CIBA - TM Series 2017 - No. 6

CIBA

TRAINING MANUAL

AQUACULTURE NUTRITION AND FEED TECHNOLOGY

From 19th to 28th January, 2017

ICAR - Central Institute of Brackishwater Aquaculture (CIBA)

Chennai 600028, Tamil Nadu

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CIBA-TM Series 2017-No.6

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NUTRIENT REQUIREMENT OF CULTIVABLE BRACKISHWATER 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.

All animals including fish and shrimp requires food to supply the energy that they need for movement and all the other activities that they engage in for growth. However, they are 'cold-blooded' and as their body temperature is the same as the water they live in, they do not therefore need to consume energy to maintain a steady body temperature and they tend to be more efficient users of food than other farm animals. The nutrient requirement of different species of finfish and shellfish vary in quantity and quality according to the nature of the animal, its feeding habits, size, its environment and reproductive state.

Fish and 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 and fish feeds on a variety of food items and derive their balanced nutrition for healthy growth. When they are cultured in confined pond they should be provided with a balanced diet as close to natural food as possible. This is the reason for understanding the nutritional requirement of candidate species which assumes paramount importance in developing the feeds for the candidate species.

Nutritional requirement of different brackishwater species

Protein

Protein is the most important nutrient in the diet of shrimp and fish. Protein requirement of aquatic organism is higher than terrestrial animals. Fish and crustaceans require food protein in the form of essential amino acids for maintenance of life, growth and reproduction and the requirement of protein depends on animal characteristics i.e., species, physiological stages, size as well as dietary characteristics, i.e., protein quality (digestibility and biological value), energy level etc. Scarcity of carbohydrate and abundance of protein and lipid in the natural aquatic food web is also probably responsible for the common trend of aquatic organisms to use protein as an energy source.

Protein is required in the diet to provide indispensable amino acids and nitrogen for synthesis of non-indispensable amino acids. A deficiency of indispensable amino acid creates poor utilization of dietary protein and hence growth retardation, poor live weight gain and feed efficiency. In severe cases, deficiency reduces the ability to resist diseases and lowers the effectiveness of the immune response mechanism. Experiments have shown that tryptophan deficient fish become scoliotic, showing curvature of the spine, and methionine deficiency produces lens cataracts.

Protein requirement vary with the age of the fish and crustaceans. Younger animal generally require higher levels of protein (5-10% more protein) than older animals. Carnivores require high dietary protein (40-50%) than omnivores (25-35%). The protein requirement 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 *Fenneropenaeus indicus* it ranges from 30-43%, which are the most important 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. Among the brackishwater finfishes, requirement of protein for Asian seabass (*Lates calcarifer*), milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) is 40-45%, 40% and 27-35%, respectively.

Amino acids

The growth of fish and shrimp is directly related to the quality of protein in terms of amino acids. After digestion of protein, amino acids are metabolized at tissue level to form new proteins for growth, maintenance and energy. Among 25 amino acids present in protein 10 amino acids must be supplied in the diet since fish and shrimps cannot synthesize them and termed as essential amino acids (EAA). These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. A large proportion of the amino acid consumed by a fish are catabolized for energy and fish are well adapted to using an excess energy in this way. 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.

Table 1 Nutrient requirement of different brackishwater shrimp and fishes

Nutrient	*P. monodon*	*P. indicus*	*L. vannamei*	*Crab (Scylla species)*	*L. calcarifer*	*Mugil cephalus/ Liza tade*	*Etroplus suratensis*
Energy (Kcal/kg)	2800-4300	3500-4000	3000-3500	4000-4200	4000-4500	4000-4500	4000-4500
Protein %	35-45	30-43	32-36	30-35	45-55	27-35	30-32
Lipid %	5-15	6-10	5-7	5-10	6-18	6-9	6-8
Carbohydrate %	20-25	25-30	30-40	25-28	10-20	30-40	30-40
Phospholipid %	0.1-2.0	0.1-2.0	1.5-5*	0.5-2.0	-	-	-
Cholesterol %	0.5	0.5	0.05-0.15*	0.5	-	-	-

*There is a significant interaction between phospholipids and cholesterol: as phospholipid levels increase, cholesterol requirement decreases. Cholesterol requirement is 0.35% when no phospholipids are present in the diet, but only 0.13% when phospholipid level is 3% of diet

Table 2 Essential Amino Acid requirement in shrimp feed

Amino acid	As % of protein		% of feed at protein level in feed			
	Tiger shrimp	vannamei	36.0	38.0	40.0	45.0
Arginine	5.8	6.3	2.09	2.20	2.32	2.61
Histidine	2.1	2.2	0.76	0.80	0.84	0.95
Isoleucine	3.5	3.1	1.26	1.33	1.40	1.58
Leucine	5.4	5.3	1.94	2.05	2.16	2.43
Lysine	5.3	4.5	1.91	2.01	2.12	2.39
Methionine	2.4	1.7	0.86	0.91	0.96	1.08
Methionine+						
Cystine	3.0	2.8	1.44	1.52	1.60	1.80
Threonine	3.6	4.1	1.30	1.37	1.44	1.62
Tryptophan	0.8	0.6	0.29	0.30	0.32	0.36
Valine	4.0	3.8	1.44	1.52	1.60	1.80

Lipid

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. Apart from its major role to supply energy lipid also act as precursors to many reactive substances. Phospholipids are responsible for the structure of cell membranes (lipid bi-layer). Fatty acids are the main active components of dietary lipids. Deficiency of essential fatty acid result in general reduction of growth and a number of deficiency signs including depigmentation, fin erosion, cardiac myopathy, fatty infiltration of liver and 'shock syndrome' (loss of consciousness for a few seconds following an acute stress). The quantitative requirement of fat in the diet of shrimp is in the range of 5 to 10%. Fat levels of 6-8% are adequate in most of the fish diets. However, the quality of fat in terms of fatty acids is more important. Carnivorous fish such as seabass can utilize lipids more effectively and lipid level as high as 20 % can be used in their diet. However, lipid level should be adjusted in diet considering the technological problems in feed manufacture and storage. Fish oil and soya oil are generally used as lipid source during feed formulation.

Fatty acids

Fish and shrimps are unable to synthesize fatty acids of the n-3 and n-6 series and must be provided in their diets. Aquatic animals require higher n-3 fatty acids than terrestrial animals. Among aquatic animals, marine habitat requires more HUFA than freshwater counterparts. 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 for *P.monodon* and other penaeid shrimps. The n3 fatty acids are more essential than the n6 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% and 1.0% in the diet of larvae and juvenile shrimp. Fresh water fish show requirement for n6 and n3 essential fatty acids (EFA), whereas marine fish show requirement of n3 and also HUFA. Studies in *Fenneropenaeus 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. Marine fish oils are rich dietary source of n-3 series while plant oils are rich in n-6 fatty acids.

Phospholipids

Shrimp require phospholipids for growth, moulting, metamorphosis and maturation. Lipids of squid, clam, shrimp, fish and polychaetes are excellent natural source of 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 contains lecithin. Phospholipids are found to be involved in the transport of lipid, especially steroids in the haemolymph.

Steroids

Shrimps grow through the process called moulting and 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%. Cholesterol is not essential for finfish. Many natural feed ingredients, such as prawn head waste and squid are good sources of cholesterol, which can be included in the feed formulations.

Carbohydrate

Carbohydrate is an inexpensive source of energy in shrimp/fish diet. Among the different types of carbohydrates available, shrimps are found to utilize disaccharide and polysaccharide better than monosaccharide. Omnivorous fishes have enzymes to digest carbohydrates while carnivorous fishes have poor ability to digest carbohydrate. Polysaccharides are better utilized than monosaccharide. Generally carbohydrate utilization by fish is found to be lower than that of terrestrial animals. Fish can utilize dietary carbohydrate up to 40%. For carnivorous fish carbohydrate level in the diet may be in the range of 10-20 %. Depending upon the total energy content required in the diet, carbohydrate can be used from 10-40% level. 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 hence improve water stability of diet. Corn flour, wheat flour, tapioca flour and other grain flours are good source of starch in shrimp and fish diet. Another polysaccharide, cellulose is required in the diet as roughage for improving the feed efficiency in fish and shrimp. Cellulose level in the diet of shrimp should be between 1-3 % and in fish it may be up to 10 %.

Mineral requirement

Micronutrient such as vitamins and minerals significantly influence the growth and survival of fish and shrimp and these cannot be synthesized by these organisms.

Fish and shellfish can absorb minerals directly from aquatic environment through gills and body surfaces or by drinking. Hence, dietary requirement of minerals is largely dependent on the mineral concentration of the aquatic environment. About 20 inorganic elements (macro and micro) are required to meet the metabolic and structural functions in the body of animals. The aquatic organisms regulate the mineral needs through dietary source and also through internal regulatory mechanisms in the kidneys and gills. In saline water, calcium (Ca) is abundant, which is absorbed by most aquatic animals. Since the availability of phosphorus (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. Mono and dicalcium phosphate contain more available P than tricalcium phosphate. 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. The requirement of magnesium (Mg) in shrimp and fish ranges between 0.04-0.3%. The requirement of zinc (Zn) ranges from 15-30 mg/kg diet for fishes and 80-120 mg/kg diet for shellfishes. The requirement of iron (Fe) ranges from 150-200 mg/kg diet for fishes and 60-100 mg/kg diet for shrimps. Major deficiency symptoms of manganese (Mn) in fishes are cataracts and abnormal curvature of the backbone and malformation of tail. A dietary supplementation of 11-13 mg/kg restores normal growth in fishes. In shrimps, the requirement goes up to 40-60 mg/kg which may be due to periodic ecdysis.

Trace minerals 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. 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.

Vitamin requirement

Micronutrient such as vitamins and mineral significantly influence the growth and survival of fish and shrimp and this cannot be synthesized by these organisms. Even though, some vitamin such as niacin can be synthesized by number of animals but are typically insufficient to meet physiological demand. Hence, supplementation of vitamins in feed becomes necessary for most of the aquatic organisms. Usually, vitamins are used at higher doses as a safety margin in crustaceans compared to that of fish. Unlike domestic higher animals, the recommended doses of vitamin for aquatic animals are higher, as many.

Vitamin (mg/kg)	**Fish**	**Shrimp**
Thiamin	10	120
Riboflavin	20	40
Pyridoxine	10	120
Pantothenic acid	40	100
Niacin	150	150
Folic acid	5	5
Vit B_{12}	0.1	<0.1
Choline	3000	600
Inositol	400	2000
Vit C	100	1000
Vit E	30	200
Vit A (IU)	2500	5000
Vit D (IU)	2400	1000
Vit K	10	40

Table 3 Vitamin requirement of fish and shrimp

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 problem during feed manufacture. Many fishes cannot synthesize Vitamin- C from glucose due to absence of enzyme L-gulonolactone oxidase. Major role of vit C is in the formation and maintenance of intracellular material having collagen or related basal constituents in bones and in soft tissues. Among the 11 water soluble vitamins, three (vit C, inositol and choline) are required in large quantities. Sources of choline include cottonseed meal, fish meal, shrimp meal, soybean meal and yeast. Stable form of vit C is available commercially.

NUTRITION AND FEEDING OF PACIFIC WHITELEG SHRIMP

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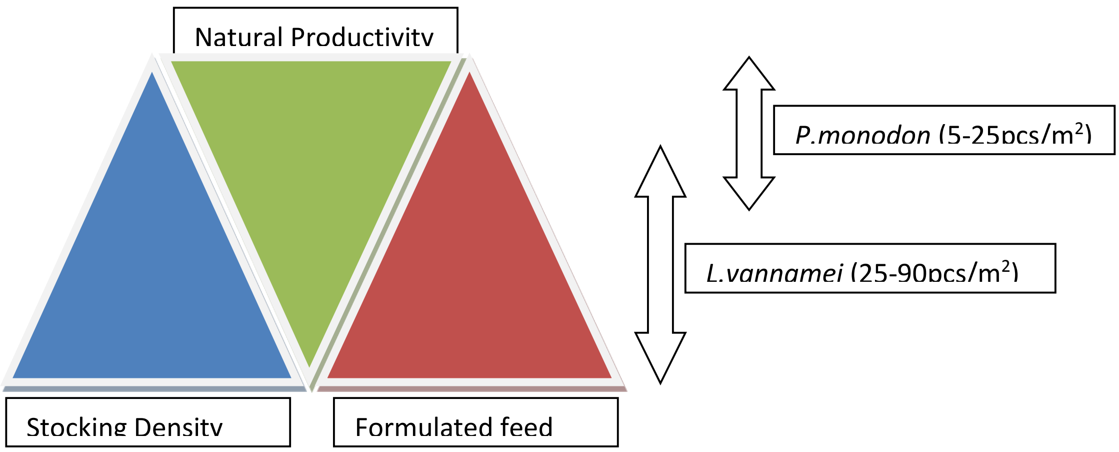
Whiteleg shrimp (*Litopenaeus vannamei*, formerly *Penaeus vannamei*), also known as Pacific white shrimp, is a variety of shrimp of the eastern Pacific Ocean commonly caught or farmed for food. It is the major species of farmed shrimp. Whiteleg shrimp are native to the eastern Pacific, from Sonora in Mexico to northern Peru. The main sources of whiteleg shrimp are Thailand, Indonesia, Vietnam, Ecuador, Mexico, Brazil and India.

Shrimp farming is now synonyms with *Litopenaeus vannamei* culture. Since, it is endowed with many positive traits for current farming, it can readily accept low protein and low fishmeal diet, trouble-free seed production, suitable for intensive and super intensive farming, resistant to wide range soil and water quality fluctuation, increased disease resistance through SPF and good customer acceptance. Nutrition and feeding of Whiteleg shrimp have received a great deal of attention (Cuzon et al., 1985; Pedrazzoli et al., 1998) over the last 20 years. Scientists, farmers, feed manufacturers, as well as suppliers of feed ingredients, all contributed to the better feeding of juveniles raised under semi-intensive or intensive conditions.

Feeding habits

Litopenaeus vannamei is an omnivorous scavenger, feeds voraciously on organic detritus and benthos (copepods, polychaeta, ostracods, nematodes and insects). Since, it can graze/nibble on microbial flocs, plankton sediments and faeces very effectively than *Penaeus monodon*, gives better feed conversion efficiency, improved water quality parameters and minimized organic load.

Figure: 1. Schematic demonstration of significance of formulated feeds with stocking density and natural productivity



Significance of formulated feed for white shrimp

Nutrients inputs and stocking densities are directly proportional. During high stocking density maintaining good water quality is real challenge; it requires feeds with high cohesive property, high digestible protein, optimal essential amino acids and fatty acids. Different levels of stocking densities are being practiced 20 to 90 pcs/m2, intended at 10-50t/ha/yr biomass production. Feed represents 40 to 60% of total production cost; hence, a range of feed formulations must be aimed to produce most economical and nutritionally rational as well. Currently essential nutrients and their digestibility and utilization are best known and with the recent advancement in analytical and experimental facilities in nutrition, feeds can be more precisely formulated to expand the industry further.

Protein nutrition

Proteins are primary nutrients for the structure and function of all living organisms. Since proteins are continually being used by the animal for growth and repair of tissues, a continuous supply of proteins or its constituent amino acids is considered obligatory. Protein is a major and the expensive component of formulated aqua feeds. Hence, nutritional studies of shrimp often start with investigating the optimal dietary protein level. As a consequence, the most researched nutrient in terms of the number of penaeid shrimp species being studied is proteins.

Protein requirement varies from 18-50% under different stocking densities and primary productivity. Ideally, 32% crude protein in diets found to very effect for white shrimps at varied stocking densities 32% protein diet was found to induce superior growth in juvenile and sub adult *L. vannamei* as compared to 16% and 48% protein diets. However, the 48% protein diet had higher Feed Efficiency (FE) on an isonitrogenous basis.

Table: 1. Protein requirement for white shrimp

Dietary crude protein	Reference
15	Aranyakananda 1993
25	Velasco et al., 2000
30	Cousin et al. 1993; Colvin and Brand, 1977
32	Kureshy and Davis 2000
36	Smith et al. 1985
40-55	Sturmer et al. 1992; Treece and Fox, 1993

The lower weight gain, which resulted from feeding the diet containing 48% protein, is possibly due to the low energy to protein ratio of the diet, which would cause shrimp to utilize protein as a source of energy. Using protein as an energy source is relatively inefficient as compared with lipids and would reduce the amount of protein available for tissue deposition (Kureshy and Davis, 2002).

Among all penaeid shrimps, white shrimps utilize plant protein very effectively and nearly 2/3rd of dietary protein comes from plant by products. Plant proteins includes soya bean meal, rapeseed meal, ground nut/peanut meal, wheat gluten and corn gluten are widely used as source of protein in India. Protein that is utilized for energy and not deposited for growth contributes to the release of nitrogen metabolites into the culture medium (Cho et al., 1994) and in 1994, Briggs and Funge-Smith reported that, 80% of nitrogen from feed added to ponds and not retained as shrimp biomass. According to Wu (1995), Nitrogenous wastes are dietary in origin with estimates of up to 52-95% of feed nitrogen being excreted as waste, depending on the species and the diet; thereby high protein and unbalance amino acid diet leads to environmental deterioration. Adequate dietary intake of high-quality protein is required to support rapid growth of fish and crustaceans, but shrimp do not have a dietary requirement for protein per se, but rather for amino acids.

Amino acid requirements

Optimal dietary amino acid profile will depend on the amino acid requirement of an animal for protein synthesis and the use of individual amino acids as energy substrates or for other purposes (Ronnestad and Fyhn, 1993). Deshimaru and Shigeno (1972) suggested that the amino acid composition of the food should be very similar to that of the animal's proteins. Mente *et al.,* (2002) found notable differences between the FAA concentrations in whole animal and in tail-muscle tissue. Tryptophan is a candidate amino acid that limits the rate of protein

Table: 3. Essential Amino Acid requirement for white shrimps, L. vannamei

Amino acids	**(% of Protein)**
Arginine	6.3
Histidine	2.2
Isoleucine	3.1
Leucine	5.3
Lysine	4.5
Methionine	1.7
Met+Cys	2.8
Phenylanine	4.1
Threonine	4.1
Tryptophan	0.6
Valine	3.8

**Taurine conditionally required by some marine fish but no evidence for L. vannamei*

synthesis, Lyndon *et al.,* (1992, 1993) found a significant increase in tryptophan levels in white muscle 12 h after a meal in cod, and correlated this increase with the protein synthesis rates at around this time. Forster et al. (2002) reported that 50% of the fish meal could be replaced in the diet of L. vannamei with soy protein concentrate supplementation with lysine alone, could be further substituted up to 75% level supplementation with arginine, methionine, and phenylalanine.

Lipid nutrition

Lipids constitute a broad group of naturally occurring molecules which include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. Dietary lipids are a highly digestible and concentrated source of energy which supply 9 kcal/g, about double of that supplied by either carbohydrate or protein (Mead et al., 1986). The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules. Although penaeid shrimp, like other animals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet.

It has been demonstrated that shrimp have a limited ability to synthesize *de novo* the n-6 and n-3 families of fatty acids (FA), including the polyunsaturated linoleic (18:2n-6, LOA) and linolenic (18:3n-3, LNA) acids. They also have a limited ability to elongate and desaturate these polyunsaturated fatty acids (PUFA) to highly unsaturated fatty acids (HUFA) such as arachidonic (20:4n-6, AA), eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids (Kanazawa, Teshima & Ono, 1979a; Kanazawa *et al.*, 1979b; Kayama *et al.*, 1980). Consequently, these FA are considered EFA. For *F. chinensis*, Xu et al., (1993) observed that n-3 or n-6 HUFA had greater value than PUFA of the same family following this trend: DHA>AA>LNA>LOA.

Phospholipid (PL)

The importance of phospholipids (PL) in penaeid shrimp nutrition, including *Litopenaeus vannamei* (Boone), has been demonstrated by many researchers (Coutteau, Camara & Sorgeloos, 1996; Coutteau, Kontara & Sorgeloos, 2000; Lim *et al*., 1997; Gong *et al*., 2000a; González-Félix *et al*., 2002a). Phospholipids are a class of lipids and are a major component of all cell membranes as they can form lipid bilayers. Most phospholipids contain a diglyceride, a phosphate group, and a simple organic molecule such as choline; one exception to this rule is sphingomyelin, which is derived from sphingosine instead of glycerol. They maintain cell structure and function, and have regulatory activities within the membrane and outside the cell. For instance, they serve as second messengers in cell signaling, an essential process in regulating cell growth, proliferation, differentiation, metabolism, nutrient uptake, ion transport, and even programmed cell death. In addition, there is evidence that PL containing choline, sphingomyelin, and their metabolites are important mediators and modulators of transmembrane signaling (Zeisel, 1993). PL act as emulsifiers and facilitate the digestion and absorption of FA, bile salts and other lipid-soluble matters. They also have a role in the transport of lipids, not only in the transport of absorbed lipids from the gut epithelium into the hemolymph, but also in the transport of lipids between tissues and organs (Coutteau *et al*., 1997) since they are constituents of lipoproteins. Shrimp growth increased with PL levels up to 3-5% of diet (Gang et al ., 2001).

Table: 4. Essential fatty acid, phospholipid and cholesterol requirement for L. vannamei

Nutrients	**(% of diet)**
Linoleic acid	0.1
Linolenic acid	0.1
Arachidonic acid	0.2
Eicosapentanoic acid	0.4
Docosahexanoic acid	0.4
Phospholipids (% of diet)	1.5-5*
Cholesterol (% of diet)	0.05-0.15*
Choline (in the form of Choline chloride, ppm in the diet)	1000**

* There is a significant interaction between phospholipids and cholesterol: as phospholipid levels increase, cholesterol requirement decreases. Cholesterol requirement is 0.35% when no phospholipids are present in the diet, but only 0.13% when phospholipid level is 3% of diet.

Carbohydrate nutrition

Carbohydrates are classified among simple (glucose, trehalose) and complex (starch, glycogen, chitin, cellulose) and the bulk of organic matter in the environment is provided by carbohydrates. Carbohydrate is the cheapest source of energy. Digestibility will be moderate if it is processed well. Critical component of feed processing because, only under optimal moisture and temperature it gets gelatinized completely which gives natural binding to the pellets that makes pellets more durable and water stable.

Carbohydrates utilization by shrimp varies with carbohydrate complexity and processing procedures. These differences are represented by the range of digestible energy values reported for Pacific white shrimp, *Litopenaeus vannamei*, for typical ingredients processed under different conditions: whole wheat 3,571-3,857 kcal/kg, corn flour 3,037-

3,917 kcal/kg, rice flour 3,093-4173 kcal/kg, and milo/sorghum 2,821-3,785 kcal/kg. Although these values are considerably lower than those of lipids, which contain about twice the energy of carbohydrates and proteins, the cost per energy unit is much lower for carbohydrate sources. Among botanical origin we can cite potato, corn (several forms), wheat, cassava, sago palm, rice basically. Digestibility of starch from various botanical origins is given in Table 7. Starch is rich in amylose and is poorly digestible compared to starch rich in amylopectin. Native potato starch is less digestible than pre-cooked one. Wheat native starch is well digestible (92%). Corn starch with 76-99% amylopectin is equally digestible.

Table: 3. Vitamins requirements

Vitamins	**Per KG diet**
Vitamin A (Retinol acetate)	2600 IU (0.89mg)
Vitamin D3 (Cholecalciferol)	2000 IU (0.05mg)
Vitamin E (alpha-tocopherol)	100 mg
Vitamin K	NA
Water soluble vitamins	
Vitamin C	40-120mg (100 mg c
Choline	1000 mg without lec
Inositol	400 mg
Niacin	80 mg
Thiamine	40 mg
Riboflavin	60 mg
Pyridoxine	60 mg
Pantothenic acid	180 mg
Folic acid	6 mg
Biotin	1 mg
Vitamin B12	0.1 mg

Chitin:

It is the major structural component of the exoskeleton of shrimps and one of the carbohydrates that shrimp meet in natural environment might be chitin. Minimum of 0.5 % chitin is recommended in shrimp feeds because dietary chitin has growth promoting effect (Akiyama et al. 1992). Glucosamine, a monomer of chitin, has inhibitory effect on growth by diminishing the growth-promoting effect of cholesterol.

Vitamin and Mineral nutrition

Vitamins are organic compounds required by shrimp in very small quantities to maintain essential functions. Quite often they function as coenzymes in chemical reactions and hence are described as the "spark plugs" of cells. The qualitative and quantitative vitamin requirements of shrimp have not received major attention. The difficulties in determining the vitamin requirements are

Exemplified by the wide variations in vitamin supplementation utilized by researchers and the commercial industry. As there is considerable debate regarding the absolute requirements for shrimp, vitamin values can be tempered with those established with other species. Shrimp feeds are generally supplemented with a vitamin premix in a sufficient quantity to exceed the estimated vitamin requirements, including losses due to feed processing. Levels above those required for maximum growth may facilitate the ability of shrimp to respond to stress. Feed processing and storage can degrade many of the vitamins, and due to the slow feeding habits of shrimp, considerable leaching can occur prior to consumption. As exemplified in studies, the use of vitamin premixes may not be required under some culture conditions if the animals have access to natural foods and there are no environmental stresses. Although this concept is well accepted for both fish and shrimp, a wide variety of factors affect the quantity, quality, and accessibility of natural foods. The decision to reduce feed costs through the utilization of nutritionally incomplete feeds is a viable strategy. However, it is difficult to predict and manage the availability and nutritional quality of natural foods. Consequently, if nutritionally incomplete feed is used, farmers increase the risk of suboptimal production due to nutritional inadequacies.

Vitamin-C

Lightner, Colvin, Brand & Danald (1977) first demonstrated the essential role of dietary vitamin C in preventing the development of the black death syndrome in penaeid shrimp. Recommended dietary ascorbic acid (AA) levels for shrimp using ascorbic acid-polyphosphate (ApP) are 20 and 120–130 mg/kg for the postlarvae of tiger shrimp, *Penaeus monodon* (Fabricius), and white shrimp, *Penaeus vannamei* (Boone), respectively. A significant effect was shown to be produced on stress sensitivity for both the species by high dietary AA levels, and moreover, *P. vannamei* displayed a higher resistance to *Vibrio harveyi* infection (Kontara, Merchie, Lavens, Robles, Nelis, De Leenheer & Sorgeloos 1997; Merchie et al. 1997).

Minerals

A variety of studies with shrimp illustrate the nutritional importance of the inorganic components of the diet. The quantitative mineral requirements of penaeid shrimp have not been established, but are reasonably well studied. Practical diets generally contain a substantial amount of endogenous minerals. Consequently, complete mineral premixes are not necessary. With the exception of phosphorus, macro minerals are generally not supplemented to commercial feeds, as there is no evidence these minerals are required in feed under normal production conditions. Since many of the trace minerals can have low biological availability or are found at relatively low levels, a number of trace minerals are commonly supplemented. Phosphorus is the most costly and problematic mineral. Its biological availability varies with the source, with the water-soluble forms having higher availability to shrimp.

Table: 4. Minerals requirements

Minerals	**Gram/Kg diet**
Calcium	23.0
Phosphorus	9.0
Total phosphorus	15.0
Magnesium	3.0
Sodium	6.0
Potassium	10.0
	Mg/Kg diet
Iron	36
Copper	32
Zinc	72
Manganese	12
Selenium	0.2
Cobalt	0.6
Iodine	1.2
Chromium	0.8
Molybdenum	0.2

Feed additives

Free amino acids: used singly or in mixtures to reduce dietary protein levels and nitrogen excretion, to overcome dietary amino acid deficiencies resulting from fish meal replacements, or as feeding stimulants

Feed enzymes: used singly or in mixtures to increase carbohydrate and mineral digestibility and reduce nutrient (e.g. phosphorus) loss to the aquatic environment.

Chemoattractants and/or feeding stimulants: used to increase feed palatability and stimulate feed intake (especially when applied to plant-based rations containing low levels of marine protein sources), increase growth (by minimizing the time the feed remains uneaten in water and thereby minimizing nutrient loss through leaching), and reduce feed wastage

Probiotics: live micro-organisms (e.g., bacteria and fungi) and/or their processed products are used as dietary supplements or added directly to the water to stabilize or enhance a healthful and appropriate microbial community in the gastrointestinal tract of the cultured shrimp and/or within the culture system, so as to improve growth, survival, and/or disease resistance. However, it is important to mention here that although good scientific evidence exists concerning the beneficial effects of probiotics on shrimp performance and health under Clearwater hatchery conditions, this has not always been the case under practical pond grow-out conditions, where resident aquatic/sediment microbial flora already exists.

Immunostimulants: used to stimulate the nonspecific immune system mechanisms of shrimp and thus increase disease resistance

Miscellaneous: Other additives that can be used in shrimp feeds include antioxidants, mold inhibiting compounds, pigments, and to a lesser extent chemotherapeutants and hormones

Feeding Management

Feed management starts immediately after the introduction of post larval shrimps. During first 30 days of culture (DOC), feeding based on assumption and majority of the feed goes as nutrient for planktons rather for shrimps; which in turn feed and shelter for post larval shrimps. Initially, culturing adequate planktons is critical for better survival, good health and also reducing size variations of the post larvae. Second month onwards daily feeding must be controlled by introducing feed trays/check trays since 85% of total feeding left. Minimum of 4 feed trays for one acre pond is recommended. The dimension of the feed trays is 80cmX80cmX10cm, and made up of steel frame with nylon mesh. Physical size of feed also very important in controlling feed conversion efficiency and reducing pollution.

Table: 5. The recommended physical dimension for white shrimps

Name	Dimension (mm)	Shrimp size (g)	% feed required
Starter I	0.3-0.6	0.02-0.5	1
Starter II	0.5-1.0	0.5-2.0	3
Starter III	0.8-1.2	2.0-5.0	8
Pre-grower 4s	1.6 x 3-4	5.0-15.0	30
Grower 4	1.8/2.0 x 4-5	15.0-25.0	30
Finisher 4L	2.0/2.2 x 4-5	25.0-harvest	28

The role of feed boy is very important for white shrimp farming. He should have adequate knowledge of demand feeding, indication of over/under feeding, moulting on feed consumption, effect of cold weather and other climatic conditions. He should not follow feeding guides all the time since, it is based on a rough theoretical calculations. Actual feeding may vary greatly with temperature, dissolved oxygen, plankton bloom (natural productivity), pond bottom and pollution.

