



## AQUACULTURE FEED

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First Printed 1993  
First Reprinted 1995  
Second Reprinted 1997

Price Rs. 30/-

Printed at Cherry's Printers, Kochi - 17, Phone : 345681

## PREFACE

The Marine Products Export Development Authority has been promoting the commercial aquaculture of shrimp as a means for boosting India's seafood exports. At the same time, it is recognised that in order to have a sustained share in the world seafood market, aquaculture of other species, which are in great demand should also be encouraged. It was in this context that a publication titled AQUACULTURE FEED was brought out at the time of INDAQUA '93, India's first aquaculture exposition. The publication has been well received and we are now coming out with a reprint of this publication. We are confident that this publication will continue to generate interest in those who intend to take up aquaculture of these species to exploit the abundant natural resources available in this country for augmenting export production.

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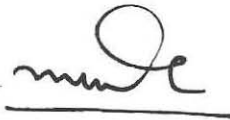
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## FOREWORD

Feed is one of the most essential inputs in aquaculture. The recent development in technology of manufacture of good quality feed has opened up a new dimension in fish production. Therefore, it was decided by the Organising Committee of "INDAQUA" - the First Aquaculture Show in India to bring out a handbook on Aquaculture Feed. The sincere effort taken by Dr. M. Paulraj, CMFRI to bring out this handbook is well appreciated. Dr. G. Santhana Krishnan, Shri D. Vincent and Shri M. Shaji have coordinated this publication. I am sure that this handbook will be very useful to all fish/prawn farmers in India.



(M SAKTHIVEL)  
CHAIRMAN  
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COCHIN - 36  
February 93

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## **1. INTRODUCTION**

Successful and sustainable aquaculture of finfish and crustaceans (prawns, lobsters and crabs), depend upon the provision of nutritionally adequate, environmental - friendly and economically viable artificial feeds. Feed is the major operational input, and feed costs normally range from 30 to 60 per cent of the operational expenditure in finfish and prawn culture systems. In view of this, artificial feeds should scientifically formulated, optimally processed, and judiciously supplied, considering the specific nutritional needs of the cultivated species and the intensity of culture operations.

## **2. IMPORTANCE OF FEED**

In extensive and semi-extensive systems, natural food contributes substantially to the nutrition of the cultured animals. Thus in these systems, the exogenous food supply needs to provide only nutrients which may be deficient in the natural food. Natural food production can also be increased through systematic and judicious application of organic and inorganic fertilisers. The semi intensive carp culture and extensive prawn culture, widely practised by farmers in India are examples. Fish or prawn production from such systems depends on the natural biogenic potential on the quantity and quality of natural food produced in the pond and artificial feed supplied, and on the physico-chemical characteristics of the water and soil. In general,

production rates exceeding 1 tonne/ha/yr are rarely achieved without artificial feeding in prawn ponds; whereas, in fish culture systems substantially higher levels of production could be achieved through judicious application of fertilizers.

In the intensive systems of fish and prawn culture, where very high stocking densities are maintained, such as, in cage culture, race-way culture, recirculatory systems and in pond culture systems, the cultured animal has to rely almost exclusively on the artificial feeds fed to them, benefiting very little from natural food, because it is insufficient to sustain high densities.

Thus at high densities, inadequate feeding leads to poor growth and nutritional diseases; and due to poor condition, increased susceptibility to infection. Artificial feeds fed to fish and prawns grown in such systems, need to be designed and manufactured in order to provide all the nutrients in adequate levels and balanced proportions to promote optimum growth.

### **3. FACTORS AFFECTING FEED DESIGN, PRODUCTION AND FEEDING**

The diversity in food and feeding habits of aquatic species - herbivores, detritivores, omnivores and carnivores, calls for careful diet design, manufacture and supply to suit the individual species during the different phases in growth.

Herbivores - grass carp, silver carp, milk fish, many tilapias

Omnivores - common carp, rohu, mrigal, mullets, channel catfish, maghur, most species of prawns

Carnivores - trout, salmon, murrels, groupers, sea bass, sea breams, eels, kuruma prawn

Some of the factors which often complicate the feed design production and feeding are :

- \* Cultured species and their genetic origin

- \* Size and stage in lifecycle-larvae, fry, fingerlings, juveniles, sub-adults, adults, brood-stock etc.
- \* Changes in feeding habit with growth
- \* Reproductive state
- \* Health status
- \* System of culture - extensive, semi-intensive, intensive, pond, cage, race-way, recirculating system etc.
- \* Feeding zones - surface feeder (e.g. silver carp, catla), column feeder (rohu), bottom feeder (common carp, prawns)
- \* Stocking density
- \* Environmental conditions
- \* Form of feed and particle size

Effective feed formulations are those which contain adequate levels and balance proportions of essential nutrients and additives, optimally processed and produced in a form easily acceptable and palatable to the species under culture. A nutrient balanced feed would be better ingested, digested and metabolised more efficiently for tissue synthesis resulting in minimal digestive and metabolic waste discharge into the environment. On the other hand, a poor quality feed may cause severe water quality problems by build up of toxic ammonia, nitrite, hydrogen sulfide, increased BOD (biochemical oxygen demand) and COD (chemical oxygen demand), thereby inducing stress in fish and prawns and predisposing them to pathogenic infections. Leaching of nutrients couples with digestive and metabolic wastes, invariably lead to plankton blooms, which could substantially deplete dissolved oxygen levels especially at night and early morning hours.

Since feed is often being blamed for inconsistent growth and poor production from culture systems, it is necessary to critically examine the factors affecting the performance of feed in relation to culture. The factors that affect the performance of

artificial feed in a culture system can be broadly grouped into four major categories: (i) feed dependent factors (ii) animal dependent factors, (iii) feeding management dependent factors and (iv) environment dependent factors.

The feed dependent factors are the most important among the factors affecting the efficiency of a feed and its cost. A proper diagnosis of these factors is of utmost importance in developing quality controlled least-cost diets.

#### **4. BALANCE OF NUTRIENTS**

Nutrients in feeds are broadly grouped into (i) energy yielding nutrients and (ii) non-energy nutrients. Proteins, lipids and carbohydrates are the nutrients that furnish the required energy for maintenance, activity, moulting, growth and reproduction. The non-energy nutrients are the vitamins and minerals, which have diverse physiological and biochemical functions and are extremely important in deciding the efficiency of a diet. Essentially, an ideal feed should have adequate levels of bio-available energy, a blend of essential amino acids and fatty acids, and adequate levels of vitamins and minerals. Besides, all these nutrients must be found in balanced proportions in the diets offered to the cultured animal for its effective utilization.

##### **4.1 Energy**

As in all other animal species, the primary function of food in fish and crustaceans is to satisfy energy requirements for maintenance, growth and reproduction. Two major advantages in the energy utilization of fish and crustaceans are that (i) they do not expend energy to maintain a body temperature different from that of their environment (thermal regulation) and (ii) the excretion of waste nitrogen requires less energy in fish and crustaceans than it does in homeothermic land animals. Ammonia which is the principal end product of protein catabolism, in these aquatic animals diffuses into the water through the gills, while in homeothermic land animals energy is required to convert ammonia to less toxic metabolites.

Fish also require less energy to maintain position and move. Excess or insufficient dietary energy levels result in reduced growth rates.

Energy is stored in the chemical structure of the complex molecules of feed materials. The energy needs of an animal are supplied by lipids (fats), proteins and carbohydrates. The gross energy (the total energy produced on combustion) levels of these nutrients are 9450 kcal/kg of lipid, 5650 kcal/kg of proteins and 4100 kcal/kg of carbohydrates. Digestible energy of a food is the gross energy of the food consumed by the animal less the energy of the faeces excreted. The energy available for growth is what remains after the energy for metabolism, reproduction, etc. has been supplied.

While formulating diets, digestible or metabolizable energy values of nutrients are more important than gross energy levels. Since the metabolizable energy levels are difficult to measure in fish and prawns, digestible energy values are considered for feed formulation. Digestible energy data of nutrients from ingredients for fish are not easily available. The approximate digestible energy values of nutrients for fish suggested by ADCP (1983) are as follows:

Carbohydrate of legumes	2.0 kcal/g
Carbohydrate of non-legumes	3.0 "
Protein (animal)	4.25 "
Protein (Plant)	3.8 "
Fats	8.0 "

Using the above data, if the proximate composition is known, the approximate digestible energy of a feed ingredient could be calculated for balancing energy levels in artificial feeds. Since protein, lipids and nitrogen free extract (soluble carbohydrates) mainly provide energy, it is necessary to determine their levels in feed ingredients used for compounding feeds. Digestible energy in feed is normally expressed represented as kcal/kg.

*Example : A feed is compounded with fishmeal, soyabean meal and rice bran in the ratio 3:3:4*

Fish meal :

Crude protein	60%
Lipids	6%
Nitrogen free extract (NFE)	3%

Soyabean meal :

Crude protein	45%
Lipids	4%
NFE	35%

Rice bran:

Crude protein	12%
Lipids	6%
NFE	52%

The digestible energy level in 1 kg of feed can be calculated as follows using the data given in ADCP (1983):

Fish meal	300 g;
Soyabean meal	300 g;
Rice bran	400 g

$$\text{DE of protein} * (180 \times 4.25) + (135 \times 3.8) + (48 \times 3.8) \\ = 765 + 513 + 182 = 1460 \text{ kcal}$$

$$\text{DE of lipids} (18 \times 8) + (12 \times 8) + (18 \times 8) = 144 + 96 + 144 = 384 \text{ kcal}$$

$$\text{DE of NEF} = (9 \times 3) + (105 \times 2) + (208 \times 3) = 27 + 210 + 624 = 861 \text{ kcal}$$

$$\text{Total estimated digestible energy} = 1460 + 384 + 861 = 2705 \text{ kcal / kg feed}$$

For a fish to grow at optimal rate, adequate level of energy must be ensured, considering its varying energy demand during the growing period.

Digestible energy requirements (kcal/kg feed) of fish and prawns:

Salmon and trout	2800 - 3300
Channel catfish	2700 - 3100
Common carp	2700 - 3100
Indian and Chinese carps	2800 - 3100
Tilapia	2500 - 3400
Sea bass, Sea breams	
Grouper and yellow tail	2700 - 3700
Prawns	> 3200

#### 4.2 Protein and Amino Acids

Protein, generally, is the major as well as the most expensive component in fish and prawn diets. Growth of fish and prawns is also primarily determined by the level of protein and its constituent amino acids. The diversity in feeding habits exhibited by the fish and prawn species in nature is reflected in the variation in their protein and essential amino acid requirements. Important functions of proteins are (i) source of energy (ii) source of amino acids required for synthesis of diverse kinds of proteins. Thus protein is utilized for maintenance, the repletion of depleted tissues, and for growth or formation of new additional protein. As pure proteins, as peptides, as amino acids and as complexes with carbohydrates (glycoproteins), lipids (lipoproteins), and minerals (metalloproteins), they have functions in the transport of metabolic substrates. Other functions include precursor of hormones, enzymes, immunogenic molecules, lubricants and protective agents, antifreeze and anti-heat shock substances, and constituents of venoms and toxins. Some of the amino acids are important as feed attractants and some as osmolytes to maintain osmoregulation.

Protein is useful to the animal only when it can be

digested and the degradation products - peptides and amino acids absorbed. If protein in the diet is insufficient, it is withdrawn from the tissues to carry on the vital life functions, thereby resulting in rapid reduction in growth. On the other hand, if the diet contains excess protein proportionately less will be used to make new protein and the rest will be metabolized to produce energy. The utilisation of dietary protein is mainly influenced by its amino acid pattern, by the level of protein intake, by the digestible or metabolizable energy content of the diet, by the level of non-protein nutrients, by the physiological state of the animal and its health status, and by the physico-chemical characteristics of the culture environment.

The optimum dietary protein level (Table 1) required for maximum growth in farmed fish and prawn is substantially higher than that of terrestrial animals. At high levels of dietary protein, a proportion is deaminated and the carbon residues burned as energy.

Feeding trials with fry, fingerling and yearling fish have shown that gross protein requirements are highest in initial feeding fry and that they decrease as fish size increases. According to Halver (1980) fry of salmon and trout should have about 50% protein in the diet, fingerling about 40% protein, and yearling about 35% protein in the diet.

Balarin and Haller (1982) summarised the dietary protein requirement of tilapia as follows:

1g	35-50 %	Protein
1-5g	30-40) %	Protein
5-25g	25-30 %	Protein
>25g	20-25 %	Protein

In the common carp (*Cyprinus carpio*) it has been shown that temperature and metabolizable energy (ME) level in the diet affected the protein requirement for promoting maximum growth:

At 23°C	ME	3.7	Kcal/g	38.0 %	Protein
At 23°C	ME	3.4	Kcal/g	35.0 %	Protein
At 25°C	ME	2.7	Kcal/g	38.4 %	Protein
At 25°C	ME	3.06	Kcal/g	33.0 %	Protein
At 28°C	ME	3.82	Kcal/g	35.0 %	Protein

In complete grow-out feeds, as required for semi-intensive and intensive prawn culture, protein level in the range 35-40% would be adequate for most species of prawns. *Penaeus japonicus*, being a carnivorous species, needs relatively high dietary protein level (50 to 55%). In general, marine prawns require relatively high levels of animal proteins, especially of marine origin. Diets compounded with a mixture of fish meal, squid meal, clam meal, mussel meal, crab meal, prawn meal, prawnhead meal, squilla meal etc. as predominant protein source have been efficiently utilised by prawns. A number of other animal protein sources such as slaughter house waste, meat meal, meat and bone meal, poultry waste meal, blood meal, silkworm pupae are also used in some diets. Among the plant protein sources soyabean meal is the best followed by groundnut cake. In the diet of fresh water prawns, plant protein sources could be used in greater proportion than marine prawns and the protein required in grow out diets is in the range 25-35%. Protein sources should be selected considering the amino acid balance required in the diets.

Table 1 Protein requirement of selected fish and prawn species

Species	Common name	Protein content (g/kg feed)
<b>Fish</b>		
<i>Cyprinus carpio</i>	Common carp	a) 330-380 b) 250-350
<i>Ctenopharyngodon idella</i>	Grass carp	a) 360-420 b) 300
<i>Labeo rohita</i>	Rohu	a) 400-450 b) 300
<i>Catla catla</i>	Catla	a) 470 b) 300
<i>Cirrhinus mrigala</i>	Mrigal	a) 400-450
<i>Hypophthalmichthys moltirix</i>	Silver carp	a) 370-420 b) 300
<i>Ictalurus punctatus</i>	Channel catfish	a) 520 b) 320-360
<i>Clarias sp</i>	Catfish	357
<i>Anguilla Japonica</i>	Japanese eel	a) 540-550 b) 450
<i>Channa micropeltes Sarotherodon mossmbicus (g)</i>	Murrel Tilapia	520 400
<i>S. nilotica (g)</i>	Tilapia	280-300
<i>Tilapia zilli (e) (g)</i>	Tilapia	350
<i>Salmo gairdneri (f)</i>	Rainbow trout	400-450
<i>Chanos chanos (h)</i>	Milk fish	400
<i>Liza parsia (h)</i>	Mullet	b) 350
<i>Liza macrolepis (h)</i>	Mullet	a) 400
<i>Epinephelus salmoides (h)</i>	Grouper	400

<i>Lates calcarifer</i> (h)	Sea bass	387
<i>Chrysophrys major</i>	Red Sea bream	550
<i>Dicentrarchus labrax</i>	European Sea bass	500-600

### Prawns

<i>Penaeus indicus</i> (h)	Indian white prawn	350-400
<i>Penaeus monodon</i> (h)	Tiger prawn	360-450
<i>Penaeus merguensis</i>	Banana prawn	420
<i>Penaeus japonicus</i>	Kuruma prawn	450-550
<i>Metapenaeus monoceros</i>		550
<i>Macrobrachium rosenbergii</i>	Giant freshwater prawn (Scambi)	250-350

- a) Fry - fingerlings
- b) Production feeds
- c) Elvers
- d) Young eels
- f) Cold-water fish
- g) Can be cultured in fresh, brackish and sea water
- h) Can be cultured in brackish and sea water

#### 4.2.1. Essential amino acids

Protein requirement is significantly affected by the quality of dietary protein. Protein quality is dependent on the levels, balance and bio-availability of essential amino acids. The ten amino acids found to be essential in the diet of a great majority of cultivated aquatic animals are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The quantitative amino acid requirements have been established only for a few species of fin fish (Table 2). For prawns, their tissue amino acid profiles are used as guidelines for balancing amino acids in feeds (Table 3).

The amino acid profile of a protein describes the relative proportions of the essential amino acids within that protein. A protein with a good profile is one whose amino acid composition approximates to the quantitative essential amino acid requirements of the cultured animal.

**Table 2 Quantitative dietary amino acid requirements of selected fish species**

Amino acid	Requirement % of dietary protein				
	Common Carp	Rainbow trout	Channel catfish	Japanese eel	Tilapia <sup>1</sup>
Agrinine	4.2	3.5	4.3	4.0	2.82
Histidine	2.1	1.6	1.5	1.9	1.05
Isoleucine	2.3	2.4	2.6	3.6	2.01
Leucine	3.4	4.4	3.5	4.8	3.40
Lysine	5.7	5.3	5.1	4.8	3.78
Methionine	3.1 <sup>a</sup>	1.8 <sup>c</sup>	2.3 <sup>a</sup>	2.9 <sup>a</sup>	0.99
Phenylalanine	6.5 <sup>b</sup>	3.1 <sup>d</sup>	5.0 <sup>e</sup>	5.2 <sup>b</sup>	2.50
Threonine	3.9	3.4	2.3	3.6	2.93
Tryptophan	0.8	0.5	0.5	1.0	0.43
Valine	3.6	3.1	3.0	3.6	2.20

<sup>a</sup> cystine absent, <sup>b</sup> tyrosine absent, <sup>c</sup> cystine present, <sup>d</sup> tyrosine present, <sup>e</sup> phenylalanine + tyrosine

Source : New, 1987 <sup>1</sup>. Jauncey *et al.*, 1983.

