently these coarse materials are further powdered to fine particle size in a micro-pulverizer. Experiments have shown that grinding ingredients to 200 – 300 micron particles have the best digestibility and good pellet compacting property. It is fairly easy to grind oil cakes and grains to fine powder. However, marine protein sources, with high oil content (above 15%) very often pose problems of grinding, as they form cakes due to the oil content and choke the grinding (blades) and the sieve. Such materials can, however, be powdered by mixing with low oil containing materials like grain flours. Different kinds of grinding machines such as hammer mill, pulverizer, flour mill and impex pulverizers are employed for grinding feed ingredients.
Sieving

The powdered ingredients are passed through a standard mesh sieve for obtaining the desired particle size. In case the grinding equipment does not have an inbuilt sieving mechanism, the materials should be subjected to sieving. Feed materials that are commercially available in fine powder form may also be sieved to screen the presence of extraneous materials and metal pieces, which might otherwise inadvertently enter the pelleting equipment and cause damage. Sieving the ingredients helps in preparing feed pellets with uniform and attractive physical appearance.

Vibrating or gyratory type of sieve assemblies are available which are generally employed for sieving feed materials.

Mixing

The powdered ingredients after weighing according to the formulation are mixed together and homogenized into a feed mixture. The liquid materials such as fish oil may be added at the end and further homogenized. Materials, which are heat sensitive and get destroyed, may not be added in the feed mix at this stage. Water required for increasing the moisture may also be added. Binders, which need mixing with water, should also be incorporated at this stage. Horizontal or vertical types of batch mixtures are employed for mixing feeds. For proper mixing of different feed ingredients into a homogeneous mass, the mixing time may be 20 to 30 minutes.

FEED PRODUCTION

The final form of the feed is produced in the form of pellets. For shrimp compact sinking pellets are produced. For finfish floating pellet feeds are preferred even though sinking pellets are equally good for fish. However, for fish species such as Asian sea bass that feed the moving or live prey floating pellets are more desirable. The following technologies are for commercial production of pellet feeds.

Pelletization

Pelletization is a process in which the feed mixture is compacted into predesigned cylindrical pellets. Pelletization is done mainly using two types of machines namely, extruder and pelletizer.

Extruder technology

The basic components in an extruder are a barrel fitted with a die plate and a screw shaft conveyer, which is connected to a high-speed motor. The feed mixture is fed into an extruder by proper arrangement of water/steam injection facility. The extruder operates at high pressure (14-98 kg/cm^2) and steam (Pressure 5 - 7 kg/cm^2) injection. Depending upon the characteristics of the feed mixture and moisture content, the pressure develops before the material passes through the die. Because of this the temperature rises and the material is forced through the die and the pressure suddenly drops. The temperature of the material rises to 110 - 130°C for a short spell of time and cooks the food, gelatinizing the starch present in the feed mixture. This imparts good binding and water stability to the resultant pellets. However, the pellets expand as they come out of the die due to sudden drop of pressure and air gaps develop inside the pellet, which makes them float or sink very slowly. This is an excellent process for producing floating pellets for finfish culture. By adjusting the pressure in the barrel and moisture in the feed, it is possible to prepare sinking pellets by extruder. The new generation extruders are made with twin screw-barrel arrangement, which are more suited for sinking pellet feed manufacture. The resultant pellets are dried as the moisture will be high.

The characteristics of extruder pellets are

1. Reduction in pellet disintegration and loss in water.
2. Increases starch digestibility due to good cooking

3. Can be worked with higher moisture and oil (fish oil) levels in the feed.
4. Extruder pellets float or sink slowly. The texture of pellet surface may not be smooth
5. Due to higher working temperature, disintegration of vitamins such as A, B, C, D, E and pantothenic acid in feed may take place.
6. Making charges for extruder pellets are higher due to high cost of extruders

Pelletizer technology

Pelletizer is primarily used for making sinking pellets. The basic principle of pelletizer is that the finely ground feed mixture is pelleted by compression process. The main components are a pair of rollers and a die, which are driven by a high-speed motor. The pelletizer works with a combination of high pressure (42 - 1800 kg/cm^2) between rollers and the die, steam (0.5 - 3.5 kg/cm^2) and moderate temperature (75 - 95°C). Moisture is the limiting factor in the pelletizer. It works satisfactorily at 15% moisture and higher moisture levels choke the die. Because of this reason starch present in feed cannot be fully gelatinized for binding. Hence, additional binder, which works on the principle of thermo plasticity, has to be used. Conditions for proper reaction between binder and feed ingredients during pelleting should be standardized. Several steam conditioners in series are used to prolong contact between steam and ingredients for producing pellets with good water stability.

Wet pelletizer

For small and laboratory scale production of feed pellets, wet pelletizer which can work with high moisture levels (30%) is used. This is similar to noodle or spagatti making machine. Moist pellet can be successfully produced in this machine. Feed mixture is wetted with water (30%) and steam cooked in batches and passed through the wet pelletizer. Starch is well gelatinized and acts as an effective binder in this process. Because of higher moisture content, the pellets should be dried for longer period.

Drying

After pelleting, the feed should be dried to reduce the moisture content below 10%. This is essential for good shelf-life of the feed. Different types of dryers are used for

drying feed pellets. There are horizontal conveyer type, vertical hopper type and fluid bed dryers . Dry steam or hot air (heated either electrically or otherwise) is used for drying feed at temperatures 70-80°C. Higher temperature is not desirable. Feed pellets with low moisture obtained through a pelletiser should be dried in cooler dryer.

Packing

The dried feed is cooled before packing. Polythene lined high guage paper or HDP bags are used for packing shrimp feed to prevent damage to the feed quality during transit and absorption of moisture on storage.

Incorporation of special additives

Feed components that are heat sensitive and are likely to be destroyed during processing are sprayed on to the finished product. Vitamins, flavour and feed attractants are some of the additives come under this category. These are added to the feed in liquid form either based in oils or aerosols or sprayed on to the pellets.

Quality control and storage

Storage of shrimp feed is of paramount importance. Absorption of moisture and atmospheric oxidation of PUFA rich lipids are two important factors, which can deteriorate quality of the feed. Moisture absorption, especially, in high humid conditions leads to mould growth which can contaminate the feed with aflatoxin which are toxic to shrimp. Lipid oxidation leads to accumulation of peroxides, which are also toxic and render the feed rancid. Preservatives such as calcium propionate and antioxidants like ethoxyquin, BHA (Butylated hydroxy anisole) and BHT (butylated hydroxy toluene) may be used. Besides, the feed should be stored in cool, dry and well ventilated premises.

Feedstocks may be best stored for 3 to 4 weeks safely. However, storage of feed for longer period may be resorted at lower temperatures of 10°C or below.

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CHAPTER – 9

BROODSTOCK NUTRITION AND FEEDING

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Introduction

One of the most important and fundamental approached to aquaculture development is to ensure a year-round, rather than seasonal, supply of high quality stocking material. For this purpose, broodstock that produce good quality fertile eggs with high hatchability, survival and growth rates must be maintained and nurtured in captivity. The nutritional status of broodstock thus maintained has a profound effect on the gonadal growth, maturation, spawning and fecundity. The quality and quantity of feed as well as the feeding regime are important. However, precise information on the nutritional requirements for gonadal maturation in various species of cultivable aquatic animals is scanty. Most of the works are on freshwater fishes and information on brackishwater and marine species are very meager.

Major dietary nutrients for broodstock (energy, proteins & lipids)

The energy costs of reproduction are three fold –

Energy cost for production of primary sex products (egg & sperm).

Energy cost for development of secondary sexual characters such as breeding colours, morphological features, release of pheromones, secretion of mucus for attaching eggs or nest building. Energy cost of reproductive behavior such as migration, territorial defense of spawning area, courtship behavior and parental care.

Dietary energy is partitioned and utilised for maintenance, growth and reproduction. The maintenance requirements of an animal are met first and then excess energy is divided between growth and reproduction. The relative partitioning of energy between growth and reproduction varies according to species (viviparous, oviparous, parental care), sex (males require less energy than females) and age (juveniles use more energy for somatic growth).

Reproductive effort is the proportion of the energy income of the fish that is devoted to reproduction at the same time reducing the energy available for body maintenance and somatic growth. Non-reproducing females use the dietary energy for somatic growth and also for storage (lipid).

In fishes having short life span and having only one breeding opportunity, the total energy obtained from the diet is used for both somatic growth and gonadal growth. When food becomes inadequate, the ovaries continue to grow at the cost of body growth. On the other hand, in long-lived species of fish (e.g. winter flounder, *Pseudopleuronectes americanus*), at low feeding levels, the somatic growth is favoured over the ovarian growth, which would delay reproduction. Brackishwater cultivable species such as mullet, milkfish, seabass and grouper fall under this category. Research has shown that in rainbow trout, ration size and feeding rate influence the size of eggs, number of eggs produced per female, and quality of eggs produced. Females fed low-ration (0.4% of b.wt/day) had lower fecundities and fewer fish produced mature gamates than those fed high-ration (1 %). Fishes that are under-fed spawn at a smaller size and there can be reduction in number of fish reaching maturity and delay in onset of spawning. The energy cost associated with maturation of testes is much less compared to ovarian maturation. Diana (1983) estimated that in pike (*Esox luscious*, a freshwater carnivore), the annual reproductive effort of males aged between 1 and 3 years was 7 to 10% and for females, it was 14 – 16%. In their first year of life, the pike allocated about 42% of their energy income to growth but after sexual maturation, less than 10% was allocated for growth.

The mean energy content of newly spawned eggs or ripe ovaries for 50 teleost species is 23.48 kilo joules per gram dry weight (Wootton, 1979).Since most of the currently cultured species are oviparous (i.e., fertilization and early development occur externally) and the embryo is free-living, it must be endowed with all the nutrients required for complete development. This is accomplished by packing in the egg all the nutrients (protein, lipids) needed for metabolism and tissue formation. These materials are synthesized and placed in the egg during the process of vitellogenesis, which occur prior to ovulation. Vitellogenin is a very high-density lipoprotein containing about 80% protein and 20% lipids (mainly phospholipids) synthesized by the liver and rich in essential fatty acids. It is a precursor of the two egg

components, lipovitellin and phosvitin.On the other hand, sperm does not contain the quantity of materials that ova do, and, consequently, the energetic 'cost' for production is lower.

Generally, the optimal dietary protein level for growth is also the optimal level for reproduction. This has been demonstrated in *Oreochromis niloticus* (DeSilva & Radampola, 1990). Amino acid requirements of broodstock fish are also generally similar to that for optimal growth. In a classic study of Red sea bream, *Pagrus major*, Watanabe *et al.* (1984) found that a dietary protein level of 45% was optimal for (a) number of egg produced, (b) number of viable eggs and (c) number of larvae hatched out. They also demonstrated that
replacing white fish meal with cuttle fish meal, remarkably increased egg viability, hatching rate and percentage of normal larvae (Table 1). Since dietary protein quality has a significant influence on the success of reproduction, broodstock diets should contain good quality protein. Similarly, Cumaranatunga and Thabrew (1989) have shown in Nile tilapia that feeding fishmeal resulted in better ovarian growth and larger oocytes than legume meal. This is due to higher levels of vitellogenic proteins and lipids in fishmeal. Marine oil supplements are necessary in broodstock feeding. Fish are incapable of synthesizing linoleic (18:3 ω6), linolenic (18:3 ω3), arachidonic (20:4 ω6), eicosapentaenoic (EPA) (20:5 ω3) and docosahexaenoic (DHA) (22:6 ω3) highly unsaturated fatty acids and are therefore essential fatty acids (EFA). Since these HUFA affect growth and survival of fishes, the quality of the eggs are also affected if the mother fish receive EFA deficient diets. EFA deficient diets fed to red sea bream gave very few buoyant eggs (Table 1). Similarly, substitution of corn oil in place of squid liver oil deteriorated egg quality. In case of *Siganus*
guttatus, fishes that received Pollack liver oil in their diet laid eggs for more than five months but did so only for two months when fed diets without Pollack liver oil.
Fish eggs contain a large amount of phospholipids in their yolk. It is therefore advisable to include a source of phospholipids such as lecithin or soybean oil in the diet of broodstock.

