Aquafeed Preparation and Feeding Management in Inland Saline Aquaculture

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Organised by

ICAR-Central Institute of Fisheries Education, Kolkata Centre

&

Fish Nutrition, Biochemistry and Physiology Division, ICAR-CIFE, Mumbai

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"Aquafeed Preparation and Feeding Management in Inland Saline Aquaculture"
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Foreword

ICAR-Central Institute of Fisheries Education as a premier institution in the field of fisherieshas to prioritize its activities to address the national issues related to aquaculture. The salt affected regions of India are presently converting to inland saline aquaculture farms and it is the need of the hour to create awareness on scientific and sustainable practices for inland saline regions among the stake holders. The prestigious National Agriculture Higher Education Project (NAHEP) funded by World Bank and Govt. of India is financially supporting ICAR-CIFE for conducting research on various aspects of inland saline aquaculture and providing hands on training and skill development programmes for farmers, students and other stakeholders. The Skill Development Programme on "Aquafeed preparation and feeding management in inland saline aquaculture" is a customized training programme for creating awareness among students regarding the inland saline aquaculture and feed and feeding management in the delicate system.

I am pleased to see this training manual, which is meticulously designed by the scientists of FNBP division and ICAR-CIFE, Kolkata Centre to highlight the advancements made in the field of fish nutrition with special reference to inland saline aquaculture.I hope this manual will be of great help for the trainees in acquiring the basic knowledge on the subject. The manual contains all the relevant aspects of aquaculture nutrition*i.e.*food and feeding habits of commercially important fish species, nutrient requirement of fish and shellfish in different culture systems, feed and feeding strategies in freshwater, brackish water and inland saline aquaculture, potential feed ingredients for aqua feed, fish feed formulation and manufacturing,digestion and absorption of nutrients, importance of live food in inland saline aquaculture, larval nutrition, feed additives, drugs and chemicals used in aquafeed, application of nutrigenomics in fish nutrition, diseases of fin fish, quality control and storage of feed and feed ingredients and physiological homeostasis in fish reared in inland saline water.I convey my complements to all who have contributed directly or indirectly in organizingthis course and bringing out a manual.

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(Gopal Krishna)

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CONTENTS

Food and Feeding Habits of Cultivable Species in Inland Saline Area

Parimal Sardar and Dilip Kumar Singh

1. INTRODUCTION

In many aquaculture operations today, feed accounts for more than one-half of the variable operating cost. Therefore, knowledge on food, feeding habit and nutrition requirement of culturable fish species is essential for successful aquaculture. In culturing fish in captivity, nothing is more important than sound nutrition and adequate feeding. If the feed is not consumed by the fish or if the fish are unable to utilize the feed because of some nutrient deficiency, then there will be no growth. An undernourished animal cannot maintain its health and be productive, regardless of the quality of its environment.

2. POTENTIAL SPECIES FOR INLAND SALINE AREAS

Inland saline aquaculture is culture of fish in saline ground water and it occurs in several countries in and around the world. Researches showing that species like finfish such as tilapia, especially GIFT, common carp, anabas, Asian sea bass and trout, and shellfish like shrimp and oysters are potential species for inland saline aquaculture. The wide range of euryhaline species have been evaluated for culture in the inland saline water are finfish (e.g., Lates calcarifer, Sparus auratus, Dicentrarchus labrax, Argyrosomus japonicus), crustaceans (e.g. Penaeus monodon, Litopenaeus vannamei, Marsupenaeus japonicus) and molluscs (e.g. Saccostrea glomerata), diadromous species such as salmonids (e.g. Oncorynchus mykiss) and salt-tolerant freshwater finfish (e.g. Oreochromis niloticus, Bidyanus bidyanus) and crustaceans (e.g. Macrobrachium rosenbergii) (Allan et al., 2009).

3. FOOD AND FEEDING HABITS OF FISH

While carp form the most important species farmed in freshwater in India, it is the shrimp from the brackishwater sector which contributes the bulk of the production. Among the carps, the three Indian major carps, namely, catla (*Catla catla*), rohu labeo (*Labeo rohita*) and mrigal carp (*Cirrhinus mrigala*) contribute over 90 percent of the total Indian aquaculture production. Three exotic carps, namely, silver carp (*Hypophthalmichthys*

molitrix); grass carp (*Ctenopharyngodon idellus*) and common carp (*Cyprinus carpio*) now form a second important group. In spite of the fact that the country also possesses several other cultivable medium and minor carp species which show high regional demand, including, *Labeo calbasu, L. fimbriatus, L. gonius, L. bata, L. ariza, Puntius sarana, Hypselobarbus pulchellus, H. kolus* and *Amblypharyngodon mola*, as well as several others, commercial farming of these species has yet to take off. The study of this species in inland saline waters is not yet done.

The other finfish species of importance include climbing perch (*Anabas testudineus*), murrels (*Channa striata* and *C. marulius*), etc. The brackish water aquaculture sector is mainly supported by shrimp production. The giant tiger prawn (*Penaeus monodon*), which is responsible for the bulk of production followed by the recently introduced white leg shrimp, *Litopenaeus vannamei*. Although India possesses several other potential species of finfish and shellfish, the production of these, is still very low key. In brackishwater ponds common fish species include Milkfish (*Chanos chanos*), Asian seabass (*Lates calcarifer*), Mullets (*Mugil cephalus, Liza parsia, L. tade*), pearl spot (*Etroplus suratensis*) etc.

Depending on the food habits fish can be categorized as i) herbivorous ii) carnivorous iii) omnivorous iv) detrivores and v) planktivores. Omnivores are again classified as a) omni-harbivores and b) omni-carnivores.

I. Herbivores*:* The fishes that prefer only plant materials as their food are called herbivores. Examples are *Ctenopharyngodon idella*, *Hypopthalmichthys molitrix* etc.

II. Carnivores*:* The fishes that prefer only animal materials as their food are called carnivores. Examples are *Channa* sp., *Lates calcarifer, Penaeus monodon* etc.

III. Omnivores: The fishes that prefer both plant and animal matters as their food are called omnivores. Examples are *Cyprinous carpio*, *Macrobrachium rosenbergii* etc.

i) Omni-herbivores: The omnivores that consume both plant and animal matters but shows more preference on the plant matter as their food are called omni-herbivores. Examples are *C yprinous carpio, Cirrhinus mrigala* etc.

ii) Omni -carnivores: The carnivores that consume both plant and animal matters but shows more preference on the animal matter as their food are called omni-carnivores. Examples are *Clarias batrachus, Heteropneustes fossilis* etc.

IV. Detrivores: The fishes that fed largely on detritus (a mixture of sediments, decaying organic matter and bacteria) as their food are called detrivores. Examples are *Cirrhinus mrigala*, *Chanos chanos* etc.

V. Planktivores: The fishes that fed largely on planktons (either phytoplankton or zooplankton or both) as their food are called planktivores. Examples are *Hypopthalmichthys molitrix, Catla catla* etc. In general almost all common fishes are planktivores.

Depending on the preference of feeding zone the fish can be categorized as i) pelagic feeder and ii) bottom or benthic feeder. Pelagic feeders are again categorized as a) surface feeder and b) column feeder.

I. Pelagic feeder: The fishes that prefer upper zone of aquatic system for inhabiting and feeding are called pelagic feeder. Examples in pond condition (2-3 m depth) are *Catla catla*, *Labeo rohita* etc.

i) Surface feeder: The fishes that prefer top layer of aquatic system for inhabiting and feeding are called surface feeder. Examples in pond condition (2-3 m depth) are *Catla catla*, *Hypopthalmichthys molitrix* etc.

ii) Column feeder: The fishes that prefer middle layer of aquatic system for inhabiting and feeding are called column feeder. Examples in pond condition (2-3 m depth) are *Labeo rohita*, occasionally *Ctenopharyngodon idella* etc.

II. Bottom or benthic feeder: The fishes that prefer bottom layer of aquatic system for inhabiting and feeding are called bottom or benthic feeder. Examples in pond condition (2-3 m depth) are *Cirrhinus mrigala*, *Cyprinous carpio* etc.

Following table shows the food and feeding habits of some commonly culturable species.

Feeding habits of some common cultivable species

Species (Commonname)	**Stage**	**Food habits**	**Preferable food items**	**Feeding Zone**
Catla catla (Catla)	Fry & Fingerlings	Planktivores	Preferably zooplankton (water fleas), planktonic algae and vegetable debris	Surface feeder
	Growers & adult	Omniherbivores	Crustacean, algae, rotifers, plants, insects, vegetable debris	

Labeo rohita (Rohu)	Fry & Fingerlings	Planktivores	Vegetable debris, microscopic plants.	Column feeder
	Growers & adult	Omniherbivores	Vegetable debris, microscopic plants, decayed higher plants, detritus and mud	
Cirrhinus mrigala (Mrigal)	Fry & Fingerlings	Detrivores	Decayed plants and animal matters, algae, detritus, mud etc.	Bottom feeder
	Growers & adult	Detrivores & Omniherbivores	Decayed plants and animal matters, algae, detritus, mud etc.	
Hypopthalmichthys molitrix (Silver carp)	Fry & Fingerlings	Planktivores	Initially mainly zooplanton such as rotifer, nauplii of copepods, water flea etc. and gradually shifted to mainly phytoplankton and secondarily zooplankton	Surface feeder
	Growers & adult	Omniherbivores	Predominantly phytoplankton, vegetable debris and occasionally zooplankton	
Ctenopharyngodon idella (grass carp)	Fry & Fingerlings	Planktivores	Initially Prefer planktonic algae, tender aquatic plants and occasionally zooplankton but gradually shifted to many kind of aquatic vegetation	Column feeder but occasionally surface feeder

	Growers & adult	Herbivores	Aquatic weeds, land grasses, kichen vegetable waste etc.	
Cyprinus carpio (common carp)	Fry & Fingerlings	Planktivores	Zooplanton such as rotifer, nauplii of copepods, water flea etc.	Bottom feeder
	Growers & adult	Omnivores	Shows equal preference towards plant and animal matter	
Clarias batrachus (Magur)	All stages	Omnicarnivores	Shows more preference towards animal matter	Bottom feeder
Heteropneustes fossilis (Singhi)	All stages	Omnicarnivores	Shows more preference towards animal matter	Bottom feeder
Pangasius pangasius(Pangus)	All stages	Omnicarnivores	Shows more preference towards animal matter	Bottom feeder
Pangasianodon hypophthalmus (Sutchi pangus)	All stages	Omnicarnivores	Shows more preference towards animal matter	Bottom feeder
Anabas testudineus (Koi)	All stages	Omnicarnivores	Shows more preference towards animal matter	Column feeder but occasionally surface feeder
Channa sp. (Murrels)	All stages	Carnivores	Mainly prefer animal matter	Bottom feeder but occasionally surface feeder

Oreochromis niloticus (Nile tilapia)	All stages	Omnivores	Shows equal preference towards both plant and animal matter	Column feeder
Macrobrachium rosenbergii (Freshwater prawn or giant river prawn or scampi)	All stages	Omnicarnivores	Shows more preference towards animal matter	Bottom feeder
Salmo trutta fario (Brown trout)	All stages	Carnivores	Mainly prefer animal matter	Bottom feeder
Oncorhynchus mykiss (rainbow trout)	All stages	Carnivores	Mainly prefer animal matter	Bottom feeder
Penaeus monodon (Giant tiger prawn or tiger shrimp)	All stages	Carnivores	Mainly prefer animal matter	Bottom feeder
Litopenaeus vannamei (Pacific white leg shrimp)	All stages	Carnivores	Mainly prefer animal matter	Column feeder

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Nutritional Requirements of Fish and Potential Feed Ingredients used in Aquafeed Preparation

G.H. Pailan

In current trend of intensified aquaculture, provision of adequate nutrition play pivotal role not only to increase yield significantly but also improves product quality, immunity and reproductive performances. As feed contributes single largest input cost (50-60%), artificial feeds in the form of supplementary feed for semi-intensive aquaculture and complete feed for intensive aquaculture needs to be formulated and processed appropriately to become cost effective and eco-friendly, that should have optimum quality for effective nutrient utilization. The formulation of such a feed requires detailed knowledge of nutritional requirements of different stages of finfish and shellfish as one of the prerequisites. Different nutrients required for finfish and shellfish are protein and amino acids, lipid and fatty acids, carbohydrates, minerals and vitamins. Among these except minerals and vitamins all other nutrients can generate energy through metabolic process. Energy is not nutrient but the property of nutrients is required for fish and prawn to maintain normal life processes and optimum growth obviously derived from protein, lipid and carbohydrates.

Generally the spawn remain for 15 days in a “nursery pond” where they are stocked at 10 million per hectare where they reach a body weight of about 130 mg each. The fry so produced are then transferred to “rearing ponds” where they are stocked at a rate of 300 000 fry per hectare for about 45 days during which period they grow to an average weight of 15 g. These fingerlings are then stocked in the “production ponds” at a density ranging from 3000-13000 fingerlings per hectare and grown for about a year before harvest. For poly culture of these species, the relative proportions of the different species can vary from one farm to another. Generally, a ratio of catla : rohu : mrigal : calbasu = 3 : 5 : 1 : 1 is considered good. This three pond system i.e. nursery, rearing and production-culture continues to be practised throughout the Indian subcontinent. The natural productivity of all the three ponds is enhanced by fertilisation and the fish yield by supplementary feeding. It is general contention that catla and mrigal grow faster than rohu and the fishes attain after one year respectively about 1.5 - 2.0 kg for catla and mrigal and 1 kg for rohu and 700 g for calbasu. However field data indicate much variations depending upon management practices.

Unlike totally feed-based aquaculture as it is practised under intensive farming conditions, semi-intensive pond culture of Indian major carps relies upon natural productivity to a large extent. Although the techniques for the culture of IMC have been standardised, it is felt that there is ample scope for increasing the production through large scale adoption of better management practices, optimisation of the supply of various nutrients through supplementary feeding and through capacity building on-farm training. To maximize utilisation of water resources, over the last decades, transition from traditional extensive culture systems towards more and more intensive culture has occurred, both by increasing the stocking density of ponds and by increasing inputs with the provision of traditional feed mixtures composed of locally available cereal brans and oil cakes. As the density exceeds the natural carrying capacity of ponds, shifts from natural food dependence to nutritionally adequate exogenous feed becomes vital.

There is a relatively significant amount of literature on the nutrition and feeding of Indian major carps. Studies have been undertaken both in the laboratory and in the field on these aspects. Application of data obtained under laboratory conditions on the nutrient "requirements of fish" to "what is needed for a pond" is often difficult, unless proper consideration is given to the fluctuating nutrient availability in a dynamically complex pond ecosystem, still deemed as a "black box". Until then, simple transposition of data obtained under controlled laboratory conditions to field situations may lead to possible excessive supply of nutrients adversely affecting both productive efficiency and environmental quality.

Protein and amino acids requirements

Protein

Proteins are large, complex molecules made up of various amino acids that are essential nutrient in the diets, since they play crucial roles in all biological processes of which control of growth becomes the most important. Protein is the most expensive nutrient in fish feed. A continuous dietary supply of protein is essential because it can not be stored, rather continuous breakdown and synthesis takes place which is called protein turnover. Fish in captivity need to utilize their dietary protein with the utmost efficiency, as the breakdown products of protein metabolism (mainly ammonia) will directly pollute their living environment. So feed ingredients having higher digestible crude protein (DCP) should be chosen for formulation and feed preparation for fish. Unlike domestic animal and poultry, fish have a high protein requirement because fish utilizes significant portion of

protein for energy needs and at the same time fish appears to be efficient in converting protein into growth-PER around 2.2-2.7 which is higher than terrestrial animals. Protein requirement in fish is influenced by various factors such as i) maturity stage: young one needs more protein than adult ii) water temperature: protein requirement increases with higher water temperature iii) protein: energy ratio: High digestible energy to dietary protein often causes cessation of feed intake before sufficient protein is consumed as fish fed on energy satiation iv) quality of protein: quality depends on amino acid pattern, balance and digestibility. If quality is low, quantitative requirement is more v) species: carnivores require more protein than herbivores and omnivores vi) feeding rate also alters the protein requirement.

Requirement of protein has been studied in fry and fingerlings of Indian major carps based mainly on weight gain of fish fed purified diets. Like most teleosts, under controlled laboratory conditions, the optimum dietary crude protein level ranges from 30-45%. Although no clear differences between the species have been reported, protein requirements appear to decrease with increasing fish size. While young fish (below 1g) appear to require 40 to 45 % protein in their diets, older fish (>5g) appear to need 30 to 35% protein. The digestibility values have been determined for several protein sources of plant or animal origin and found to be generally high (74-94%).

Data on protein requirement of finfish and shellfish available in the literatures are depicted in Table 1.

Table 1: Estimated dietary protein requirement of finfish and shellfish

Species	**Crude protein level in diet (%)**
Cyprinus carpio	45-54 (Young) 31-38 (adult)
Ctenopharyngodon idella	41-43 (Spawn) 38-40 (Fry) 30-33 (Fingerlings & Adult)
Hypophthalmichthys molitrix	36 – 42 (young) 30 (Adult)
Labeo rohita	40 - 45 (Spawn) 35-40 (Fry) 28-30 (Fingerlings &Adult)
Catla catla	40 - 47 (Spawn)

	35-40 (Fry) 28-30 (Fingerlings &Adult)
Cirrhinus mrigala	40 - 45 (Spawn) 35-40 (Fry) 28-30 (Fingerlings &Adult)
Mahsher	40-45
Catfish (magur)	40-52 (Spawn) 35-40 (Fry) 26-32 (Fingerlings &Adult)
Salmon & trout	35-55
Tilapia	30-56
Snakehead	50-55
Anabas testudineus	38-40
Eel	40-45
Asian seabass	40-50
Milkfish	35-40
Mullet	35-40
Macrobrachium rosenbergii	42 (Post larvae) 40 (Juvenile) 35-38 (Grower) 25-30 (Adult)
Penaeus monodon	35-46
Penaeus indicus	30-45
P. merguiensis	42-52

Some data on protein digestibility of common ingredients used in the diets of Indian major carps*

Ingredient	Crude protein, %	Protein digetibility, %		
		Rohu	Catla	Mrigal
Rice bran / polish	8.8	90	90	91
Wheat bran	10.5	93	93	93
Yellow corn	6.0	86 - 96	86 - 96	86 -96
Potatoe starch	6.5	86 - 96	83 - 98	85 -98

Mustard oilcake	37.3	78
Linseed meal	39.0	82
Sesame seed meal	38.2	76
Soybean meal	49.5	84
Fishmeal	52.4	80
Silkworm pupae meal	70.6	85

* Data from Erfanullah and Jafri (1998) and Hossain et al. (1997)

Amino acids

The amino acids are the building blocks of proteins. Therefore, the first need regarding protein requirements of fish is to fulfill the indispensable amino acid requirement of the animal, and secondly to supply dispensable amino acids or sufficient amino nitrogen to enable protein synthesis.Beyond the gross percentage of protein, quantity and availability of essential amino acids determines the protein quality and the availability of critical or limiting amino acids are big concerns. Fish need of critical amino acid supplements to the prepared feeds. So during fish feed formulation along with fulfilling the protein requirement balancing of amino acids should be the prime criteria. About 20 amino acids have been isolated from natural proteins. Ten of these are indispensable for fish and prawn. The animal is incapable of synthesizing indispensable amino acids and must therefore obtain these from the diet. Essential amino acids for fish are histidine, isoleucine, lysine, leucine, methionine, arginine, phenylalanine, threonine, tryptophan and valine. Among these lysine and methionine are limiting amino acids as most of the ingredients especially plant feed ingredients are deficient of these amino acids. Amino acid requirements for growing vary among species of fish under captive conditions and these also vary with the stage of life cycle of same species. High requirement of essential amino acids for fish larvae in comparison to requirement of juvenile is reported.

Generally information on protein requirement will be of limited value unless optimum needs of 10 essential amino acids are known. Quality, rather than quantity of dietary protein is the major concern in the development of diet. So, recent trends of replacement of high quality costly protein ingredients by cheaper protein ingredients should be carefully evaluated.

The indispensable amino acid (IAA) requirements have been worked out for at least two species (rohu and catla), using purified diets. Considerable similarity is seen with the requirement values found for the common carp. Given that there is a strong correlation

between essential amino acid requirement pattern and the content of the same amino acids in whole body tissues of these fish, a relatively similar IAA requirement profile for the three major carps (rohu, catla and mrigal) has been suggested.

The requirements of essential amino acids for different fish species are shown in Table 2.

Table 2: Essential amino acid requirement of finfish and shellfish

EAA/IAA (% of Protein)	Rohu	Catla	Mrigal	Seabass	Common carp	Tilapia	Milk fish
Arginine	4.8	5.8	5.3	5.2	4.3	4.0	3.6
Histidine	2.5	2.3	2.1	2.0	2.1	1.7	2.0
Lysine	6.2	5.7	5.9	4.0	5.7	4.1	4.5
Isoleucine	2.4	3.0	2.8	4.0	2.5	3.1	4.0
Leucine	3.7	4.6	4.3	5.1	3.3	3.4	5.1
Methionine*	3.6	2.9	3.2	2.5	3.1	3.2	2.4
Valine	3.6	3.8	3.5	3.6	3.6	2.8	3.6
Phenylalanine**	3.7	4.0	4.0	4.2	6.5	5.5	3.5
Thereonine	5.0	4.3	4.1	4.5	3.9	3.8	3.8
Tryptophan	1.0	1.1	1.0	0.6	0.8	1.0	0.5

EAA/IAA (% Protein)	Salmon	Rainbow trout	Catfish	Tiger prawn	Prawn
Arginine	5.0	5.0	4.3	5.8	3.7
Histidine	1.8	1.8	-	2.1	0.7
Lysine	5.0	4.5	5.1	5.3	3.2
Isoleucine	2.3	2.0	-	3.5	0.6
Leucine	4.0	3.5	-	5.4	1.0
Methionine*	4.0	2.8	2.3	2.4	1.2
Valine	3.3	3.2	-	4.0	2.1
Phenylalanine**	5.3	4.5	5.0	4.0	1.7
Thereonine	2.3	2.0	2.0	3.6	1.6
Tryptophan	0.5	0.5	0.5	0.8	0.5

*Cysteine-0.7-0.8% ; **Tyrosine-1.0-1.2% needs to be supplied /supplemented

The amino acid requirements of grass carp and silver carp are slightly lower and of freshwater prawn are almost similar to Indian major carps.

Lipid and essential fatty acids requirements

Lipid

Next to protein lipid forms the major dietary component of finfish and shellfish. Lipid is a complex mixture of simple fat, phospholipids, steroids, fatty acids and other fat soluble substances such as carotenoids, vitamin A, D, E & K. Dietary lipids are important sources of energy and fatty acids (both essential and non-essential) that are needed for normal growth and survival of fish. Although fish have a low energy demand, and is thus susceptible to deposition of excessive lipid. Lipids do have a role as carriers for fat-soluble vitamins (vitamin A, D, E and K). Lipids are also important in the flavour and textural properties of the feed consumed by fish. It has a protein sparing effect as per energy contribution is concerned.

Triglycerides (fat & oil) are the major componenet of lipid in fish feed. Carnivores require more lipid than herbivores and omnivores. Depending on the quality and quantity of dietary protein, requirement of dietary lipid, which contain the appropriate levels of essential fatty acids should range from 5-8% and 3-8% for carps and freshwater prawn respectively. Lipid requirement for carnivores may ranges from 6-12% and in salmon, trout it may reach up to 15% to make energy dense diet.

Phospholipids represent the second largest lipid components after tryglycerides. In conjunction with proteins, phospholipids form the basic lipoprotein structure of biological membrane and acts as emulsifying agents. It also helps in lipid transport (especially steroid) and brain function. Phospholipids are dietary essential for larvae of fish and prawn for their better growth and survival. 2-4% dietary supplementation of phospholipids (lecithin/cephalin) is necessary for carps and freshwater prawn only at young stages but supplementation of phospholipid to brood stock diet has no value on larval quality. In marine shrimp it 0.1-2%. Soya lecithin is good source of phospholipid for fish and prawn. For larval stages of all the three Indian major carps, the essentiality of dietary phospholipids is also established (Paul *et al.*, 1998).

Cholesterol is used as a precursor of important steroids, moulting hormones (ecdysone), and vitamin D. In carps cholesterol can be synthesized via a complex process, which utilizes acetate as a substrate but freshwater prawn like other crustaceans is presumably unable to synthesize sterol *de novo*. The requirement of the freshwater prawn for dietary cholesterol is approximately 0.5-0.6%. In shrimp diet it is 0.5-1%.

Most of carp fish are grown under pond conditions using locally available ingredients, little work has compared the effects of different fish oils. In catla fed purified, fat-free diets, supplementation of even saturated lipid results in positive growth response. Lipid requirement in different finfish and shellfish species is shown in Table 3.

Table 3: Dietary lipid requirement of finfish and shellfish

Species	**Lipid level in diet (%)**
Indian and Chinese carp	5-8
Tilapia	6-10
Salmon & trout	8-15
Catfish	6-12
Macrobrachium rosenbergii	3-8
Penaeus monodon	6-15
Penaeus indicus	6-10

Essential fatty acids

The fatty acids which cannot be synthesized in animal's body are termed as essential fatty acids (EFA) need supply through diet. In general, freshwater fish require either dietary linoleic acid (18:2*n*-6), or linolenic acid (18:3*n*-3), or both, whereas marine fish require dietary eicosapentaenoic acid (20:5*n*-3) and/or docosahexaenoic acid (22:6*n*-3) at 1% level of each in diet for normal growth, reproduction and health. However, it is reported that in the freshwater food fish 1% levels of dietary docosahexaenoic acid significantly improved egg hatchability. It is reported that omega three (ω-3) or *n*-3 fatty acids are more beneficial. Carps and prawn appear to require a mixture of PUFAs like linoleic acid (18:3, ω 6) and linolenic (18:2, ω 3) at 1% each of the dry matter of diet, which can be reduced by feeding ω 3 and ω 6 HUFAs (22:6, ω 3, 20:5, ω 3 and 20:4, ω 6) at a dietary level of 0.1-0.5% because HUFAs can be synthesized from PUFAs but bioconversion in opposite direction is found to be lacking. HUFAs are the physiologically more active form. The requirements for essential fatty acids (mainly ω 3 PUFAs and HUFAs) are greater in larvae and brood stock than juvenile and grow out stage for better hatchability and survivality of larvae, which is less tolerant to stress. Due to small size, *Artemia* naupli is the most suitable live food for larval stages of freshwater prawn and *Artemia* naupli and rotifer for carp larvae but it is deficient of most essential EPA and DHA and other n-3 PUFAs, so, *Artemia* naupli (presently known as *Artimia* enrichment or

boosting) and rotifer should be enriched by these fatty acids that result better survival of larvae. It is necessary when larvae is developed in indoor condition, but at nursery ponds with sufficient fertilization there is no need of this type of enrichment.

Adding sunflower oil or cod liver oil supplying w6 or w3 fatty acids resulted in significant improvement in growth and feed efficiency and a mixture of both oils was found to be superior to either lipid source separately in carps. As in other teleosts, the fatty acid composition of body lipid reflects the composition of dietary lipids. In the context of pond culture, it is necessary to understand what changes are induced by the supply of natural w-6 or w-3 sources along with the effects of supplemental feed.EFA requirement in different finfish and shellfish species is shown in Table 4.