**Table 3 Essential amino acids profile of prawns**

Amino acids	Percentage of total amino acids		
	<i>P. monodon</i>	<i>P. indicus</i>	<i>P. japonicus</i> <sup>a</sup>
Arginine	9.16	9.94	8.16
Histidine	1.52	1.91	2.40
Isoleucine	3.86	4.01	4.58
Leucine	8.04	7.24	8.04
Lysine	6.83	6.62	8.46
Methionine	2.01	1.82	2.90
Cystine	0.85	0.89	1.13
Phenylalanine	4.02	4.01	4.83
Tyrosine	3.05	2.93	4.20
Valine	3.70	3.09	4.44

Source: Cherian *et al.*, 1991 <sup>a</sup> Modified

Important points to be considered while balancing amino acids in feeds are:

- \* Methionine is spared by cystine, thereby the level of cystine in the feed proteins should be considered while adjusting methionine levels in diets.
- \* Phenylalanine is spared by tyrosine in fish, but in prawns phenylalanine is converted into tyrosine by an irreversible pathway. So, adequate level of phenylalanine must be ensured.
- \* Methionine may undergo oxidation, during processing of ingredients and feeds, to methionine sulphoxide or methionine sulphone. The bio-availability of sulphone and sulphoxide to fish and prawn is largely unknown. According to Halver (1980) methionine sulphoxide may have some biological value for fish which have some capability of reconverting it to methionine and thus partially make up for some of the methionine oxidized during processing. It is essential to determine the level of methionine after preparing feeds of a particular composition.
- \* Lysine is a basic amino acid, having two amino groups. The second amino group (epsilon) must be free and reactive for effective utilization of lysine. During the manufacture of feeds the second amino group in Lysine may react with non-protein molecules present in the feedstuff to form additional compounds that render the lysine biologically unavailable. So, the bio-available lysine in finished feeds is more important than chemically measurable lysine in feed ingredients.
- \* Isoleucine and leucine must be found in balanced proportions. Excess of either one as in blood meal may affect the effective utilisation of feeds.
- \* There are also reports that arginine and lysine should be

found in balanced proportions usually in the ratio of 1:1.

By and large, the balance of bio-available amino acids in the finished feeds is the most important factor deciding the quality of a protein.

#### 4.2.2 *Supplementing diets with amino acids*

Supplementation of the deficient amino acid or amino acids to feed proteins, is one way of utilising feed proteins with limiting amino acids. But fish and prawns utilize free amino acids at varying degrees of efficiency.

In the case of common carp, a diet based on a mixture of amino acids and a diet in which trypsin hydrolysate of casein was used, were found to be ineffective. Addition of methionine, cystine or lysine, the most limiting amino acids, to a diet based on fish meal, resulted in substantial reduction in growth compared to a diet based on fish meal. Similarly, addition of free arginine, cystine, tryptophan or methionine to casein had little effect on growth or food conversion.

Trout and salmon were able to utilize free amino acids for growth. A zein-gelatin diet supplemented with lysine and tryptophan was shown to be markedly superior to an unsupplemented zein-gelatin diet for rainbow trout. Soyabean meal supplemented with five or more amino acids, including methionine and lysine, was a superior protein source to soyabean meal in which methionine and lysine were only supplemented. Diets containing fish meal, meat and bone meal, and yeast and soyabean meal could be improved by supplementing with cystine (10g/kg) and tryptophan (5g/kg) together. Fish meal can be entirely replaced in diets of rainbow trout by a mixture of poultry by-product meal and feather meal supplemented with 17g lysine HCl, 4.8g DL-methionine and 1.44 g L-tryptophan/kg.

In the case of prawns, diets based exclusively on synthetic amino acids were poorly utilised. However, when squid meat

extract was supplemented with methionine it produced better growth in *Penaeus japonicus*. The nutritive value of casein for *P. indicus* was improved by supplementing a mixture of L-amino acids - phenylalanine, lysine, cysteine, tryptophan, taurine, glycine, proline and glutathione.

Addition of a peptide fraction isolated from shortnecked clam containing taurine, glycine, alanine, and arginine in the ratio 7:3:1:1 to *Penaeus japonicus* diet induced maturation. But inclusion of free amino acids in the diet did not result in any positive response. Another polypeptide containing aspartic acid, phenylalanine, proline, taurine, betaine and glutathione also proved somewhat useful in ovary maturation, but free amino acids were not effective.

#### 4.2.3 Non-protein nitrogen utilization

Studies with a mullets species has shown that urea nitrogen (2% in the diet) can replace partly protein nitrogen in the diet. In common carp diets containing either 9.5% ammonium citrate or 2.4% urea were utilized quite effectively. In clarias betrachus inclusion of urea (3% weight of the feed) substituted approximately 1/5 of the dietary protein, did not affect the performance of the fish.

#### 4.3. Lipids and fatty acids

Lipids are important in the diet as a source of energy, essential fatty acids, sterol, phospholipids, as carriers of fat-soluble vitamins (Vitamin A,C,D and K) and in lipid transport. Phospholipids and sterol esters play a vital role in biomembranes. Sterols are important as precursors of steroid hormones, which have diverse physiological functions in growth promotion, moulting in crustaceans, in sex control and maturation. Highly unsaturated fatty acids are important in brain and nerve activity and also as precursor of prostaglandin. Lipids also provide flavour and textural properties of the feed consumed by fish.

The total lipid recommended in fish and prawn feeds is

given in Table 4. Carnivorous fish such as sea bass, sea bream and trout utilize lipids more effectively; lipid level as high as 20% can be used in their diets; but in most commercial feeds about 10-12% is added. In complete feeds for prawns, about 6 to 10% lipid containing blend of essential fatty acids and phospholipids should be ensured. However, feeds for extensive and semi-intensive prawn culture need to have less than 6% lipids, as the natural food of prawns are quite rich in lipids. Lipid levels should be adjusted in diets considering the technological problems in feed manufacture and storage. Dietary lipids are a highly digestible source of energy, and in most ingredients, 85-90% of lipid is digested by fish and prawns when incorporated at optimal levels.

High quality lipids, capable of satisfying the essential fatty acid requirements of the fish, are added at higher levels since lipids spare protein in carnivorous species. Protein level in rainbow trout has been decreased by approximately 15% by inclusion of fish oil.

#### *4.3.1. Phospholipids:*

Prawns require a source of phospholipids rich in phosphatidyl choline with some quantity of phosphatidylethanolamine and phosphatidyl inositol for normal growth, moulting, metamorphosis and maturation. Soyabean lecithin at dietary level of 1-2% promote growth in prawns. Lipids of marine animals squid, clams, prawns, fish and polychaetes are excellent natural source of phospholipids.

#### *4.3.2 Cholesterol:*

Prawns also require a source of sterol and cholesterol. It has been found to be most effective at dietary levels of 0.25 to 0.5%. Marine invertebrate meals and oils-squid, shrimp, clam, crabs and mussels, are good sources of cholesterol. Sterols are not essential in the diet of fish.

### 4.3.3 Essential fatty acids (EFA)

About 100 different kinds of fatty acids have been isolated from lipids of various animals and plants.

A few of these fatty acids are essential in the diet of fish and crustaceans, as they are not biosynthesised by these animals. The fatty acids that are essential for normal growth, moulting (crustaceans) and maturation are polyunsaturated and highly unsaturated fatty acids (HUFA):

Linoleic acid (18:2 n-6)

Linolenic acid (18:3n-3)

Arachidonic acid (20:4 n-6)

Eicosapentaenoic acid (10:5 n-3)

Docosahexaenoic acid (22:6 n-3)

]

HUFA

There is wide variation in the dietary essential fatty acids requirement of aquatic species. Water temperature, salinity, and capability of individual species in utilising essential fatty acids (EFA) are factors that affect the EFA requirement. In general, aquatic animals raised in freshwater, brackish water and sea water have a higher requirement for n-3 fatty acids than terrestrial animals. But in *Tilapia zili* grown both in fresh water and sea water 18:2 n-6 and 20:4 n-6 are superior to n-3 fatty acids. Most of the marine fish require highly unsaturated fatty acids of the n-3 series. In some species, like rainbow trout 18:3 n-3 alone is adequate in the diets when reared in both fresh and sea water; but n-6 fatty acids are important in maturation of rainbow trout. Cold water species have a greater requirement for n-3 fatty acids than warm water species. In prawns, reared in sea water 20:5 n-3 and 22:6 n-3 are superior, but a blend of 18:2 n-6, 18:3 n-3, 20:5 n-3 with a greater proportion of the HUFA promote maximum growth. Dietary n-3 and n-6 fatty acid requirements of fish and prawns are given in Table 5.

#### 4.3.4 EFA Deficiency symptoms

In fish EFA deficiency caused poor growth and feed conversion, erosion of caudal fin, swollen livers, altered permeability of biomembranes, fatty degeneration of liver, decreased red blood cell volume, decreased blood cell counts, hematocrit and haemoglobin levels and increased accumulation of 20:3 n-9 into phospholipids, EFA deficiency also affect maturation, spawning, hatchability of eggs and larval survival.

In prawns EFA deficiency resulted in poor growth, poor food and protein conversion, poor protein retention, delayed or incomplete larval metamorphosis and high mortality in larvae.

#### 4.3.5 Lipid sources

Lipids extracted from clams, squids, mussels, prawns, krill, and fish are the best lipid sources for inclusion in fish and prawn diets. But the most easily available source of HUFA is fish oil-sardine, shark liver and cod liver oils (Table 6). Vegetable oils which contain mostly the n-6 fatty acids include soyabean oil, corn oil, groundnut oil, sunflower oil and safflower oil. All the oils, particularly the marine oils must be stabilised with antioxidants.

#### 4.3.6 Negative aspects of lipids

The polyunsaturated fatty acids are highly susceptible for oxidation during storage of feeds. The products of lipid oxidation may react with other nutrients such as amino acids and vitamins and reduce their biological availability. Antioxidants should be added to prevent this undesirable effect.

Another negative aspect relates to the presence of cyclopropenoic fatty acids in cotton seed oil and oils from plants of the order Malvales. these fatty acids are suspected to interfere with certain enzymes and affect normal lipid and protein metabolism. The oils containing cyclopropenoic fatty acids should be excluded from feeds.

Table 4 Lipid requirement of selected fish and prawn species

Species	Lipid level (g/kg feed)
<b>Fish</b>	
Common carp	80 - 180
Indian and Chinese carps	50- 80
Rainbow trout:	
Fry	150
Fingerlings	120
Production feed	90
Tilapia :	
Fry to 0.5	100
0.5 to 35 g	80
> 35 g	60
Channel catfish	80 - 120
Catfish (Clarias sp.)	100
Eel	120 - 150
Milkfish, mullets	60
European sea bass	120 - 130
Red sea bream	80
Grouper 140	
<b>Prawns</b>	
Prawns (in general)	50 - 100
Commercial feeds for prawn	40 - 100
Kuruma prawn	80 - 100
Tiger prawn	60 - 100
Indian White prawn	80 - 100
Giant fresh water prawn (scambi)	30 - 60

Table 5 Essential fatty acid requirements

Species	EFA	Dietary level (%)	Remarks
<b>Fish</b>			
Rainbow trout (Fry, fingerlings to adult)	18:3n-3 or 20:5n-3 + 22:6n-3	0.8-1.6	Adequate in both fresh and sea water 20:5n+22:6n-3 superior to 18:3n-3
Rain bow trout (brood stock)	n-3 plus n-6 18:2 n-6)	1.0	n-6 fatty acids very important in maturation
Common carp	18:2 n-6 +	1.0	20:5n-3+22:6n-3 superior to 18:3n-3
	18:3n-3	1.0	
	20:5n-3 +	0.5	
	22:6n-3		
Eel ( <i>Anguilla japonica</i> )	18:2n-6 +	0.5	
	18:3n-3 or	0.5	
	20:5n-3 +	0.5	
	22:6n-3		
Tilapia zilli	18:2n-6 or	1.0	n-6 fatty acids are superior to n-3 fatty acids both in fresh water and sea water
	20:4n-6 or	1.0	
	18:2n-6 +	1.0	
	20:4n-6		
Tilapias (other species)	18:2n-6 +	1.0	
	18:3n-3	1.0	

Channel catfish	20:5n-3 + 22:6n-3	-	Lipid containing 18:2 n-6 depressed growth
Mullet, milkfish	lipids containing a mixture of 18:2n-6 18:3n-3, 20:5n-3 and 22:6n-3	-	In milkfish fatty acids profile in the tissue lipids vary with salinity and food
Sea breams, Sea bass Grouper	20:5n and 22:6n-3		1.2
<b>Prawns</b>			
Kuruma prawn	n-3 n-6 20:5n-3+22:6n-3 (ratio 2:1)		1.0 Addition of 18:2n-3 or 18:2n-6 to fish oil improved growth
Indian white prawn	18:2n-6+18:3n-3 + 20:5n-3+22:6n-2		1.2 May vary with salinity
Tiger prawn	As above	-	
Fresh water prawn (scambi)	n3+n-6 HUFA levels as low as 0.075% increase growth by 50-60%		Diet containing 3% shrimp head oil produced larger shrimp and double the biomass as that of control
Penaeus chinensis	20:4n was equal to 22:6-3 in efficacy		

**Table 6 Polyunsaturated fatty acid levels in various lipid sources found in India**

Lipid source	EFA as % of total fatty acids					Total HUFA n-3 & n6
	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3	
1. Corn oil	50.02	2.8	-	-	-	-
2. Cotton seed oil	52.22	0.68	-	-	-	-
3. Coconut oil	4.22	0.9	-	-	-	-
4. Gingelly oil	47.16	0.9	-	-	-	-
5. Groundnut oil	33.07	3.64	-	-	-	-
6. Linseed oil	22.29	41.06	-	-	-	-
7. Mustard oil	16.71	26.3	-	-	-	-
8. Rapeseed oil	16.0	7.0	-	-	-	-
9. Safflower oil	71.9	1.12	-	-	-	-
10. Soyabean oil	51.8	7.38	-	-	-	-
11. Sunflower oil	57.46	0.34	-	-	-	-
12. Cod-liver oil	3.18	0.51	-	10.41	12.51	24.3
13. Pawn-head oil	2.92	1.16	3.29	5.88	14.84	24.6
14. Indian oil sardine	1.53	9.01	0.08	8.25	10.73	26.65
15. Shark liver oil	3.20	1.27	-	3.04	10.67	26.30
16. Soyabean lecithin	60.1	7.3	-	-	-	-

Source: Chandge and Paulraj, (1990)

#### 4.4. Carbohydrates

Carbohydrates are a cheap natural source of energy. Digestible carbohydrates, at optimal levels spare protein for growth, especially in omnivorous and herbivorous species. The carbohydrates that are of importance in the nutrition of fish and prawns are:

Monosaccharides	:	Glucose and fructose
Disaccharides	:	Sucrose and maltose
Digestible polysaccharides	:	Starch and glycogen
Structural polysaccharides	:	Chitin, cellulose and several others
Oligosaccharides	:	Raffinose and Stachyose

#### 4.4.1 Carbohydrate utilisation in fish

Carnivorous fish species (trout, sea bass, sea bream, eels) have poor ability to digest carbohydrates, and formulated feeds for these species contain carbohydrate level less than 20%. Omnivorous and herbivorous species (crayfish, channel catfish, tilapia, mullets, milk fish) are capable of utilising 40 to 45% carbohydrate in the form of gelatinised (cooked) starch. The traditional feed mixture of oilcakes and cereal brans used in Indian carp culture systems contain as high as 45% carbohydrate.

Glucose, sucrose and maltose are utilised by fish, but at varying degrees. In view of its availability, starch is the most widely used carbohydrate source in fish feeds. Raw starch is not digested as well as the cooked starch. Cooked starch from wheat flour, maize flour and tapioca are better digested.

The poor utilization of starch in carnivores is due to low amount of amylase produced.

Cellulose and chitin are digested by fish which hosts microorganisms in the digestive tract. These gut microbes produce extracellular cellulases or chitinases. Cellulase activity has been demonstrated in the digestive tracts of several estuarine species and also in carp and channel catfish. Similarly, chitinolytic activity has been detected in some species.

#### 4.4.2. Carbohydrate utilisation in prawns:

In prawns carbohydrates are important in energy

production, chitin synthesis and non-essential fatty acid synthesis. A variety of digestive enzymes have been recorded which have carbohydrate utilising capacity.

Chitin (0.5%) or its precursor glucosamine (0.8%) when included in diet has been shown to improve growth and feed efficiency in prawns. In prawns chitin is required for the formation of exoskeleton as well as peritrophic membrane, in which the faecal pellets are enveloped. Prawns moult at frequent intervals, and they invariably consume the moult. Thus a greater proportion of chitin is recycled. Exoskeleton from prawns, crabs and mantis-shrimp can be used in the diet as source of chitin. Extracellular chitinolytic activity has been recorded in some species of prawns. Cellulose digestion is not clear. Crude fibre levels in commercial prawn feeds are less than 5%.

Glucose is not well digested by prawns. Maltose, sucrose and glycogen are well utilised. The most commonly used carbohydrate is gelatinised starch. Wheat starch, maize starch and tapioca starch are well utilized. Potato starch is poorly digested. Carbohydrate levels in commercial semi-intensive prawn feeds is in the range of 25-30%. In feeds for extensive culture, starch level in the range 35-40% is being used. In *Penaeus indicus* starch digestibility was maximum upto a dietary level of 20%. Exceeding this there was steady decline in starch digestibility.

Seeds of leguminous plants contain good levels of oligosaccharides such as raffinose and stachyose, which the fish and prawns are incapable of digesting due to the absence of specific enzymes. Nutritive value of soyabean meal is partly affected by these. Processing the soyabean meal either to remove or modify these would result in more acceptable soyabean meal for aqua-feeds. Soaking of the soyabeans for 48 hours (induce germination) prior to processing for meal production helps breakdown the oligosaccharides by enzymatic hydrolysis. The nutritive value of pulses and other legume seeds which contain

oligosaccharides also could be improved by this process (chow and Halver, 1980).

#### 4.5. *Vitamins*

Vitamins are essential for normal growth, maintenance and reproduction of animals. Eleven water-soluble vitamins and four fatsoluble vitamins are required in the diet of fish and prawns.

At low stocking densities, under extensive culture conditions, natural foods may provide a good proportion or all of the vitamins required by fish and prawns. However, high stocking densities, as in semi-intensive and intensive systems, where natural food is limited to sustain the entire population, vitamin supplementation of the diets is important. Specific quantitative vitamin requirements are yet to be determined for most species of cultured fish and prawns. Those species for which vitamin requirements have been determined, the data is still incomplete due to severe methodological constraints. The existing data for fish mainly relate to fry and fingerlings and those of grow-out stages and broodstock are mostly empirical recommendations. In the case of prawns data are available for only a few vitamins, that too, for post-larval and juvenile stages of few species.