Trace nutrients for broodstock (vitamins, minerals & pigments)

Vitamin E plays an important role in reproductive physiology in fish as it does in birds and mammals. This has been confirmed in freshwater Ayu (*Plecoglossus altevilis*),

Carp (*Cyprinus carpio*) and rainbow trout broodstocks. Dietary vitamin E in the broodstock diet is mobilized and transported to the oocytes wherein it greatly helps in hatching and survival of the young. The concentration of this vitamin is high in the eggs and low in the tissues of the

female spawner after breeding. During larval development, the level drops rapidly. Hence the importance of dietary vitamin E is more during early maturation of the female fish and less critical during final maturation and spawning activity. The Gonado-Somatic Index in carp fed α-tocopherol deficient diet was drastically reduced and the ovaries contained oocytes without yolk granules or yolk-vesicles. Low and deficient levels of trace elements such as manganese, zinc and iron lowered the percentage of both eyed and viable eggs in 1981). Phosphorus deficiency in red sea bream broodstock resulted in increased deformities in the rainbow trout (Takeuchi *et al.*,larvae (Watanabe *et al.*, 1984). It has been demonstrated that if the parent fish are fed diets containing pigments such as β-carotene, canthaxanthin, asthaxanthin or natural pigment sources such as krill oil there is good improvement in the egg quality, particularly if fed just prior to breeding (Table 2). This is particularly applicable for fishes that continue to accept feed even while breeding.

A summary of the known nutritional requirements of fish broodstock is given in Table 3.

Brackishwater fish broodstock nutrition and feeding

Mullet

Mullet broodstock reared in outdoor earthern ponds obtain their nutrition both from the supplementary feed provided and other algal and diatom communities on which they graze. Shehadeh *et al.* (1973) reported that the feaces of *Mugil cephalus* broodstock contained 52% algae (*Lyngbya*), 17% diatoms (*Navicula*) and 31% sand. Mullets are bottom feeders. Rangaswamy (1973) also reported that the stomachs of *M. cephalus* from Pulicat Lake consisted of sand grains (51.9%), decayed organic matter (32.9%), diatoms (6.7%), dinoflagellates (2.5%), foraminifera (2.7%), algae (1.5%) and other miscellaneous items

(1.8%) indicating their bottom feeding habit.

In tanks (RCC or polythene lined), where access to the bottom soil is not possible mullets can be made to attain maturity solely on the nutrition from the formulated feeds.

They readily accept moist feed ball containing groundnut oil cake and rice or wheat bran in equal proportions. At SEAFDEC, grey mullet broodstock have been successfully maintained and bred using commercial trout pellets (Purina) fed at 2 % of the fish body weight. Nash and Shehadeh (1980) fed *M. cephalus* broodstock formulated feed pellets containing wheat middling, cottonseed meal, soybean meal, fishmeal and vitamins (Table 4). The composition of mullet eggs is a good indicator of the spawners nutritional requirement. The amino acid and fatty acid profile of spawned eggs of *M. cephalus* is given in Table 5, which shows that even though the fatty acid content of the trout feed provided was much lower than that in the eggs, it was adequate to provide the nutritional needs of the maturing fish. Female *M. cephalus* have the tendency to accumulate lipids in their muscle and liver and in the form of adipose tissue in the body cavity and around the intestine during the active feeding period before the onset of breeding season. This stored form of energy is mobilized for maturation of the ova and formation of the yolk (vitellogenesis). Relatively less storage lipid is seen in males since the energy requirements for testes maturation and production of sperms is less.

Majority of adult mature mullets caught from the wild during their breeding season (October to February) appear to have reduced feeding activity due to the distended gonads in their abdominal cavity. They would not accept feed due to the stress and changed environment. It is therefore advisable not to provide any feed. At CIBA the captive stocks of *M. cephalus* is fed on a specially formulated sinking feed pellet of 3 mm diameter, having 30 to 33 % good quality plant and animal proteins and 5 to 8% lipid (fish body oil). The feed formula is given in Table 6. This feed is given at the rate of 5 % of total fish biomass once daily. The given feed is invariably consumed within 30 minutes. This feed was found to be highly satisfactory with about 70% of the stock attaining maturity.

Milkfish

Milkfish (*Chanos chanos*) feeds on phytoplankton but readily accepts artificial feeds in confinement. Studies on feeding of milkfish broodstock with artificial diets are meager. Commercial fish diets and formulated diets have been used in the development of milkfish brood stocks (Liao and Chen, 1979; Lee, 1983; Marte *et al.*, 1984). A variety of feeds including rice bran, wheat flour, soybean meal and formulated eel feed are fed to

milkfish broodstock given at the rate of 5 % biomass once a day (Kuo, 1984; Lin, 1985). Occasionally algae, yeast and vitamin E and B were also given. Milkfish that matured and spawned naturally in SEAFDEC's floating cages were fed crustacean feed pellets containing 42 % protein (Lacanilao and Marte, 1980). Natural and induced maturation, followed by either spawning or stripping, has also occurred in milkfish fed Purina trout chow and compound feeds containing 32 – 40 % protein (Lee *et al.*, 1986; Liao and Chen, 1984). Lam (1984) suggested that a high protein diet might be important for milkfish maturation and spawning. Crear (1980) found that brine shrimp were a major component of the diet of mature milkfish at Christmas Islands.

Seabass and grouper

In Malaysia, Seabass (*Lates calcarifer*) spawners are fed small low-grade fish (not trash fish) at a rate of 2 % body weight once daily. These small fishes are without spines and with soft scales. The feed fish is washed and rinsed with the head portion and intestine intact and fed to broodstock (Ali, H. M., 1986). Ponds stocked with seabass were given trash fish once a

day (afternoon) by Kuo (1984). Kungvankij (1986) fed seabass brood fish with fresh cleaned trash fish given daily at the rate of 5 % of total biomass. Maneewong (1986) mentioned that sardine or anchovy with intestine and head removed are used for feeding after chopping into bite-size pieces at 1 % of body weight once a day in the morning.

In the case of European Seabass, *Dicentrarchus labrax*, feeding squid a few times each week starting two months prior to spawning help in obtaining high quality eggs. Dietary lipids have been found to be important because long-term deficiencies in ω3 HUFA can induce early gonadal atresia, lower fecundity and subsequent reduction in egg survival. Other trace nutrients such as vitamin C and E and carotenoids are also very important. The overall requirements for trace nutrients are satisfied by a mix of high-quality food items such as fresh squid, cuttlefish, shrimp, krill and fish. Marine lipid and vitamin supplements may be included. Although semi-moist and dry compounded diets are sometimes used as maturation diets for this species, they are generally accompanied by a fresh component such as squid, shrimp or fish. The composition of experimental broodstock diet for seabass and grouper (Meyers, 1987) is given in Table 7. At CIBA's Muttukadu Field Centre, Seabass and Grouper (*Ephenephalus tauvina* and *E.*

malabaricus) are maintained in separate 100 tonne RCC tanks and fed *ad lib* on frozen whole
tilapia. Feeding time is usually after tank cleaning and water exchange (11.00 – 12.00 hrs). The excess food, which falls to the bottom of the tank, is removed.

Shrimp broodstock nutrition and feeding

Nutrition plays a major role in shrimp reproduction. The nutritional status of the shrimp will undoubtedly affect their reproductive potential. A number of aspects of reproduction are affected, by deficiency feeding, such as, the time to first maturity; fecundity; egg size and egg quality (egg composition, hatchability and larval survival).The criteria generally used to assess the reproductive performance of shrimps are –

Spawning frequency
Percentage of shrimps that spawn
Spawns per shrimp
Fecundity
Hatch rate
Larval survival and Protozoea length

Even though about 20 species of shrimps are being matured and spawned in captivity, the nutritional requirements for penaeid shrimp reproduction are not clearly defined. Shrimp broodstock collected from the wild and cultured broodstock often show variable responses in the hatchery. The reasons are not easily explainable but indicate some nutritional differences in the stocks. This lack of knowledge is an obstacle for the development of dry broodstock feed which would provide the entire requirement of the animal and aids the production of high quality nauplii, consistently. Therefore in commercial hatcheries, the conventional diets offered to shrimp broodstock and spawners consist of fresh or frozen seafood such as shrimp, squid, crab, fish muscle, *Artemia* biomass (brine shrimp), mussel, oyster or clam meat, bloodworms and polychaete worms. The shrimp spawners prefer these fresh feeds and they often exhibit excitation when worms are placed in the tank. Though these items are expensive, particularly polychaete and bloodworms, they are all rich in protein. Squid contains high levels of sterols whereas worms are rich in long-chain, highly unsaturated fatty acids (HUFA). Such fresh diets do have disadvantages of varying nutritional

quality, unpredictable supply problems and may also deteriorate the water conditions in addition to
introducing disease causing pathogens.

Artificially formulated dry diets for shrimp broodstock on the other hand offer many advantages – reliable supply, minimal preparation time, known nutrient content and reduced tank fouling. It also offers and opportunity to orally administer drugs, hormones or vitamins. Studies on the specific dietary nutrient requirement of shrimp broodstock are scanty. Lester (1990) studied the effect of varying dietary lipid levels ranging from 7.8% to 13.9% on reproductive performance of *Penaeus stylirostris* and found that dietary lipid levels affect reproduction in terms of number of females mating, hatch percentage, number of nauplii per spawn and length of protozoea I. His results indicate that a total dietary lipid level of 10 to 11 percent is better than 7.8% and 13.9%. Marsden and his coworkers (1997) formulated and tested an artificial moist diet (Table 8) in comparison to control diet consisting of squid and mussel meat. The moist diet improved significantly spawning frequency and larval survival, these parameters were found to be more sensitive to nutrition. However, no effect was seen on fecundity or hatch rate. Protein and lipid requirements of broodstock shrimps are generally higher than that required for maximum growth. The general dietary levels recommended for shrimp broodstock is given in Table 9, that can be used as guideline for preparation of broodstock feeds. Commercially available shrimp broodstock pellets can also be used but preferably limited to 50% of the total diet. Feeding more than 50% may lead to decrease in reproductive
performances. Lester (1990) compared the effects of combinations of commercial feed and fresh feeds on reproductive performance of *P. stylirostris*. His treatments were, A commercial feed alone.
Commercial feed + 40% fresh diet of squid. Commercial feed + 40% mixed fresh diet of squid, bloodworms, shrimp & brine shrimp. Mixed fresh diet of squid, bloodworms, shrimp & brine shrimp (equal proportions). He found that the mixture of fresh diet (Diet 4) was the best followed by Diet 3 which
contained dry feed and mixed fresh meat. The daily feeding rate in hatcheries is 15 to 25 % of shrimp broodstock body weight given in four installments. If commercial dry diet is used, it may be given during daytime.

Concluding remarks

Fresh diets offered to broodstock of fish and shrimp have many disadvantages but they are indispensable at the present level of knowledge. Most of the cultivable aquatic species of interest are cultured for market exclusively on artificial formulated feeds. However, when they are maintained for breeding purposes the emphasis is on the use of natural fresh feeds. The fastidious nature of the species concerned and the many unknown nutrients in natural feeds has made their use a regular feature in broodstock husbandry. The use of compounded dry feed can at the most be up to 50% only, without affecting performance. The overall husbandry conditions also, such as, stress, water quality, rearing temperature, photoperiod and management procedures in addition to the nutritional aspects, determine the success of the broodstock in terms of reliable seed productions.

Table 1: Effect on the spawning and egg quality of red sea bream *Pagrus major* of broodstock diets of different composition (Watanabe *et al.*, 1984).