Table 4: Essential fatty acids requirement of finfish and shellfish

Species	**EFA**	**% required in diet**
Carp	18:2w6 18:3w3 Or 20:5w3 or 22:6w3	1 1 0.5-1
Eel	18:2w6 18:3w3	0.5 0.5
Channel catfish	18:3w3	<1
Magur & Singhi	18:2w6 18:3w3	1 1
Trout	18:3w3 or 22:6w3	0.8-1 0.5-1
Salmon	18:2w6 18:3w3 22:6w3	1 1 0.5-1
Tilapia	18:2w6 Or 20:4w6	0.5-1 0.5-1
Milkfish	20:6w3	1
Seabass	20:5w3 22:6w3	0.5 0.5

Macrobrachium rosenbergii	18:3w3 18:3w6 Or 22:6w3	0.5-1 0.5-1 0.5-1
Penaeus monodon	20:5w3 22:6w3	0.5-1 0.5-1

Carbohydrate requirement

Carbohydrate is the inexpensive source of energy in fish diet. No definite dietary requirement of carbohydrate has been demonstrated in fish and shellfish, however, the maximum inclusion level of carbohydrate in fish diet is related to the dietary protein and lipid level. Depending on the total energy content required in the diet, dietary carbohydrate can be quantified. If carbohydrates are not provided in the diet, more protein is catabolized for energy. Thus for protein sparing action it is important to provide the appropriate concentration of carbohydrates in the diet of fish and shell fish species being cultured. Generally fish has the limited capacity to utilize dietary carbohydrates. However, carbohydrate utilization is more in herbivores and omnivores than the carnivores. In general carnivores cannot utilize more than 20 % dietary digestible carbohydrate, however, up to 30% digestible carbohydrate can be incorporated in carnivore diet. Herbivores and omnivores can utilize about 25-50% digestible carbohydrate. Gelatinization of carbohydrates improves its utilization. Carps and prawn utilize complex polysaccharides like starch more efficiently than simple sugars like glucose. Using starch as carbohydrate source in fish diet has dual advantage. Besides being energy source, it can act as binder upon gelatinization by cooking and improves water stability of the diet.

By the supply of carbohydrates at a dietary level of about 30%, some protein sparing action has been found. Indian major carps appear to have amylolytic activity in their digestive tracts thus capable of digesting complex carbohydrates. Although all Indian cyprinid species appear capable of utilising carbohydrates well, data of Erfanullah & Jafri (1998) suggest that mrigal might tolerate less carbohydrate than the other two major carps. Some cellulase activity has even been reported in the intestine of rohu. Rohu are able to utilise complex polysaccharides more efficiently than simple sugars. These data go in favour of using rice bran or other plant by-products generally rich in carbohydrates. However, there is very little information available on the metabolic utilisation (postprandial hyperglycemia, insulin secretion) of ingested carbohydrates for any of the

three major carp species, neither under laboratory conditions nor under pond culture conditions. : Indian major carps have very long intestine to facilitate utilization of various plant derived feedstuff rich in carbohydrates and they also have very high amylase activity. Some difficulties seem to arise with high levels of dietary starch as exemplified by high plasma glucose concentration and prolonged post-prandial hyperglycaemia. These phenomena were initially thought to be due to impairment of glucose phosphorylation capacity which is catalysed by a group of enzymes called hexokinases.Until recently glucokinase was considered absent in fish but data now available confirmed existence of this enzyme in the liver of carp, trout ,seabream as well as its regulation by dietary carbohydrate supply.

Gluconeogenesis from dietary amino acids or from body protein degradation is also considered high in fish.Persistent gluconeogenesis is another possible mechanism by which high post-prandial hyperglycaemia is maintained in fish. Whether with changes with dietary carbohydrate level, hepatic glucose 6 phosphatase activities and its gene expression gets affected is, however, not yet clearly known.

Crude Fibre:Crude fibre is non digestible complex carbohydrate containing lignocellulose (cellulose and lignin), hemicelluloses and pectin) in carp diet ranges between 4-8%. Other CF content in fish diet may be chitin, laminarine. CF cannot be digested but acts as roughages and provides the bulk for proper digestion and egestion of faeces i.e. it improves feed efficiency. In case of fish and prawn, uo to 8% CF is permissible. For both fish and prawn less than 4% CF is desirable. In shrimp diet carbohydrate up to 6%, but less than 3% is desirable.Dietary carbohydrate level of finfish and shellfish is depicted in Table 5.

Table 5: Dietary digestible carbohydrate level of finfish and shellfish

Species	**Carbohydrate level in diet (%)**
Indian and Chinese carp	22-26 (Spawn) 30-35 (Fry & Fingerlings) 40-50 (Adult)
Tilapia	35-40
Rainbow trout	25-30, desirable <20
Salmon	25-30, desirable 6-15
Catfish	30-35
Sturgeon	25-30
Eel	25-30

Turbot	15-20
Milkfish	35-40
Sea bass	25-30
Asian seabass	20-25
Sea bream	25-30
Red drum	20-25
Yellow tail	10-15
Macrobrachium rosenbergii	25-40
Penaeus monodon	20-25
Penaeus indicus	25-30

Energy requirements

Energy is not nutrient but is a property of nutrient, which is released during metabolic oxidation of nutrient. Energy is required to carry out various physical, physiological and biological activities of animals. Protein, lipid and carbohydrate are the energy source of animals. The energetic value (or physiological fuel value) of protein, lipid and carbohydrate for finfish and shellfish are 5.6 kcal/g, 9.4 kcal/g and 4.1 kcal/g respectively. The total digestible energy content of the diet varies with the proportion of protein, fat and carbohydrate. The energy needs for maintenance activity must be satisfied before any growth, and reproduction in animals. Energy is partitioned by an animal between each of the various physiological processes involved in maintenance, growth and reproduction. So for growth of animal extra energy over maintenance level should be provided. If the dietary energy is less than maintenance requirement, which otherwise will be fulfilled by depletion of body reserve resulting reduction of growth. Similarly for reproduction of animal extra energy over maintenance and growth level should be provided, otherwise part of the energy provided for somatic growth will be diverted or else body reserve will be depleted towards reproductive growth. In this situation about 30% of energy provided for somatic growth diverted or 60% of body energy reserve is depleted for gonadal maturation. Thus if gonadal growth can be prevented by any means, somatic growth can be maximized by giving same level of energy provided for maintenance and growth. Provision of excess dietary energy over maintenance, growth and reproduction diverted towards either deposition fat or excretion as nitrogenous waste rather than somatic growth.

All of the energy lost due to standard metabolism, heat of nutrient metabolism and physical activity appears as heat. The maintenance requirement can be determined by measuring the heat produced. The heat production can be measured directly in a calorimeter or it can be estimated by measuring oxygen and then multiplied by energy equivalent. The factor most commonly used is 3.42 kcal/mg O_2. Maintenance energy can also be estimated by measuring energy loss during starvation. Digestion efficiency in fish decreases as feeding level is increased. So the feeding level at which the increased efficiency of energy utilization should be optimized.

Fish require 10-30 fold lower maintenance energy than terrestrial animal, because fish are poikilotherms, fish maintain body balance against buoyancy which needs less energy, finally, fish are ammoniotelic. The maintenance energy requirement for fish ranges between 41.84-292.88 kcal/kg. Digestible energy requirements for maximum growth of finfish and shellfish range between 2800-4063 kcal/kg feed, while the gross energy range between 4000-4800 kcal/kg diet. The energy content of egg in fish is 6.45 kcal/g dry weight and the total energy stored in egg is 8-15% of gross body energy.

There are several factors like very temperature, swimming against water flow, body size, crowding, dissolved oxygen level, waste accumulation and stress, growth rate, species etc. can alter the energy requirements of fish. Feeding rates should be adjusted to compensate for these factors to avoid overfeeding, but still providing sufficient energy for optimum growth.

A minimum dietary gross energy level of 15 MJ/kg is considered essential for efficient nutrient utilization in mrigal. For juvenile rohu, 16MJ/kg gross energy or a protein / energy ratio of 22 mg / kJ is reported to be required for optimal growth and feed efficiency (Das et al. 1991). A definite protein-sparing effect of fats has been shown. However, a higher supply of non-protein energy in the form of fats above 15% does not appear to induce any further beneficial effects in rohu. Dietary energy requirement of finfish and shellfish is shown in Table 6.

Table 6: Dietary energy requirement of finfish and shellfish

Species	**Digestible energy (kcal/kg)**	**Gross energy (kcal/kg)**
Indian and Chinese carp	3100 (Fry & Fingerlings) 2800 (Adult)	-
Tilapia	200-3400	-

Trout & Salmon	3240-4110	-
Catfish	2580-3760	-
Milkfish	2500-3500	-
Macrobrachium rosenbergii	3200-3400	4000
Penaeus monodon	2800-3600	4300
Penaeus indicus	3500	4000

Protein to Energy Ratio (P: E)

Fish eat on energy satiation, thus protein and energy in the fish diet should be balanced in such a way so that fish is satisfied by energy requirement and at the same time optimum level of protein is consumed by the fish. Excess energy relative to protein content in the diet may result in high lipid deposition resulting fatty fish and due to less protein consumption, fish achieve less muscle growth. On the other hand less energy relative to protein content in the diet may result in high protein intake and fish satisfy their energy from protein. In this condition nitrogenous waste in the form of ammonia is more but protein accretion is less which results less growth of fish. Thus, excess protein is not only wasteful and uneconomical but also causes stress to fish and aquatic pollution as well. If diets contain low amount of available non-protein energy, protein the expensive nutrient, which potentially could be used for growth has to be used to obtain energy. But practically to economize the formulation, protein should be adjusted in such a way so that most of it can be only utilized for growth purpose and most energy should be obtained from non-protein sources i.e. lipid and carbohydrate to spare protein for energy purpose. Thus fish diet should have optimum protein: energy ratio (P: E) and at the same time optimum protein : lipid, protein: starch (carbohydrate) and optimum lipid: carbohydrate ratio should be maintained in the diet. P:E is higher in small fishes. Optimum P:E of fish diet is shown in Table 7. The use of protein as an energy source by carps and prawn can be minimized at a 1:4 ratio of lipid to carbohydrate and growth and survival to be highest at a dietary protein: starch ratio of 1:1 or 1: 1.5.

Table 7: Dietary P: E for optimum growth of finfish and shellfish

Species	**P:E (mg/kcal)**
Indian major carp	95-109 (Fingerlings) 113 (Fry)
Common carp	108
Tilapia	103
Rainbow trout	92-105
Catfish (magur)	88
Macrobrachium rosenbergii	90-98

Vitamin requirements

With intensification of aquaculture system, the nutritional importance of vitamins has grown steadily in recent years. Vitamins are organic compounds, which are essential for transformation of energy but it itself cannot supply energy to the body and trace amount of it is required from exogenous source for normal growth, reproduction and health. Vitamins are classified into two groups i.e. fat soluble vitamins (vitamin A, D, E, K) and water soluble vitamins (vitamin C and vitamin B-complex includes thiamin, riboflavin, pyridoxine, niacin, Pantothenic acid, inositol, folic acid, cholin, biotin and B_{12}). Supplementation of vitamin C and E in brood stock diet increased viable egg production. Larval diet should be over fortified with vitamins particularly with vitamin C, which protect larvae from stress. Brine shrimp naupli is deficient of vitamin C but rich in other vitamins should be enriched with vitamin C. But for larvae and brood stock other kinds of vitamins should also be supplemented. Excess fat soluble vitamins in the diet result in abnormal growth and liver disease.

Carp species lack gulono-lactone-oxidase, the terminal enzyme for the conversion of glucose to ascorbic acid and thus depend on dietary supply of vitamin C. It has been shown that both rohu and catla fry require ascorbic acid at a dietary level of about 100 mg / kg diet. Although other vitamins have not been adequately studied, for practical purposes, data obtained for common carp can be extended to Indian major carps because of the relative phylogenetic closeness of these species and similarities in gross nutritional requirement between different teleosts. The requirements of vitamins for finfish and shellfish are given in the table 8.

Commercial grade vitamin premix (Table 9) is generally used in preparation of feed, which should not exceed inclusion level of 2% for carp and prawn diet. Generally it remains 1%.

Table 8: Dietary vitamins requirement of finfish and shellfish

Vitamin	**Common carp & other carps**	**Catfish**	**Seabass**	**Rainbow trout**	**Prawns**
Thiamine (mg/kg)	2-3	1-3	Trace	1-2	50-100
Riboflavin (mg/kg)	7-10	9	Trace	3-30	30-58
Pyridoxine (mg/kg)	5-10	3	5-10	1-15	30-50
Pantothenic acid (mg/kg)	30-40	25-50	15-19	10-50	50-100
Niacin (mg/kg)	30-50	14	-	1-150	100-150
Folic acid (mg/kg)	15	Trace	-	5-10	5-10
Cyanocobalamin (mg/kg)	Not required	Trace	Trace	0.02	0.02-0.1
Myo-Inositol (mg/kg)	200-300	Trace	Trace	200-500	200-300
Choline (mg/kg)	1500-2000	400	-	50-3000	400-2000
Biotin (mg/kg)	1-1.5	Trace	-	-	1.0
Ascorbate (mg/kg)	30-50 650-700 (IMC)	60	700	100-500	50-100
Vitamin A (I.U./kg)	1,000-2,000 2000 (IMC)	1000-2000	-	2000-15000	5000-10000
Vitamin E (I.U./kg)	80-200 98-131 (IMC)	500-1000	-	2400	100-200
Vitamin K (mg/kg)	2-3	30	-	30-50	5-20
Vitamin D3 (I.U./kg)	NR	Trace	-	10	1000-2000

NR = not required

Table 9: Recommended level of vitamins in carp and prawn diet

Vitamin	Quantity/kg diet
A	5000-10000 IU
D_3	100-200 IU
E	100-200 IU
K	200-400 mg
C	50-100 mg
Thiamine	30-50 mg
Riboflavin	30-50 mg
Pyridoxine	0.02-1 mg
B_{12}	0.5-1 mg
Biotin)	400-2000 mg
Cholin	5-10 mg
Folic acid	200-300 mg
Inositol	100-150 mg
Niacin	50-100 mg

Mineral requirements

Minerals are required for various life processes including the formation of skeletal tissue, respiration, digestion, and osmoregulation. Finfish and shellfish unlike most terrestrial animals can absorb some minerals (especially macro elements) from the aquatic environment in which they inhabit. The minerals required by the fish are categorized in two groups such as macro or major elements (Ca, P, Cl, Mg, Na, K) and trace or minor elements (Co, Cu, I, Fe, Mn, Se, Zn, Al, Cr, Va). Calcium and phosphorus are closely related in metabolism. P and Ca are required for bone formation. Ca plays a major role in blood clotting, muscle activity and nerve transmission. P is involved in energy transformation, permeability of cellular membrane, genetic coding, general control of reproduction. Phosphorus level in water is generally low; therefore it has to be compensated through dietary supplementation. Trace minerals form aquatic source can fulfill the requirement partly for fish and prawn so need to be supplemented through diet. Calcium and phosphorus ratio should be 2 or 1.5:1 in the diets of carps and prawn. P and trace minerals (Fe, Mn and Zn) deficiency in brood stock diet reduced viable egg production.

Although minerals have not been adequately studied, for practical purposes, data obtained for common carp can be extended to Indian major carps because of the relative phylogenetic closeness of these species and similarities in gross nutritional requirement between different teleosts. Metabolic activity and mineral deficiency symptoms of finfish and shellfish is shown in Table 10. Mineral requirement of finfish and shellfish is shown in Table 11.

Table-10: Summary of information metabolic activity and deficiency symptoms in fish

Mineral Elements	**Principal metabolic activities**	**Deficiency symptoms**
Calcium	Bone and cartilage formation; blood clotting; muscle contraction	Not defined
Phosphorus	Bone formation; high energy phosphate esters; other organo-phosphorus compounds	Lordosis, poor growth
Magnesium	Enzyme co-factor extensively involved in the metabolism of fats, carbohydrates and proteins	Loss of appetite, poor growth, tetany
Sodium	Primary monovalent cation of inter cellular fluid; involved in acid- base balance and osmoregulation	Not defined
Potassium	Primary monovalent cation of intra-cellular fluid; involved in nerve action and osmoregulation	Not define
Sulphur	Integral part of sulphur amino acids and collagen; involved in detoxification of aromatic compounds	Not defined
Chlorine	Primary monovalent anion in cellular fluids; component of digestive juice (HCI); acid-base balance	Not defined
Iron	Essential constituent of haeme in haemoglobin, cytochromes, peroxidases, etc.	Microcytic, homochromic anaemia
Copper	Component of haeme in haemocyanin (of cephalopods); co-factor in tryosinase and ascorbic acid oxidase	Not defined

Manganese	Co-factor for arginase and certain other metabolic enzymes; involved in bone formation and erythrocyte regeneration	Not defined
Cobalt	Metal component of cyanocobalamin (B_{12}), prevents anaemia; involved in C_1 and C_3 metabolism	Not defined
Zinc	Essential for insulin structure and function; co-factor of carbonic anhydrase	Not defined
Iodine	Constituent of thyroxine; regulates oxygen use	Thyroid Hyperplasia (goiter)
Molybdenum	Co-factor of xanthine, oxidase, hydrogenases and reductases	Not defined
Chromium	Involved in collagen formation and regulation of the rate of glucose metabolism	Not defined
Fluorine	Component of bone appetite	Not defined

Table-11: Mineral requirement of finfish and shellfish

Mineral Elements	**Carps**	**Channel catfish**	**Rainbow trout**	**Tilapia**	**Eel**	**Prawn**
Calcium (%)	0.1-0.5 0.19 (IMC)	<0.1	<0.1	-	0.27	0.1-0.5
Phosphorus (%)	0.6-0.7 75 (IMC)	0.45	0.6	0.9	0.3	0.6-0.7
Magnesium (%)	0.05	0.04	0.05	0.06	0.04	0.05
Sodium (%)	0.1-0.3	-	-	-	-	0.1-0.3
Potassium (%)	0.1-0.3	Trace	Trace	Trace	Trace	0.1-0.3
Sulphur (%)	0.3-0.5	-	-	-	-	0.3-0.5
Chlorine (%)	0.1-0.5	-	-	-	-	0.1-0.5

Iron (mg/kg)	50-150	30	Trace	Trace	170	50-150
Copper (mg/kg)	1-4	5	3	3.5	Trace	1-4
Manganese (mg/kg)	13	2.4	13	12	Trace	13
Cobalt (mg/kg)	5-10	-	-	-	-	5-10
Zinc (mg/kg)	15-30	20	15-30	20	Trace	15-30
Iodine (mg/kg)	100-300 mg	1.1	1.1	Trace	Trace	100-300mg
Molybdenum	(trace)	-	-	-	-	(trace)
Chromium	(trace)	-	-	-	-	(trace)
Fluorine	(trace)	-	-	-	-	(trace)
Selenium	(trace)	0.25	0.15-0.3	Trace	Trace	(trace)

Commercial grade mineral premix (Table 12) is generally used in preparation of feed, which should not exceed inclusion level of 2% for carp and prawn diet. Generally it remains 1%.

Table 9: Recommended level of minerals in carp and prawn diet

Minerals	**Quantity/kg diet**
Ca	10-18 g
P	18 g
Mg	0.8-1 g
Na	6 g
K	9 g
S	0.2 g
Mn	20 mg
Zn	50-100 mg
Fe	5-20 mg
Co	10 mg
Se	1 mg
Cl	Traces
Mo	Traces

Cr	Traces
Fl	Traces
Cu	25 mg

Basically the finfish and shellfish must be given a diet containing optimum level of high quality protein with adequate essential amino acid balance, optimum P:E and adequate balances of essential fatty acids, vitamins and minerals over a prolonged period to maximize growth.

1. Feed Ingredients

Broadly the feed ingredients can be classified as energy (<20% protein) and protein rich (>20% protein) ingredients. As per the origin, it is categorized as plant ingredients and animal ingredients. As per the quality is concerned animal protein is better than plant protein. Ingredients should be selected in a formulation on the basis of local or regular availability and cost without much more deviation of quality of feed. These informations should be gathered on the basis of regular survey. The composition of the feedstuffs are known to vary regionally, seasonally and also with soil fertility and type of processing and storage method adopted. Therefore, it is desirable that each batch of feed ingredient should be analysed for actual nutrient content prior to feed formulation. For reference information chemical compositions of different feed ingredients of fish and prawn available in India are given in different literatures.

1.1. Fish meal

Fishmeal is a generic term for a nutrient-rich feed ingredient derived from fishes and used primarily in diets for domestic animals. There are several processing methods to produce good quality fishmeal, but the basic principle involves separation of the solids from the oil and water. Cooking, pressing, drying and grinding the fish make fishmeal. Addition of fishmeal to animal diets increases feed efficiency and growth through better food palatability, and enhances nutrient uptake, digestion, and absorption.Fishmeal of high quality provides a balanced amount of all essential amino acids, phospholipids, and fatty acids (e.g. DHA or docosahexaenoic acid and EPA or eicosapentaenoic acid) for optimum development, growth, and reproduction, especially of larvae and brood stock. The nutrients in fishmeal also aid in disease resistance by boosting and helping to maintain a healthy functional immune system. High-quality fishmeal also allows for formulation of nutrient-

dense diets, which promote optimal growth. Protein content in fish meal varies from 50-70% depending on the quality of fishmeal.

Cost: 50 Rs/kg

1.2. Soybean meal

Soybean meal is the by-product after the normal removal of the oil from soya by mechanical or solvent extraction method. It is one of the most important protein sources as feed of productive farm animals and as partial replacement of fish meal. Protein content of soybean meal, dehulled soybean meal and full-fat soybean meal is 44%, 48%, 36% respectively. It has one of the best amino acid profiles compared to other vegetable meals. However, limiting amino acid are methionine and cystine while arginine and phenylalanine are higher. It also Contains endogenous anti-nutrients, including protease (trypsin) inhibitor, phyto-haemagglutinin and anti-vitamins which interfere with the nutrient utilization. It is a good source of choline but low in vitamins B_{12}, nicotinic acid and pantothenic acid. Digestible energy ranges from 2,572-3,340 kcal/kg. maximum inclusion level is 30%.

Cost- 34 Rs/kg

1.3. Mustard oil cake

The most common variety are the white mustard, black mustard and Indian or leaf mustard. Mustard seeds contain oil (30-35%), which is usually extracted by cold pressing. Protein content is 30-35% with metheonine is the main limiting amino acid. The most deleterious antinuutritional factors (ANF) present are glucosinolate and erucic acid which resulted in histological changes of liver, and depress growth. Recommended level of inclusion is 5-10% and often required dietary supplementation of iodine when inclusion rate is higher.

Cost- 30 Rs/kg

1.4. Rapeseed meal

It is the by-product of rape seed after oil extraction. It contains the protein between 35-40% and often used as substitute of soyabean meal. Limiting amino acid are methionine, cysteine and lysine. Remarkable level of vitamin B6, niacin and panthothenic acid and minerals mainly phosphorus and potassium. Contain 12% crude fiber and ANF glucosinolate and erucic acid.

Cost-15 Rs/kg

1.5. Groundnut oil cake:

It is byproduct of ground nut after oil extraction. It contains 40 % but can reach over 50% when nuts are decorticated. High arginine content but deficient in lysine, methionine, threonine and tryptophan. Contain no antinutritional factor but are more prone to aflatoxin (B1) presence.

Cost- 35-40 Rs/kg

1.6. Cottonseed meal

Cotton is grown for its fibre for manufacture of textiles and the cotton seeds after oil extraction resulted in meal which is useful feedstuffs. It is rich in protein 35-40% and contain ANF gossypol & cyclopropenoic acid which decrease protein digestibility. Gossypol binds lysine thereby decreasing its bioavailability. However, Heat treatment allows 90% gossypol inactivated. New variety called glandless variety increased cotton yield and no gossypol. Gossypol can be detoxified by iron salts (1:1) which bound to gossypol and making it non-toxic bound form.

Cost- 22-15 Rs/kg

1.7. Sunflower meal

Protein content of sunflower meal is 25-26%. It is rich in methionine and arginine than soybean meal and deficient in lysine. Lower ANF polyphenol but this is high in fiber content (25-27%) that limit its uses in feed formulation. It is useful source of niacin and choline. In general, the vitamins are slightly higher than soybean meal and contain tannin, protease and arginine inhibitor as ANF's.

Cost- 25 Rs/kg

1.8. Brewery grains

It is the by-product of beer brewing produced after fermentation of wheat, rice, maize etc. the mash is pressed to separate the liquid and the solid fraction remained is brewery grains. It is limited in methionine and cystine and contains protein 27% CP. The crude fiber content is high and therefore in limited use. Inclusion level is 5-10%.

1.9. Rice bran

This has been the most popular ingredient of the practical diets for fin fishes especially carps. It has crude protein value of 10-12%, crude fibre 12-18%, total lipid 7-12%, ash 8-12%. It is a good source of energy and B group vitamins. Deoiled rice bran is better in terms of nutritional profile and this also keeps away the problem of rancidity.

Cost- 10-15 Rs/kg

1.10. Wheat flour and wheat bran

This is a good source of energy having crude protein 10-14%, crude fibre 12-18%, ash 6-18%. It is a good source of phosphorous, potassium, magnesium and zinc. Amongst vitamins, niacin, pantothenic acid and biotin are in good amounts. For prawn feeds, ground whole wheat flour is widely used. Inclusion of this in feeds foments gelatinization hence improving the feed stability.

Cost- 20-30 Rs/kg

1.11. Corn gluten

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

Cost- 35 Rs/kg

2. UNCONVENTIONAL FISH FEED INGREDIENTS

Unconventional feed resources refer to all those feed ingredients that are not traditionally used in animal feeding and are not normally used in commercially produced rations for livestock.

Characteristics of unconventional feed resources:

i) They are end products of production and consumption that not have been used, recycled or salvaged.

ii) They are mainly organic and can be in solid, slurry or liquid form.

iii) Their economic value is often less than the cost of their collection and transformation for use and consequently they are discharged as wastes.

2.1. Shrimp meal:

Inclusion in shrimps and other crustaceans feed is 10% (omnivorous) to 20% (carnivorous). Shrimp meal has 40% CP. It is used as a pigmentation agent (Astaxanthin).

Chitin has growth promoting effect. Shrimp meal lipid contains 10% cholesterol (The shrimps are not able to synthesize cholesterol).

Cost- 40 Rs/kg

2.2. Blood meal

Boiling the blood followed by drying in oven or sundry or smoke. Dry cassava powder can be used as a binder and boiling water to gelatinize it. It is high in protein content (85%). Low level of methionine, isoleucine, and arginine but great access of leucine and rich in lysine. Antagonism exists between leucine and isoleucine.

Cost- 22-27 Rs/kg

2.3. Poultry offal meal

Poultry processing generates a lot of wastes such as offal, blood and heads of birds which is can be processed to form poultry by-product meal. It has Protein content around 60%, lipid 14-30%. Essential amino acid content is significant when mixed with plant products.