Dietary vitamin requirements are affected by the size, age, growth rate, physiological conditions, health status, nutrient composition of the diet, feed hydro-stability, environmental conditions and also the availability of vitamins from natural food and gut microbial contributions. Loss of water-soluble vitamins during processing and storage of ingredients and finished feeds, and leaching from feeds when introduced into water are factors that affect the availability of vitamins to the cultured animals.

##### 4.5.1. *Water-soluble vitamins*

###### 4.5.1.1. *Vitamin C (Ascorbic acid):*

Fish and crustaceans are incapable of biosynthesis of

ascorbic acid, and therefore, diet is the only source of vitamin C. Some important functions of this vitamin in animals are protecting enzymes and hormones from oxidation and inhibition, growth regulation, RNA synthesis, reactants in enzyme systems such as in the hydroxylation of epinephrine and tryptophan, in collagen synthesis, biological reducing agent in hydrogen transport, detoxification of aromatic drugs, and erythrocyte maturation. In crustaceans, vitamin C influences the alkaline phosphatase activity during synthesis of chitin and sclerotization of the epicuticle.

Deficiency of ascorbic acid results in metabolic disorders, spinal deformities in fish (dislocation of vertebrae and atrophy of the spinal cord), deformities in the gills, vertebrae, internal and external haemorrhage, fin erosion, increased susceptibility to pathogenic bacterial infection and reduced absorption of calcium from surrounding water by gills and skin.

In crustaceans, vitamin C deficiency led to the inhibition of alkaline phosphatase activity resulting in poor chitin synthesis and sclerotization of the epicuticle. In prawns reduced food intake, poor conversion of food, high incidence of post-moult deaths, dystrophy of muscle and hepatopancreas, blackening of gills and in extreme cases black-death disease, whitened or blackened lesions, and high rates of mortality.

The recommended dietary levels of ascorbic acid for fish and prawns are presented in Table 7. The dietary requirement is in the range of 100-150mg/kg for carp and channel catfish. Ascorbic acid levels in prawn diets is dependent on its stability. About 90% of unprotected forms of ascorbic acid is oxidized during processing and storage of feed and further loss occurs while feeding due to leaching of the vitamin in the water. This necessitates inclusion of very high levels of ascorbic acid in the diets, often in the range 4 to 12 g/kg feed. However, if it is in the coated forms (gelatin or silicone coated), the requirement is about 1 to 1.5g/kg. A recent breakthrough has been the introduction of heat-stable and water-stable ascorbic acid derivatives for use

in aquaculture feeds. L-ascorby 1-2 sulphate has been successfully used in diets of salmonid fish, whereas ascorbyl-polyphosphate (stay-C) containing a mixture of tri-, di- and monophosphate esters of L- ascorbic acid is widely used in prawn and fish feeds. The minimum vitamin C activity of derivative is 15% and therefore stay -C level in feeds should be regulated to the required vitamin level. To maintain an ascorbic acid level of 200 mg/ kg of feed 1330mg/kg Rovimix stay - C (Roche) has to be added.

**Table 7 Recommended vitamin levels (per kg of feed)**

Vitamin	Salmon, trout	Carp	Prawns
<b>Fat - Soluble</b>			
Vitamin A (I.U.)	1000-2000 3500	2000	5000-10000
Vitamin D3 (I.D)	1600-2000 3000	-	1000-2000
Vitamine E (I.U.)	30-50 100	100	100-200
Vitamin K (mg)	10	4	5-20
<b>Water - Soluble</b>			
Ascorbic acid (mg)	300	30-50	200-400a
Cyanocobalamin (mg)	0.01-002	b	0.02-0.1
Biotin (mg)	0.4	0.6-1.0	1.0
Choline (mg)	800	500-600	400-2000
Folic acid (mg)	5-10	15	5-10
Inositol (mg)	200-400	440	200-300
Niacin (mg)	150	28	100-150
Pantothenic acid (mg)	60	30-40	50-100
Pyridoxine (mg)	10-20	5-10	30-50
Riboflavin (mg)	20	4-7	30-58
Thiamine (mg)	10	2-3	50-100

- a) Asorbic acid level required when Ascorbyl polyphosphate is used as the source
- b) Gut microbial population synthesize the vitamin in adequate levels
- c) Recommended level would vary depending upon the culture systems and ingredient composition and stability of the vitamins.

Sources of ascorbic acid: Citrus fruits, cabbage, liver, kidney tissues, fresh insects, fish tissues and goose-berry (amla) are good natural sources. Synthetic ascorbic-acid is added to feed as a dry powder. Coated forms of ascorbic acid-ethylcellulose coated, fat-coated, silicon coated, gelatin coated-have better stability than crystalline ascorbic acid.

#### 4.5.1.2. Choline :

A source of methyl groups, involved in a number of transmethylation; as phosphatidyl choline has an important structural role in biomembranes, as acetylcholine functions as an important neurotransmitter; choline is also a lipotropic and antihæmorrhagic factor, essential for good growth and good FCR.

Deficiency leads to poor growth and food conservation, loss of appetite, increased gastric emptying time, hæmorrhagic kidneys and enlarged livers in fish. In prawns, choline deficiency resulted in poor growth, poor appetite, passive activity (lethargy), dystrophy of muscle, and hepatopancreas, and post-moult deaths. If adequate level of lecithin is included in the diets, lecithin can partly off-set the requirements of choline.

Dietary requirement:

Trout and salmon 600-800mg/kg diet; carp 500-600mg/kg diet; recommended level in prawn diet 400-2000mg/kg.

Sources: Wheat germ, beans, brain and heart tissue are rich sources. Choline is added to feeds as a 70% choline chloride solution or as 25 to 60% dry powder. Choline is stable in multivitamin premixes and stable during processing and storage in pellets and extruded diets. Loss from pellets in water is less than 10% after 60 minutes.

#### 4.5.1.3 Inositol :

It is a structural component in living tissues and a component of inositol phosphoglycerides, it has lipotropic action by preventing accumulation of cholesterol, important in maintaining normal lipid metabolism and membrane function.

Deficiency leads to poor growth and food utilization, increased gastric emptying time, edema and dark colour in fish. In prawns reduced growth and feed efficiency occurred.

Dietary requirement:

In fish diets 200-400 mg/kg; recommended level in prawn diets 300mg/kg. Inositol is added to feeds as a dry powder of mesoinositol.

#### 4.5.1.4. Thiamine:

Functions as a coenzyme in carbohydrate metabolism; essential for good appetite, normal digestion, growth, fertility and functioning of nervous tissue.

Deficiency leads to impaired carbohydrate metabolism, nervous disorders, poor appetite, poor growth, increased sensitivity to shock.

Dietary requirement:

Thiamine requirement of aquatic animals is much higher than terrestrial animals mainly due to leaching of the vitamin from the diets. Thiamine requirement is affected by carbohydrate level and energy density of

the diet. Channel catfish require about 1mg/kg, salmon and trout 10-12mg/kg, carp 2-3 mg/kg and recommended level in prawn diets 50-100 mg/kg.

Sources: Dried Peas, beans, cereal bran and dried yeast. Thiamine is easily lost by holding diet ingredients, too long in storage or by preparing the diet under slightly alkaline conditions. High moisture content as in moist or frozen diet increases the chance of enzymatic hydrolysis and subsequent destruction of thiamine. Thiaminases occurring in freshwater fish, shrimp tissues and certain molluscs, reduce the biological availability of thiamine. Thiaminase is inactivated by heating.

Thiamine is added to feeds as thiamine mononitrate or thiamine hydrochloride. Thiamine mononitrate is stable only in vitamin premixes, that do not contain trace elements and choline chloride. Thiamine losses in the pelleting or extrusion process range from 0 to 10% and during storage of feeds 11 to 12%. But thiamine loss of about 68-100% can occur when feed is in water within 2 hrs time.

#### 4.5.1.5. Riboflavin:

Riboflavin is found in the tissue coenzymes, flavin mononucleotide and flavin adenine dinucleotide - coenzymes for several enzymes, including glutathione reductase and D-amino oxidase, important for the degradation of pyruvate, fatty acids and amino acids, and conversion of tryptophan to nicotinic acid.

Deficiency leads to poor appetite, poor growth and increased mortality in fish and prawns; cataracts, short-body, dwarfism, fin necrosis, snout erosion, spinal deformation, photophobia and haemorrhages in some species of fish.

Dietary requirement:

Salmon and trout 10-12mg; carp 4-7mg; channel catfish 10mg; prawns 30-50mg/kg diet.

Sources : Liver, kidney, heart, yeast, germinated grain, ground nut, soyabeans and eggs are good natural sources. Commercially available riboflavin is added to feed as a spray-dried powder. Processing loss is about 26% and storage losses in pelleted feed are slight; about 40% may be lost when pellets are introduced in to water. In carp diets both riboflavin and riboflavin tetrabutryate are found to be effective.

#### 4.5.1.6. Pyridoxine:

Required for many enzymatic reactions in which amino acids are metabolised. It has a role in synthesis of serotonin, porphyrin, and messenger RNA.

Deficiency in diet affect amino acids and protein metabolism and causes epileptic fits, general nervous disorders, rapid and gasping breathing with flexing of opercles, poor appetite and anaemia in fish.

Dietary requirements:

In fish diets 10-20mg/kg, prawn diets 30-50mg/kg.

Sources : Good natural sources are yeast, whole cereals, egg yolk, liver and glandular tissues. Pyridoxine hydrochloride is the commercial form used in vitamin premix. It is very stable in premix and in finished feeds. Storage losses are 7-10 % after 10 months.

#### 4.5.1.7. Pantothenic Acid:

Plays a stellar role in general metabolic pathways. It has a significant role in fatty acid oxidation and synthesis; synthesis of cholesterol and phospholipids, phosphate energy transfer, pyruvate oxidation etc.

Deficiency symptoms observed are poor growth, impairment of reproduction, clubbing together of gill filaments

and lamellae (clubbed - gill disease), distended opercles, necrosis, and fish become sluggish. In prawns apart from poor growth and feed efficiency, unusual partial moulting observed.

Dietary requirement:

Salmon and trout 40-50 mg; carp 30-40mg; channel catfish 25-30mg; prawns 50-100mg/kg diet.

Sources: Good natural sources are cereal bran, yeast, liver, fish flesh and spleen. Pure panto thenic acid is unstable, hygroscopic and viscous. In feeds calcium D - pantothenate (92% activity) is better than calcium dl-pantothenate (46% activity). Calcium pantothenate is relatively stable in moist and dry diets Processing losses during pelleting or extrusion can be as high as 10%.

#### 4.5.1.8 Niacin :

Component of coenzymes NAD and NADP, required for synthesis of high energy phosphate bonds and serve as hydrogen acceptors.

Deficiency symptoms are loss of appetite, skin and fin erosions, deformed jaws, poor feed conversion and haemorrhages in fish. In prawns poor survival, poor growth, poor food intake, poor feed conversion and black lesions in the body and gills.

Dietary requirements:

Fish in general 50-100mg/kg; carp 25-30mg; prawn diets 100-150mg/kg.

Sources: Rich natural sources are yeast, liver, kidney, heart, legumes etc. In many animals, the amino acid tryptophan can be converted to niacin or nicotinic acid. Gut microbial synthesis is another source of this vitamin. In feeds it is added as niacinamide or nicotinic acid as a dry powder. Processing losses of niacin in extruded diets is about 20%; about 50% of the vitamin from feed is lost in water within 24 hours.

#### 4.5.1.9. Biotin :

Intermediate carrier of carbon-di-oxide in several specific carboxylation and decarboxylation reactions; required for the biosynthesis of long-chain fatty acids, purine and the metabolism of odd-chain carbon fatty acids, has a role in blood glucose regulation and cell membrane function. Deficiency leads to loss of appetite, skin dis-order, muscle dystrophy, spastic convulsions and fragmentation of erythrocytes, depigmentation and dark colouration in fish.

Recommended dietary level :

0.4 to 1.0mg/kg diet for fish and 1mg/kg diet for prawns.

Sources : Yeast, liver, glandular tissues, milk products egg yolk are good natural sources. Heating of egg destroys avidin present in raw eggs rendering biotin bio-available. diet should be protected from conditions which promote oxidation. D-biotin is stable in multivitamin premixes. Feed processing losses are about 15%.

#### 4.5.1.10. Folic Acid:

Folic acid is required for normal blood cell formation, involved in one carbon transfer mechanisms as in the metabolism of amino acids, biosynthesis of purines and pyrimidines.

Deficiency leads to poor food intake, poor growth, lethargy, fragile fins, anaemia, dark skin pigmentation and infraction of spleen and reduction in erythrocyte numbers in fish.

The dietary requirement:

Range between 5-10mg/kg for fish and 5-10mg/kg for prawns. Intestinal microflora of carp are capable of synthesising folic acid.

Sources: Yeast, green vegetables, liver, kidney, glandular tissues,

fish tissues and viscera are good sources. Insects contain xanthopterin which has folic acid activity. Folic acid is added to feed as a dry dilution. Storage losses are as high as 43% in three months.

#### 4.5.1.11. Cyanocobalamin (B<sub>12</sub>):

Cobalt containing vitamin involved with folic acid in haemopoiesis, required for normal maturation and development of erythrocytes; synthesis of labile methyl compounds; required for normal cholesterol metabolism and in purine and pyrimidine syntheses; extremely important as a growth factor.

Deficiency leads to pernicious anaemia characterised by fragmented erythrocytes, poor appetite, poor growth and food conversion. In carp and channel catfish gut microbes synthesize cyanocobalamin. In channel catfish gut microbes synthesized about 1.4ng of cyanocobalamin/g weight.

Dietary requirements:

Fish 0.015 to 0.02 mg/kg dry diet; prawns 0.02 - 0.1 mg/kg diet.

Sources: Fish meal, prawn meal, crab meat, fish viscera, liver, kidney, glandular tissues, slaughter-house wastes and poultry byproducts. In mild acid conditions gets easily destroyed by heating.

### 4.5.2 *Fat-soluble vitamins*

#### 4.5.2.1. Vitamin A:

Vitamin A occurs in two forms, vitamin A1 (retinol) found in marine fishes and vitamin A2 (retinol 2) in freshwater fishes. Vitamin A is essential for maintaining epithelial cells, has a role as a visual pigment, promotes growth of new cells and in maintaining resistance to infection. It is also involved in calcium transport across biomembranes, in reproduction is also involved in calcium transport across biomembranes, in

reproduction and embryonic development and in cellular and sub-cellular membrane integrity.

Deficiency of vitamin A leads to poor growth, poor vision, cataracts, keratinization of epithelial tissue, xerophthalmia, night blindness, haemorrhage in eye and fins and abnormal bone formation. Hypervitaminosis (high dosages) results in enlargement of liver and spleen, abnormal growth, skin lesions, hyperplasia of head cartilage and fusion of vertebrae.

Dietary requirements :

Fish 1000-2000 I.U./kg; recommended level for prawns 5000-10000 I.U./kg feed.

Sources : Code-liver oil, shark-liver oil and many other fish oils. Vitamin A is added in feeds as acetate, plamitate or propionate esters to enhance vitamin A stability. About 20% lost during extrusion of feed; at room temperature storage loss is about 53%. B- Carotene is also converted into vitamin A by some species.

#### 4.5.2.2. Vitamin D:

Vitamin D<sub>3</sub> (cholecalciferol) is most effective, vitamin D is essential for maintaining calcium and inorganic phosphate homeostasis and alkaline phosphatase activity.

Deficiency leads to reduced weight gain, lower body ash, lower body phosphates, lethargy and poor appetite. Excess dietary levels also impair growth and cause lethargy and dark colouration in fish.

Dietary requirements :

Fish requires between 1000 and 1000 I.U., recommended level for prawns 1000-2000 I.U./kg feed.

Sources: Fish liver oil is a rich source of vitamin D. Shark liver

oil contains about 25 I.U./g, cod-liver oil 100-500 I.U./g and albacore tuna liver oil 200000 I.U./g. One I.U. is equal to 0.025g of crystalline vitamin D. In feeds cholecalciferol is added as a spray dried powder.

#### 4.5.2.3. Vitamin E:

Vitamin E is composed of a class of compounds classed as tocopherols  $\alpha$  - tocopherol is the most important one. Vitamin E is important as an extracellular and intra-cellular antioxidant, thus protects oxidisable vitamins and unsaturated fatty acids. Vitamin E along with selenium and vitamin C prevents muscular dystrophy and helps in normal reproductive activity.

Deficiency leads to poor growth, exophthalmia, clubbed gills, muscular dystrophy, depigmentation etc. In common carp fed oxidised silkworm pupae loss of flesh on the back of the fish was prevented by supplementing tocopherol acetate.

#### Dietary requirement :

Varies with the polyunsaturated fatty acid levels in the diet. In fish dietary level recommended range from 30-50mg/kg diet and for prawns 100-200 I.U./kg.

Sources : Vegetable oils are good sources. Synthetic tocopherol in esterified acetate or phosphate form is commonly used in diet. Vitamin E acetate is stable during feed preparation and storage.

#### 4.5.2.4. Vitamin K:

Vitamin K is required for normal blood clotting mechanisms; energy metabolism and ameliorates aflatoxin toxicity. Deficiency leads to anaemia and haemorrhagic gills, eyes and vascular tissues.

Dietary level in fish feeds : 10mg/kg; prawn feeds 5-20mg/kg.

Source: Green and leafy vegetables, alfalfa leaves, soyabeans and liver. Synthetic components added to feeds are either menadione sodium bisulfite (50% K3) or menadione sodium bisulfite complex (33%K3). Alkaline pH, heat, moisture and trace minerals increase the rate of destruction of menadione salts in pelleted feeds.

#### 4.6. Minerals

Minerals are important (i) to provide strength and rigidity to bones in fish and exoskeleton in crustaceans (ii) to maintain acid-base equilibrium and osmotic relationship with the aquatic environment (iii) for normal interaction activities involving nervous and endocrine systems, (iv) as components of blood pigments, enzymes and organic compounds in tissues and organs indispensable for metabolic processes involving gas exchange and energy transaction. There are about 21 recognised elements which perform essential functions in the body. Calcium, phosphorus, potassium, magnesium, sodium, chlorine and sulphur are required in relatively higher levels and are termed macroelements and iron, copper, zinc, manganese, cobalt, selenium, iodine, nickel, fluorine, vanadium, chromium, molybdenum, tin and silicon are termed micro or trace elements. Diet is the main source of minerals, although some minerals are absorbed from the environment. Non-availability of adequate quantities of minerals affect growth, moulting (in crustaceans) and may cause irrecoverable deficiency diseases.