	Control	Low protein	Lowphosphorus	EFA deficient	Cuttle fish meal
	Fishmeal 45 % CP				
Egg					
Egg produced (x 04/fish)	100.5	72.7	84.1	116.5	173.5
Buoyant gg %	80.9	54.4	62.1	23.9	88.5
Abnormal egg %	30.7	70.7	67.9	93.7	2.7
Av No. of oil globules	1.7	3.5	3.1	6.2	1.0
Hatched larvae					
Rate of hatching %	69.4	23.6	26.3	0.9	93.9
Deformity %	23.3	84.1	75.5	-	1.9
Normal larvae %	62.4	3.8	6.2	-	97.6
Final productivity of fish seed from total eggs produced %	24.3	0.1	0.3	-	78.9

Table 2: Effect of broodstock diets of different composition fed before breeding season on the spawning and egg quality of red sea bream (Watanabe *et al.*, 1984).

	High protein (fish meal)	Fish meal Cuttle fish meal	β-Carotene + Canthaxanthin	Krill oil extract	Frozen raw Krill	Corn oil
	55 % CP, 10 % lipid	45 % CP, 10 % lipid	0.1 % + 0.3 %	9 %		10%
Egg						
Egg produced (x 10^4/fish)	149.5	121.6	120.4	90.1	202.1	58.7
Buoyant egg %	49.1	68.6	56.4	69.6	82.7	18.2
Abnormal egg %	77.5	22.1	37.0	20.9	8.1	94.0
Av No. of oil globules	2.4	1.5	1.8	1.2	1.1	3.4
Hatched larvae						
Rate of hatching %	83.1	93.7	77.4	67.5	90.3	27.3
Deformity %	14.8	4.7	15.0	8.4	2.0	43.2
Normal larvae %	51.6	82.2	74.3	88.2	91.2	24.0
Final productivity of fish seed from total eggs produced %	21.1	52.8	39.1	41.4	68.1	1.2

Table: 3 Summary of the known nutritional requirements of fish broodstock. [Source: Kanazawa (1988)]

Species	Requirement
Ayu (*Plecoglossus altevilis*)	Vitamin E increases spawning success, egg survival, hatchability and larval survival. Requirement in broodstock is 34 mg/kg diet. Phosphorus increases spawning success. 20:5(ω3) and 18:3(ω3) fatty acids are probably required.
Carp (*Cyprinus carpio*)	Vitamin E increases GSI and is required for vitellogenesis and for proper maintenance of ω6 fatty acids in oocytes. ω3 fatty acids are probably required.
Rainbow trout (*Oncorhynchus mykiss*)	Based on transfer of nutrients to eggs, requirements are 10000 – 20000 IU/kg diet for vitamin A, 100 mg/kg diet for Vitamin E although lower for vitamin D. Low-protein/high-energy diets are as good as high-protein/low-energy diets for broodstock development. Trace minerals, particularly manganese, are required. 20:4(ω6) and 18:2(ω3) fatty acids are EFAs for broodstock. EFAs are also required for high quality sperm.
Red sea bream (*Pagrus australis*)	Vitamin E is required and probably has the same effect as for ayu. ω 3 fatty acids are required for buoyant (viable) eggs. β-Carotene and other carotenoids are important for egg viability. An unknown component in cuttlefish meal enhances spawning success.

Table 4: Ingredient composition of two feeds used for *Mugil cephalus* broodstock (Source: Nash & Shehadeh, 1980).

Ingredients	Level of inclusion
DIET 1	
Wheat middlings	55 %
Cotton seed meal	14 %
Soy bean meal	14 %
Tuna fish meal	14 %
Propylene glycol (antifungal)	1.4 %
Vitamin B complex	1.4 %
Vitamin Mix	0.2 %
DIET 2	
Wheat middlings	4 parts
Soy bean meal	1 part
Fish meal	1 part
Dried *Ulva*	1 part

Table 5: Amino acid and fatty acid profile of spawned eggs of *M. cephalus* reared in outdoor seawater tanks (Tamaru *et al.*, 1992).

Amino acid profile (mg/100 mg dry wt)		Fatty acid profile (mg/100 mg dry wt)		
			Spawned eggs	Purina trout chow ***
Thr	2.77	14:0	0.21	0.54
Cys	0.91	16:0	1.64	1.34
Val	2.96	16:1ω7	3.21	0.51
Met	1.05 *	18:0	0.42	0.40
Ile	2.36	18:1ω9	4.27	0.95
Leu	4.42 **	18:2ω6	2.15	0.92
Phe	2.53	18:3ω3	0.15	0.13
His	1.29	18:4ω3	0.07	0.10
Lys	3.77	20:1ω9	0.13	0.06
Arg	3.16	20:4ω6	0.40	0.07
		20:5ω3	0.58	0.61
NEAA	23.52	22:1ω11	0.06	0.00
		22:6ω3	1.51	0.50
Total protein	48.47	Total	14.80	6.13

lowest EAA
highest EAA
* floating pellet (fed at 2% of fish body wt.)

Table 6: CIBA mullet broodstock feed formulation.

Ingredients	Percent Composition
Fish meal	15
Soya flour	17
Groundnut oil cake	20
Cotton seed cake	5
Wheat flour	15
Maida	2.2
Lecithin	1
Fish oil	5 (1 % in low-lipid feed)
Vit/Min mixture	2
Additives	0.705
Wheat bran	Qty. to make up to 100

PROXIMATE COMPOSITION:

	High-Lipid	Low-lipid
Crude protein %	31.61	31.33
Crude lipid %	8.83	5.21
Crude fibre %	8.22	9.72
Total ash %	14.11	14.29
Moisture %	7.60	8.4

Table 7: Ingredient composition of experimental broodstock diet for seabass and grouper (Source: Meyers, 1987).

Ingredients	Level of inclusion	
Fresh ground fish	46	%
Fish meal (local)	20	%
Extracted soybean meal	12	%
Wheat pollards	4	%
Fresh mussel meat	4	%
Fish oil	4	%
Soybean lecithin	3	%
Vitamin mixture	2	%
Seaweed binder	5	%
Composition of vitamin mixture		
Thiamine HCl	120	mg/kg dry diet
Riboflavin	40	mg/kg dry diet
Pyridoxine HCl	120	mg/kg dry diet
Cyanocobal amine	0.02	mg/kg dry diet
Folic acid	5	mg/kg dry diet
Niacin	150	mg/kg dry diet
Calcium pantothenate	100	mg/kg dry diet
Biotin	2	mg/kg dry diet
Vitamin C (Sodium ascorbate)	1000	mg/kg dry diet
Inositol	800	mg/kg dry diet
Choline chloride	1200	mg/kg dry diet
Vitamin A	5000	IU
Vitamin D	1000	IU
Vitamin E	200	mg/kg dry diet
Vitamin K	40	mg/kg dry diet

Table 8: Artificial moist diet for shrimp broodstock (Marsden *et al.*, 1997).

Ingredient	G/100 g dry diet
Squid (*Loligo* sp.) meal	41
Minced mussel (*Perna canaliculatus*)	22
Calf liver	11
Artemia enrichment (dry Selco)	4
Binder mix	10
Milled mollusc shell (*C. deltoides*)	2
Lecithin	3
Vitamin mix *	5
Mineral mix **	3
Cholesterol	1.1
Asthaxanthin	4×10^{-3}
β-Carotene	4×10^{-3}

Vitamin mix	Mg/100 g diet
p-amino benzoic acid	15.80
Biotin	0.63
Inositol	632.00
Nicotinic acid	63.20
Ca-pantothenate	94.80
Pyridoxine HCl	19.00
Riboflavin	12.60
Thiamin HCl	6.32
Folic acid	1.26
Cyanocobalamine	0.13
Choline HCl	948.00
Menadione	6.34
Na-ascorbate	3160.00
Calciferol	1.90
Tocopherol	50.00

Mineral mix	G/100 g diet
K_2HPO_4	0.70
$Ca_3(PO_4)_2$	0.95
$MgSO_4.7H_20$	1.10
$NaH_2PO_4.2H_20$	0.28

Proximate composition (% dry matter)

	Expt. Moist diet	Control (Squid : mussel = 1.3 : 1)
Dry matter	21.5	20.0
Crude protein	54.6	73.5
Ash	17.0	8.2
Lipid	10.7	7.8
n-3/n-6 ratio	4.5	45.1

Table 9: Recommended dietary nutrient levels for omnivorous shrimp {Source: BOBP & Tacon (1987)}.

Nutrient level	Grower (10g to harvest)	Broodstock (> 10 g +)
Crude Lipid %	10	10
Marine:Plant lipid *	5:1	5:1
Cholesterol % **	1.0	1
Crude Protein (% min)	35	45
Carbohydrate (% max)	35	25
Crude Fibre (% max)****	3	2
Major minerals		
Ca(% max)	2	2.5
P Av.(% min)	1.2	1.4
K (% min)	0.7	0.9
Mg (% min)	0.08	0.13
Trace Minerals (mg/kg, min)		
Fe	60	100
Zn	80	120
Mn	40	60
Cu	8	12
Co	0.8	1.2
I	4	6
Cr	0.6	1
Se	0.17	0.25
Vitamins (mg/ kg, min) ***		
A (IU)	8000	12000
D3 (IU)	2400	4000
E	240	400
K	10	14
Thiamin	66	90
Riboflavin	66	90
Pyridoxine	66	90
Pantothenic acid	220	300
Nicotinic acid	330	450
Biotin	0.54	0.75
Folic acid	12	18
B12	0.08	0.12
C	1500	2500
Choline	2400	3200
Inositol	1500	2100

* Marine lipids - shrimp head oil, marine fish body oil, marine fish liver oil, marine invertebrate oils. Plant lipids - soya bean oil rich in phospholipids or soy-lecithin

** purified cholesterol or natural source (shrimp-head oil).

*** suggested vitamin levels are 2 –5 times greater than the recommended dietary requirements to compensate for the loses due to processing, storage and leaching.

**** excluding chitin

CHAAPTER - 10

NUTRITION AND FEEDING OF LARVAE OF SHRIMP AND FINFISH

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INTRODUCTION

The growth and survival of every organism depends on the type of nutrition it receives in its life cycle. The requirement of nutrients varies throughout the life cycle of an individual. At early stages the requirements of nutrients is comparatively high which declines with age which is related to the basal metabolic rate. Also the requirements depend upon the feeding habits that change accordingly to the morphology of digesting organs and processes. The classic examples of varied nutritional habitats are seen in crustacean predominantly which distinctly change at every metamorphism in the life cycle.

Larval nutrition in finfish and shellfishes had been the most studied because of its commercial importance as against other species. Their study had been most critical, complicated and cumbersome in nature. Each species has its own preferences for food which in most cases is the reflection of their adult hood feeding habits. The larval stages in certain cases a quick one i.e. one after another is completed in hours (crustacean), minute size and abiotic factors which significantly affect the growth and survival rate. All these problems hinder in the understanding of the performance of the formulated feeds on the larvae.

According to Kuronuma and Fukusho (1984) the principles guiding the feeding of larvae in tanks or enclosures, are:

- that the food given is consumed completely
- that the food is well digested, keeping the animal healthy and growing normally, and
- that the production and supply of this kind of food is economically feasible.

On the basis of the above criteria, four larval or 'hatchery' feeding strategies are currently available for the mass propagation of marine fish and shrimp larvae, through metamorphosis, to the post-larval or fry stage. These include:

- The exclusive use of a succession of live planktonic food organisms (ie. algae, diatoms, flagellates, yeasts, rotifers, copepods, brine shrimp nauplii and metanauplii).
- Use of selected live and/or frozen plankton in conjunction with 'fresh' and/or frozen fish, mollusc or crustacean tissue preparations.
- Use of selected live and/or frozen plankton in conjunction with dry feed materials or formulated complete artificial diets.
- The exclusive use of microencapsulated, microparticulate or flaked complete artificial larval diets.

Generally, the finfishes and shellfishes larval nutrition mainly depends on two main processes:

Morphological processes **Digestive processes**

MORPHOLOGICAL PROCESSES

Studies have shown that there are diversified morphological differences in larval and adult stages. The first and foremost differences is the mouth size changes which effect the capacity of the fish / shellfish to ingest food. Success of survival ability of the organism depends on the selection of particle size in accordance with the mouth size. Certain species of fish and shellfish are unable to even feed on live *Artemia* nauplii or even nauplii of rotifer which are smaller (95-350um) (Nellen, 1986). Apart from the size of the mouth in larval stages, the digestive tract morphology has significant role in determining the specific feed requirement. The digestive tract is simple, relatively short gut and epithelial cells show no regional differentiation in the lining of digestive tract which makes the organism more sensitive towards feed preferences. Most of the epithelial gut lining shows absorptive enterocytes with many microvilli on their luminal surface with few secretory cells.