2.4. Poultry feather meal

Contain mainly keratins, indigestible in nature and hydrolyzed by high thermo treatment. Limiting amino acids are methionine, lysine, isoleucine and histidine but rich in cysteine. 17-20% inclusion level in diet.

2.5. Meat and bone meal

It is the dried and rendered product from mammal tissues. It does not contain horn, hair, hide trimmings, stomach contents, added blood. Protein 50-56%, ash content 35%, lipid 8-10% mainly saturated or mono-unsaturated fatty acids. Methionine and isoleucine are low.

Cost- 25-50 Rs/kg

2.6. Crab meal

It is produced by drying the waste from crab processing or whole crab. Protein content is 32% whereas crab protein concentrate contain 89% protein high in lysine and low in methionine. High ash content of 9-21% due to presence of exoskeleton. These are rich source of carotenoids in feed. Recommended level of inclusion is 5-10%.

2.7. Krill meal

Krill autolysis rapidly so it is steam dried on board. Protein content varies from 58-67%and amino acid profile of krill protein is similar to that of fish meal. It is rich source of n3 fatty acid and carotenoids. However, it contains high level o fluoride. Recommended inclusion rate in 40% of animal protein.

Cost- 50 Rs/kg

2.8. Fish protein concentrate

Here the insoluble fish protein is converted into polypeptides and amino acids by enzymatic and chemical hydrolysis. It contains high level of protein (85%) with rich in sulphur containing amino acid but low in tryptophan. Enzymes and chemical used are papain, trypsin, pepsin and hydrochloric acid. It also acts as a feed stimulant because of free amino acid presence.

2.9. Squid meal:

Drying is the common method of processing of squids. It has high protein content of 70-90% its amino acid profile is similar to that of fish meal and shrimp meal and much higher than soybean meal. It acts as chemo-attractants (glycine and betaine) and also have growth promoting properties.

Cost- 35-50 Rs/Kg

2.10. Earthworm meal

These are detritivorous terrestrial oligocheate worms. They live in the soil and feed on decaying leaves and other organic matter. It is commercially produced by heaping animal, human wastes or refuge in a land with enough moisture in the soil or swamps. It contains high protein content of 60 %. It is low in methionine, cystine and tryptophan. Processing is either by oven drying, smoking over a kiln or pulverising with a pepper grinder. The chemical score is 71%.

2.11. Housefly meal

When there is a supply of damp decaying organic matter, houseflies thrive. The nutrient quality analysis of processed larvae is Moisture 8%, protein 45%, fat 15%, Ash 8% and chitin 25%.

2.12. Tadpole meal

Frogs and toads breed at the onset of the rainy season with the first rains acting as stimulus for reproduction. Eggs are laid in stagnant pools or any body of water and later hatch into tadpoles. Tadpoles can be cultured like fish and harvested before they can metamorphose. The harvested tadpoles can be processed by oven drying or smoking over a kiln. Proximate analysis showed that the meal contained 50% crude protein, 11% lipid, 3.8 % fibre and 26% ash. The chemical score of tadpole meal is 62%.

2.13. Silk worm pupae meal

It is the by-product after the silk has been wound off from the cocoon and can serve as a feedstuff. It contains 72% crude protein. The limiting amino acids are lysine, arginine, methionine and histidine. It has high chitin level so unutilized by the animal. However, it acts as chemo-attractant due to high content of unsaturated fatty acid. Inclusion level is 5-15%.

2.14.Leaf meals

Aquatic macrophytes and terrestrial plants are abundant in the tropics, growing freely without cultivation. All contain diverse levels of protein, which can produce an inexhaustible and inexpensive source of nutrient for fish. Examples of plants with nutritionally valued leaves are Azolla (25% CP), *Eichhornia (*27% CP), Alfalfa (20% CP), *Leucaeena* leaf meal (25% CP), sweet potato leaf (23% CP), *Sesbania* leaf (25% CP), Mulberry leaf (28% CP), *Colocasia esculenta* leaf (27% CP), *Simmondsia chinensis* (Jojoba) (30% CP).

Cost- Locally available

2.15. Distillery by-products

Distillers' grains are simply grains minus the starch. They are obtained from the de-alcoholised fermentation residues which remain after the grains are fermented by the yeast. Protein content varies from 28-32%. However, quality of protein is not satisfactory due to imbalance in several amino acids. This are very good source of water-soluble vitamins like B_2, niacin, pantothenic acid, folic acid and choline. Distillers are relatively high fiber content that limits its uses as a protein or energy source. Inclusion level is 5-10%.

Cost- 25-20 Rs/Kg

2.16. Fish soluble

This is the water remaining after the oil is removed from the liquid pressed out during the manufacture of fish meal. The condensed and dried fish soluble when included in small quantities an aqua feed serves as an attractant. It is high in B group vitamin and contains an unidentified growth factor.

Cost- 37 Rs/Kg

2.17. Fish silage

It is prepared from trash fish, waste fish head, viscera prawn waste small crabs and mixed with a mixture of acids to bring down the pH to 4. This causes liquefaction and prevents bacterial decomposition. Biological fish silage is prepared by introducing lactic acid bacteria into 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 in fish feeds.

3. CONCLUSION

With the ever increasing demand of fish it has become very important to turn the fish production from aquaculture for which feed inputs would be required to sustain stocks at higher densities. Traditionally used feeds are cheaper but not nutritionally balanced hence would fail to support high stocking densities. Knowledge about feed formulation has to be spread with a thought towards the acceptance pattern of the Indian farmers. Cost factor is a major deterrent that keeps farmers away from the formulated feeds so techniques should be developed to effectively utilize locally available ingredients.

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Protein and Energy Requirement of GIFT and *P. vannamei* Reared in Inland Saline Aquaculture

N.P. Sahu, Shamna N, and Parimal Sardar

1. Introduction

There are about 1000 million ha land which is adversely affected by sodic soil (CSSRI, 2011; Sandeep *et al.,* 2013) and over 380 million ha of land is affected due to secondary salinity in over 20 countries (Ghassemi *et al.,* 1995; Lambers, 2003). Inland saline waters adversely affect the agricultural outputs and environment in different parts of the world such as USA, Australia, India, China and Israel (Allan, et al., 2001). In India, salinization of inland waters, particularly in the north-west region is increasing at an alarming rate due to both natural and anthropogenic activities (Dhawan et al., 2010). The estimated saline affected land is about 10.1 million ha, which is distributed mainly in the arid and semi-arid regions of Rajasthan, Haryana, Punjab, Gujarat, Uttar Pradesh, Delhi, Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu and Andaman & Nicobar. Haryana, Punjab, Rajasthan and Uttar Pradesh and covers about 40% of the country's total inland saline soil (Singh et al., 2017).

2. Nutritional requirement studies

The knowledge about the nutritional requirement of different species is crucial for formulation of cost effective quality feed in a species-specific manner. There is a wide variation in the nutrient requirement among the aquatic species. Hence, it is necessary to determine the optimum dietary nutrient level of various species to develop the cost-effective diet for intensification of the culture practice.Among the different nutrients, protein is the most important for aquatic organism and requirement study of protein should get the first priority (Lovell, 1975).Protein requirement varies with different species, different life stages within the same species, sex, physical and physiological activities, environmental factors (temperature, salinity) etc. The optimization of protein requirement is necessary to develop cost-effective and environmentally friendly feed.

- **Protein requirement of GIFT Tilapia reared in ISW**

The dietary protein requirement of different species of tilapia varies from 20 to 56% (Winfree and Stickney, 1981; Shiau and Huang, 1990; El-Sayed and Teshima, 1992; FAO, 2019) in relation to different conditions. Generally, the requirement of protein decreases

with age and size. The protein requirements of tilapia fry, juvenile, and adult were found to be 30-56%, 30-40%, and 28-30%, respectively (Winfree and Stickney, 1981; Jauncey, 1982; Al Hafedh, 1999; Siddiqui et al., 1988; Twibell and Brown, 1998). In the case of Nile tilapia, protein requirement ranges from 25 to 45% (De Silva and Perera, 1985; Siddiqui et al., 1988; El-Sayed and Teshima, 1992; Omar, 1994; Abdel-Hakim et al., 2001).

The protein requirement of GIFT tilapia reared in inland saline water at 5ppt and 10ppt was found to be34.20% and 37.37% respectively

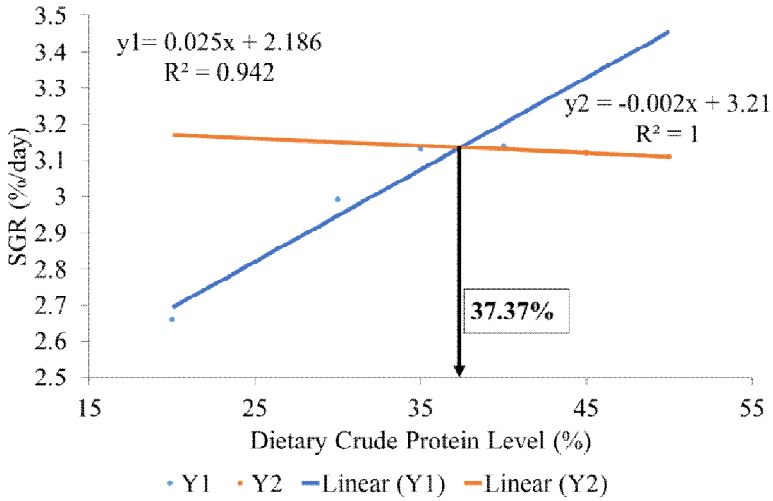

Figure 1: The optimum dietary crude protein requirement in relation to specific growth rate of GIFT fingerlings reared in ISW of 10 ppt and fed with graded levels of dietary crude protein for the period of 60 days

- **Protein requirement of *P. vannamei* reared in ISW**

The optimal dietary protein requirement of *P. vannamei* has been reported from 20 to 45% depending on the shrimp size, salinity, and dietary nutrient levels (Velasco et al., 2000; Martinez-Cordova et al., 2003; Perez-Velazquez et al., 2007; Venero et al., 2008; Jatobá et al. 2014; Shahkar et al., 2014; Sui et al., 2015; Yun et al. 2015; Yun et al., 2016). In inland saline condition, the first study on protein requirement of *P. vannamei* based on broken line analysis of specific growth rate, 35.66% was the optimum dietary protein requirement of L. vannamei reared in ISW at 10 ppt salinity which is shown in Fig 2.

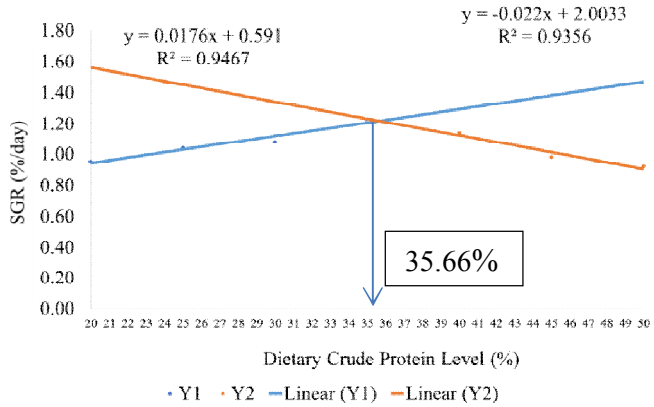

Figure 2: The optimum dietary crude protein requirement in relation to specific growth rate of *Penaeus vannamei* juveniles reared in ISW of 10 ppt and fed with graded levels of dietary crude protein for the period of 60 days

- **Protein:Energy ratio of GIFT tilapia reared in ISW**

Lipids and carbohydrates can spare protein for energy production, thus directing protein towards growth of fish. Therefore, by establishing optimum dietary protein-energy ratio (P: E), dietary protein inclusion can be minimised in the diet to support growth without much of its breakdown (trans-deamination) for energy production, which is met by other non-protein energy sources like lipid and carbohydrate. Studies have demonstrated that feed providing balanced ratio of protein to energy can spare dietary protein for energy metabolism and increase its utilization for fish growth (Nankervis et al., 2000). It also controls higher Specific dynamic action (SDA) and reduces ammonia excreted in the habitat of fish (by reducing excess deamination).The results a study to find out the P:E ratio for GIFT concluded that a diet containing 35% crude protein, 10% ether extract and 400 Kcal DE/100g with P/E ratio of 87.5 mg protein/Kcal DE is optimal for growth and nutrient utilization of GIFT fingerlings reared in inland saline water at 10 ppt.

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Aquafeeds and Potential Feeding Management Applicable to Inland Saline Aquaculture

Shamna N

The feeds used in aquaculture can be broadly classified into commercial feed and farm-made feed. The commerxcial feeds are prepared in companies while the far-made feeds can be prepared in the farm level. Another major classification of feeds includes dry feeds and non-dry feeds. The dry feeds contains 8-12% moisture only while the non-dry feeds have higher moisture level and can be further divided into semi-moist, moist or wet feeds. Based on the life stages of fish the feeds can be again classified into larval feeds, grow-out feed, brood stock feed and designer feed.

Types of feed

Based on the moisture contents aqua-feed are of three types such as i) wet feed ii) moist feed and dry feed.

A. Wet feeds

Moisture levels in the wet feeds range from 45 to 70%. These feeds are prepared using ingredients with high moisture level such as trash fish, fishery waste, slaughter house waste (poultry offal, beef liver) etc. These feeds are made at the farm shed on day to day basis and fed mainly to carnivorous fish. The major disadvantages of these feeds are 1) transportation and storage 2) irregular availability of fresh raw fish.

B. Moist feeds

Moisture level in the moist feeds range from 25 to 45%. These feeds are also made at the farm shed on day to day basis and fed to fish. Some examples of moist feeds are i) moist ball ii) cooked paste iii) cooked ball etc. The major disadvantages of these feeds are transportation and storage problems.

C. Dry feeds

Moisture content in the dry feeds range of 7 to 13%. These feeds are relatively easy to manufacture, transport, store and convenient to dispense into culture systems. Some examples of dry feeds are i) mash feed ii) pellets: a) steam compressed sinking pellets b) Extruded pellets: 1) sinking pellets 2) slow sinking pellets 3) floating pellets.

Over the years many technologies of larval feed development have evolved especially with the advancement in pharmaceutical industry regarding microencapsulation. Larval feed production technology is different from that of starter feed production due to extremely small size of larval feeds (less than 400 micron) and high surface area to volume ratio of such small particles. Leaching of water soluble nutrients is a major problem.Larval feeds needs to be palatable and its nutrients must be easily digestible to larvae whose digestive system may not be completely functional at first feeding. Due to its small size, larval feed requires very high water stability. But at the same time feed binding methods to impart water stability decrease the nutrient availability and digestibility.

Three major categories for larval feed production methods are

1. Microbound
2. Microencapsulated
3. Complex particles

First two methods are differentiated by type of binder used. Microbound particles are held together by binder from inside and these are produced in appropriate size/cake/flake with different processing methods. Microencapsulated feeds are surrounded by a layer of material, a capsule that retains the feed-ingredient mixture inside the particle. These particles can have slow release of the material inside or prevent leaching of the water soluble nutrients. Complex particles consist of embedded particle placed inside large carrier particles.

Microbound feeds are small particulate feeds ranging in size from 50-700 µm, held together by an internal binder (Complex carbohydrate or a protein having adsorptive and adhesive properties). It differs from microencapsulated feeds which are characterized by a distinct wall or capsule surrounding a central core of material.

Feeding situation like species, water temperature, culture system, feeding habits determines types of larval feed. Palatability, nutrient stability, nutrient availability and particle stability are important traits of a microbound feed. Palatability is affected by factors such as smell, flavour and texture. Low particle stability degrades water quality and it may result in gill damage, bacterial contamination, low level of oxygen due to disintegration of particles.

Classes of microbound feeds

3 major classes according to production process are -

1. Crumbled

2. On-size
3. Complex particles

Crumbled feed produced by manufacturing a pellet, flake or cake that is fractured into smaller pieces and sifted for desired size. On-size feeds are manufactured directly to correct size particle. Complex particle is produced by combining 2 or more techniques including microencapsulation. Crumbled feeds can be pelleted feeds or flaked feeds or cake feeds.

On-size feed can be -

1. Microextruded marumerized feeds
2. Particle assisted rotationally agglomerated feed
3. Spray beadlets

Microextruded marumerized (MEM) feeds

MEM is a two step process adapted from pharmaceutical industry to make small, preshaped larval feeds. Feed can be characterized as smooth and spheroid with high density. The process decrease nutrient leaching by decreasing the surface area to volume ratio.

Particle assisted rotationally agglomerated feed

PARA uses a marumerizer without extruded noodles. Wet mash is placed with a charge of inert particles. Low pressure agglomeration resulting in low density feed with slow sinking rate.

Spray beadlets

These are small micround feeds that trap high molecular weight, water soluble nutrients such as starch and protein within gels of calcium alginate and gelatin. Particles are produced by spraying slurry of dietary components and a selected gelling agent into a curing bath.

Complex particles

Complex particles are combination of two or more manufacturing techniques to exploit the advantages and overcome the limitations of individual methods. For example Microcapsules or crumbled cake particles may be embedded within larger MEM, PARA or

spray beadlets. Pelleted feeds can be produced through either steam pelleting, cooking extrusion or cold extrusion. Steam pelleting is a process by which solid pellets are formed by forcing die after preconditioning with steam to a temperature of 70-85^{0}C and moisture content of 15-18%.Cooking extrusion is a process by which a feed mash is moistened with 20-25% water, precooked at 100-150^{0}C , then forced through a die under high heat and pressure.

Strategies

Inland saline water is ioninc imbalanced water and hence, the proper estimation of ions in the culture system has to be done. According to the requirement of salinity, the water can be diluted by addind freshwater or concentrated by adding salts.The major deficient ions like potassium can be fortified in the water or addition of potassium in feed can also be adapted. The level of magnesium varies with zones; hence, have to be taken care of. It is observed in a feeding trial that 50% reduction in the potassium fortification in water could be achieved by 1% supplementation of potassium chloride in feed in *P. vannamei.* Feed can be provided in bags, trays or baskets.

Conclusion

The protein and energy requirement of species reared in inland saline aquaculture can be higher, and hence the requirement should be met. Feeding rate and feeding frequency can be same as that of existing practices. High energy low protein diet will be an eco-friendly diet for inland saline eco-system.

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Effect of Anti-nutritional Factors and its Mitigation

Dilip Kumar Singh and Parimal Sardar

1. INTRODUCTION

Aquaculture has become the fastest growing food production sector of the world, with an average annual increase of about 10%. To sustain such high rates of increase in aquaculture production, a matching increase in the levels of production of fish feed is required. Aquafeed production is currently one of the fastest expanding agricultural industries in the world, with annual growth rates more than 30% per year. Fishmeal and fish-oil are mainly used as the feed for aquatic animals, but fishmeal production requires raw materials either from capture fisheries or aquaculture production. So, the aquafeed industries are turning towards alternative protein sources from plants for feeding the fishes. However, one of the major concerns of including plant ingredients is the presence of Antinutritional factors. Anti-nutritional factors (ANF) are compounds which act to reduce nutrient utilisation and or food intake (These antinutritional factors play a great role in limiting the wider use of many plants. They are natural compounds capable of precipitating deleterious effects in man and animals (Osagie, 1998). The levels of toxic substances in plants vary with the species of plant, cultivar and post-harvest treatment such as soaking, drying, and autoclaving and seed germination. These anti-nutritional factors are also known as 'secondary metabolites' in plants, and they have been shown to be highly biologically active (Zank, 1991).

Antinutritional factors can have adverse effects on the health of the consuming organism. Consuming improperly processed foods especially legumes which are reported to contain very high concentrations of anti-nutritional factors can cause adverse health effects. There are reports from time to time of deaths after consumption of some beans despite cooking and also reports of renal and liver failure. There is a wide distribution of biologically-active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition. Some of these plant chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed in appropriate amounts after adequate processing.

2. CLASSIFICATION OF ANTINUTRITIONAL FACTORS

They could be broadly divided into four groups:

i. Factors affecting protein utilization and digestion, such as protease inhibitors, tannins, lectins

ii. Factors affecting mineral utilization, which include phytates, gossypol pigments, oxalates, glucosinolates

iii. Antivitamins

iv. Miscellaneous substances such as mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitizing agents, phytoestrogens and saponins

Antinutrients may also be classified according to their ability to withstand thermal processing, the most commonly employed treatment for destroying them.

i. Heat-labile factors include protease inhibitors, phytates, lectins, goitrogens, and antivitamins.

ii. Heat stable factors are represented by saponins, non-starch polysaccharides, antigenic proteins, estrogens and some phenolic compounds.

3. MAJOR ANTINUTRITIONAL FACTORS AND THEIR EFFECTS

3.1. Saponins

Saponins cause haemolysis of RBC and decrease liver cholesterol production and decrease bile acids. Saponin is found to have inflammatory effects in several fish species when given alone or as the feed ingredient. However, there are reports that the saponin given along with broad beans and some of the other legumes did not result in inflammation but tended to reduce the fish's utilization of nutrients in the feed. No effect on fish growth, feed intake or feed utilization, though a trend was observed. Neither there was impact on the distal intestinal expression of genes encoding the brush border enzymes ALP and Malt, nor were expression levels of Casp3, CD4, TGFβand TNFα. Numerically but statistically insignificant higher body mass gains of fish fed with saponin fractions tended to have inferior performance. High expression levels of GH and IGF1 significantly correlate with growth and nutrient utilization.

3.2. Tannins

Tannin exhibits in 2 forms, hydrolysable tannin, and condensed tannins, which exhibit antitrypsin and antimycotic activities. They also reduce vitamin B12 absorption. Ten μM tannic acid has a protective effect on DNA damage for short periods whereas at longer incubation times, this compound accelerates the haemolytic event. Total leucocyte

count and respiratory burst activity were significantly increased in tannin-fed groups. Quebracho tannin at a level of 2% in the diet did not have any adverse effects on carp up to 84 days of feeding. Tannic acid had no apparent adverse effects in the initial period of about 28 days, after which adverse effects such as reduction in feed intake, metabolic growth rate and oxygen consumption became apparent. De-oiled copra meal can be incorporated into carp diets up to 20% in the raw condition and up to 30% in the treated condition without any deleterious effects on growth performance of *Labeo rohita* fingerlings.

3.3. Oxalates

Oxalic acid is a metabolic product formed through several pathways in plants and animals. Oxalates of monovalent ions, such as sodium, potassium or ammonium are well soluble in water while those oxalates formed with divalent ions, such as calcium, magnesium and iron are almost insoluble. Oxalate has long been considered an anti-nutritional factor in humans and consumption in the form of plants containing high amounts of oxalate, such as spinach, beet or rhubarb, has been recommended not to exceed an upper limit. Documented adverse effects of dietary oxalate in mammals include the binding of oxalate with calcium in the intestinal lumen to form insoluble calcium oxalate making calcium unavailable for absorption and excreting it with the faeces. This may lead to low blood calcium levels and in cases of long-term exposure, the bone material may be excessively mobilized to compensate for the shortages of minerals. Further, long-term exposure to a high-oxalate diet may lead to the formation of Ca or Mg-oxalate stones in the kidney, which can cause urine flow problems or kidney failure. For ruminants, dietary oxalate consumption should be less than 2% to avoid oxalate poisoning and less than 0.5% for non-ruminants. Soluble oxalate content in some potential fodder crops used in aquaculture diets is within these critical ranges: saltbush *Atriplex halimus* 1.0 – 3.0%, rice *Oryza sativa* 1.0 – 2.5%, *Medicago sativa* 0.9-1.1%; *Jatropha curcas* 2.4%. Oxalate supplementation (0%, 0.5%, 1.5% and 2.5%) in common carp had a positive effect on growth, and it is hypothesized that this may be due to antimicrobial effects exerted in the intestine. Oxalate increased mineral and lowered lipid content. However, the exact mechanisms of these effects on the fish still need to be researched.

3.4. Gossypol

Gossypol reduces iron absorption and complex with protein, resulting in methionine deficiency. Monogastric animals, such as pigs, birds, fish, and rodents, are more susceptible to gossypol toxicity than ruminants. Immunotoxicity of gossypols are demonstrated by macrophage chemotaxis induced by *Edwardsiella ictaluri* challenge was increased in channel catfish (*Ictalurus punctatus*) fed cottonseed or receiving gossypol, but catfish were unaffected by gossypol in another study. Gossypol also increased serum lysozyme activity in channel catfish following an *E. ictaluri* challenge.

3.5. Phytates

Phytate is an organic chelate which binds to P, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+} and amino acids. In human nutrition, phytic acid is a commonly used additive because of its antioxidant properties which make it a versatile food preservative. However, in animal nutrition, phytate has been described mainly as an ANF. In general, fish fed with a diet high in PA show a lowered growth performance. The latter can be attributed to various factors such as a reduced bio-availability of minerals, impaired protein digestibility caused by the formation of PA–protein complexes and depressed absorption of nutrients due to damage to the pyloric cecal region of the intestine. For example, it was observed decreased growth rates in rainbow trout fed a diet containing 5 g kg^{-1} synthetic PA. Similar studies with chinook salmon and carp show the same results. Also, for PA, the sensitivity is species dependent. Salmonids seem to be able to tolerate dietary levels of phytate in the range of 5–6 g/kg, while carp appears to be sensitive to these levels. It seems to be advisable to maintain the level of phytates below 5 g/kg in fish feeds. A significant concern about the presence of phytate in aquafeeds is the negative effect on the overall growth performance due to its interference with mineral uptake. Phytate chelates with di- and trivalent mineral ions such as Ca^{2+}, Mg^{2+}), Zn^{2+}, Cu^{2+}, and Fe^{3+} preventing their availability for fish growth. PA was also reported to interact with other compounds. Formation of complexes with protein and carbohydrate (starch) reduce their bioavailability and digestion. For instance, the creation of PA–carbohydrate complex influences digestion rate of starch. PA–protein complex will inhibit digestive enzymes. Fish can absorb phosphorus directly from the water. Nevertheless, the main provider of phosphorus is the diet since absorption via the water is believed to be very low. Within plant-based feeds, 50–80% of the phosphorus occurs in the form of the calcium or magnesium salt of PA. This organic form of phosphorus must first be

hydrolysed within the gastrointestinal tract by the enzyme phytase to inositol and phosphoric acid before it can be utilised and absorbed by the animal. For monogastric animals, this form of phosphorous is not available because they lack the enzyme phytase in their digestive tract. When phosphorous is deficient, it not only affects hard tissues such as bone and cartilage but also has a negative influence on various aspects of the intermediate metabolism. Phosphorus deficiency is responsible for rickets, leading to skeletal malformation. Therefore, optimising the dietary inclusion level is critical at all times. High dietary phytate levels (2.2%) have also been reported to have a negative effect on fish growth and feed efficiency in channel catfish.

3.6. Phytoestrogens

Phytoestrogens are a broad class of estrogenic compounds found in plants that have the potential to disrupt fish sexual development and gamete quality when they are concentrated and released into the environment due to human activity. Numerous studies have reported biologically active levels of phytoestrogens (particularly genistein, equol, and -sitosterol) in the effluent from wood pulp mills or sewage treatment plants or in agricultural soils. At these concentrations, phytoestrogens can disrupt sexual differentiation and induce production of egg yolk protein in males. The potential for fish populations to experience widespread and acute contamination from phytoestrogens is significant; undiluted plumes of pulp and paper mill effluent have been reported dozens of kilometres downstream from point sources.