Fish and crustaceans can absorb minerals directly from the aquatic environment through the gills and body surfaces. Therefore the dietary requirements of minerals is largely dependent on the mineral concentration of the aquatic environment in which the fish and prawns are cultivated. It is generally believed that calcium, sodium, potassium and chlorine requirement might be satisfied through absorption from the environment. Phosphorus is deficient in water and thus essential in the diet. Since the digestive system of prawns is not very acidic,

mineral supplements which are water-soluble are most available; whereas the finfish as a group show considerable variation in the utilization of mineral elements.

#### 4.6.1. Calcium and Phosphorus

Under low-density (extensive) culture conditions, natural food and the water provide these elements in required levels. But under high density systems, as in semi-intensive and intensive stocking, the dietary levels are very important. Calcium (Ca) is absorbed from seawater but freshwater is low in calcium. Calcium is also available to the animals from the feed ingredients, with the ingredients of animal origin contributing a greater proportion than plant ingredients. Since phosphorus (P) levels in natural waters is low, diet is the major source of phosphorus. Phosphorus from ingredients of animal origin (fishmeal, prawn meal etc.) is better absorbed in most species; but some species, such as carp, do not effectively absorb phosphorus from feed. Plant source of phosphorus is poorly available as it is bound in a complex with phytin. Phosphorus availability varies with the source and species also (Table 8).

**Table 8 Provisionability factors for phosphorus**

Type of ingredient	% availability factor (to apply to the total phosphorus in the feed)	
	Stomachless fish (cryprinids, carp etc)	Other fish and Prawns
Plants	30	30
Plant products	57	58
Wheat germ	30	70
Animal products	97	90
Microbial products (yeast, bacteria etc.)	90	90
Monobasic sodium, potassium	95	95

or calcium phosphates

Dibasic calcium phosphate	45	70
Tribasic calcium phosphate	15	65
Fishmeal	10-33	60-72
Rice bran	25	19

Major portion of calcium (99%) and phosphorus (80%) are found in bones, teeth, scales in fish and exoskeleton in crustaceans. The level of phosphorus in the diet influences the calcium retention in the body. Glucose in diet helps to improve absorption of dietary phosphorous in fish. Phosphorus absorption from the diet is also increased by increasing temperatures.

The dietary calcium and phosphorus requirement are as follows:

	Available phosphorus %	Calcium
Sea bass, sea bream etc.	0.7	
Rainbow trout grouper	0.7 - 0.8	0.5
Channel cat fish	0.4 - 0.5	
Common carp	0.6 - 0.7	
Indian and Chinese carps	0.5 - 1.0	0.5
Red sea bream	0.68	0.34
<i>Penaeus japonicus</i>	1.04	1.24

Fishmeal is a rich source of calcium and phosphorus. But it is in the form of tri-calcium phosphate which has low availability to fish like carp. The availability of inorganic phosphorus depends on the solubility of the salt concerned. The more soluble the more available. Thus mono and di-calcium phosphate are better source than tri-calcium phosphate, especially in the case of stomachless fish like carp.

Phosphorus availability of common feed types varies from 33% in cereal products to 50% for fish meal and mineral

by-products. Soyabean meal has an intermediate phosphorus availability of 40%.

The calcium and phosphorus ratio in *P. japonicus* diet is given as 1.2 : 1; when this ratio was changed to 2:1 growth was reduced and pigmentation decreased.

#### 4.6.2 Magnesium

Magnesium is closely associated with calcium and phosphorous, both in its distribution and its metabolism. Functions include component of skeletal structure, organs and muscle tissues, where it plays vital roles as enzyme co-factors (metalloenzymes), structural component of cell membranes, and in extracellular fluids.

Fish and crustaceans are capable of extracting magnesium from the environment. However, since fresh water contains very low levels of the element non marine fish depend greatly upon dietary sources to meet their requirement. Magnesium requirements - rainbow trout 0.06-0.7%, carp 0.04-0.5%, prawns 0.08-0.1% in the diet. Deficiency causes loss of appetite, poor growth, sluggishness and convulsion followed by tetany in fish. Excess of dietary Ca and P increases the magnesium requirement.

#### 4.6.3 zinc

Zinc is involved in nucleic acid synthesis and also a component of metallo-enzymes. Deficiency results in short body dwarfism, cataract, poor appetite, poor growth, mortality, skin and fin erosion. Plant proteins containing phytic acid bind zinc rendering it unavailable. Dietary requirement of rainbow trout 15-30 mg/kg diet; in prawn diets 50-100 mg - larger levels may be required to prevent calcium antagonism. Zinc sulphate ( $ZnSO_4$ ) is commonly used as a source of Zinc. Fish can tolerate relatively high levels of zinc (upto several hundreds mg).

#### 4.6.4. Iron

Component of haeme in haemoglobin, cytochromes, peroxidases etc., and involved in respiratory processes including oxidation reduction and electron transport. Essential to maintain haemoglobin hematocrit value, and mean corpuscle diameter. A minimum dietary iron concentration of 150 mg/kg is required to prevent iron-deficiency symptoms such as hypochromic, microcytic anaemia in red sea bream and common carp. Ferric citrate, ferrous sulphate are used in mineral premix. Ferrous iron is better absorbed than ferric iron.

#### 4.6.5. Copper

Component of haeme in haemocyanin of crustaceans; co-factor in tyrosinase and ascorbic acid oxidase. Inclusion of 3.0 mg/kg copper in the diet improved the growth of common carp fingerlings. Copper requirements of channel catfish do not exceed 1.5 mg/kg and the recommended level for prawn is 25 mg/kg.

#### 4.6.6. Manganese

Co-factor for arginase and certain other metabolic enzymes; involved in erythrocyte regeneration and bone formation. In rainbow trout and carp 12-13 mg/kg manganese in the diet improved growth. Manganese sulphate ( $MnSO_4$ ) and manganese chloride ( $MnCl_2$ ) are found to be good sources of manganese in fish diets. Manganese deficiency leads to abnormal curvature of the backbone and malformation of the tail in rainbow trout.

#### 4.6.7 Selenium :

Component of the metalloenzyme glutathione peroxidase, which contributes to the antioxidant defence mechanisms of the fish. Dietary selenium and vitamin E function synergistically in the prevention of oxidative damage. Deficiency of selenium causes muscular dystrophy and exudative diathesis.

Selenium levels between 0.15 and 0.38 mg/kg seems to be adequate. High dietary levels (13mg/kg) result in uncoordinated spiral swimming behaviour 12-24 hours before death in fish.

**4.6.8 Iodine**

Constituent of thyroxine; regulates oxygen use. In Chinook salmon 0.6 mg iodide/kg dry diet is recommended. Deficiency causes thyroid hyperplasia (goitre).

**4.6.9 Other mineral elements**

Cobalt is an essential component of vitamin B<sub>12</sub> (cyanocobalamin). Addition of cobalt chloride or cobalt nitrate to the feed enhanced the growth and haemoglobin formation in carp.

Sulfur is very important as a component of sulfur amino acids, methionine, cystine, taurine, glutathione, heparine etc. About 200 mg of sulfur is recommended per kg of prawn feed.

Sodium and potassium are added in relatively higher levels (Table 9). Molybdenum, chlorine, fluorine and chromium are required in trace levels.

**Table 9 Mineral requirements in diets (per kg feed)**

Calcium (g)	: trout and salmon 0.2-0.3 common carp 0.28 red sea bream 3.4; Japanese eel 2.7; Indian and Chinese carps 5-18; other fish (in general) 5; prawns 10-18
Phosphorus (g):	trout and salmon 7-8; common carp 6-7; tilapia 9; red sea bream 6.8; Japanese eel 2.9; channel catfish 4-7; Indian and Chinese carps 5-7; sea bass, sea bream 7-8; prawns available P 9, total phosphorus 18; Ca: P ratio 1:1 - 1: 6:1.
Magnesium (g) :	trout and salmon 0.5-0.7; carp 0.4-0.5; fish (in general) 0.5; prawns 0.8-1.0

Copper (mg)	: trout and salmon 3; channel catfish 1.5; fish (in general) 1-4; prawns 25.
Manganese (mg)	: trout and salmon 12-13; carp 4; fish (in general) 20-25, prawns 20;
Zinc (mg)	: trout and salmon 15-30; fish (in general) 30-100; prawns 50-100
Iron (mg)	: carp 150 fish (in general) 50-100 prawns 5-20
Cobalt (mg)	: fish (in general) 5-10; prawns 10
Selenium (mg)	: trout and salmon 0.1-0.4; prawn 1
Salt (g)	: channel catfish 5-10
Sodium (g)	: fish (in general) 1-3; prawns 6
Potassium (g)	: fish (in general) 1-3; prawns 9
Sulfur (g)	: fish 3-5; prawns 0.2
Chlorine (mg)	: fish 1-5
Molybdenum, chromium, Fluorine	- trace levels

Source: ADCP (1983), Cho et. al., (1985) New (1987)

**Table 10 Ingredients used for mineral premix**

Mineral	Ingredients used
Calcium	: Calcium carbonate, calcium phosphate, di-calcium phosphate, calcium lactate
Phosphorus	: mono sodium, potassium or calcium phosphates, dicalcium phosphate
Magnesium	: magnesium carbonate, magnesium sulphate
Sodium	: sodium chloride
Potassium	: potassium chloride, potassium sulphate
Zinc	: zinc sulphate ( $ZnSO_4 \cdot 7 H_2O$ ), zinc oxide
Copper	: copper sulphate ( $CuSO_4 \cdot 5H_2O$ ), copper oxide
Manganese	: manganese sulphate ( $MnSO_4 \cdot H_2O$ ),

	mangenuous oxide
Iron	: ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) ferrous gluconate, ferrous carbonate
Iodine	: potassium iodide, potassium iodata, ethylene diaminedihydro iodide (for freshwater prawns)
Cobalt	: cobal chloride, cobalt sulphate
Selenium	: sodium selenite.

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## 5. FEED INGREDIENTS AND THEIR POTENTIAL NUTRITIVE VALUE

Information on the raw materials available in different parts of the country and their nutritive value is important for identifying ingredients for incorporation into practical feeds of aquatic organisms. While data on potential nutritive value of a large variety of feed ingredients are available, data on their biological value for fish and prawns are rather limited.

### 5.1 *Ingredients of animal origin*

#### 5.1.1 Fisheries by-products :

Fish meal, fish protein concentrate, fish solubles, prawn head meal, small prawn (shrimp) meal, crab meal, squilla (mantis-shrimp) meal, squid and cuttle fish waste meal, clam meal, mussel meal, gastropod meal, marine animal lipids including fish oil and fish liver oil; crab shell, prawn shell etc. are important ingredients for aquaculture feeds.

#### Fish meal

Fish meal is perhaps the most abundant animal protein source commercially produced and marketed in several countries. The quality of fish meal depends upon the species, size, maturity stage, the freshness of the source material and the process employed in manufacturing the meal. The best meals are manufactured by steam cooking fish, pressing to remove water and oil followed by drying. Fish meals prepared from fresh single species of fish are the best ingredients in the diets of prawns and carnivorous finfish species. In India, quality fish meal production is extremely limited and is provisionally given as less than 10000 tonnes per year.

The fish meal as marketed in India is pulverised dry fish. Most of this fish powder is prepared from beach dried miscellaneous fish and crustaceans, often termed trash fish. Some major quality problems associated with dry fish powder area:

\* The trash fish is invariably spoiled before and during processing due to enzymatic and microbial degradation,

thereby the attractability is lost; lipids become rancid and unsaturated fatty acids undergo oxidation.

- \* Variation in species and size composition of the trash fish renders it difficult to produce sustained quality fish meal.
- \* High levels of acid-insoluble ash (in some samples as high as 16%) representing sand and silica particles.
- \* High levels of salt (in some samples as high as 12%) much more than the preferred salt level (less than 2%).
- \* Moisture content also highly variable.
- \* High levels of chitin, when crustacean materials dominate.
- \* One of the reasons for the relatively poor performance of indigenous prawn feeds is the use of poor quality fish meal.

The protein content of fish meal often range from 50 to 65% and fish protein concentrate 70 to 80% (not produced in India). The protein level in dry fish powder ranges from 40 to 55%. The major variation is brought about by the ash levels. Ash content ranges from 17 to 30%. Fish meal prepared from unspoiled fish is a good source of calcium 2.2 - 7%, phosphorous 1.9 to 3.8, iron, copper and zinc and other trace elements; rich source of vitamins choline, biotin, pantotenic acid, niacin, cyanocobalamin and all the essential amino acids. The level of fish meal in prawn feeds ranges from 20-40%, carnivorous fish feeds 20-40% and omnivore feeds 5-15%. Inosine, phosphate and certain L-amino acids in fish meal have roles as chemoattractants in fish and prawns. Fish protein concentrate and fish solubles are excellent ingredients in prawn feeds manufactured for semi-intensive and intensive culture.

Over cooking of fish meal causes protein degradation, while under cooking causes microbial contamination, mainly by *Salmonella* bacteria. Adulteration with urea (nitrogen content 46%) to boost the crude protein level is one problem with low grade fish meals. Quality standards for fish meal and fish oil (cho et al., 1985) is given in Table 11.

### Fish solubles

Fish solubles are the water material remaining after the oil is removed from the liquid pressed out during the manufacture of fish meal. The condensed and dried fish solubles when included in small quantities in aqua feeds serve as an attractant. It is also high in B group vitamins and contains an unidentified growth factor.

### Fish silage

Acid silage is prepared from ground trash fish, waste fish head, viscera prawn waste, small crabs and mixed with a mixture of acids such as HCl/H<sub>2</sub>SO<sub>4</sub> and propionic or formic acid to cause liquifaction and to prevent bacterial decomposition. Biological fish silage is produced by introducing lactic acid bacteria into a ground fish carbohydrate mixture. The lactic acid bacteria produce the acid necessary to preserve the fish. The resulting liquid product can be used as an ingredient mainly in fish feeds.

### Crustacean meals

Meals obtained from small prawns, prawn-heads, mantis shrimp, crabs and krill (euphausids) are important ingredients for prawn feeds. Fresh crustacean meals are good attractants for prawns. Crude protein level varies from 30 to 50% depending upon the size and species. Ash content ranges from 25 to 40% and chitin is found to be in levels as high as 16%. Good source of cholesterol, carotenoid pigments, chitin, calcium, iron, manganese, choline, niacin, pantothenic acid and cyanocobalamin meal should be prepared from fresh materials. In prawn feeds, inclusion rates range from 5 to 15% and meals from small prawns upto 25%.

### Molluscan meals

Fresh meat or meals prepared from fresh small squids and cuttle fish, and their wastes, clams, mussels and snails are excellent ingredient for prawn feed. Availability in commercial quantities and high cost restrict their use to about 5 to 10%. These ingredients are found to have excellent attractant and growth promoting properties when added in diets. Their essential amino acids, fatty acids, vitamin and mineral profiles are superior to most other ingredients.

### 5.1.2. Poultry feather and by-product meals

Meal prepared from poultry viscera, feet, heads and undeveloped eggs has crude protein in the range of 45 to 60%. It is a good source of all the essential amino acids; calcium, phosphorus, iron and zinc; choline, niacin, pantothenic acid riboflavin and cyanocobalamin.

Poultry feathers are collected, ground and cooked under pressure to produce feather meal. Crude protein levels range from 75 to 85%, 2-3 % lipids, 1-2 % fibre, 0.75 % phosphorus and 1.5 to 1.6 % sulphur but poor source of other minerals. Severely deficient in several essential amino acids. It is a better source for prawn than fish diets.

### 5.1.3. Slaughter house wastes

Dried and pulverised or spray dried blood meal is a high protein ingredient containing 75-85% crude protein. It leucine/ isoleucine ratio is unbalanced. It is a poor source of most of the minerals, but rich source of iron containing as high as 2784 mg/kg dry matter Niacin and cyanocobalamin are found in good levels. Spray-dried blood meal has 9-11 % lysine with an availability of 80-90 %.

Meat meal is prepared by drying meat wastes, pulverised and used as an ingredient. Fresh meat waste can also be used in moist diets. The material should not be spoiled or containamited before use.

Meat and bone meal prepared from cattle wastes have relatively high fat content 8-11 %. about 30% ash, 4-5% phosphorus, 8-10% calcium and 45-50 % protein. Collagen levels are high and hence the protein digestibility is low.

Liver meal is a rich source of B group of vitamin.

### 5.1.4. Milk by-products

Whole milk power and skimmed milk powder have essential amino acids profile close to that of chicken egg protein. Can be used in feeds if available at reasonable cost. Dried whey, a by-product from the production of cheese is utilized in diets of

salmonids upto 10%; they contains high level of lactose (about 65%) and 13% crude protein.

#### 5.1.5. *Chicken egg*

Without shells has about 46% crude protein, 43% lipids and 4% ash. Good source of all the essential amino acids, pantothenic acid, cyanocobalamin, riboflavin, iron and zinc. Particularly beneficial as an ingredient in hatchery and nursery feeds for fish and prawns.

#### 5.1.6. *Silkworm pupae*

Can be used as an ingredient in fish and prawn feeds at low level. But, high level of chitin, and the lipid which is prone to rancidity, pose problems. Solvent extraction of lipids may improve the quality.

### 5.2. Ingredients of plant origin:

#### 5.2.1. *Oil cakes and meals*

Soybean oil cake:

Among the major plant protein sources, soybean is considered as the best protein source, in terms of its protein content and amino acids profile. The energy content will vary the level of residual oil and percentage fibre in the meal. Methionine is the first limiting amino acid. Lysine and threonine levels are less than that of fish meals. With essential amino acid supplementation, it is a potential partial replacement for fish meal. Contains protease inhibitors, the enzyme urease; haemagglutinins and glycosides, but these are destroyed by heat. The heat treatment results reduced biological availability of both lysine and cystine and partial destruction of arginine, tryptophan, histidine and serine. Phytates, lipoxidase antivitamin A, antivitamin D are other anti-nutrients found in soybeans. About 50 to 70% of the phosphorus is present in the form of phytic acid which is biologically not available. During processing phytate protein-mineral complexes from resulting in decreased availability of calcium, zinc, copper, manganese, molybdenum and iron. Among the vitamins choline is found in

relatively high levels; but niacin, riboflavin, pantothenic acid and thiamine are significantly reduced (losses of 10-75%) during heat treatment.