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^oC 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^{o}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^{o}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.

Feedstock 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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CURRENT STATUS OF AQUA FEED INDUSTRY IN INDIA AND CIBA'S INITIATIVE IN NEWER FEED TECHNOLOGIES

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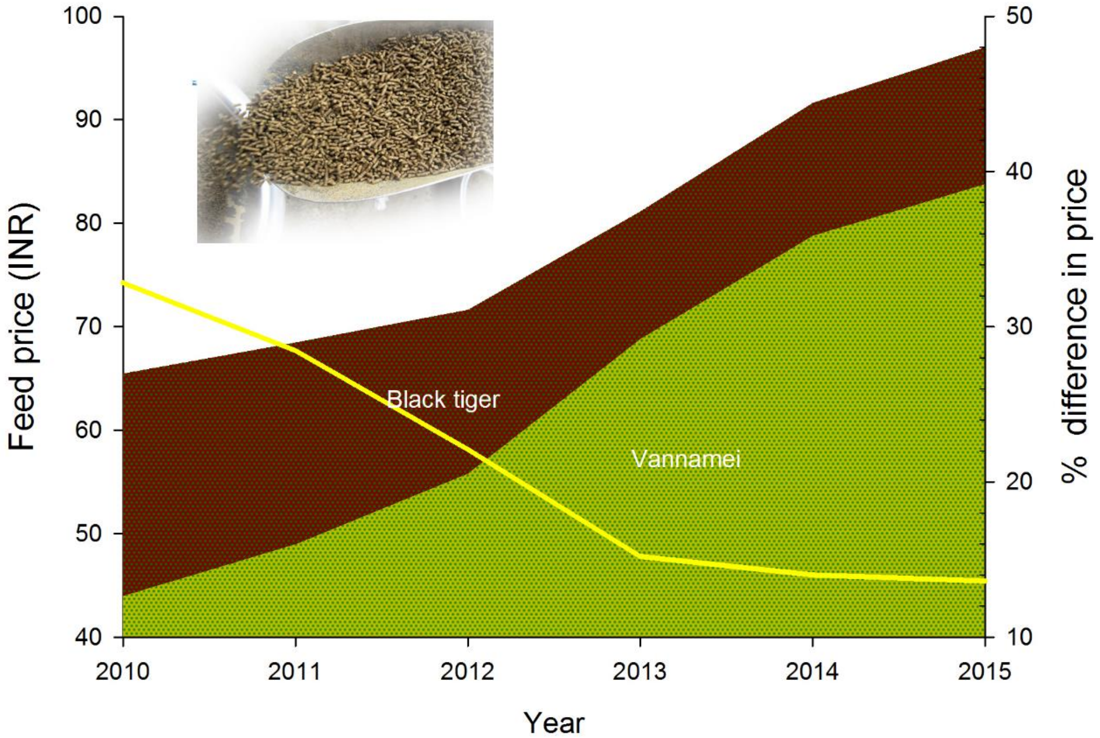
Aquaculture has emerged as one of the fastest growing food producing sector in the current decade. It is estimated that global fish production would increase by 19% between 2014 and 2024 with the main driver being formulated feed. India ranks second in aquaculture production. Indian aquaculture is mainly contributed by freshwater aquaculture while the brackishwater aquaculture is poised for vertical and horizontal expansion during the years to come. The rapid expansion of aquaculture has to be supported by the availability of cost effective sustainable technologies.

Aqua feeds in India have evolved commercially in late 90's for shrimp culture and then for fishes. In the brackishwater aquaculture sector majority of the feed used is scientifically formulated compounded feed produced by multinational / Indian company. There are some small players with a capacity to produce 1 tonne per hour operating to cater to the need of local area/ undertaking job works for the nearby farmers. In the fresh water aqua feeds traditional feeding using farm made feed comprising rice bran and locally available plant protein source is the predominant feeding system where in about 80-85% of the potential is unexplored for manufactured feeds. The sinking feed for Carps and the extruded floating feeds for fish are making a steady and positive impact in changing traditional feeding system with the focus on increasing the productivity on a sustainable manner in the longer run.

Brackishwater aquaculture is synonymous with shrimp aquaculture in India and tiger shrimp, *Penaeus monodon* is the main species cultured in the brackishwater till 2010. During the year 2011-12, a total of 135778 MT of tiger shrimps were produced from an area of 115342. After the introduction of the pacific white shrimp in 2009 there is steady and sharp increase in culture area as well as production and *L. vannamei* has become the main species cultured in brackishwater. The growth of *L.vannamei* production has been stupendous. From a production of about 18247 MT from 2930 ha in 2010-11, the *L.vannamei* production reached 353413 MT in 2014-15 registering an increase of 1837% in production. The boom period for vannamei farming began in the late 2011, when shrimp prices were very good, and the positive trend continued till early to 2014. However, due to recurrent problem in diseases such as white spot virus and running mortality syndrome there is a sudden decline production of farmed shrimp. This decreased production coupled with spiraling hike in all input prices particularly the feed prices has made a significant impact on the profitability of the shrimp farming. The cost of production of shrimp has increased from Rs. 180 per kg of shrimp in the year 2010 to Rs 280 per kg. This sharp increase in price is mainly due to the steady increase in the cost of feed. The feed cost per kilo of shrimp produced alone reached an all-time high of Rs. 135 per Kg.

This scenario resulted in considerable difficulties for the small and medium scale farmers as they could not able to cope up with the double blow of increased feed price and a drop in shrimp price.

This increase in price has not been commensurate with farm gate price offered to the farmer. This has made serious dent in the profit margin and farmers started looking for ways and means to reduce the cost of production of shrimp. The feed cost has been considered as one of the major factor for the increased cost of production and the feed cost is mainly determined by its protein content. In this context it is pertinent to note that *L. vannamei* is an omnivorous scavenger and is less aggressive and less carnivorous than *P. monodon.* The available research information on the nutritional requirement of this species also indicates that it requires less protein than the tiger shrimp. Further it can also accommodate more plant protein sources in the diet than the tiger shrimp. These inherent advantages should have resulted in availability of more cost effective feeds. However the analysis of actual scenario revealed an interesting picture. All the major feed companies in India maintained their protein content for tiger shrimp in the range of 38-40% while the crude protein content in vannamei feed was kept in the range of 34-36%. The average price of shrimp feed for both the species during the period from 2010 to 2015 is given below.



During the year 2010 the cost of vannamei feed was Rs. 44 per kg against Rs. 66 per kg for the tiger shrimp feed and the price difference between these two feeds was Rs 22 per kg. Even though the nutrient contents were maintained uniformly over the years the price difference between these feeds showed a consistent decrease. Currently, the cost of vannamei feed has reached its peak at Rs.85 per kg while the tiger shrimp feed costs about Rs. 98 per kg and the price difference narrowed down to Rs. 13 per kg. This showed that the cost of vannamei feed available to the farmer at present is high against its nutrient content and the feed cost has to be brought down to make the vannamei farming more profitable and affordable by the shrimp farmer.

As per the available information, during 2014 a total of 1.25 million metric tons of aqua feed has been produced in India comprising of about 600,000 tons of shrimp feed and 650,000 tons of fish feed. Out of the total shrimp feed produced vannamei contributed about 80-85%. The total shrimp feed is produced by the eight major feed mills and 30 small feed mills.

Availability of cost effective feed is the felt need of the farmers at present and they started exploring the possibilities of producing their own feed in order to reduce the cost of production. Many big farmers and farmer's group/cooperatives are contemplating to establish smaller feed mill to produce feed for their own requirement. In addition they can also cater to the small farmers located in their region with the availability of cost effective feed. This direct marketing channel from the feed mill to the farmer would result in availability of cost effective feed. Central Institute of Brackishwater Aquaculture (CIBA) has developed the indigenous shrimp feed technology and helping them in such endeavours. During the year 2015, CIBA has entered into MOU for feed technology transfer with four such entrepreneurs. Feed mill with a production capacity of 1-2 tons per hour is being established in the state of Gujarat, Andhra Pradesh, Kerala and West Bengal in India. There is a great scope for establishing many such feed mills in the coastal states of India. This would result in availability of cost effective feed for small and medium farmer. Brackishwatrer aquaculture techonology is evolving with an array of new array of feed technologies and farming system and the details are given below.

Indigenous Shrimp Feed Technology

CIBA has taken up research program on a mission mode approach and has made rapid strides in the area of shrimp feed development. Knowledge on nutritional requirements of candidate species is essential for the development of feed and hence to begin with the nutritional requirement of the shrimp species viz., *P. monodon and P. indicus* have been extensively researched upon.

The feed technology was developed through formulation of several test feeds using indigenous ingredients to meet the dietary requirements of tiger shrimp (*Penaeus monodon*) and Indian white shrimp (*Fenneropenaeus indicus*). These test feeds were first evaluated in laboratory experiments and then in yard experiments on three size groups namely 2-5 g, 5-10g and 15-25g for developing three grades of feed namely Starter, Grower and Finisher. The feed formulations which gave best growth and feed conversion ratio (FCR) were selected for up-scaling. A pilot-scale Ring-Die pellet feed mill of capacity 500kg per hour was set up at the Muttukadu experimental station of CIBA. The pellet mill was integrated with a hammer mill, micro-pulverizer, sieve assembly, ribbon mixer, steam boiler and crumbler for completing the feed processing. All the machinery is of indigenous origin.

The multi locational demonstration of indigenous shrimp feed technology resulted in commercialization of CIBA developed shrimp feed technology to the following firms.

- M/S Bismi Feeds (P) Ltd, Deen complex, OSM Nagar, Mayiladuthurai, Nagapattinam District, Tamil Nadu

- M/S. Pisiculture care Unit, Village Madhubati, P.O. Kamarpukr, Hooghly District, West Bengal

The feed produced by M/S Bismi feeds using CIBA shrimp feed technology has gained popularity among the farmers in Tamil Nadu and has become the sought after brand. The feed produced using CIBA shrimp feed technology is being sold @ Rs 4-6 lower than the reputed commercial brand shrimp feeds. The reduced cost of shrimp feed is due to the use of indigenous raw materials and machineries.

Feed for vannamei : Vannamei *Plus*

CIBA has developed a cost effective grow out feed using indigenous ingredients. This feed has been tested in a farmer pond at Gujarat and compared against the commonly used commercial shrimp feed. The cost of the commercial feed available to the farmer was about Rs.78 per Kg while the cost of feed (inclusive of cost of ingredients + processing and transportation cost) to the farmer was Rs. 54 per kg. At the end of 117 days of culture the results showed that shrimps have attained an average final body weight of 27.1 g in the pond fed with CIBA feed while the control pond shrimps have attained the final body weight of about 24.6g. The total quantity of shrimp harvested was about 2017kg in the CIBA feed fed pond while it was about 1913 kg in the control pond fed with commercial feed. The FCR obtained was 1.68 with CIBA feed and 1.79 in the commercial feed. Interestingly the feed cost to produce one kg shrimp was Rs.91 with CIBA feed against Rs.140 in the commercial feed. This could be potential saving for the shrimp farmers and would pave the way for considerable reduction in production cost with better profitability for small and medium farmers. These results showed that the indigenous *vannamei* grow-out feed developed by CIBA could be cost-effective and able to be an import substitute to bring down the cost of production and increase the profitability of Indian shrimp farmers.

This feed technology has been commercialized to the following firms

1. Sai Aqua Feeds
2. Poshak Bio Research Pvt Ltd.,
3. BT revelations
4. M/S. M.K Feeds
5. Kavya Aqua farm
6. West land marine Pvt 21imited

Specialty feeds

The nutritional requirement of shrimp varies depending on the environment in which it is cultured. In India the shrimp farming is being practiced from low saline (1 to 5ppt) to high saline conditions (35-45ppt). Considering these specific dietary requirements for improving the growth and FCR under high and low salinity, trials have been carried out and feeds for low and high saline conditions have been developed. These feeds are ready for field testing and commercialization.

The other specialty feeds developed were to address stress and attractability. Under intensive / semi intensive farming, shrimps are subjected to considerable stress which in turn affects the growth and FCR. To address this, various stress busters have been explored and feed with specific additives to mitigate the stress have been developed. Extensive research efforts on palatability and attractability lead to the development of cost effective feed with good attractability and palatability.

Low protein and Low fish meal based feeds

Fish meal is the mainstay in shrimp feed and CIBA has initiated major research programme on replacement of fishmeal with alternate protein sources with reasonable rate of success. This has led to development of **Organic shrimp feed** wherein, there restriction on the usage of fish meal and fish oil. Similarly there are exclusive feeds for feeding the shrimp under BIOFLOC system. Since the bioflc contributes considerable amount of micronutrients it is apt to reduce the protein content and use more of plant protein based ingredients to meet the protein requirement. His would help in reducing the cost of feed and will pave the way for sustainability.

Broodstock Feed for Etro brood Plus

Pearlspot is a popular aquarium and food fish, promoted as potential candidate for brackishwater farming. Farmers produce pearlspot seeds in large ponds in traditional manner by feeding the adults with commercial carp feeds and farm made feeds. Seed production with such a low fecund fish in this manner is labour intensive, less productive and uneconomical. CIBA developed a broodstock diet (Etro brood Plus) to provide specific nutrients vital for maturation and spawning, and demonstrated for the first time that pearlspot can be made to spawn more than 4 times/year (average) in 1000 L tanks with an average fry yield >2200/spawning. CIBA also developed a larval feed and standardised a feeding strategy to rear the pearlspot larvae with maximum survival and fast growth in the absence of parental care.

Milkfish brood Plus and Mullet brood Plus

Institute has developed a unique formulation for Milkfish Broodstock using novel and speciality ingredients and the feed has been found to be effective for Milkfish breeding and this has been tested and validated. The feed developed is also being used for Milk fish Broodstock by Aditya Fish Hatcheries under the Collaborative research cum technology transfer program.

Shrimp Larvi Plus : Shrimp Larval Feed

Development of inert diet for rearing shrimp larvae has been a challenging task. This is mainly because, besides providing a balanced nutrition package in a tiny particle, which should be digested and assimilated, the diet particles should be designed to keep them suspended in the rearing medium so that the filter feeding larvae can utilize it. Live food organisms such as brine shrimp nauplii are still indispensable for rearing shrimp larvae. Nevertheless, artificial diets are also supplemented to overcome the uncertainties associated with mass production of live food organisms, their nutritional imbalances and cost. Increasing costs of imported brine shrimp cyst have pushed the cost of production of shrimp seed. With fluctuating international market for shrimp the farmers are looking for reduction in input costs of in shrimp production. Use of inert diet supplements for feeding shrimp larvae would certainly reduce the cost of production of shrimp seed. It is in this context CIBA has taken up research project for developing inert diet supplements for shrimp larvae.

Shrimp Larvi Plus has been successfully developed for feeding the larvae and post larvae of Shrimp. IT has been tested in vannamei larvae in commercial hatcheries and the technology is being commercialized with M/S. MARITECH.

Seabass Plus : Feed for seabass

Seabass Plus is a formulated pellet feed developed by CIBA for the nursery and grow-out culture of Asian seabass, *Lates calcarifer* and contains 40-50% protein. It meets the dietary requirements of Asian seabass for optimum growth with good feed efficiency. CIBA ***Seabass Plus*** uses indigenously available raw materials. The feed processing technology for different grades was standardized in a Ring-Die pellet mill and also in a Twin-Screw Extruder at the Pilot-scale feed mill of CIBA at Muttukadu. The feeds were systematically developed through formulation of several feeds using selected indigenous feed ingredients and evaluation of the test feeds in laboratory and yard trials and selecting the best performing formulation. Using the selected formulation, the feed was processed into granules and pellets with indigenous feed mill and processing technology to suit the growing stages of Asian seabass in nursery and grow-out systems. The feeds were extensively tested in the institute facilities and also in selected farmers' fields in different states such as Andhra Pradesh, Maharastra and Tamil Nadu. CIBA Bhetkiahar resulted in excellent growth and survival of fry in nursery and grow-out systems in these field trials. The fish was successfully grown to 1kg in 8-9 months with an impressive FCR of 1.5 to 1.8. It is believed that the performance and cost effectiveness of this indigenous feed will go a long way in propagating large scale farming of Asian seabass. **Seabass Larvi** **Plus** : Feed for seabass larva developed and tested in commercial Hatcheries.

Feed for Crab : Scylla ***Plus***

Similar to the feed development programme of shrimp and fish considerable research has been carried out in the development of feed for mudcrab. In this context feed for fattening and grow out culture has been developed and field tested with highly encouraging results.

.CIBA developed low cost indigenous automatic feeder for *L. vannamei*

Automatic feeders are more suitable for *L. vannamei* culture due to its feeding behavior as it is a column dweller and it needs continuous feeding. Entrepreneur farmers have imported automatic feeders and it is costly. These prompted CIBA to design and develop a low cost feeder for the use for small farmers.

The design of an automatic feeder developed at CIBA consist of four major components, a feed hopper, a mechanism for feed distribution an electrical power supply/ solar power and a control unit for operating (frequency and time of distribution). Each component has been designed with utmost care. The hopper bottom has been designed such that the angle of repose of the hopper bottom is greater than the angle of repose of the materials for easy dispensing from the hopper. Motor unit was fixed with variable speed regulator to control the dispensing of the feed. Two timers with digital display was set such that the dispensing of the feed and the duration of feeding could be adjusted easily by the farmers. This has been successfully demonstrated in the shrimp farm at Marakanam. Tamil nadu.The cost is only Rs 15,000/- and it is easily operated and adjustable with digital display. The provision for incorporation of solar energy using DC motor has been done. Trial demonstration showed that the radius of dispersion in 26 m and quantity of feed can be adjusted as per the need.

Conclusion

The feed development programme at CIBA matched the pace and growth of aquaculture industry. The research planning in the areas of aquaculture nutrition are in tandem with the priority of the farming community and have led to development of indigenous feed and processing technology for brackishwater shrimp and fish. The successful commercialization of CIBA shrimp feed technology and its presence in the market is the testimony to the strength of feed development programme of this institute. The state of the art facilities established in the area of feed mill, nutrition analytical laboratory and wet lab experimental facilities coupled with trained scientific acumen will meet the existing and emerging challenges in nutrition and feed development of this sector and will pave the way for sustainable brackishwater aquaculture.

GENETIC IMPROVEMENT PROGRAMMES IN AQUACULTURE

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Genetic improvement through selection has been an important contributor to the dramatic advances in agricultural productivity that have been achieved in recent times (Dekkers and Hospital, 2002). Genetic improvement of any population of a species involves periodic evaluation, selection and culling of animals. Especially in aquaculture sector, any new species identified for genetic improvement program should be domesticated first and reproduction of that species should be mastered to perfection. Otherwise, limited numbers of founders at the beginning of domestication process lead to limited genetic variability in hatchery broodstock populations (Sbordoni et al 1986, Bierne et al 2000, Goyard et al 2003). Unlike livestock which have been domesticated years ago, aquatic species are yet to be domesticated save a few species. Genetic improvement programmes for sustainable aquaculture production is reflected in FAO's *Code of Conduct of Responsible Fisheries* (FAO, 1995). Genetic improvement is possible only when breeding programmes are well-designed which leads to substantial response in the character or trait under selection. Some examples which come to our mind especially when dealing with aquatic species is the genetic improvement programme of Atlantic Salmon (Gjoen and Bensen, 1997), rainbow trout (Gjerde, 1986), Nile Tilapia (Eknath and Acosta,1998) Rohu Carp in India (Reddy *et al* 2002) and Pacific White Shrimp in Columbia (Gitterle, 2005). Several shrimp lines selected for their resistance to pathogens or for their growth rates are now commercially available and certified free of the four following viruses: Taura Syndrome Virus, Infectious Hypodermal and Hematopoietic Necrosis Virus, White Spot Syndrome Virus, and Yellow Head Virus (Moss *et al* 2005). All these programmes have revealed the effectiveness of genetic selection in aquaculture when economically important traits are selected for. However, it is important to note that aquaculture production is using only a miniscule portion of genetically improved species, be it finfish or shellfish. A considerable portion of aquaculture production depends largely on species that have not undergone any systematic genetic improvement. Genetic improvement programmes are of long-term nature and the inputs in terms of money, labour and personnel are very substantial. There has however been a school of thought that unless genetic improvement programmes are initiated, one cannot increase aquaculture production.

CANDIDATE SPECIES FOR AQUACULTURE

When we compare the aquatic species with the terrestrial animals, the foremost thing that comes into the mind is the amount of variation available in traits in aquatic species. Gjedrem (1997) observed that the coefficient of variation in fish/shellfish species for growth rate is 20-35% whereas it ranges from 7-10% in farm animals. This variability reflects higher genetic variability and ultimately translates into higher genetic response on application of selection. Another feature of aquatic species is that fertilization is external (which would aid in production of either half- or full-sib families with relative ease) and fecundity is very high which enables us to apply a very high level of selection intensity during selection for a particular trait. These two aspects of aquatic species coupled with short generation intervals make them excellent candidates for genetic improvement.

DESIGN OF BREEDING PROGRAMMES

One of the most important aspects of a genetic improvement programme is that the individual fish/shellfish should be amenable to identification or tagging. Refstie and Aulstad (1979) have listed the requirement for identifying fish.The method should be applicable to small animals, should be inexpensive and less laborious.

1. Whatever tags are placed should not in anyway influence growth.
2. The tags should be such that they should be readable past the recording.

Some of the tags used in aquaculture are:

1. Passive Implant Transponder Tags or PIT tags for fish
2. Visible Implant Elastomer Tags for shrimp
3. Visible Implant alphanumeric tags for fish and shellfish
4. Floy tags
5. Coded wire tags

In addition to the above physical tags, genetic tags could be utilized to identify the pedigree of an individual. This is through genetic markers (microsatellites). Tagging is very beneficial in that we can go for communal rearing of fish/shellfish so that there are common environmental effects while recording a trait. This is extremely helpful while analyzing the data statistically.

The breeding programmes could be either individual-based or family-based depending upon the trait in question. There have been instances where initial individual selection is practiced after which some other economic traits are added as selection criteria, depending upon the necessity.

GLOBAL SELECTION PROGRAMMES

Before we attempt to discuss selection programmes at a global level, a short introduction about the concepts of selection would be pertinent. Selection is of two types- natural and artificial. The survival of the fittest is seen in the process of natural selection whereas artificial selection is carried out by man. Artificial selection has been in vogue since long in plants and terrestrial farm animals. Selection is therefore defined as the choice of the individuals to be used as parents for the next generation (Falconer and Mackay, 1998). During selection, no new genes are produced. When selection is carried out, the frequency of the desirable genes increases. Selection is usually practiced on quantitative traits like growth rate. All quantitative traits follow a normal distribution and exhibit continuous variation. These traits are governed by many genes (polygenic) and are analysed by estimating population parameters like mean, standard deviation etc. While carrying out matings after selection, one has to bear in mind that inbreeding should be avoided. Some of the matings that result in an increase in inbreeding coefficient and are to be avoided are: Sire-daughter, dam-son, full-sibs and half-sibs. Depending upon the type of selection to be practiced one can go in for either mass selection (or individual selection) where the individual's own performance is taken as a basis for selection. Family selection is used when the heritability of a trait is low. However, family selection results in fewer families being represented among selected parents resulting in build-up of inbreeding. Within family selection operates on a larger portion of the additive genetic variance.

Presently there are more than 40 family-based selection programmes all over the world. It is observed that half of these programmes target salmonid species. In shrimps, genetically improved strains are available for Pacific White Shrimp. In fishes, the GIFT is a good example of genetically improved Tilapia. In Norway, Canada, Chile, Faroe Islands, Iceland, Scotland and Ireland, genetically improved Atlantic Salmon is available. In Greece, there is a genetic improvement programme in place for the European Seabass (Morten *et al* 2007). In Australia, a selection programme in Kuruma Shrimp is underway and it has been possible to obtain a genetic gain of 11% per generation for growth (Lyons *et al* 2007). The domestication and selective breeding in tiger shrimp was initiated in late nineties. However, after three generations, a National Consortium was formed with pooled resources and the private has a major role to play in this project.

In Norway, selective breeding in Atlantic Salmon was initiated in 1975 and in 2007 the programme was at F_8 generation with an average genetic gain of 10-12%. The growth period of Salmon in freshwater has been reduced from 16 to 8 months and the growth period in sea water has been reduced from 24 to 12 months. The total growth period has thus been reduced from 40 to 20 months and the fish now attains a body weight of about 5 kg within a year (Morten, 2007).

There are genetic improvement programmes in tiger shrimp in the private sector in USA. There is another domestication programme at AQUALMA in Madagascar which in in vogue since long. However, the details of the performance of these improved strains are not available in public domain.

GENETIC IMPROVEMENT PROGRAMMES IN INDIA

In India there is a genetic improvement programme in *Labeo rohita* in place at the ICAR-Central Institute of Freshwater Aquaculture (CIFA) Bhubaneswar. It started as a collaborative programme between CIFA and the AKVAFORSK, Norway. An average genetic gain of 17% per generation after 5 generations of selection has been reported.

The CIBA, Chennai, CIFE, Mumbai in collaboration with AKVAFORSK, Norway made attempts to domesticate and improve tiger shrimp for growth and white spot disease resistance. This project was executed from 2004 to 2009. The results of the project indicate that growth and pond survival are traits that are controlled by additive gene action which implies that selection for these traits could elicit good response. The resistance to WSSV was having very little additive genetic variance implying that selection cannot bring any improvement in this trait. (Hayes *et al* 2010). However, such traits could be improved by detecting Quantitative Trait Linked loci (QTLs). Later from 2009-2013, CIBA, Chennai, CIFA, Bhubaneswar in collaboration with NOFIMA: The Norwegian Institute of Food Fisheries & Aquaculture Research, Norway were involved in a project to find markers for WSSV resistance in tiger shrimp and Aeromoniasis resistance in rohu carp using next generation sequencing technologies on *Illumina* platform. A high density linkage map in tiger shrimp using SNPs was constructed in this project, the first time globally (Baranski *et al* 2014).

The project included transcriptome sequencing, detecting SNPs in transcriptome and association studies using genotypes generated on a DNA chip. Mapping populations were developed for rohu carp and tiger shrimp which resulted in identification of Single Nucleotide Polymorphism (SNPs). Theses SNPs were used to unravel QTLs for resistance to WSSV in tiger shrimp (Robinson et al 2014). At CIFA, these QTLs in rohu are being used in the ongoing selective breeding programme. In tiger shrimp, 9 genes have been identified which contain QTLs related to resistance to WSSV. There is a plan to hone in on the QTLs so that these could be effectively used in a future breeding programmes. The CIFA, Bhubaneswar in collaboration with World Fish Centre, Malaysia, has initiated a selective breeding program in *Macrobrachium rosenbergii* in 2007. Founder stocks for the program were collected from Gujarat, Kerala and Orissa. Diallele crossing of these stocks have led to choice of the synthetic base population. The families are grown separately in hapas in a pond till they reach the tagging stage. The juveniles are tagged with visible implant alphanumeric tags and communally reared in a pond. Already 3 generations of selection have been completed alongwith performance testing of the selected animals in farmers' ponds.

BIOTECHNOLOGICAL TOOLS USED IN GENETIC IMPROVEMENT PROGRAMMES

The role of DNA based genetic markers in breeding programmes is gaining importance. Various types of markers are available viz. allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellites, single nucleotide polymorphisms (SNPs) and ESTs. The application of these markers has allowed for rapid strides in aquaculture, investigation of genetic variability, inbreeding, parentage assignments, strain and species identification as also construction of high resolution genetic linkage maps for aquaculture species.

A considerable number of microsatellites have been detected in aquatic species, but these markers offer no value if they are not related to or situated close to certain desirable trait(s). The marker that is fast gaining considerable importance is the single nucleotide polymorphism (SNPs). It has been found that SNPs exhibit polymorphism both between as well as within families. Such markers can be useful for identification of families. In case family-specific markers are found, they could be used for parentage assignment. The entire population can be grown in a common environment and the animals that grow fast and are selected can be assigned to their respective families. This is known as genetic tagging.

Based on DNA markers at a large number of sites in the fish genome, a genetic linkage map is constructed. This could then be used to detect QTLs, if any, controlling disease resistance. By identifying markers associated with high performance QTLs in different species, it would be possible to improve that trait by incorporating these QTLs into the breeding programme. The breeding programmes would greatly benefit by such a practice and is termed *marker assisted selection* or MAS. This would ensure greater genetic gain by increasing the efficiency of the selection. In Australia, at CSIRO, Li *et al* (2006) had identified a QTL for growth in Kuruma Shrimp which is located close to ***fatty acid elongase*** gene. The SNP markers were used to detect the QTL. The shrimps that carry this gene grow fast and are heavier compared to their counterparts.

Sex-specific linkage maps are available for almost all commercial species of fish and shrimp. Recently, several EST markers have been developed for shrimp species (Maneeruttanarungroj *et al.,* 2006; Tassanakajon *et al.,* 2006; Tong *et al.,* 2002). An EST database developed from Thailand for *P. monodon* has about 40,000 EST sequences. The NCBI has huge collection of EST database for commonly cultured fish and shrimp species. Quantitative trait loci for total length and carapace length have been mapped on to the male linkage map of *M. japonicus* using interval mapping method (Li, Y *et al.,* 2006). A microsatellite marker is found to be associated with WSSV susceptibility in *Penaeus monodon* (Mukherjee and Mandal, 2009). An allele at microsatellite locus RS0622 (AY132778 in NCBI database) was found as associated with resistance to WSSV in *Fenneropenaeus chinensis* (Dong *et al.,* 2008). Sporadic reports of SNPs in candidate genes of economic traits exist in aquaculture species (Glenn *et al.,* 2005, Hemmer-Hansen *et al.,* 2011, Prasertlux *et al.,* 2010 and Yu *et al.,* 2006). Their utility for MAS has to be validated.