With the onset of exogenous feeding, marked morphological changes take place in the larvae. There is gradual initiation of mucosal fold and development of regional differentiation in the intestinal region. Micromolecules (protein / lipid) of nutrients are absorbed by pinocytosis (engulfing of molecules by surface membrane epithelial cells) and the nanomolecules are intercellular broken down and absorbed. In shellfishes, the larvae subsist on embryonic food initially. Subsequent larval stages depend on the filtering mechanism mostly phytoplankton – marine alga (*Chaetoceros sp., Skeletoema* sp., etc.) depending upon their preferential feeding status. Later larval stages start feeding on live organisms (Rotifer or *Artemia* sp.) by holding like the adult forms Morphologically the digestive tract is very simple and nutrients are usually absorbed by method of pinocytosis. Once the exogenous feeding starts the digestive tract morphologically develops and enzyme secretion initiates. Fishes with teeth start developing them and the intestinal coiling starts simultaneously (Stroband and Dabrowski, 1980). Thus, the mucosal development in larvae facilitates greater digestion with the help of more digestive enzyme secretion from the gastrointestinal tract. These developmental changes are visible with the increasing feeding frequency and prey size.

DIGESTIVE PROCESS

It is well known that in early phase of larval development, the secretion of digestive enzyme is limited because most of the epithelial cells are absorptive. Subsequent development of gastrointestinal complexity is correlated with changes in the activities of digestive enzymes. In case of most fishes, the enzyme activities depend on the development of gastrointestinal tract. Certain enzymes like trypsin level increases upto day 12 and decreases to day 16. Thereafter it increases to day 25. Similarly, pepsin which is active at low pH, increases from day 16 onwards. However, enzymes like chymotrypsin and amylase activity largely remains unchanged in fishes throughout the larval development. Thus, the relative gut length, gut passage rate, tryptic activity and the ability to reabsorb digestive enzymes in the hind gut –all increased with age from larvae to adults stage. It has been established that proteolytic activity increased when exogenous proteases from live prey contribute in the stomach of larvae (Lauff and Hofer, 1984; Walford and Lam, 1993). Studies on barramundi seabass (*Lates calcarifer*) larvae shows that the exogenous proteolytic enzymes and endogenous trypsin secretion induced by the ingested live food is sufficient to cause

rapid breakdown of rotifers. As it is known that during the early stages due to short larval gut length and retention time of the food, the extracellular digestion based on the tryptic activity may be insufficient to achieve complete hydrolysis of the protein. These exogenous enzymes not only help in digesting the food but also activate the endogenous enzyme activities by cleaving the inactive zymogens form.

So these studies on barramundi and other fishes it is concluded that exogenous enzymes help in compensating to a certain degree in addition to the pinocytotic activity occurring at a high degree in the rectal cells which tend to absorb protein macromolecules which are subsequently digested intracellularly. So considering all these drawback in digestion mechanism in larval cycle, development of larval diets which are species specific with better efficacy in terms of maximum nutrient availability at minimum loss is one of the challenges faced by nutritionists World wide.

FEEDING BEHAVIOUR

The success of a hatchery largely depends on the larval nutrition. Despite of its importance in the overall growth, survival and development of the aquatic larvae, little is known about the absolute nutrient requirement. As stated earlier, the basic problem of lack of knowledge lies in the smallness of the organism associated with its handling sensitivity and weighing (or measuring). In addition to this, most species larvae depend on live feed for which they depend on their vision – an important aspect for capturing the live prey (Sbikin, 1974). So inducement for artificial feeds is quite difficult as these feeds are more or less static which denies the acceptance by the larvae. However best formulated feeds are given to the larvae, there is poor acceptance, conversion, growth and survival compared to live feeds. Now how far the visual parameter helps in the ingestion of food by larvae is still unresolved completely. Also studies have been shown that amino acids of live food are catabolised at a lower rate (i.e. easily available and assimilated) and so used in greater extent for protein synthesis As against the artificial feeds (dry diets), the digestion and assimilation is energy consumption process and so poor efficiency and growth is invariably obtained. But in hatchery operations there is a necessity of using artificial feeds when secondary cultures of food organism (live feeds) unpredictably collapse. Even then major hatchery operations prefer to depend solely on live feeds only.

NUTRIENT REQUIREMENTS

Unlike the larger size groups, larval nutrient requirements had been more elusive for most culturists. Ultimately they had to depend on live feeds and thus it becomes indispensable. However, larval nutrition had attracted most researchers for its challengers it poses.

Most larval feeds available for various species are not complete replacement for live feed but effectively are used as supplement However most studies have shown the effect of enriching live food organism with various nutrients usually fatty acids (Lavens *et al.*, 1991). This enrichment helps in higher production of fish / shellfish. Studies (Kanazawa, 1991 b, Wilson 1991, Kissil 1991, Cho & Convey 1991, Luquet 1991) on larvae showed that there is higher requirement of protein and fatty acids than in adults. Over fortification of larval diets with vitamin has shown better results of survival (Wilson 1991).

Amongst the live foods that have been most intensely investigated with respect to their nutritive suitability are the brine shrimps and rotifers. In brine shrimp *Artemia* species; the main factor affecting its quality as a food source for marine larval organisms is its content of the omega 3 (ω3) Essential Fatty Acid {Eicosapentenoic acid (EPA) and Docosahexaenoic acid (DHA)}. Simple methods have been developed (Lager et al., 1986) to incorporate particulate products by having the nauplii consume particles of a desired composition. This bioencapsulation is known as *Artemia* enrichment or boosting. Similarly, methods have been evolved to enrich rotifers by culturing them in specific media such as ω – yeast by feeding with a mixture of homogenized lipids and Baker's yeast or a marine alga – *Chlorella* spp., all of which are rich in ω 3 PUFA. Such enriched feed improves the quality of seed, survival rate and growth (Guillaume *et al.*, 1991, Hung, 1991).

Amongst the EFA – EPA/DHA, are the most important for larval stages and their quantity varies from species to species. In most species the larvae have specific type of these EFA requirement. Studies on Mahimahi (Coryphyaena hippurus) showed disproportionately conservation of DHA in starved fish larvae and when fed *Artemia* enriched with DHA had greater stress resistance (Ako *et al.*, 1991). In another study of sole larvae (Solea solea) DHA deficient but with EPA showed better survival and growth (Howell & Tzouman, 1991). Thus, each species has its requirement for a specific

nutrient. But incorporation of all these essential components in feed, improves significantly the growth and survival suggesting that these components are indispensable. Now reasonably doubts arise, how these larvae are able to survive in natural and pond condition. There the conditions are different. The natural live feed has an array of nutrients available which enables the cultured organisms to obtain its entire nutrient requirement without supplementation. These live feeds in turn help the natural stocks of fish and shellfish larvae to flourish. But to enhance the production of larvae in hatchery, condition are simulated and the stocks quality / quantity is improved by different operation techniques.

Thus; larval feed development largely depends on:

- Selection of nutrient specific to the species
- Nutritional balance of formulation
- Retention of nutritional components
- Homogeneity of particles
- Particle size and distribution
- Density of particles
- Water solubility
- Storage stability
- Packing requirements

Apart from providing a balanced diet other problems related to larval rearing is the weaning of larvae. Some of the larvae tend to grow faster naturally than the other in the stock, which have to be segregated time to time for higher survival ability and production. These fast growing ones are not necessarily due to nutritionally balance feed but could be due to number of other factors that the hatchery operator usually faces.

Feeding the sea bas larvae in CIBA hatchery

1. Live Feed

1.The following live feed are very important for feeding the larvae

1 Algae	1 Green unicellular algae like *Chlorella* sp *Tetraselmis* sp *Nannochlorpsis* or *Isochrysis* sp are needed for feeding the live feed (zooplankton), Rotifer and for adding to seabass larval rearing tanks for water quality maintenance.
2Rotifer	2 Rotifer (*Brachionus plicatilis*) or B.rotundiformis is the most prefereed diet for the fish larvae in their early stages. The size of the Rotifers vary from 50 – 250 µm. The early stage larvae (upto 7 days) are fed with small sized rotifer i.e. less than 120µm and later assorted size rotifer can be fed.
3Artemia	3 Brine shrimp, *Artemia* in nauplii stage are required for feeding the larvae from 9th day. Artemia with its natural nutrient profile required for larval development of fish is used in all the hatcheries. *Artemia* cyst are kept for hatching and the freshly hatched nauplii are given as feed for fish larvae upto 21 days and afterwards *Artemia* biomass can be given.

4 Whatever good the culture system may be in many cases, Rotifer or Artemia nauplii produced in the hatchery may not be having all the nutrients required for the larvae, (especially the unsaturated fatty acids), the cultured Rotifer/Artemia are enriched with nutrient rich media and then fed to the larvae.

2 Feeding

Feeding the larvae should be done with utmost care. Under feeding will lead to starvation and cannibalism in seabass larvae. Excessive feeding that too feed like rotifers will remain in the tank and excrete toxic metabolites deteriorating conditions in the tank. Feed rationing and feeding depends upon the larval density and conditions of the larvae.

Rotifer (*Brachionus plicatilis*) are given as feed to the larvae from 3rd day. Rotifer is maintained in the larval rearing tanks at concentration @ 2o nos./ml initially. From 4th day to 9th day the rotifer concentration is increased to 30 – 50 nos./ml gradually. And concentration is increased to 6 – 10 nos/ml from 9th to 15th day of rearing. Every day after water exchange, the food concentration in the tank should be assessed and fresh rotifers should be added to the required concentration.

In the early stages (3 – 5 days) the larvae may not be in a position to ingest the large sized rotifers. Hence after collecting the rotifers from the tanks small sized rotifer less than 100 μ should be sieved using suitable mesh size bolting cloth nets. Rotifers collected are passed through bolting cloth net of 100 micron and the rotifers passes through are collected and fed to the early larvae. From 6th day assorted size rotifer can be given as feed.

Artemia nauplii are given as feed along with rotifers and green water from 10th day. By this time the larvae will be around 4 mm TL in size. Larvae can be feed exclusively with Artemia from 16th day to 24th day. The density of the brine shrimp nauplii in the rearing medium is maintained @ 2000 nos./l initially and gradually increased to 6000/l as the rearing days progress. The daily ration of Artemia nauplii feeding is adjusted after assessing the unfed Artemia in the rearing tank at the time of water exchange and the larval density.

Feed density/quantity to be given to seabass

Larvae at different days of rearing

Days	Feed				
Larval age	*Chlorella/ Tetraselmis/ Isochrysis* Conc. Thousand cells/ml	Larval age	*Chlorella/ Tetraselmis/ Isochrysis* Conc. Thousand cells/ml	Larval age	*Chlorella/ Tetraselmis/ Isochrysis* Conc. Thousand cells/ml
3 – 8	20	3 – 8	20	3 – 8	20
4 –15	20	4 –15	20	4 –15	20
16 –25	-	16 –25	-	16 –25	-
26th day onwards	-	26th day onwards	-	26th day onwards	-

By 21st day the larvae will be around 10 – 11 mm TL in size after completing larval development stages. From 25th day the larvae can be fed with *Artemia* sub adult (biomass) along with cooked minced fish/shrimp meat. The fry can also he weaned slowly to artificial feed.

Under circumstances, when the rotifers could not be fed with marine *Chlorella* adequately, the nutritional quality of such rotifers may be poor. In such case, the rotifers can be enriched with special enrichment media. Enrichment is done by keeping the rotifers in emulsified enrichment medium like SELCODHA or cod-liver oil for 18 - 24 hours. By this process, the animals will ingest the enrichment media which is rich in Poly unsaturated Fatty Acids (PUFA), required for larval growth. The animals are washed and fed to the larvae. In this way Rotifers *Artemia* nauplii/*Artemia* biomass can also be enriched and fed. Moina a cladoceran can also be fed to the seabass larvae after 21 days.