Relatively little is known about the effects of phytoestrogen exposure on fish sperm quality. Sperm motility and concentration decreased in a dose-dependent manner in rainbow trout, *Oncorhynchus mykiss*, fed diets experimentally enriched with genistein, an isoflavone. The male fighter fish exposed to waterborne genistein and - sitosterol (a phytosterol) at environmentally relevant and pharmacological concentrations for four weeks but found that neither dose of either phytoestrogen affected sperm quality. It was found that -sitosterol disrupted transcription of steroidogenic acute regulatory (StAR) protein in goldfish, *Carassius auratus*, which could interfere with steroidogenesis. Genistein had a significant negative effect on sperm motility, ATP content, and in vitro fertilization rates in both species. The aforementioned studies all focus on phytoestrogen disruption of sperm maturation, but little work has been done on acute sperm exposure to phytoestrogens, spontaneous swimming activity, latency to respond to a perceived intruder (mirror reflection),

intensity of aggressive response toward a perceived intruder, probability of constructing a nest in the presence of a female, and the size of the nest constructed. A few changes in spontaneous swimming activity, the latency to respond to the mirror, and nest size, and modest changes in the probability of constructing a nest have been found in *Betta splendens*. There were significant decreases, however, in the intensity of aggressive behaviour toward the mirror following exposure to several concentrations, including environmentally relevant ones, of 17 beta-estradiol, genistein, and equol. This suggests that phytoestrogen contamination has the potential to significantly affect the behaviour of free-living fishes.

3.7. Protease inhibitors

Protease inhibitors involved in the activation of complement, blood coagulation and inflammatory response in mammals. Seed extracts did not affect gastric proteases whereas they significantly inhibit intestinal proteases. Inhibition of alkaline proteases showed that pancreatic proteases of *L. argentiventris*were more sensitive to seed protease inhibitors than those of *L. novemfasciatus*. Legume seeds showed the highest inhibitory capacity on alkaline proteases causing inhibition higher than 50% of total proteolytic activity. Protease inhibition on digestive extracts was assessed using different relative concentration of seed extracts and represented by constructing dose- response curves. To reduce the inhibitory effect, seed extracts were acid-treated before the inhibition assay. Results showed that acid treatment did not affect the inhibitory capacity of seeds on alkaline proteases in both species. However, when the action of gastric enzymes was simulated on seed extracts, the inhibitory capacity was reduced significantly, mainly in the case of *L. novemfasciatus*. The responses of fish enzymes to heat-treated seed extracts were also tested. Only higher temperatures were capable of reducing the inhibitory capacity of seed, with the specific response to the snapper species. The use of biochemical assays allows us to quantify the action of inhibitors on total proteolytic activity. Also, zymograms obtained by substrate-SDS-PAGE provided qualitative information about the number and type of proteases affected by each inhibitor. Each seed extract produces a characteristic profile of inhibition of alkaline protease. The results obtained are important for the future formulation of feeds for these snapper species.

3.8. Indigestible oligosaccharides

Most research concerning indigestible oligosaccharides has been conducted on humans. The data available for fish feed is much more limited. Most of the experiment was conducted with soybean and its derived products, including SBMR. Those experiments showed that indigestible oligosaccharides have some unwanted physiological effects when incorporated in the fish diet. Indigestible oligosaccharides have long been considered as undesirable indigestible factors that promote flatulence. Both raffinose and stachyose are considered ANFs because their fermentation in the gut of monogastric animals causes discomfort (flatulence), inducing stress in the animals. Oligosaccharides also increase the viscosity of the chime n the digestive tract, interfering with the absorption of other nutrients. In general, it can be stated that the oligosaccharides negatively affect the nutrient utilization of the feed. For example, in salmon and rainbow trout, the removal of oligosaccharides from SBM significantly improved the utilization of other nutrients. Additional experiments showed that in fact, it was the soluble carbohydrates, extracted with alcohol from SBM, that lowered the digestibility of lipids, protein and minerals. According to the sensitivity to oligosaccharide is highly dependent on fish species. It was observed that rainbow trout tolerates higher levels of oligosaccharide than Atlantic salmon.

3.9. Miscellaneous anti-nutritional factors

Glucosinolates are broken down to Isothiocyanates and VTO by the enzyme, myrosinase, and thus they inhibit the binding of iodine to T3/T4, resulting in the goitrogenic effect. Cyanogens produce HCN and glucans by the beta-glucosidase enzyme. The HCN inhibit ETC. Toxic amino acids such as mimosine interfere with the action of thyroxin and inhibit transaminases. Alkaloids such as caffeine, nicotine and cocaine affect the nervous system. Lectins are another group of ANFs, which include agglutinins such as soy agglutinins, ricin etc. They cause agglutination of RBC by binding to carbohydrate receptors on RBC. They also bind to the intestinal mucosa. However, they are inactivated by pepsin in gastric fishes.

Detoxification methods for major Antinutritional factors

Sl. No.	Antinutritional factor	Detoxification method
1.	Saponins	Solvent extraction
2.	Tannins	Dehulling/Milling

3.	Oxalates	Treat with boiling water
4.	Gossypol	Moist heat treatment
5.	Phytates	Enzymatic degradation
6.	Phytoestrogens	Solvent extraction
7.	Protease inhibitors	Heat treatment
8.	Indigestible oligosaccharides	Preparation of protein concentrates

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Fish Feed Formulation and Preparation

G. H. Pailan

1. INTRODUCTION

Present trend of intensified aquaculture can be sustained by feeding with nutritionally balanced feed. By selecting various ingredients in correct amounts, a compounded feed which is nutritionally balanced, palatable, pelletable and easy to store can be prepared. Based on the formula mash and pellet feed can be prepared. From the mash feed wet ball, cooked paste and cooked ball can be prepared. As feed is the single largest input cost being around 50 to 60% in aquaculture production, judicious formulation is required to develop economic, eco-friendly and quality feed to sustain the aquaculture operation. Appropriate feeding method should also be employedin feed-based aquaculture system to avoid feed wastage and environmental pollution.

2. FEED FORMULATION

Feed formulation is the process of quantifying the amounts of feed ingredients that need to be combined to form a single uniform mixture for animals including fish that supplies all nutrients at their required level. A feed ingredient is a constituent or any combination/mixture added to and comprising the feed. Nutrient is a substance of feed ingredient or feed that provides nourishment, which is essential for the maintenance of life, growth, reproduction and health protection. No single ingredient is expected to fulfil the nutrient requirement of any cultured organism. Supplying adequate nutrition for various aquaculture species involves the formulation of diets containing about 40 essential nutrients. Among these, balancing of at least crude protein and protein to energy ratio (P:E) are important because protein helps in growth and fish fed on energy satiation.

2.1. Traditional method

In traditional method, there is no systematic formulation. Different ingredients are mixed at different ratios depending upon the availability. In this method balancing of nutrients is not generally checked and generally additives are not added Examples of some traditional formulae are given below:

Ingredients	**Ratio**	**CP (%)**
Mustard oil cake (MOC) or ground nut oil cake (GNOC) and rice or wheat bran	1:1	20-22
MOC:GNCor SBM:DORB:Wheat flour	2.5:2.5:3:2	25-28
MOC, deoiled rice bran (DORB) and Linseed oil cake (LOC)	4:5:1	25
Sunflower oil cake (SOC): DORB: Silkworm pupae (SWP)	13:6:1	25-30
MOC: LOC: DORB: SWP	9:3:7:1	25-28

2.2. Improved method

2.2.1. Pre-requisite information needed for feed formulation

- Nutrient requirements of the fish species at different life stages
- Food and feeding habits of fish
- Locally available feed ingredients, their cost, nutrient composition and quality (digestibility)
- Type of feed to be prepared
- Feed additives need to be added

2.2.2. Methods of feed formulation

Diets are formulated to balance protein, lipid, energy, vitamins, minerals, energy and protein: energy ratio at required levels of fish. As balancing of all nutrients and energy is cumbersome process during formulation, at least balance of protein and energy should be made through formulation of aquafeed. If the energy and protein content of the diet is balanced, then other nutrients are automatically almost augmented. However, marginal supplementation of some critical nutrients is required to avoid deficiency, if necessary. Different mathematical techniques are used to balance the nutrient content of the diet that are i) Pearson's Square Method followed by Trial and Error Method and ii) least cost formulation such as a) Graphic solution b) linear programming c) Solver function on excel and d) use of computer assisted readymade software. Method of feed formulation for food fishes and ornamental fishes are same except consideration of some special additive for later one, for example, dietary supplementation of carotenoid is especially considered for ornamental fish to enhance and augmentation of bright colouration in this fish.

2.2.2.1. Pearson's Square Method for balancing of crude protein (CP)

Through Pearson's square methods individual nutrient as well as energy can be balanced separately in the feed and finally all nutrients and energy can be balanced using trial and error method. Different feed supplement and feed additives can also be considered during feed formulation by this method both for food and ornamental fishes. Here Pearson's Square Method for balancing crude protein only be discussed.

Example I without additive

Formulate a diet to balance 30% crude protein (CP) by using the ingredients such as fish meal (60 % CP), ground nut oil cake (GNOC) (40 % CP), de-oiled rice bran (DORB) (12 % CP) and maize meal (9 % CP).

Pearson's Square Method

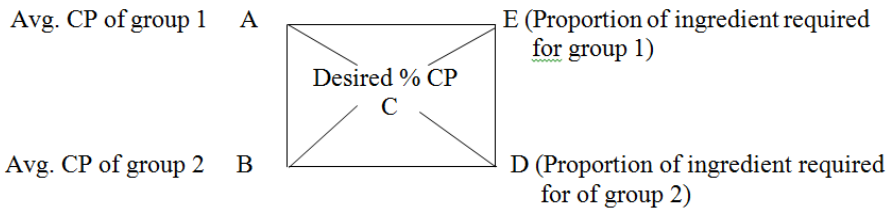
Through this method protein level is balanced in the formula by the calculation of following steps.

Step-1

Fix the amount of selected ingredients considering economic point of view. Suppose the fishmeal is fixed at 15% inclusion level. So, fishmeal (15%) will contribute 9% (i.e. 15 X 0.60 = 9.0)protein in the diet. Therefore, the other 85% (100-15 = 85%) of the non-fishmeal ingredients will have to makeup rest 21% (30-9 = 21%) of the protein. If this part of the formulation is treated separately on fresh 100% basis, the non-fishmeal portion of the diet must contain 21 X 100/85 = 24.70 % of CP.

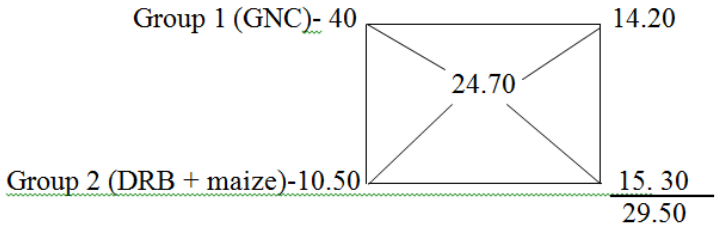
Step-2

All the non-fishmeal ingredients should be divided into two groups. One group should contain more than 20% CP termed as protein rich ingredients. Other group should contain those ingredients having less than 20% CP termed as energy rich ingredients. Accordingly GNC (40% CP) should be placed in protein rich group (group 1) and DRB (12% CP) and maize (9% CP) should be placed in energy rich group (group 2) in the square as follows:



Step-3

So group-1 contains GNC and average protein is 40%. Similarly group-2 contains rice bran and maize (50:50) and average protein = (10+9)/2 = 10.5%.So put the value in respective place. And difference of A-C and B-C should be put in D and E, respectively ignoring the sign.



Step-4

The proportion of each ingredient should be calculated on percent basis as follows

GNC = (14.20 X 100)/29.50 = 48.14%,

DRB + Maize (50:50) = (15.30 X 100)/29.50 = 51.86%

So, DRB= 51.86/2 = 25.93%

andMaize = 51.86/2 = 25.93%

Step-5

These ingredients, however, should actually constitute only 85% of the mixture. Therefore, the amount of GNC, DRB and maize to be incorporated in the final mix is 40.92% (48.14 x 0.85), 22.04% (25.93 x 0.85) and 22.04% (25.93 x 0.85), respectively.So for balancing the 30% CP level, the inclusion level of ingredients in diet will be as follows:

Ingredients	**Inclusion level (%)**
Fish meal	15
GNC	40.92
DRB	22.04
Maize	22.04
Total	100

Example II with additive

Formulate a diet to balance 30% crude protein (CP) by using the ingredients such as fish meal (60 % CP), ground nut oil cake (GNOC) (40% CP), de-oiled rice bran (DORB) (12 % CP) and maize (9 % CP) with the supplementation of 2% vitamin-mineral mixture and 1% carboxymethyl cellulose (CMC) as binder.

Pearson's Square Method

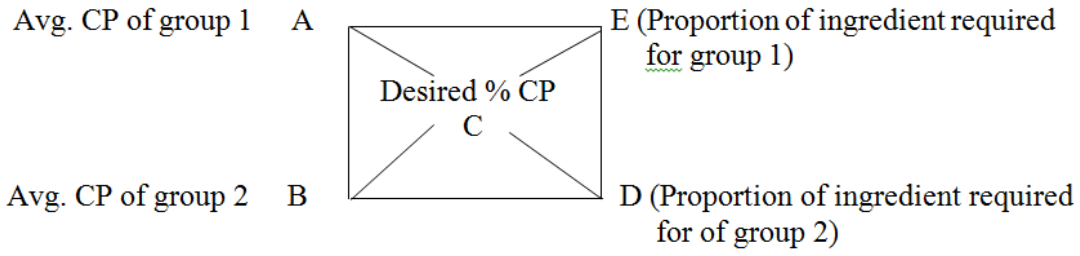
Through this method protein level is balanced in the formula by the calculation of following steps.

Step-1

3% additives (vitamin-mineral mixture and CMC) will not provide any protein. Fix the amount of selected ingredients considering economic point of view. Suppose the fishmeal is fixed at 15% inclusion level. So, fishmeal (15%) will contribute 9% (i.e. 15 X 0.60 = 9.0)protein in the diet. Therefore, the other 82% (100-18 = 82%) of the non-fishmeal ingredients will have to makeup rest 21% (30-9 = 21%) of the protein. If this part of the formulation is treated separately on fresh 100% basis, the non-fishmeal portion of the diet must contain 21 X 100/82 = 25.61% of CP.

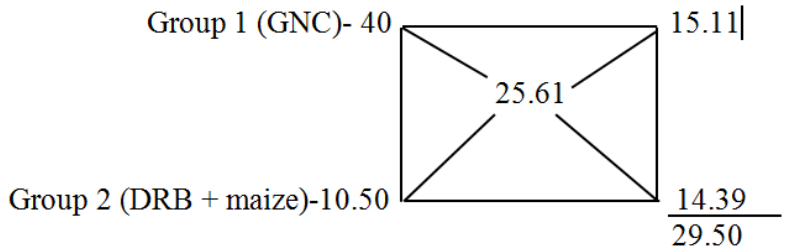
Step-2

All the non-fishmeal ingredients should be divided into two groups. One group should contain more than 20% CP termed as protein rich ingredients. Other group should contain those ingredients having less than 20% CP termed as energy rich ingredients. Accordingly GNC (40% CP) should be placed in protein rich group (group 1) and DRB (12% CP) and maize (9% CP) should be placed in energy rich group (group 2) in the square as follows:



Step-3

So group-1 contains GNC and average protein is 40%. Similarly group-2 contains rice bran and maize (50:50) and average protein = (10+9)/2 = 10.5%.So put the value in respective place. And difference of A-C and B-C should be put in D and E, respectivelyignoring the sign.



Step-4

The proportion of each ingredient should be calculated on percent basis as follows:

GNC = (15.11 X 100)/29.50 = 51.22%,

DRB + Maize (50:50) = (14.39 X 100)/29.50 = 48.78%

So, DRB= 48.78/2 = 24.39%

andMaize = 48.78/2 = 24.39%

Step-5

These ingredients, however, should actually constitute only 82% of the mixture. Therefore, the amount of GNC, DRB and maize to be incorporated in the final mix is 42.00% (51.22 x 0.82), 20.00% (24.39 x 0.82) and 20.00% (24.39 x 0.82), respectively. So for balancing the 30% CP level, the inclusion level of ingredients in diet will be as follows:

Ingredients	Inclusion level (%)
Fish meal	15
GNC	42
DRB	20
Maize	20
Vitamin-mineral mixture	2
CMC	1
Total	100

3. FEED PREPARATION

Different methods are employed for preparation of different types of feed. Thus, first we should know about the different types of aqua-feed used for feeding the fish.

3.1. Preparation methods of different types of Feed

3.1.1. Preparation methods of mash feed

i) Formulation ii) procurement of ingredients iii) Grinding of ingredients iv) weighing of ground ingredients as per formula v) mixing of ground ingredients to form mash feed vi) bagging vii) storage.

3.1.2. Preparation methods of farm-made steam compressed sinking pellets

i) Formulation ii) procurement of ingredients iii) Grinding of ingredients iv) weighing of ground ingredients as per formula v) mixing of ground ingredients vi) conditioning and cooking vi) preparation of pellets using pellet machine vii) drying of pellets (for farm made pellets drying can be done under sunshine but for factory made pellets drying can be done using mechanical cooler and dryer). In factory made feed additives and oil can be added to the pellets by top dressing method viii) bagging ix) storage.

3.1.3. Preparation methods of sinking pellets

i) Formulation ii) procurement of ingredients iii) Grinding of ingredients iv) weighing of ground ingredients as per formula v) mixing of ground ingredients (without feed supplements, feed additive and oil if any) vi) Adding some water to make a dough vii) keeping the dough in a plastic bag viii) cooking of dough under steam with the help of

pressure cooker up to 14-16 sities or 15-20 minutes ix) cooling the dough x) adding feed supplements, feed additive and oil xi) preparation of pellets with the help of hand or motor operated simple pelletizer xii) drying of pellets in room temperature under ceiling fan viii) packing ix) storage.

3.1.4. Preparation methods of wet ball

i) Formulation ii) procurement of ingredients iii) Grinding of ingredients iv) weighing of ground ingredients as per formula v) mixing of ground ingredients to form mash feed vi) addition of water to make dough vii) preparation of small wet balls and used immediately.

3.1.5. Preparation methods of cooked paste

i) Formulation ii) procurement of ingredients iii) Grinding of ingredients iv) weighing of ground ingredients as per formula v) mixing of ground ingredients to form mash feed vi) take in a large cooking container vii) addition of water vii) cooked up to preparation of paste viii) cooked paste should be immediately used after cooling.

3.1.6. Preparation methods of cooked ball

i) Formulation ii) procurement of ingredients iii) Grinding of ingredients iv) weighing of ground ingredients as per formula v) mixing of ground ingredients to form mash feed vi) take in a large cooking container vii) addition of water vii) cooked up to preparation of paste viii) cooling of paste viii) preparation of small balls from cooked paste and use immediately.

4. CONCLUSION

Aquaculture system mostly depends upon supplemented feeds for optimum growth and production of fish. No single ingredient can be expected to meet all nutrient requirements of cultured organisms. Through feed formulation where appropriate feed ingredients are selected in correct amounts and blended to produce a diet which is nutritionally balanced with the required quantities of essential nutrients.

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IMPORTANCE OF LIVE FEED IN INLAND SALINE AQUACULTURE

S. Munilkumar

Introduction

Fishes,prawns and other cultivable aquatic animals at the time of their first feeding are quite fragile and delicate creatures. It is most **'critical phase'** of their life when they need right type of nourishment for their survival and growth. If this requirement is not met, they perish.

In nature, most fishes and prawns during their **'critical phase'** receive their nourishment by feeding on various types of phyto and zooplankton. Such a diet does not only provide a very wide spectrum composition of food but because of its auto digestion characteristics, also facilitates better nutrient assimilation in the larvae. This is why live food organisms are called as **"living capsules of nutrition"** for fish and prawn larvae.

Past Practices and Present Trend

In the past, for taking care of **'critical phase'** of fishes and prawns one had to look for a suitable and practical substitute for natural plankton, taking into account their nutritional quality as well as production costs in the selection process. Later on, some aqua hatcheries, located close to the sea, rivers and large water bodies, started making use of wild zooplankton for the purpose both as sole food source and as a supplementary diet. Though, wild zooplankton provided the larvae a complete and balanced diet but the practice proved problematical on several scores such as

i) Poor survival and storage difficulty;
ii) Presence of bigger zooplankton like *Cyclops,* even in small number, causing serious damage to the larvae;
iii) Risk of bringing in parasites (viz. *Argulus, Lernaea* etc.) to the hatchery;
iv) Variations in quality and quantity of collected zooplankton etc.

As an answer to these problems, the idea of culturing these planktonic organisms was conceived. Consequently, culture techniques for a number of species of algae, rotifers, cladocerans and other such live food organisms were developed. Today, these live feeds are cultured world over and used as larval feed in aqua hatcheries.

Important Live Food

Algae:

Algae are chlorophyll bearing unicellular or multicellular plants. When multicelluar, they may be colonial or filamentous. Most of them are aquatic. Besides chlorophyll, they also show various carotenoid pigments which impart different colours to algae. According to the nature of photosynthetic pigments, algae are further classified into three divisions such as Chlorophyta (green) algae, Phaeophyta (brown algae) and Rhodophyta (red algae). While brown and red algae are mostly marine forms, green algae i.e. Chlorophyta is mostly freshwater and free floating type. Brown algae contain iodine and algin. Some red algae are the source of agar jelly, used in the preparation of ice creams and culture media. Chlorophyta, i.e. green algae serve as initial food producers and the first link in the aquatic food chain, both in freshwater and marine ecosystems.

Use of micro-algae as a possible source of protein food was recognized by the researchers in mid 20th century. In the beginning, the main attention had been on the production of single cell protein (**SCP**) for human consumption. Later on, however, many new applications came to be recognized viz wastewater treatment, nutrient recycling, bio-conservation of solar energy, etc. In recent years, mass culture of unicellular algae such as diatoms (viz. *Chaetoceros* and *Skeletonema*) and small phytoplankters (viz. *Isochrysis, Tetraselmis and Chlorella*) is becoming quite popular for feeding larvae of fishes, prawns, shrimps and molluscs in aqua hatcheries.

Importance of micro-algae in aqua hatcheries not only owes to its nutritional attributes but more so for its small size ranging from 5 to 25 microns meeting the feed size requirements ideally well for early stages of various aquatic animals. Today, micro-algae is used as an essential food source for rearing all stages of marine bivalve molluscs (clams, oysters, scallops), gastropods (abalone, conch), larvae of fishes (code, halibut, tilapia) and shrimps (*Penaeus* sp). Micro-algae also constitute an important source of food for live food organisms (rotifers, copepods, cladocerans, brine shrimp etc.) used in aqua hatcheries. Importance of micro-algae as larval food is also because it stimulates enzymatic synthesis and on-set of feeding in young larvae. Besides, it also acts as water conditioner by stripping off the nitrogenous substances.

Infusoria

Infusoria refers to microscopic single celled animalcules belonging to the Class Ciliata of Phylum Protozoa. Besides being small in size, they are soft bodied and nutritionally very rich and, therefore, serve ideally as starter diet for early stages of fish larvae. *Paramoecium* and *Stylonychia* are the most common forms of freshwater infusoria while *Fabrea* and *Euplotes* are of marine ones.

Rotifers

Popularly called "wheel animalcules"rotifers are an important group of live food organisms for use in aqua hatcheries. Brachionus, which is the most known form of all rotifers, serve as an ideal starter diet for early larval stages of many fish and prawn species. Depending on the mouth size of the cultured organisms, small (50 to 110 micron length) or large (100 to 200 micron length) rotifers are used.

Maintaining large cultures of rotifers and their production on a predictable basis is a major problem. The food of rotifers appears to the key element in their mass production. Presently, fresh baker's yeast is mostly used as the main diet ingredient for rotifers. However, its freshness, a criterion that is difficult to evaluate, can greatly influence the dietary value of the yeast for the rotifers and as a consequence, determine success of rotifer culture. Several measures are taken to deal with the problem such as supplementation of baker's yeast with micro-algae, improving the nutritional quality of rotifers through vitamin C supplementation, treatment with antibiotics to prevent bacterial contamination and use of probiotics, i.e. the addition of beneficial bacteria in rotifer culture.

Artemia

Of the live food used in aqua hatcheries, *Artemia,* commonly called brine shrimp, constitute the most widely used organism. It is an organism closely related to shrimp belonging to the Order Anostraca of the Class Crustacea and Phylum Arthropoda. The biggest plus point of using *Artemia* is that one can produce live food "on demand"from a dry and storable powder i.e. dormant *Artemia cysts* which upon immersion in seawater regain their metabolic activity and within 24 hours release free - swimming larvae (nauplii) of about 0.4 mm length. *Artemia,* has high nutritive value and high conversion efficiency. All the life stages of *Artemia,* i.e. cysts (after decapsulation), nauplii, juveniles, sub-adults are used as feed. Today, in majority of the commercial aqua hatcheries, *Artemia,* nauplii is virtually used as a sole diet. Frozen adult *Artemia,* are widely used by aquarists, fish breeders and aquaculturists. *Artemia* biomass is also used as food additive for domestic

livestock or extraction of pharmaceutical products as also in making protein rich food products. It is even used for human consumption in some countries. Owing to its great utility value, *Artemia* trading is a growing business in several parts of the world. An important characteristic that influences the suitability of Artemia in aqua hatcheries is the size of nauplii, which can vary greatly from one geographical source to another. This is one of the reasons why the local strains of *Artemia* in India are not performing so well in aqua hatcheries and, therefore, hatchery operators here mostly have to bank on imported *Artemia* cysts.

Cladocerans

Cladocerans called "water fleas",Cladocera is an Order of sub-class Branchiopoda and Class Crustacea of the Phylum Arthropoda. Two cladocerans, namely *Daphnia* and *Moina* are important as live food. *Daphnia* is found in freshwater ponds, tanks and lakes, all over the world. It swims by rapid jerks of the two large antennules. *Daphnia* contains a broad spectrum of digestive enzymes such as proteinases, peptidases, amylase, lipase and even cellulase which serve as exoenzymes in the gut of fish and prawns. Being larger in size than *Moina,* it serves as live food for advanced stages of fishes. *Moina* are primarily inhabitants of temporary ponds or ditches. It is smaller in size (0.5 to 2 mm) than *Daphnia* containing 70% more protein and therefore, goes well as a replacement for *Artemia* in aqua hatcheries.

Tubifex

Tubifex is a type of worm of Class Oligochaeta under the Phylum Annelida. Clusters of these worms are commonly seen in sewage drains. When disturbed, they jerk into the mud. Though not for larval and post-larval stages, but *Tubifex* make an ideally suited diet for brooders of various ornamental fishes.

Chironomid Larvae

Chironomid is an insect belonging to the Order Diptera of the Class Insecta of Phylum Arthropoda. The larvae of this insect make a staple item of food to the young ones of all carnivorous fishes.