Modern technology complements traditional or conventional breeding but cannot replace it. Till date, whatever progress in plants and farm animals has been achieved could be attributed to conventional trait-based methodology. For a genetic improvement programme to be effective, the basic requirements are tagging, accurate data and pedigree recording.

IMPORTANCE OF NUTRITION IN CULTURE AS WELL AS BREEDING PROGRAMMES

Nutrition is an important aspect in culture as well as in genetic improvement programmes in aquatic animals. The expression of phenotype is a result of the genetic makeup as well as the environment in which it is nurtured. Provision of an apt nutrition to an animal would result in the maximum expression of the genotype in terms of production performance. This is where the nutritionist has a stellar role to play. The nutritionist needs to ensure that appropriate nutrients in the required quantity are provided to the aquatic animals so that the full potential of the genotype could be realized. That is the reason as to why any programme whether it is culture or genetic improvement is incomplete without the involvement of a nutritionist

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BRACKISHWATER FISH SEED PRODUCTION AND FARMING – AN APPROACH TO AUGMENT FISH PRODUCTION IN INDIA

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Introduction

Aquaculture is considered as one of fastest growing food sectors, which is not only producing fish for consumption but also generating employment opportunities in various allied sectors. Brackishwater aquaculture is mainly focused on production of shrimps at larger extent and finfish species to some extent. Sustainability of brackishwater aquaculture is mainly depends on the culture of many fish species instead of depending single or few species. Availability of fish seed in adequate quantity is a critical factor for expanding the finfish farming for up scaling the fish production. Wild seed availability is seasonal and location specific, which may not be able to meet the huge demand from farmers. Development of suitable techniques for fish seed production in fish hatcheries would help to solve these issues. Important candidate species for brackishwater aquaculture has to be identified based on certain essential factors.

Species selection

The fish should have the following characteristics for the economical cultivation.

- It should rapidly grow in a short period of culture

-It should withstand wider ranges of salinity and temperature

-May be an herbivore fish, feeding on plankton, benthic algae decaying plants etc., but carnivore fish also preferred for mono species culture

-It should have good market value

-Adequate seed should be available from hatcheries according to the demand

-The fish should have small head and less bone so that the edible part will be more

Some of the important cultivable bracksishwater finfishes are

Grey mullets: *Mugil cephalus, Liza parsia, Liza tade*
Milkfish: *Chanos chanos*
Seabass : *Lates calcarifer*
Pearlspot: *Etroplus suratensis*
Cobia : *Rachycentron canadum*
Red snapper*: Lutjanus argentimaculatus*

Grey mullet *Mugil cephalus*

Grey mullets are the preferred food fishes in India due to its white tender meat. *Mugil cephalus* inhabits estuarine, freshwater, coastal and marine water bodies. It occurs in lagoon, with juvenile fishes most common in impounded areas, around mangroves, in seagrass beds, and offshore. Mullets are herbivore fish and it consumes the decaying organic matter. It is one of the ideal candidate species for brackishwater culture and can withstand salinity from 0 to 35 ppt. The fish can grow 500gm in six months period under pond conditions with low cost feed such as rice bran and ground nut oil cake. Among the mullets, *M. cephalus* grows faster than other species. *M. cephalus* is distributed in the Indo-pacific region, Hawaii, Israel, Iran and Egypt. In India mullet is being cultured by traditional method. In India mullet is being cultured in Kerala, West Bengal and certain parts of Tamil Nadu as polyculture mode along with other finfish and shrimp species. In monoculture, mullet can be stocked @ 6000-7000 fingerlings/ha to achieve a production of 3.0-3.5 tonn/ha.

First maturity of grey mullet can be observed in 2-3 years old fish. In natural condition, mullet maturation and spawning noticed during October to January in the east coast of India and during June-July in the west coast. Longer darker period and low temperature directly linked with the maturation of *M.cephalus*. Females with initial oocyte diameter of 600 µm and oozing males can be selected for induction of spawning through hormonal manipulation. Carp Pituitary extracts and LHRHa @ 20mg/kg and 200µg/kg body weight are used as priming and resolving doses for spawning. After ovulation, stripping of ovulated eggs is common practice followed. The stripped eggs are fertilized by mixing with milt obtained from males using bird feather by dry method. The floating fertilized eggs can be stocked in the incubation tanks for hatching. The newly hatched mullet larvae can be stocked in the larval rearing tanks to grow them to fry size in the hatchery. The first feeding of rotifers starts 65-70 h after hatching as presence of rotifers in the gut of striped mullet *Mugil cephalus* was observed first 70 h post hatch. Rotifer feeding is continued till 25th day along with the *Artemia* nauplii in larval rearing of *M. cephalus*

Milk fish *Chanos chanos*

The milkfish *Chanos chanos* is an important food in South East Asia. They occur in Indo-Pacific region. In Philippines, milkfish seed are collected from the backwater, low lying region areas and raised for grow out culture either in ponds or in cages. Milkfish is an ideal fish for pen culture and can be cultured even in fresh water. Milkfish can grow to 500gm in 5-6 months. It is a plankton feeder and the diet of the milkfish either supplied wholly by natural productivity or is fed wet particulate diets. Milkfish sold fresh, frozen, canned, or smoked. The milk fish is a national symbol of the Philippines. The fish can withstand maximum salinity up to 35 ppt. In India milkfish seed is available in short period of three months in Andhra Pradesh coast and near Rameshwaram in Tamil Nadu. Milkfish can be cultured along with other fishes and shrimps in ponds. In monoculture pond, milkfish can be stocked @ 7000-8000 fingerlings/ha and formulated feed can be provided @ 3-5% body weight daily. After 6 months culture, milkfish can attain the mean body weight of 500g with the productivity of 3.5-4.0 tonnes/ha.

Milkfish mature in seawater at the age of 3 years. However, brood fishes with age of 5 plus years are usually selected for breeding purposes. Milkfish require higher temperature and longer day period for maturation, which is usually coincide with summer period. Milkfish can be bred through LHRH hormone treatment. Usually fertilized eggs are collected from the spawning tanks after spontaneous spawning takes place in the tank. The fertilized eggs stocked and larval rearing conducted in the hatchery for seed production.

Pearl spot *Etroplus suratensis*

Pearl spot is an endemic to Indian sub-continent countries such as India, Sri Lanka, Bangladesh and Pakistan. It can withstand the salinity from 0 to 30 ppt but can grow better in lower salinities up to 15 ppt. It is an ideal candidate species for brackishwater farming. It is a detrivore fish but can accept the artificial diet. It can attain the growth of 150-200gm in 6-9 months depending upon the salinity. *E.suratensis* is mostly preferred for polyculture.

Seabass *Lates calcarifer*

Asian seabass *Lates calcarifer* is highly priced fish in India, which is distributed in Indo-Pacific region and in Australia. It can withstand the salinity from 0-40 ppt. The fish can be cultured in freshwater, brackishwater and marine condition in earthen ponds and cages. Under pond condition, the fish can attain the growth of 800-1000gm in 6-8 months period either with trash fishes or artificial diet. With the stocking density of 4000-4500 nos/ha, seabass production can be achieved between 3.0 to 3.5 tonn/ha. The fish is being widely cultured in Australia, Thailand, Singapore, Malaysia, Philippines, Indonesia and India. In the recent days, seabass is also cultured in Europe. In India, the seed production technology of seabass has been standardized by Central Institute of Brackishwater Aquaculture, Chennai. In India, seabass farming is practiced in many states by obtaining either hatchery produced seed or wild source seed. Seabass can be farmed in the sea cages and also in the shallow backwater net cages.

Seabass is protoandous hermaphrodite fish and they live as males (2-3kg) in early stage and later they become females (above 4.0 kg). Female seabass (5.0-7.0 kg) with oocyte diameter of above 450 µm can be selected for induction of spawning and can be administered LHRHa hormone @ 70 µg kg^{-1} body weight. Males (2.0–3.0 kg) in oozing condition can be administered with LHRHa @ 35 µg kg^{-1} body weight and can be maintained together with female in the spawning tanks in the female and male ratio of 1:2 Spawning can be effected spontaneously in the evening between 18–20 h after 32–34 h of hormone administration. The average size of fertilized eggs was 780 µm (776–782 µm). Fertilised eggs can be incubated in 500 litre conical FRP tanks and provided with slow flow through (1.75 l/minute) of seawater (salinity, 32.0 ppt; temperature, 27.0–29.0 ºC) with continuous aeration for hatching. Hatching can be observed at 16 h after fertilization. Asian seabass *Lates calcarifer* larvae are voracious feeder and feed has to be supplied adequately in the hatchery production. Rotifer is used as initial feed for seabass larvae soon after mouth opening (mouth size, 250 µm) at 48 h of hatching (1.6±0.2 mm). Rotifer is supplied for seabass larvae up to 9th day post hatch (dph) and from 10th to 15th dph; rotifer is supplied along with *Artemia* nauplii. Usually, rotifer is supplied to the seabass larvae at the rate of 20-30 nos./ml in the rearing tank. Seabass larvae can reach to fry size (1.2-1.5 cm) in 25 days rearing in the hatchery.

Cobia *Rachycentron canadum*

Cobia is distributed worldwide in warm marine waters, except for the central and eastern Pacific, resulting in a very large potential area suitable for the production. They can be found throughout the water column and are caught in both coastal and continental shelf waters, although they are typically considered to be an offshore species. Wild-caught cobia do not support a major commercial fishery and are uncommon throughout its range and generally considered incidental catch.

Cobia is one of the most preferred marine fishes in the cages because of its rapid growth rate. The fish can grow 4-6 kg in one year under ideal condition in the cages. It can be cultured in deeper ponds with good water exchange. Cobia tolerates the salinity range from 15 to 35 ppt. It is widely farmed in Vietnam, Mexico, USA, Taiwan, China and other South East Asian countries. Cobia matures after attaining the age of 3 years. Sexes are separate. By applying hormone treatment with LHRHa and HCG, cobia can be induced to spawn. Cobia larvae reach to three inch size fingerlings in 45 days period rearing in the hatchery and these fingerlings can be stocked in the cages or ponds for grow out culture.

Red Snapper *Lujanus argenimaculatus*

Lujanus argenimaculatus is widespread in the Indo-West Pacific from Samoa and the Line Islands to East Africa, and from Australia northward to the Ryukyu Islands. Has dispersed into the eastern Mediterranean (off Lebanon) via the Suez Canal, but is not well established there. Maximum size reported is 120 cm with an average size of 80 cm. An important market species throughout the Indo-Pacific region, but never found in large quantities. Caught mainly with nets (red fishes gillnetting, snapper gillnetting), hand lines, bottom long lines, and trawls. Juveniles and young adults found in mangrove estuaries and in the lower reaches of freshwater streams. Eventually, they migrate offshore to deeper reef areas, sometimes penetrating to depths in excess of 100 m. Habitat frequently consists of areas of abundant shelter in the form of caves or overhanging ledges. Snapper mainly feeds on fishes and crustaceans. Spawning occurs throughout the year, at least in lower latitudes. Under culture condition, snapper can be weaned to artificial diet and highly suitable for cage based culture

Conclusion

Finfish farming can be one of the best options to diversify the brackishwater aquaculture to achieve the sustainability. A number of species available in this group are highly suitable for farming in different water bodies such as backwater, lagoons, open sea cages and various systems such as pen, cage and ponds. Farmers can select the suitable species for culture based on their resource and investment capacity by choosing either low cost herbivores species such as milkfish, pearlspot, grey mullets or the high valued carnivore species such as seabass, cobia and red snapper and groupers.

POTENTIAL FISHMEAL ALTERNATES IN FORMULATED AQUAFEEDS FOR SUSTAINABLE AQUACULTURE

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Aquaculture supplied more than 63 million metric tonnes of fish to the world total production of 154 million metric tonnes in 2011 (FAO, 2012). At present only two third of this farmed fishes are produced from artificial feeding. However the percentage of non-fed species in world production has declined gradually from more than 50% in 1980 to the present level of 33.3%, indicating the importance of the formulated feed in global aquaculture industry and the further increase in demand for feed ingredients. Since feed is the single largest operational expense (about 60%) in aquaculture, feed cost determines the profitability of the aquatic organism that is grown. Even though whole feed is a matter of concern, it is a blend of several ingredients and the ingredients are the one which determining the cost and quality of the feed as whole. Except few, most of the aquafeed ingredients are, for the most part, by-products of food processing obtained when high-value food for humans is extracted from a raw material. Fishmeal has been considered as a key protein source for use in aquaculture feeds for both carnivorous and omnivorous species, and many aquaculture formulations still have fishmeal included at level more than 50%. But, excessive dependence on any one particular ingredient could increase the risk associated with supply, price and quality fluctuations. Furthermore, based on the volumes of fishmeal and fish oil used in aquaculture, especially for carnivorous species, production of these species is still viewed as a net fish consumer rather than producer, and this practice has raised concerns about the sustainability of these industrial productions. As an approach to reduce risk, the identification, development and use of alternatives to fishmeal in aquafeeds remains a high priority research. Considerable efforts have been made in the past in evaluating a wide range of potential alternatives to fishmeal for use in aquaculture feeds. This training manual chapter enlightens its nutritional significance in aquafeed and challenges in its replacement.

Fishmeal and its nutritional value

Physically fishmeal is a brown powder obtained after cooking, pressing, drying and milling fresh raw fish and/or food fish trimmings. Fishmeal is produced from short-lived, marine, fast-growing harvesting stocks of fish for which there is little or no demand for human consumption and some are manufactured from by-products of seafood processing companies. The whole fish used in fishmeal production are mainly small, bony and oily such as anchovy, horse mackerel, menhaden, capelin and sand eel. Trimmings otherwise called fish processing waste from the seafood processing are also used, and they constitute around 25% of the raw material for fishmeal production. Global production of fishmeal is concentrated around a few top producers; top ten manufacturers in 2007 made up approximately 80% of the global production. Today Peru is the largest producer, China the second, Chile the third and then the Nordic countries Norway, Denmark and Iceland follow as the most important producers. There are approximately 300 dedicated plants worldwide that produce about 6.3 million tons of fishmeal annually.

Although fishmeal is not the cheapest source of protein, it is widely used because of its unique properties like highly digestible protein and its component amino acids, and fat and its component fatty acids - especially the omega 3 fatty acids EPA and DHA which have been shown to improve animal health as well as productivity. Fishmeal is not only the source of protein, it also excellent source of other essential nutrients and unidentified growth factors. Nutritional profile of a premium quality fishmeal is given in Table 1. Fishmeal contains typically 60% to 72% protein, 10% to 20% ash and 5% to 12% fat (Table 1), which is high in the health promoting omega-3 very long chain polyunsaturated fatty acids EPA and DHA, often referred to as 'omega-3s'. The high quality and quantity of essential nutrients, especially of well-balanced amino acids, essential fatty acids, and energy content makes fishmeal an indispensable ingredient in diets of most aquaculture species.

Table 1 Proximate composition (as % as-fed basis) of different types of fishmeal used in aquafeeds

Ingredient	Moisture	Protein	Fat	Fibre	Ash
Anchovy	7.9	66.7	6.8	1.0	15.9
Menhaden	6.5	63.4	9.9	0.9	19.9
Herring	6.5	63.4	9.9	0.9	19.9
Tuna	7.9	72.3	8.4	0.7	10.1
Sardine	7.7	62.0	7.9	0.6	14.7

*Source: Tacon et al., 2009

<u>Alternates not only for fishmeal, but also for other ingredients:</u>

Considerable efforts have been made in the past in evaluating a wide range of potential alternatives to fishmeal and fish oil for use in aquaculture feeds. Fluidity of the ingredient price, increasing understanding on the nutrient requirements of the target species, growing competition for food by human and other livestock, increasing awareness on sustainability issues, forced the aquafeed industry to seek for alternates not only for fishmeal, but also for all other ingredients (NRC, 2011). Over the years, poultry nutritionists have been able to develop more than 80 alternative ingredients to fishmeal that can be used in poultry diets on a least-cost basis. But, aquaculture has widely experimented only 12 to 15 commonly used ingredients, and it limits the flexibility to the formulator in maximising the cost benefits in the feed formula.

<u>Potential feed ingredients as alternates for fishmeal</u>

Possible alternatives for fishmeal in aquafeeds can be broadly categorised in to three major categories:

1. Vegetable by-products
2. By-products of terrestrial and aquatic animal processing industries
3. Single cell proteins

1. Vegetable by-products

Oil meals

The most important protein supplements of plant origin which could be potential alternates for fishmeal are the oilseed meals, produced from the cake remaining after oil has been extracted. Oils may be mechanically expelled or extracted by using solvents. Most common oil meals are from soybeans, cottonseed, canola, rapeseed, peanuts, sesame seeds, sunflower seeds and coconuts (Table 2).

Table 2 Proximate composition (as % as-fed basis) of oil meals as potential alternates of fishmeal

Ingredient	Moisture	Protein	Fat	Fibre	Ash
Mustard	10.15	32.2	8.9	8.1	9.2
Rapeseed	8.30	34.7	7.5	12.3	6.7
Coconut	8.7	21.5	3.5	14.8	7.1
Cottonseed	10.0	32.9	1.7	21.8	6.0
Groundnut	8,3	31.8	2.2	27.0	4.7
Safflower	8.9	42.7	1.4	13.0	7.1
Sesame	7.6	45.0	4.8	6.7	13.0
Soybean	10.3	44.7	1.3	6.0	6.7
Sunflower	10.0	23.3	1.1	31.6	5.6

*Source: Tacon et al., 2009

Vegetable protein concentrates

Vegetable protein concentrates are processed, concentrated form of by-products high in protein content. There are several types of protein concentrates and their composition highly varies with the processing methods used. They could be potential alternates on par with fishmeal in view of its high protein content. Some common vegetable protein concentrates and their compositions are presented in Table 3.

Cereal by-products

a. Dried distiller's grains (DDGS):

Distillers Grains are a cereal by-product obtained after the removal of ethyl alcohol by distillation from the yeast fermentation of a grain or a grain mixture by condensing and drying at least 3/4 of the solids of the resultant whole stillage by methods employed in the grain distilling industry. The predominating grain shall be declared as the first word in the name. Dried Distillers Grains with Solubles (DDGS) is wet distiller's grain that has been dried with the concentrated thin stillage to 10-12 % moisture (Table 4). DDGS have an almost indefinite shelf life and may be shipped to any market regardless of its proximity to an ethanol plant. Drying is costly, as it requires further energy input.

b. Corn gluten meal

Corn gluten meal is the dried residue from corn after the removal of the larger part of the starch and germ. It may contain fermented corn extractives and/or corn germ meal.

Table 3 Proximate composition (as % DM basis) of vegetable protein concentrates as potential alternates of fishmeal

	Soy protein concentrate	Canola protein concentrate	Corn protein concentrate	Potato protein concentrate	Rice protein concentrate
Dry matter	93	90	90	90	91
Crude Protein	59	63	76.2	76	69
Crude Fat	5.4	8	4.5	1.5	10
Crude Ash	7.9	5.9	1.3	2	4
Crude Fibre	1.5	4.7	1	6.3	3
NFE	19.2	14.8	7	4.2	6

Table 4 Proximate composition (as % as-fed basis) of cereal by-products as potential alternates of fishmeal

Ingredient	Moisture	Protein	Fat	Fibre	Ash
DDGS	8.2	28.4	8.5	9.4	4.9
Corn gluten meal	8.6	56.1	4.0	2.9	2.1

*Source: Tacon et al., 2009

2. By-products of terrestrial and aquatic animal processing industries

Advances in breeding, production and processing technologies have improved the livestock production industry as source of food for humans. On processing these livestock's, varieties of rendered animal by-products are produced in large quantities and available in variety of forms for use in aquafeeds. Various by-products of animal processing industries and their compositions are presented in Table 5.

a. ***By-products of marine origin:***

Condensed fish solubles

Condensed fish solubles is obtained by evaporating excess moisture from the stick water, aqueous liquids, resulting from the wet rendering of fish into fishmeal, with or without removal of part of the oil.

Crab meal

Crab meal (Crab process residue meal) is the undecomposed ground dried waste of the crab and contains the shell, viscera and part or all of the flesh..

Shrimp meal

Shrimp meal (Shrimp process residue meal) is the undecomposed, ground dried waste of shrimp and contains parts and/or whole shrimp.

b. *By-products of terrestrial origin:*

Blood meal

Blood meal, flash dried (Animal blood meal flash dehydrated) is produced from clean, fresh animal blood, exclusive of all extraneous material such as hair, stomach belchings and urine .

Hydrolyzed poultry feather meal

Hydrolyzed poultry feathers meal is a product resulting from the treatment under pressure of clean, undecomposed feathers from slaughtered poultry, free of additives and/or accelerators.

Meat and bone meal

Meat and bone meal is the rendered product from mammal tissues, including bone, exclusive of any added blood, hair, hoof, horn, hide trimmings, manure, stomach and rumen contents.

Table 5 Proximate composition (as % as-fed basis) of various animal meals and by-products as potential alternates of fishmeal

Ingredient	Moisture	Protein	Fat	Fibre	Ash
Animal meal by-products of marine origin					
Fish soluble	7.4	55.9	6.5	3.2	12.6
Shrimp head meal	8.8	46.6	6.4	11.1	26.5
Crab meal	7.1	33.9	2.8	10.7	41.9
Squid viscera meal	10.3	50.3	18.6	1.5	9.8
Animal meals by-products of terrestrial origin					
Blood meal	9.0	85.5	1.4	0.9	5.3
Feather meal	8.4	84.0	4.2	1.0	3.6
Meat and bone meal	7.5	50.1	10.6	2.4	28.8
Poultry by product meal	7.4	59.0	12.4	2.6	15.3
Terrestrial invertebrate meals					
Silkworm pupae meal	11.1	55.1	23.2	5.5	3.8
Maggot meal	3.0	43.2	23.0	1.0	16.2
Earthworm meal	7.4	56.4	7.8	1.6	8.8
Polychaete worm meal	8.0	55.0	15.0	1.0	12.0

*Source: Tacon et al., 2009

Poultry by-product meal

Poultry by-product meal consists of the ground, rendered, clean parts of the carcass of slaughtered poultry, such as necks, feet, undeveloped eggs and intestines

c. *Terrestrial invertebrate products*

The average reported proximate and essential amino acid composition of some terrestrial invertebrate products which have been successfully used in compound aquafeed. Insect larvae/pupae have been used as traditional supplementary feed items by small-scale farmers in many Asian countries and, together with snails and annelids, offer a potential non-conventional feed source for use by small-scale farmers. In general, invertebrate meals are good dietary sources of animal protein, lipids and energy.

3. Single cell proteins

Single cell protein (SCP) is a term applied to a wide range of unicellular and filamentous algae, fungi and bacteria which can be produced by controlled fermentation processes. In contrast with conventional plant and animal feed proteins, these microorganisms offer numerous advantages as protein producers, including: (1) their production can be based on raw carbon substrates which are available in large quantities as wastes from other industries which otherwise cause an environmental hazard; (2) the majority of microorganisms cultured are highly proteinaceous (3) they have a short generation time, (4) they can be cultivated in a limited land space and produced continuously with good control. Some common single cell proteins and their compositions are presented in Table 6.

Table 6 Proximate composition (as % as-fed basis) of various single cell proteins as potential alternates of fishmeal

Ingredient	Moisture	Protein	Fat	Fibre	NFE	Ash
Brewer's yeast	7.6	46.1	1.3	2.9	34.0	8.1
Fungal biomass	8.5	44.4	9.4	16.9	16.1	4.7
Spirulina	6.4	62.1	4.8	0.5	17.3	8.9
Chlorella	5.7	47.2	7.4	8.3	20.8	10.6
Methanol Substrate	6.4	73.1	5.7	0.4	2.7	11.7

*Source: Tacon et al., 2009

Fish in and fish out (FIFO) ratio

"Fish In Fish Out" (FIFO) ratio is nothing but a numerical ratio which expresses the amount of wild fish used to produce a unit amount of fish through aquaculture. The recent cited figures range from 3:1 to 10:1. The most recent figure published by Tacon and Metian (2008) Salmon in 2006 as 4.9:1, meaning that, it needs 4.9 tonnes of wild fish to produce 1 tonne of farmed salmon. It can be easily understanding how they derived up with this figure. If we take 1 tonne (1000 kg) of wild fish, it is assumed that this would yield 225 kg of fishmeal and 50 kg of fish oil. If we say that on average salmon diets contained 30% fishmeal and 20% fish oil, this means that one could produce 250 kg of salmon feed by using up all of the 50 kg of fish oil. Salmon then have a feed conversion ratio (FCR) of 1.25 which therefore will give a harvest volume of 200 kg of salmon. So our starting 1000 kg of wild fish have been turned into 200 kg of salmon which is a FIFO ratio of 5:1 (1000:200).

But recently International Fishmeal and Fish oil Organisation (IFFO) indicated the earlier values are misinterpreting, and given an explanation (Jackson, 2010) on FIFO ratio clarifying the misunderstanding on the use of fishmeal and fish oil use in aquaculture. In the previous FIFO ratio worked out by Tacon and Metian (2008), while all the fish oil from 1000kg wild fish was used to produce the salmon feed, there was 150kg of fishmeal left over. They assumed it as waste hence there is no more fish oil; in their calculation this is just thrown away and wasted. Realising this as fictional, IFFO rectified this by smartly demonstrating to use the left over fishmeal in production of shrimp (which needs more fishmeal) and carp. By doing this they brought down the FIFO ratio of Salmon as 2.27 from 4.49, shown by Tacon and Metian (2008). They also indicated the potential for further reduction on considering the use of waste from seafood processing industries for fishmeal production. They claim that, FIFO can be brought down by judicious allocation of fishmeal and fish oil among a range of farmed species groups rather than in single. For example, fishmeal and fish oil can be shared for production feed for salmon, shrimp and carps. In this, Salmon is a major consumer of fish oil and shrimp is consumer of more fishmeal. On the other hand, carps need only little of fishmeal. The worked out comparisons of FIFO calculation by Tacon and Metian (2008) and IFFO are presented in Table 7.

Table 7: Comparison of "Fish In Fish Out" (FIFO) ratio worked out by Tacon and Metian (2008) and International Fishmeal and Fish oil Organisation (IFFO)

	Salmon alone	**Salomon + Shrimp + Carp**		
		Salmon	Shrimp	Carp
Weight of pelagic fish used(kg)	1000	1000		
Weight of Fishmeal (kg)	225	225		
Weight of Fish oil (kg)	50	50		
Fish oil in the diet (%)	20	20	2	0
Fishmeal in the diet (%)	30	30	20	5
Requirement of oil (kg)	50	35	15	0
Requirement of fishmeal (kg)	75	52	150	23
Feed that can be produced (kg)	250	175	750	450
FCR	1.25	1.25	1.7	1.8
Fish biomass produced (kg)	200	140	441	250
Fishmeal left as waste (kg)	150	0		
Fish oil left as waste (kg)	0	0		
Total Biomass produced	200	831		
FIFO	5	1.2		

Source: Jackson, 2010 (www.iffo.net/downloads/100.pdf)

From this example it is clear that there is surplus fishmeal with salmon and surplus fish oil with shrimp production. The excess fishmeal after use in both the species can be used for carp which usually needs little. This indicates that mutual sharing among the two species may be more efficient. It also indicated that calculating the FIFO ratio based on just one type of farming does not give the correct picture. Considering this issue in reality, IFFO derived the following new formula to calculate FIFO ratio.

$$\text{FIFO Ratio} \quad = \frac{\text{Level of fishmeal in the diet} + \text{Level of fish oil in the diet}}{\text{Yield of fishmeal from wild fish} + \text{Yield of fish oil from wild fish}} \times FCR$$

For example FIFO ratio of salmon will be like this

$$\text{FIFO Ratio} \quad = \frac{30 + 20}{22.5 + 5} \times 1.25 = 2.27$$

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PRINCIPLES OF FEED FORMULATIONS FOR SHRIMP/FISH AQUACULTURE

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INTRODUCTION

Feed cost is the major input cost in aquaculture which assumes 50-70% of cost of production. Hence the feed should be nutritionally well balanced to maximize the growth rate and feed conversion efficiency. Feed formulation is a scientific method of combining available feed ingredients to meet the pre-established nutrient requirements to prepare a compounded feeds for the candidate species intended for aquaculture in a cost effective manner. It is essentially the application of nutritional science to a practical situation in a feed manufacturing process. The main objective of feed formulation is to utilise the knowledge of nutrient requirements, cost effective available feed ingredients, and their inclusion levels based on their nutritive composition, digestibility and antinutritional factors. Feed formulation usually follows a certain sequence of 'trial and error' steps, involving lot of educative guesses and skills. The nutritionist who attempts to formulate a feed must have in depth knowledge of the biology of the target species, feeding behaviour and the purpose for which feed is being prepared.

Formulation and preparation of compounded feeds is based on several scientific and economical factors.

A. Information of candidate species

1. Nutrient requirements of the candidate species including protein, energy, fat, amino acids, fatty acids, vitamins, minerals for each stage of culture.

2. Feeding habits of the candidate species- type of natural foods, type of feeding habits (herbivore, detritivore, omnivore or carnivore), feeding station (bottom, column, surface), visual or olfactory feeder, rapid or slow feeder. Preferred physical texture, method of feeding (swallowing or nibbling), anatomy of mouth parts and organs of prehension.

B. Information of ingredients

1. Availability of ingredients, nutrient composition, cost, seasonal, geographical variations, type of availability (dry or wet), processing (solvent extracted, mechanical extracted).