3. Use of micro diets in sea bass larval rearing.

Micro diets to sea bass larvae were introduced as early as 9th day and the larvae accepted the micro diet. Exclusive use of microdiets reusled in poor survival and weight gain. However , if the micro diets are fed along with live feed a significant improvement in survival and larval metamorphosis was noticed. The major advantage in use of microdiets along with the live feed is the minimal size variation in larvae accompanied with better survival indicating that co feeding minimizes the cannibalism in larvae. The larval metamorphosis also quick in co feeding than sole live feeding. The larvae which are reared under co feeding regime were easier to wean them in to compounded diets in the nursery styage. Co feeding rotifers and microdiet allowed exclusion of artemia in the larval rearing protocols from day 9th to day 25th resulted in better survival and earlier metamorphosis than the protocol of co feeding artemia with micro diet.

Conclusion

At present almost all commercial marine shrimp and fish hatchery operations rely on the exclusive use of a succession of live food organisms or zooplankton, and in particular the rotifer Brachionus plicatilis (size range: 100–400um, wet weight c. 0.003mg) and the brine shrimp Artemia salina (newly hatched nauplii size range: 420–520um, wet weight 0.01–0.03mg, dry weight 1.6–3.3ug; hydrated decapsulated cyst size range: 200–270um), for the larval culture cycle. Despite the economic efficacy of a well managed shrimp or marine fish hatchery using a live food feeding regime, there are a number of disadvantages associated with an intensive live food hatchery feeding strategy.

In the past, attempts to replace live food organisms with inert or artificial complete diets have resulted in reduced larval survival, delayed larval development, and often total larval mortality .To a large extent, this has been due to the use of inadequate feed management techniques ie. infrequent feed presentation and water exchange, poor understanding of larval feeding behaviour and physical feed requirements), poor feed water stability and a consequent loss of water soluble nutrients and increased water pollution. However, recent improvements in our understanding of larval nutrition ie. high dietary requirement for highly unsaturated fatty acids ,phospholipids requirement, larval digestive physiology , possible dietary requirement for purified enzyme preparations/ enzyme 'triggers' and for soluble or highly digestible dietary feed ingredients, larval and larval feed manufacturing has stimulated renewed interest in the development of artificial larval diets to replace live food organisms during the hatchery cycle.

CHAPTER-11

FEED MANAGEMENT IN SHRIMP AND FINFISH AQUACULTURE

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What is feed management ?

Feed management means control and use of feed for aquaculture operation in such a manner that the utilization of feed is optimum with minimum wastage, negligible impact on environment, achieving best feed conversion ratio (FCR) and maximum growth of fish and shrimp and production. Such feed management practice if adopted, aquaculture production will be not only economical and profitable but also sustainable and eco-friendly. A best can produce poor results if the feed management is poor. On the other hand a moderate feed can produce best results under good feed management.

Most of the feed suppliers provide feeding charts for feeding fish and shrimp during the period of culture operation. These tables may be prepared based either some experiences or based on theoretical models. Since most of the feeding charts are based on size of fish and biomass in the culture pond still errors occur because accurate estimation of biomass in a pond is very often not possible correctly. In many farms excess feeding may occur due to this error. In some cases farmers may be over enthusiastic in achieving faster growth may over feed the stock leading to poor feed management.

Rate of feeding

Even though there are some investigations on the quantities requirements of feed in relation size and stage of the growing fish/shrimp still research on these aspects is needed for making the feeding tables more accurate. Generally the method of calculating the daily ration is based on the body weight of fish. The quantity of ration varies from 100% of body weight for larvae and fry and gradually reduced to 50%, 20%, 10%, 5% and 2-3% as the fish/shrimp grow marketable size. Suppose if W grams is the average weight of the stoked animal and if there are A number of animals in the pond then the total biomass in the pond is W x A grams which is equal to W x A/1000 kg. If feed is to be given at 10% of body weight then the quantity feed required per day is

$$\frac{W \times A}{1000} \times \frac{10}{100} \text{ kg}$$

In pond to estimate the biomass accurately is not possible. Generally periodically (once a week or 10 days) using a suitable net, sampling of the fish/shrimp and the average weight of the animal is calculated. Total biomass is calculated by multiplying the average weight by the number of animals surviving at that time. This is mainly by done by counting the number of animals caught per each netting and estimating the total number of animals taking into account the area covered by each netting and the total area of the pond. Some times the number of animals surviving in the pond is approximately estimated by giving a margin of 5 –10% mortality per month on the total number of animals initially stocked.

The alternative method of feeding is not by calculating the daily ration but by leaving the fish on self-demand feeding conditions. When the fish is hungry it will approach the demand feeder for its food requirements. It was observed that fish quickly learn how to obtain feed. The growth of fish also is good with best FCR and minimum wastage of feed. This method works best with finfish farming. Mechanical demand feeders and feed bags suspended at different places in pond are used in this method feeding.

Floating pellet feeds for finfish have the advantage in controlled feeding. Since the feed floats on the surface of water, the active feeding by fish can be directly observed and the consumption of feed can be monitored. Based on the observations the quantity of feed to be broadcast can be regulated.

Schedule and frequency of feeding

The total quantity of feed required in a day should not be fed at time. Scheduling and frequency of feeding greatly help in successful feed management. Time schedules for feeding the fish may be fixed such that larger ration may be given when the fish is expected to be most hungry. If night feeding is limited the morning feeding should have larger ration. There should be a minimum of three time schedules of feeding in a day – morning, noon and evening. Some species are more active during night and should receive comparatively larger portion of the ration. Observations and experiences show that frequent feeding of small portions of the ration seems to help in better utilization of the feed and there by lead to efficient FCR. The daily ration can be offered at every 2-4-hour interval in divided doses. There must also a mechanism in each case to monitor the feed consumption and offering of the next scheduled dose should be regulated

according to the consumption from the previous feed offered. Regular observations and experience help in mastering the management of feeding in a culture farm.

Feeding shrimp in grow-out ponds

The quantity of feed required in a day for feeding shrimp is estimated based on biomass in the culture pond. To start with feed is offered at 15 – 20% of body weight. As the shrimps grow, it is gradually reduced and brought down to 2-3% towards the end of the culture period. A model chart for feeding is given in Table 1. The entire quantity of feed required for a day in a pond should not be put at one time. The shrimps should be offered feed at every 3 - 4 hours in small doses. This helps in better utilization of feed and reduces wastage. Shrimps are active feeders during night, hence large doses may be offered in the evening and during night. Keeping the feed in bamboo or velon screen trays kept inside the pond at different locations is a good practice (Fig. 1). These are known as check trays. Periodically these check trays can be lifted up to check the feed consumption. A part of the feed may also be broadcasted for proper distribution. Instructions of the feed supplier with regard to feeding may be followed. Excess feeding leads to uneaten feed at the pond bottom. This will cause pollution of pond water and stimulates algal blooms, which may cause stress to shrimp. Under these conditions mass mortality of shrimp may occur. Feeding a little less does not do any harm, but feeding a little excess may be harmful and can cause heavy loss. Feed management needs experience and skill to obtain best results. Water quality in culture pond is also linked to feed management. If the water quality (such as dissolved oxygen, ammonia, nitrite, nitrate, hydrogen sulphide) in the pond is poor, even the best feed may give poor performance.

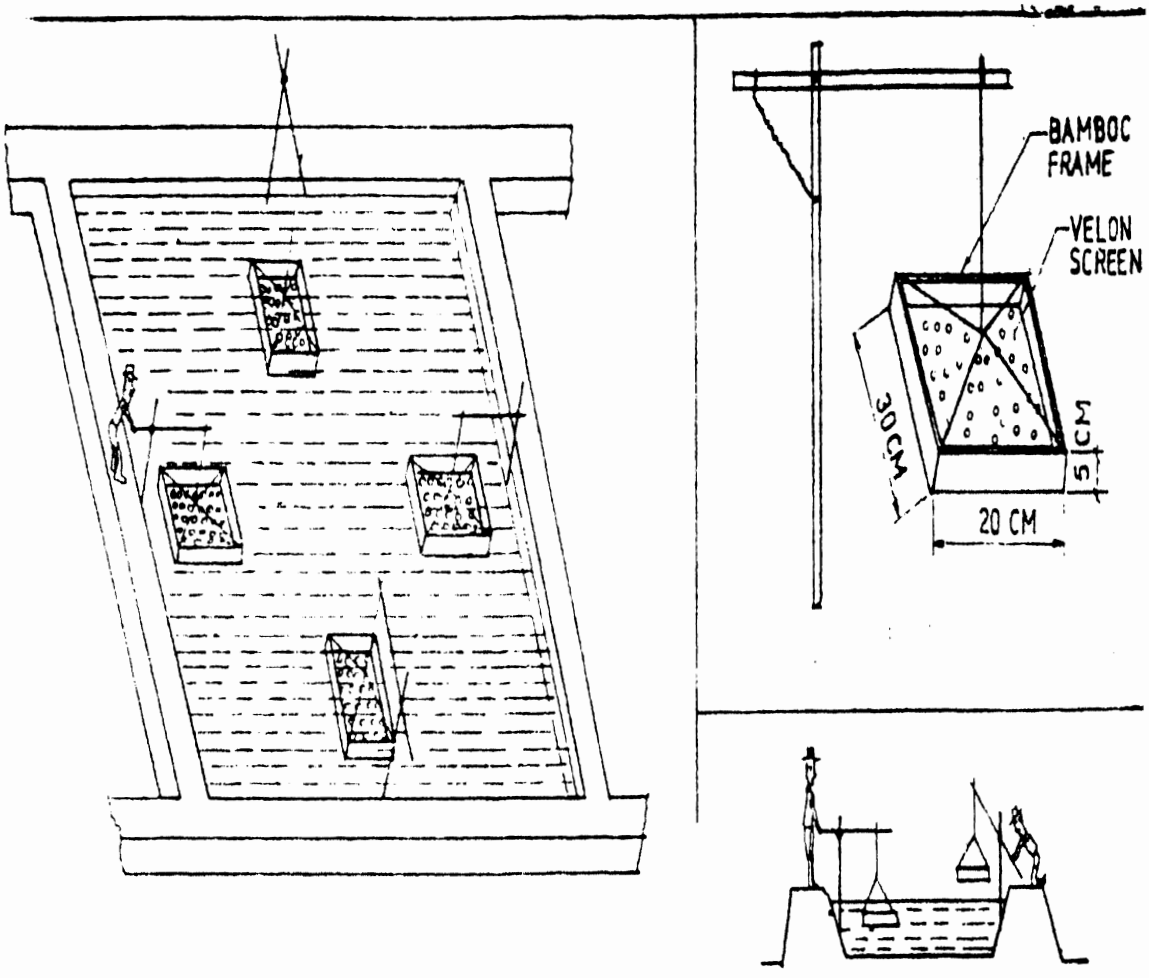
Shrimp feeds should be stored properly. Absorption of moisture during storage leads to mould growth and lowers the quality. Certain kinds of fungi (*Aspergillus* sp) produce aflatoxin, which is very toxic to shrimps. Feedstocks required for use of one month may be purchased at a time and stored in a cool and well-ventilated place. For longer shelf-life, the feed may be stored at lower temperature of 10^0 C.