Conclusion

Although a good deal of progress has been made in understanding the dietary requirements of larval and post-larval stages of cultivable fishes, crustaceans, and molluscs, but commercial culture of early stages of these animals still require use of live food. Important groups of live food *viz.* various species of micro-algae, the rotifer *Brachionus*, and the anostracan brine shrimp (*Artemia*), are available for use in aqua hatcheries. Techniques for culture of these live food organisms need to be popularised so as to meet the requirements for successful operation of aqua hatcheries in different parts of the country.

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Feed Additives, Drugs and Chemicals in Aquafeed

Shamna N

1. INTRODUCTION

Efforts to intensify aquaculture of valuable aquatic animal species can lead to increased stress, limited growth performance and poor welfare in farmed fishes and shellfishes. Feed additives are well known for their function in boosting aquafeeds and safeguarding general health of aquatic animals. There are several functional feed additives including probiotics, prebiotics, synbiotics, immunostimulants, organic acids, nucleotides and medicinal herbs have been found to possess beneficial immunostimulant and anti-stress relieving properties. Oxidative damage (oxidative rancidity) and microbial attack lead to major problem in feed storage. The absence of natural antioxidant protection in rations rich in polyunsaturated fatty acids is highly prone to oxidative decomposition which in turn may cause a reduction in the nutritive value (Lall, 2002). Furthermore, the use of natural feed additives to serve these purposes increases the consumer confidence of farmed fish.

2. ADDITIVES

Feed additives are substances which are added in trace amounts to a diet or feed ingredient to

a) preserve its nutritional characteristics,
b) facilitate ingredient dispersion or feed pelleting
c) facilitate feed ingestion and consumer acceptance of the product,
d) supply essential nutrients in purified form or
e) facilitate growth,

The feed additives make feed more efficient by enhancing its pelletability, palatability, attractiveness. These are antibiotics, colouring materials, flavours, hormones, medicines, binders etc. The following are the major categories of feed additives-

2.1. Functional amino acids

Dietary amino acids are crucial for fish as energy substrates, endogenous protein synthesis and to regulate metabolic pathways. Recently, there are several studies to use amino acids to increase disease resistance, immune response and reproduction as well as aquatic animal welfare. The commonly studied ones are arginine, glutamine, methionine,

cysteine and taurine, histidine and branched chain amino acids like leucine, isoleucine and valine. This has led to a boost of commercially available functional fish feeds that aim to optimize fish performance and quality of the product.

2.2. Essential fatty acids (EFA)

The fatty acid cannot be synthesized within the body are called as essential fatty acids. For fresh water fish, linoleic acid (18:2 n-6) and a linolenic acid (18:3 n-3) are essential fatty acids, whereas arachidonic acid (AA 20:4 n-6), eicosapentaenoic acid (EPA 20:5 n-3) and docosahexaenoic acid (DHA 22:6 n-3) are essential for marine fish. Depending on the species, EFA are supplemented @ 0.5-1% level in the formulated diet, especially in broodstock diet to increase viable egg and in larval diet to increase survivality. The arachidonic acid serves as precursors for biologically active eicosanoids, which are in turn important mediators in inflammatory reactions and partly also in the regulation of immune response.

2.3. Phospholipids

Phospholipids are the most important constituents of plasma membrane, maintain the permeability of plama membrane. It is an essential component in crustacean diet. Phospholipid (Lecithin) is commonly supplemented @ 2-4% in fish diet for optimum growth and survival whereas in the formulated feed of marine shrimp supplemental level is 0.2-2%. Soya lecithin is preferable phospholipid for fish diet.

2.4. Cholesterol

Shellfish cannot synthesize cholesterol in their body. Need dietary addition @ 0.5%. The main function of cholesterol in crustacean is the synthesis of moulting hormone ecdysone.

2.5. Vitamins

Some vitamins are not synthesised or synthesised in insufficient amounts to meet the physiological needs of the animal and therefore must be obtained from the diet. In aquatic animal nutrition, vitamins are among the most expensive ingredients used in complete diet formulation. Vitamins play essential roles in the organism, acting principally as cofactors for enzymes; thus, inadequate supply leads to reduced enzyme activities and in turn to both non-specific responses such as poor growth, low survival and increased

susceptibility to infections and diseases as well as more specific deficiency signs and symptoms. Vitamin C is one of the important nutrients because it is a powerful antioxidant and immunomodulator for fish/ shrimps. Vitamins C and E are major antioxidant additives used in the food industry and have been shown to reduce the oxidative stress in animals.

2.6. Trace minerals

Minerals serve as essential components for enzymes, vitamins, hormones, pigments, and co-factor in metabolism, catalysts, and enzyme activators. Shrimp can absorb or excrete minerals directly from the aquatic environment via gill and body surfaces. So, the dietary requirement of minerals is largely dependent on the minerals concentration of the aquatic environment in which the shrimp is being cultured. The micro-elements or trace minerals, such as chromium, cobalt, copper, iodine, iron, manganese, molybdenum, selenium and zinc, are required in small quantities and participate in a wide variety of biochemical processes. They are involved in cellular metabolism, formation of skeletal structures, maintenance of colloidal systems, regulation of acid–base equilibrium, immunity enhancer, stress releaser, disease resistance and other physiological functions. They are important components of hormones and enzymes, and serve as cofactors and/or activators of a variety of enzymes. The microelements have especially been strengthened by the importance of their roles in immune defence and antioxidative protection.

2.7. Acidifiers

Organic acids have been used for decades as food preservatives in livestock feeds due to their antimicrobial properties, as well as their ability to enhance growth, nutrient utilization and disease resistance of aquatic animals. In the intestinal tract of aquatic animals, organic acids inhibit the growth of Gram-negative bacteria by penetrating through the cell wall and releasing protons in the cytoplasm. Thus, the bacteria consume a large amount of adenosine triphosphate (ATP) to excrete protons in trying to keep the balance of intracellular pH, thus depleting cellular energy and subsequently leading to death. Organic acids and their salts can also contribute in nutritional ways; they are components in several metabolic pathways for energy generation, such as for ATP generation in the citric acid cycle or carboxylic acid cycle. It appears that organic acids and/or their salts have good potential as dietary supplements to improve growth performance, feed utilization, nutrient digestibility, disease resistance as well as the alteration of the gut microbiota populations in

several aquatic animal species. Examples for commonly used organic acids in feed are formic acid, propionic acid and citric acid.

2.8. Binders

The stability of feed in water ensures the proper delivery of nutrient to fish and pellet durability for preserving physical form during storage. Hence, the aquaculture feeds should be stable to prevent disintegration during handling, shippingand in water column, particularly for shrimp rearing. Binders are helping to attain this property in aquafeed. Binders used in formulated feeds are mainly complex polysaccharides. Though a wide range of binders are available in the market, most commonly used binders in formulated feed are Carboxymethyl cellulose (CMC): 2-4%, Gum acacia: 2-5%, Na or K bentonite: not > 2%, Agar: 2-5%, Wheat gluten: 10-12%, Starch powder 2-5% etc. Heat treatment during diet preparation results in gelatinization of starch that along with energy supply also acts as binder.

2.9. Probiotics

The probiotics can be defined as live micro-organisms, which administered in adequate amounts deliberate a health benefit on the host. Probiotics are used in aquaculture to improve growth performance, nutrient utilization, decrease diseases and enhance immune system. Probiotics include Gram +ve, Gram -ve bacteria and many other organisms like yeast, bacteriophages and unicellular algae. LAB, *Enterococcus, Streptococcus* which are the main Probiotics of GIT. Lactose is converted into lactic acid by LAB, reducing the pH in the GIT and naturally preventing the colonization by many pathogenic bacteria.

2.10. Prebiotic

Prebiotics are non-digestible food ingredient that stimulate the growth or activity of beneficial gut probiotic bacteria in host. Non-digestible carbohydrates can be classified according to molecular size or degree of polymerization (number of monosaccharide units). Polysaccharide used as prebiotics should be resistance to gastric acidity, resistance to hydrolysis by digestive enzyme, fermented by gastrointestinal microflora. Increase the abundance of intestinal bacteria related to health. The most commonly prebiotics are used in aquafeed are mannanoligosaccharides (MOS), inulin, galacto-oligosaccharides (GOS),

arabinoxylooligosaccharides (AXOS), levan, fructo-oligosaccharides (FOS), xylose-based oligomers (XOS).

2.11. Antioxidants

Antioxidants are food additives used to extend the shelf life of foods by delaying the onset of oxidation. Unsaturated fats and oils are particularly susceptible to oxidation and their degradation products are responsible for "rancid" off notes and flavours. Antioxidants are used to improve the stability of colours, flavours, vitamins and other nutritive components such as long chain polyunsaturated fatty acids. Natural antioxidants are vitamin C and E and synthetic antioxidants are butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) and ethoxyquin. The supplemental doses are 1) Vitamin C: 100-300 mg/kg, 2) Vitamin E: 300-390 mg/kg, 3) BHA: 0.02%, 4) BHT: 0.02% 5) Ethoxyquin: 0.015%. Better approach is the supplementation of vitamin C and E along with any one of synthetic antioxidants.

2.12. Feeding stimulants

The compounds which stimulate gustatory receptors cells which results enhanced feed intake called feeding stimulants. Fish reject unpalatable feed resulting less feed intake. Thus plant based formulated feed should be supplemented with feeding stimulants which increases palatability of feed, thus increases feed intake. Feeding stimulants are also called gustatory stimulants or palatability enhancers or flavoring attractants. Medicated feed also need to be supplemented with feeding stimulants. It is also effective to train young fish or larvae to accept formulated diet after weaning from live foods. Chemicals identified as feeding stimulants are amino acids, betaine, quaternary ammonium bases, nucleoside (inosine) and nucleotide (IMP, AMP, UMPetc), organic acids (propionic acid), bile salts etc. Effectiveness of different compounds differs among different species. L forms of amino acids are more effective than its D-isomer. Mixtures of several aminoacids are more effective than individual amino acids. Mixtures of amino acids and other stimulants are more effective, e.g. betaine plus amino acid mixture. There is species specific sensitivities to different classes of taste stimuli. Feeding stimulants of common carp is a mixture containing betaine and L form of amino acid mixture such as proline, alanine, cysteine, glutamic acid and glycine. A mixture of proline, alanine, cysteine, glutamic acid and glycine at 0.1-0.5% acts as feeding stimulant. Addition of betaine to this mixture @ 0.25% and nucleotide rich spirulina or yeast @ 1% gives better result and also improves immunity.

2.13. Carotenoids

Supplementation of artificial feeds with a balanced mixture of natural carotenoids can enhance the antioxidant status and the pigmentation in fishes. As fish cannot synthesize these pigments, they rely on dietary supply of carotenoids to achieve their natural skin pigmentation. Thus, carotenoid rich nutritionally balanced artificial feed should be formulated and prepared for getting good colouration to ornamental fish as it is one of the most important quality criteria for species such as Koi carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*). Commonly occurring carotenoids in freshwater include beta-carotene, lutein, taraxanthin, astaxanthin, tunaxanthin, alpha-, beta-doradexanthins, and zeaxanthin.

3. CONCLUSION

For getting success in aquaculture venture of ornamental and food fishes, additives plays a major role. The farm-made feeds can also be supplemented with additives to enhance the water stability and efficiency of the feed.

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Feed and Feeding Strategies in Brackishwater Aquaculture

Debasis De & T.K.Ghoshal

Brackishwater aquaculture has shown phenomenal growth in the last decade in India producing protein rich health food and earning valuable foreign exchange. Feed is a major input in Brackishwater aquafarming. Preparation of nutritionally adequate feed for involves understanding the dietary requirements of the species, proper selection of feed ingredients, formulation of feeds and appropriate processing technology for producing water stable pellet feeds. Depending upon the type of farming, a wide range of feedstuffs are used for feeding stocked fish and shrimp. While no feed is used in traditional farming systems, supplementary and adequate feeds are used in improved extensive aquaculture.

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

i) Feed formulation and nutrient content of feed ingredients
ii) Feed manufacturing process and physical characters of the feed
iii) Feed handling and storage
iv) Feeding strategies-type of feed, feed application methods, feeding regime
v) Aquatic environment and natural food availability

Feeding management in fish culture systems

Feed management means use of feed in such a way that utilization of feed is optimum; wastage is minimum thereby negligible impact on environment, achieving best feed conversion ratio and maximum growth and production of fish and shrimp. A very good quality feed can produce poor result if the feed management is poor, whereas, a moderate feed can produce very good results under good feed management.

The foremost critical factor is selection of appropriate feeds and planning of optimal feeding regimens. Suitable feed should fulfill the nutritional requirements of species under culture. Proteins, lipids, carbohydrates, vitamins, minerals and water are the six major classes of nutrients, which are used for building, maintenance of tissues and supply of energy. The requirement for these nutrients varies depending on the species according to their feeding habit, habitat in which they live in and the stage in their life cycle. Our aim should therefore be to produce nutritionally balanced feed with optimum protein energy ratio. It should also ensure that nutrients are not lost in water during the feeding process.

Therefore, aquaculture feeds of different formulations are processed using the special technologies to ensure the diet remains intact in water before ingestion, and nutrients are prevented from dissolving. These general categories of feeds used in aquaculture are wet feeds with moisture contents of 50-70 percent, semi moist formulated feed with moisture contents of 20-40 percent and dry pelleted feeds with moisture contents of less than 10 percents. Since problems are associated with the distribution, handling, utilization, storage and quality of wet feeds and moist feeds, more and more dry feeds are manufactured either by steam pelleting or by extrusion pelleting.

Following points should be strictly followed while feeding the fish for maintaining good pond hygiene and to reduce wastage of feed and to avoid accumulation in pond bottom.

1. Pond biomass should be assessed regularly and ration should be offered as per biomass of the pond.
2. Time, frequency and method of feeding should be proper.

Ration size

The size of daily food ration, the frequency and timing of meals are the key factors influencing the growth and feed conversion. Hence, the optimal feeding regimens must be determined as per the feeding behaviour, appetite and functioning of the digestive systems. Fish lose weight when their food intake falls below that required for maintenance. When ration size increases, the growth rate increases. Generally the method of calculating the daily ration is based on the body weight of fish.

$$\text{Ration size(Kg)} = \frac{\text{ABW(g)} \times \text{Stocked nos.} \times \text{Survival(\%)} \times \text{Rate of feeding(\%)} \times 1\text{kg}}{1000 \times 100}$$

The quantity of ration varies from 100% of body weight for larvae and fry and gradually reduced to 50 %, 20%, 10%, 5% and 2-3% as the fish/shrimp grow marketable size. Ration size is also estimated by various methods using the feeding charts, feed equations, growth prediction and check tray etc. Besides the ration size, the optimal feed particle size also affects the growth and feed conversion efficiency. Large fish can ingest small particles, but it requires more energy to capture the required equivalent weight or smaller food particles. This results in measurable reduction in food conversion efficiency.

Attention should also be given to the influences of feed shapes, colors and textures of pellets on ingestion rates.

Feeding methods

Production of high quality fish at least-cost depends on an effective feeding method. Various techniques exist, from hand feeding to mechanized feeding. They depend on diverse range of factors such as labour costs, scale of farming, species under farming, the type of system i.e. for hatchery or grow out systems. Often farmers use a combination feeding methods such as hand feeding to mechanized feeding. Feed bag suspended at different places in ponds is most common method of feeding to the fish. In mechanical feeding system, demand feeder is used in which fish approaches to the feeder for its feed requirements when they feel hungry. It was observed that fish quickly learn how to obtain feed. The growth of fish is good with best FCR and minimum wastage of feed in self-demand feeding system. This method works best with finfish farming. A reliable and least-cost feeding system should ensure the effective distribution and spread of adequate feeds in aquaculture ponds.

Schedule and frequency of feeding

The total feed required in a day should not be fed at a time. Scheduling and frequency of feeding greatly help in successful feed management. Time schedule for feeding the fish may be fixed in such a way that larger ration may be given when the fish is expected to be most hungry. For optimum growth & feed conversion, feed should ideally be 1 % of the body wt. If ration for a day is 5% body wt., fish need to be fed 5 times,1% of body wt. each time. Most of the Brackishwater fishes are fed 3-4 times a day. High feeding frequencies reduces starvation & stunting thereby resulting in uniform in size. There should be a minimum of three time schedules of feeding in a day- morning, noon and evening. Frequent feeding of small portion of ration help in better utilization of the feed and thereby lead to efficient FCR. There must also be a mechanism in each case to monitor the feed consumption and offering of next dose of feed should be regulated on basis of consumption from the previous feed offered.

Handling and storage of feeds

Optimizing handling and storage procedures on farms is an essential component of good management practice. High quality feed can readily spoil and denature if stored under inadequate conditions or for too long a period. In correctly stored feeds may not only be unappetizing to fish or lacking in essential nutrients but also may contain toxic and antinutritional factors. This can lead to abnormal behaviour, poor feeding response and

growth. Hence different feed types such as wet feeds, moist feeds and dry feeds must be handled and stored under appropriate conditions.

Water quality

The interrelationships between feeding and water quality in aquaculture is complex. By providing optimal species-specific requirements such as temperature, dissolved oxygen, pH and salinity, adequate feeding to satiation, improved growth and high survival can be ensured. When the water quality parameters fall below optimal levels, the species under culture will be stressed and feeding and growth will be impaired. Accumulation of left over feed together with excretory products is associated with high BOD, NH_3, H_2S, CH_4 and harmful effects of eutrophication. This is a critical issue in management since effluent quality can be linked directly to feeds and feeding practices and is regulated under water pollution control laws in many countries. Thus, feeding regimes should be designed to minimize the nutrient loss and faecal output and to maximize the nutrient retention and health status of the cultured fishes.

Feeding management in shrimp culture system

Proper feed management is essential for successful and profitable shrimp farming. As feed alone costs 50-55% of total culture expenditure, strict supervision on feeding is required. Following points should be strictly followed while feeding the shrimp for maintaining good pond hygiene and to reduce wastage of feed and to avoid accumulation in pond bottom.

1) Proper feeding guidelines should be followed to fix ration size for shrimp culture pond
2) Check tray should be monitored daily
3) Time and frequency of feeding should be proper.

Shrimp appetite will vary due to the environmental conditions i.e., water quality, water temperature, sunny/overcast days and physiological conditions such as disease and moulting. Feed should never be given in excess as uneaten feed pollutes the water. As shrimps are the nocturnal feeder, larger doses may be offered in the evening and during night. Regular observations and experience helps in mastering the management of feeding in a culture farm. Generally during new moon and full moon moulting of shrimp takes place and they become sluggish and reduce the feed intake. Quantity of feed offered should be reduced at the extent of 30-50 % during that period.

Ration size

Generally the method of calculating the daily ration is based on the body weight of shrimp (Table 7). First fifty days of culture blind feeding is generally practiced as per the Table 6. Daily ration should be divided and given 2 to 5 times a day (Table 4, 6,7). The feeding activity and quantity of feed consumed may be checked by keeping feed in check trays (size: 80 cm x 80 cm)@ 6 nos./ha in different places in pond. After one month of stocking, consumption of feed should be checked by using check trays.Besides the ration size, the optimal feed particle size also affects the feed intake and growth of shrimp. Feed particle size should vary as per body weight of shrimp (Table 5). Feed should be broadcasted evenly in a periphery of about 2 meters from dyke in all sides of the pond.

Table 1: Feeding Schedule for shrimp

Feed type	Shrimp weight (g)	Time of feeding				
		6.00 AM	11.00 AM	6.00 PM	10.00 PM	2.00AM
Starter	Up to 4.0	30 %	-	35%	35 %	-
Grower	4 – 25	25 %	15 %	30 %	30 %	-
Finisher	> 25	25 %	15 %	20 %	25%	15%

Table 2: Recommended shrimp pellet size

Feed type	Size of shrimp (g)	Pellet size
Starter	0-4.0	0.5-1.0 mm crumble
Grower	4.0-25.0	2 - 2.3 mm x 4 - 5 mm
Finisher	>25	2-2.5 mm x 6 – 8 mm

Table 3: Feeding schedule for first fifty days of shrimp farming

Age (Days)	Feed increment per day (g)	No. of meals per day	Feed per day per lakh PL-20 (Kg)
1	-	2	2.0

2-10	400	2	2.4-5.6
11-30	600	3	6.2-17.6
31-50	500	4	18.1-27.6

Table 4: Feeding schedule after 50 days of culture based on check tray performance

Days of culture	Expected ABW (g)	% of ABW to be used as feed	Feed % in Check tray	No. of meals per day
51-55	6-7	5.0-4.8	2.0	4
56-60	7-8	4.8-4.6	2.2	4
61-65	8-9	4.6-4.4	2.2	4
66-70	9-10	4.4-4.2	2.4	4
71-77	10-12	4.2-4.0	2.6	4
78-83	12-14	4.0-3.7	2.7	4
84-90	14-16	3.7-3.5	2.8	4
91-97	16-18	3.5-3.2	2.9	4
98-104	18-21	3.2-2.9	3.0	4
105-110	21-24	2.9-2.7	3.2	4
111-117	24-27	2.7-2.5	3.3	5
118-124	27-30	2.5-2.2	3.5	5
125-131	30-33	2.2-2.0	3.6	5
131-133	33-36	2.0-1.8	3.7	5

Check tray monitoring:

Quantity of feed to be kept in check tray depend upon pond size and average body weight of shrimp and can be determined using the following formula

$$\text{Quantity of feed in each check tray (g)} = \frac{1600}{\text{Area of pond}} \times \frac{\text{Feed \% in check tray}}{100} \times 1000$$

The check trays should be observed after 2 hr of feeding .Depending on the quantity of feed consumed in the check tray, the next dose should be increased or decreased. Special

care should be taken during moulting, shortage of dissolved oxygen and stressed condition due to heavy rain, high temperature, unfavourable pond bottom and water quality. If tray monitoring is done properly and check tray feed is consumed within 2 hours, survival % can be accurately estimated by the following formula

$$\text{Survival \%} = \frac{\text{Total quantity of feed consumed per day}}{\text{Stocked shrimp x ABW x \% ABW feed}}$$

Success of feed management depends on the farmer's experience and observation on the feeding behaviour and feed intake of shrimp. Following a strict feed management, survivability up to 80 % and average weight of 30 g can be achieved in culture duration of 120 days. Progressive farmers may have small scale feed mill to prepare shrimp feed using locally available feed ingredients for tiger shrimp culture and may get a good economic return. Central Institute of Brackishwater Aquaculture (CIBA), Chennai and it's regional centre at Kakdwip extend technical guidance to set up feed mills in Tamil Nadu and West Bengal for preparation of shrimp feed using ingredients available in the country.

Conclusion

- Judicious feed management is important factor in achieving good feed efficiency and reducing feed wastage.
- Freshly prepared good quality feed proven with best potential FCR, could reduce feed waste.
- Feed with poor water stability, which have lost their nutritional potency and are poorly accepted by the fish or shrimp should be rejected
- Appropriate particle size of the feed should be designed for a particular stage of life cycle
- The ration size and feeding Schedules should be regulated with reference to feeding guides, response of fish and environmental conditions.

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Quality Control and Storage of Ingredients and Finished Aqua-Feeds

Manish Jayant and Dilip Kumar Singh

1. INTRODUCTION

Feed storage is keeping of ingredients for longer time and without significant alterations in their physical form, chemical composition and feeding value. Feed most often represents the greatest percentage of the total cost of raising fish and shrimp, and substantial amounts can potentially be wasted through spoilage and breakage. All feeds whether moist, semi-moist or dry are susceptible to degradation with time. The degree to which feed formulators and manufacturer can reduce wasted feed and realize its full purchase value is ultimately dependent on how well the basic principles of feed storage and handling are understood and applied.

Dry feeds are perhaps the most stable, but if exposed to high humidity; it will take on water increasing susceptibility to attack by molds. The main aim of feed storage is-

- To know the effective storage methods of feed and feed ingredient.
- To know the loss of different nutrients under adverse storage condition.

Semi-moist feed will degrade once removed from their vacuum packs even though they are highly stabilized.

Do's and don't in feed storage room:

- For a good storage provide a building storage that is secured and adequately locked.
- Don't accept deliveries of raw materials that are visibly lumpy.
- Purchase required quantity of ingredients so that you do not need to keep great quantity in stock.
- Always keep the store clean. Make small sack.
- Ensure that ingredients are clearly and neatly labeled.
- Don't walk over the feed bags.
- Feed should not be stored in direct sun light. This would adversely-affect the vitamin and lipid quality of the feed.
- Feed should be used within 2-3 months of manufacturing.
- Feed should be stored on wooden spacers not more than 5 bags high to maintain air circulation.
- Feeds store should be 100% water proof and damp proof.

- Proper ventilation should be provided.
- Dry feed should be stored under cool and dry condition with temperature of < 20°c and humidity < 70%.

Bag storage of feed:

Precaution should be taken before and after filling the bags.

- Bags should be thoroughly cleaned before use for avoiding contamination.
- Bags should be disinfected against insects and fungi.
- Repeated water soaked bags should not be used for filling high density materials like grains and cakes as water soaking for long time weaken the load or pressure bearing ability of the fiber.
- Bag should be stacked away from the walls and at least 50cm space should be left between the walls and stack to facilitate inspection.
- A strong platform of wood or steel should be used as base for stacking the bags and space between the floor and surface platform should be 5-7cm for facilitating aeration, cleaning and fumigation.
- Passage for the movement of a person (50cm) should be provided at a distance of 4-5m in the stacks.
- The bags are stacked in criss-cross system. The height of stack is determined on the basis of the height of store or godown.

2. PROBLEMS DURING FEED STORAGE AND ITS CONTROL

Major problems faced while storing the feed are:

a) Insects
b) Rodents and birds
c) Micro-organisms
d) Deteriorative changes in feed stuffs

A) INSECTS

Insects feed on most feed ingredients and contaminate them with faeces, webbing, body parts, foul odours, and micro-organisms. Beetles and moths are the most destructive of the grain insects, and many are capable of destroying an entire store of feed.

Factors affecting insect infestation of feedstuffs

Major factors affecting population growth of most insect species are: temperature, relative humidity, and moisture content of the feed ingredient. The nutritive content and certain physical properties of feeds will also determine the vulnerability of such materials to attack. Only a few insect species are able to attack sound kernels of feed grains. High moisture content (16 percent or more) renders feed grains soft and susceptible to attack. Meals pressed into cakes or hard flakes are more resistant. Insects appear to eat small particles more readily than large ones.

a. Temperature

All insects attacking stored feedstuffs have an optimum zone of temperature at which populations increase most rapidly (Table 1). Most of the important insect pests are tropical species with an optimum temperature of about 28°C. It is, therefore, evident that losses from insect infestation will be greatest in the tropics.

b. Relative humidity

Up to 70% relative humidity, there is progressive increase in insect multiplication. Beyond 70% relative humidity, mould formation sets in and complicates the situation. The moisture content of feedstuff is closely related to the relative humidity. A low moisture content coupled with low humidity will provide protection against insect infestation.

Insect infestations sometimes cause excessive heating of grain. When the insect population reaches a certain density, their metabolic activities release more heat than can be dissipated. In localized areas where the insect population is extremely dense the temperature may reach 45°C. Associated micro-organisms, mainly fungi, may raise this to nearly 75 °C, causing extensive spoilage and, occasionally, spontaneous combustion.