Levels of incorporation in feeds for carps, tilapia, channel catfish is as high as 50%; sea bass, grouper, trout 10-20% and prawns upto 40% Dehulled and solvent extracted soybeans yield a meal with a crude protein level of 46-48% mechanically extracted soybeans produce a meal of about 38-42% protein.

Cotton seed oil cake:

Protein content varies from 29 to 42% depending on the amount of hull removed. The content as well as biological availability of lysine, threonine, tryptophan and methionine is lower than that of soybean oil cake. It is a good source of thiamine and vitamin E. The presence of the polyphenolic pigment gossypol and cyclopropenoic fatty acids adversely affects its nutritional value. Glandless variety of cotton seed is almost free from gossypol. Crude fibre level are high. Low-gossypol meals contain less than 0.40% free gossypol.

Groundnut oil cake:

It is commonly obtained from groundnut kernels, after decortication of husks or pods, Crude protein ranges from 35 to 42%. It is lower in lysine, tryptophan, threonine and methionine than soybean cake. It is a good source of magnesium, sulphur and potassium. Good source of vitamins, niacin, pantothenic acid, thiamine, while choline and vitamin E levels are low. Highly prone to fungal growth and mycotoxin (aflatoxin) in highly humid area.

Sunflower oil cake:

The protein quality of sunflower oil cake is regarded to be lower than that of soybean cake, with lysine being especially deficient. But levels of methionine and cystine considered to be higher than that of soybean cake. Heat treatment during processing severely depresses the availability of arginine, threonine, leucine, lysine and tryptophan. It is higher in crude fibre than soybean cake. B group vitamins and carotene are found in good levels.

#### Safflower oil cake:

The protein is poorer in essential amino acids than soybean and safflower cakes. Vitamin profile is somewhat superior to soybean cake.

#### Rapeseed oil cake:

Composition has been shown to vary depending on the growing conditions. Crude fibre level can be as high as 16%. The available lysine and threonine content is approximately 10% lower than that in soyabean cake; but methionine and cystine levels are higher. The presence of erucic acid and glucosinolates adversely affects its nutritional value.

#### Gingelly oil cake:

The hull of the gingelly seeds accounts for 15 to 20% of the whole seed, which contains high level of oxalic acid and phytic acids. These acids impart a bitter taste to the oil cake and complexes with calcium and other minerals, rendering them nutritionally unavailable. Dehulled, expeller processed oil cake is high in methionine, cystine and tryptophan, but low in lysine. Niacin and pyridoxine levels are tryptophan, but low in lysine. Niacin and pyridoxine level are quite high.

#### Linseed oil cake:

The hull has a mucilage coating which contains a water dispersible carbohydrate which has low digestibility. Amino acids profile is inferior to soybean cake with very low level of lysine and methionine; the presence of pyridoxine antagonist, linatine, induces pyridoxine deficiency; contains an enzyme and a glucoside that produces prussic acid, but during processing the enzyme gets destroyed.

#### Coconut oil cake:

Has low protein content (average 24.6%) prone to rancidity; high fiber content (average 14.5%); deficient in methionine and cystine; rich in potassium and iron; good levels of choline and niacin.

Mustard oil cake.

Used in carp diets; non-detoxified meal contains toxins as in rape seed.

### 5.2.2. *Cereal products*

Ground broken rice, wheat, sorghum, millets and maize can be used as ingredients considering their cost, availability and carbohydrate levels. Among the cereal by-products the following are important.

Rice bran :

Crude protein 10-12% crude fibre 12 to 18% depending on the level of husk or hulls; total lipids 7 to 12%; ash 8 to 12%; good source of energy and B group vitamins. Deoiled rice bran is better in terms of its nutritional profile and keeping quality lipid; is prone to rancidity.

Wheat flour and bran :

Good source of energy; crude protein 10-14%; crude fibre 12-18%; ash 6-18%; good source of phosphorus, potassium, manganese and zinc; niacin, pantothenic acid and biotin. Ground whole wheat flour is widely used in prawn feeds. Gelatinisation improves water stability.

Corn gluten :

Crude protein 20 to 30%; arginine and lysine levels are low; good source of iron and zinc; niacin and vitamin E.

Sorghum and millets:

Crude protein 8 to 12%; poor profile of amino acids, minerals and vitamins; can be used as an energy feed.

### 5.2.3. *Root crops*

Tapioca, sugar beet molasses, and meals made from potatoes and sweet potatoes have been used for their qualities, in

aquaculture feeds. Presence of hydrocyanic acid should be monitored in tapioca.

#### 5.2.4. Yeast

Brewers yeast is the dried sterilised, unextracted yeast (*Saccharomyces*) resulting as a by-product from the brewing of beer. Crude protein 40-45%; crude fat 1%; crude fibre 2-7%; ash 6-9%; good source of phosphorus, potassium and iron; rich source of B group vitamins biotin, choline, niacin, folic acid, pantothenic acid, pyridoxine, riboflavin and thiamine. Lactic yeast is a good ingredient for prawn feed.

#### 5.2.5. Molasses (Dehydrated)

Crude protein 8 to 10%; ash 10-16%; fibre 6-10%; potassium, copper, iron and manganese levels high.

#### 5.2.6. Alfalfa

Crude protein 13-17%; crude fibre 25-30%; good source of minerals-calcium, potassium, iron, manganese and zinc, and vitamins choline, biotin, niacin, pantothenic acid, riboflavin and vitamin E.

#### 5.2.7. Spirulina

Crude protein 55 to 65% with good levels of most of the essential amino acids, calcium, phosphorus, iron, magnesium, potassium, vitamin B<sub>12</sub> 1.5µg/g and folic acid 0.39µg/g.

#### 5.2.8. Miscellaneous ingredients

Leaf meal from ipil - ipil plant is used in the diets of fish and prawn; inclusion level range from 4 - 5% in practical grow-out diets. Ipil - ipil leaves and seeds contain the glucoside mimosine which gets reduced in level if soaked in water and dried. The dried

powdered leaf is used in feeds for prawns and fish.

Sea weed meals are good sources of trace minerals and vitamin A and can be used in low levels 2 - 3% in prawn diets.

Yeasts grown on paraffins, industrial wastes such as molasses, fruit wastes and milk wastes are receiving increasing importance as fish feeds. Amino acid levels vary according to the source. Some are high in lysine but low in methionine.

New sources of protein include earthworm meal, algal meal, insect meal, aquatic weeds meal, leaf-meal from wild plants, silage of plant and animal origin. Some of these require extensive biological evaluation before use in compounded feeds.

**Table 11 Quality standards for fish meal and oil**

1. Fish meal		2. Fish oil	
Crude protein	> 68%	Peroxide value	< 5 meg/kg
Lipid	< 10%	Anisidine value	< 10 meg/kg
Ash (total)	< 13%	Total pesticides	< 0.4 ppm
Salt (NaCl)	< 3%	PCBs	< 0.6 ppm
Moisture	< 10%	Nitrogen	> 1%
Ammonia - N	< 0.2%	Moisture	< 1%
Antioxidant		Antioxidant (Liquid)	500 ppm
(sprayed liquid form)	200 ppm	Deaerate and mix antioxidants bub-	
Processing: steam processed;		bling nitrogen gas before storage in	
ground finer than 0.25 mm		airtight containers.	

Source: Cho et al., 1985

Table 12 Proximate composition of ingredients

Ingredients	As % dry matter			
	Crude Protein	Total Lipids	Crude Fibre	Ash
Fish meal	52-60	5-12	1-4	22-38
Fish meal (Anchovies)	58-64	6-8	1-3	26-28
Prawn meal (small prawns)	58-65	4-7	4-7	21-26
Clam meal ( <i>Meretrix</i> sp)	52-58	6-9	-	7-9
Clam meal ( <i>Villorita</i> sp)	38.9-58	7-12	-	5-9
Prawn-head meal	34-45	4-7	11-18	36-44
Slaughter-house Waste	54-65	9-26	-	5-9
Blood mead	79-88	1-2	-	4-6
Meat meal	44-52	8-11	2-4	24-31
Poultry waste	52-56	16-35	-	9-18
Silkworm pupae	48-53	26-30	6-8	7-11
Silkworm pupae (Solvent extracted)	77.6	1.0	4.3	7.3
Brewer's yeast	44.2	1.4	3.0	6.8
Spirulina	55-68	6-8	1-3	8-10
Alfalfa	16-24	2-4	16-30	10-16
Groundnut cake	41-43	3-8	6-8.5	4-8
Soybean cake	42-48	2-7	6-8	5-7
Cotton seed cake	36-44	4-8	16-22	6-9
Sunflower oil cake	38-47	4-6	14-16	6-7
Coconut oil cake	22-28	6-9	12-14	18.22
Rice bran (deolied)	15-17	1-2	14-16	16-20
Rice bran	12-14	5-9	15-20	14-28
Wheat flour	9-12	3-4	6-8	4-6
Wheat bran	12-14	6-8	10-14	6-8
Maize	10-12	4-6	4-8	6-9
Sorghum	8-10	2-5.3	1.8-3	2-4
Horse-gram	16.20	1.1-2.2	6-8	7-9
Tapioca chips	1.5-2.5	0.4-0.6	2-6	2-4

Table 13 Amino Acids (%) profile of selected ingredients on dry matter basis

Ingredients	CP	ARG	HIS	ISO	LEu	LYs	MET	CYs	PHE	TYR	THGR	TRY	VAL
Fish Meal (Anchovy)	65	4.11	1.76	3.38	5.43	5.43	2.16	0.66	0.03	2.44	3.0	0.82	3.5
Shrimp Meal	44	2.79	1.07	1.86	2.98	2.41	0.91	0.66	1.76	1.47	1.58	0.4	2.03
Chicken egg white	85	4.96	1.93	5.18	7.4	5.24	3.3	2.1	5.35	3.41	3.78	1.31	6.36
Whole egg	47	3.06	1.15	2.99	4.2	3.23	1.54	1.14	2.70	1.99	2.35	0.76	3.44
Blood Meal	85	3.88	5.59	0.98	11.86	8.06	0.95	0.78	6.36	2.44	3.93	1.13	8.13
Meat Meal	52	3.6	1.02	1.75	3.19	3.23	0.7	0.65	1.81	0.96	1.64	0.34	2.52
Poultry													
by-product Meal	56	3.77	1.01	2.38	4.0	2.89	1.06	0.92	1.84	0.94	1.94	0.46	2.86
Hydrolysed feathers	85	7.05	0.99	4.06	6.94	2.32	0.55	3.24	3.05	2.32	3.96	0.52	6.48
Soybean flour	50-52	7.7	2.7	5.0	7.8	6.5	1.4	1.5	5.0	3.5	4.1	1.5	5.1
Soybean Meal	46	2.81	0.90	1.65	2.75	2.47	0.44	0.83	1.79	1.36	0.71	1.62	1.06
Groundnut Meal	42	4.55	0.95	1.76	2.7	1.77	0.42	0.73	2.04	1.51	1.16	0.48	1.88
Sunflower Meal	46	4.42	1.23	2.25	3.83	1.92	1.16	0.74	2.36	1.39	1.93	0.61	2.6

CP = Crude Protein

## 6. ADDITIVES

A variety of substances are added in aquaculture feeds to protect the labile nutrients, to improve nutrients availability and utilization by the cultured animals. Binders, antioxidants, mould inhibitors, antimicrobial agents, attractants, growth promoters, medicants, pigments etc are often included. The substance selected and used should not have any harmful effect to the cultured animal or to the consumers, should not react with the feed ingredients and alter the nutritional quality of the feed negatively, and should not reduce the desirable qualities of the meat produced by way of affecting its taste, appearance, flavour and texture. The substance should be available in sufficient quantities at reasonable cost.

### 6.1. Binders

Binders are essential to provide the desired water stability to the feeds, to prevent disintegration of feeds and leaching of nutrients into the water. The selection and inclusion levels of a binder are dependent on the binding capacity and cost. Gelatinised starch from tapioca, wheat flour and rice flour are quite adequate in most feeds. Recommended levels of binders guar gum, gum-acacia, are 1-2%; gelatin, collagen, alginates, carrageenan and agar 2-5%; and wheat gluten (10-12%) and starch from wheat flour and tapioca could be used in relatively high levels (upto 30%). A mixture of some of these binders may be more effective and economical to use. Carrageenan and chitosan can be used in larval feeds also.

### 6.2. Antioxidants

Antioxidants are essential to protect the fatty acids, and other oxidizable components in the feeds. They can be added to lipids or the vitamin premix during manufacture of feeds. Lipids containing polyunsaturated fatty acids on exposure to air, light, heat and moisture, are highly prone to oxidation. The oxidation products may combine with vitamins and amino acids and reduce their bioavailability in feeds. Marine lipids (fish oil) and oil-rich fish meals should be specially protected from oxidation

by adding antioxidants. The factors which accelerate lipid oxidation in feed ingredients and finished feeds are lipolytic enzymes, hematin in fish and meat meals, peroxides, ultra violet light, high temperature, and trace metals notably copper, iron, cobalt and zinc. The most commonly used antioxidants in feeds are (i) ethoxyquin 0.015%, (ii) butylated hydroxyanisole 0.2%, (iii) butylated hydroxy toluene 0.2%. Natural antioxidants which go as components of feed include ascorbic acid and vitamin E.

### **6.3. Antimicrobial agents.**

Feed ingredients and formulated diets, being highly nutritive, under unfavourable conditions of storage, are susceptible for the growth of microorganisms - bacteria, yeast and fungi. The biggest problem is with fungi which grow rapidly at moisture content exceeding 13 %, relative humidity of 70 to 90 % and temperature 25°C such as found in tropics. *Aspergillus* spp, *Fusarium* spp., and *Penicillium* spp. are associated with this spoilage and cause extensive damage to feeds and ingredients. They alter the nutritional status, cause bad flavour and taste, staleness, and produce highly toxic and carcinogenic mycotoxins (aflatoxins, trichothecenes, ochratoxin and zearalenone, T-2 toxine etc.). Thus fungi infestation of feeds affects the shelf-life and results in substantial economic loss. Preservatives are useful in controlling fungi. The most widely used preservatives are sodium and calcium propionates at levels ranging from 0.1 - 0.25%. Sorbic acid, potassium sorbate, sodium sorbate, propionic acid, calcium sorbate, menadione, sodium benzoate etc, can also be used as preservatives.

In aquaculture systems, the cultured animals are predisposed to pathogenic infection. In order to achieve maximum production and feed efficiency these infections should be prevented or controlled. Certain broad spectrum antibiotics are used in medicated feeds to control such out-breaks of diseases. Some of these are oxolinic acid, oxytetracycline, terramycin etc.

### **6.4. Chemo - attractants and feeding stimulants**

These are substances that, induce feeding behaviour in

animals and help to improve feed intake. Free amino acids and nucleotides are the most important ones. Some species of finfish are known to require specific feeding stimulants (Table 14). In general, a mixture of L-amino acid, glycine - betaine, inosine or inosine - 5 phosphate are considered as 'Universal feeding stimulants' for fish (Mackie and Mitchel, 1985). Inosine and inosine phosphate are found in relatively high levels in finfish and invertebrate tissues. During spoilage of fish inosine will get decomposed to inactive hypoxanthine. Squid, shrimp, clam, mussel and ptychaete extracts are known to be excellent natural attractants and feeding stimulants for prawns and carnivorous fastidious fin fish such as sea bass and red sea bream. Artemia powder is an attractant for European sea bass and mussel meat for *Lates calcarifer*. Among individual amino acids, glycine and taurine were found to be attractants for *Penaeus indicus*. A mixture of L - amino acids, containing alanine, glycine, proline, taurine and betaine were found to be feed attractants for *Penaeus japonicus*. Trimethyl ammonium hydrochloride (TMAH) a volatile substance is also considered to improve feed intake in prawn.

**Table 14 Feeding stimulants identified for fish and prawn species**

Feeding stimulant	Fish/Prawn
1. Free L-amino acids present in all animal tissue	Rainbow trout, sea bass, European eel, Japanese eel.
2. D-amino acids	Not effective
3. Glycine, L-proline and L-alamine mixture	Most effective feeding stimulant for European eel, Japanese eel and sea bass
4. Inosine and inosine-5' monophosphate	Specific feeding stimulants for turbot and krill
5. Inosine 5' - phosphate plus L-amino acids	Yellow tail
6. Glycine-betaine plus	Red sea bream, Dover sole, Puffer fish

- |  |              |
|--|--------------|
| 7. L-alanine, glycine,<br>L-histidine, L-proline plus \      | Japanese eel |
| uridine 5' monophosphate                                     |              |
| 8. L-alanine, glycine, proline<br>and taurine; betaine, TMAH | Prawns       |

### 6.5. Pigments

Colour of fish and prawn is a very important characteristic affecting market price. Cultured animals should have the natural colour and appearance, as that of the wild ones. Carotenoids are the most important among substances which render colour. Fish and crustaceans can not synthesize the pigments, but can alter the molecules by oxidation. In the case of crustaceans, carotenoids have an important function in reproduction. Addition of carotenoids to brood stock diets resulted in shortening of the maturation period, an increase in egg numbers, enhanced hatchability of the eggs and survival of the larvae. The carotenoids may also have a role in the immune system in helping prevent disease problems. Addition of 30-35 ppm astaxanthin improved pigmentation in *Penaeus monodon* after eight weeks, with light-brown and greenish brown individuals. Crustaceans and polychaetes are very good source of carotenoids.  $\beta$  - carotene and its oxidative derivatives - cryptoxanthin, zeaxanthin, canthaxanthin and astaxanthin are the preferred carotenoids; but their efficacy in improving colour differs markedly.

### 6.6. Anabolic agents

In order to improve growth above the physiological maximum, the species must be either genetically manipulated or given a substance which will act pharmacologically to improve the metabolic or digestive efficiency, and to promote protein deposition and hence growth (Matty, 1988). The most successfully used hormone for growth promotion in fish culture is 17  $\alpha$  methyltestosterone, which is effective in doses as low as 2 mg/kg diet. The other potential growth promoting hormones are thyroxine, insulin, triiodothyronine, growth hormone and

recombinant bovine somatotropin.

Addition of synthetic enzyme preparaton containing amylolytic and proteolytic activity at levels of 0.01, 0.02 and 0.1% improved the growth of carp (*Cyprinus carpio*), the growth increase being 26% for 0.1% and 12-13% for 0.01 and 0.2% enzyme extract.

The antibiotic terramycin act as a growth stimulant in carp. Addition of 6000 to 10,000 units per kg of dry diet ensured an additional production of 5-15 %. Another antibiotic drug called monesin (inophores) has been shown to promote growth in shrimp.