Farmers should look for feeds that are as fresh as possible. Fresh feeds generally give good fishy smell. Stale smell indicates that the feed is not fresh. Water stability of feed also affects the performance of the feed. It will not disintegrate fast but also causes water pollution leading to economic loss. The feed should be stable under water at least for 2 hours. Feed should not be too hard also as it not properly assimilated the animal. Feed with poor water stability leads to poor FCR and higher cost of production

Table 1: Rate of feeding of shrimp and quantity of feed to be given in culture pond

Week after stocking	Weight of shrimp (g)	Survival expected %	Rate of feeding % of body weight		Quantity of feed to be given per day (kg)	
			$5/m^2$ *	$10/m^2$ *	$5/m^2$ *	$10/m^2$ *
1	0.5	90	nil	--	nil	2 .0
2	1.0	89	nil	--	nil	4.0
3	2.0	88	4.0	6.0	3.5	10.5
4	2.9	87	3.8	5.5	4.8	13.9
5	3.9	85	3.6	5.0	5.9	16.6
6	5.0	84	3.4	4.8	7.1	20.2
7	6.2	84	3.2	4.6	8.3	23.9
8	7.5	83	3.0	4.4	9.3	27.4
9	9.0	82	3.0	4.0	11.0	29.5
10	11.0	80	3.0	3.8	13.2	33.4
11	14.0	78	2.8	3.4	15.2	37.1
12	16.0	76	2.5	3.2	15.2	38.9
13	18.5	75	2.4	2.8	16.2	38.9
14	20.0	74	2.3	2.7	17.0	40.0
15	22.5	73	2.2	2.5	18.0	41.0
16	25.0	72	2.0	2.3	18.0	41.4
17	28.0	71	2.0	2.1	19.8	41.7
18	31.0	70	2.0	2.0	21.7	43.4
19	33.0	70	1.9	2.0	22.0	46.2
20	35.0	70	1.8	1.9	22.0	46.2

The above figures are only a guideline. The actual figures should be calculated by periodic sampling and recording the average weight and estimated survival.



Arrangement of feed check trays in a shrimp farm for monitoring the feed consumption

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CHAPTER - 12

NUTRITION AND FEEDING OF CRABS

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Introduction

Although crab fattening and crab culture have been in vogue for several years, nutritional studies and investigations on feed development have started only recently. The commercially important species of crabs for aquaculture in India are the mud crabs (*Scylla serrata* and *Scylla tranquebarica*) and Portunid crabs (*Portunid pelagicus*).

Nutrient requirements

Protein requirement in the diet of crabs has been studied by Chen et al. (1994) in Chinese hairy crab, *Eriocheir sinensis*, and suggested a protein requirement of 46.5 to 53.6% for 6-10 g juveniles using semi-purified diets. However, using shrimp meal diet, Mu et al. (1998) recommended 39.0 – 42.5% protein in the diet of the same crab. In the case of mud crabs, *Scylla serrata*, and *Scylla tranquebarica*, the protein requirement has been estimated to be 37% (Ahamad Ali, 2000-unpublished). Sheen and Wu (1999) suggested a dietary lipid (cod liver oil + corn oil in 2:1 ratio) requirement of 5-13.8% for mud crab, *S. serrata*. They have also reported that the diet deficient in n-3 and n-6 fatty acids caused protracted molting in the crab. The crab also requires approximately 0.51% of cholesterol in its diet (Sheen, 2000). At CIBA for developing suitable formulated feed for the culture of mud crab different lipids were evaluated in its diet. Fish oil, sunflower oil, groundnut oil and palm oil were incorporated at 6% level in a standard diet with 35% protein. The diets were fed to juvenile crabs in 65-day feeding trial in the laboratory. The results have indicated that the diet with fish oil produce highest weight increase in the crab. This was followed by the diet with sunflower oil, groundnut oil and palm oil in the decreasing order.

Cholesterol is an important lipid nutrient required for crustaceans for numerous physiological functions including moulting. The dietary requirement of cholesterol for mud crab *Scylla tranquebarica* was also determined at CIBA using purified diets, with

cholesterol levels ranging from 0 to 1.4%. Feeding experiments were conducted in the laboratory on the juvenile crabs for a period of 120 days with test diets have shown that the crabs fed with 0.5% cholesterol diet gained highest weight with good moulting frequency. The results indicated that cholesterol requirement in the diet of crab is 0.5%.

The effect of dietary lecithin was studied on the growth and feed conversion ratio (FCR) in the mud crab *Scylla tranquebarica* at CIBA by preparing test feeds with five different levels of lecithin. The test feeds were fed to juveniles of the crab in a 45-day feeding trial. The results have shown that the crabs fed with test feed containing 1.0% lecithin gave better weight gain and resulted in better FCR compared to the lower levels of dietary lecithin. Higher levels of dietary lecithin have no beneficial effect.

Formulated feeds for crab culture

Crab fattening is practiced in pens and cages or in protected ponds. The water crabs are generally fed with feeds such as low value fish (trash), molluscan meat such as clams, oysters and mussels, wherever these are available at reasonable prices. Uses of formulated feed for crab culture is at to come into practice.

With a view to study the effect of feeding formulated feeds to water crabs, laboratory feeding trials were conducted at CIBA with three formulated feeds having different protein and lipid levels and control feed prepared with fresh fish tissue. The feeds were fed separately to freshly molted crabs (*Scylla tranquebarica*), held in 1000 l tanks in triplicate during the intermoult period (25 days). The experimental results showed that at the end of feeding trial there is a significant increase in dry matter percentage in the animals, which ranged from 42.5 to 36.9%. The crabs fed with feed having low protein-high lipid have shown better performance. Trials conducted in earthen pond on water crabs fed with pellet feed gave very encouraging results.

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CHAPTER – 13

METHODS AND APPROACHES TO TRANSFER INNOVATIONS OF SHRIMP NUTRITION AND FEED TECHNOLOGIES

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Coastal aquaculture immensely contributed to food and nutritional requirements, export earnings and generates substantial income and employment to large number of people. With declining catches from open sea and prospect of high foreign exchange earnings from farmed shrimp exports and status of second position in global aquaculture next only to China, India hopes to achieve the annual growth rate of over 4% in fisheries. The contribution from the inland sector is nearly 55%. The information plays a key role to run the shrimp farming in a profitable way. Along with critical inputs, good environmental conditions, sustainable market and appropriate policy support of government transferring innovations ensure profitable farming in the long run. Proper feed management is essential for successful and profitable shrimp culture (Ali. 2002). Kumaran et al (2004) reported by conducting a study on awareness and perceptions of shrimp farmers on feeds and feed management practices through extension education programmes.

Innovations

Anything that is perceived as new is called as innovation. It is the successful exploitation of ideas. While research and development plays a central role in directing the development of ideas into commercially viable products, the ideas for innovations flow from different directions both within and outside organisation. Concepts like complete feeds, ingredients like steam-dried fishmeal and technologies like extrusion progressed from ideas to products. Anyone in or associated with an organisation could spark the idea for an innovation. In fact, sparks for innovation could originate from a pellet mill operator explaining why he is unable to meet a production target or from a team of college students taking a tour of the feed mill. Apart from various functional groups within an organisation, suppliers also provide information, knowledge and unique insight that accelerate the process of innovation. Forming trustworthy

relationships and having a mechanism for continuous dialogue with various stakeholders are keys to leveraging the resources for innovation.

Diffusion and adoption of innovations

The main function of aquaculture extension is to communicate the different stakeholders the latest technologies emanating from research institutions and other organisations. The diffusion and adoption of new ideas and practices has received increasing attention in the present context of decreased returns over the increased cost of production. Roger's (1966) theory of diffusion of innovations is based on the five stages in the adoption process viz., awareness, interest, evaluation, trial and adoption. According to this theory, an individual before finally adopting a new idea passes through a mental process comprising of all or some of these stages. Since the time taken to pass through a mental process is not the same for all individuals, adaptors can also be categorised into five groups based on the time taken for adoption. These categories are innovators, early adopters, early majority, late majority and laggards.

Characteristics of innovations

Five characteristics of innovations have also been delineated, having a bearing on the rate of adoption. These are:

1. Relative advantage

   It is the degree to which an innovation is superior to the ideas it supersedes.

2. Compatibility

   It is the degree to which an innovation is consistent with existing values and past experiences of adopters

3. Complexity

   It is the degree to which an innovation is relatively difficult to understand and use

4. Divisibility (Trialability)

   It is the degree to which an innovation may be tried on a limited basis

5. Communicability

   It is the degree to which the results of innovation may be diffused to others

The knowledge of the process of adoption and diffusion and its implications may be of immense value to extension workers in pushing through successfully an innovation in the field of coastal aquaculture.

A suggested strategy for the introduction of an innovation

Pre-introduction phase

1. Care should be taken to see that the innovation is simple, compatible with current cultural practices and there is a genuine need for the same

2. Its economic advantages should be very large and manifest

3. Its social consequences, if any, should be anticipated and provisions made to deal with them.

4. The adoption stages from awareness to evaluation should be adequately arranged by wide publicity and suitable demonstrations

5. All stakeholders should be fully involved through their opinion leaders and a mood of mutuality should always prevail on the part of change agents.

During the introduction

1. As far as possible, on the spot guidance should be provided to the potential adopters.

2. Attention should be focused more on opinion leaders and active rejecters

3. The users should be encouraged to be self-dependent and appreciative of new ideas.

4. A constant evaluation of the programme should be made and suitable changes brought out.

After the introduction

1. An effective follow up should be ensured till complete adoption

2. The experiences gained should be utilised in bettering the present programme and in planning future strategies of change.

3. Success should be measured more in terms of creation of favourable attitude towards innovativeness rather than success in a particular programme

4. Maximum responses and reactions of adopters should be obtained to ensure an effective feedback and to complete the process of communication.

Methods to transfer innovations of shrimp nutrition and feed

Methods are the ways and means of relating the extension workers and farmers for transferring innovations. The utility of extension worker lies in the fact that he helps in facilitating the learning process and transferring innovations. Many methods are used in transferring the innovations to the stakeholders. An extension worker seldom resorts to one method in any method in any given meeting. For instance, he could conduct a field-trip, make use of resource person and have a learner give a demonstration. The table 1 indicates a list of some of the methods used and classified according to individual, group and mass bases which illustrates the extensiveness of choices available.

Table 1. Methods of transferring innovations

Individual methods	Group methods	Mass methods
1. Farm and home visit	1. Demonstration	1. Film
2. Personal letter	2. Role playing	2. Radio
3. Telephone calls	3. Panel	3. Television
4. On farm trials	4. Symposium	4. Newspaper
5. Circular letter	5. Study tour	5. Farm publications
6. Case study/success story	6. Group discussion	6. Exhibition
	7. Conference	7. ICT initiatives
	8. Training	
	9. Farmers interaction meet	

Guidelines in selecting and making use of different methods

1. Consider the target audience
2. Easy to see, understand, handle and transport
3. The message should be simple and direct
4. Key points to be emphasised
5. Attractive extension materials
6. Good learning situations
7. Time and place should be chosen properly

Approaches in transferring aquacultural innovations

Different approaches can be followed in transferring innovations to the aqua farmers. These are outlined as under:

1. Entrepreneurial approach

The potential entrepreneurs could be identified for field testing of transferable technologies. Upon success of the field testing, the entrepreneurs can be motivated to adopt the innovations.

2. Participatory approach

The beneficiaries need to be involved right from need analysis to taking up the technologies in their own farms. CIBA implemented a project through participatory approach wherein scientists demonstrated that using the feed formulated by CIBA, the

cost of production of one kg of tiger shrimp was found to be Rs.100.98 whereas the cost of production with commercial feed reported to be Rs. 157.36 (Ponnusamy et al., 2003).

3. Fee-for-service approach

Some private firms set up diagnostic labs in aquaculture cluster areas for seed, feed and soil and water quality analysis and charge the service rendered by them.

4. Group approach

The groups like aqua club, creek association need to be formed to serve the interests of some stakeholders. Norwegian feed suppliers, Nor Aqua formed a benchmarking club named as TEAM09 for salmon farmers which is designed to give producers the opportunity to compare on-farm performance between production units of similar size and circumstance. The key aim of club is to achieve an average FCR of 0.9:1. It is important for member farmers to assess their own production performance against their peers and learn how to interpret comparative figures and findings effectively so that worthwhile conclusions can be drawn, allowing appropriate action to be taken (Anonymous, 1999).

5. Digital communication approach

The following are some internet sites for browsing aquaculture related information and innovations such as www.feedware.com, www.aquafeed.com, www.was.org, www.aquaculturemag.com, members.magnet.at/aquaculture/webring.ht, www.aquanic.org, naca.fisheries.go.th/, www.ag.auburn.edu/dept/faa/icaae1.html, www.stir.ac.uk/aqua/fishing.