C. Processing at feed mill

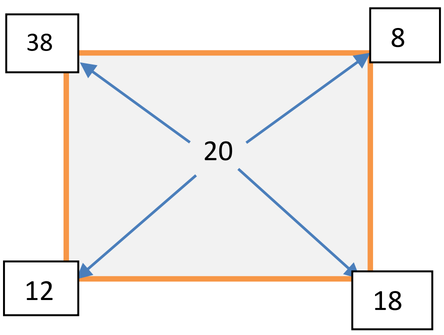
1. Type of feed preparation- mash, crumbles, pellet, flakes. Sinking pellet or floating pellet, type of machinery used. Based on this information, binder addition, heat labile additives addition in feed formulation varies.

Approaches for feed formulation

<u>A. Pierson square;</u>

Method was one of the earliest tools in feed formulation. The method facilitates the calculations involved in blending two ingredients or products with different nutrient concentrations to yield a product with a specified nutrient concentration. Until the advent of computers, this method was a mainstay in feed formulation. In the following example the goal is to produce a 20% crude protein (CP) product by blending two ingredients, a 38% CP ground nut oil cake (GNC) and a 12% CP wheat (Houser and Akiyama, 1997).

1. Place the % CP of the GNC in the left hand corner.
2. Place the % CP of the wheat in the lower left hand corner.
3. Place the desired % CP in the middle.
4. Subtract the smaller number from the larger number diagonally in this case, 38 minus 20 and 20 minus 12 the results , parts of each ingredient that are required in the formulation, are noted in the right corner on the same horizontal line as the ingredient.
5. Add the total of the two numbers on the right hand side.



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6. Divide the parts for each ingredient by the total parts and multiply by 100 to determine the percent of each ingredient needed.

8/26=30.8%GNC ; 18/26=69.2% wheat

Proof: 30.8*0.38=11.70

69.2*0.12= 8.3

Total 20

If more ingredients are to be included, the above product which is actually a blend of two ingredients may be combined with a third ingredient in a second Pierson Square to produce a blend of 3 products. Extensive information on the use of multiple Pierson Squares in feed formulation is reviewed by Church and Nipper 1984.

B. Graphics solution to feed formulation:

In order to gain a basic understanding of what is happening in feed formulation using linear programming, it is useful to visualize the process graphically. A two dimensional graph, with the Y axis representing the percent WHEAT in the feed, and the X axis representing the percent GNC in the feed is constructed

Suppose that the following nutrient values have been determined for WHEAT and GNC

	CP (%)	Fat (%)	CF (%)
WHEAT	12	1.5	3
GNC	38	3	12

Solving for protein minimum

1. Set up an equation describing the relationship of WHEAT and GNC to the protein restriction.

Eg: (12*%WHEAT) + (38*%GNC)≥20.0

2. In order to find one point on the graph, set the % WHEAT =0 and solve the equation for % GNC.

0*WHEAT+0.38*%GNC)≥20.0

%GNC≥20.0/ 0.38

%GNC≥52.64

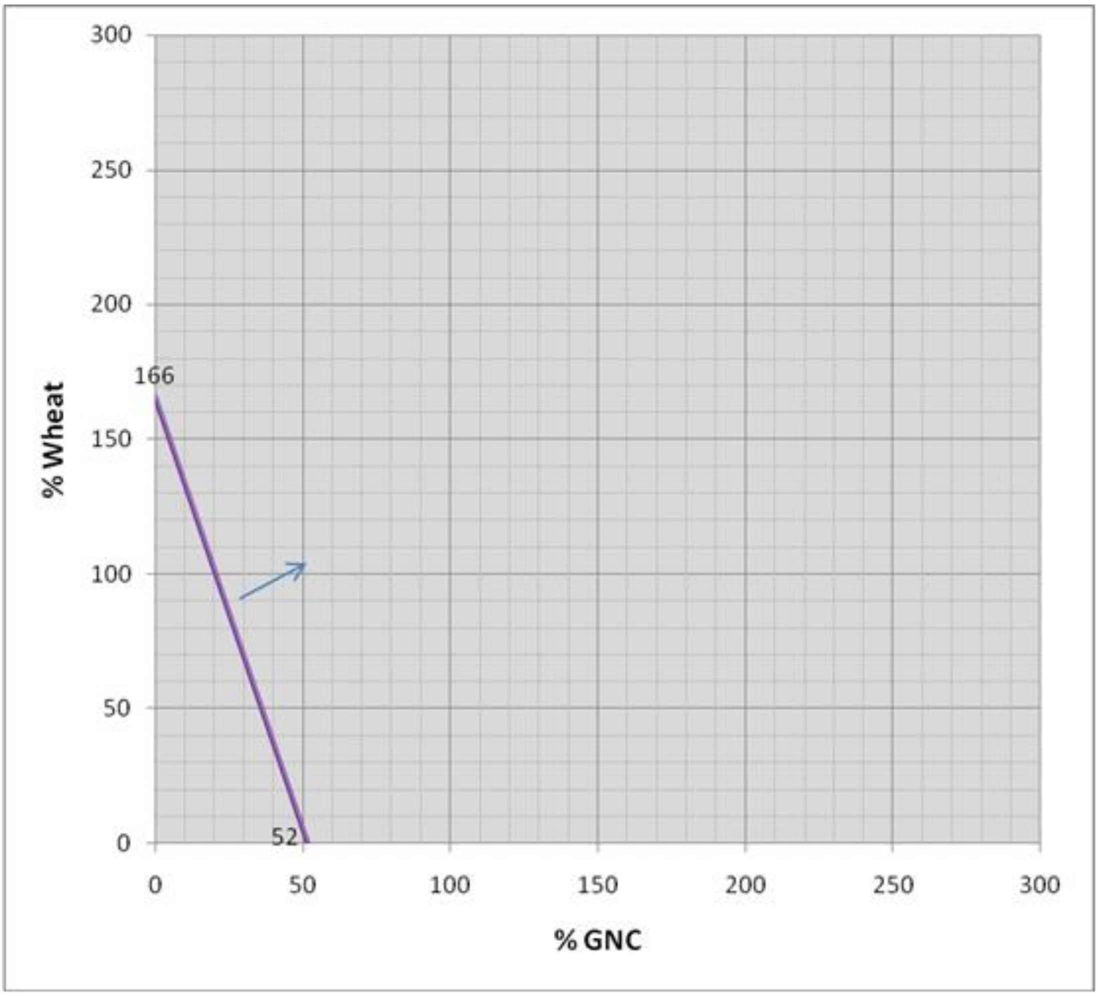
Expressing the result, if no WHEAT is included, it will take a minimum of 52.64%

GNC to provide 20.0% protein in the feed. This concept may be easily proven

38% CP in GNC* .5264 ≥ 20

3. Two points are needed to define a line. Set the % GNC = 0 and solve the equation for % WHEAT.

Any WHEAT/GNC ration on or to the right of the line in Figure 1 will satisfy the requirement of 20%. If the cost of WHEAT is lower that the cost of GNC, the point on the line nearest the Y axis will be chosen. If GNC is less expensive than WHEAT, the point on the line nearest the x axis will be chosen, obviously the percent



wheat plus the percent GNC cannot exceed 100.

Therefore, if no GNC is used, it will require 166.7% WHEAT to produce a feed containing 20% protein. Obviously it is impossible to provide remember this is a mathematical concept; we are not yet dealing with a bag of feed.

4. Two points that define the range of % WHEAT and % GNC that will satisfy the minimum restraint of 20.0% protein in the feed have been defined. The results are presented in Figure 1

Solving for fat minimum

Set up an equation describing the relationship of WHEAT and GNC to the fat restriction. Following the procedure used to determine possible levels to satisfy the protein minimum set % WHEAT = 0 and solve the equation for % GNC.

(0*WHEAT) + (0.03*%GNC) ≥ 2.0

0.03*GNC≥ 2.0

66.7 %GNC≥ 2.0

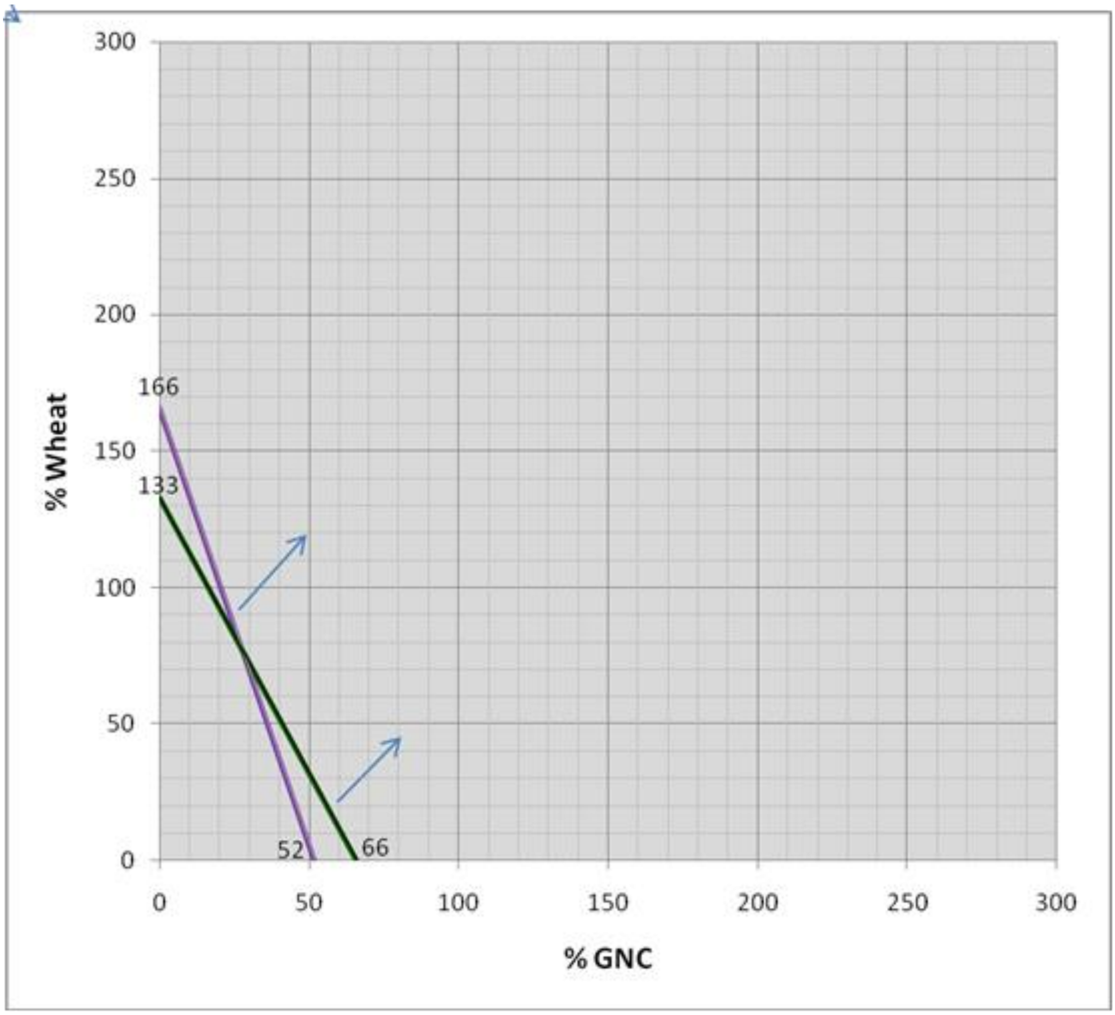
Set % GNC =0 and SOLVE THE EQUATION FOR % wheat.

0.015*%WHEAT+0*%GNC≥2.0

0.015*%WHEAT≥2.0

%WHEAT≥2.0÷0.015

%WHEAT≥ 133.4



Accordingly two points that define the range of % WHEAT and % GNC in which the minimum restraint of 2.0% fat in the feed will be satisfied have been defined. The results are present in figure 2 in order to meet both the 20 % minimum protein requirement and the 2% fat requirement. The ratio of WHEAT to GNC must be on or to the right of the protein line and above the fat line.

Solving for 7% fiber maximum

1. Set up an equation describing the relationship of to the fiber restriction.

Example: (3.0*%WHEAT) + (12*%GNC) ≤ 7.0%fiber

2. Following the procedure used to determine possible levels to satisfy the protein minimum, set % WHEAT=0 and solve the equation for % GNC.

0*WHEAT+0.12*%GNC ≤ 7.0

%GNC ≤ 7.0÷0.12

%GNC ≤58.4

3. Set %GNC=0 and solve the equation for % WHEAT.

$$0.03*\%WHEAT+0*\%GNC \leq 7.0$$

$$0.03*\%WHEAT \leq 7.0$$

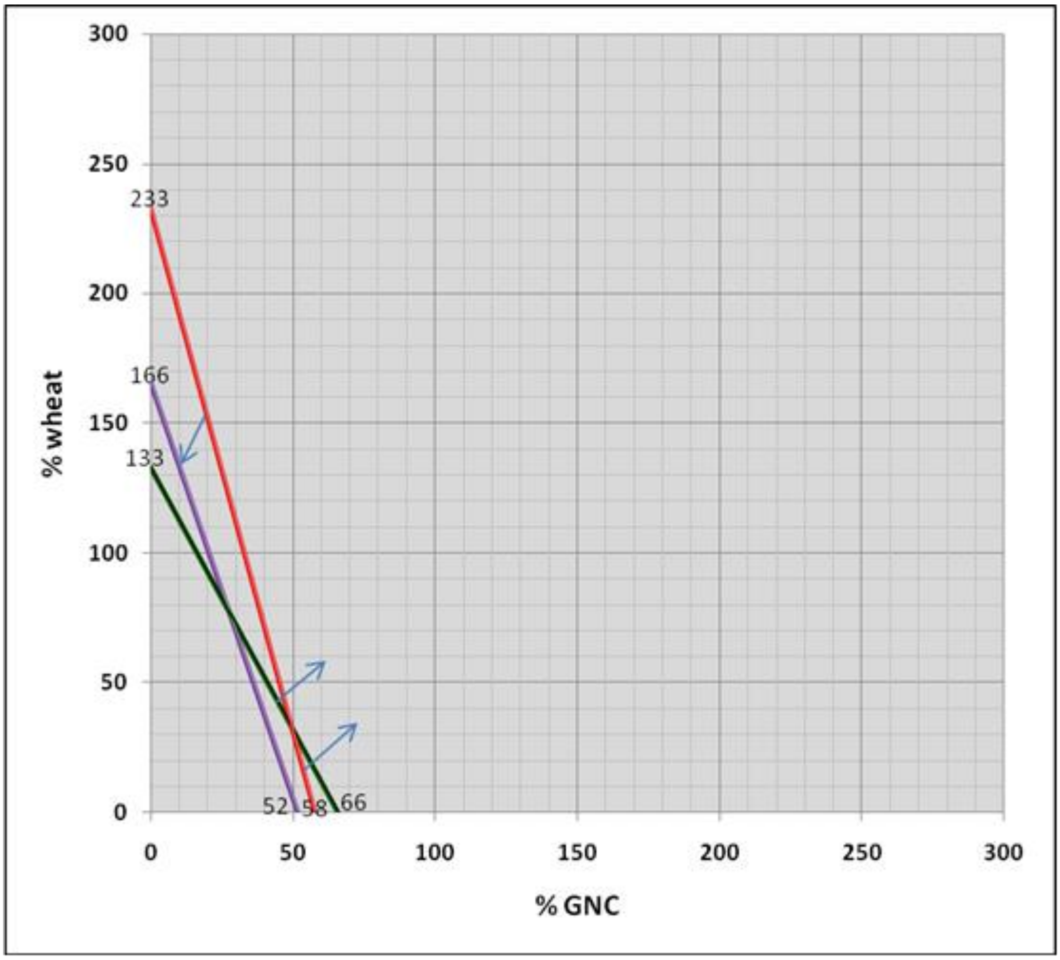
$$\%WHEAT \leq 7.0 \div 0.03$$

$$\%WHEAT \leq 233.4$$

1. To points that define the range of %WHEAT and %GNC that will satisfy the maximum restraint of 7.0% fiber in the Feed have been determined .The results are presented in figure 3.

The ratio WHEAT:GNC That will satisfy 20% crude protein a 2% fat maximum and 7% fiber maximum falls within the plane enclosed by protein, fat and fiber lines(fig 3).If the cost of WHEAT is less expensive than the cost of GNC , the apex of the triangle nearest to the Y axis is chosen. If the contrary is true, the apex of the triangle nearest to the X axis is chosen. Least cost formulation, then, is the mathematical process whereby all the possible combinations of WHEAT and GNC that meet the restrictions within the plane indicated are determined and the apex yielding the lowest cost ratio of WHEAT and GNC is chosen (Houser and Akiyama, 1997).

If the formulator wishes to include restrictions that the sum of % WHEAT and GNC must equal 100, a line that links the 100% values on the WHEAT and GNC would have to fall on this line. The result is presented in fig 5. Obviously, a third ingredient could be plotted using a third



axis. A three dimensional figure would result and its volume would contain the possible solutions.

In the example presented, only the nutrient side of the equation was restricted. However the ingredient side can also be restricted. Using linear programming, it is possible to include many ingredients and many nutrient restrictions to accomplish the same objective as has been shown with this graphic presentation

C. Linear programming/least cost formulation:

In aquaculture feeding the number of possible ingredient sources and the number of nutrients in each for which requirements have been estimated are extensive. It follows therefore that a large number of arithmetic calculations are required to produce a least-cost diet. Although 'hand' formulations using a simple calculator may be sufficient (if not a little tedious and time consuming) for the scientist or farmer wishing to formulate a feed for use within his or her own laboratory or farm, this is not the case for the commercial feed manufacturer where time is money and profits are made or lost in the area of feed formulation as the raw material cost is currently representing 80% of finished feed.

To meet this demand the animal feed manufacturing industry has been employing the computer technique of linear programming since the mid-nineteen fifties. Linear programming is essentially a mathematical tool by which resources are evaluated or selected to achieve an optimal solution to a problem. The value of linear programming is in the number of ingredients and number of requirements or restrictions which can be handled in a short period of time.

ESSENTIAL NUTRIENTS REQUIRED
(Proteins, amino acids, fatty acids, carbohydrates, vitamins, minerals, energy, energy/protein balance)

↓

SELECTION OF INGREDIENTS
(Composition, digestibility, quality control, and cost)

↓

BASAL FEED FORMULATION
(Fixed part of the feed - ie. level of fish meal, fish oil etc.)

↓

FIXED SUPPLEMENTS
(Vitamins, minerals, antioxidants, mould inhibitors etc.)

↓

BINDER OR FILLER

↓

FINAL FORMULATION/CALCULATION OF ESSENTIAL NUTRIENT LEVELS

↓<T

QUALITY CONTROL ← MANUFACTURED FEED → FEEDING

Figure 1. Procedure for diet formulation (based after Cho, Cowey and Watanabe, 1985)

Certain data must be supplied to the computer together with the programme to be executed. These data are:

- the detailed feeding standard which has to be satisfied (ie. dietary nutrient requirements), together with any permitted deviations in each nutrient;
- the detailed nutrient composition of each potential ingredient;
- any restriction on the proportion of the final mix that any one ingredient may

represent, this can be a maximal or minimal value(constraints)
- the cost per unit weight of each ingredient.

The formula calculated by the computer will be that meeting the specification at the least cost of ingredients, hence any extra mixing or handling charge due to the inclusion of a certain ingredient must be added to the cost per unit weight of that ingredient or else added to the cost of the formula.

When using a computer it must be borne in mind that the adequacy of a diet compounded to the resultant formula will be affected by two main factors: the extent to which the feeding standards (ie. nutrient requirements) adopted adequately represent the biological needs of the fish or shrimp and the accuracy with which the amounts of nutrients in the ingredients available to the fish or shrimp are known.

Conclusion

Complete diet formulations which have been tested and proven under practical rearing conditions (ie. working diet formulations as opposed to un-tested or hypothetical diet formulations) may be available in literature.

However, it should be stated that each of the individual practical diet formulations presented must be considered as being unique and as such should not be copied or applied on face value by persons wishing to formulate their own rations; the nutrient content, physical characteristics (ie. particle size), digestibility, and cost of individual feed ingredients varying considerably from one factory or country to another (depending on the manufacturing process employed and the quality of the raw material used) and also culture conditions it was tested.

ENGINEERING PROPERTIES OF SHRIMP AND FISH FEEDS

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Introduction

Aquaculture is one of the fastest growing food production activities in the world. It plays a significant role in many countries by providing a higher income, better nutrition, and better employment opportunities. Aqua-feed technology is moving in tandem with the aquaculture growth with the usage advance techniques for the improvement of digestibility, feed conversion ratio, control of pellet density, greater feed stability in water, better production efficiency and versatility. Inspite of these, it is the fact that the informations on impact of the feed quality on the biological response of the fish and shrimp has not been adequate. The physical and mechanical properties of feeds are very important to understand its behavior during processing, transporting, packaging and pneumatic conveying, without generation of excessive amount of dust and fine particles.. Besides, there is a lack of standardization of equipment and measurements for describing physical properties of fish feed. With this as background the important engineering properties which include physical and mechanical have been complied.

Dimension and Mass

Purpose: The purpose of this experiment to ensure that the feed we produce is of similar dimension throughout the batch. This ensures that the quality of feed being made is uniform and the machinery is working properly without any malfunction.

Procedure: Ten samples from a batch were taken, numbered and kept separately. Each of the samples were individually measure for height and diameter (in mm) using a vernier caliper (digital or manual), and for mass (g) using a precision balance. All the readings were tabulated. Mean and Standard deviations were calculated. A smaller value of SD will indicate a better quality feed.

Area, Volume and Expansion Ratio

Purpose: This experiment confirms similarity in lateral area, base area, surface area and volume throughout the batch of feed produced. This also ensures that the quality of feed being made is uniform.

Procedure: Dimensions obtained from the above experiment are used to calculate the lateral area, surface area, base area and volume using the given formula below.

Lateral Area (A_L) $= 2\pi rh$

Surface Area (A_S) $= 2\pi rh+2\pi r^2$

Base Area (A_B) $= \pi r^2$

Volume (V) $= \pi r^2 h$

Where,

r – radius of the feed particle

h – height of the feed particle

The expansion ratio of the feed is analyzed by finding the ratio between the lateral area, base area, surface area and volume of the feed produced to that of the die used for manufacturing it.

Sphericity

Definition: Sphericity expresses the characteristic shape of a solid object relative to that of a sphere of the same volume.

Purpose: Sphericity is a physical property that can be measured on a spherical feed, especially extruded feeds.

Procedure: 10 samples of a specific feed were analyzed for largest circle inscribed by the feed and smallest circumscribed circle. It is found by using the formula given below

$$\text{Sphericity } (\varphi_s) = D_i/D_c$$

Where,

D_i - diameter of largest inscribed circle

D_c - diameter of smallest circumscribed circle

Porosity

Definition: It is defined as the ratio of measure of void spaces in feed pellet over the total volume of the feed.

Procedure: Porosity of a feed can be experimentally determined by taking a known volume of feed pellets and crumbling them. The difference in volume between the crumbled feed and initial volume will give us the volume of void in the feed (V_V). The porosity of the feed is obtained by the following equation

$$\Phi = V_V / V_T$$

Where,

V_V – Volume of Void in the feed

V_T – Total volume of feed particle

Angle of Repose (AOR)

Definition: Angle of repose is the measure of the angle between the surface of contact (ground) and the maximum slope the pile of feed can attain before the heap starts to coast down. Or simply it can be said as the steepest angle at which a stable sloping surface formed of loose feed material.

Purpose: The angle of repose is used in the design of equipment for the processing of the particulate feed material. For example, it may be used to design an appropriate hopper or silo to dispense the feed in an automatic feed dispenser machine.

Procedure: Feed of considerable quantity (say 5 Kg) was taken and hopped into the repose apparatus. The material is poured through a funnel to form a cone. The tip of the funnel should be held close to the growing cone and slowly raised as the pile grows, to minimize the impact of falling particles. Stop pouring the material when the pile reaches a predetermined height or the base a predetermined width. Rather than attempt to measure the angle of the resulting cone directly, divide the height by half the width of the base of the cone. The inverse tangent of this ratio is the angle of repose. AOR is measured in degree (°). Theoretically smaller the size of feed, lower the angle of repose will be as smaller particle size tend to have less surface area for friction to hold on to adjacent particles to maintain the heap.

Coefficient of Friction (COF)

Definition: It is described as the ratio of the force of friction between two particles and the force holding them together. It is obtained as tangent of Angle of Repose.

Purpose: Angle of Repose and Coefficient of Friction are co-related to each other. It is useful in designing a hopper for automatic feed dispensing machine.

Procedure: There is no need for experimental method for acquiring the data. The Coefficient of Friction is obtained as tan (AOR). It is a dimensionless unit.

Bulk Density

Definition: It is defined as the mass (g) accumulated by the feed to occupy a volume of 1 litre.

Purpose: Bulk density plays a major role in aqua feed manufacturing. This is the prime characteristics that decide whether a feed will be a floating feed, sinking feed, slow sinking or fast sinking feed. This parameter gives a rough estimate of how much volume a specific feed would occupy a known volume of space. Nutritional content such carbohydrates, protein, lipids and moisture of the feed greatly affect the bulk density of a feed. It is expressed in Kg/m^3.

Procedure: For experimental analysis of Bulk Density, a known volume (1 litre) container was taken and weighed. Some quantity of feed was taken into the container till it has become full; feed was manually compacted to its maximum extent without causing physical damage to the feed. The container was then weighed. Subtracting the obtained value with initial weight of the container yielded the result in g/litre which is proportionally equal to Kg/m^3.

True Density

Definition: It is defined as the mass (g) accumulated by the powdered feed material to occupy a volume of 1 litre. True density is the density of the solid material excluding the volume of any open and closed pores

Purpose: True density of a material varies with the method a feed that is being made. True density of a feed can also be obtained with the formulated feed mixture before pelletizing or extruding it into feed. It is expressed in Kg/m^3. It generally indicates the density of true material before processing.

Procedure: Some amount of feed is taken and grounded to powder by any available means. A known volume (1 litre) container was taken and weighed. The powdered feed was transferred into the container. It was compacted to the maximum extent till the required volume was attained. The container was then weighed. Subtracting the obtained value with initial weight of the container yielded the result in g/litre which is proportionally equal to Kg/m^3. True density of a feed will mostly be higher than its bulk density.

Moisture Content (M_c)

Definition: Moisture content of a material is defined as ratio of mass of water present in a sample to the mass of solids present in it.

Purpose: Moisture content of a feed is a direct indicator for spoilage. That is why feed after milling is dried to a certain extent to obtain its equilibrium moisture content. Thus estimation of moisture content gives us an idea about the quality of feed.

Procedure: A known mass of feed sample say 10 gram was weighed initially and taken in a crucible. It was kept in a drying oven at 105° C for 30 minutes. The crucible is taken and carefully kept in a desiccator to avoid atmospheric moisture absorption. The feed was weighed and the value was noted down. The same procedure was repeated every half an hour till a constant value of mass was measured. The moisture content of the feed was identified using the formula given below.

$$\mathbf{M_c\ (\%)} = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100.$$

Sinking Velocity (V_s)

Definition: The velocity at which the feed travels (sinks) in the water per unit time.

Purpose: Sinking velocity of a feed is determined by the bulk density of the feed. More the density, faster the feed sinks to the bottom. This experiment is suitable for sinking feeds with higher bulk densities. This also infers whether the feed is a fast sinking one or slow sinking one. It is measured in m/s.

Procedure: A tall glass jar is graduated with 20 cm scale on the outside and filled with water till the 20 cm mark. Individual feed pellets are dropped one at a time with stop watch timer starting at the moment of feed starting to sink from the surface of water and stopped once it reached the 20^{th} cm mark and the time is noted down. The procedure is repeated for 10 to 20 samples per batch and the average time taken is recorded. From the obtained result we can calculate the velocity in m/s

Floatability (T_F)

Definition: It is defined as the time a feed pellet floats on the water surface before it starts to sink

Purpose: Floatability determines how long a feed floats on the surface of water before it starts to sink and gradually degrade. Feed with less bulk densities tend to have higher floatability time. It is determined in seconds

Procedure: Experimentally the floatability can be determined by timing a feed pellet from the moment of impact on the surface of the water till it starts to sink using a stop watch. Multiple samples from a batch are tested for its floatability time to obtain a mean value of T_F.

Texture Properties

Purpose: Properties such as hardness, fracturability, cohesiveness are some of the textural properties that can analysed on the feed pellets. All the properties are expressed in N or Kg load.

Definition: (i) Hardness: The maximum load a pellet can withstand before it breaks.

(ii) Fracturability: The minimum load required to create a fracture on the surface of the pellet

(iii) No. of. Fractures: The number of fractures that are created on a pellet before it is completely crushed

(iv) Cohesiveness: The load that is required to make a secondary deformation from the point of fracture.

Procedure: A sample of feed pellet is positioned on the load cell and programmed appropriately for the type of test that is about to be conducted. Once programmed and started, the probe lowers down gradually at a load and breaks the pellet automatically. The results are generated descriptively with numerical data as well graphical representation of it on the software provided. Multiple samples from a batch are tested for statistical analysis of result.

Rate of Dispersion

Definition: It is defined as the amount of feed that is being expelled from the Automatic feeder machine per unit time.

Purpose: It is used to determine the rate of dispersion of Automatic feeder machine with respect to varied feed size at varied machine operating time.

Procedure:

A plain tarpaulin sheet with a surface area more than 80 m² was laid down in the ground with machine at the center of the sheet. 2 Kg of feed was taken and hopped into the drum and the machine operating time was set initially for 3 seconds. The feed gets dispersed from the dispenser pipe with constant mass flow rate. Once the machine operational time stops, the dispersed feed spread over the sheet was collected and measured for its weight (g). Follow this procedure with different machine operating time (5 seconds, 7 seconds and 10 seconds) to identify the rate of dispersion of the machine.

Rate of Dispersion (g/sec) =

$$\frac{Weight\ of\ feed\ dispersed\ (g)}{Operational\ Time\ (seconds)}$$

Range of Broadcasting or radius of influence

Definition: It is defined as the measure of throw distance of the feeder machine from its center.

Purpose: To identity the minimum and maximum dispersing radius of machine with different feed size at different operational time.