Table 1: Environmental Constraints of Major Insect Pests Infesting Feedstuffs

Species		**Minimum for increase to epidemic numbers**		**Optimum range for increase**
Scientific name	**Common name**	**Temp, °C**	**Rel. Hum, %**	**Temp, °C**
Sitophilus spp.	Weevils	15	50	26-30
Sitotroga cerealella	Grain moth	16	30	26-30
Tribolium spp.	Flour beetles	21	*	30-33
Oryzaephilus spp.	Saw-tooth grain beetles	21	10	31-34

Cryptolestes spp.	Flatgrain beetles	21	50	30-33
Cadra cantella	Tropical warehouse moth	17	25	28-32

Species breeding rapidly even in driest conditions (Sources; Howe, 1965; Cockerel[1] *et. al.,* 1971)

Losses Due to Insect Attack

- **Weight loss**

Weight loss in infested feed is not always evident unless it involves sacked grain or oil cakes when the appearance of frass on the surface of the sack points to the feeding activity of a large insect population.

- **Quality loss**

Insect tend to accelerate harmful chemical changes for example, secretion of enzyme lipase by the insects will enhance deteriorative chemical processes. Many feedstuffs contain a high percentage of fat which tends to break down during storage. This breakdown is accelerated by insect attack, especially when the insects break off small particles, introduce micro-organisms, or raise the temperature or moisture content. Evidently, the insects use the fat in the material they eat. The breakdown of fat causes an increase in free fatty acids which cause off-flavours. The free fatty acid involved in product rancidity is assumed to be oleic acid, and the quantities released are the result of oxidation of fats in certain feedstuffs. Scavenging insects, such as cockroaches, may cause contamination with pathogenic bacteria such as *Salmonella.*

Control

Climate is the most important factor determining the effectiveness of a storage system for feed ingredients because of the close relationship between insect growth and ambient climatic conditions. An important relationship also exists between insects and micro-organisms in stored feedstuffs.

Total eradication of insect populations from tropical warehouses for feed ingredients is not possible. The degree of infestation can, however, be brought to manageable proportions through a programme of vigilance and effective control measures. Heavily infested ingredients should not be brought into the store. Infested material, if accepted, should be kept separate until fumigated (this is to be done as soon as possible) to totally eradicate the pests.

Chemical treatment for store room:

Surface treatment: These are used only for application on the floor, walls and other exposed surface and never mixed with food items.

Fumigation: The method of creating a lethal environment with the use of volatile chemicals producing diffusible gaseous state when exposed to air.

B) RODENTS AND BIRDS

Rodents consume feeds & contaminate it by excreta, hair, and dead bodies. Each rat - 10,000 droppings, 4 liters of urine, 5 lakhs of hair / year and eats - 8.5% of its body weight/day.

Losses caused by birds are 0.85% of the stored material. They consume feeds & contaminate by excreta and feathers. Each bird can consume on an average 25 g grain/ day.

Control

Good house-keeping involving the sweeping up of spilled material and check of rodents which may carry insects in their furs and can keep away birds.

C) MICRO-ORGANISMS

Micro-organisms are biological contaminants of the natural environment and are present in all feedstuffs. Bacterial and field fungi do not thrive at moisture levels below 20%, post-harvest processing of commodities and animal renderings involving heat, chemical and mechanical extraction, and dehydration eliminate most of the original contaminating micro flora. Fungi spores, which are resistant to harsh processing treatment may remain dormant in the processed feedstuff until more favorable conditions once again, permit their proliferation.

Factors Affecting Fungal Growth in Feedstuffs:

Adventitious storage fungi grow at moisture content (15 to 20 %) in equilibrium with a relative humidity of 70 to 90% and are considered the principal spoilers of feedstuffs in storage. When the relative humidity falls below 65 percent no growth occurs.

Under favorable conditions, fungi can raise the temperature in their immediate environment to 55°C with concomitant increase in moisture content of the affected feedstuff to as high as 20 percent. When this occurs, secondary spoilage by bacteria takes place. The most common fungi involved in the spoilage of feedstuffs belong to

the Aspergillus spp. and the Penicillium spp. These grow at temperatures up to 55° C and at a minimum, relative humidity of 65 percent. Fungal attack leads to:

1. Mycotoxin production

Mycotoxins are compounds produced by fungi growing in infested agricultural commodities. They are toxic to both humans and animals. The aflatoxins, a group of highly toxic and carcinogenic metabolites produced by Aspergillus flavus are perhaps the most important among mycotoxins contaminating feedstuffs. Feedstuffs known to be contaminated by A. flavus include: groundnut cakes, maize, sorghum, sunflower, cottonseed cakes, copra, and cassava.

2. Heating and moisture increase

Mould growth in feedstuffs is accompanied by rising temperatures and moisture content. Aspergillus glaucus, which has a minimum moisture requirement of 14.5 percent, is the first significant species involved during mould infestation of feed grains. Temperature elevation that accompanies this initial attack flavours the proliferation of a second species, A. candidus, which raises the moisture level of the infested grain to 18 percent or higher. At such high moisture levels, A. flavus activity becomes intense and total destruction of the wholesomeness of the feed grain becomes complete.

Fungal activity in stored feed- grains is not often apparent until after serious damage is done. This is because such activity takes place not near the surface where temperature gradients produced by such activity are quickly abolished, but within the interior of the storage container. Silos for grains should, therefore, be equipped with temperature sensors to provide early warning of trouble. Similar preventive measures are not possible for bagged material. The common practice of storing bags of grains in large piles to minimize and control insect infestation actually promotes fungal activity, especially in the tropics. The "sweating" of bags within a large stack is evidence of serious fungal damage to the stored grain. The surface of such bags will feel warm to the touch, and when a hand is inserted into the grain, it will actually feel hot.

3. Staleness

Feedstuffs that are damaged by fungi tend to become lumpy. Feed grains suffer discolouration while damaged maize turns a dark brown with some blackened kernels being evident. The grain also exhibits a characteristic bluish sheen. Staleness or mustiness is another characteristic of commodities damaged by fungi.

Control

The prevention of mould contamination of stored feedstuffs depends mainly on the successful control of insect infestation, because the destructive activities of insects often create conditions favourable to mould; viz., increased moisture and temperature and the destruction of the protective hulls of feed grains expose their moist interiors. There is no effective way of eliminating mould, although effective measures have been developed to control their growth in compound feeds. These measures include the use of propionate and, more recently, gentian violet.

3. DETERIORATIVE CHANGES IN STORED FEEDSTUFFS

Most stored feedstuffs undergo some chemical changes altering flavour and nutritive value. These changes are usually deteriorative in nature and are associated with the lipid content of the feedstuffs. Lipids tend to break down during storage into free fatty acids.

Factors Affecting Deteriorative Processes

I. Environmental factors

Environmental factors that determine the extent of deterioration of stored feedstuffs also affect the rate of growth of its insect and microbial population. These include ambient temperature and relative humidity. Other environmental factors relate to the general cleanliness of the storage areas and to design features of the storage building; e.g., protection against rain and insulation against scavenger pests.

II. Insects and micro-organisms

The increase of fatty acids as a result of lipid breakdown is of particular importance in the storage of fish meal, cereal-brans, and oil seed derivatives; e.g., copra, groundnut, and palm kernel meal. This increase in free fatty acids in improperly stored feedstuffs results in rancidity.

III. Rancidity

There are three major chemical processes that give rise to rancidity: oxidation, hydrolysis, and ketone formation. Due to the relative unimportance of the other two processes in stored feedstuffs only oxidation of fats will be described here.

Rancidity resulting from lipid oxidation is the most important deteriorative change occurring in stored feedstuffs. Feedstuffs containing lipids which are highly unsaturated (e.g., rice bran and fish meal), are especially susceptible to oxidation.

Factors affecting lipid oxidation

The chief factors increasing the rate of lipid oxidation in stored feedstuffs are as follows:

a. Enzymes
b. Hematin
c. Peroxides
d. Light
e. High temperature
f. Trace metal catalysis

Control

Lipid oxidation can be inhibited by adding compounds known as anti-oxidants. Two commonly used feed anti-oxidants are ethoxyquin and butylated hydroxytoluene which sequester free radicals formed during oxidative processes. Cereal grains contain effective quantities of natural anti-oxidants (e.g. α-tocopherols) which impart considerable stability to their lipid contents, unless the kernels are damaged by storage pests.

4. STORAGE OF FEED

4.1. Dry feed:

For reasons of cost and convenience, dry diets are presently the most widely used feeds in aquaculture. The feed include extruded feeds, hard pellets, crumbles, and flakes. It is easy to overlook the fact that even the most durable crumbles and pellets can break down into dust and fines when subjected to sufficient amounts of compression and abrasion. It is important to give ample consideration to moving the feed as little as possible and as gently as possible. Dry feed should be stored under clean dry ventilated conditions within a room with a concrete floor and walls, and used within two months. Bags should be stored on wooden pallets and never be allowed to rest directly against the concrete floor or walls. A good storage facility should also provide adequate containment for control of pests.

4.2. Moist feed:

Moist feeds undergo rapid spoilage during storage unless the moisture content is at or below a permissible level, the pasteurized and chemical agents added to prevent yeast and mould growth. Despite their widespread use in aquaculture, there is still no commercial production of moist fish feeds. Problems associated with handling and storage of the final product are the most difficult to overcome. Stored in rigid containers even

which will not prevent the "caking" up of moist pelleted feed resulting from handling and prolonged storage. Some moist feed diets contains humectants like propylene glycol and sodium chloride which lower water activity to prohibit bacterial growth. Fungicides like propionic acid or ascorbic acid maybe added to retard mold growth. They should be packaged in hermetically sealed containers and preferably stored at low temperatures to reduce the opportunity for mold growth. They also need to be over fortified with vitamins because moisture enhances oxidative loss of vitamins.

5. PREVENTION OF SPOILAGE FROM RANCIDITY:

There are three major chemical processes that give rise to rancidity: oxidation, hydrolysis and ketone formation. Feedstuff containing lipids which are highly unsaturated are susceptible to oxidation. Factors increasing rate of lipid oxidation in stored feedstuff are-

- Enzymes
- Peroxides
- Light
- High temperature
- Trace metal catalysts.

Prevention:

Lipid oxidation can be inhibited by adding compounds known as anti-oxidants. Blocking of hydrogen supply. Addition of anti-oxidant such as Vitamin C and Vitamin E. Avoid direct contact with oxygen.

Level of antioxidant use in the feed:

Blocking of hydrogen supply: Addition of anti-oxidant such as Vitamin C and Vitamin E. Avoid direct contact with oxygen. The U.S. Food and Drug Administration permits the following levels of antioxidant in finished feed are:

(a) Ethoxyquin (1,2 dihydro-6-ethoxy-2,2,4- trimethy quinoline): 150 ppm,

(b) BHT (butylated hydroxytoluene): 200 ppm, and

(c) BHA (butylated hydroxyanisole): 200 ppm.

6. FISH DISEASE VECTORS IN FEED AND QUALITY CONTROL:

Several ectoparasites of fish have been shown to transmit viral disease-causing agents of fish (Ahne, 1985). Some of the possible fish disease vectors in feed are Viral hemorrhagic septicemia disease which is one of the most dangerous viral diseases of fish

caused by VHSV, Epizootic haematopoietic necrosis virus (EHNV) causing agents like Ranavirus and Epizootic ulcerative syndrome (EUS) by *Aphanomyces invadans*.

Raw Materials:

The purpose of quality control of raw materials is to ensure that minimum contract specifications are met. It provides knowledge concerning the exact composition of raw materials and the level's of toxic substances normally present so that mixed feeds of the required nutritive value can be safely processed from them. The least cost formulations are possible only when the composition of each raw material is known with a high degree of precision and when good quality control is maintained.

Preliminary inspection:

Materials received at the mill should be subjected to a thorough physical inspection to determine the following:

(a) Evidence of wetting - mould growth confirms water damage;

(b) Presence of scrap metals, stones, dirt, or other non-biological contaminants.

(c) Presence of insects.

Sampling:

Sampling of bagged ingredients is done with a spear probe. The probe is inserted diagonally and as horizontally as possible, from one corner of the bag to the other. In lots of 1-10 bags, all bags are sampled. In larger lots, 10 percent of all bags are sampled. Samples submitted to the quality control laboratory should be placed in tightly sealed containers. Materials received in bulk are sampled by using a scoop, according to the size of the consignment. For smaller than 10 t consignments, two samples per ton are taken. Larger consignments, up to 100 t, require one sample per ton or one sample for every two tons depending on the size of the consignment.

Testing of ingredient: For feedstuffs likely to contribute to both the protein and energy content of the final product, proximate analysis is usually conducted which determines the moisture, crude protein, lipid, crude fibre, ash, and nitrogen-free extract content of the feedstuff. Certain feedstuffs contain natural toxins that, at high enough levels, are growth inhibitory and sometimes fatal to the animal consuming them. Principal among these are:

(a) Urease

(b) Gossypol

(c) Isothiocyanates

(d) Aflatoxin

Finished Products:

Preliminary inspection: Most modern mills are equipped with sieves and magnets along the material flow lines for removal of tramp metal, rocks, and other scrap contaminants. Any detection of foreign contaminants should be brought to the attention of the mill supervisor who could then determine if the contaminants originated in the raw material or if they were the result of improper maintenance within the mill premises.

Sampling:

To detect product in homogeneity and significant ingredient separation during the manufacturing process, sampling should be obtained during bagging-off time by taking a handful from every fifth bag of 40-50 bags and pooling the individual samplings. Testing for variability is best conducted by probing the bottom, middle, and top of a bag with a short probe. Samples submitted for chemical analysis should be placed in tightly sealed containers. Maximum permissible storage time for selected feed stuff.

7. SAFETY OF FARM FISH PRODUCTS- HARMFUL RESIDUES

It is important for processors, food-service establishments and retailers to implement best practices in providing appropriate unit-processing operations to control pathogens and prevent cross contamination in their establishments. Aquaculture farms must also follow good management practices both to limit the potential for human food poisoning and to reduce the opportunity for disease in their growing waters. For aquaculturists, it is crucial to remember that some human bacterial pathogens are also pathogenic to fish.

Three plants of fresh catfish fillets obtained during each of the four seasons were screened for selected human pathogens. *V. cholerae* was found in 10-45% of the samples in summer and 15-35% of the fillets in fall. In summer, *P. shigelloides* was found in 10% of samples at one plant. It is clear that *V. cholerae* cause chlorella (Fernandes et al., 1997).

In the few samples where pesticide residues were detected, they were far below U.S. Food and Drug Administration "action limits." For example, the average concentration of DDT and its breakdown products in catfish was more than one hundred times lower than the FDA action limit for those compounds. Even the highest level of DDT found in catfish was still only 6% of the FDA action limit.

The same samples were also analyzed for nine "heavy metals" that are indicators of pollution and potential food safety problems. Most of the metals were found in only a small percentage of the samples and all were far below recommended safety limits. For

example, the average residue of mercury in channel catfish was more than one hundred times lower than the FDA action limit.

Most chemical contaminants are associated with soils, and fish farmers avoid sites with a history of pesticide use. Even if the soil does contain low levels of pesticides, they are present only in the topmost layer of soil, which is removed and used to form levees during pond construction. Also, nearly all catfish ponds in Mississippi use pesticide-free groundwater to fill ponds, making it impossible to contaminate ponds with water-borne pollutants. If consumers are concerned about levels of pesticide and metal residues in seafood, farm-raised catfish, trout, and crayfish are healthy alternatives.

8. CONCLUSION

Fish feed plays an important role in production of fish at a satisfactory level. It is also necessary to meet seasonal peak supply and to increase shelf-life of fish feed so that feed produced during lean period can be supplied during peak period. If it is not stored properly then some microbial and environmental alteration will occur. Farmers buy commercial feed stored for different period of time before reaching them but they don't know the nutritive value of different stored feed.

Feed most often represents the greatest percentage of the total cost of raising fish and shrimp, and substantial amounts can potentially be wasted through spoilage and breakage. Even so, practical information about proper storage and handling of the most common types of feed is difficult to find, and usually only addressed in the literature in a general sense. Specific storage conditions and handling procedures are usually left to assumption.

Storage is an important step as the total feed produced cannot be broadcasted or sell all-together. Proper storage conditions and good handling are important for keeping the quality of fish feed. Good quality fish feed provides better growth survival and in turn better profit to the farmers.

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Application of Nutrigenomics in Fish Nutrition

Sujata Sahoo

Nutrigenomics is a branch of functional genomics, endeavors to resolve the influence of dietary chemicals upon the genome and to increase our understanding of how dietary components influence metabolism (Müller and Kersten, 2003; Mutch et al., 2005). It can be defined as the study of genome-wide effects of diet or components thereof on the transcriptome, proteome and metabolome of cells, tissues or organisms at a specific moment in time. It encompasses epigenomics study as well.

Until now there are twelve bony fish species (eg: Zebra fish, Medaka. Rainbow trout and Puffer fish etc.) And one cartilaginous fish species (elephant shark) whose genome is fully sequenced and available in public databases. This genomic information served as a portal for biologists to study the functional genomics, understanding gene and gene product roles through holistic approaches.

Transcriptomics

It is defined as the monitoring of the complete set of RNA transcripts produced by the genome in particular cells, tissues or organism at any given time. Gene expression profiling (GEP) is the measurement of the expression of thousands of genes at once, to create a global picture of cellular function. The development of Microarray technology made this task much easier. Apart from Microarray, serial analysis of gene expression (SAGE, SuperSAGE) method is also used for gene expression profiling.

In nutritionresearch GEP can be used for a) identification and characterization of molecular pathways that may be altered by nutrients; b) pinpointing specific mechanisms that trigger such beneficial or negative effects and;c) identification of specific genes altered by nutrients that might prove valuable as molecular biomarkers or nutrient sensors and in gene discovery.

Although not of aquaculture importance, zebrafish being completely sequenced and commercially available microarray chip, is the model organism for nutrigenomics study. Work with zebrafish nevertheless provides valuable information, since GEPs following various manipulations have established biomarkers that may be appropriate for research with aquaculturally important species. Later by RT-PCR, effect of any nutrient on biomarker genes can be studied. This also provides a very valuable tool to study various immunostimulants. Mannan oligosaccharides modulate the immune response, in trout and

carp, can positively affect growth, survival, and feed efficiency, while enhancing the activity of lysozyme and complement proteins (Staykov et al., 2005). ArunKumar and co-workers (2012) evaluated theimmunomodulatory effect propolisethanolic extract when fed to *Labeorohita* by semiquantitative expression profiling of IFN gamma gene. Propolis extract is included in three levels(1%, 2% and 3%) in the diet and one diet was prepared with no propolis (control) extract with all other nutrients being same. 1-2% propolis extract showed enhanced expression than control where as 3% propolis extract showed less expression than control. NBT assay, total leukocyte count and serum lysozyme activity also supported these findings. Similarly Adnan and co-workers(2014) showed enhanced gene expression of defensinand hepcidin (antimicrobial peptides) in *labeorohita* fed with fucoidan at 2% level.

GEP can identify useful marker genes to assess micronutrient status and requirements, as well as identifying other unknown beneficial and negative effects of dietary nutrient concentrations. Selenium biomarkers have already been established for fish (Thisse et al., 2003) using microarrays, and include glutathione peroxidase, thioredoxin reductase and the cell cycle regulator p53. In an order to study effect of vitamin C on collagen synthesis, Yadii and coworkers (2014) fed *Pangasianodon hypophthalmus* juveniles, seven iso-nitrogenous and iso-lipidic diets with variable level of vitamin C (0.0, 50.0, 100.0, 200.0, 500.0, 1000.0 and 2000.0 mg/kg feed) for 60 days. Real time PCR was performed to evaluate the proly-4-hydroxylase expression responsible for collagen synthesis. Results suggest that dietary vitamin C has positive impact on the collagen biosynthesis in this fish and also increase the gene expression of vitamin C dependent proly-4-hydroxylase. Highest expression was in observed in both bladder and muscle with vitamin C dose of 500mg/ kg feed. Since more than 40 micronutrients are required in the diets of growing animals along with macronutrients, transcriptomics will be a better approach compared to traditional methods in terms of expenditure, time and facilities. A 42-day feeding trial was conducted by Tyagi and co-workers (2014) to evaluate the responses of different dietary proteins on the gene expression of Acetyl-CoA carboxylase. Casein was used as an animal source and soybean meal was used as a plant source at different levels (24%, 32%and 40%). The diets were T1 (24%), T2 (32%), T3 (40%)from animal source and T4 (24%), T5 (32%)and T6 (40%) from plant source.The gene expression of acetyl-CoA carboxylase was increasing with increasing protein level, it was highest at 32% of plant as well as animal protein, and then it became constant. Sarvendra and co-workers (2014) studied the expression of MyoD gene in skeletal muscle of

Pangasianodon hypophthalmus fed for 60 days with different level of proteins (20-45%). Gene expression was shown an increasing trend on 15th and then on 30th day than after decreased in expression on 45th day. Highest expression of MyoD gene reported on 45th day at 35% level of protein with substantially highest level of % weight gain (132.83±32.48) and SGR (1.83±0.31) with significantly. Similarly A 60 day feeding trial was undertaken to determine the effect of different carbohydrate level on the gene expression of two important enzymes during the carbohydrate metabolism in striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) juveniles. Similarly to study the effect of graded level of carbohydrate on amylase gene expression, four purified (isonitrogenous and isolipidogenic) diets with varying carbohydrate level i.e. 20% (T1), 30% (T2), 40% (T3) and 45% (T4) were prepared and fed to *Pangasianodon hypophthalmus* (Sauvage, 1878) juveniles in a 60 days trial (Bhumarkar et al., 2014). Amylase mRNA expression was studied by qPCR and found to be highest in T1 followed by T3, T2 and lowest in T4 on 60th day. Treatment wise the maximum expression was found on 45th day of experiment.

Proteomics

It is defined as the study of the complete set of proteins in a cell or tissue at a specific point in time. There are many methods to study proteomics. Most common is protein profiling using 2D-PAGE and mass-spectrometry. Protein Chips and Reversed phase protein microarrays are the recent advanced techniques in this field. After genomics and Transcriptomics, proteomics is the next step in the study of biological systems. It is more complicated than genomics because an organism's genome is more or less constant, whereas the proteome differs from cell to cell and from time to time. Transcriptomics provides a rough estimate of gene expression but cannot be correlated with protein expression. Usually post translational modification makes a protein functional. Alternate gene splicing, Protein degradation and association with other molecules are the other reasons to study proteomics. Proteomics confirms the presence of the protein and provides a direct measure of the quantity present.

Proteomics study in fish nutrition still is in its preliminary stage. Vilhelmsson et al.(2004) studied the effect of fasting, quality of proteins and total replacement of fish meal by plant proteins on liver proteomes. Pathways involved in energy generation, bile acid synthesis & transport, cellular protein degradation are shown to be involved. Liver proteome also showed different profiles.

Metabolomics

It is defined as the identification and quantification of large sets of metabolites from cells or biological fluids and their alteration following physiological disturbance. Transcriptomics and proteomics analyses reveal the set of gene products being produced in the cell, data that represents one aspect of cellular function. Conversely, metabolic profiling can give an instantaneous snapshot of the physiology of that cell. Although the metabolome can be defined readily enough, it is not currently possible to analyse the entire range of metabolites by a single analytical method. Mass Spectrometry and NMR Spectroscopy based methods are currently used to study metabolomics. Using an ^{1}H NMR spectroscopy approach, Rani et al.(2014) studied the metabolic pattern in liver tissues of Arctic charr (*Salvelinusalpinus*) fed a commercial diet with unknown composition (ST), a diet with all protein from fish meal (FM) and a diet with most of the protein from zygomycetes biomass (FZ). Signals for acetate, β-alanine, choline, creatine, formate, glucose, inosine, lysine, SN-glycero-3-phosphocholine and two unknown metabolites were higher in fish fed diets FM and FZ than in fish fed diet ST. These results show that the metabolic profile in liver of Arctic charr will remain unchanged if fish meal protein is replaced with zygomycete protein, suggesting similar physiological responses to both feed ingredients. In contrast, feeding a commercial diet altered the metabolic fingerprint compared with diets FZ and FM, suggesting important differences in ingredient composition and the physiological response to this diet.

Metabolomics study in fish nutrition is in its infancy stage with little or no work in this area. Thus this area has a lot of scope for future research.

Epigenomics

Epigenomics is defined as the detection and examination of DNA methylation patterns both spatially and temporarily. Epigenetic modifications are reversible modifications on a cell's DNA or histones that affect gene expression without altering the DNA sequence. Chromatin Immunoprecipitation (Chip) along with DNA microarray (Chip-Chip) and Next generation Sequencing (Chip-Seq) are the methods to study Epigenomics. Epigenetic modifications are responsible for activation and suppression of (certain) genes which can get inherited across generations. Thus we can achieve lifelong remodeling of fish epigenomes by nutritional, phytochemical, and metabolic factors. This is called as nutritional reprogramming. Epigenetics study in fish has done as a model organism. As an epigenetic factor, butyrate regulates the transcription via influencing core histone acetylation. Butyrate is a histone deacetylase inhibitor and induces histone

hyperacetylation. Rimoldy et al, (2013) evaluated the epigenetic effects of butyrate used as a feed additive in European seabass as well as butyrate beneficial effect on intestinal inflammation and liver. Effect of macro and micro nutrients on epigenetics needs to be addressed in future.

Conclusion

Nutrigenomic approaches clearly promises a new era for the aquafeed ingredient industry. This area provides evolving technologies that willlead to development, refinement and modification of bioactive and natural compounds that more accurately target specific health benefits both to cultured animals and consumers.

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Breeding and Culture of Ornamental Fishes

B.K. Mahapatra

Ornamental fish keeping is one of the important hobbies in the world. The colorful, quiet, mute and decibel-free, eco-friendly pets in the world are a thing of beauty and joy to the young and old alike and so dear to the hobbyists the world over. In the U.K., the number of aquarium keepers far exceeds that of cats' and dogs' being 50% of the households that own them as pets. Germany and France are next only to the UK followed by Italy with the fourth highest pet ownership in Europe. Spain, Netherlands, Belgium and Switzerland are other European countries where fish are maintained as popular pets. Fish is kept as a hobby by 25% of the households in the USA. However, these countries have to be importing the fish, the main exporters being located in Asia – Indonesia being a home to 35% of the world's ornamental fish species. Production & trade of ornamental fish, particularly cultured fresh water fish and plants forms the backbone supporting the global ornamental fish industry. The Global Ornamental fish trade is estimated at around US $ 9 BILLION (FAO 2000). The US leads the import market followed by EU and Japan. The Asian countries export more then 50% of the aquarium fishes globally. Singapore is the epicenter of all the Asian trade and is the largest exporter of ornamental fishes in the world. India is the natural abode of the entire gamut of ornamental fishes available in the subcontinent. Due to ignorance about this wealth of the trade and lack of technical competence of this million-dollar sector, India only managed to export ornamental fishes worth around 30 million rupees.The North Eastern states contribute around 85% of the total market. Inability to recognize our natural resources, unavailability of easy funding, absence of local exporting agencies, lack of suitable low-cost breeding technologies and transportation facilities are the major hurdles in the speedy growth of this potential industry in India. Such a vast and important industry has the potential to contribute to the sustainable development of aquatic resources, but may face challenges due to increased attention to environmental and social issues.