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PRACTICAL –1

ANALYSIS OF SHRIMP/FISH FEEDS FOR PROXIMATE COMPOSITION

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Introduction

Information on the proximate principles in formulated feeds (shrimp or fish feeds) is important to evaluate their nutritional quality. The feed samples are usually analysed for the following important parameters, by standard methods.

1. Moisture
2. Crude protein
3. Crude lipid
4. Crude fiber
5. Total ash
6. Acid-insoluble ash
7. Nitrogen-free extract
8. Gross energy

Determination of Moisture content

This procedure is used to determine the amount of moisture (as a percentage) in various materials including feed ingredients, complete feeds etc.

Materials

Glass petridishes
Analytical balance
Hot Air Oven
Dessicator

Procedure

Accurately weigh (W_1) out 2 to 5 grams of finely ground sample into clean dry preweighed (W_0) glass petridishes and dry at 105°C in an Oven (overnight). Remove samples from oven, cool in a dessicator and reweigh (W_2). The difference in weight represents the moisture content of the sample and is expressed as a percentage of the original sample weight.

Calculation

Moisture content % = $[(W_2 - W_0) \div W_1] X 100$

Determination of Crude protein by TECATOR KJELTEC System

The crude protein content in the feed is determined in the KJELTEC System by measuring the total Nitrogen content in the sample and converting this to crude protein using the factor 6.25. This factor is based on the assumption that the average protein contains about 16% nitrogen. The principle of this analytical technique is similar to the conventional micro-kjeldahl method and can be divided into three stages.

1. The digestion of the sample with concentrated H_2SO_4 in the presence of a catalyst (Hg), to convert all the N present to $(NH_4)_2SO_4$.
2. The liberation of NH3 from the digest by addition of excess NaOH and steam distillation into saturated boric acid.
3. Determination of the NH_3 liberated by back titration to the end point with standard HCl.

Materials

Electronic balance
Sample mill
Digestion block
Kjeltec distillation Unit
Digestion tubes
Conical flasks, burettes, measuring cylinder.
Concentrated H_2SO_4
Kjeltabs (digestion mixture tablets)
Boric acid (4%)
Standard HCl
Mixed indicator (bromocresol green plus methyl red)
NaOH (40%)

Procedure

Sample digestion: Finely ground samples of feed are transferred quantitatively (0.2 g) into three digestion tubes. To this tube, one Kjeltab (catalyst) and 5 ml of concentrated H_2SO_4 are added. Blank tubes, with catalyst and acid but no sample, are also similarly prepared. The sample is digested in a heating block set at 420°C for 3 hours (sample becomes clear). The cooled sample is then ready for steam distillation.

Distillation: when the distillation unit is operated on automatic mode, the addition of water, alkali and steam are preset. A conical flask with 25 ml of 4% boric acid plus few drops of indicator is placed at the receiver end. The digested sample in the tube is fixed with the steam tube inserted into the sample. Closing the sample compartment

automatically starts the distillation, by consecutively adding distilled water, alkali and steam. The machine turns off steam after 3 minutes and the unit is ready for the next sample. The flask is then taken out for titration. The liberated NH_3 in the boric acid is titrated against 0.2 N standard HCl and the titer value recorded.

Calculation

$$\text{Crude protein} = \frac{\text{(sample titer - blank titer) X 0.2 X 14.007 X 6.25 X 100 \%}}{\text{wt of sample (in mg)}}$$

Determination of Crude lipid by SOXTHERM System

This procedure involves the extraction of ether soluble components from a sample of feed. The extract is referred to as 'crude' lipid since other ether soluble materials are also extracted along with lipid.

Materials

SOXTHERM extraction unit
Extraction beakers
Extraction thimbles and thimble holder
Dessicator
Oven
Electronic balance
Boiling chips
Petroleum ether (BP 40° - 60°C, 140 ml/sample)

Procedure

1. Quantitatively (W_0) take about 5g of ground sample (in triplicate) into preweighed thimbles and cover with a wad of cotton.
2. Place a few boiling chips into the extraction beaker, dry at 105°C, cool and record the empty weight (W_1).
3. Place the thimble with sample into the beaker and add 140 ml of petroleum ether. The beakers are then loaded on to the extraction unit.
4. Start the preset programme in the controller unit
5. The programme runs through the following steps -

Boiling	30 minutes
Solvent reduction A	5X15 ml
Extraction	80 minutes
Solvent reduction B	8 minutes
Solvent reduction C	5 minutes

6. When the display shows 'Program Finished' return to stand-by position by pressing ENT-key.
7. Remove the beakers from the extraction unit and dry at 105°C for at least 1 hour in a oven before recording the exact weight of beaker plus lipid (W_2)

Calculation

Crude lipid content % = $[(W_2 - W_1) \div W_0] \times 100$

Determination of crude fiber using the TECATOR FIBRETEC System.

Crude fiber is the term applied to the indigestible carbohydrate portion of feed consisting principally of cellulose, hemicellulose and lignin. It is defined as the loss on ignition of the dried residues remaining after the consecutive digestion of feed sample with dilute acid and alkali under specific conditions (Weende method).

Materials

FIBERTEC System - Hot and Cold Extraction Units
Hot plate
Filter Crucibles with stand and holder
Suction pump
Sample mill
Electronic balance
Hot air Oven (105°C)
Muffle Furnace (500°C)
Dessicator
Sulphuric acid solution (1.25%), 0.255 N
Sodium or Potassium hydroxide solution (1.25%), 0.313 N
n-Octonol
Acetone

Procedure

Weigh (Wo) and transfer quantitatively triplicate samples of ground feed (<1 mm) into clean preweighed filter crucibles (Porosity No. 1, 40 - 90 μ). Transfer the crucibles with sample onto the Hot-Extraction unit and digest the sample for 30 minutes with 150ml of hot acid solution with a few drops of octonol. Filter out the acid solution and wash thrice with hot distilled water, using suction. Then the sample is digested for 30 minutes with hot alkali solution with drops of octonol, to dissolve alkali-soluble matter from the sample. The procedure of filtration and washing is repeated. The crucibles are then transferred to the Cold-Extraction Unit and samples washed with 25 ml portions of acetone, to remove lipids, and each time sucked dry. The crucibles with final residue are then dried at 100°C for six hours, cooled and weight recorded (W_1). Ashing of the residue is done at 500°C for 3 hours and the final weight of crucible with ash is recorded

(W_2). (NOTE: Samples with high fat contents are to be defatted in the Cold-Extractor before Hot-Extraction).

Calculation

The fiber content of the sample is calculated by the formula -
% Crude fiber = $[(W_1 - W_2) \div W_0] \times 100$

Determination of Total Ash

The ash content of a sample is the inorganic residue remaining after the organic matter has been burnt away.

Materials

Silica crucibles
Electronic balance
Muffle furnace

Procedures

1. Weigh out accurately triplicate samples (W_0) into clean, preweighed (W_1) silica crucibles.
2. Char the samples over a hot plate and transfer the crucibles into a Muffle furnace set at 450°C.
3. After six hours, cool the crucibles and record the weight of crucibles with ash (W_2).

Calculation

Total ash content % = $[(W_2 - W_1) \div W_0] \times 100$

Determination OF Acid-Insoluble Ash

The content of Acid-Insoluble Ash is an index of the sand or siliceous matter in the feed.

Materials

Conical flasks, Measuring cylinder, funnels
Electronic balance
HCl (10%)
Whatman No.42 filter paper circles
Silica or Porcelain crucibles
Muffle furnace

Procedures

1. Weigh 200 to 300 mg of ash (W_1) and transfer to a conical flask (two replicates per sample).
2. Add 25 ml of hot 10% HCl solution and simmer over a Bunsen burner for 10 minutes.
3. Cool and filter through Whatman No.42 ashless filter paper. Wash residue till acid free.
4. Transfer the filter paper with residue to a preweighed crucible (W_2).
5. Incinerate at 450°C overnight, cool and reweigh the crucible (W_3).

Calculation

Acid-Insoluble ash content = $[(W_3–W_2)÷W_1]$ x total ash % in feed

Determination of Nitrogen-Free Extract

The content of Nitrogen-Free Extractives (NFE) or crude soluble carbohydrate is computed by the following formula –

% NFE = 100 - (% moisture + % crude protein + % crude lipid + % total ash + % crude fiber)

Determination of Gross Energy

Gross or total energy content of a feed material can be obtained by summation of the known energy values contributed by the protein, lipid and carbohydrate fractions of the sample. The generalised physiological energy value of proteins, carbohydrates and lipids are 19, 15 and 36 kilojoules per gram, respectively.

Energy contributed by crude protein = protein content of sample % X 19 kj/g = x
Energy contributed by crude lipid = lipid content of sample % X 36 kj/g = y
Energy contributed by carbohydrate = NFE content of sample % X 15 kj/g = z

Gross energy content (GE kj/g) = [x + y + z] ÷ 100

PRACTICAL - 2

PROCESSING AND PRODUCTION OF SHRIMP FEED

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To prepare a batch of shrimp feed using small scale feed mill equipment. The feed is formulated using indigenous ingredients with 38% crude protein.

MATERIALS

Feed ingredients

The following ingredients may be used for formulating feed.

1. Dry fish
2. Prawn head waste
3. Squid waste
4. Squilla
5. Soybean meal
6. Wheat flour
7. Fish oil
8. Vitamin and mineral mixture

Feed formulation

Fish meal	30 kg
Prawn head waste	5 kg
Squid waste	5 kg
Squilla	5 kg
Soybean meal	25 kg
Wheat flour	25 kg
Fish oil	3 kg
Vitamin mineral mixture	2 kg
Total	100 kg

METHOD

Grinding

First grind individually dry fish, prawn head waste, squid waste, squilla in a hammer mill to reduce the size. Then powder all the ingredients in a micro pulverizer seperately.

Mixing

Weigh out the feed ingredients as per the formula and put in the mixer except vitamin and mineral mixture. Homogenise the feed mix for 15 minutes. Add 35 litres of water and further homogenise for another 10 minutes.

Steam cooking

Load feed mix in trays and keep the trays in steaming chamber. Allow the temperature to reach 95 - 100°C and keep for 5 minutes. Take out and cool the feed mixture.

Incorporation of vitamins

Add the vitamin and mineral mixture to the steamed and cooled feed mix and thoroughly homogenize in a dough mixer.

Pelletisation

Pelletise the feed in a wet pelletizer fixed with 3 mm diameter die. Collect the pellets in aluminum trays.

Drying

Restore the trays loaded with moist feed into an electrical tray dryer. Adjust the temperature at 75-80°C and allow the feed to dry until the moisture content is less than 10%.

Checking quality of feed

The dry feed pellets may be physically examined for visual appearence such as uniformity, colour and smell. The pellets should have surface without cracks. The feed may be sampled and analyzed for proximate composition. The water stability of the pellets may also be tested after twenty four hours of preparation.

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PRACTICAL -3

DETERMINATION OF WATER STABILITY OF SHRIMP FEED PELLETS

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PRINCIPLE

Water stability of dry shrimp/fish feed pellets is determined by the loss in weight of pellets kept in water for a specified time interval by different methods. The loss in weight of pellets indicates the stability, higher the loss poorer the stability.

Materials

1. Oven.
2. Nylon mesh
3. Wire guaze basket
4. Sieve (2.4mm)
5. Bowl shaped sieve (0.5mm)
6. Aerator
7. Balance
8. Plastic pool
9. Glass beaker (1L)
10. Glass jar (10L)
11. Water bath
12. Glass tube
13. Glass rod
14. Nylon string
15. Magnetic stirrer
16. Aquaria
17. Stop watch

METHODS

. Cone shaped pouches made of nylon mesh (1mm mesh size), are thoroughly washed and dried at 70°c to constant weight in an oven. Uniform size feed pellets of 5 mm length are prepared. 2 - 3 grams of pellets are taken in each pouch and initial weight is recorded. Nine such pouches are taken for each sample. Petridishes for each sample are placed at different points in the bottom of plastic lined pool (or tank) with 300 l seawater (depth of the water 90 cm). Water temperature, salinity and pH of the seawater are recorded. Slowly the pouches with the feed pellets are lowered in the pool and placed on the petri dishes. At different intervals of time (1, 2, 3, 4, 5, 6, 8, 12 and 24

hrs) one pouch with pellets is slowly taken out of the water. The pellets are examined for their physical shape. Wash the adhering salt on the pellets by diping in fresh water for 5 mins. The pouch with pellets is dried at 70°c to constant weight. Difference in the initial weight and final weight of the pellets gives loss in weight at different intervals of time.