Glucosamine, the chitin precursor, has been found to promote growth in prawns. Substances like zeolite (1-2%), thyroproteins and phytosterols (0.1%) are also suspected to mprove growth performance. Enzymes, like papin at a level of 0.1 to 0.2% and bile satls 20 g/tonne of feed have shown to promote growth in prawns. Multi-enzyme preparation will be more useful than individual enzymes.

In the estuarine grouper 17  $\alpha$  methyltestosterone accelerated growth at a dietary concentration of 9 mg/kg, the average growth increment over that of the control was 43.4%. Another feed additive nitrovin (trade name Payzone) containing not less than 2.2% of the growth promoting compound has accelerated growth by an average of 62.8% over that of the control, at a concentration of 1 g/kg feed (Chua and Teng 1979).

### 6.7. Miscellaneous additives

Aspirin is used in tilapia fry (100 mg size) diet at a concentration of 1 g/kg feed, as an anti-stress factor before transferring the fry to seawater race-ways (34-36 ppt salinity). Sorbital (liver-health), glycerololeate (water-oil emulsifier), fish autolysate (anti-stress), carnitine (for better utilization of lipid), sodium polyphosphate (antioxidant, anti-viral effect), are some of the additives used in fish feeds.

## **7. MANUFACTURE OF AQUACULTURE FEEDS**

### **7.1. Feed types**

Artificial feeds used in aquaculture are of the following types

#### *7.1.1. Wet feeds*

Feeds containing moisture levels in the range 45-70% are prepared from high moisture ingredients such as trash fish, fishery waste, slaughter - house waste etc. The feeds are made at the farm shed on a day - to - day basis and fed mainly to carnivorous fish such as sea bass, sea bream, eels etc.

#### *7.1.2. Moist feeds*

Moist feeds contain moisture level in the range 25-45% and are made from a mixture of high moisture ingredients, as in wet feeds, and dry pulverised ingredients. Semi-moist diets contain moisture in the range 15 to 25% and have a minor contribution from high moisture ingredients.

Wet, moist and semi-moist diets are considered to be more palatable to most species because of the soft consistency, and good growth and feed efficiency are achieved.

The major disadvantages of these feeds are (i) the transportation and storage under refrigeration until use to prevent spoilage, (ii) irregular availability of fresh raw fish and other animal wastes in adequate quantities, (iii) introduction of pathogens, particularly from fishery wastes, if not adequately pasteurised, (iv) improper transportation and storage damages certain labile vitamins and lipids, and favour propagation of fungi and bacteria in such feeds, (v) unconsumed feeds may affect the water quality.

#### *7.1.3. Dry feeds*

Dry feeds contain moisture in the range 7-13%. They

are relatively easy to manufacture, transport, store and convenient to dispense into the culture systems. Other advantages include bulk purchase and storage of ingredients, option to select a wide variety of ingredients with specific nutritional characteristics, and the possibility of a quality controlled, least-cost, feed production programmed. Dry feeds can be produced in different sizes to suit the specific needs of the grow-out stages of the cultured species, weaning diets for larvae to specialised diets for broodstock. Dry feeds also permit production of specialised feeds, such as medicated feeds incorporated with specific medicines/antibiotics, required to control any disease out-breaks, hormones incorporated feeds to reduce monosex individuals or for growth promotion or to induce maturation in fish and prawns.

Dry feeds may be either prepared using dry ingredients alone or a mixture of wet and dry ingredients, adequately processed and dried. Dry feeds may be made as meals, pellets, flakes etc.

Meals or mashes are simple mixtures of dry ground ingredients, these could be made into a dough or paste or balls just before feeding at farm site and the feed could be fed to fish and prawns in trays or baskets or feed bags tied to poles in ponds, as is practiced in carp culture systems in India. These type of feeds have poor water-stability and cause water quality problems due to the feed breaking-up and dissolving in water.

Two types of dry pellets are normally produced, floating pellets and sinking pellets, floating pellets for fish species which predominantly feed in the water surface or column, e.g. tilapia, trout, grouper, sea bass, carp etc. Sinking pellets are required for bottom feeders like prawns. Floating pellets enable observation on feeding activity and, to some extent, the health of the fish. But, overheating as required in the extrusion manufacturing process of floating pellets may reduce their nutritional value. Pellets could be crumbled and graded through screens of different mesh to obtain granules required for feeding the different growth stages of fish and prawns.

#### 7.1.4. Larval feeds

The various types of artificial diets that have been considered for weaning larvae are :

##### Minced diets

A feed paste is prepared by homogenising wet or wet and dry ingredients with additions of mineral and vitamin premix and binders and fed as such.

##### Wet microparticulate diets

A custard diet prepared with chicken eggs, prawn flesh, clams, fish solubles, vitamins, minerals, feeding stimulants and wheat flour and homogenising to get fine particles. The desired particle size can be obtained by sieving.

##### Dry microparticulate diets

Preparation of a water-stable matrix of dry ingredients or a mixture of dry, moist and wet ingredients followed by suitable drying (freeze-drying, vacuum drying, oven-drying), grinding and sieving to get desired particles. This is the most widely used type of artificial diet for larval rearing.

##### Spray dried diets

Well mixed finely ground materials are sprayed into air and then dried. Particles ranging in size 50-100 microns are produced and used for rearing marine fish larvae in Japan (Kuronuma and Fukusho, 1984). Ingredient composition of a spray-dried larval feed is given in Table 15.

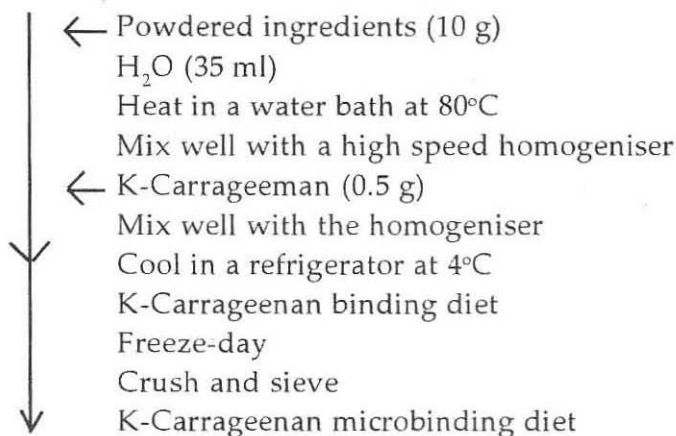
##### Microbounds diets

Powdered diets with a binder, Carrageenan, agar, zein, alginic acid and gelatin microbound diets have been proposed (Kanazawa, 1986). An outline of the procedure for microbound

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diet preparation is given in Fig. 1.

Fig 1 Preparation of a microparticulate diet bound with carrageenan



**Table 15 Ingredient composition of spray dried and vacuum dried larval feeds and their characteristics**

<i>1. Spray-dried feed ingredient</i>	<i>Percentage inclusion</i>
Shrimp (fresh)	20
White fish (fresh)	30
Wheat gluten	15
Milk powder	10
Egg yolk	5
Cow liver	5
Soybean oil	5
Grape sugar	5
Vitamin mixture	5

*Characteristics of feed granules:*

Shape	:	Nearly spherical
Size	:	50 to 100 microns
Solubility	:	Stable for 24 hrs. in water
Swelling	:	15-20% increase in size
Sinking	:	5-10 cm/min.

<i>2. Vacuum-dried feed ingredient</i>	<i>Percentage inclusion</i>
Frozen fish	30.6
Shrimp (fresh)	30.6
Egg yolk	10.2
Wheat Gluten	20.5
Grape sugar	2.0
Vitamin mixture	6.1

*Characteristics of feed granules:*

Shape	:	Nearly spherical
Size	:	240-420 microns
solubility	:	Not dissolved in water for 10 hrs.
Swelling	:	20-30% increase in size
Sinking	:	1.5 cm/min

*Source:* Kuronuma and Fukusho (1984)

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**Microcoated diets**

Prepared by coating microbound diets with some materials such as zien and cholesterol-lecithin.

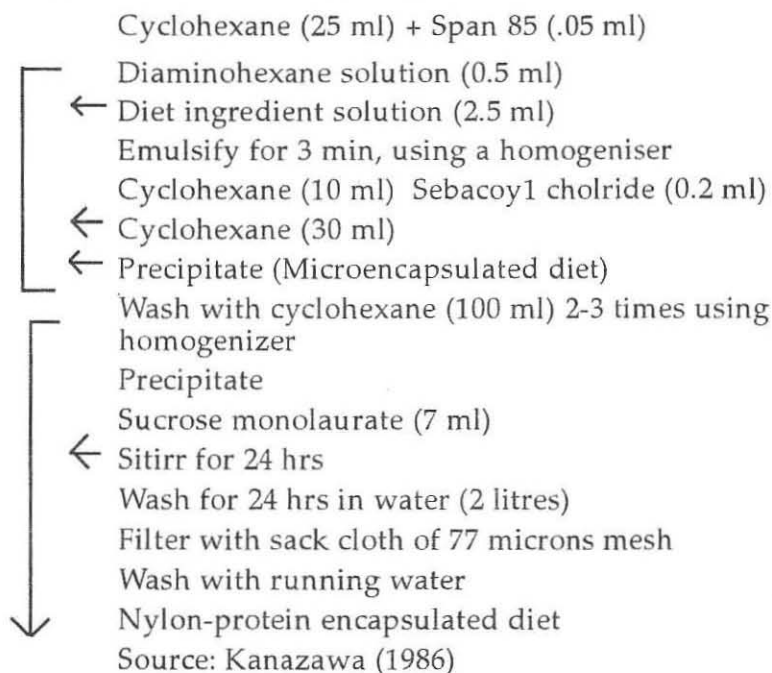
**Microencapsulated diets**

The concept of a miniature packaging assembly (a microcapsule) in which liquids or particulate dietary components are enclosed in a carefully engineered wall, with release under specific macro or microenvironmental conditions has broad

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applications in fish culture (meyers, *et al.*, 1975). Release of the internal nutrient components at active sites can be accomplished by rupture (enzymatic action, pH change or bacterial action) of the capsule wall. A major advantage of capsulated diets is that there is minimal loss of nutrients within the aqueous environment thus minimising organic load in the system and alterations in oxygen and pH levels. The capsules can be produced in a range of size (5 to 700 micron capsules are produced by Frippak) thereby, suitable sizes can be offered to the fish larvae and fry as they grow. Depending upon the capsule wall material Kanazawa (1986) proposed the following categories: nylon-protein microencapsulated diet (MED), gelatin gum acacia MED, egg albumine MED, glycopetide MED and chitosan (MED). An outline of the procedure for nylon protein MED preparation is shown in Fig. 2.

### Fig 2 Preparation of nylon-protein microencapsulated diet



## Flake diets

Flake diets prepared through a double drum dryer processing unit is a potential feed for fish (Meyers, 1979). Flakes can be reduced to small particle sizes by grinding and sieving without reducing the basic stability characteristics. Ingestion rates of the feed could be enhanced by using suitable binders, flavours and colours. Larval stages of striped bass, perch, and Atlantic silverside have been reared on flake particles.

### **7.2. Selection of ingredients and additives**

The choice of a feed ingredient or an additive is dependent on (i) its likely contribution in the finished feed, (ii) its easy availability in sufficient quantities to meet the feed production requirement round the year, (iii) its cost (iv) stability during processing, storage and feeding, (v) its amenability to processing, and (vi) its antinutritional factors status.

Acceptability or palatability of feeds also depend upon the ingredients and additives. For instance, inclusion of higher levels of marine protein and lipids sources prepared from fresh raw material improves the acceptability of feed leading to increased feed intake in carnivorous finfish and marine prawns. Pelletability of the feed and its durability are also affected by the physical characteristics of the ingredients.

### **7.3. Formulation of feeds**

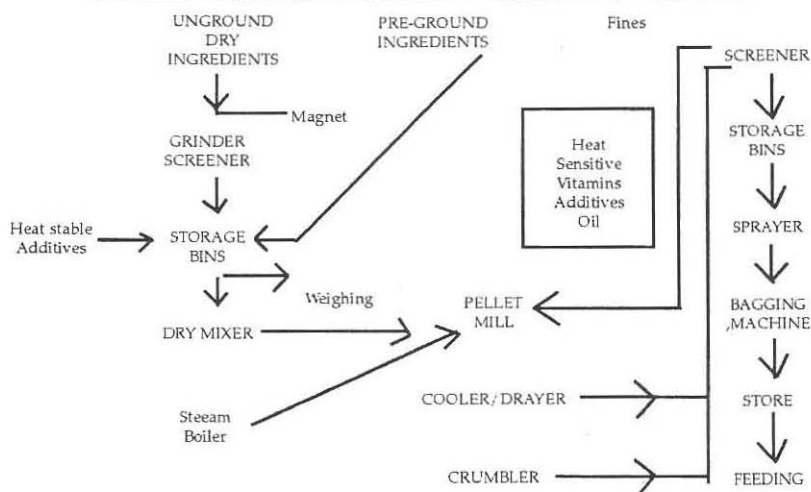
Feed formulation is a specialised exercise and the best formulae are those designed through linear programming (LP) techniques using a computer. The LP techniques allow use of a large variety of ingredients and additives to achieve nutrients balance in feeds at least-cost. However, ingredients and nutrient parameters are selected. For more details please refer to Chow et al. (1980), Hardy (1980) and New (1987).

### **7.4. Feed Processing**

The major steps in dry feed manufacturing process are (Fig. 3)

- \* Procurement of quality ingredients and additives
- \* Storing in ware-house after proper labelling/marketing
- \* Preserving labile ingredients, e.g. oils, vitamins in preferably low-temperature (or cool place)
- \* Accurate weighing of the ingredients (ground and unground) and additives in proportions required for the select formula
- \* Preparing vitamin and mineral premixes
- \* Grinding of unground materials
- \* Microgrinding
- \* Screening
- \* Mixing
- \* Pelleting/extrusion
- \* Cooling/drying
- \* Crumbling
- \* Screening particle segregation
- \* Bagging.
- \* Storage.

FIG-3 FLOW-CHART FOR STEAM-PELLETING



Source: Cho *et al.* 1985 with minor modifications

#### *7.4.1. Premix preparation*

In order to facilitate uniform dispersion of vitamins, minerals and additives that are normally required in small quantities in formulated feeds, a premix is prepared. Each of the ingredient that is to be used in premix preparation must be finely ground ( $< 200\mu$ ). A carrier material also should be ground to uniform level and the two must be mixed thoroughly in a batch type mixer. A dietary ingredient is used as a diluent (wheat flour) to prepare the premix to a weight corresponding to 0.5% of the entire feed mix. The premix is then blended with the feed mix equal to 3-5% of the total mix (30-50 kg/tonne of feed) and the mix is ready for use in the final feed mix.

#### *7.4.2. Grinding*

Grinding of ingredients helps in size reduction and some moisture is removed due to aeration. Drying of feed materials before grinding will improve the grinding efficiency of the mill. Grinding improves feed mixing properties, pelletability, acceptability and digestibility.

Coarse ingredients are conveyed over a permanent magnet in feed mills which removes any metal pieces. Hammer mill and attrition or plate mill are the machinery used for grinding. Hammer mills are most efficient to grind, dry low-fat ingredients; but proper screen selection and feed intake permits grinding many other material. Grinding efficiency of the mill depends upon the number of hammers, their size, arrangement, sharpness, the speed of rotation, the horse power of the motor, the size of the screen mesh used and the type of the material being ground. By selecting the appropriate screen desired particles can be obtained. Grinding of dry fish and other animal by-products may be difficult, but by mixing with other dry ingredients improved grinding can be achieved. Microgrinding of ingredients is necessary for prawn starter feeds.

#### *7.4.3. Mixing*

The objective of mixing is to obtain a homogeneous dispersion of nutrients and additives, so that every unit weight

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of the feed fed to the animal has the same nutritive profile. Feed mixing includes all possible combinations of solids and liquids. Dry powdered materials mix adequately more quickly than moist ingredients or mixtures of the two. The mixing time for a batch of dry or moist ingredients varies according to the design of the equipment. Microingredients such as vitamin and mineral premix and additives should be premixed to an amount 3 to 5% of the total feed mix before incorporation in the mixer. Accurate mixing requires the addition of ingredients in a sequence from batch to batch. The usual practice is to add large volume ingredients first, then those of smaller amount. At least 50% of the total feed mix should be added in the mixer before addition of the feed mix containing the microingredients. Lipids should be sprayed on to the dry feed mixture to ensure their homogeneous distribution. Liquids should be added after all the dry ingredients have been mixed.

Mixing may be either a batch or continuous process. Mixing equipment include horizontal continuous ribbon mixers, non-continuous ribbon mixers, vertical mixers, liquid mixers or blenders. Horizontal types are preferred for aquaculture feeds as they enable to blend small quantities of liquids such as lipids mollasses and also in mixing ingredients with different particle sizes.

Bowl shaped mixers with paddles are the most suitable for moist feeds. Ingredients such as condensed fish or fermentation solubles, molasses or fish oils are often premixed in a bowl type variable speed mixer, blending the liquid with the dry ingredients.

When lipid levels used in feed are high as required for carnivorous fish such as trout, sea bass and groupers, oils could be sprayed on to the pellets after pelleting.

Heat sensitive vitamins, enzymes and other additives can also be sprayed after cooling the pellets. Chemical binders, if used, should be dissolved in cold or hot water and the solution is added to the feed mix.

#### 7.4.4. *Pelleting*

Pelleting involves forcing a soft feed through the holes in a metal die plate to form compact pellets and then cut into a pre-determined size.

Specialised pellet mills for aqua-feed manufacture have a conditioning unit mounted above them, where liquids such as water and molasses can be added to improve pelletability. Instead of water steam may be passed into the feed through a steam injection system.

The general process involves passing a feed mixture through the conditioning chamber, where 4 to 6% water as steam may be added. Moisture provides lubrication for compression and extrusion and the presence of heat causes some gelatinisation of a raw starch in the ingredients, resulting in adhesion.

From the conditioner feed material falls into the centre of the pelleter itself. In the pelleter two or more rollers and feed ploughs push the material through the holes of the die plate. Stationary knives positioned on its outer side cut the pellets to a pre-set length. Within about 20 seconds of the entering the pellet mill, feed attains moisture of 15-16% (ingredients 10-12%) at 80-90°C. During subsequent compression and extrusion, friction further increases feed temperature to nearly 92°C. Pellets, discharged on to a screen belt of a horizontal tunnel drier, are aircooled within ten minutes and dried to moisture below 10%.