SPECIES OF COMMERCIAL IMPORTANCE

It is unfortunate that the endemic ornamental species were until recently neither promoted nor its potential realized, the species dominating the market being mostly of South American origin. It is, however, true that these species are universally popular. The

top ten "brands" are the tetra, guppy, goldfish, catfish, molly, gourami, platy, loach, cichlid and the barb. Of the 30-35 species that are the favourite of aquarists, only a few are of Asian origin – *Brachydanio rerio* and *Puntius conchonius* being the most common (Table 1). There is a growing preference for keeping large-sized fishes in the aquaria probably on account of their hardy nature and visibility. The hill stream fishes belonging to the genera *Balitora*, *Barilius*, *Garra*, *Homaloptera*, *Lepidocephalus*, *Nemacheilus*, *Psilorhynchus* are considered to be coldwater species, these are generally found in warmer waters too and could be easily acclimated to the stagnant water conditions found in the aquaria. A higher level of dissolved oxygen is, however, a basic requirement of these species. Some of the other endemic species from the south are *Aplocheilus lineatus*, *A. blockii*, *Danio malabaricus*, *D. aequipinnatus*, *Macropodus cupanus*, *Oryzias melastigma*, *Pristolepis marginata*, *Puntius melanampyx*, *P. mahecola*, *P.arulius*, *P. narayani*, *P. setnai*, *Etroplus maculatus* and *E. canarensis* that are known to have an immense potential for export (Table 2). The wrasses (*Labridae)*, damsels (*Pomacentridae*), parrot fish (*Scaridae*), surgeons (*Acanthuridae*), trigger fish (*Balistidae*), goat fish (*Mullidae*), squirrel fish (*Holocentridae*), butterfly fish (*Chaetodontidae*) and rock cod (*Serranidae*) that inhabit the Lakshadweep lagoons rich in corals are amongst the most sought after marine ornamentals.

BREEDING AND SEED PRODUCTION OF THE ORNAMENTALS

In India, Kolkata, Mumbai, Chennai, Cochin, Madurai are the most important breeding centres for ornamental species with more than 150 full time and 1500 part-time breeders in the whole country. There is an urgent need to develop captive breeding techniques for all the important exportable species as the native stocks will soon be under pressure mainly due to heavy exploitation and loss of habitats and also to maintain an year round supply. Moreover, farm bred fish are far more amenable to aquaria. A few of the indigenous freshwater that have been bred are *Colisa sota*, *C. fasciata*, *Oreichthys cosuatis*, *Gagata cenia*, *Danio dangila*, *Nandus nandus*, *Puntius melanampyx*, *Puntius melanostigma*, *Puntius filamentosus*, *P. vittatus*, *Parluciosoma daniconius*, *Pristolepis marginata*, *Garra mullya*, *Nemacheilus triangularis*, *Danio malabaricus,Esomus danricus, Etroplus maculatus* and *Macropodus cupanus.* Of the marine species, the clown fish and sea horses are of considerable importance. A well-developed, disease-free broodstock, nutrient and balanced feed, appropriate temperature and water quality coupled with properly prepared nursery facilities are an essential requirement for successful spawning and larval rearing of ornamentals just as in case of other species of fish.

Ornamentals include both oviparous (egg layers) and viviparous (live bearers) species. Oviparous fishes lay floating, adhesive or non-adhesive eggs that may be scattered, laid in bubble nests or deposited on substrates or in shallow pits. Parental care is also known and while female cichlids incubate them in their mouths, the father mothers the offsprings in case of sea horses. The breeding and rearing of egg layers is a little difficult but it is rather easy to raise the live bearers and the neo-hobbyists or small entrepreneurs should first begin with handling the live bearers. As an example, the breeding and culture techniques of the swordtail, *Xiphophorus helleri*, a highly popular ornamental fish are described here in sufficient details.

BREEDING TECHNIQUES OF AQUARIUM FISH

One of the attractions of ornamental fish keeping is that the fishes may multiply in captivity. Although many techniques used for ornamental fish breeding are not much difficult, the breeding methods for specific ornamental fish species are closely guarded secrets. Farmers have operated almost entirely on their own, developing their own methods and rely on many years of experimentation. The chapter provides an overview of freshwater ornamental fish breeding. Because of the great diversity of species, only basic information is presented on breeding technology. Emphasis is placed on providing a summary of essential biological characteristics and requirements that characterize wide variety of species. The aim is to provide specific guidelines to assist in the development of appropriate management practices for freshwater ornamental fish breeding.

Sexing Fish

In order to breed a species, the aquarist primarily needs to be able to distinguish between the sexes. Determining the sex of a fish is an important step in knowing whether one has a pair. The sexes can be easily distinguished by primary (shape of sex organs) and secondary differences (size, shape, color [sexual dichromatic], fin development). Males are frequently more colorful, larger, and have more elaborate fins. In some species, the males are slightly larger and the females are slightly rounder in the belly.

Selecting the Parent Fish

Once males and females have been distinguished, a suitable pair or spawning group should be chosen. There are several important traits to seek in choosing the parent fish.

- Choose fish that display good markings like strong coloration, good fin development, etc., that should produce attractive young ones.
- Only use healthy fish for spawning because unhealthy fish, if they will spawn, may produce unhealthy or deformed young ones.
- Be sure that the pair is compatible. Many species cannot be put together in a breeding tank and expected to get along and produce young ones. In fact with many cichlids, pairs form only after a group has been raised together for months or years. In certain species, one partner will bully the other to death if there is no compatibility.
- Make sure that the pair is both of the same species because hybrids are usually sterile. With some cichlids and Killifish, females of different species look similar.

Conditioning the fish to breed

In the wild, breeding is stimulated by a change in the environmental surroundings of the fish, and this can be created to some extent in aquaria. Presumably the circumstances that trigger breeding are multifactor, consisting perhaps of a combination of factors such as food availability, water temperature, length of daylight, and changes in water chemistry. A varied diet, with an increased level of protein is recommended for conditioning. Many species can be conditioned using a well-balanced flake food, though conditioning with live foods such as brine shrimp, insect larvae, and flying insects gives better results. A small increase in the ambient temperature can prove to be beneficial, while more lighting proves a stimulus for coldwater fish species, which are normally exposed to seasonal changes. The condition of the water is significant and introducing a pair to a fresh tank may produce success. If possible, the sexes should be separated three weeks before being re-introduced. The fishes, which prove reluctant to breed, it has been possible experimentally to inject them with specific hormones to stimulate reproductive activity, but such techniques are not available to the average aquarist.

Breeding the fish

Different groups of fishes reproduce in different ways. An understanding of how the various species go about breeding is indispensable to undertake breeding programme. In general the fishes can be divided into two broad categories – **Egg layers and Livebearers**. Within this basic grouping, different species have their own ways of

ensuring the survival of at least a proportion of their offspring.

(i) Livebearers

Livebearers are fish that bear live young ones. There are two types of livebearers: ovoviviparous, where the eggs form and hatch within the female before birth; and viviparous, where no eggs are formed, and the young are nourished through an umbilical-like cord or from secretions by the female. Livebearers are often prolific, easily bred species.

Spawning tank

The live bearing fishes are the easiest of all aquarium fishes to breed; indeed, the only problem usually encountered is that of saving the young from the cannibalism of their parents. Various traps have been designed for the relatively rapid separation of the young from their mother at birth. The most satisfactory arrangement is a screen of mosquito netting on a stainless steel or wooden frame, which can be wedged across the tank so as to confine the female to one end while allowing the young to pass. Despite of all these devices, the more natural method is having plants in abundance to provide shelter for the young, and removing the mother at the earliest chance. The best plants for young livebearers are masses of *Myriophyllum, Ambulia, Nitella, Utricularia, etc.*

Guppy

Black molly

Sword tail

Platy

Fig-1: Live bearer ornamental fishes

Fig-2: Breeding of live bearer ornamental fishes

Raising the fry of Livebearers

Livebearer young are quite large, and young fishes can feed on dry or other prepared food straight away. If they are given only prepared food, growth will be poor, but a mixture of live and dry food is quite satisfactory. In the early stage, feeding of live food is very important for good development. Later it matters much less, although the fishes will still do better with a good proportion of live food. Suitable first live foods are micro worms, newly hatched brine shrimp, shredded earthworms, daphnia, newly hatched mosquito wrigglers or shredded white worms. Suitable dry foods include any fine powder food, such as dried shrimp finely ground, fine cereals, and liver or egg powder.

(ii). Egg layers

Most aquarium species are egg layers with external fertilization. Within this group, fishes can be divided into five groups - **egg-scatterers, egg-depositors, egg-buriers, mouth-brooders, and nest-builders**; depending on how they lay and handle their eggs.

Egg-scatterers

These species simply scatter their adhesive or non-adhesive eggs to fall to the substrate, into plants, or float to the surface. The egg-scatterers either spawn in pairs or in groups. There is no parental care given and even they eat their own eggs, so large amounts of eggs are produced. The Characins and Cyprinids lay their eggs this way.

Spawning tank

Because egg scatterers often eat their own eggs, the spawning tank has to be set-up so that the eggs fall out of the reach of parents. For egg scatterers like Barbs and Danios, which lay non-adhesive eggs, the spawning tank can be furnished with a substrate consisting of two layers of marbles or nylon netting just above the tank floor. As the eggs are laid, they fall through the marbles or the netting out of the reach of the parents. After spawning is over, the eggs or the parents can be removed.

For egg scatterers that lay adhesive eggs like Tetras, the spawning tank should be furnished with a substrate. The tank should be planted with fine-leafed plants. The eggs are laid amongst plants, and adhere to the fine-leaves. The parents should be removed after spawning.

Egg-depositors:

In this case, the eggs are either laid on a substrate, like a stone or plant leaf or even individually placed among fine leafed plants like Java moss. Egg- depositors usually lay less egg than egg-scatterers. Egg-depositors fall into two groups: those that care for their eggs, and those that don't care. Among egg depositors that care for their eggs are cichlids and some catfish. Cyprinids, various catfish, and Killifish make up the majority of egg-depositors that do not care for their young ones. These species lays their eggs against a surface, where the eggs are abandoned. These species do not usually eat their eggs.

Spawning tank

For those egg-depositors that care for their young ones, the parents can remain in the tank after spawning. Substrate spawners, depending on the species, should be given a tank furnished glass panes, broad-leafed plants, or flat stones for spawning sites. Some species such as Discus and Angelfish prefer vertical surfaces. For cavity spawners, flowerpots turned on their side, coconut shells, and rocky caves are suitable spawning sites. The tank should be furnished with either live or plastic plants to give the fish a sense of security.

Egg-depositors that do not care for their young ones should be given a tank furnished with fine and broad-leafed plants, Java Moss, or artificial spawning mops. After spawning either the parents or plants with the eggs should be removed. If the plants containing eggs are removed, new plants should be placed in the tank for future spawning.

***Egg-buriers*:**

Fishes in this group usually inhabit waters that dry up at some time of the year. The majority of egg buriers are annual Killifish, which lay their eggs in mud. The parents mature very quickly and lay their eggs before dying when the water dries up. The eggs remain in a dormant stage until rains stimulate hatching.

Spawning tank

A peat-moss substrate is one of the best substrates for egg-burying species. The peat moss can be removed after spawning and placed in a plastic bag to be stored for weeks to months (depending on the species). A new peat-moss substrate can be placed in the tank for further spawning. In order to initiate hatching, the stored peat can be immersed in soft water.

Mouth-brooders:

Mouth-brooderscarry their eggs or larvae in their mouth. Mouth brooders can be broken up into ovophiles and larvophiles. Ovophiles or egg-loving mouth-brooders lay their eggs in a pit, which are sucked up into the mouth of the female. The small number of large eggs hatch in the mother's mouth, and the fry remain there for a period of time. Many cichlids and some labyrinth fish are ovophile mouth-brooders. Larvophile or larvae-loving mouth-brooders lay their eggs on a substrate and guard them until the eggs hatch. After hatching, the female picks up the fry and keeps them in her mouth. When the fry can feed for themselves, they are released.

Spawning tank

Ovophile mouth-brooders can be bred in the main aquarium because the eggs are protected in the mouth cavity. However, it is better to separate mouth-brooders with eggs because of their potentially aggressive behavior. There are no special breeding tank requirements other than the usual tank set-up for the species. Larvophile mouth-brooders should be placed in a separate breeding tank because the eggs are not protected in the mouth, but laid on a surface where they are open to predators.

Nest-builders:

Many fish species build some sort of nest for their eggs. The nest ranges form a simple pit dug into the gravel or the elaborate bubble nest formed with saliva-coated

bubbles. The Gouramis, Anabantids and some catfish are the most common of this type of spawners. Nest builders practice brood care.

Spawning tank

Nest-builders should be provided with material with which to build their nests. For bubble-nest builders, fine leafed and floating plants should be provided, and the tank should have no water current to disturb the nest. Species that build nests in the substrate should be given fine gravel or sand.

Raising the fry of Egg layers

When the eggs hatch, the larvae that emerge look nothing like the parent fish. Instead, the larvae have a large, yellow yolk sac and are barely able to swim. The larva feeds on the egg sac until all the yolk is absorbed. Once the yolk sac is absorbed, the fry starts feeding on external food. The fry of small fish can be first fed with Infusoria, "green water," or egg yolk. Later these fry can be fed larger foods like white worms, Daphnia, *Artemia* nauplii, and ground flakes. These foods are good as a first food for slightly larger fry such as those of cichlids. Once the fish grow larger, larger foods like brine shrimp, larger Daphnia, flakes, insect larvae, and chopped *Tubifex* worms are accepted. The fry should be fed several times a day. Many species need periodic sorting by size, so that larger fish do not cannibalize smaller fish.

Breeding and Culture of Gold Fish (*Carassius Auratus)*

Common gold fish, *Carassius auratus*, belongs to the order: Cypriniformes and family Cyprinidae. It is an omnivorous fish and feeds on a wide variety of live feed and accepts artificial feeds also. Colour of gold fish ranged from Red, Orange, Silver, Black, Brown, White and many more. More than 30 varieties of gold fish are available. The most common varieties are Oranda, Lion head, Fan tail, Telescope eye, Bubbles eye, Albino, Pearl scale and several others.

Fig-3: Gold fish

Required water parameters such as pH 7.0 to 8.0, temp 25 to 30 ^{o}C, dissolved oxygen 5 to 7 ppm, dissolved free carbon dioxide 0 to 4 ppm and total alkalinity 80 to 100 ppm. Electric aerator (pump) raise dissolved oxygen level of water to 6-7 ppm which is necessary for breeding. Partial water exchange (25 to 30 %) is very much essential from breeding tank. Breeding carried out in "Gamlas", 40 to 60 l capacity which is made up of clay or cement or in rectangular glass aquarium of 50 l capacity. General fecundity of gold fish ranged from 500 – 700 depending upon the size. Sex ratio is kept 2:1 (male:female) to ensure successful breeding. Eggs are generally released during night hours. Fertilized eggs are transparent and grayish in colour and unfertilized eggs are transparent white. Eggs are sticky in nature; substratum may be maintained with soft weeds, tiles, corrals etc., for settlement of eggs.

Fertilized eggs hatch in 4 to 7 days depending upon water temperature. No parental care is seen. Parents eat hatchlings. As a result parents will be removed after breeding.

Sex Determination:

In case of male gold fish white bumps or tubercles develop on the operculum and pectoral fin. Main ray of pectoral fin have thick edge in case of male but thinner edge in case of female. Fins become more pointed in case of male but look rounded in female. Vent assumes a concave shape with a small opening in male and vent becomes convex and large opening in case of female. Abdomen is seen to be smaller in male but large in case of female.

Brooders

Base for adhesive eggs Eggs on mop (inside water) Eggs on mop (outside water)

Newly hatched ones Gold fish fry in rearing tank Gold fish ready for marketing

Fig-4: Different stages in gold fish breeding

Culture of common gold fish:

The culture of common gold fish is being taken up normally in cement tanks of dimension 10' x 5' x 2' or 12' x 6' x 2'. Preferable temperature for culture is 15.5 to 24 ^{o}C. pH range 7.0 - 8.0 and prefer moderate hardness of 50 – 75 mg $CaCO_3$ per litre and oxygen level of 5 to 7 ppm. Generally 300 fry (23 mm) of gold fish are stocked in each cement tank of dimension 10' X 5' X 2'.

The newly hatched young ones depend upon their yolk size as a food source for a couple of days. When the fry become free swimming they are being fed with *Artemia*, *Daphnia*,

Moina, Tubifex worm and other planktons. Young ones of 2 – 3 days old feed with egg yolk and dried milk powder. After 10 days the young ones start feeding the tubifex worms and maintained till their disposal.

Breeding of Tiger Barb (*Barbus tetrazona)*

Out of more than 30 commercially important species of freshwater ornamental fishes reported from Indian waters tiger barb, Barbus tetrazona is one of them. This species is hardier and active and does not require much of attention in regard to its basic needs. Their large scales, bright colors, schooling behavior and ease of maintenance and of breeding them have made the fish popular in the aquarium trade. Though there are 1078 barb species reported from all over the world, only 70 barb species are commercially important because of their color pattern.

Fig-5: Tiger barb

Maturity of tiger barb:

The tiger barb which is four banded usually attains sexual maturity at a total body length of 20-30 millimeters (2-3 cm) (or) at approximately 6-7 weeks of age. Although tiger barbs are not sexually dimorphic, males display a bright red coloration on fin rays and snout while females tend to be more round in the abdominal region and slightly less colorful. Females are usually larger than males. They can obtain a maximum length of 7 cm and body depth at 2 cm. All related barbs mate in a ratio of one male to one female with the male displaying aggressive behavior while the female is submissive.

Brood stock conditioning:

Conditioning the sexes in separate tanks is an important step in the seed production process. Tiger barbs for use as brood stock (2 to 3 cm body length) are first collected from

a production ponds or natural water bodies and graded with size graders. Sexually mature females are identified by full round abdominal region and sexually matured males are identified by bright red colors on the fin rays. The selected brooders are then placed sex-wise in separate circular or square or rectangular conditioning tanks. Rectangular tanks are more conductive for removing and selecting brooders. A stocking density of one fish per four liters of water is recommended. The conditioning tank should be provided with sufficient aeration and water exchange at a rate of 20% per day. The separated fish are conditioned by a diet of frozen blood or tubifex worms of Artemia. High quality flakes or a prepared taste are given as feed at least twice or thrice per day for a period of two weeks. Since wild tubifex causes infection to broodstock utmost care should be exercised to prevent this, through needed cleaning etc. During conditioning good water quality should be maintained as the conditioning of diets can lead to fouling of the water. Lack of proper conditioning will result in greatly reduced number of successful synchronized spawning.

Spawning of Tiger Barb:

Submerged aquatic plants or roots are often chosen by the females as the substrate on which they deposit eggs. During actual spawning event, the male clasps the female with its fins during which eggs and sperms are released over the substrate. The behavior may last for several hours or until all the eggs are released. An average of 300 eggs can be expected from each female per spawn. Tiger Barb will consume the eggs greedily after spawning. Therefore parents must be removed as soon as possible (Vogt and Wermuth, 1961; O'Cornel, 1977 and David, 1983). Spawned eggs are adhesive, negatively buoyant in freshwater and on an average 1.18±0.05 mm in diameter. The eggs will hatch in 3 days if a temperature of 25º to 27º is obtained.

Male and Female Tiger barb

Fig-7: Young ones of tiger barb

Induced Breeding of Tiger Barbs:

Sometimes the tiger barbs can not breed under normal captive conditions. To induce these fishes to breed hormonal intervention is needed. The hormones commercially available to induce them to breed are Ovaprim, Ovatide and Chorulon. The injection can be given inside the peritoneal cavity or just below the dorsal fin in intramuscular region. Since the fishes are very small, care must be taken to avoid any injury to the individual fishes. Adequate care should be taken to calculate the dosages are required. Now days, some hormones are mixed with the supplementary feed and given orally to avoid any injury to these small animals during injection.

Rearing of Young Ones:

The newly hatched fry are non-spawning for two days. They obtain nutrition from the yolk sac. So much so, the fry do not require supplemental feeding at this time. Three days after hatching, yolk sac is usually absorbed. Newly hatched brine shrimp Artemia approximately 0.02 inches in size are introduced as the first feed and it is used exclusively for next two days. The fry should be fed to satiation three or four times per day. After feeding with brine shrimp exclusively for two days, commercial feed can be introduced for the fry to feed upon. Once the fry gets adjusted to consume a commercial feed and consumes it for two days and are approximately 5 mm (0.2 inches) in length, they can be transferred to prepared outdoor nursery tank or directly stocked into a grow out pond or tank (Clyde et al.,1998).

Disease Management:

The three most common disease problems encountered by commercial barb farmers are caused by protozoans (*Trichodina*), monogenera (*Dactylogyrus* and *Gyrodactylus*) and

fungi (*Saprolegnia*). Thes are found in all gill and soft tissue such as fin rays. *Trichodina* can be controlled by giving 25 ppm formalin bath. For treating monogenera parasites one hour bath with formalin of 25-30 ppt sodium chloride dip is recommended. For treating fungal disease dip treatment of 250 ppm formalin on a few consecutive days is recommended.

Breeding of Angel Fish (*Petrophyllum Scalare)*

Angel fish breeding has progressed into an art with the development of the veil finnages, superveil finnages and the many color varieties. It is remarkable that all of these forms came from the original standard silver angel fish from the wild.

Fig-8: Angel fish

Sex identification:

It is difficult to identify male and female in the angel fish but at the time of spawning genital papillae are the reliable identification of sex determination. These look like little nipple-like projections and are called ovipositors. The female's ovipositor is larger and more blunt than the males which is slender and more pointed. These protuberences, which appear at the vent, are used respectively for depositing eggs and fertilizing them.

Male & female angel fish

Set up for mass scale breeding

Baby angel

Angel ready for marketing

Fig-9: Different stages of Angel breeding

Spawning tank:

Large aquarium tank of 80 to 100 l capacity is ideal for spawning tank. Spawning tank water requires a slightly acidic pH level of 6.6 to 6.8. The fish can spawn at higher pH of 8 but fish tend to spawn more readily at the lower pH levels mentioned above. It is especially important to keep the water acidic if you are going to keep the eggs with the parents. Maintaining the pH 6.6 to 6.8 for hatching provides an optimum pH condition for hatching eggs. The tank is furnished with slates or glass plates that are slanted at angle to lay eggs upon. An air stone giving mild aeration may be placed at the corner.

The pair will select a spawning site and thoroughly clean it about two or three days before actual spawning takes place. When the cleanliness of spawning site finally meets the approval of the parent fish, the female will make a few test runs. She will pull her ventral fins of feelers close to the lower sides of her abdomen and her anal fin will be situated so that her entire lower line is relatively straight. Her ovipositor will then be able

to make full contact with the slate; glass plate or whatever chosen for a spawning site. The male will then make a few practices run too before the actual spawning takes place.

When spawning actually takes place, the female will pass over the site and eggs are deposited which adhere to the surface. The male then moves in and scoots along over the string of eggs just laid and fertilizes them, his fins taking the same position as the females so he can press closely to insure a higher fertilization rate.

The male and female angel fish will take runs passes over the spawning site until several hundred or more eggs have been laid, depending on the size and condition of the female prior to spawning. The parents will hover closely over the spawn and fan continuously with their pectoral fins to create a circulation of water over and around the eggs. Some fertilized eggs will turn white in a matter of hours and will be removed by the parents.

Hatching Eggs:

For the successful hatching of the eggs it is recommended to use very soft water preferably rain water or distilled water because it has naturally low pH of 6.2-6.5.

When the spawning is over the glass plate should be removed from the spawning tank and place it in a 30-50 l tank with sponge filter and a piece of slate leaned up against a side wall. An air stone should be placed in the jar in such a way that somewhat vigorous stream of air bubbles does not hit the eggs directly. Few drops of 10% Methylene blue was added to prevent the fungal attack on eggs. Hatching should occur in about 36-48 hours depending on the temperature. There will be a period after hatching and before free swimming when the fry will stick together. At this time increase the aeration so all the fry will have access to sufficient oxygen.

Do not put food in the tank till they fry are free swimming. After about 3-5 days when they are free swimming, introduce newly hatched brine shrimp into the tank for the fry to eat. Rearing tanks for baby angels that are two weeks and older incorporate normal dechlorinated tap water. Ten liter for every hundred liter of water is changed daily from the bottom of the tank where all the detritus accumulates. These rearing tanks are not treated to lower acidity.

Feeding Schedule: **One week to three weeks**

Angel young ones do not need any type of feeding until they are in free swimming stage. It takes about four to six days depending on the temperature. When the young fry became free swimming feed them newly hatched brine shrimp (Artemia) or *Moina.*

Brine shrimp is fed directly to the young at first to make sure that no excess is floating around in the tank for hours at a time. Three or four feedings per day should be sufficient. Any brine shrimp floating around after 20 minutes is a sign that you are feeding too much. Remember, feeding in light quantities decreases overfeeding and associated problems such as ammonia and disease.

Three weeks to five weeks:

After three weeks the fry attain a size in which they will accept finely crushed flake foods. Flake foods are provided in small quantities as a supplement.
After three weeks brine shrimp can be fed and eaten within 15 minutes of adding it to the aquarium.

Five weeks to seven weeks:

At five weeks of age, the young angel fish are introduced to dry foods. A small amount is fed twice daily until the seventh week. During this time, the small angel fish will attempt to eat the dry flakes but small angel fish will attempt to eat the dry flakes but they will usually spit it out soon after taking it into their mouths. Some will eat the flakes and some will not. Around the seventh week the angel fish begin accepting dry flakes and there should be few flakes, if any remaining on the bottom of the tank like the previous weeks.

Six weeks to adult hood:

At about six weeks of age, the young angel fish have reached a size in which they will begin accepting blended beef hurt cubes. Baby brine shrimp can still be given to the young angels for upto three months but beef liver and flakes are all that is necessary for quick growth.

Breeding of Gouramies:

Gouramies although closely related to Bettas, do not have their fighting depositors. A under good conditions they are friendly community fish. In all gouramies, the pelvic fins are shaped as long as thread-like feelers, which can be moved in all the directions. Popular

aquariums varieties of gouramies are the giant gourami (*Colisa fasciata*), dwarf gourami (*Colisa lalia*), pearl gourami (*Trichogaster leeri*), blue or three spot gourami (Treichogaster trichopterus), moonlight gourami (*Trichogaster microcephalus*), snakehead gourami (*Trichogasrter pectolaris*), chocolate gourami (*Sphaericthys osphronemoides)* and kissing gourami (*Helostoma temmincki*).

For describing the breeding of gouramies, a typical example of blue or three spot gourami is presented below.