B. Weigh wire gauge basket and approximately 2g uniform size pellets. Slowly immerse and suspend the basket with pellets in the glass jar containing seawater. Continously aerate the water with an aerator. At different intervals of time, 2, 4, 6, 8, 12 and 18 hrs, remove the basket, dry at 70°c and weigh. Water stability is calculated using the formula

$$\% \text{ Water stability} = \frac{\text{Final Weight x \% dry matter}}{\text{Initial Weight x \% dry matter}} \times 100$$

C. Weigh the pellets of the feed (about 1 g) exactly in the bowl shaped sieve. Fill up to the rim of 1L glass beaker with seawater and place in a water bath at 28°c temperature. Place the bowl on the beaker and fill seawater gradually till the seawater level is just below the rim of the sieve. A continuous flow of air is bubbled through a glass tube (3mm bore) from bottom of the beaker and directly under the mesh sieve. The feed pellets are gently agitated continously. Small particles of feed pass through the mesh and settle at the bttom of beaker. Continuously agitate the water for 16 hrs. At end of the trial, dry the sieve for 24 hrs at 100°c and weigh. Calculate the weight loss in feed.

D. Weigh uniform size (2mm dia; 20mm length) feed pellets approximately (with known moisture content 10 g in a wire gauge basket. Slowly immense the wire gauge basket with pellet in 7L seawater in an aquaria with continuous aeration. At different intervals of time, take out the wire gauge basket with residual feed pellets, dry at 100°C and weigh the feed. Calculate water stability

$$\text{Water stability \%} = \frac{\text{Final weight x \% dry matter}}{\text{Initial weight x \% dry matter}}$$

E. Weigh 100 g dry feed pellets. Put the weighed pellets in 1L beaker contianing distilled water. After 30 mins, pour through sieve (2.4mm mesh size) and collect intact pellets in the sieve. Dry the sieve with residual pellets at 150°c. Calculate the loss in weight of pellets.

F. Tie a few pellets by strings at the middle of each pellet seperately. Suspend the tied pellets in 1L beaker with distilled water. Adjust the suspension of pellet in water such that it is just below the water surface. Note the time of fall of each feed pellet from the string and the average of which gives the stability of the feed in water. Higher the time, the better is the stability of pellet.

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PRACTICAL - 4

ANALYSIS OF AMINOACIDS BY HPLC

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INTRODUCTION

Amino acid analysis

The amino acids are analyzed by Shimadzu High Performance Liquid Chromatographic system in tissues, feed ingredients and feed samples. Separation of amino acids is done in a column packed with a strongly acidic Na^+ type cation exchange resin (styrene - divinyl benzene copolymer with sulfinic group) under gradient elusion. The amino acids are detected and quantified using a fluorescent detector after post column derivitization of the amino, NH_2 functional group with O-phthalaldehyde and 2-mercaptoethonol, which results in a fluorescent compound.

Reagents Required

1. Mobile Phase A: Dissolve 19.6 g sodium citrate, 70 ml ethanol and 14.2 ml perchloric acid in 800 ml triple distilled water. Adjust pH of the buffer to exactly 3.20 $\pm$ 0.01, make upto 1000 ml. Filter through 0.47 u nylon membrane filter.
2. Mobile Phase B: Dissolve 58.8 g sodium citrate and 12.4 g boric acid in 400 ml triple distilled water, add 30 ml of 4N sodium hydroxide. Adjust pH to 10.0 and make upto 1000 ml. Filter through 0.47 u nylon membrane filter.
3. Mobile Phase C: Dissolve 4 g sodium hydroxide in 500 ml distilled water and filter through 0.47 u nylon membrane filter.

4. Boric acid - carbonic acid buffer (pH 10.0): Dissolve 40.7 g Na_2CO_3, 13.6g H_3BO_3 and 18.8 g K_2SO_4 separately in triple distilled water and make upto 1000 ml.

5. Brij-35 solution: Dissolve 5 g Brij-35 in a wide mouth bottle in distilled water with warming, make up to 50 ml.

6. Sodium hypochlorite solution (>7%)

7. Reaction reagent I: Mix 500 ml of boric acid carbonic acid buffer with 0.2 ml sodium hypochlorite solution. Filter using Whatman No.1 filter paper. Prepare fresh before use.

8. Reaction reagent II: Dissolve 400 mg o-phthalaldehyde in 7 ml ethanol, add 1ml 2-mercaptoethanol and 2 ml Brij-35 solution, make up to 500 ml with boric acid-carbonic acid buffer. Filter using Whatman No.1 filter paper. Prepare fresh before use.

9. 6N Hydrochloric acid.

10. 1% hydrochloric acid.

11. Sodium hydroxide pellets

12. Diluent: Dissolve 9.8 g sodium citrate tribasic in 400 ml distilled water, add 8 ml perchloric acid and 50 µl n-caprylic acid. Adjust the pH of the solution to 2.2 and make upto 500 ml.

13. Standard amino acid (Sigma) containing 10 ug of each amino acid per ml. Use undiluted.

Acid hydrolysis of protein sample

a) Weigh accurately 50 mg of dry powdered sample (<25 mg protein) into a labelled clean glass ampule (10 ml capacity)

b) Wash down the sides of the ampule by adding 5 ml of 6N HCL.

c) Evacuate and seal the ampule.

d) Hydrolyse at 110°C for 22 hours, cool.

e) Centrifuge at 2000 rpm for few minutes.

f) Break seal, filter contents through acid washed filter paper. rinse the filter paper several times with 1% HCL. Pool the filtrate and make up volume to 25 ml with 1% HCL.

g) Evoporate 5 ml aliquot of the filtrate in a flash evoporator. remove traces of HCL by placing over NaOH pellets in a vacuum descicator.

h) Dissolve contents and make up volume to 5 ml with diluent. Filter sample through 0.22 u membrane filter before injection.

Instrument conditions for amino acid analysis

Column: Shimpack ISC - 07/S1504 Na

Mobile phase: Pump A = Mobile phase A - 0.4 ml/min

Pump B = Mobile Phase B - Gradient

Column temp: 60°C

Derivatization: Reaction reagent I 0.2 ml/min

Reaction reagent II 0.2 ml/min

Detection : Fluoresence detector, FLD-6A

Duration of run : 55 min

Procedure

1. Prepare the HPLC system for analysis
2. After obtaining the stable baseline, load the appropriate programme (File No.5) containing gradient parameters in the Master pump A.
3. Set the analysis file in the chromatopak

The Retention Times of amino acids under standard conditions is as follows:

Retention times (min)

Asp	8.58
Threo	10.50
Ser	11.30
Glu	12.48
Pro	13.74
Gly	18.13
Ala	19.35
Cys	22.60
Val	24.27
Meth	25.89
Isoleu	27.90
Leu	28.62
Tyro	31.27
Phe ala	33.40
Hist	39.62

Lys	41.70
Tryp	43.94
Arg	47.07

4. Run 1: Analysis is done without any injection to record the gradient profile.5. Run 2: Edit the analysis file and specify the calibration parameters. The Response Factor (F1) for each amino acid is obtained during the this calibration run by the external standard calibration curve method using a single point calibration curve.

Inject 20 µl of amino acid standard and start the analysis. Record the chromatogram after substraction of the gradient profile obtained during Run 1.

The various amino acids are identified based on the retention time specified in the ID table and their corresponding Response Factors (F1) are automatically calculated by the chromatopak.

6. Run 3: Inject 20 µl of diluent and record the sample blank chromatogram.

7. Run 4: Inject 20 µl of the previously prepared sample and analyze. The concentration of the amino acids in the injected sample is quantitatively estimated by the chromatopak.

8. After analysis of each samples, flush the column with Mobile phase C for 20 min.

9. Before shut down, flush all flow lines and column with distilled water for 30 min.

PRACTICAL - 5

MEASUREMENT OF CALORIFIC VALUE BY BOMB CALORIMETER

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The semi micro oxygen bomb calorimeter is intended for measuring heats of combustion of solid or liquid samples ranging in size from 25 to 200 milligrams, liberating from 50 to 1200 calories when burned in oxygen. Combustion is accomplished in a 22 ml stainless steel bomb immersed in a measured quantity of water held in a glass dewar. Temperatures in the calorimeter are sensed by a thermistor and read from a microprocessor based thermometer which is built into the calorimeter case.

Weighed quantity of sample (0.025-0.2 g) pressed in to a pellet in a pellet press and exactly reweigh the pellet. Cut a 10 cm length of fuse wire and bind it to the hook terminals, then catch the wire loop in a small allen wrench n\and rotate the wrench to form a five-turn helical coil. The coiled wire will concentrate heat on the sample and serve also as a rigid support for holding the sample in a place while handling the bomb prior to ignition. Place the pellet in a sample holder of the bomb. Set the bomb head on the cup; then slide the cup into the body sleeve and attach the screw cap. Close the bomb after clamping these parts together by hand, set the bomb in the bench socket and tighten the screw cap firmly with the octagon wrench furnished with calorimeter. Handle the bomb carefully during this operation so that the sample will not be disturbed.

Close the needle valve and pressure relief valve on the oxygen filling connection; then open the oxygen tank valve no more than one-quarter turn from the closed position. Attach the filling hose to the gas inlet tube on the bomb head by pressing the coupling onto the valve cap. Push the coupling downward until it rests firmly against the collar on the valve cap. Insert the pin wrench through the eye of the valve cap and open the cap one-half turn from the closed position. Leave the pin wrench in the cap. The bomb is now ready to be filled with oxygen. Open the valve on the filling connection slowly and observe the gage as pressure rises in the bomb and connecting hose. When the pressure reaches 35 atmospheres, close the control valve immediately and use the pin wrench to close the valve cap on the inlet tube connection to release the residual pressure in the filling hose. After the pressure gage returns to zero, remove the pin wrench and lift the filling line from the bomb head.

The calorimeter bucket consists of the Dewar flask with its attached spacer ring. The bucket should be filled with distill water (400-450 ml) which just covers the small hole (gas inlet/outlet) on the bomb head inlet valve. The temperature of the water should be within +/- 1^0C of room temperature and be consistent from test to test. After filling the Dewar, set it in the stainless steel air can and gently push the spacer ring down as far as it will go. Attach one of the ignition wires to the socket in the central terminal on the bomb head and push it down as far as it will go. Attach the second wire to the socket provided on the bomb hanger. Complete the ignition circuit by connecting one wire to the 10 cm terminals on the ignition unit and the other to the common terminal. Set the bomb in the loop of the bomb support and carefully lower it into the Dewar to hang from the top rim of the flask. Position the bomb in such a way that stirrer can operate freely without striking the bomb.

Install the thermistor in the cover opening and press the bushing firmly into place to anchor the probe in its proper position. Place the cover on the calorimeter with the orienting pin in the alignment hole. Turn the stirrer drive by hand to be sure that it runs freely, then slip the drive belt first onto the motor pulley and then onto the stirrer pulley. The bomb is now ready for ignition. Connect the motor and ignition unit to an electric outlet and start motor by setting *101.

After ignition a distinct temperature rise should appear. Remove the drive belt and thermistor probe and open the calorimeter. Lift the hanger and bomb out of Dewar, remove the ignition cord and open the valve cap with the pin wrench, releasing pressure slowly. After all pressure has been released, open the bomb and examine the inside and if there is any evidence of incomplete combustion, the test is not valid and will have to be repeated. After making the necessary corrections for fuse and acid, the data can be stored.