Die hole size usually range from 1.5 mm upto 9.5 mm. The thickness of pellet dies can be up to about 90 mm.

For small scale operation, a dough of the feed mix can be prepared and extruded through a mincer extruder and the feed strands could be steamed, dried, crumbled and graded for use.

#### 7.4.5. *Floating pellets*

Floating pellets, with bulk density ranging from 0.25 to 0.3 g/cc could be produced by the extrusion process in which

expansion rather than compression takes place. In this process water and steam are applied to the feed mix in the conditioning unit to raise the moisture from 10-12% to 25 - 30% and the feed is conveyed into a pressure - sealed cylinder. Steam injection increases gelatinisation of the starch in the ingredients. Extrusion of the feed to atmospheric pressure through holes in the die plate at the end of the cylinder puffs up the feed. The extruded feed strands are cut to pre-determined length outside of the die plate by a rotating knife. The pellets are dried in a high temperature oven at about 120°C to a moisture content suitable for storage (10-13% moisture).

Raw starch is essential for a good pellet and about 90% of the starch gets gelatinised during the 30 to 60 seconds period, when the feed is in the expansion cylinder. High temperatures partially destroy heatlabile vitamins and decrease the availability of some amino acids. Heat sensitive additives can be sprayed on the pellets after extrusion.

#### 7.4.6. *Cooling/Drying*

The temperature and the moisture from the dry pelleting processes should be reduced within few minutes after extrusion for proper storage and handling. This can be done by collecting the pellets and spreading them in thin layer on a concrete or tiled floor and blowing air over them or by solar drying. Alternatively, they could also be dried in an oven with temperature regulation and air-circulation. But in commercial feed production vertical or horizontal type cooler-dryer with air circulation are used. The temperature is reduced to ambient levels, and the moisture preferable is below 10%, but not exceeding 13%.

#### 7.4.7. *Crumbling*

Crumbling of the pellets is necessary to produce granules or crumbles for feeding small fish and prawns. The cooled pellets are ground on corrugated rolls (roller mill) and the output sifted and graded.

#### 7.4.8. *Fat spraying*

High lipid levels as required in the diet of carnivorous fish may affect the pelleting. So lipid is sprayed onto the feed in mixtures placed in the production flow after the pelleting in commercial operations.

#### 7.4.9. *Bagging*

The finished dry pellets or granules should be bagged for storage and the bags must be suitably marked to show the product specification and date of manufacture, if sold.

#### 7.4.10. *Storage*

Storage of feed ingredients and finished feeds (pellets, granules or flakes) is an important step in maintaining the quality of the feeds.

Moist and semi-moist feeds and feed ingredients as far as possible should be used immediately after preparation. Freezing is necessary if it has to be stored or transported. A cold storage may be required for the purpose.

Proper storage of feeds and ingredients is necessary to prevent theft, physical and chemical damage, infestation by insects or microorganisms and rodents (rats). The accumulated economic loss can be enormous due to the quality changes.

Fungal growth is very rapid when temperature exceeds 25°C and humidity exceeds 85 per cent, and produce toxins besides staleness. It is important to protect feeds and feed ingredients by adding cost effective preservative, (Refer section on additive). To prevent the entry of insects and rodents proper screens should be provided in the warehouse ventilators. Vitamins and vitamin premixes should be kept in the coolest place possible either in the manufacturers containers or in air-tight-light-proof containers and used within six months. Lipids should be stabilised with antioxidants (Refer section additives) and kept in sealed dark plastic containers in a cool place. Dry ingredients and feeds should be used within 2-3 months.

#### 7.4.11. *Quality control*

The quality of the feed depends upon the raw material quality and quantity, processing conditions and shelf life. The efficacy of a feed in promoting growth primarily depends on the above aspects. Besides balance of nutrients, feed should be water-stable, attractive, palatable and should have appropriate size to suit the growing stages. Poor feed quality can be due to (i) poor quality raw material (ii) adulteration with urea, (iii) high acid-insoluble ash representing sand and silica (v) high ash levels (vi) use of rancid lipids (vii) inadequate vitamin levels in premix (viii) low levels of highly unsaturated fatty acids (ix) low levels of essential amino acids; imbalance of calcium, phosphorus ratio (x) presence of antinutritional factors (xi) old feeds-prolonged storage of feed lowers its nutritional quality (xii) high moisture levels (exceeding 13%) and (xiii) high salt content.

### 8. FEEDING STRATEGIES

In order to achieve maximum growth and best feed efficiency of a given feed from a culture system the feeding strategies employed-feeding rate (feeding level, ration size), feeding frequency, and feeding methods are extremely important.

#### 8.1. **Feeding rate:**

The amount of feed offered (ration size) should be regulated with reference to the biomass of the fish or prawns being cultured, as well as observation on their daily feed intake. Normally, farmers, without considering the feed intake of the prawns or fish, supply feeds according to the biomass. This often leads to over-feeding, feed wastage and deterioration in water quality, resulting in poor production and FCR. Daily observations on feed intake in check nets or trays for prawns is very important to decide on the amount of feed to be offered on subsequent days. Factors that may affect feed intake are: poor water quality, low oxygen level, high temperature, high ammonia, nitrite and hydrogen sulphide levels toxic metabolites, over sized or undersized feed particles and deterioration in health of the animals. Increasing temperature and salinity enhance the energy

requirements of fish and prawn. To meet this increased energy demand they consume more food. But with increasing temperature and salinity dissolved oxygen levels decrease; thereby relatively less oxygen will be available to the animals. Dissolved oxygen levels are also dependent upon the BOD (biochemical oxygen demand) COD (chemical oxygen demand) and also on the density of phyto - and zoo-plankters. So, when dissolved oxygen content reaches sub-optimal levels feeding should be either stopped or ration size should be reduced, depending upon the severity of the situation. In farms, in which adequate aeration is provided to maintain dissolved oxygen levels, factors other than dissolved oxygen listed above affect feed intake.

Fish fry and fingerlings, and prawn post-larvae and juveniles require more food to meet the demand for fast growth, so the ration size should be adjusted to reflect this. Differences between species in the food requirements and rearing temperatures make it difficult to give any generalised chart for feeding.

Both over-feeding and underfeeding affect growth, production and FCR. The daily feeding rate for prawn (% of biomass) varies from 25% to 2% during the crop period, maximum during the post - larval stage (PL20) to the minimum when the animals reach marketable size. Feeding at constant rate should be avoided. Feeding rates for fish also vary according to water temperature and size (Appendix 4). The amount of feed to be given daily can be calculated as follows:

$$\text{Feed to be given} = \frac{\text{Number of animals} \times \text{Average weight} \times \text{Feeding (\% biomass)}}{100}$$

If the pond contains 70,000 prawns of average weight 12 g and the feeding rate is 4%, the amount of feed to be given will be

$$\frac{70,000 \times 12 \times 4}{100 \times 100} = 33.6 \text{ kg/day}$$

## 8.2. Feeding frequency

The number of meals offered daily and time feeding are also factors that affect growth and feed efficiency. Frequent feeding reduces starvation and stunting, enables uniform growth and results in minimum feed wastage.

In the case of fish the daily ration (total amount of feed fed per day per pond, cage etc) of feed is offered in several meals, often 4 to 6 times a day, and as the fish grow the number of meals are reduced to either one or two. Feeding in finfish is mostly done during the day, between 6 AM and 6 PM but during fry stage a continuous feed supply is maintained.

In the case of prawns the number of meals offered per day range from 4 to 6 for post larvae and juveniles, to 3 to 6 times a day for sub-adults and adults. The total amount of feed to be offered each day is divided into small quantities and fed (Appendix 4). In the case of burrowing prawn species like *Penaeus semisulcatus* and *P. japonicus* late afternoon evening and night feeding is preferred. In the case of tiger prawn (*P. monodon*) and white prawn (*P. indicus*) about 40-45% of the daily ration is offered between 6 AM and 11 AM and the balance between 2 PM and 12 PM.

## 8.3. Size of the feed

Properly sized feeds must be fed to satisfy the specific demands of the growing stages (Examples are given in appendix 4). If a particular feed contains under-sized or over sized particles, screening should be done to remove such feed particles. Dust or fine particles, if present, often clog the gills of the fish and prawns and cause damage to the gills. Clogging of gills by feed dust also provides a substrate for propagation of a variety of microorganisms which cause further damage to the animal. The wasted feed particles also pollute the water.

Every individual in the cultured population must obtain a share of the feed offered, at every meal time (time of feeding). So, the number of feed particles in the daily ration should at least correspond to the estimated number of surviving individuals.

For instance, if 1,50,000 *Penaeus indicus* are estimated to be present in a pond, each of the prawn should get a feed particle. Supposing that the total biomass of prawns is 1000 kg and the ration offered 6% of the biomass, the quantity of feed to be offered in a day is 60 kg. If these 60 kg of feed are distributed at 4 feeding times, equally, then each meal size is 15 kg. Each of this 15 kg feed must have about, 1,50,000 particles for all the prawns to get a granule or pellet. Thus artificial feeds must have adequate number of particles to satisfy almost the entire surviving population.

#### 8.4. Feeding methods

Feeding method also should ensure that each of the cultured organism gets a share of the feed being supplied. Dry pellets, granules and crumbles for fish and prawns could be broadcasted. Fish in general, are fast feeders and good palatable feeds are consumed within 15 to 30 minutes by most species. But, prawns being slow feeders, it is necessary to evenly distribute granules and pellets. Broadcasting the feeds from pond bunds is quite adequate for small ponds (<0.5 ha). But in larger ponds (>0.5ha) broadcasting the feeds from bunds should be supplemented by distribution in slightly interior areas using a small boat.

Moist dough feeds and meals should not be broadcasted; but kept in earthen or plastic trays, placed in the peripheral areas of the pond bottom. Adequate number of feeding trays (30-40/ha) must be used to create maximum opportunities for the prawns to feed.

Feeding method adopted in semi-intensive carp culture systems in Andhra Pradesh is quite unique and the same could be adopted in other carp farming states. The outline of the method is as follows: Feed mixtures (de-oiled rice bran mixed with either groundnut cake, cotton seed cake, mustard cake or sunflower cake, often in the ratio 3 to 4:1) is kept in perforated fertilizer bags tied to bamboo or casuarina poles. About 20 to 30 bags are used per hectare. Feed mixture is transported in a catamaran or a small raft and equally distributed in the feeding

bags. Fish browse on the feed through the perforations in the bags and within two hours most of the feed kept in the bags is consumed (Rao, 1992).

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## APPENDIX - I. EXAMPLES OF FEED FORMULAE FOR PRAWNS

Formula 1 Species : *Penaeus indicus*

Ingredients	g/kg
Fish meal@	310
Prawn meal@	150
Soybean meal	150
Groundnut oil cake	100
Squid-head meal@	30
Chicken egg	10
Yeast	11
Wheat flour	100
Tapioca flour	50
Fish oil	30
Soybean oil	20
Lecithin	10
Vitamin premix@	8
Mineral permix*	21

@ Fish meal (anchovies) prawn meal (small prawns) and squid-head meal were prepared from fresh material by steam-cooking followed by pressing to remove water and oil, and by drying (55-60°C).

ζ Mineral premix composition:  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  8g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  5g;  $\text{KH}_2\text{PO}_4$  4g;  $\text{Na}_2\text{H}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  2g;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  0.6 g;  $\text{FeSO}_4$  0.6 g;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.6 g;  $\text{Co}(\text{NO}_3)_3$  0.1 g;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  0.1 g.

\* Vitamin premix composition:  $\beta$ -Carotene 0.14 g; calciferol 0.01 g; tocopherol acetate 0.25 g; menadione 0.01 g; ascorbic acid (uncoated) 4 g; thiamine hydrochloride 0.1 g; calcium pantothenate 0.1 g; folic acid 0.01 g; p-aminobenzoic acid 0.1 g; choline chloride 1.5 g; inositol 0.9 g; biotin 0.002 g; cyanocobalamin 0.01 mg

## AQUACULTURE FEED

Proximate Composition: Crude protein 39.5%; Total lipids 8.5%; ash 14.5%

Form of Feed: Granules for postlarval and 2mm pellets for juveniles.

Survival: 90-100% and FCR 1.6 to 1.8.

Source: Paulraji (CMFRI) unpublished

### Formula 2 Species: *Peaneus indicus*

Ingredients	g/kg feed
Fish meal	300
Prawn-head meal	100
Squilla (mantis shrimp meal)	100
Groundnut oil cake	100
Gingelly oil cake	100
Code - liver oil	20
Soybean oil	30
Vitamin mix	3
Mineral mix	7.5
Wheat flour	110
Tapioca flour	109.5
Agar agar	20
Proximate composition:	
Crude protein	36.0
Lipid	12.0
Ash	18.0
Fibre	6.0
NFE	28.0

#### Vitamin premix (mg/kg diet):

Thiamine mononitrate 45; riboflavin 30; nicotinamide 100; pyridoxine hydrochloride 20; calcium-d-pantothenate 50; biotin 0.1; cyanocobalamin 0.01; choline chloride 400; ascorbic acid 400; inert carrier  $\alpha$  cellulose 1955 mg.

#### Mineral premix (g/kg diet):

$K_2HPO_4$  1.75;  $Ca_3(PO_4)_2$  3.39;  $NaH_2PO_4 \cdot 2H_2O$  0.69;  $MgSO_4 \cdot 7H_2O$  2.65;  $MnSO_4 \cdot 5H_2O$  0.032;  $FeSO_4 \cdot 7H_2O$  0.013

Source: CMFRI

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**Formula 3 Species: *Penaeus monodon***


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Ingredients	Dietary level (%)
Squid head meal	10.0
Fish meal	20.0
Soybean cake	34.0
Shrimp meat	24.0
Wheat flour	8.0
Aquamix (vitamin mix) <sup>1/</sup>	2.0
Kelco alginate	2.0

<sup>1/</sup> Details not given

Source: Manik et al., 1980

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**Formula 4 Species: *Penaeus monodon* & *P. merguensis* Dry Pellets**


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Ingredients	Dietary level (%)
Fish meal	27.0
Meat & bone meal	10.0
Soybean meal	15.0
Exp. sesame cake meal	5.0
Exp. groundnut meal	5.0
Maize	4.0
Coconut cake	10.0
Extracted rice bran	10.0
Leaf meal	10.0
Tapioca	5.0
Vitamin mix No. 2	8.0
(+BHT 0.02% and ethoxyquin 0.015%)	1.0

Source: Kanazawa, 1984.

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**Formula 5 Marine shrimp (Practical Pond Diet)**


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Ingredients	(%)
Fish meal (61% CP)	15.0
Ext. soybean meal	36.0
Shrimp waste meal	10.0
High-gluten wheat flour	20.0
Rice bran	12.0
Fat*	2.0
Binder (Hemicellulose or Lignin Sulphonate: by-products of wood processing)	2.0
Vitamin mix *	0.5
Trace mineral mix*	0.5
Dicalcium phosphate	1.0
Coated vitamin C	0.038

Source: NRC, 1983 \* Details not given

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**Formula 6 Marine shrimp (Moist/Dry Pellets)**


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Ingredients	(%)	(%)
Animal viscera	60	-
'A' grade fish meal	-	40
Rice bran	20	35
Wheat flour	20	25

Source: Krin 1986 (cited by New 1987)

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**Formula 7 Freshwater Prawns (Dry Pellets)**


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Ingredients	1 (%)	2 (%)
Fish oil	3.0	3.0
Shrimp meal	25.0	10.0
Fish meal	10.0	4.0
Peanut meal	5.0	2.0
Soybean meal	5.0	2.0
Broken rice	25.5	39.0
Rice bran	25.5	39.0
Guar gum	1.0	1.0

Source: New and Singholka, 1982.

## Formula 8 Freshwater Prawns (Dry Pellets)

Ingredients	(%)
Fish meal	20.0
Soybean meal	9.0
Rice bran	45.0
Coconut oil cake	20.0
Tapioca	5.0
Pfizer premix A	1.0

Source: Manik, 1976

## Formula 9 Freshwater Prawns (Dry Pellets)

Ingredients	(%)
Shrimp head meal	30.0
Soybean meal	4.0
Ricebran	35.0
Coconut oil cake	20.0
Tapioca	9.0
Agar	1.0
Pfizer premix A	1.0

Source: Manik, 1976

## Formula 10 Freshwater Prawns (Dry Pellets)

Ingredients	Waldron's Prawn No.1	Waldron's Prawn No.2
	(%)	(%)
Alfalfa	4.00	4.00
Corn	56.75	56.75
Soybean meal	27.00	25.00
Meat and bone meal	11.00	8.00
Tuna meal	-	5.00
Vitamin mix*	1.25	1.25

\* Details not available

Source: Corbin, et al., 1986

## APPENDIX - 2 EXAMPLES OF FEED FORMULAE FOR FISH

## A. Carps and Tilapia

## Formula 1 Indian carps (Catla, rohu and mrigal) fry

Ingredients	Level of inclusion	
	g/kg dry feed	
	1	2
Groundnut cake	200	610
Sesame cake	350	-
Fish meal	100	-
Rice bran	340	370
Dicalcium phosphate	5	15
Table salt	3	3
Mineral mix (a)	1	1
Vitamin mix (b)	1	1

## a. Mineral premix:

mg/kg in the final diet

$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$	Cu	=	10
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	Fe	=	100
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	Mn	=	50
ZnO	Z	=	50
$\text{COCl}_2 \cdot 6\text{H}_2\text{O}$	Co	=	0.05
KI	I	=	1

## b. Vitamin premix:

mg/kg of diet

Vitamin A	5000 I.U
Vitamin D	600 I.U
Thiamine ( $\text{B}_1$ )	10
Ribloflavin	20
Pantothenic acid	30
Niacin	50
Vitamin C	200

Source: Chow, 1982.