Procedure:

A plain tarpaulin sheet with a surface area more than 80 m² was laid down in the ground with machine at the center of the sheet. 2 Kg of feed was taken and hopped it in the drum and the machine operating time was set initially for 3 seconds. The feed gets dispersed from the dispenser pipe with constant mass flow rate. Once the machine operational time stops. The distance of the dispersed feed spread over the sheet was measured using a tape from the machine center to the minimum distance dispersed to maximum distance the feed got dispersed. Take at least three different feed dispensed readings at minimal and maximum and make it average minimum and maximum dispensing radius. Follow this procedure with different machine operating time (5 seconds, 7 seconds and 10 seconds) to identify the various dispensing radius from the machine.

Uniformity of distribution

Definition: It is defined as the capacity of the machine spreading uniformly over the 360º

Purpose: To identity the spreading distribution of the feed over the feeding area by the machine so that to avoid the feed stress at a particular segment of feeding area.

Procedure:

A plain tarpaulin sheet with a surface area more than 600 m² was laid down in the ground with machine at the center of the sheet. The tarpaulin sheet is divided into 24 segments (each 15º) and from the center of the machine a circle was drawn at a radius of 1m, 3m and 6m so that a quadrant will be formed with 24 segments and concentric circles drawn as seen in the figure. 2 Kg of feed was taken and hopped it in the drum and the machine operating time was set initially for 3 seconds. The feed gets dispersed from the dispenser pipe with constant mass flow rate. Weigh the amount of feed dispensed at each quadrant inside the segment to identify the uniform distribution. Simultaneously we can measure the number of feed spread at each quadrant in the segment.

ECONOMICS OF AQUA-FEED INDUSTRY IN INDIA

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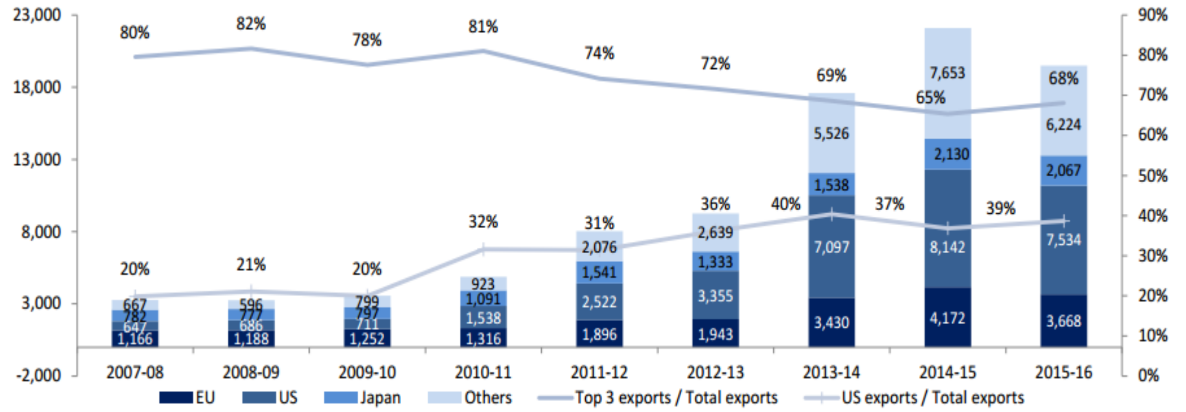
Indian Shrimp production and trade:

Global supply of fish (especially shrimp) increased during 2016 with the gradual recovery of Thailand and Vietnam from disease outbreaks; India also lost share of global business to these South East Asian countries. Coupled with flat demand, realizations fell for individual countries. Depreciation of the Euro, weaker economic condition in China and devaluation of the Yen adversely impacted global demand for seafood. Nevertheless, despite drop in demand from EU, Japan and China, shift in consumer preferences in the US—from red meat to healthier seafood, lead to overall flat global demand for seafood.

Aquaculture occupies an area of about one lakh hectares and with the introduction of *P. vannamei* the production has increased to about 5 lakh tonnes in the year 2014-15. This has also brought in an income of Rs.23,000 crore out of Rs.30,000 crores of export value of sea-food exports.

Consuming 123,114 tonne of frozen shrimp exports from India in FY2016, US is India's largest market for frozen shrimp. While shrimp exports to US contribute to ~34% of sales in volume terms, in value terms it stands at ~39% of total exports (exports to US stood at $1.33 billion in FY2016). In value terms, exports to US are followed by exports to EU, SE Asia and Japan in that order.

The graph below shows the export shares of top three importers has been on increase from 2010, while the share of exports to USA has been decreasing till 2015 and has increased in the last year by three per cent.

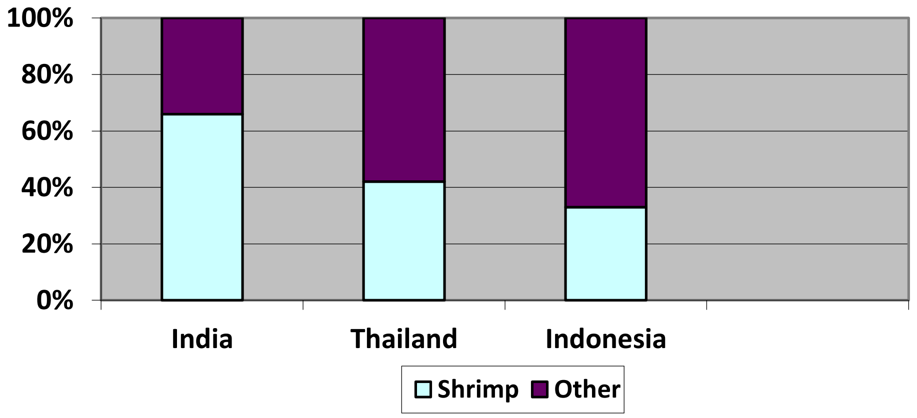


Aquafeed scenario in global context

The major countries with aquafeed manufacturing capabilities and the average feed costs are presented in the following table.

S.No	Country	Aquafeed production (Million tonnes)	Averagae cost of feed – Finisher grade (USD)
1	China	17300	850
2	Viet Nam	2800	1333
3	Norway	1789	1100
4	Chile	1243	1250
5	Indonesia	1230	700
6	India	1160	702
7	USA	1002	550
8	Brazil	940	750
9	Thailand	930	735
10	Canada	760	1000
11	Bangladesh	740	750
12	Peru	524	947

The share of shrimp and other aquaculture species in demand for aqua feed is given below in the chart:



Shrimp aquaculture and feed use in India

In India Brackishwater Aquaculture started as a traditional system of farming system with the use of rice-bran and farm feeds till it developed into scientific farming since late 1980s. Expansion of shrimp farming area with advent of scientific farming has led to increased demand for feeds. This growth has led to use of pelleted feed produced industrially. Aqua-feed manufacturing units have come up in Tamil Nadu and Andhra Pradesh, mainly by multi-national companies. ICAR-CIBA has developed its own feed technology which has been given to many entrepreneurs supporting the small scale manufacturing units of about thousand tonnes per annum.

Scientific view of feed production sector:

About 20 feed plants operate in India. Companies like CP, Avanti, Water Base, Highashimaru, Grobest, Godrej Growel and Cargill have been producing feeds suitable for brackish water Aquaculture. Most of their plants are located in Andhra Pradesh. Few plants are there in Tamil Nadu and Kerala states. There are two distinct shrimp cycles (crops) in India. The first cropping starts in February/March and ends in May/June. The second cropping starts in August/ September and ends in November/December. Typically, the first crop produces a higher yield than the second crop. Better water quality following the 2–3 months "between crop holiday" is cited to be the reason for such a difference. However, in recent years and with better management, this is becoming less common. Monthly sale quantum of feeds picks from April to August while rest of the months the time is hardly 1/3rd or 1/4th of the peak demand.

Problems and Constraints:

Feed Industry has been helping scientific shrimp farming all along. Around 50 thousand hectares in Kerala and West Bengal all together are traditional and extensive farming system. These farms follow auto stocking of seeds/ seeds from wild catch. Some farmers rarely make supplemental stocking with hatchery as they are not disease proof. These systems have very large shallow water ponds and using commercial feeds in these areas is neither technically manageable nor economically viable.

Small and medium scale farmers, without any credit support from formal credit institutions they always suffer for want of money. Many a time, they are at the mercy of the feed dealers/ consultants who arrange feed on credit. Sometimes they are forced to take the other products also which may not be required. Sometimes, the feed suppliers also play dual role of buying the products from the farmers. The farmer is affected in realizing the better price for their farm production. Small and medium farmers also face lack of power as they do not have collective bargaining power. Feed manufacturers hike the price increasing the profit margins during the peak demand periods.

Recommendations:

The following aspects need attention for better utilization of feed technologies for cost effective shrimp production.

1. Increased use of domestic/ indigenous technology for feed production will help the farmers to realize better utilization of local materials at cheaper costs thereby leading to higher profits.
2. Import tariffs on certain farms of feed components need to be reduced.
3. Payments for import of feed ingredients are to be scientifically reviewed periodically taking into consideration all requirements of farmers.

4. There should be a central laboratory facility accessible to farmers to check the quality of the feed and verify the claims of the manufacturers.
5. Locally available ingredients which do not have competitive use from human point of view should be used in Aqua-feeds.
6. Feeding schedule/ timings need to be standardized as per climatic variations to increase feeding efficiency.
7. Making cheaper credit available to the farmers will help in relieving the farmer from the clutches of the dealers/ other intermediaries.
8. The standards for feed formulations and immediate required quantity of components should be arrived at by the research institutions and made available to the farmers and the industry.
9. With increased income of development economies like India and African countries increase the market scope for shrimp produced in the country apart from usual export designations of Europe, USA and Japan.
10. The feed being the major component of cost in shrimp farming needs to be taken care of by the Research and Developmental organizations, development departments and regulatory authorities.

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LIVE FEEDS IN AQUACULTURE NUTRITION: IMPORTANCE, TECHNOLOGY AND RESEARCH AVENUES

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Introduction

The main critical bottleneck in the hatchery production of fish and shellfish is the larval rearing which includes the transition from an endogenous to an exogenous feeding by the larvae. There are two types of fish larvae: *precoicial* and *altricial*. In *precoicial* larvae, when yolk sac is exhausted, they look as mini adults, having fully developed fins and mature digestive system including functional stomach. These fishes can ingest and digest formulated feeds as a first feed. *eg*: salmon and trout. In case of *altricial* larvae, when yolk sac is exhausted, they remain in relatively undeveloped state. The digestive system is rudimentary, lacking a stomach and much of the digestion takes place in the hind gut epithelial cells. Such a digestive system seems to be incapable of processing formulated diets in a manner that allows survival and growth of the larvae comparable to those fed on live feed. Fishes, shrimps and other cultivable aquatic animals at the time of their first feeding are quite fragile and delicate creatures. It is the most *critical phase* of their life when they need right type of nourishment for their survival and growth. If this requirement is not met, they perish. So it's necessary to give live feeds at this stage.

Mainly live feeds consist of phytoplankton and zooplankton grazed upon by economically important fishes. Importance of live feed is due to several factors as rich essential nutrients, broad spectrum composition of food, better intake due to the movement, auto-digestion characteristics, facilitate better nutrient assimilation in larvae, stimulate feeding behaviour due to soft texture and attractability and ample scope for enrichment. This is why live feed organisms are called ***living capsules of nutrition***.

History of live feed culture

The culture of microalgae seems to have its origins in the late 1800s and was enabled by the methods developed by bacteriologists (Bold 1950). A significant advance in marine microalgal culture was reported by Gross (1937), who tried to culture diatoms and dinoflagellates. Methods for marine algal culture continued to advance during the middle of the twentieth century with the development of artificial media (Provasoli *et al*. 1957) and the development of *'f' medium* for the enrichment of seawater (Guillard & Ryther 1962). Improved methods for monospecific algal cultures allowed expansion of hatcheries for molluscan aquaculture and enabled culture of live invertebrates as feed for larval fish and crustaceans.

Another extraordinarily important advance was made in the 1960s, when Japanese researchers discovered that rotifers, *Brachionus plicatilis*, previously considered a pest in culture ponds, could be used as a first food for larvae of both freshwater and marine fish species (Hirata 1979). This advance clearly allowed the culture of many more species whose larvae hatched at such a small size that their mouth gapes were insufficient for the ingestion of the larger

Artemia prey. After that many fruitful experiments and attempts have been conducted in producing a nutritionally superior live feeds to finfish and shellfish larvae to get maximum survival through a number of techniques, like enrichment.

Important live food used in brackishwater aquaculture

1. Microalgae

Microalgae are essential food source in rearing of all stages of marine bivalve molluscs (clams, oyster, scallops), the larval stages of some marine gastropods (abalone), larvae of marine and brackishwater finfishes (cod, tilapia, milk fish) and shrimps (*Penaeus*). Their size varies from few micrometres to 100 µm. The most commonly used species in mariculture and brackishwater aquaculture are diatoms (*Skeletonema costatum*, *Chaetoceros gracilis*, *Thalassiosira pseudonana*) flagellates (*Isochrysis galbana*, *Tetraselmis*, *Chlorella*) etc.

2. Rotifers

Rotifers are popularly called as wheel animalcules. *Brachionus plicatilis* and *B. rotundiformes* are the most important species used in hatcheries for larviculture. Depending on the mouth size of cultured organisms, small (50-100 µm) or large (100-200 µm) rotifers are used. The rotifers have been indispensable as a live feed for mass larval rearing of many aquatic organisms. The nutritional value of rotifers mainly depends on their food. So there is lot of scope for enrichment.

3. Artemia

The brine shrimp, Artemia is used as live feed in the aquarium trade, marine and brackishwater finfish and crustacean larval culture. It is an organism closely related to shrimp belonging to order Anostraca of the class Crustacea. There are more than 50 geographical strains of Artemia has been identified. The biggest plus point of using artemia is that one can produce live feed (Artemia nauplii) ***on demand*** from dry and storable cyst ie the dormant cyst which upon immersion in seawater regain their metabolic activity and within 24 hours release free swimming nauplii of about 0.4mm length. Frozen adult Artemia are widely used by aquarists, fish breeders and aquaculturists.

4. Copepods

Copepods are diverse group of animals belonging to the class Crustacea with more than 10,000 species in different ecological niches. Three major groups of free living copepods are Cyclopoids, Calanoids and Herpecticoids. Copepods are nutritionally superior natural live feed for most of the marine and Brackishwater finfishes. Copepods are better live feeds for aquaculture compared to Artemia or rotifers because of the adequate nutritional value, size variation, and swimming behaviour. Obtaining enough copepods of desired stages at a specific time has been one of the barriers for extensive use in aquaculture and experimental work with fish larvae or other copepod-feeding organisms.

1. Polychaete worms

Polychaetes are annelid worms which form an indispensable component of the maturation diet of penaeid shrimp broodstock in hatcheries all over the world due to their high nutritive value. In India, almost all shrimp hatcheries use polychaete worms to promote maturation and spawning of broodstock/spawners. Since polychaete abundance is considered an indication of pond productivity and availability of natural food, inoculation of polychaetes in shrimp ponds is a common practice in many countries.

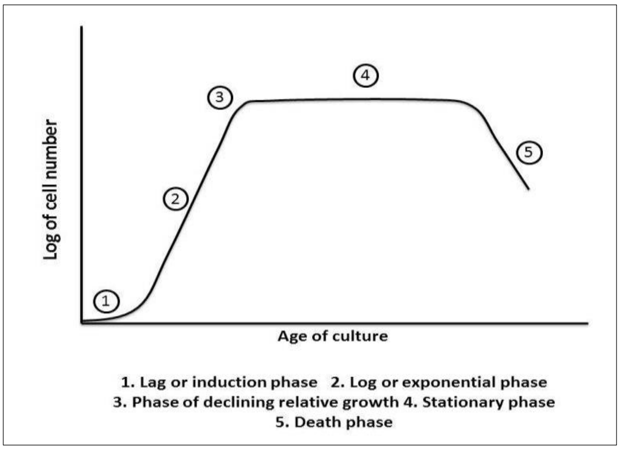
1.Microalgae

Mass-cultured microalgae are the primary food source for larval and juvenile bivalves, and for the larvae of many crustacean and fish species in mariculture. They also are the primary diet of zooplankton reared as food for late-larvae and juveniles of some crustacean and fish species. Microalgae varied in their proportions of protein (6.6-62%), carbohydrate (5.5-23%) and lipid (7-33%). All species had similar amino acid composition, and were rich in the essential amino acids.

- **Algal culture techniques**

Algae can be produced using a wide variety of methods, ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks. Various chemical media are available for indoor and outdoor cultivation. (Guillard's F/2 medium, Walne medium etc). For growth of microalgae in indoor laboratories certain factors are essential like, nutrients through specific media, light intensity (5000-6000 lux), aeration/agitation, temperature (24±1^{0}C), CO_2 (for better growth) etc. The nutritional quality of microalgal biomass is directly related to the culture conditions. There are five different stages in the algal growth (Fig 1).

Fig 1. Growth phases in the micro algal life



cycle

Fig 2: Indoor microalgal laboratory

The terminology used to describe the type of algal culture includes:

✓ **Indoor/Outdoor**. Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators and competing algae (Fig 2), whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods.

✓ **Open/Closed**. Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc.

✓ **Axenic (=sterile)/Xenic**. Axenic cultures are free of any foreign organisms such as bacteria and require a strict sterilization of all glassware, culture media and vessels to avoid contamination. The latter makes it impractical for commercial operations

✓ **Batch, Continuous, and Semi-Continuous**. These are the three basic types of micraolgal cultures

✓ ***Batch culture:*** The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase and the larger culture volumes are then brought to a maximum density and harvested. The following consecutive stages might be utilized: test tubes, 2 l flasks, 5 and 20 l carboys, 160 l cylinders, 500 l indoor tanks, 5,000 l to 25,000 l outdoor tanks (Fig 3).

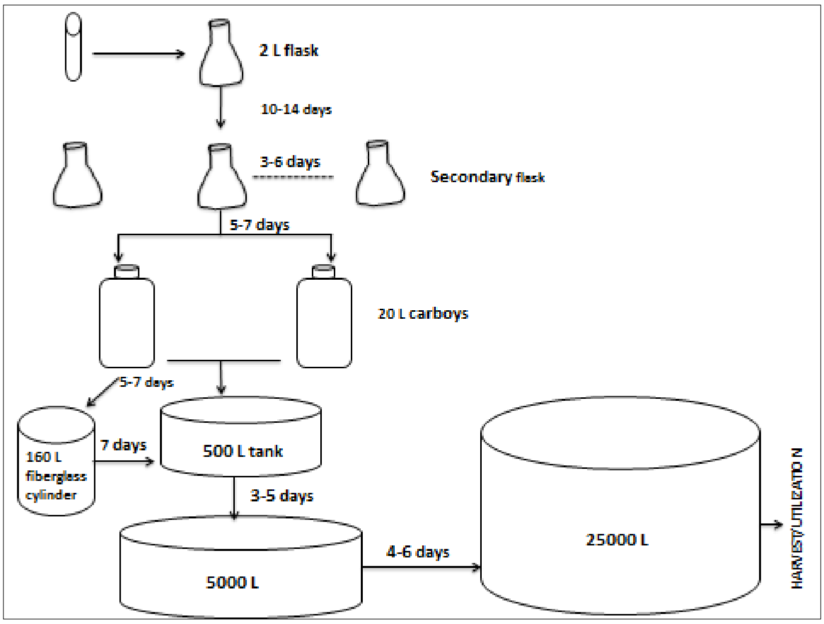


Fig 3. Diagrammatic representation of batch culture of microalgae

✓ ***Continuous culture:*** The continuous culture method, *i.e.* a culture in which a supply of fertilized seawater is continuously pumped into a growth Chamber and the excess culture is simultaneously washed out, permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished:

- *Turbidostat culture*, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.
- *Chemostat culture*, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (*e.g.* nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant.

✓ ***Semi-continuous culture:*** The semi-continuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment. The culture is grown up again, partially harvested, etc. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable. Competitors, predators and/or contaminants and metabolites eventually build up, rendering the culture unsuitable for further use. Since the culture is not harvested completely, the semi-continuous method yields more algae than the batch method for a given tank size.

✓ **Algal production in outdoor ponds:** Large outdoor ponds either with a natural bottom or lined with cement, polyethylene or PVC sheets have been used successfully for algal production. The nutrient medium for outdoor cultures is based on that used indoors, but agricultural-grade fertilizers are used instead of laboratory-grade reagents

➢ **Nutritional properties of microalgae**

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Although there are marked differences in the compositions of the micro-algal classes and species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. Expressed as percentage of dry weight,

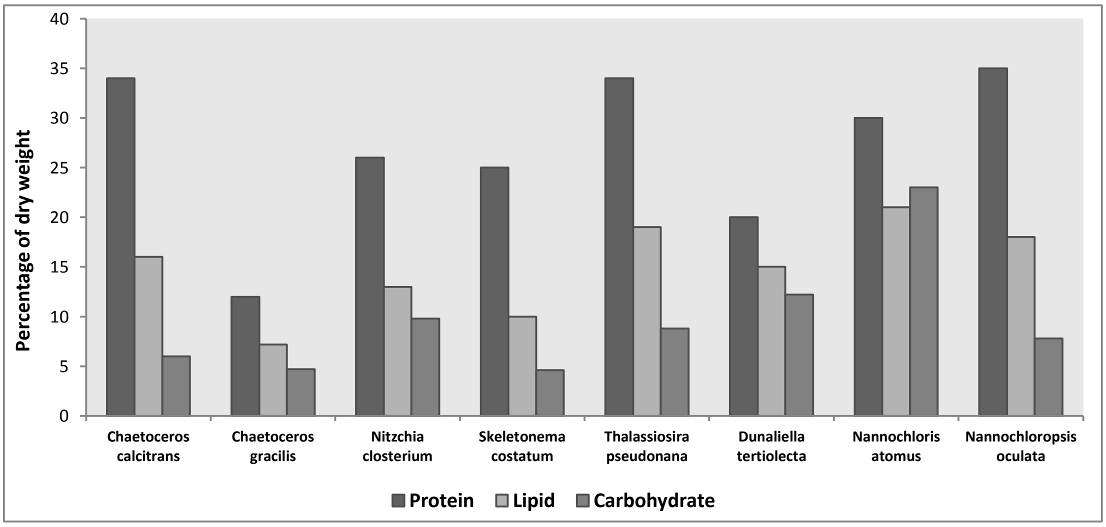


Fig 4. Concentrations of protein, lipid and carbohydrate in some species of micro-algae commonly used in aquaculture

the range for the level of protein, lipid, and carbohydrate are 6.6-52%, 7.2-23%, and 4.6-23%, respectively (Fig 4). The content of highly unsaturated fatty acids (HUFA), in particular eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA), and docosahexaenoic acid (22:6n-3, DHA), is of major importance in the evaluation of the nutritional composition of an algal species to be used as food for marine organisms. Significant concentrations of EPA are present in *Nannochloropsis* sp, *Nitzschia* sp, *Chaetoceros calcitrans*, *C. gracilis*, *S. costatum* and *Thalassiosira pseudonana* whereas high concentrations of DHA are found in the *Pavlova lutheri*, *Isochrysis galbana* and *Chroomonas salina*. Micro-algae can also be considered as a rich source of ascorbic acid (0.11-1.62% of dry weight). Spirulina is a blue green alga with immense nutritional values. It has been declared a '*Super food*' for the 21st century by the World Health Organization.

➢ **Isolation of microalgal strains and culture collections**

Apart from the commonly mass cultured and used microalgae in aquaculture, many of potential microalgal species occur natural waterbodies like backwaters and estuaries, which can be utilized for better larval survival and health. Isolation and maintenance of culture collections are the perquisites of many of the microalgal research. Isolation and identification of new species of marine microalgae are essential for achieving specific traits like high lipid content (Slocombe *et al*., 2013) or any other bioactive compounds especially in the field of aquaculture. Thus, there is a high demand to develop novel isolation and identification protocols. However, standard procedures such as single-cell isolation of strains, DNA extraction, purification, amplification, sequencing and taxonomic identification is essential for the microalgal research. Presently, a number of new cost effective high throughput methodologies are used by researchers all over the world.

1. Rotifers

The success of rotifers as a culture organism is due to their planktonic nature, tolerance to a wide range of environmental conditions, high reproduction rate (0.7-1.4 offspring. female^{-1}.day^{-1}). Moreover, their small size and slow swimming velocity make them a suitable prey for fish larvae that have just resorbed their yolk sac but cannot yet ingest the larger *Artemia* nauplii. However, the greatest potential for rotifer culture resides in the possibility of rearing these animals at very high densities (i.e. densities of 2000 animals.ml^{-1} have been reported by Hirata (1979). Even at high densities, the animals reproduce rapidly and can thus contribute to the buildup of large quantities of live food in a very short period of time. The inclusions of specific nutrients essential for the larvae in rotifers are possible due to the filter-feeding nature of the rotifers (i.e. through bioencapsulation). *Brachionus plicatilis* and *B. rotundiformes* are the most important species used in aquahatcheries for larviculture. *Brachionus calyciflorus* and *Brachionus rubens* are the most commonly cultured rotifers in freshwater mass cultures. They tolerate temperatures between 15 to 31°C.

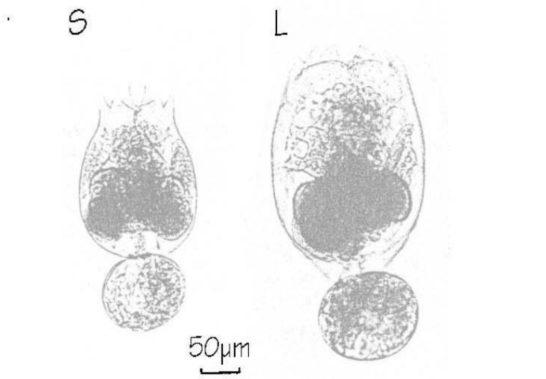
✓ **Culture procedures**

Intensive production of rotifers is usually performed in batch culture within indoor facilities; the latter being more reliable than outdoor extensive production in countries where climatological constraints do not allow the outdoor production of microalgae. Basically, the production strategy is the same for indoor or outdoor facilities, but higher starting and harvesting densities enable the use of smaller production tanks (generally 1 to 2 m^3) within intensive indoor facilities. In some cases, the algal food can be completely substituted by formulated diets.

Normally *B. plicatilis* is mass cultured in 10-15 ppt saline water where maximum reproduction occurs. Continuous culture method is commonly used. The tank with fertilizer media and seawater (Ammonium sulphate: Single super phosphate: Urea 10:1:1) inoculated with chlorella. Then rotifers are inoculated. When it reached 10×10^6 cells/ml peak, yeast is added (1g/ 1 million rotifers/day). When rotifer concentration reached 100-150 no/ml about 25% of the culture is harvested.

✓ **Nutritional value of cultured rotifers**

The nutritional value of rotifers for larval fish depends on the rotifers food source. Highly unsaturated fatty acids (HUFA) are essential for the survival and growth of the larvae. Rotifer feeds containing DHA and EPA can be valuable for marine and brackishwater fish larvae. Depending upon their food source, rotifers are composed of about 52-59% protein, up to 13% fat and 3.1% n3 HUFA. Depending upon their food source, rotifers are composed of about 52-59% protein, up to 13% fat and 3.1% n3 HUFA.



***Brachionus rotundiformis* (S-type) and *Brachionus plicatilis* (L-type) (modified from Fu *et al.*, 1991).**

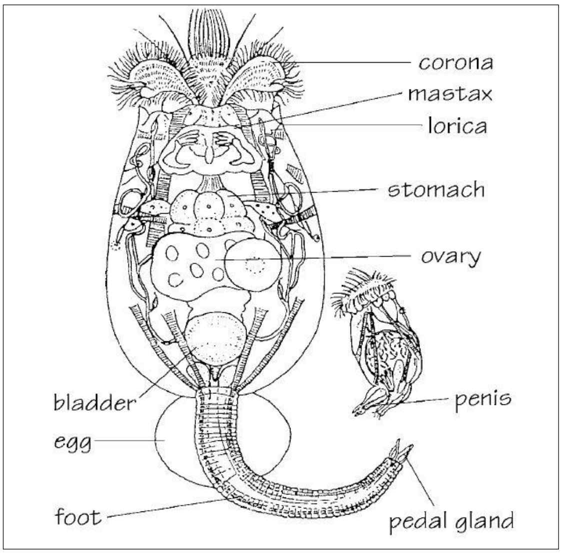


Fig 5 & 6. S & L type rotifers and morphology of rotifer.

The high content of the essential fatty acid eicosa pentaenoic acid (EPA 20:5n-3) and docosa hexaenoic acid (DHA 22:6n-3) in some microalgae (*e.g.* 20:5n-3 in *Nannochloropsis occulata* and 22:6n-3 in *Isochrysis galbana*) have made them excellent live food diets for boosting the fatty acid content of the rotifers. Rotifers submerged in these algae (approximately 5×10^6 algae ml^{-1}) are incorporating the essential fatty acids in a few hours and come to equilibrium with a DHA/EPA level above 2 for rotifers submerged in *Isochrysis* and below 0.5 for *Tetraselmis*. However, the culture of microalgae as a sole diet for rotifer feeding is costly due to the labour intensive character of microalgae production. Generally rotifers are enriched in commercial enrichment media and then fed to the fish larvae in “green water”. This “green water”, consisting of 10^6 algal cells.ml^{-1}, which is applied to maintain an appropriate HUFA content in the live prey before they are eventually ingested by the predator

2. Artemia

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp *Artemia* constitute the most widely used food item. Annually, over 8000 metric tons of dry

Artemia cysts are marketed worldwide for on-site hatching into 0.4 mm nauplii. Indeed, the unique property of the small branchiopod crustacean *Artemia* to form dormant embryos, so called 'cysts', may account to a great extent to the designation of a convenient, suitable, or excellent larval food source that it has been credited with. Those cysts are available year round in large quantities along the shorelines of hypersaline lakes, coastal lagoons and solar saltpans scattered over the five continents. After harvesting and processing, cysts are made available in cans as storable **'on demand'** live feed. Upon some 24-h incubation in seawater, these cysts release free-swimming nauplii that can directly be fed as a nutritious live food source to the larvae of a variety of marine as well as freshwater organisms, which makes them the most convenient, least labour-intensive live food available for aquaculture. Approximately 90 % of the world's commercial harvest of brine shrimp cyst comes from the Great Salt Lake in Utah. All the life stages of Artemia, the decapsulated cyst, nauplii, juvenile, and sub adults are used as feed.