## Formula 2 Indian carps (rohu) fry

Ingredients	Dietary level g/kg	Proximate composition	
		%	
Groundnut oil cake	600	Protein	29.00
Rice bran	300	Fat	12.00
Blood meal	100	Carbohydrate	9.80
		Crude Fibre	17.27

Source: Mohanty and Narayanaswamy 1986

## Formula 3 Carp poly culture (catla, rohu, common carp, silver carp)

Ingredients	g/kg	Proximate composition(%)	
Fish meal	225	Crude protein	35.80
Groundnut cake	225	Total lipid	11.80
Rice bran	400	NFE	19.45
Tapioca powder	150	Crude fibre	7.
		Ash	18.55

Source: Nandeesh et al., 1986

## Formula 4 Common Carp and Tilapia Hybrids (Dry Growers Pellets)

(*T. nilotica* x *T. aurea*)

Ingredients	'Standard'	'High Protein'
	(%)	(%)
Fish meal (CP 70%)	25	35
Sorghum meal	55	45
Wheat meal	20	20

Source: Viola and Arieli, 1982 (Israel)

**Formula 5 Tilapia (*T. nilotica*) and Common carp  
(Fingerling Test Diets)**

	A (%)	B (%)
Ext. cottonseed meal	53.0	-
Ext. soabean meal	-	50.0
Ext. rice bran <u>a/</u>	43.0	46.2
Bone meal <u>a/</u>	3.0	3.5
Table salt	0.2	0.2
DL-methionine	0.3	-
L-lysine	0.4	-
Vitamin mix <u>b/</u>	0.1	0.1

*Source: Chow, 1982 a (Egypt)*

a/ These two ingredients were boiled with water equivalent to 100% of the whole diet to promote a binding capability. Other ingredients were added, the mixture extruded through the 2mm die of a mincer, sun dried and crumbled.

b/ A poultry vitamin mix providing (per kg diet): A 5000 I.U., D<sub>2</sub> 600 I.U., B<sub>1</sub> 10 mg, B<sub>2</sub> 20 mg, Pantothenic acid 30 mg, Niacin 50 mg, Pyridoxine 2 mg, Vitamine c 200 mg.

*Source: New 1987*

**Formula 6 Tilapia (*T. nilotica*)**

Ingredients	(%)
Cottonseed oil cake	82.0
Wheat flour	8.0
Cattle blood meal	8.0
Bicalcium phosphate	2.0

*Source: Pullin and Lowe - Mc Connell,, 1982.*

Formula 7 Tilapia (*T. mossambica*)

Ingredients	(%)
Rice bran	74.59
Coconut oil cake	18.65
Wheat flour	2.10
Cattle blood	4.66

Source: Pullin and Lowe-Mc Connell, 1982

## Formula 8 Tilapia (In Freshwater) Dry Feeds (Experimental)

Ingredients	Animal Size		
	Fry-0.5 g (%)	0.5-35 g (%)	35 g (%)
Brown fish meal	30	10	5
Hydrolysed feather meal	15	5	3
Meat meal	5	5	5
Soybean meal	5	12	4
Groundnut meal	10	12	10
Cottonseed meal	5	20	20
Rice bran	10	20	20
Dried distillers solubles	10	10	10
Vitamin premix	2	2	2
Mineral premix	4	4	4
Lipid supplement	4	-	-

Source: Jauncey and Ross, 1982

**B. Brown Trout****Formula 1. Moist Pellets (Solberg)**

Ingredients	%
Raw minced industrial fish	50.0
High-fat fish meal	21.2
Extracted soy meal	22.2
Fish oil (with 0.2% Antioxidant)	3.0
Lecithin	2.0
Potassium iodide	0.2
Sodium chloride	0.24
Decalcium phosphate	0.136
Thiamin	0.02
Vitamin E	0.004
Alginate H 120 (binder)	1.0

*Source: Sedgwick, 1982*

**Formula 2. Moist diet**

Ingredients	g/kg feed
Fish meal (CP 68%, ash 13%)	480
Soybean meal (CP 60%)	100
Corn gluten meal (CP 60%)	120
Whey (CP 12%)	70
Brewer's yeast (CP 45%)	50
Vitamin premix (a)	20
Mineral premix (b)	10
Fish oil with antioxidant	150
Add:	
Raw fish (fresh, ground, pasteurised)	500g/kg dry mix
Formic acid	3g/kg dry mix

(a) Vitamin Premix (g/kg premix)

Vitamin A (as acetate) 500,000 1.5; Vitamin D<sub>3</sub> 300,000 1.4; Vitamin E 10,000 1.4; Vitamin K (menadione sodium bisulfate) 3; Vitamin B120.0003; ascorbic acid 40; biotin 0.05; folic acid 1; naicin 20; pantothenic acid (as d-Calcium pantothenate) 15; pyridoxine (as HCl salt) 3; riboflavin 5; thiamine (as HCl salt) 3; choline chloride (50%) 300; Wheat middling added to adjust the total premix to 1 kg.

(b) Mineral premix (g/kg premix)

Copper (as Cu SO<sub>4</sub> · 5 H<sub>2</sub>O) 2.5; Iron (as Fe SO<sub>4</sub> · 7H<sub>2</sub>O) 6.3; Manganese (as Mn SO<sub>4</sub> · H<sub>2</sub>O) 8.6; Iodide (as KI) 0.8; Zinc (as Zn SO<sub>4</sub> · H<sub>2</sub>O) 14.4; Salt (99% NaCl) 300; Wheat middlings: added to adjust the total premix to 1kg.

Source: Cho et al., 1985.

### Formula 3. Dry Diets

Ingredient	Production diet	Broodstock diet
	kg/100kg	kg/100 kg
Fish meal (CP 68%; ash 13%)	46	35
Feather meal hydrolysed (CP 80%; ash 4%)	8	8
Soybean meal (solvent extracted, dehulled CP 48%)	9	9
Corn gluten meal (CP 60%)	8	7
Brewer's dried yeast (CP 45%; ash 7%)	5	5
Alfalfa meal (CP 17%) and Crude Fibre 24%)	-	6
Whey, spray dried (CP 12%; ash 10%)	8.5	7
Wheat middlings (CP 17%; fibre 8%)	-	14
Vitamin premix <sup>a</sup>	1.5	2
Mineral premix <sup>b</sup>		
Fish oil with antioxidant	3	2
Fish oil with antioxidant	10	4
(Sprayed on pellets/granules)	10	4

a and b - Vitamin and mineral premix composition as in moist diet (Formula 2)

Source: Cho et al. 1985.

## C. Mulletts, Milkfish, Sea bass, Grouper etc

Fpormula 1. *Chanos chanos*, *Liza macrolepis* and *Liza parsia*.

Ingredients	g/kg
Fish meal	200
Prawn-head meal	40
Soybean meal	150
Groundnut oil cake	250
De-oiled rice bran	150
Tapioca flour	100
Wheat flour	50
Fish oil	20
Soybean oil	30
Mineral premix@	8
Vitamin premix	2

@ Mineral premix composition: Calcium biphosphate 1.2 g; calcium lactate 2.7 g; ferrous sulphate 0.1 g magnesium sulphate 0.2 g; dibasic potassium phosphate 2.4 g; sodium biphosphate 0.8 g; sodium chloride 0.2 g; aluminium chloride 0.05 g; zinc sulphate 0.15 g; cuprous chloride 0.05 g; manganese sulphate 0.05 g; potassium iodide 0.05 g; cobaltous chloride 0.05 g.

b Vitamin premix composition: Choline chloride 0.4 g; inositol 0.15 g; L-ascorbic acid 0.25 g; nicotinc acid 0.1 g; calcium pantothenate 0.1 g; riboflavin 0.2 g; thiamine hydrochloride 0.15 g; pyridoxine hydrochloride 0.15 g; folic acid 0.01 g; cyanocobalamin 0.010 mg; biotin 0.01 mg; cholecalciferol 0.2 g; menadione 0.01 g; tocopherol acetate 0.2 g; cellulose - added to adjust the total mix to 5 g.

Form of feed: Semi-moist dough (25-30% moisture)

Proximate composition: Crude protein 35.5%; Total lipids 6%; ash 12.5%; crude fibre 9%.

Response parameters: Survival 85-95% and FCR 1.8 to 2.2

Source: Paulraj (CMFRI), unpublished

**Formula 2. Microparticulate diet (MBD) for fish larvae**

Ingredient	g/100g dry diet
Chicken egg yolk	24.0
Short-necked clam powder	8.0
Bonito extract powder	5.0
Milk casein	10.0
Egg albumin	15.0
Soluble yeast powder	10.0
Amino acid mixture	5.0
Vitamin mixture	8.0
Mineral mixture	6.0
Bonito egg lecithin	3.0
Squid liver oil	4.0
Soybean oil	2.0

Source: Kanazawa, 1986

**Formula 3. Milkfish *Chanos chanos* fry**

Ingredients	Percentage
Fish meal	30
Shrimp head meal	8
Soybean meal	10
Meat and bone meal	6.7
Corn gluten meal	10.2
Rice bran	12.1
What flour	15.9
Code-liver oil	2.6
Vitamin mix	1.0
Mineral mix	3.5
Proximate composition:	
Crude protein	40.7
Crude fat	8.4
Ash	15.5
Crude fibre	4.2
Nitrogen-free-extract	31.2

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**AQUACULTURE FEED**

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Vitamin mix: (mg/kg diet) Thiamine-HCl 20, Riboflavin 40, Pyridoxine-HCl 30, Cyanocobalamin 0.1, Niacin 200, Pantothenic acid 600, Biotin 2, Inositol 600, Folic acid 50, Choline chloride 1000, P-aminobenzoic acid 100, ascorbic acid 2000, B-carotene 20, Vitamin D 10, Vitamin E 100, Vitamin K 10, BHT 10, cellulose 5792.9

Mineral mix:  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  12.938,  $\text{CaCO}_3$  8.750,  $\text{KH}_2\text{PO}_4$  6.250,  $\text{KCl}$  0.625,  $\text{NaH}_2\text{PO}_4$  3.750,  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$  0.188,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.313,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.875,  $\text{KIO}_3$  0.006,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  0.010,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.281,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  0.001, Cellulose 0.004.

Source: Alava and Lim, 1988

**Formula 4. Processed moist diet for fry and fingerlings of sea bass (*Lates calcarifer*) and grouper (*Epinephelus tauvina*)**

Ingredients	Levels
Crude palm oil	5.0
Fish meal (55% protein)	44.0
Soybean meal	9.0
Yeast feed grade	1.0
Vitamin premix (a)	0.6
Fresh live-stock blood	35.0
Groundnut oil cake	20.0
Rice bran	25.4

(Protein approximately 40% by dry weight)

- (a) Composition of vitamin premix (mg per premix): Thiamine - HCl 2.0, Riboflavin - 3.0, Calcium pantothenate 6.0, Niacin 12.0, Pyridoxine - HCl - HCl 2.0, Folic acid 5, Biotin 0.2, Cyanocobalamin 0.1, Choline chloride 60, Vitamin C-50, Vitamin A 500 I.U., Vitamin D<sub>3</sub> 25 I.U., Vitamin E 20 I.U., Vitamin K 0.5 mg.

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Source: ADCP 1983

**Formula 5. Sea Bass (Juvenile Test Diets)**

Ingredients	1	1
	(%)	(%)
Trash fish	50.0	50.0
Fish meal	5.0	7.0
Wheat pollards	35.5	29.5
Soybean meal	7.0	11.0
Vitamin mix No. 1	2.5	2.5

*Source: Chow, 1984*

**Formula 6. Sea Bass and Gouper (Moist Pellets)**

Ingredients	(%)
Freshly minced fish	50.0
Fish meal	17.5
Soybean	7.5
Rice bran	17.0
Groundnut meal	5.0
Wheat flour	2.5
Vitamin mix No. 2	0.5

*Source: Abu Hassan et.al., 1984*

**Formula 7. Experimental brood stock diet for Sea bass and grouper**

Ingredients	Level of inclusion (%)
Fresh ground fish	46
Fish meal (local)	20
Extracted soybean meal	12
Wheat pollards	4
Fresh mussel meat	4
Fish oil	4
Soybean lecithin	3
Vitamin mix <sup>a</sup>	2
Seaweed binder	5

- a) Composition of vitamin mix (mg/kg dry diet:) Thiamine HC1 120, Riboflavin 40, Pyrodoxine - HC1 120, Cyanocobalamin 0.02, Folic acid 5, Niacin 15, Calcium pantothenate 100, Biotin 2, Vitamin C (sodium ascorbate) 1000 Inositol 800, Choline chloride 1200, Vitamin A 5000 I.U., Vitamin D 1000., I.U. Vitamin E 200, Vitamin K 40.

**Formula 8. Sea Bass and Grouper (Semi-moist grow-out feed)**

Incredients	(%)
Ground fresh fish	46
Fish meal (local-56% protein)	25
Soymeal (Ext.)	10
Meat and bone meal	4
Wheat pollards	4
Fish oil (local)	3
Seaweed binder	5
Mussel meal	2
Vitamin mix (composition given in 7 above)	1

*Source: Meyers, 1987*

APPENDIX - 3

COMPOSITION OF VITAMINS AND MINERAL MIXTURES

1. Vitamin Mix No.1 (Freshwater prawns, Marine shrimp and Sea bass)

Ingredient		mg/g of premix x 1/
Vitamin	A	500 I.U.
Vitamin	D <sub>3</sub>	100 I.U.
Vitamin	B <sub>1</sub>	0.1
Vitamin	B <sub>2</sub>	0.3
Pyridoxine		0.2
Vitamin	B <sub>12</sub>	0.001
Nicotinic Acid		2.0
Calcium Pantothenate		0.6
Folic acid		0.05
Vitamin	K	0.2
Vitamin	C	5.0

*Source: Chow, 1984*

## 2. Vitamin Mix No.2 (Marine shrimp)

Ingredient		mg/kg of dry diet
Thiamin	HC1	120
Vitamin	B <sub>2</sub>	40
Pyridoxine	HC1	120
Nicotinic acid		150
Calcium pantothenate		100
Folic Acid		5
Biotin		1
Vitamin	B <sub>12</sub>	0.02
Inositol		4000
Choline chloride		1200
Sodium ascorbate (Vit. C)		5000
Vitamin	E	200
Vitamin	K	40
Vitamin	A	5000 I.U.
Vitamin	D	1000 I.U.

Source: Kanazawa, 1984

3. Vitamin Mix 3 (Marine Percoidae)  
(Provisional)

Ingredient		mg/kg dry diet
Vitamin A		6000 I.U.
Vitamin B <sub>1</sub>		20
Vitamin B <sub>2</sub>		20
Vitamin B <sub>6</sub>		20
Vitamin B <sub>12</sub>		0.02
Folic acid		5
Inositol		600
Niacin		150
Pantothenic acid		50
Vitamin C		200
Vitamin D <sub>3</sub>		2500 I.U.
Vitamin E		200
Biotin		1
Vitamin K		10

Source: New, 1986 a

## APPENDIX - 4

EXAMPLES OF FEEDING RATE FEEDING FREQUENCY AND FEED  
PARTICLE SIZE FOR PRAWNS AND FISHExample 1: Feed ration size and feeding schedule for prawns *penaeus indicus* and *Penaeus monodon* (dry granules and pellets)

Size of prawn (g)	Ration size % of biomass	Feeding frequency (No. of meals/ day)
0.01-0.1	30-20	6-4
0.1-1.0	20-12	5-4
1-3	12-7	5-4
3-5	7-5	4-4
5-8	5-4.5	5-4
8-12	4.5-3.5	5-4
12-16	3.5-3.0	4
16-20	3.0-2.5	4
20-25	2.5-2.0	4
25-30	2.0	4
>30	2.0	4

**Preferable timings of feeding**

06-6.30 a.m.	30% of the ration
10-10.30 a.m.	10% of the ration
05-05.30 p.m.	30% of the ration
10-10.30 p.m.	30% of the ration

**Feeding method: broadcasting**

- Note: 1. Feed manufacturers usually provide feed particle size and feeding rate (% of biomass) charts for their feeds;
2. A feeding chart is given as a guideline; but it should be supplemented by day-to-day observation of feed intake through check-trays in each of the ponds; growth rate of prawns, prevailing environmental conditions (especially dissolved oxygen levels, plankton blooms) and health of the prawn. Based on these observations feeding rate should be adjusted in ponds.

## 2. Feeding rate for Tilapia (*T. nilitica*) in tanks and cages at 27-31°C for a commercial pellet

Fish size (g)	Feed rate (%) Biomass/day)
5	30, reducing to 20
5-20	14 reducing to 12
20-40	6 reducing to 4.5
40-100	7 reducing to 6.5
100-200	4 reducing to 2
200-300	1.8 reducing to 1.5

Source: Pullin and Lowe - McConnell, 1982 cited by New, 1987

## 3. Feeding rate of Channel Catfish fed with commercial feed

Fish size (g)	Feeding rate (% Biomass/day)			
	15.5°C	21°C	24 - 29°C	32°C
27	1	3	4	2
91	1	3	3	2
227	1	3	3	1.5
427	1	1.4	1.4	0.7
863	0.8	-	-	-

Source: Cited by New 1987

## 4. Trout feed sizes and feeding frequency

Fish size (g)	Feed type	Feed size (mm)	Feeding per day
1	Starter Granule	0.5	6-8
3	Starter Granule	1.0	5
10	Starter Granule	1.5	4
20	Grower Granule	2.0	4
>50	Grower Granule	3.0	3
>50	Grower Pellets	2.4 x 3.0	3
100	Grower Pellets	3.4 x 4.0	3
<200	Grower Pellets	4.8 x 5.0	2
>200	Grower Pellets	6.4 x 6.0	1-2

Source: Cho et al. 1985

## 5. Experimental feeding guide for Carp

Temperature °C	Pellete size Fish weight (g)	% Body weight/ Biomass per day					
		1.5mm 5	1.5mm 5	2.7mm 5-20	4mm 50-100	5mm 100-300	5mm 300-1000
-17		6	5	4	3	2	1.5
17-20		7	6	5	4	3	2
20-23		9	7	6	5	4	3
23-26		12	10	8	6	5	4
26		19	12	11	8	6	5

Source: Cited by New 1987

## Quality control parameters for feeds and feed ingredients

1. Moisture
2. Crude protein
3. Non-protein nitrogen (in protein sources)
4. Crude fibre  
Chitin (crustacean and insect material) plant and microbial products
5. Total lipids Phospholipids  
- essential fatty acids free fatty acids, iodine number acid insoluble ash
6. Total ash
7. Nitrogen-free extract
8. Energy content
9. Amino acids profile of protein
10. Fatty acids profile of lipids
11. Sterol profile
12. Minerals  
calcium, phosphorus, magnesium sulfur and trace elements
13. Vitamins
14. Sodium chloride in marine protein sources.
15. Gossypol in cotton seed meal
16. Thioglucosides in rape seed, mustard and other potential ingredients
17. Hydrocyanic acid in tapioca
18. Mycotoxins
19. Microbial counts
20. Test for water stability
21. General examination for: Natural smell, colour, size of feed particles, fungal growth, insect infestation.

Ref: Publications suggested for reading: CMFRI Special Publication No.8; New 1987, Chow, 1980.