Artemia mainly feeds on algae. It prefers to take *Dunaliella* and *Scenedesmus* but cannot digest species like *Chlorella* due to thick cell wall. Artemia attains sexual maturity in a fortnight after hatching and reproduce continuously throughout its life span of 30-45 days. Artemia occurs in two strains, one bisexual (presence of male and female) and other parthenogenitic (only female). In aquahatcheries artemia cysts are hatched into nauplii following the standard technique involving various steps like, hydration of cyst, decapsulation of cysts, hatching of decapsulated cysts and harvesting nauplii.

By bio-encapsulating specific amounts of particulate or emulsified products rich in highly unsaturated fatty acids in the brine shrimp metanauplii, the nutritional quality of the *Artemia* can be further tailored to suit the fish or shrimp requirements. Application of this method of bioencapsulation, also called *Artemia* enrichment or boosting, has had a major impact on improved larviculture outputs, not only in terms of survival, growth and success of metamorphosis of many species of fish and crustaceans, but also with regard to their quality, *e.g.* reduced incidence of malformations, improved pigmentation and stress resistance.

The pattern of total lipids and fatty acid composition, as well as the metabolization of fatty acids in the *Artemia*, seemed to differ widely from strain to strain, and even from batch to batch, as a consequence of the fluctuations in biochemical composition of the primary producers (mainly unicellular algae) available to the adult population. Appropriate techniques have thus been developed to improve the lipid profile of deficient *Artemia* strains, taking advantage of the indiscriminate filter-feeding behaviour of *Artemia*. A number of other compounds also appear to be variable from strain to strain: nutritional components such as total amount of free amino acids, pigments, vitamin C, minerals and trace elements.

3. Copepods

Most of the commercial fish and shellfish species are reared using rotifers and Artemia nauplii since they can be cultured in large quantities at high densities. Unfortunately, using rotifers and Artemia during this early period in life history does not always promote optimal larval growth since these live preys may contain an inadequate fatty acid profile and, in some cases, be of an inappropriate size. Obtaining enough copepods of desired stages at a specific time has been one of the barriers for extensive use in aquaculture and experimental work with fish larvae or other copepod-feeding organisms. Therefore, establishment of reliable production methods for copepods that can meet the quantitative requirements of larval fishes is essential.

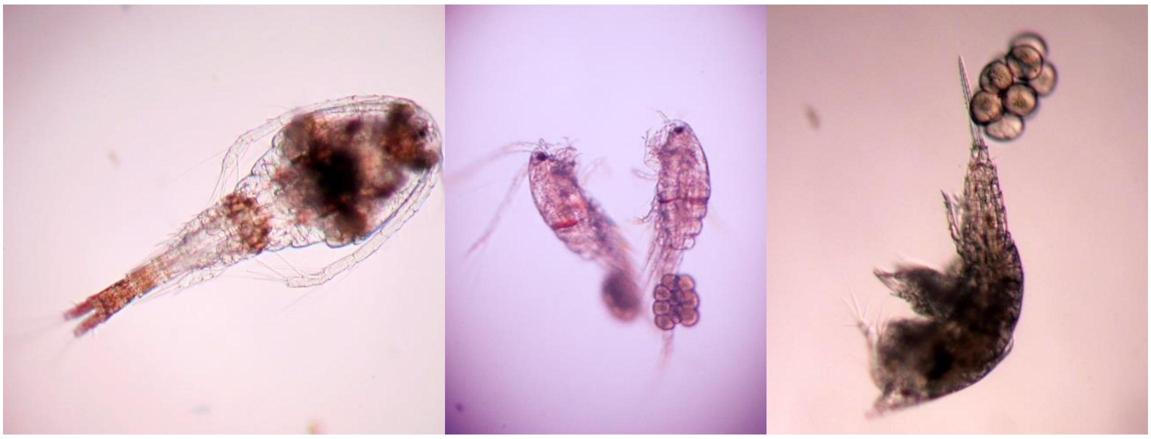


Fig 7. Copepod species used for larval nutrition

Copepods are nutritionally superior to all other live feeds commonly used in marine and Brackishwater aquaculture. This is the one of the main reasons by which copepod culture has got immense importance in finfish larval nutrition. A number of beneficial effects have been linked to copepod nutrient composition in relation to early larval nutrition. In particular, emphasis has been put on lipid composition, and the content and ratio of the polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA)

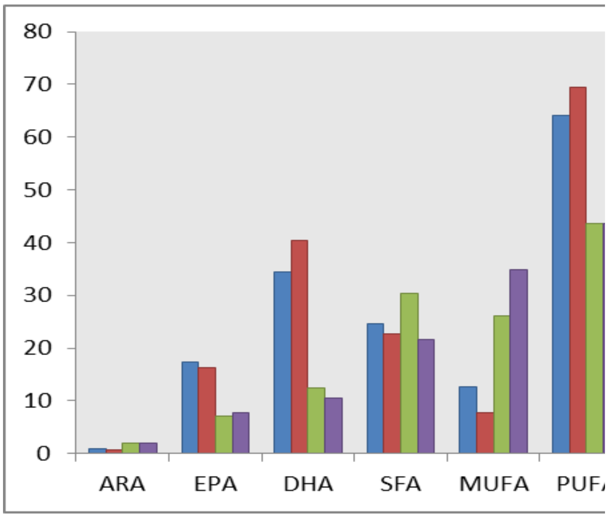


Fig 8. Fatty acid composition of various live feeds (Source: Meeren *et al*., 2008)

In contrast to rotifers, copepods are more difficult to culture on a commercial basis. Only a few species of copepods, such as *Tigriopus japonicus,* have been mass cultured successfully. A system for the mass culture of a benthic marine *harpacticoid* copepod, described by Sun and Fleeger (1995), should be useful for aquaculture. Other species of copepods considered to be promising for mass culture are *Acartia clausi*, *A. longiremis, Eurytemora pacifica, Euterpina acutifrons, Oithona brevicornis, Pseudodiaptomus inopinus, P. marinus, Microsetella norvegica and Sinocalanus tenellus.* Evjem et al. (2003) reported that copepodid and adult stages of the marine copepods *Temora longicornis* and *Eurytemora sp*. had a total lipid content varying between 7% and 14% of dry weight (DW). The predominant fatty acids of all copepods are docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and the saturated fatty acid 16:0.

Many temperate copepods produce **resting eggs** as a common life-cycle strategy to survive adverse environmental conditions, which is analogous to *Artemia* and *Brachionus* sp. These characteristics can be made useful in aquaculture. Researches are going to increase the production rate of copepods since low production rate is a constraint in the wide use of copepods in aquatic larval rearing.

Conclusion

Live feed are inevitable for larval rearing of many species of finfish and shellfish, the research on new vistas in live feed nutrition is the need of the hour. Apart from commonly used live feeds like some microalgae, artemia and rotifers there are many more live feed organisms with high nutritional profile are yet to develop. The high cost of Artemia cysts has increased fish production costs and cheaper alternative diets with similar nutritional quality needed to be maintain the cost competitiveness of the fish in the global market. The industrial development of aquaculture has been hampered by the lack of suitable live feeds for feeding the fish at their various production stages. The availability of on-grown live food would not only offer farmers and exporters a better alternative option for feeding to their fish, but more importantly, the possibility of enhancing the fish performance and quality through bio-encapsulation. There are several potential live feed organisms which are to be addressed for mass culture techniques. The researches in the area of live feed nutrition will enhance the successful and effective larval rearing of many marine and brackishwater fishes and shellfishes.

For further reading

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- *Manual on the Production and Use of Live Food for Aquaculture*, FAO FISHERIES TECHNICAL PAPER 361.
- *Live Feeds in Marine Aquaculture*, 2003.Edited by Josianne G. Støttrup and Lesley A. McEvoy. Blackwell Science Ltd.
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NUTRIENT DEFICIENCIES AND EFFECT ON FISH AND SHELLFISH HEALTH

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INTRODUCTION

Nutrition involves the chemical and physiological processes which provide the nutrients necessary for maintenance, normal function and proliferation of cells. Hence, nutrition encompasses ingestion, digestion and absorption of nutrients and their transport within the body, as well as removal of excess nutrients and metabolic wastes. Nutritional requirements of terrestrial animals have been studied intensively; whereas, studies on nutritional requirements of aquatic animals have been started recently.

Fish nutrition is the study of nutrients and energy sources essential for fish health, growth, reproduction and immunity. Dietary requirements are affected by species, size or life stage, feed intake, energy density of the diet, interactions of dietary nutrients, physiological status and environmental factors of the animal.

NUTRITIONAL REQUIREMENTS OF FISH AND SHRIMP

The essential nutrients for fish are amino acids, fatty acids, vitamins, minerals and energy-yielding macronutrients (protein, lipid and carbohydrate). Diets for fish must supply all essential nutrients and energy required to meet the physiological needs of growing animals.

LIVE FEED AND SUPPLEMENTED DIET

A gap in nutritional requirement of fish and shrimp is due to lack of appropriate diets. It is difficult to control nutrient concentration in live feed because the organism having its own metabolism. Formulated feeds have technical limitations such as high leaching and low digestibility.

FISH AND SHELLFISH IMMUNITY

Vitamins, minerals, proteins and essential fatty acids are essential nutrients of fish food. Nutritional status is considered one of the important factors that determine the ability of fish to resist diseases. Balance between macro and micronutrients, including amino acids, polyunsaturated fatty acids (PUFA), vitamins and trace elements, perform specific and essential role in development of immune system at the larval stage and maintain optimal health of larvae as well as bigger fish and shrimp. Eicosanoids involved in the regulation of the immune system by their direct effects on cells such as macrophages and lymphocytes or their indirect effects via cytokines. Vitamin deficiencies result in depressed immune function and slow or no recovery from disease in larval developmental stages.

Table 1. Nutritional requirements of fish and shrimp

Species	Nutrient requirements
Penaeus monodon	Protein 40%, Lipid 8-12% n3-HUFA 2.6%, Carbohydrate 20%
Littopenaeus vannamei	Protein 30-35%, Lipid 3-7% Carbohydrate 40%
Penaeus japonicus	Protein 40%
Lates calcarifer	Protein 45-55%, Lipid 6-18% Carbohydrate 10-20%, Fatty acid 1.72% HUFA (EPA and DHA) 1.72%
Gilt head seabream	n3-HUFA 1.6%
Chanos chanos	Protein 30-40%, Lipid 7-10% Carbohydrate 25%, n-3 PUFA 1-1.5%
Oreochromis niloticus	Protein 35%
Fish larvae	Protein 32-45%, Lipid 4-28% Carbohydrate 10-30%

EFFECT OF NUTRIENT DEFICIENCY IN FISH AND SHELL FISH

Proper balance of macro and micronutrients, including amino acids, polyunsaturated fatty acids (PUFA), vitamins and trace elements, which are essential for the development of immune system starting at the larval stage, is required. Deficiencies in these nutrients may impact developmental stages and development of lymphoid organs. Adequate nutrition is essential for cells of the immune system to divide and synthesize effector molecules. The quantitative need for nutrients to maintain a normal immune function is relatively small compared to the requirements for growth and reproduction. Composition and physical characteristics of the diet may modify the microorganisms in the gastrointestinal tract and the integrity of intestinal epithelium. The presence of lectins, protease inhibitors, and oligosaccharides and fibre can affect the gut physiology and gut microflora and thus affect the nonspecific immune response

CONCLUSION

Nutrients perform specific and essential role in development and maintenance of immune system at the larval and grow-out stages. No complete replacement of live feed has been achieved so far because formulated feeds do not meet all the requirements and result in poor growth and survival of larvae. Partial replacement can be done by reducing the live feed with formulated diet for developed larval stage. The nutritional requirements, larval digestive system, metabolism, physiology, feeding, behaviour, environment and disease resistance aspects need to be taken into consideration for development of micro-diet for fish larvae. Use of high quality feed ingredients free from contaminants, proper nutrient balance in feed formulation, prevention of micronutrients loss during feed processing, better handling, storage and feed management have good potential to improve health of aquatic animals.

Table 2. Role of nutrients on fish and shrimp immunity

Nutrients	Function	Species
Methionine	Optimal growth and survival	*P.monodon*
n-3-HUFAs (arachidonic acid, EPA, DHA	Significantly higher final weight and instantaneous growth rate	*P. vannamei*
fed 12% or 16% lipids (fish oil/corn oil, 1:1)	Higher plasma lysozyme and alternative complement activities	*Epinephelus malabaricus*
Dietary chitin	Stimulates the innate immune response by increasing complement activity, cytotoxic activity, respiratory burst and phagocyte activity,	Gilthead sea bream
n-3 and n-6 PUFA	Nonspecific immunity	Freshwater and marine fish
Vitamin C	Collagen formation, wound healing, hematopoiesis, detoxification of compounds	Freshwater and marine fish
Vitamin E	Decrease the production of lipid peroxides and reactive oxygen species which are considered toxic and destroy cells of the immune system	Freshwater and marine fish
Vitamin B	Enzyme activators and play a key role in carbohydrate, protein and lipid metabolism	Freshwater and marine fish
Iron (Fe)	Primary nonspecific host defences against bacterial infections	Freshwater and marine fish
Mn, Cu, Zn and Se	Prevention of lipid peroxidation	Fishes

Table 3. Effect of Nutrient Deficiency

Nutrient deficiency	**Effect**	**Fish Species**
PROTEIN		
Lysine	Dorsal/caudal fin erosion, increased mortality	Trout and Carp
Tryptophan	Scoliosis, cataract	*Oncorhynchus nerka*
Methionine	Reduceed growth and survival	*P. monodon*
LIPID		
Essential fatty acid (EFA)	Reduced growth and feed efficiency, reddening of fins	*Lates calcarifer*
EFA	Swollen pale liver, fatty liver	*Oreochromis niloticus*
EFA	Decreases complement activity, haemolytic and agglutination activity	Gilthead sea bream (*Sparus aurata* L.)

EFA	Reduced spawning efficiency (decreased hatching rate/survival)	Red sea bream (*C. major*)
CARBOHYDRATE		
Simple sugars	Deleterious effects on growth of juveniles	*P. vannamei*
MINERAL		
Ca	Anorexia, poor growth and feed efficiency	Japanese seabream (*Pargus major*)
Iron	Microcytic anaemia	Freshwater and marine fish
Mn and Zn	Depressed natural killer activity of leucocytes	Rainbow trout
VITAMIN		
Vitamin C	Morphological abnormalities (mouth and opercular regions)	Newly hatched larvae and hatchery reared fry and juveniles of *Chanos chanos*
Vitamin D	Poor growth, high mortality, reduced appetite and darkening of midgut	*Marsupenaeus japonicus* *P. vannamei*
Riboflavin (vitamin B2)	Sluggishness, photophobia, cataracts, stunted body, reduced growth, feed efficiency and survival, dark colouration	*Lates calcarifer*
Choline	Reduced growth and survival	*P. japonicus*

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SIGNIFICANCE OF SOIL AND WATER QUALITY MANAGEMENT IN BRACKISHWATER AQUACULTURE

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A pond with good soil and water quality means availability of nutrients in optimum quantity will produce healthier animals than a pond with poor soil-water quality. Hence, it is essential to understand the pond soil and water characteristics, optimum requirements including the available nutrients, and their management to increase the productivity of the ponds in general and thereby augmenting aquaculture production. Poor environmental conditions in pond bring in a state of stress that is unfavourable for the cultured animals but favourable for the disease causing agents. Soil and water quality can be maintained within the optimal range by giving importance starting from site selection and suitable pond preparation methods.

1. Soil and water requirements

Pond soils may have negative effects on aquaculture production if one or more of their properties are outside the optimum range for aquaculture. Understanding of the soil parameters helps to decide the management strategies to be followed in terms of liming, manuring, fertilization, water management etc. for increasing nutrients use efficiency. Water quality and quantity determines the success or failure of an aquaculture operation. Day-to-day management of ponds requires only an estimation of the topping-up rate of the water supply, to combat evaporation and seepage losses. An annual water budget should be calculated for a potential farm site so that the supply is adequate for existing and future needs. The optimum ranges of important soil and water requirements to brackishwater aquaculture are given in Table 1.

2. Soil and water treatments during pond preparation

Even if the site is good with optimum soil characteristics, problems may still crop up by the large quantity of inputs like feed and fertilizers, which lead to excessive phytoplankton production, low dissolved oxygen, high ammonia, poor bottom soil condition and other problems. Most of these problems can be avoided by proper management practices during pond preparation. Maintenance of good water quality is essential for both survival and optimum growth of shrimps. Water treatment is an important step during pond preparation for the maintenance of good water quality at later stage.

2.1 Pond preparation

The main objectives of pond preparation are to provide the animal with a clean pond base and appropriate stable water quality.

Pond drying:

- Emergency harvest:
  - ✓ Do not discharge water from white spot disease (WSD) affected ponds. Remove aeration devices and implements and disinfect by evenly distributing calcium hypochlorite to provide a minimum final free chlorine concentration of 10 ppm. Allow the system to stand for a minimum of 24–48 hours at this minimal chlorine concentration.
  - ✓

Soil parameter	Optimum Range
pH	6.5-7.5
Organic carbon (%)	1.5-2.0
Available nitrogen (mg/100g)	50-70
Available phosphorus (mg/100g)	4-6
Calcium carbonate (%)	>5.0
Electrical conductivity (dS/m)	>4
Depth to sulfidic or sulfuric layer (cm)	50-100
Clay content (%)	18-35
Textural class	Sandy clay, sandy clay loam and clay loam

Water parameter	Optimum range
Temperature (°C)	28-32
pH	7.5 – 8.5
Salinity (ppt)	15-25
Transparency (cm)	30-40
Total suspended solids (ppm)	<100
Dissolved oxygen (ppm)	4.0 - 7.0
Total ammonia-N (ppm)	<3.7
Free Ammonia (ppm)	<0.1
Nitrite-N (ppm)	<0.25
Nitrate-N (ppm)	0.2-0.5
Dissolved-P (ppm)	0.10-0.20
Chemical oxygen demand (ppm)	<70
Biochemical oxygen demand (BOD_5) (ppm)	<10
Hydrogen sulphide (H_2S) (ppm)	0.002

Table 1: Soil and water requirements for brackishwater aquaculture

Emergency harvest:

Do not discharge water from white spot disease (WSD) affected ponds. Remove aeration devices and implements and disinfect by evenly distributing calcium hypochlorite to provide a minimum final free chlorine concentration of 10 ppm. Allow the system to stand for a minimum of 24–48 hours at this minimal chlorine concentration.

CIBA's work on the duration of viability of WSSV in pond sediments has revealed that the virus remains viable and infective up to 19 days in the sediment despite sun-drying under experimentally simulated pond drying conditions, and up to 26 days post emergency harvest due to WSD under actual field conditions. Drying pond sediment for at least three to four weeks can help in prevention of WSD.

Following the harvest after a crop, the deposits of organic debris in the pond bottom should be removed or treated, ploughed, tilled and levelled. All parts of ponds should be thoroughly sun dried for at least three weeks for microbial decomposition of soil organic matter and mineralization of organic nutrients.

Investigations on duration of drying period revealed that farms practicing drying duration of 3, 5, and 10 days were affected with white spot, running mortality syndrome (RMS) and white gut diseases and harvested within 60-70 days of stocking whereas the farms that adopted a drying period of 30 to 45 days had successful harvest.

Lime application: can be done based on the pH of the soil and the availability of lime type. In soils with chronically low pH it may be beneficial to apply half the total dosage before slight tilling in order to neutralize underlying soil layers.

Fertilisation: Application of inorganic nitrogen fertilizers to supply nitrogen will increase soil organic matter degradation during fallow periods between crops. Urea can be spread over pond bottoms at 200 to 400 kg ha^{-1} at the beginning of the fallow period to accelerate decomposition of organic soil. Agricultural limestone should not be applied until a few days after urea is applied to prevent a high pH. Sodium nitrate can be applied @ 20 to 40 g m^{-2} to wet soil to encourage organic matter decomposition in wet areas.

Water treatment

- Filter source water first through coarse screens to remove larger aquatic animals and debris and then pump into a supply/settling canal for allowing the suspended particles in the source water to settle before pumping the water into culture ponds. Then, pass the water through a series of progressively finer screens (150–250 µm mesh size) before being introduced into the reservoir.
- Chlorinate water in the reservoir with sufficient chlorine (10 ppm) to kill any potential vectors or carriers in the water collected in the reservoir. For one ha reservoir pond of one meter depth, 150-160 kg of calcium hypochlorite providing 65% active chlorine would give a final concentration of 10 ppm .Vigorously aerate reservoir at least 48 h for de-chlorination to remove residual chlorine.

3. Soil and water management during culture period

The sediment – water interface is an intricate system where complex chemical and microbial changes occur plays another important role in brackish water aquaculture. The two most important nutrients in pond aquaculture are nitrogen and phosphorus because these two nutrients often are present in short supply and limit phytoplankton growth. These two nutrients are added to ponds through fertilizers, manures, and feeds.

3.1. Nutrients dynamics

Fertilizer nitrogen usually is in the form of urea or ammonium, and urea quickly hydrolyzes to ammonium in pond water. Ammonium may be absorbed by phytoplankton, converted to organic nitrogen, and eventually transformed into nitrogen of shrimp protein via the food web. Ammonium source may be oxidized

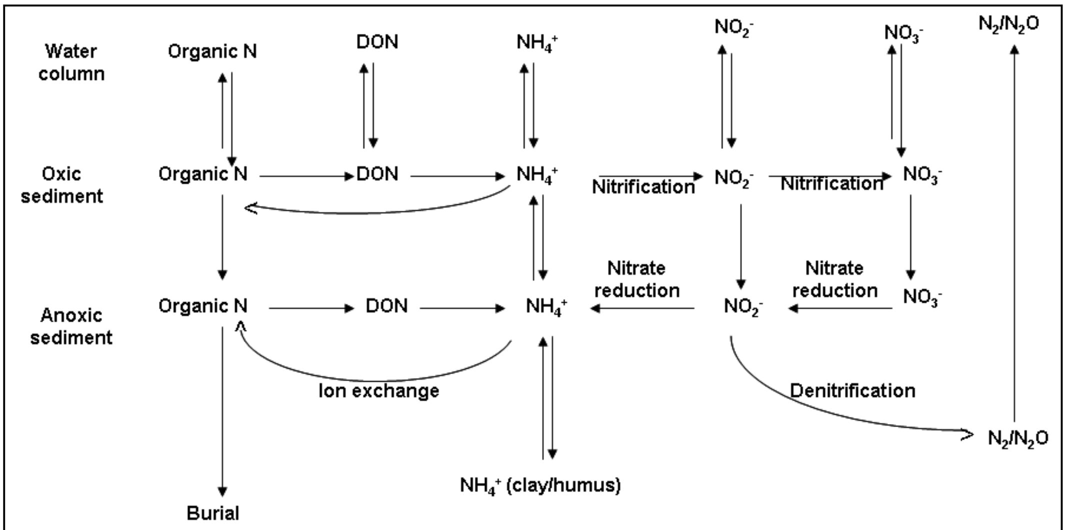

Fig.1 - N cycling in the sediment (Source: Herbert, 1999)

to nitrate by nitrifying bacteria, and nitrate may be used by phytoplankton or denitrified by anaerobic microorganisms in the sediment. Nitrogen cycling in sediment and water column showing different forms is shown in Fig.1.

Soils that are near neutral in pH have less capacity to adsorb phosphorus and a greater tendency to release phosphorus than do acidic or alkaline soils. Phosphate is released from iron and aluminum combination when reducing conditions develop from oxygen depletion. A dynamic equilibrium exists between sediment and overlaying water so that a small amount of phosphorus is maintained in solution. Loss of phosphorus from the water is a desirable event in ponds with large inputs of feed and high levels of aquaculture production because phosphorus from feeds often is a key factor contributing to excessive phytoplankton growth.

The ratio of major fertilizing elements carbon, nitrogen and phosphorus has immense importance in mineralization and aquatic productivity. Carbon to nitrogen ratio is useful in identifying sediments containing excess nutrients and in turn affects the rate of release of nutrients from decomposing organic matter. In general, soil C: N ratios between 10-15 are considered favourable for aquaculture. Manipulation of C: N ratio reduces the accumulation of inorganic nitrogen by feeding bacteria with carbohydrates, and through the subsequent uptake of nitrogen from water, by the synthesis of microbial proteins. Ratios of N: P greater than 15:1 resulted in increased proportions of diatoms. Silicate fertilization may be beneficial when silicate concentrations are low and a greater percentage of diatoms are desired. The values of gross primary productivity were the direct function of values of fertilizer mineralization indices for carbon, nitrogen and phosphorus.

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3.2 Minerals supplementation

In order to maintain the optimum concentration of minerals and ionic balance, modifications in mineral supplementation through water and diets are available. Water modification approaches are more effective compared to the dietary modification strategies though the cost of ionic fortification is comparatively high when the culture area is large. Leaching of the water soluble minerals from the diets is a limitation for dietary supplementation. Ionic levels in the low saline water ponds have to be raised to their concentration in seawater diluted to the same salinity of production ponds. In order to calculate the desired mineral levels at different water salinities, the water salinity (in ppt) is to be multiplied by the factors shown for each mineral (Table 2).

Table 2. Calculation of mineral requirement for different saline waters

	Sodium (Na)	Potassium (K)	Magnesium (Mg)	Calcium (Ca)	Chloride (Cl)	Sulphate (SO_4)
Sea water (34.5 ppt)	10500	380	1350	400	19000	2700
Factor of each mineral to be multiplied with the water salinity	304.5	10.7	39.1	11.6	551	78.3

**For example, seawater with 35 ppt salinity has 400 ppm Ca, with 4 ppt salinity well water the desirable levels would be 46.4 ppm Ca (4 * 11.6)

3.3 Soil and water parameters monitoring during culture period

In order to understand the condition of the pond bottom, soil pH, organic matter and redox-potential (E_h) for oxidized/reduced pond bottom condition have to be monitored regularly. The E_h of pond sediment should not exceed -200 mV.

The water parameters that should be monitored routinely in ponds during culture period are temperature, pH, salinity, dissolved oxygen and transparency.

- The pH should be in optimum level of 7.5 to 8.5 and should not vary more than 0.5 in a day.
- Variations in salinity not exceeding 5 ppt in a day will help in reducing stress on the shrimp.
- The optimum range of transparency is 25-35 cm.
- The un-ionized form of ammonia nitrogen should be less than 0.1 ppm.
- Any detectable concentration of hydrogen sulphide is considered undesirable.

Periodical water exchange as and when required will help in maintaining the water quality in optimal range. The use of aerators results in mixing of water at surface and bottom and breakdowns the DO and thermal stratification. Only the scientifically proven chemicals and probiotics are to be used by the farmers. The discharge water from the shrimp ponds has to be allowed into a treatment system pond before letting it into the environment so that the suspended solids may settle at the bottom.

4. Conclusion

The two-pronged approach of combining pond management and health monitoring is the key for successful and sustainable aquaculture. It is important to know how much shrimp/fish can be supported by the pond environment (carrying capacity of pond). In general, nutrient requirement of aquaculture ponds depends upon the soil condition and biological productivity and such information is sparse. The promotion of natural planktonic or benthic microbial and microalgae communities present in the pond environment helps in the utilization of nutrients through autotrophic and heterotrophic processes, thus improving water quality. Understanding the nutrient interaction will help reduce the cost of production by reducing the cost of feed, fertilizer, etc. and also reduce the adverse environmental impact.

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BIOFLOC MEAL COULD BE A SUSTAINABLE INGREDIENT IN AQUA FEED FORMULATIONS

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Aquaculture is growing fast and contributes about 50% to the global total fish production, and expected to grow at much faster rate to supply the increasing demand of fish for human consumption (FAO, 2016). Fish culture involving formulated feed usage is increasing in geometric proportion, and it is creating huge pressure on ingredient supply, especially fishmeal. Therefore, finding sustainable alternate feed ingredients is the biggest challenge for the aqua nutritionist. We expect biofloc meal could be a sustainable ingredient for future.

Significance of biofloc in shrimp and fish nutrition:

Providing good water quality through biological processing involving consortium of bacteria and microalgae ('BIOFLOC") and use of bioflocs as a fresh feed (in situ feeding) within the system is considered to be a low cost sustainable feeding tactic, and is receiving popularity in farming of white leg shrimp around the world. Biofloc is found to be a nutritionally rich and balanced with good amount of protein, minerals and other micronutrients. More importantly, it is interesting to note that bioflocs or its attached microorganisms could exert a positive effect on the digestive enzyme activity of shrimp or fish. Bioflocs not only act as a feed, but also manages the water quality with no any additional cost. Even though bioflocs are known to be consumed a lot by the white leg shrimp as fresh feed, it may not be consuming all the available biofloc biomass, and many will be will discarded during water exchange. Furthermore, other cultured species of shrimp and fish are either physiologically or anatomically restricted to show that much attraction towards consumption biofloc. In such situations, most of the floc biomass will be thrown in to the receiving water body. Here there is an immense scope for harvesting these nutrient dense feed particles with advent of various filtration and separation technologies, and further processing it could add a potential feed ingredient to the aquafeed ingredient basket.

Concept of biofloc formation and biofloc meal production as a sustainable approach:

Feed is a major input in any shrimp farm and it never been completely utilized by the shrimp for its growth. Naturally, complete utilization of feed consumed by any animal is impossible, and shrimp is not an exception. Considering a global average of FCR 1.4, one can calculate that, to produce 1kg fresh shrimp (with 80% water) we need 1.4kg dry feed (with 10% water). In equivalent figures of dry matter, this will be like this; to produce 1 kg of dry shrimp biomass we need 6.3 kg of organic matter. Eliminating shrimp biomass (1 kg), about 5.3 kg of organic matter is thrown as waste either on the pond bottom or in to the nearby water body during water exchange. However, under existing practices, even in zero water exchange systems, except the nutrients in the shrimp biomass, nothing is recovered back. Instead, nutrients are simply thrown as sludge or along with the discharged water. The concept of having biofloc to recycle both the organic and inorganic nutrients by utilizing the services of microalgae and bacteria, and providing biofloc itself as a natural feed for the shrimps is a novel concept, and it would be eco-friendly, cost-effective, and having a lot of potential for further intensification by increasing the shrimp stocking density in a unit area provided. On the other hand, shrimp being an aquatic animal which passes water through their gill lamellae for oxygen uptake from water, sustaining plenty of suspended particles in the ambient water may lead to gill congestion (Xu et al., 2012). Therefore, it is assumed that constant removal of flocculated biomass from the system would be favourable for shrimp. Furthermore, it also makes space for fresh algal and bacterial cells to grow, which in turn increase the productivity of the system. The removed suspended particles may be concentrated, dewatered and dried to produce nutrient rich biofloc meal. This is not only a nutritious meal, but also a sustainable ingredient from the aquaculture practice itself.

Harvesting of bioflocs using radial settler

Biofloc and biofloc meal:

Biofloc is a clumpy assemblage of microorganisms (bacteria, micro algae, cyanobacteria, fungi, protozoans, micro-zooplanktons, etc.) with dead particulate organic matter suspended in the water column of an intensively aerated or agitated aquaculture system. Research showed that, bacteria and some microalgae produces exopolysaccharides (EPS) such as uronic or pyruvic acid (Shipin et al. 1999) under stressed condition. These polymers act as adhesives to aggregate the dispersed cells of bacteria, microalgae and other particulate organic matter to form a clumpy mass called bioflocs (Frølund et al. 1996). These dense flocculated assemblages will tend to settle down at the bottom of rearing system.

Biofloc meal is nothing but a concentrated, dewatered and dried form of biofloc harvested from the system. Harvesting biofloc would be a costly process, which may add cost to the product. It needs to be simplified in an economical way. While the existing processes are scaled up to a larger scale, the price can be significantly reduced. The drying can be either spray drying or drum drying. Spray drying will preserve the nutritional value of the meal. Freeze-drying would be an option for keeping the nutrients of the biofloc meal intact, and the meal can be used as ingredient in speciality feeds such as brood stock and larval feeds.

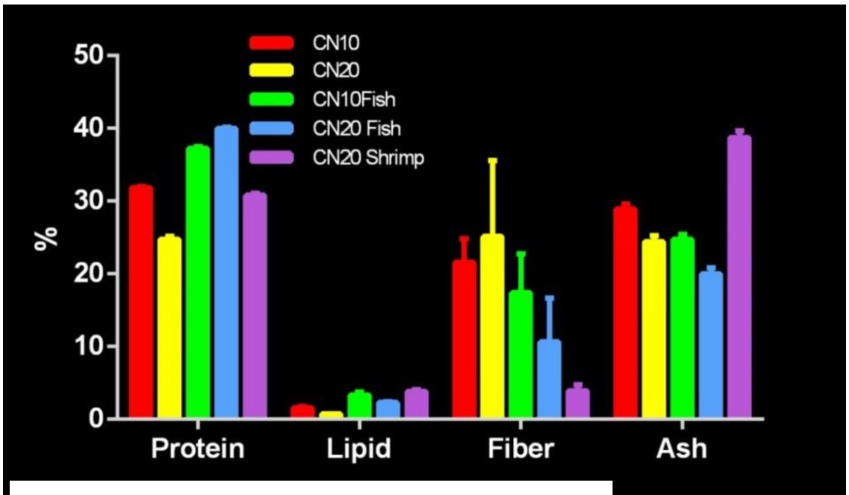
Culturing and harvesting of Biofloc

Self-circulating out-door microcosm tanks were used for producing bioflocs. Bioflocs were produced against various C:N ratios and presence of fish and shrimp. Radial flow settlers in combination with filter meshes were used for harvesting the biofloc from the water.

C:N ratios were adjusted using formulated feed and jaggery.

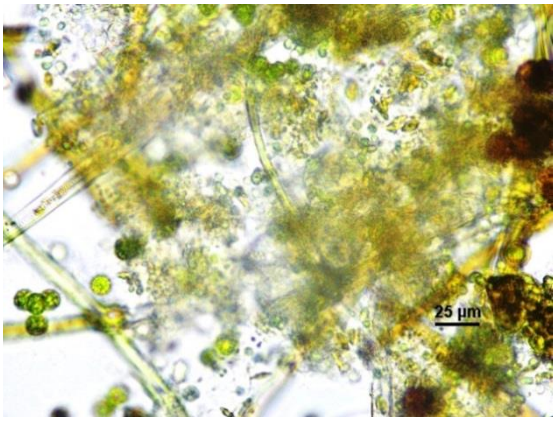
Nutritional value of biofloc meal:

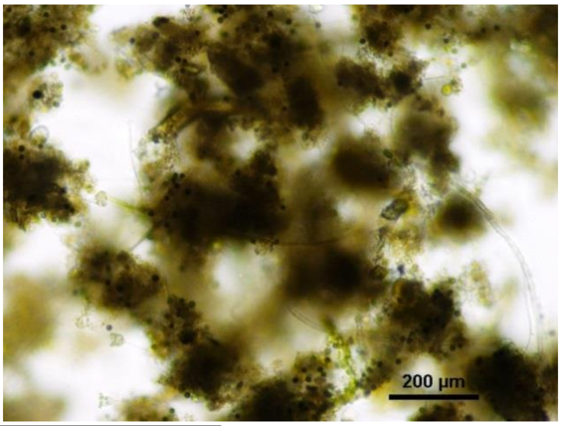
Since biofloc is formed of various groups of microorganisms and organic particulate matter by recycling the dissolved nutrients from feed, naturally it will become nutrient dense material. Reported protein and lipid content of biofloc collected from different culture conditions ranges from 25 to 50% and 0.5 to 15% respectively (Khun et al., 2009). Bioflocs were found to be good source of nutrients like protein, lipids, vitamins and micro nutrients. Bioflocs may also have potential to provide exogenous enzymes to the cultured animal and some probiotic effects. There are differing judgment about the adequacy of



Nutritional *characteristics of the biofloc produced under varying C:N ratio*

bioflocs to provide the limiting amino acids methionine and lysine.





Green biofloc viewed under microscope

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SOLAR POWERED FEED DISPENSING TECHNOLOGY FOR SHRIMP AQUACULTURE

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Introduction

The major component of scientific shrimp farming is to provide artificial feeds at right quantity at right time according to the requirement and feeding is one of the most critical aspects of shrimp husbandry. Development of a shrimp feed of high quality to meet the nutritional requirements of the shrimp which is manufactured from high quality, digestible ingredients, with appropriate size and palatability for the shrimp is the most important one. Along with that the method of feeding is also important since it influences the overall feed quantity consumption, soil and water quality of the pond and ultimately the success of the culture itself. It may not be an exaggeration to state that feeding method and management is as important as the development of the feed. It is very important to have control of how much feed is to be given in order to achieve the optimal growth rate without overfeeding. Excess feeding leads to waste feed, which results in excess costs and poor water quality, which in turn could lead to stress of the aquatic animal. Feeding is labour-intensive and also expensive. Feeding frequency is dependent on labour availability, farm size, as well as the species and sizes.

Automatic feed dispensing and *L. vannamei* culture

The introduction of *L.vannamei*, heralded the rapid adoption of high intensive farming with the availability of SPF stock. The stocking density allowed as per Coastal Aquaculture Authority guidelines for *L vannamei* is 60 /sq.m and the potential productivity level is around 10-12 t /ha accordingly the feeding management requires greater attention. The automatic feeders may be more suitable in L. *Vannamei* due to its feeding behaviour which is a column dweller compared to tiger shrimp*, P. monodon* which is a bottom feeder. The bio security requirement for shrimp farming could be best met with the utilisation of automatic feeder. The feed quality could be well maintained with these systems.

It has been reported that automatic feed dispensing enhances the nutrient use efficiency, better FCR, optimal conversion, reduces the days of culture, which will ultimately result in profitable and economical shrimp farming. These devices are indeed will help to overcome labour problems in the industry and introduce a semi-automatic process in the shrimp aquaculture industry. One of the most important challenges for engineers is the designing of proper feeding devices for shrimp farm use which could ensure optimum feeding rate at desired frequency with uniform distribution. Automatic feeders are therefore particularly appropriate to intensive systems of shrimp and fish culture systems and the feeding of nursery fry tanks which require frequent, small doses of feed. Though some models are available in the market, the cost is prohibitive .This prompted ICAR - CIBA to design and develop a system specific, low-cost, energy efficient, easily operated automated feed dispensing unit for precision feeding efficient nutrient use efficiency .

The automatic feeder should be synchronised with the existing feed management for the shrimp and the following three factors are to be taken care of

Feeding rate - Adjustments to the feeding regime with appropriate quantity based on life stage

Feeding frequency- Feed is presented frequently that are appropriate for the number and size of the shrimp in the population being fed

Feeding distribution- It is distributed evenly over the water/culture area to ensure that all the shrimp have equal access to the feed;

Feeding rate and automatic feeding

Feed tables have been developed that give a recommended feed rate, expressed as percent of bodyweight per day (% BW/Day), for animals of different sizes. To calculate the daily feed allowance for a population of shrimp, the total biomass of the shrimp population is to be multiplied by the recommended feed rate Accurate information about the average weight and total number of shrimp in the population is required to correctly calculate the daily feed allowance. The shrimp population should be sampled at least every week to determine the average shrimp body weight.

Demand-based feeding is an alternative to using a feed table. With this method, the feed allowance is adjusted up or down depending on the feeding activity of the shrimp. At each feeding, the technician estimates the amount of feed that the shrimp can consume during the time interval between feedings. If a significant amount of feed remains from the previous feeding, the amount fed at the next feeding should be reduced by at least 10 percent. If all of the feed has been consumed between feedings, the feed amount can be increased by 10 percent. This approach to feeding ensures that the feed rates will be appropriate for the conditions in the pond.

Feeding frequency and automated feeding

The number of feedings per day is determined by pellet stability, shrimp feeding behaviour and by the rate at which the feed is consumed, digested and metabolized by the shrimp. Dividing the daily feed ration into multiple feedings, spaced several hours apart, improves feed conversion ratios and growth rates. In addition, feeding only what the shrimp can consume in 3 or 4 hours reduces losses of nutrients due to leaching. It is not clear whether or not there is any benefit derived from feeding the shrimp throughout the 24-hour period. While *L. vannamei* are active at night, they may not be actively feeding during this time period. Robertson et al. (1993) reported that *L. vannamei* receiving four feedings a day during daylight hours performed as well as, or better than, shrimp fed around the clock. Small shrimp metabolize their food faster than large shrimp, and generally require more feedings per day. Post larval shrimp require frequent feedings because they have very high metabolic rates, but are not able to store much feed in their guts. Ideally, post larvae should be fed every 2-3 hours. Longer intervals between feedings may result in heavy losses. Automatic feeders, which dispense small amounts of feed at programmed intervals or on a continuous basis, can be used to make sure the shrimp, are fed in a timely manner. As the shrimp grow the feeding frequency can be decreased. Four feedings spaced three hours apart during daylight hours works well for juveniles larger than 1 gram in size.

Feeding distribution and automatic feeding

Through the automatic feeding system the uniformity in application could be achieved. The uniform application of feeder helps to achieve uniform growth.

Design consideration of the Solar Powered Automatic shrimp Feeder:

Design criteria

A simple design of automatic feeder consists of four major components, a feed hopper, mechanisms for feed distribution, an electrical power supply for the distribution mechanism and a control unit for starting and stopping the distribution mechanisms. (Fig 1.) Each component has to be designed with utmost care. The cost will be the prohibiting factor for wider adoption and hence energy efficient low-cost feeder with efficient nutrient delivery is the need of the hour. The material for the feeder assembly needs to be corrosion resistant and durable in salinity condition. The material should be light but strong enough to face the windy conditions in the coastal area.

Fabrication of the feeder

A feeder developed is simple, low-cost one and contains only standard, easily available parts and it is a timer-controlled automatic feeder.

Feed hopper

The feed hopper is PVC drum of capacity 125 kg. The material selection has been done with the cost, saline condition and durability in the mind. The material necessarily be rust free, and should not react with the feed material and should have a long life and hence the PVC was selected. The angle of repose/angle of inclination plays an important role in designing feeding devices such as demand feeder which depends on the gravitational force for feeding (Mohabatra, 2009). In the case of timer control, automatic feeder distribution mechanism is more important. Yet to facilitate the feeding to the distribution system, the hopper bottom was designed with a greater angle of repose (50^0) was designed in than the angle of repose of the materials to be dispensed (44^0). The valve which controls the flow of feed as per the timer was designed such a way that it operates smoothly. The feed dispensed through the feeder is such that feed quality is not affected viz., the feed is not broken.

Control unit

The automation is effected through two timers. The two timers with a digital display were set such that the dispensing of the feed and the duration of feeding could be adjusted easily by the farmers.

Distribution unit

A 0.5 HP motor was fixed with variable speed regulator to control the dispensing of the feed. The distribution system was four pipes fixed at the circular disc at the bottom of the feed hopper. Series of trials were conducted with different pipe diameter, pipe length at different motor speed to investigate the quantity of feed and radius of the influence. Based on the results it has been modified to three pipes of uniform length (175mm) and diameter (20mm) with a 30^0 inclination towards upward direction for wider dispersion.(Fig 5.). It is powered by the motor of the 0.5hp series motor with an rpm of 2800. Adjustment has been made for variable speed to effect uniform dispersion.

Solar power

The power crisis warrants the inclusion of alternative energy source for operation of the automatic feeder and hence 0.5hp DC motor of 2800 rpm was included. The cost and the efficiency of solar powered UPS operated automatic feeder has been proved to be very efficient and economical.

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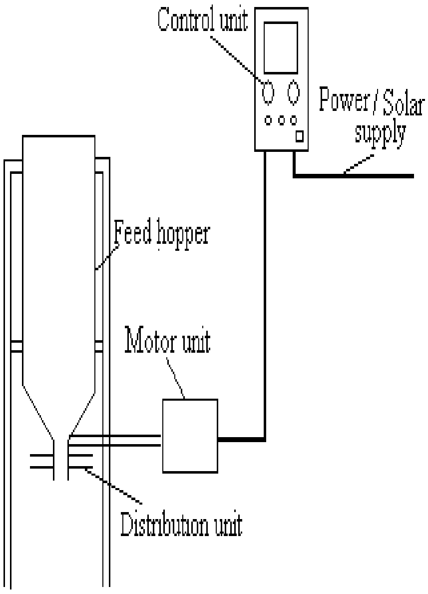

Figure 1 Components of Solar Powered Automatic Feeder

Shellfish farming in India

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Background

Crustaceans (shellfishes) are a large group of animals belonging to the sub phylum Crustacea within the phylum Arthropoda. They are free living aquatic animals except for a few forms which are terrestrial and parasitic. Amongst them commercially important shellfishes like shrimps, lobsters, crayfish and crabs are for human consumption and several tiny crustaceans like brine shrimp, copepods and cladocerans f orm valuable live food for commercial aquaculture. The global crustacean aquaculture production in 2014 was around 6.9 million tonnes (22.6 % of the total value) which is 9.3 % of the global aquaculture production excluding aquatic plants i.e. 73.8 million tonnes (FAO, 2016). The global farmed shrimp production for the year 2014 was a staggering 4.58 million tonnes which is 66.37 % of the crustacean aquaculture production and 65.1 % of the value realised from crustacean farming.

India forms the 2nd largest aquaculture producer globally with the production peaking at 4.88 million tonnes in 2014. The total volume of farmed crustaceans in India in 2014 was 3,85,739 tonnes of which farmed shrimps alone accounted for 3,77,059 tonnes (FAO,2016). Amongst the shrimps, Pacific white shrimp, *Penaeus vannamei* an exotic shrimp species constitutes the largest farmed species of shrimp in India, followed by the giant tiger shrimp, *Penaeus monodon* and Indian white shrimp, *Penaeus indicus*. Thus, shrimp farming in India is growing at a rate of close to 10% per annum over the last ten years. The progressive rise in crustacean aquaculture in India is laced with problems with respect to diseases, environmental, social issues and higher production costs which affect the industry significantly.

Current status of shrimp farming in India

In India about 1,30,948 hectares is under shrimp aquaculture with West Bengal having the maximum area under shrimp farming (53947 ha) followed by Andhra Pradesh, Kerala, Tamil Nadu, Gujarat and Maharashtra. The total farmed shrimp production in the country during the year 2014-15 was 4,34,558 tonnes from contributed by a major chunk of white leg shrimp *Penaeus vannamei* (3,53,413 tonnes) followed by tiger shrimp *Penaeus* monodon (73,155 tonnes) (MPEDA,2015). Thus the average productivity of shrimp farming in the country recorded was 3.67 t/ha/yr. The shift in culture practice from tiger to vannamei was as a result of rampant outbreaks of WSSV disease in 1994 followed by other disease outbreak in tiger shrimp. The dominance of vannamei resulted due to the availability of improved specific pathogen free seeds and higher growth performance and decline of tiger shrimp culture. However, the introduction of SPF vannamei drastically improved the total volume of frozen shrimp export from the country to 357505 tonnes amounting to ₹ 22468.12 crores (US$ 3709.76 million) during 2014-15. This accounts to around 67% of the total money earned from sea food export and 34% by volume of the total sea food export.

Tiger shrimp farming (*Penaeus monodon*)

India is well known for its traditional shrimp farming practices like the *bheries* in West Bengal and *Pokkali* fields in Kerala.

Subsequent to scientific farming introduction in late 80's and early 90's, by 2001-02 about 1,57,400 hectares with a production of around 1,02,940 tonnes of tiger shrimp culture was achieved. However, with introduction of SPF vannamei seed the production in 2009, the whole scenario of shrimp farming changed with upward export earnings. Yet, the major states which still produce tiger shrimp include West Bengal, Orissa, Kerala, Andhra Pradesh and Gujarat with West Bengal ranking first in terms of production and area under culture. The average productivity of tiger shrimp farming is around 1.49 t/ha/yr with Gujarat recording the maximum productivity of 2.47 t/ha/yr (Table I).

Table: I State wise area under culture and production of *P. monodon* during 2013-15. Source MPEDA, 2015

State	2013-14		2014-15		
	Farmed area (ha)	Production (t)	Farmed area (ha)	Production (t)	Productivity (t/ha/yr)
West Bengal	48,730	53,049	48848	53526.26	1.09
Orissa	5,523	11,075	5392	10075	1.86
Kerala	12,719	3,360	13230.15	3643.18	0.27
Andhra Pradesh	2,522	2,883	2190	2962	1.35
Gujarat	1,375	4,362	880.78	2183.82	2.47
Karnataka	94	56	688.65	498.73	0.72
Maharashtra	817	1,083	126.2	177.5	1.40
Tamil Nadu	343	916	37.84	73.3	1.93
Goa	53	14	6.8	16.1	2.36

Pacific white shrimp, *Penaeus vannamei* farming in India

The exotic shrimp *P. vannamei* was introduced into the country in the year 2009 and the availability of disease free SPF vannamei shrimp seeds replaced the traditional *P. monodon* farming leading to increased production. States like Andhra Pradesh, which is the major vannamei producing state, followed by Tamil Nadu and Gujarat in the country. During the year 2014-15, the total vannamei shrimp production in the country accounted for 3,53,413 tonnes from a total area under culture of around 50,240 hectares. The average productivity of vannamei farming in the country is 4.63 t/ha/yr (Table II).

Shrimp production systems in India

i) Traditional shrimp farming

The modern shrimp farming system in the country originated from the traditional shrimp farming practice like *Pokkali fields* and *Bheri*s. These traditional systems are practiced in the states like Kerala, West Bengal, and Karnataka. In the traditional system the tidal water is allowed to stand in the ponds in low lying coastal belts. Auto stocking occurs in these ponds along with the tidal influx and culture without any additional inputs (feeding and manuring). Harvesting of marketable size shrimps is usually done periodically using a trap during the spring tide. In the pokkali fields of Kerala the farming practice is done in two forms. In the perennial fields shrimp culture is done throughout the year using trap and culture method. In the seasonal ponds, rice cultivation is done during the monsoon period using the local salt tolerant variety of rice *pokkali* and in the summer season when salinity picks up shrimp farming is practiced. Production level in traditional ponds is generally low (up to 500Kg/ha/yr).

Table: II State wise production of *P. vannamei* and area under culture from the period 2013-15. Source MPEDA, 2015.

State	2013-14		2014-15		
	Farmed area (ha)	Production (t)	Farmed area (ha)	Production (t)	Productivity (t/ha/yr)
Andhra Pradesh	49764	210639	37560	276077	7.35
Tamil Nadu	5087	26281	5037.1	32687.87	6.48
Gujarat	707	6326	3545.4	26763	7.54
Orissa	485	2907	2340	11866	5.07
Maharashtra	908	3291	1274.51	4901.04	3.84
Karnataka	157	517	124.76	623.2	4.99
West Bengal	130	479	326	395	1.21
Goa	29	67	27.2	88.2	3.24
Kerala	Nil	Nil	5.8	11.75	2.02

ii) Zero water exchange shrimp farming

The incessant disease outbreak and SPF vannamei farming system in the country paved the way for refinement of the shrimp farming system in the country using many of best management practices principles. Amongst them is the zero water exchange system, both in saline and low saline farming, wherein the water exchange is near to zero in this system of farming. The occurrence of disease is lower compared to the traditional system. The pond water and soil quality is maintained with use of the microbial community- the probiotics periodically which maintains the favourable bacterial population in the pond. In zero water exchange farming system, the bio security is a necessity as compared to any other farming system.

Mud crab Aquaculture

In India, although mud crab culture has been practiced through ages in the traditional shrimp farming system, monoculture of mud crab started only after the live mud crab exports had started in early 1990s (Nasar & Noble 1995). Owing to its high market demand, mud crab has become a new species for culture during the last two decades. Further, it is also readily fitting in to the existing coastal aquaculture facility infrastructure already built for shrimp farming. The frequent crop failure in shrimp farming sector has also instilled the growth of mud crab farming. The advantages of mud crab aquaculture has been several fold; for example: individual animal can be sold for premium price as each animal grows up to 500 to 800 g. Mud crab can be exported as live product as it is able to survive without water for 14-18 h.

Although four species of mud crabs of genus Scylla (*S. serrata, S. olivacea, S. tranquebarica and S. paramamosain*) has been identified, *S. serrata* and *S. olivacea* have only been reported from India. About 3500 mt of mud crab has been exported from India annually in which wild catch dominates the export market (Elsemma *et al* 2015). Mud crabs are farmed in several ways. In low input production system, soft empty post molt mud crabs from the wild catches are reared in enclosures or cages until attaining marketable attributes. Sometimes it is wrongly called as fattening, although no improvement in the body weight after rearing. Owing to the financial gain realized during a short period, this method of farming was prevalent for some years. However, later years improvement in culture system was developed due to competition for limited resources and eventually led to the evolution of a farming system in which juvenile or undersized mud crabs (< 300 g BW) were used for stocking the ponds. Farmers purchase seed crabs and rear them extensively in earthen pond to commercial size on a diet of trash fish. Still there is no organized farming practice for mud crab in India.

Research and Development

The most important bottleneck in mud crab aquaculture has been the development of hatchery technology, although research groups in abroad and in India was able to mass rear mud crabs in commercially acceptable quantities in experimental hatcheries or near commercial hatcheries. The small fragile larvae with relatively long hatchery cycle, compared to shrimp hatchery production, has been problematic in sustaining the survival in mud crab hatchery. Any shrimp hatcheries can easily be transformed to mud crab hatchery; however, the only difference from the shrimp hatchery is the use of large quantities of Rotifer. The mud crab larvae requires more than 20 no/ml Rotifer, and therefore development of Rotifer at mass scale, sometimes hinders the production of larvae. However recent research findings suggest that use of Rotifer could be limited to the initial 5-7 days, and thereafter mud crab larvae are able to survive alone with Artemia nauplii. The very low larval survival was considered to be one of the constraints in the mud crab hatchery development. However, the high fecundity level of mud crab (3- 5 million zoea per female with about 500 g body weight) and low stocking density at the grow-out production system (0.5 no/m^2) indicate that one female is enough to stock 600 ha ponds, if all the zoea developed to crab instar. Therefore, low survival may not be a problem for commercial hatchery development. Nevertheless, the real problem is consistency in the successful hatchery production, there has been large variation among larval cycles (survival rate: 0-4%) and research effort should be in that direction. Scope for a backyard hatchery concept for mud crab is imperative due to the small scale nature of mud crab farming.

One of the problems experienced by the mud crab farmers during the initial phase of crab farming with hatchery produced seeds was fragile and sensitive nature of megalopa or first crab instar. In order to circumvent this issue, nursery rearing is essential in mud crab aquaculture. Central Institute of Brackishwater Aquaculture developed a nursery rearing technology for mud crabs, in which the seed crabs/megalopa reared at two phases, and within 75 days about 25 g BW crabs are produced. Further, in order to optimize the financial returns from mud crab farming, CIBA has developed a three phase/tire farming system: nursery, mid grow-out and final grow-out system. In this form of farming, farmers can take profit at any phase, and they need not wait for the long culture period. Polyculture is another viable option for mud crab farmers, a successful model for polyculture of mud crab along with finfishes have been developed. Within seven months of culture, 3500-4500 kg/ha productivity was realized with 50-60% production from mud crab. Certainly the present form of mud crab is at the transition phase between fishery based aquaculture and full-fledged aquaculture. However, the obvious potential of mud crab indicates its scope for further develop for the sustainability of brackishwater aquaculture.

Issues and challenges in shrimp farming

a. Higher cost of shrimp feed

High cost of shrimp feed has been a major concern for shrimp farmers around the country. Most commercial feed manufacturers levy high rates for shrimp feeds thus seriously affecting the profitability of the venture. As on today, most commercial formulations charge around Rs. 72 to 75/Kg of shrimp feed. The prices of shrimp feed would be higher at distant places like Haryana wherein the shrimp culture is growing steadfastly. So small and marginal shrimp farmers with small holding sizes or leased land are the worst affected due to the increased shrimp feed prices. Due to the shrimp feed which accounts to 60% of shrimp production cost, that amounts to about Rs. 220/kg.

b. Poor growth and stunting in vannamei

Stunted growth of vannamei has been a major issue presently in the Indian shrimp farming sector. Most farms in Andhra have reported poor growth of vannamei. It has been observed that several farms reported a growth of 10 to 12 grams even after 110 days of culture. The exact reasons of stunting is not known, although researchers and farmers opined towards the poor quality of seed and emerging diseases to be a major cause of the menace. Studies have shown that most hatcheries currently make use of pond reared broodstock which has the problem of inbreeding. Such inbred shrimp seed obviously will present a slower growth. Moreover, the greed to make more money has resulted in farmers skipping the basic pond preparation practices. Since, ponds are not dried between the crops and the soil not given sufficient time to release all accumulated organic matter, subsequent crops face production issues as a result of the reduced carrying capacity of the system.

c. Emerging diseases in shrimp farming

Diseases have been a major cause for setbacks in shrimp farming. Several industry sources are of the opinion that the Indian farmed shrimp production set to decline in 2016 as a result of stunted growth and emerging diseases. White shrimp farmers across the country have faced the issue of new and emerging diseases most of which does not have an identified etiological agent. Some of the most reported diseases in vannamei farms in India for which a definite etiological agent have so far not been identified are Running Mortality Syndrome (RMS), Covert Mortality Disease (CMD), White Muscle Syndrome (WMS), bacterial white spots, White Gut Disease (WGD) and muscle cramping. One of the emerging diseases namely White Faecal Syndrome (WFS) often associated with poor growth of vannamei has been identified to be caused by a microsporidian parasite called *Enterocytozoon hepatopenaei* (EHP). Additionally, this year there has been a greater incidence of white spot virus (WSSV) and IHHNV outbreaks in several coastal districts of A.P. thereby seriously affecting the production in these areas.

d. Poor quality of seed

Seed prices for vannamei in recent years have significantly dropped from 70 paisa to 90 paisa/PL in 2012-14 to 30 paisa/PL. There are even reports that certain hatcheries are willing to supply seed at much lower prices. The high demand for vannamei has resulted in establishment of a large number of hatcheries. This has resulted in several hatcheries turning to pond reared brood stock rather than the imported SPF brood stocks. Use of pond reared non SPF brood stock has resulted in inbreeding of the stocks and farmers currently receive non SPF seed with poor growth parameters. Larger farms have started to take seed from several hatcheries and rear them in separate ponds to identify the hatchery which is supplying the best quality seed. Again in this case, the marginal and small farmers are the worst affected class owing to their reduced buying power.

e. Poor cooperation among farmers

This has been a major cause of disease outbreaks in several areas. Certain farmers purposefully stock poor quality seed in high density to reduce production cost and finally end up having white spot infections. Such farms immediately practice distress harvesting by draining, thus resulting in disease spread in the whole area. The issues of common water intake and outlet have not been resolved in most farming areas.

f. Non adherence to regulations

The coastal aquaculture authority (CAA) established under the Coastal aquaculture authority act of 2005 has laid down clear cut regulations for shrimp farming in the country which involves several means of ensuring a sustainable development of the industry. The registration of all coastal shrimp farms which has been a major objective and regulation under the CAA still continue to be a challenge for the authority and the nation. Even today, several farms in coastal areas do not have certificates of registration under CAA and several registered farms does not abide by the regulations made mandatory by the authority. One of the major reason for the issue is the poor staff strength of the authority which mostly depends on state and district level committees for its functioning. The authority has also laid down regulations for hatchery operation and farming for vannamei which are not followed. One of the major regulation of CAA, namely the effluent treatment pond (ETP) still continue to be distant dream for several farms thus seriously affecting the growth and sustainability of the industry.

Future prospects

a. Species diversification in shrimp farming

Species diversification is an ideal intervention for enhancing growth and sustainability of the industry. Presently the industry is near fully dependent on vannamei for farming with a small number of farmers practicing tiger shrimp farming. Off late, farmers have been witnessing poor growth, stunting and increased incidence of white spot disease in the case of vannamei resulting in a major dilemma for the sector. SPF broodstock is presently available for tiger shrimp and farmers are willing to revert back to the species thus opening up a ray of hope to the sector. Thus, the main reasons for diversification of species is a necessity to resolve some of the compounding issues such as

- Countering some of the diseases that are rampant for now dominating in cultured species which may not be as potent in the new introduced species
- Highly dynamic international prices can be controlled with the shortage of dominating species at a time
- Improvement of domestic market with replacement of short duration small size culture able species

- Development of localized and native species culture for domestic and international market based on demand

CIBA has been promoting the farming of Indian white shrimp (*Penaeus indicus*) *Penaeus japonicus, Penaeus merguiensis, Metapenaeus kutchensis* through its field trials in several agro climatic zones for inter cropping with vannamei and the results have been favourable.

Kuruma shrimp (*Marsupenaeus japonicus*)

Cultured in Taiwan and Australia using quality seed produced through selective breeding and sold to Japan as live shrimps for US$100 per pound ($220/kg).

Western blue shrimp (*P. stylirostris*)

Once a popular choice for shrimp farming in the western hemisphere, until the IHHN virus wiped out nearly the whole population in the late 1980s. Some of the stocks that survived have become resistant against this virus and some to against the Taura virus.

Chinese white shrimp (*P. chinensis*, also known as the *fleshy prawn*)

Occurs along the coast of China and the western coast of Korea and is being farmed in China which tolerates colder water (min. 16 °C). Once a major species in the world market now for domestic market.

Indian white shrimp (*P. indicus*)

A native of the Indian Ocean and along the African shores. Though very susceptible to diseases but grows faster with lower minimal protein requirement. Domestically highly preferred culture shrimps in West Bengal and Kerala. fast-growing and have a quick turnover rate in ponds — sizes of 10-20 g at high density and 20-30 g at low density can be attained after three months with harvests of 300-400 kg/ha/crop. Can tolerate higher pond salinities making an ideal alternate crop to *P. monodon* during dry months.

Banana shrimp (*P. merguiensis*)

Almost similar to *P. indicus* occurring along the Indian Ocean, Oman to Indonesia and Australia. It can be grown at high densities and resistant to lower temperatures up to 15^{O}C with quick turnover rate in ponds of sizes of 10-20 g at high density and 20-30 g at low density can be attained after three months with harvests of 300-400 kg/ha/crop. Moreover, an ideal alternate crop to *P. monodon* during dry months.

Ginger shrimp (*Metapenaeus kutchensis)*

Greasy back shrimp with a short growing period of 2-3 months in ponds, attaining market sizes of 10-15 g. Larval rearing is easier compared to *P. monodon*, *P. indicus and P. merguiensis and adults are m*ore resistant to handling

b. Organic shrimp farming

Shrimp farming in parts of Kerala and West Bengal continue to be a traditional, improved traditional and extensive farming activity with minimum levels of input. Such farms shall be identified and brought under organic shrimp aquaculture on making a minimal level of intervention. This would increase the profitability of such low scale operations. The certification of the farms also requires the availability of organic seed and feed which can be developed shortly.

c. Bio-floc: Intensive nursery and grow-out shrimp farming

Biofloc technology is a major scientific innovation for intensive aquaculture for high productivity with a sustainable approach. Bio-floc is the assemblage of beneficial microorganisms such as heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus. As it contains predominantly heterotrophic bacterial community over autotrophic and denitrifying bacteria, this can be controlled by maintaining high carbon to nitrogen (C:N) ratio. Biofloc in combination with periphyton (BPT) increases the natural production and in turn productivity. The concept of the retention of waste and its conversion of biofloc as natural food (production of microbial biomass) within the culture system is marked with lower/minimal water exchange, high density culture, reduction of feed and avoidance of disease outbreak (such as RMS, EMS, EHP) especially for the nursery phase of shrimp culture and so on. By sheer managing the optimum C:N ratio (C:N ratio 12-15:1) in an aquaculture system by adding different locally available cheap carbon sources and/or reducing protein percentage in feed. Under optimum C:N ratio, inorganic nitrogen is immobilized into bacterial cell while organic substrates are metabolized.

Advantages of bio-floc based aquaculture technology

- Biosecurity of the system can be maintained with Zero/minimal water exchange system.
- Heterotrophic bacteria can reduce toxic metabolites (NH_3, NO_2)
- Easier management and environmental friendly approach (reduced protein requirement, fish meal usage and water/nutrient discharge), diurnal changes (pH, O_2, CO_2) in pond water is reduced
- Doubling the protein utilization as the shrimp use proteins twice - eat feed and then harvest flocs. Enhance digestion (with enzymes and growth promoters)
- Probiotic action - more diverse aerobic gut flora reducing pathogenic bacteria (*Vibrios*).
- Role in immune response by stimulating humoral and cellular immunity
- Reduced costs (15-20% lower cost of production) including 30-50% cost savings in feed
- Augmentation of natural food and improvement of FCR
- Reduced sensitivity to light fluctuation
- Major advantage growing shrimp in biofloc will not require of multiple external filtration. It reduces the start-up operational expenses
- The high protein-lipid rich nutrients in bio-floc, including fatty acids protects against oxidation, vitamins, phospholipids and highly diverse "native protein", could be utilized continuously and thereby help in building reserve energy, broodstocks gonads formation and superior reproductive performance.
- Bio-flocs can act as a natural probiotic which could act internally and/or externally against, *Vibrio* sp. and ectoparasites.

d. SPF and SPR Broodstock:

These specific pathogen free (SPF), Specific pathogen resistant (SPR) or high health stock is one of the best way to recruit the stock in hatchery. Hatcheries normally prefer wild broodstocks due to the belief that they produce more and healthier nauplii. In recent years, however, the high risk of introducing viral pathogens with wild broodstock has changed this concept and domesticated stocks either genetically improved or suspected to be resistant or tolerant to specific pathogens are used as broodstock. SPF stocks are generally maintained in highly biosecure facilities and their offspring (designated "high health" rather than SPF) are supplied to the industry. SPR shrimp are those that are not susceptible to infection by one or several specific pathogens, and Specific Pathogen Tolerant (SPT) shrimp are those that are intentionally bred to develop resistance to the disease caused by one or several specific pathogens like

1. Taura syndrome virus (TSV) 2. Yellowhead virus (YHV) 3 .Hepatopancreatic parvo-like virus (HPV) 4. *Baculovirus penaei* (BP) 5. Infectious myonecrosis virus (IMNV) 6. Necrotising hepatopancreatitis bacterium α-Proteobacterium (NHPB) 7. Necrotising hepatopancreatitis (NHP).

e. Biosecurity principle and protocols

The role of biosecurity measures is to diminish the risks arising from the entry, establishment or spread of pathogens within the system to a manageable level. Biosecurity protocols are advocated to be followed in all coastal aquaculture units to minimize the disease risk and become very important when viral /bacterial threat is imminent and moreover when specific pathogen free or specific pathogen resistant stocks are used (as in the case of *L. vannamei*).

The basic elements of a biosecurity programme in a shrimp hatchery include the physical, chemical and biological methods necessary to protect the hatchery from the consequences of all diseases that represent a high risk. Various levels and strategies for biosecurity may be employed depending on the hatchery facility, the diseases of concern and the level of perceived risk. There are two common ways by which disease transmission occurs:

- **Vertical transmission** - from mother shrimp to the post larvae in hatchery systems
- **Horizontal transmission** - from one affected shrimp to the other in farming systems.

The physical separation or isolation of the different production facilities is a feature of good hatchery design and should be incorporated into the construction of new hatcheries. In existing hatcheries with no physical separation, effective isolation may also be achieved through the construction of barriers and implementation of process and product flow controls. Water distribution and discharge from the hatchery should also be compatible to all biosecurity protocols. Seawater disinfection,vehicle tyre bath, footbaths and hands to be disinfected, waste discharge water management and infected animals disposal are some of measures should be strictly followed.

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An overview of diseases of farmed shrimp in India

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A. Introduction

Aquaculture continues to be the fastest growing food production sector with an annual average growth rate exceeding 6 % (FAO, 2014) and having enough potential to meet the growing demands for aquatic food. Aquaculture besides being the source of valuable income and high quality protein rich food also provides employment to both skilled and unskilled workers. Shrimp continues to be the largest single seafood commodity by value, accounting for 15% of all internationally traded fishery products. Farm raised shrimp is comprised of 55% of global shrimp production and this is entirely dominated by two species – the black tiger shrimp (*Penaeus monodon*) and the white Pacific white shrimp (*Penaeus vannamei*) (FAO 2014). In India, shrimp aquaculture started as a traditional practice in natural water bodies such as bheries or pokkali fields and subsequently transformed to commercial industry during 90's. Initially it was dominated by a single species, *Penaeus monodon* and the production of which reached a maximum of 1.44 lakh tones in 2006-07 (www.mpeda.com). Pacific white shrimp *P. vannamei* was introduced into the brackishwater aquaculture system of India in 2009. Because of its specific pathogen free (SPF) status, fast growth rate and culture feasibility in wide salinity range, this species was readily accepted by the farmers and subsequently has become the dominant cultured species in India.

The increasing trend in intensification and commercialization has exacerbated the epidemics of diseases and this has become a major constraint for the sustainability of this industry. Severe disease related mortality and associated economic loss due to different viral agents such as monodon baculovirus (MBV) in Taiwan, infectious hypodermal and hematopoietic necrosis virus (IHHNV) in the Americas, yellow head virus (YHV) in Thailand and Taura syndrome virus (TSV) in the Americas during the different periods have been reported. In addition to all these, the major disease outbreak due to White Spot Syndrome Virus (WSSV) has also been reported from all parts of the world. In India, it is estimated that there is an annual loss of about Rs. 300 crores due to WSD in shrimps. This section is aimed at highlighting the most significant and emerging diseases in Indian shrimp aquaculture during recent years.

B. **OIE listed diseases**

The World Organisation for Animal Health (OIE), an intergovernmental organisation established to promote world animal health with 180 Member Countries, sets out standards for the improvement of aquatic animal health and welfare of farmed fish worldwide, and for safe international trade in aquatic animals (amphibians, crustaceans, fish and molluscs) and their products. The OIE lists diseases meeting the relevant criteria such as those causing significant production losses at a national or multinational level, with proven infectious aetiology, having repeatable and robust means of detection/diagnosis etc. In 2016, nine diseases of crustaceans have been listed by the OIE. Important bacterial and viral diseases are explained in the succeeding sections, along with diseases currently plaguing aquaculture in India.

I. Bacterial diseases

1. Acute hepatopancreatic necrosis disease (AHPND)

Since 2010, early mortality syndrome (EMS), recently named as acute hepatopancreatic necrosis disease (AHPND) has caused severe economic impact in South East Asian countries. However, AHPND has not been recorded in India so far. The disease is characterised by unusually high mortality within the first 30-35 days of shrimp grow-out culture, due to a variety of pond management and pathogen related factors. Some of the farm level clinical signs include: onset of clinical signs and mortality starting as early as 10 days post stocking, moribund shrimp sink to pond bottom, shrimp often have soft shells, partially full to empty gut, hepatopancreas (HP) often appears pale to whitish due to loss of pigment, significantly emaciated HP (shrunken, small, swollen or discoloured), and HP does not squash easily between thumb and finger. The disease and the typical pathology was caused by a bacterial pathogen belonging to Vibrio group and identified the species to a special strain of *Vibrio parahaemolyticus* (VP_{AHPND}).

AHPND could be prevented by ensuring good farm biosecurity and best management practices (BMPs) beginning with good pond preparation, modifications to farm and pond designs to allow better biosecurity (e.g. use of smaller-sized ponds with plastic liners that can be fully drained, dried and disinfected between culture cycles) ensuring that all facilities and equipment are properly disinfected before stocking of PL (e.g. implementing cyclical dry-out and clean-up routines after every production cycle, involving careful cleaning and disinfection of all facilities, including the insides of air lines, pipes, water pumps and air pumps), using an increased number of reservoirs and water filtration to eliminate fish and other disease carriers, avoiding heavy chlorination of pre-treatment of water, avoiding traditional fertilization schedules with commonly used products, use of PL derived from broodstock verified to be free of AHPND (i.e. PL derived from SPF or high health (HH) broodstock), avoiding stocking ponds during the high-temperature season, stocking larger-size PL, co-culture of shrimp with finfish (e.g. tilapia) or use water from tilapia pond, avoiding overfeeding, applying “designer” pre- or probiotic preparations, applying “designer” phages that specifically target the VP_{AHPND}.

2. **Necrotizing Hepatopancreatitis (NHP)**

This disease is also known as Texas necrotizing hepatopancreatitis (TNHP), Texas pond mortality syndrome (TPMS), Peru necrotizing hepatopancreatitis (PNHP). NHP has been reported as an important disease since its first diagnosis in 1985. It has been reported to cause mass mortalities to the tune of 20-90 percent of *P. vannamei* in highly saline commercial grow-out ponds nearly every year since then. By 1993, NHP spread to Ecuador, Guatemala, Honduras, Mexico, and Peru and by 1995, coincided with warm waters with high salinity associated with El Nino, and caused severe mortalities (60-80 percent mortality) of *P. vannamei* and *P. stylirostris* throughout Ecuador. NHP has not yet been reported in Asia, but could cause significant damage were it to be transferred here with untested shrimp introduction.

Necrotizing hepatopancreatitis is caused by obligate intracellular Rickettsia-like bacterium, a member of the order α-Proteobacteria (Gram-negative, pleomorphic, rod-shaped or helical-shaped bacterium). Affected shrimp are lethargic, anorexic with empty gut and show epibiotc fouling. Exoskeleton becomes soft and show abdominal muscle atrophy. Affected ponds have increased FCR and growth of affected shrimp is retarded. The hepatopancreas becomes watery with white or black streaks. Mortality rates reach up to 90% within 30 days of the appearance of clinical signs. NHP can be diagnosed by microscopic demonstration of lipid droplets and melanisation of hepatopancreas in wet mount of preparations. It may be confirmed by histopathological examination showing atrophy and the presence of granulomata in the hepatopancreas, and haemocyte aggregations around the hepatopancreatic tubules. Intracytoplasmic Rickettsia-like bacteria may be prominently seen in the cytoplasm. Molecular diagnostic tools such as *in situ* hybridization, dot blot hybridisation, and PCR for specific α-Proteobacterial DNA are also available. NHP could be transmitted horizontally. Strict adoption of BMPs, use of SPF broodstock and stocking NHP free seed in the farms is the best way to prevent NHP. Adhering to strict biosecurity protocols and practicing BMPs will be useful in preventing NHP.

II. Viral diseases

More than 20 viruses are described so far, that are reported to infect farmed penaeid shrimp around the world. Among these, the white spot disease (WSD), infectious hypodermal and haematopoietic necrosis (IHHN), infectious myonecrosis (IMN), Taura syndrome (TS) and yellow head disease (YHD) have inflicted significant economic losses to the aquaculture sector worldwide. IMN, TS and YHD have not been reported from India yet.

1. White spot disease (WSD)

It is an acute, highly contagious disease of shrimp, caused by a large, enveloped double stranded DNA virus. It infects all life stages of decapod crustaceans of marine and brackishwater sources. WSD has been identified from crustaceans in China, Japan, Korea, South-East Asia, South Asia, India, Mediterranean, Middle East and Americas.

Clinically, the disease is characterized by lethargy, reddish to pinkish discoloration of the body, loose cuticle, swelling of branchiostegites, broken antennae, damaged appendages, and the most conspicuous feature of small to large white spots on the inner side of the carapace which spread all over the body in advanced infection. Cumulative mortalities in infected populations may reach 100 per cent within 3 to 7 days of the onset of clinical signs.

WSD is transmitted both vertically and horizontally. So far no control measures are known.

2. Infectious hypodermal and haematopoietic necrosis (IHHN)

IHHN is caused by infectious hypodermal and haematopoietic necrosis virus (IHHNV), a single stranded DNA virus. It is the smallest of the known penaeid shrimp viruses but believed to be the most stable virus of the known penaeid shrimp viruses. Most penaeid species can be infected with IHHNV in all life stages, including the principal cultured species, *P. monodon*, *P. vannamei*, and *P. stylirostris*.

Three distinct genotypes of IHHNV such as Type 1 from the Americas and East Asia (principally the Philippines); Type 2 from South-East Asia; Type 3A from East Africa, India and Australia; and Type 3B from the western Indo-Pacific region including Madagascar, Mauritius and Tanzania are identified so far. The first two genotypes are infectious to the representative penaeids, *P. vannamei* and *P. monodon*, while the latter two genetic variants are not infectious.

In *P. vannamei* it causes the chronic disease called runt deformity syndrome (RDS) characterized by lower overall crop production, shrimp with increased size variability and cuticular deformity. In juvenile *P. stylirostris,* more than 90 per cent mortality reported within several weeks of onset of infection. Gross signs of infection include white to buff mottling of the cuticle, opacity of striated muscle and melanised foci within the hypodermis. In the later stages of infection *P. stylirostris* and *P. monodon* may appear bluish in color. Infected *P. vannamei* display deformed rostrums, cuticle and antennal flagella.

3. Infectious myonecrosis (IMN)

Infectious myonecrosis (IMN) is a recently identified viral disease caused by double stranded RNA infectious myonecrosis virus (IMNV). It causes mortalities in juvenile and sub adult pond-reared stocks of *P. vannamei* and the mortality range from 40 to 70 per cent. Outbreaks of the disease seems to be associated with certain types of environment and physical stresses (i.e. extremes in salinity and temperature, collection by cast net, etc.), and possibly with the use of low quality feeds. This disease has been reported in north-eastern Brazil, Java Island, Sumatra, Bangkok, west Borneo, south

Sulawesi, Bali, Lombok and Sumbawa in South-East Asia.

Mortalities from IMN can range from 40 to 70 per cent in cultivated *P. vannamei*, and feed conversion ratios (FCR) of affected populations can increase from a normal value of ~ 1.5 up to 4.0 or higher. IMN affected shrimp presents focal to extensive white necrotic areas in the striated (skeletal) muscle, especially of the distal abdominal segments and tail fan, which can become necrotic and reddened in some affected shrimp. It is transmitted horizontally by cannibalism and via water. Vertical transmission from broodstock is suspected from anecdotal evidence but it is not known whether this occurs via transovarial mechanism or by surface contamination of newly spawned eggs.

4. Yellow head disease (YHD)

It is caused by single stranded RNA virus. YHV genotype 1 the pathogenic type and is one of six known genotypes in the yellow head complex of viruses. YHD was first noted by Limsuwan in cultured *P. monodon* adults in central Thailand during 1991. Later, it is observed to affect *P. monodon*, *P. vannamei*, *P. stylirostris*, *P. japonicus*, *P. merguiensis*, *P. setiferus*, *M. ensis*, *P. styliferus*, *Palaemonetes pugio* and *Metapenaeus affinis*.YHV1 is reported from Chinese Taipei, Indonesia, Malaysia, Philippines, Sri Lanka, Thailand, Vietnam and Mexico.

Clinical signs of YHD occur in *P. monodon* within 7–10 days of exposure and 100 per cent mortality within 3-5 days of the first appearance of clinical signs. Shrimps with YHD display yellow colouration of the dorsal cephalothorax caused by the underlying yellow HP showing through the translucent carapace. Within the ponds, infected animals, usually between 5 and 15 g, begin consuming feed at an abnormally high rate for several days then cease feeding entirely. One day after cessation of feeding, moribund shrimps may be seen swimming slowly near the edges of the pond. By the third day, mass mortality occurs and the entire crop is typically lost. Contaminated water, cannibalism of weak or moribund shrimp, animate vector, net and other equipments transmit the disease. Vertical transmission has not been reported.

5. Taura syndrome (TS) / Red tailed disease

It is caused by a nonenveloped, linear, single stranded RNA virus called Taura syndrome virus (TSV). The principal host species for TSV are *P. vannamei* and *P. stylirostris* but it can affect other penaeid species also. TS is widely distributed in the shrimp-farming regions of the Americas, South-East Asia and the Middle East.

During the acute phase of infection, shrimps appear pale red while their tail fans become bright red. They are soft shelled, lethargic and anorexic. Those with severe infections die during moulting and cumulative mortalities may reach 80-95 per cent. Recovery in chronically infected shrimps generally display multifocal, melanised cuticular lesions and may also have soft cuticles and red body coloration with normal feeding. Both horizontal and vertical transmission is reported.

C. Diseases in Grow-out systems

A number of other diseases of unidentified etiologies are constantly associated with shrimp culture practice during the past 5-6 years, and have been responsible either for direct mortality or growth reduction and thereby bringing loss to farmers. Shrimp hatcheries are also prone to losses either due to disease outbreak or other unknown factors. Some of the disease syndromes associated with current shrimp culture practice in India are discussed below.

1. Hepatopancreatic microsporidiosis

Hepatopancreatic microsporidiosis (HPM) is caused by *Enterocytozoon hepatopenaei* (EHP). It was first reported as an unnamed microsporidian from growth retarded giant or black tiger shrimp *P. monodon* from Thailand in 2004. It also has much smaller spores (approximately 1 µm in length) and is currently known to infect both *P. monodon* and *P. vannamei*. It has been found that EHP can be transmitted directly from shrimp to shrimp by cannibalism and cohabitation. EHP is confined to tubule epithelial cells of the shrimp HP and shows no gross signs of disease except retarded growth. It is likely that other penaeid shrimp and/or other crustaceans or even other marine or brackish water species in the region may also be susceptible to infection. For example, some samples of polychaetes and mollusks have tested positive by PCR, but it is still not known whether they are infected or passive carriers. *Artemia* may also be a mechanical carrier. Although EHP does not appear to cause mortality in *P. monodon* and *P. vannamei*, information from shrimp farmers indicates that it is associated with severe growth retardation in *P. vannamei*. Thus, farmers and hatchery operators monitor for EHP in broodstock, PL and rearing ponds. The best approach for maturation and hatchery facilities to avoid EHP is not to use wild, captured, live animals (e.g., live polychaetes, clams, oysters, etc.) as feeds for broodstock. Better would be pasteurization (heating at 70oC for 10 minutes). Another alternative would be to use gamma irradiation with frozen feeds. Alternatively, polychaetes could be selected and tested for freedom from shrimp pathogens and then reared as broodstock feed in biosecure settings designed to maintain their freedom from shrimp pathogens.

2. Size variation/ Growth retardation

Size variation / growth retardation in *P. vannamei* grow-out farms is another major concern during recent years. It is reported that some viruses, viz., infectious hypodermal and haematopoietic necrosis virus (IHHNV), lymphoid organ vacuolization virus (LOVV), monodon baculovirus (MBV), hepatopancreatic parvovirus (HPV) and Laem-Singh virus (LSNV) are associated with slow growth and size variation in shrimp. Feed conversion ratios (FCR) ranged from 1.7 to 2.5 when compared to 1.5 or less for normal ponds. Recently *Enterocytozoan hepatopenaei* (EHP) has been found to be associated with size variation / growth retardation.

3. White faeces syndrome (WFS)

White faeces syndrome reported since last decade, has recently been noted as serious problem for *P. vannamei* throughout the world. However, this disease has been reported from both cultured black tiger shrimp and pacific white shrimp. WFS usually occurs after 60 days of culture (DOC) and it may be accompanied by high shrimp mortality. Ponds affected with WFS show white faecal strings floating on the pond surface while the shrimps show white/golden brown intestine, reduced feed consumption, growth retardation and often associated with loose shell. The disease can cause moderate to severe economic loss by reducing the shrimp survival by 20–30 percent when compared to normal ponds. While investigating the aetiology of WFS this disease has been associated with presence of vermiform like gregarine bodies, vibriosis, *Enterocytozoan hepatopenaei*, blue green algae and loose shell syndrome. *Vibrio* spp. are significantly higher in haemolymph and intestine. The newly described microsporidian *E. hepatopenaei* and it is not causally associated with WFS. It has been estimated that the Thai production losses due to WFS in 2010 were 10–15%. The cause of white faeces syndrome and treatment is uncertain. However reduced stocking density, proper water exchange together with better management practices will be helpful in evading WFS.

4. Running Mortality Syndrome (RMS)

Since 2011, a new syndrome loosely termed as Running Mortality Syndrome (RMS) by the farming community has been affecting shrimp farms. The affected ponds suffer low level mortality on a daily basis, after a month or 40 days of culture (DOC). Mortality rate may be low (e.g. <1%/day), but the cumulative loss over phase will be high, often reaching 70%. A portion of shrimp continue to survive and can grow to fully harvestable size. Investigations conducted in ICAR-CIBA revealed no association of RMS with known shrimp pathogens. Bioassay experiments did not elicit any disease in the experimental shrimp. RMS affected shrimp showed recovery and appeared healthy and active after 6-7 days of transferring to wet lab in water with optimal parameters. The causative agent of RMS is unknown.

5. White muscle syndrome

In recent years, shrimp farms have been experiencing white muscle with muscle necrosis in the *P. vannamei* grow-out cultures associated with low mortalities. The white muscle syndrome (WMS) affected shrimps show focal to extensive necrotic areas in striated muscle tissues, displaying a white, opaque appearance. White muscle in shrimp can also be caused by the advanced infection of microsporidians belonging to the genera *Ameson* and *Agmasoma*, or dietary deficiency of selenium. The cause of WMS is not understood. BMPs will be helpful in prevention of this disease.

D. Non-infectious diseases in shrimp aquaculture

Nan-infectious disease are common in the grow-out farms, as influences of nutritional factors, environmental factors such as temperature extremes and oxygen depletion, toxicity from biotic and abiotic origins, become critical during the lengthy culture period.

1. Soft shell syndrome

Soft shell syndrome is a condition in which shrimp exoskeleton becomes soft.
Cuticle of the affected shrimps is persistently soft, loose and papery for several weeks. Affected shrimps are weak and show poor escape reflex, and these animals are susceptible to cannibalism. Severely affected P. *indicus* often show undulating gut in the first three abdominal segments. Several factors are implicated as causative agent for this condition as: sudden fluctuation in water salinity, high soil pH, highly reducing conditions in soil, low organic matter in soil, low phosphate content and pesticide pollution in water, nutritional deficiency and insufficient water exchange. The disease may be prevented or controlled through environmental and dietary manipulations by providing favourable water and soil conditions in the pond and feeding adequately with balanced diets.

2. Black gill disease

A number of abiotic and biotic reasons have been attributed to the black gill in shrimps. Presence of excessive levels of toxic substances such as nitrite, ammonia, heavy metals, crude oils etc. in the culture water may lead to black gill disease. High organic load, heavy siltation and reducing conditions in rearing pond can also cause this disease in shrimps. Attack of certain bacterial, fungal and protozoan pathogens can also cause black gill condition in shrimp. Affected shrimps have gills with black to brown discoloration, in acute cases necrosis and atrophy of the gill lamellae may be apparent. The blackening is due to the deposition of melanin at sites of massive haemocyte accumulation, followed by dysfunction and destruction of whole gill processes.

Treatment of the black gill disease depends upon the cause of the disease.

Preventive or corrective measure may be adopted to avoid or reduce the biotic abiotic factors in the rearing pond to control the disease condition.

3. Red disease

The juveniles and adult shrimps broodstock affected with red disease have reddish discoloration in body, pleopods and gills. Definite causative agent is not known. One of the reasons believed to be the cause of disease is a microbial toxin in rancid or spoiled diets or in detritus of ponds rich in organic matter. Extreme conditions of pH or salinity in pond water may also cause the disease. Healthy management of ponds along with the use of good quality feed may help in the avoidance of red disease.

4. Cramped tail disease

Affected shrimps have rigid dorsal flexure of the abdomen, which cannot be straightened. These shrimps lie on their sides at the bottom of the pond and are susceptible to cannibalism. Exact cause for this disease is not known, but environmental and nutritional causes have been suggested. Maintenance of healthy conditions in the pond with proper feeding with balanced diet may be helpful in the prevention I control of this disease.

5. Gas-bubble disease

Super saturation of atmospheric gases and oxygen in the pond can result in the gas-bubble disease, which affects the shrimps of all sizes. Presence of gas bubble in the gills or under the cuticle is the characteristic of this disease. Gas bubble disease due to oxygen is not lethal, while that of nitrogen can be lethal. The threshold saturation level to cause the gas-bubble, in the case of nitrogen is 118 % while that of oxygen is 250 % of normal saturation. The severely affected or dead shrimp due to this disease may float near the water surface. Super saturation of the gases must be avoided to prevent the disease.

E. Conclusion

The increased intensification and commercialization of shrimp aquaculture has resulted in increase in the emergence of new diseases. The emergence and spread of infectious disease is usually the result of a series of linked events involving the interactions between the host (including the physiological, reproductive and developmental stage conditions), the environment and the presence of pathogens. Among the infectious diseases listed by the OIE, only WSD and IHHNV are prevalent in India, and none of the others have been reported so far. However, new syndromes of unknown aetiology have been on the rise in brackishwater aquaculture and require attention by investigators considering morbidity and mortality associated with such syndromes. With advancements in penaeid shrimp nutrition and feed technology, nutrition-related diseases are not a serious issue in present day aquaculture. Focusing efforts on producing high quality seed, better pond manage to reduce stress and risk of infection, following routine farm biosecurity, responsible trade practices, response to disease outbreak, and improved better management practices shall aid in preventing the epidemics of diseases. Further health management is a shared responsibility, and each stakeholder's contribution is essential to the health management process.

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