Fig-10: Male & Female Giant Gourami, *Colisa fasciatus*

The three spot gourami breeds during April to August. During breeding season mature male develops dark colouration and female show buldging abdomen. While making breeding pair care must be taken to select the mature female, which is ready to spawn. This is because males of blue gourami are very aggressive in nature and tend to kill female, if she is not ready to breed. Aquarium tanks of 50-80 litre capacity can be used for breeding. The water level in the aquarium should not be more than 25 cm. One or two pieces of floating plants and beetle leaves may be floated on the water surface to hold the bubble nest. The tank should not be provided with aeration. The pairing of blue gourami is made in the ratio of 1:1. If the male in breeding condition, it will start making nest within one or two days. The bubble nests floats under the plant leaves and looks like soap foam. The male drags the female under the leaves and during courtship female releases a batch of 20-25 eggs. The male pick up the eggs and attach them in the floating nest.

Table-1. Commercially important ornamental fishes

Scientific name	**Common name**	**Distribution**	**Remarks**
FAMILY – CYPRINADAE			
Barbus everetti	Clown barb	Singapore,Indonesia, Malaysia	Rivers, ponds; 14 cm; males colourful
Barbus tetrazona	Tiger barb	Indonesia, Malaysia	Running waters; 7 cm; males colourful
Brachydanio rerio	Zebra barb	Eastern India and Bangladesh	Sluggish waters; 6 cm; males colourful
Carassius auratus	Gold fish	China, now everywhere	Over 100 colourful varieties; 30 cm/15 years
Danio aequipinnatus	Giant Danio	Sri Lanka and Eastern India	Sluggish standing waters; 10 cm; females larger
Puntius conchonius	Rosy barb	India and Bangladesh	Rivers and ponds; 14 cm
FAMILY – ANABANTIDAE			
Betta splendens	Siamese fighting fish	Thailand, Kampuchea, Vietnam	Males larger (6 cm), colourful; carnivorous
Colisa chuna	Honey gourami	India, Myanmar, Thailand, Malaysia	Males colourful; 5 cm; omnivorous
Colisa lalia	Dwarf gourami	India and Bangladesh	Males larger (5 cm), brightly coloured;
Helostoma temminckii	Kissing gourami	Malaysia and Thailand	Males fight in a kissing posture; 30 cm;
Trichogaster leeri	Pearl gourami	Malaysia, Thailand and Indonesia	Males colourful; 10 cm; omnivore
Trichogaster trichopterus	Blue gorami	Malaysia, Thailand, Myanmar	15 cm; males build bubble nests for egg-laying
FAMILY – CHARACIDAE			

Hemigrammus erythrozonus	Glow light tetra	Brazil, swamps and rivulets	Females larger; 4 cm;
Hyphessobrycon erythrostigma	Bleeding heart tetra	Brazil, Colombia and Peru	Males brightly coloured; 12 cm
Metynnis hypsauchen	Silver dollar	Brazil	Several varieties; 10 cm
Paracheirodon innesi	Neon tetra	Brazil	Females larger; 4 cm; community fish
FAMILY – POECILIIDAE ***(Live bearers)***			
Poecilia latipinna	Sailfin molly	Central America to Mexico	Females larger (18 cm); do well in salt water too
Poecilia reticulata	Guppy	Central America to Mexico	Females larger (8 cm); variety of colours
Xiphophorus helleri	Swordtail	Southern Mexico and Gautemala	Females larger (12 cm); male with swordtail
Xiphophorus maculatus	Platy	Yucatan Peninsula and Mexico	Females larger (6 cm); no sword, dorsal rounded
FAMILY – CICHLIDAE			
Cichlasoma meeki	Firemouth cichlid	Central America, Mexico, Yucatan	Males colourful, longer dorsal; 12 cm
Pterophyllum scalare	Angelfish	South America	Disc-like (12 cm long/25 cm deep); varieties
Symphysodon aequifasciatus	Discus	South America	As deep as long (20 cm); sexing difficult
FAMILY – LORICARIIDAE			
Hypostomus plecostomus	Pleco	Brazil and Peru	Need shelters, high oxygenation and filtration
Xenocara dolichopterus	Suckermouth	South America	15 cm; other species up to 60 cm
FAMILY – CALLICHTHYIDAE			

Corydoras aeneus	Bronze cory	South America	Long courtship before spawning; 7.5 cm
FAMILY – COBITIDAE			
Acanthophthalmus kuhlii	Kuhli loach	Southeast Asia	Prefer fine sandy substrate and dense vegetation
Botia macracanthus	Clown loach	Indonesia and Malaysia	42 cm; make audible sounds and have eye spines
FAMILY – BELONTIIDAE			
Macropodus opercularis	Paradise fish	Asia	10 cm; Bubble nest builders; male guards

The courtship repeated many times for a few hours to give 500-1000 eggs. Once the spawning is over remove the female from the tank. The male will take care of the eggs and young ones. Hatching takes place within 24 hours. As soon as the fry are free swimming, the male should also remove from the tank.

After 36 hours, when the fry are at free swimming state, they are provided infusoria as a feed. After 6-7 days the fry start taking brine shrimp or small Moina. At this stage they should be feed 3-4 times a day. The growth of fish is very uneven and often some "shoot fry" develop. The "shoot fry" i.e. bigger ones of the lot should be separated and reared in the different tank. Now they can be stocked in a bigger tanks and give a diet of worms and formulated feed.

Conclusion

Fishes simply being in good condition and able to breed may not be enough to trigger spawning. The final stimulus for the development of eggs and the production of sperm may be one of the environmental factors already mentioned; it could also be the "correct behavior" of a suitable mate. Achieving this correct behavior in an aquarium may mean that you have to provide a suitable tank bottom, hollow or cave, or type of vegetation before the fishes will begin to spawn. Always maintain written records of your success and failures, particularly with the more difficult species. You may be the first aquarist to get it right!

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Physiological homeostasis in fish reared in inland saline water (ISW): constraints and mitigations

S. Dasgupta

Any change in congenial environment may evoke nonspecific response from the body to cater demand made upon it. Such responses are commonly defined as stress in fish and can be characterized by physiological changes such as plasma cortisol, glucose, lactate, and electrolyte concentrations and are quantitatively related to the severity and longevity of the stressor. A stressed organism passes through distinct phases: I) an alarm phase is usually characterized by a rapid physiological response; ii) a stage of resistance, the second phase, the organism adapts to or compensates for the altered conditions causing the stress in order to regain homeostasis. The organisms may return in the physiological condition of pre-stress state or to an altered resting state. If the stress is overly severe or long-lasting, compensation may not be possible and the organism enters the final stage of exhaustion. Stress is not always harmful; acute responses to stressors may be beneficial to the fish and enhance their normal adaptive ability, whereas chronic exposure to stressors may hamper growth, immunity and survival. Aquaculture management practices and selection pressures affect the stress response, which requires that the ultimate care. Super intensive aquaculture involving very high stocking densities needs minimizing stressors arise from handling, poor water quality and disease treatment. Moreover aquaculture in inland saline water requires special management systems for ensuring optimum growth and survival of fish to enhance net production.

Salinity is an inherent physicochemical property of water and governs the activity and distribution of fishes. Salinity change in ambient water causes salinity stress if not compensated for; it influences physiological homeostasis and routine biological processes. Majority of the fish are restricted to natural habitat with relatively stable salinity at 30-40 ppt (marine) and <0.5 ppt (freshwater). The fishes have narrow salinity tolerance (stenohaline) inhabits stable saline zone, whereas, the fishes have wider salinity tolerance (euryhaline) enjoy a wide zone of variable salinity. Euryhaline fishes adopt various osmoregulatory strategies from active salt absorption to salt secretion and from waterexcretion towater retentionfor facing challenges of wide salinity fluctuation of water. Such dynamic strategies entail osmosensing, signalling downstream for encoding

information about the direction and magnitude of salinity, epithelium transport and permeability effectors. Mosaic evolution involving ancestral and derived protein functions help in developing underlie mechanisms of euryhalinity. Among many proteins preserved in euryhaline and stenohaline, a few proteins have evolved functions specific to euryhaline fish. Most euryhaline fishes have an upper salinitytolerance limit of approximately two fold of seawater (60 g kg−1). However,some species tolerate even up to 130 g kg−1 salinity by switching their special adaptive strategy.

Maintenance of osmotic homeostasis in fishes

Marine hagfish and elasmobranchs are osmoconformers and maintain NaCl concentration in body fluid equal to that of seawater. Over 25000 fishes are teleosts that maintain the osmolality of their extracellular body fluidsrelatively constant at approximately 300 mosmol kg−1 (which isisosomotic to 9 g kg−1 salinity), independent of environmentalsalinity. To maintain osmotic consistency of body fluid, teleosts in freshwater encounter passive salt loss by active absorption and passive gain ofwater by excretionof dilute urine. Conversely, marine teleosts actively secretesalt and retain water to maintain osmotic homeostasis. Many physiological mechanisms involved in these processes of steady-state osmoregulation are strikingly different in freshwater and marine teleosts.

Current model suggests that salt secretion is mediated by branchial MR cells mainly involving sodium potassium ATPase (NKA), sodium potassium 2 chloride co-transporters (NKCC), and cystic fibrosis transmembrane conductance regulator (CFTR) for Cl^- ions, and ‘‘leaky’’ tight junction for Na+ in seawater acclimated teleosts. In freshwater, Cl^- uptake is coupled to HCO_3 efflux through an anion exchanger in the branchial MR cells, whereas, Na+ uptake involves various channels such as, an apical sodium/hydrogen exchanger (NHE3), V-type H+-ATPase and sodium/chloride cotransporter (NCC) depending on the species, environment and subtype of ionocytes. The subsequent transport of three intracellular Na+ into the serum in exchange for two K+ extracellular ions is generally occurred through basolateral NKA, which couples the energy of ATP hydrolysis.

According to Nelson’s phylogeny (Nelson, 2006), the 15 most primitive orders of fish, except lampreys consist almost entirely of strictly marine species. The next 20 moderately advanced orders are composed mostly of freshwater species (with the exception of Albuliformes, Anguilliformes, Saccopharyngiformes and Clupeiformes). The remaining 27 orders are again mostly composed of marine species (with the exception of

Percopsiformes, Atheriniformes, Cyprinodontiformes, Synbranchiformes and Ceratodontiformes). This scenario suggests that earliest fishes evolved in a marine environment, invaded freshwater habitats before the origin of ray-finned fishes (actinopterygii), and reinvaded marine environments during a second wave ofevolutionary expansion after bony fishes (teleosts) had already appeared. However, many teleosts maintain internal fluid osmolality (9 g kg−1) much lower than that of a marine environment indicates origin of this clade in a freshwater or brackish (mesohaline) habitat. Euryhaline fishes have radiated either in coastal environments such as estuaries and intertidal zones subject to large and frequent salinity fluctuations, or in arid zones containing desert lakes and creeks. A range of species has found suitable for culture in saline groundwater including euryhaline finfish (e.g., *Lates calcarifer*, *Argyrosomus japonicas,* Pagrus auratus, Acanthopagrus butcheri, Salmo salar), crustaceans (*Litopenaeus vannamei*), molluscs, diadromous species and salt-tolerant freshwater species such as finfish (e.g., *Oreochromis niloticus*, *Bidyanus bidyanus*) and shellfish (*Macrobrachium* sp).

How do euryhaline fish cope up with salinity stress?

Fish perceive changes in environment's osmolality through multiple molecular senors. Molecular osmosensors include transmembrane proteins such as ion channels, the calcium-sensing receptor, phospholipase A2 and cytokine receptors, proteins that are directly regulated by intracellular calcium and other inorganic cations, and cytoskeletal proteins. In addition, osmosensing is informed by direct osmotic and ionic effects on DNA and protein stability. Prominent effector proteins regulated by salinity stress signaling networks include NKCC, CFTR, several plasma membrane ATPases, and other transporters. The regulation of these proteins in response to salinity change is reflected at the levels of expression (abundance), compartmentalization and activity. In addition, euryhaline fish achieve a switch from plasma hyper- osmoregulation (environmental salinity <9 g kg−1) to plasma hypo-osmoregulation (environmental salinity >9 g kg−1) increasing cell proliferation and turnover and via extensive epithelial remodeling of gills. Although osmoregulation is a complex physiological process involving body surface, digestive system, gills and renal organs, most sodium (Na+) and chloride (Cl–) regulation takes place in the gills.

Inland saline water and constraints in aquaculture:

In India about 8.5 million ha of salt affected land is found in arid and semi-arid regions of Rajasthan, Haryana, Punjab, Gujarat, Uttar Pradesh, Delhi, Andhra Pradesh Maharashtra, Karnataka and Tamil Nadu. Among these 40% of share goes to Haryana, Uttar Pradesh, Punjab and Rajasthan. Inland saline aquaculture is one of the best ways to resolve this problem because it involves the culture of various euryhaline and marine aquatic animals in these waters. Although the ionic composition of saline groundwater generally reflects that of seawater, the exact composition varies both locally and regionally. Inland saline waters invariably have low levels of potassium and high levels of calcium and variable concentrations of magnesium in comparison to natural sea water. Saline groundwater can contain as little as 5% of the potassium found in equivalent salinity seawater (i.e. K-equivalence) to as high as 75% K-. Therefore, it is imperative to manipulate these ions within the optimal range for the better growth and survival of the animals reared in these waters.

Mitigations:

The deficient ions can be fortifying by two ways by direct addition to the water as inorganic forms or through feed. The most common material for increasing the potassium concentration in pond water is fertilizer-grade potassium chloride (KCl), often called muriate of potash. This material is approximately 50% potassium, so a double dose is required for rectification. Another fertilizer, sulfate of potash magnesia (K2SO4Æ2MgSO4), sold under the trade name K-Mag, has been used as a source of both potassium and magnesium for treating inland shrimp ponds Periodic application of magnesium chloride to maintain the magnesium concentration above 100 mg L^{-1} in ponds with salinities of 1–6 g L^{-1} resulted in a significant increase in fish and shrimp survival and production.

Supplementation of the minerals necessary for normal osmoregulatory function into diets has been suggested for a number of fish and crustaceans. It has been hypothesized that a lack of ions at the gill–water interface could possibly be mitigated by dietary supplementation of these ions by increasing their availability and absorption in the digestive tract. The growth and feed efficiency of juvenile reddrum (Sciaenops ocellatus Linnaeus) reared in freshwater improved following dietary supplementation of NaCl. Dietary supplementation of NaCl, magnesium chloride,

potassium chloride, cholesterol and lecithin improved the osmoregulatory capacity of shrimp and fish reared in low salinity waters.

Supplementation of free amino acids (FAA) in shrimp feed has also been suggested as a potential way to improve the osmoregulatory capacity of shrimp reared in low salinity. Most euryhaline organisms, such as L. vannamei, possess a cell volume regulatory response to counteract abrupt changes in salinity that involves certain non-essential amino acids (NAA) and quarternary ammonium compounds (QAC). These NAA and QAC are present in the cytoplasm of cells and can make up a large proportion of the total intracellular osmolality. Dietary phospholipids and cholesterol probably are important for shrimp cultured in low salinity water because of their role in lipid mobilization and storage in the hepatopancreas. Phospholipids are necessary for normal cell structure and function because of their role as polar lipids that make up part of the cell membrane. Phospholipids are also involved in gill membrane function and lipid metabolism, and they serve as secondary messengers in cell signalling. In addition, phospholipidsmight also facilitate the incorporation of cholesterol into haemolymph proteins.

The energetic cost of osmotic and ionic regulation can result in less energy being devoted to growth. It has been hypothesized that supplementation of diets high in HUFA will make osmoregulation in low salinity waters more efficient for shrimp and fish. Changes in gill fatty acid composition can influence osmoregulation via modulation of water and ion permeability as well as the activity of the Na+–K+–ATPase. Higher dietary carbohydrate levels could help counteract the higher energetic cost of osmoregulation at low salinity. Dietary supplementation of carotenoid pigment astaxanthin shows improvement in growth and survival of shrimp acclimated on low saline water. .

Inland saline water has already in use for growing many species, but the water is imbalance compared to seawater for rearing fish and shrimps. Water modification and dietary modification approaches have been explored by researchers and farmers to improve the growth and survival of brackish water fish marine shrimp in low salinity waters. Future studies warrant to verify the process remediation of inland saline water for fish/shrimp culture in actual pond production trials in low salinity waters.

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PRACTICAL DEMONSTRATION

Proximate Analysis of Feed and Feed Ingredients

Dilip Kumar Singh and Arunadevi

1. ESTIMATION OF MOISTURE CONTENT

INTRODUCTION:

Moisture is the material lost by food on heating to approx. around 105°±5°C in hot air oven. It is generally considered to be water but actually these are the volatile materials which are driven off at higher temperature. Moisture content of all the ingredients should be within 7-13%. Moisture content more than 13% favors the growth of fungus/molds which results in deterioration of quality of the feeds.

MATERIALS REQUIRED:

1. Petri dish
2. Analytical balance
3. Hot air oven
4. Dessicator

PROCEDURE:

Take a petri dish

↓

Dry in hot air oven at 105±5°C for 30 minutes

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After drying place the petri dish to dessicator for 45-60 minutes

↓

When cool record the weight of the container

↓

Add about 5gm sample in the petri dish & weight properly

↓

Place the petri dish in Hot air oven

↓

Maintain a temperature of 105±5°C for 6 hrs

↓

Cool the petri dish in the dessicator for 45-60 minutes

↓

Weight up to constant weight of the petri dish

↓

Calculate the moisture content

CALCULATION:

Weight (gm) of the petri dish (A) =

Weight (gm) of the sample (B) =

Dry weight (gm) of the sample + petri dish (C) =

Dry weight (gm) of the sample (D) = C-A

Moisture content = $\frac{(B-D)}{} \times 100 / B$

2. ESTIMATION OF TOTAL ASH CONTENT

INTRODUCTION:

Ash contains minerals, toxic elements and sand particles. Ingredients having high ash content are not included in fish feed as it reduces the availability of the organic nutrients. Ash content of fish feed should be in the range of 12-17 %.

MATERIALS REQUIRED:

1. Muffle furnace

2. Silica crucible

PROCEDURE:

Take a dry silica crucible and weigh

↓

Take 5 gm of dried, powdered & moisture free sample

↓

Record the total weight

↓

Put the crucible in a furnace (550°C) for 6 hrs

↓

Transfer the crucible to the dessicator after 12 hrs

↓

Cool it down & take weight

↓

Calculate the total ash content

CALCULATION:

Weight (gm) of the silica crucible (A) =

Weight (gm) of the sample (B) =

Weight (gm) of the ash + silica crucible (C) =

Weight (gm) of the ash (D) = C-A

$$\text{Total ash content} = \frac{D}{} \times 100 / B$$

3. ESTIMATION OF ACID INSOLUBLE ASH

INTRODUCTION:

Insoluble ash generally consists of sand particles. The higher percentage of insoluble ash indicates presence of contaminants which are not desirable in the ingredients.

MATERIALS REQUIRED:

1. 10% HCL
2. Ash less filter paper (Whatman no 40)
3. Beaker
4. Funnel
5. Silica crucible
6. Muffle furnace
7. Dessicator
8. Weighing balance

PROCEDURE:

Take the ash content of sample in a 50 ml glass beaker (acid washed)

↓

Dissolve the sample by adding 25 ml of boiling 10 % HCL

↓

Allow to cool and transfer into a funnel

↓

Filter the sample solution through ash less filter paper

↓

Wash the glass and funnel with distilled water and deionized water repeatedly

↓

Remove the filter paper using forcep and transfer it to silica crucible

↓

Ash the filter paper containing sample in muffle furnace at 550-600°C for 6 hrs

↓

Remove the crucible, cool in a dessicator

↓

Weigh the silica crucible

↓

Calculate the acid insoluble ash

CALCULATION:

Weight (gm) of the silica crucible (A) =

Weight (gm) of the sample (B) =

Weight (gm) of the acid insoluble ash + silica crucible (C) =

Weight (gm) of the acid insoluble ash (D) = C-A

% of acid insoluble ash = D X 100 / B

4. ESTIMATION OF CRUDE FAT OR ETHER EXTRACT

INTRODUCTION:

The extraction of fat from substance is often tedious and requires through contact and heating with solvent. This is done in a special apparatus known as Soxhlet Extraction Apparatus. In this procedure, the apparatus is designed so that a fresh portion of solvent comes in contact with the materials to be extracted over a relatively long period of time. Crude fat content in feed should be in the range of 3-8%.

MATERIALS REQUIRED:

1. Reagent: Solvent- Petroleum ether (spirit) (B.P. 40 – 60°C)
2. Moisture free sample
3. Lipid extraction flask
4. Thimble
5. Cotton
6. Soxhlet extraction apparatus

PROCEDURE:

Take approximately 2 gm of dried powdered sample in thimble

↓

Take weight of the extraction flask

↓

Place the thimble inside the extraction flask

↓

Put some cotton in thimble to prevent overflow of sample

↓

Place the flask and properly fit in Soxhlet extraction unit

↓

Pour the solvent in required quantity (80-100 ml)

↓

Fix the heating mantle to 80 ^{o}C for about 1 hr

↓

After 1 hr increase the temperature to 180 ^{o}C and close the knob of the unit

↓

Remove the solvent completely from the flask

↓

Dry the flask for 30 minutes at 105 ^{o}C in a hot air oven

↓

Cool the beaker in the dessicator and weigh

↓

Take out the solvent and kept properly for further use

↓

Calculate the ether extract content of sample

CALCULATION:

Weight (gm) of the sample (A) =

Weight (gm) of the flask (B) =

Weight (gm) of the flask + crude fat (C) =

Weight (gm) of crude fat (D) = C-B

Crude fat % = D X 100 / A

5. CRUDE PROTEIN ESTIMATION USING KJELTECH AUTOMATIC DISTILLATION UNIT

INTRODUCTION:

Kjeltech automatic distillation unit is designed to perform safe and quick distillation such as the micro-kjeldhal distillation and direct distillation. The Nitrogen in the compound is converted into Ammonium Sulphate by digesting the compound with conc. H_2SO_4. Ammonium Sulphate thus obtained is decomposed by alkali and the liberated NH_3 is absorbed in boric acid and titrated against standard H_2SO_4.

MATERIALS REQUIRED:

Reagents

1. Boric acid (4%): A 4% solution is prepared by dissolving 4 g of boric acid in 100 ml of distilled water
2. Sodium hydroxide (40%): A 40% solution is prepared by dissolving 40 g of sodium hydroxide in 100 ml of distilled water
3. Mixed indicator: A 0.4 % solution of bromocresol green and 0.1 % of methyl red are prepared in absolute alcohol and mixed in equal volume
4. Standard 0.05 N H_2SO_4: An approximately 0.1 N solution of H_2SO_4 is prepared by diluting 2.8 ml of concentrated H_2SO_4 to 1 lit. It is standardized against standard alkali and diluted to give an exactly 0.05 N H_2SO_4.
5. Digestion Mixture: Copper sulphate and potassium sulphate are mixed in the ration of 1:9 by weight.

APPARATUS:

1. Kjeltech automatic distillation unit
2. Digestion tube
3. Burette
4. Pipette
5. Measuring cylinder
6. Conical flask

PROCEDURE:

A. Digestion:

1. Weigh .5 gm sample and put into digestion tube.
2. Add 3-4 g catalyst-mixture or digestion mixture.
3. Add 25 ml of conc. H_2SO_4.
4. Place the digestion tube in the digestion unit.
5. Adjust the temperature 80 ^oC for about 1 hr.
6. Increase the temperature to 350 ^oC for 2 hrs.
7. After digestion all samples turn to a viscous blue/green transparent solution.
8. Cool it down to room temperature
9. Pour 10 ml distilled water in the tube and mix and transfer the content to the 100 ml volumetric flask
10. Repeated washing of digestion tube is needed.
11. Make the volume 100 ml with distilled water.

B. Distillation:

1. Take 10 ml aliquot in digestion tube.
2. Place a digestion tube in the adapter of the distillation unit.
3. Take 25 ml of 4% boric acid with mixed indicator in a 250 ml conical flask.
4. Place it in the spill tray and immerse the distillate outlet capillary tube into the solution and close the door.
5. Set 2 strokes on the alkali addition set thumb wheel and press alkali addition start button. (The standard setting of the alkali pump is 20 ml 40% alkali per stroke.
6. Pass steam for 9 minutes.
7. Colour of distillate turns to clear blue.

B. Titration:

1. Titrate the distillate with standardized $^{N}/_{10}$ H_2SO_4 until the end-point (appearance of pink colour) is reached.
2. Note the volume of acid consumed in the titration.

CALCULATION:

$$CP\ \% = \{(V \times 0.0014 \times D \times 100)/W \times A\} \times 6.25$$

Where,

V = Volume of $^{N}/_{10}$ H_2SO_4 used in titration (ml)

D = Volume make in volumetric flask after digestion (ml)

W = Weight (gm) of the sample used in digestion

A = Volume of aliquot taken for distillation

6. DETERMINATION OF CRUDE FIBRE

INTRODUCTION:

Fibre is not a specific compound, but a mixture of plant components such as lignin, cellulose, hemicelluloses, pentosans and other components that are generally indigestible to fish. Practical diets will contain 3-6% crude fibre and addition of fibre is unlikely to have any significant benefits. Because fibre is indigestible, it adds to feacal matter and increases the BOB in culture system.

MATERIALS REQUIRED:

1. Fiber crucible
2. Funnel
3. Muffle furnace
4. Fat free sample

Reagents:

1. 1.25% (V/V) H_2SO_4 solution (6.44 gm of NaOH (97% purity) was weighed & taken in a beaker & mixed with 500ml. of distilled water).

2. 1.25% (W/V) NaOH solution (3.45 ml of H_2SO_4 was taken & mixed with water little quantity of distilled water. The mixed solution was transferred to a flask to make & the required quantity of distilled water was mixed up to 500ml).

PROCEDURE:

Take weight of dried fiber crucible

↓

Take approximately 1 g finely powdered dried fat-free sample in a fibre crucible

↓

Add 150-200 ml of 1.25% H_2SO_4 solution (acid wash)

↓

Boil and reflux for 35 minutes at 350 ^oC

↓

Wash twice with distilled water to remove the traces of acid

↓

Add 150-200 ml NaOH (1.25%) solution (alkali wash)

↓

Boil for 35 minutes 35 minutes at 350 ^oC

↓

Wash twice with distilled water to remove the traces of alkali

↓

Transfer the fibre crucible in a hot air oven at 100^oC and dry it completely

Cool in the dessicator and take the weight

↓

Ash the residue in the muffle furnace at 500^oC for 5 hrs

↓

Cool in a dessicator and weigh the crucible

↓

Calculate the fibre content

CALCULATION:

Weight (gm) of the sample (A) =

Weight (gm) of the fibre crucible (B) =

Weight (gm) of the fibre crucible + ash (C) =

Crude fibre % = C-B X 100/A

7. DETERMINATION OF NITROGEN FREE EXTRACT

INTRODUCTION:

Nitrogen free extract (N.F.E) in soluble portion of the carbohydrate, starch, protein, dextrin and different mono and disaccharides. It can be estimated by subtraction method after estimation of crude protein % other extract crude fibre % and total Ash matter % of dried matter of fat.

CALCULATION:

N.F.E = {100 - (crude protein% + ether extract% + crude fibre % + total Ash)}

8. ESTIMATION OF GROSS ENERGY

INTRODUCTION:

Gross energy of and feed ingredients can be estimated by using Bomb calorimeter. It works on the principle of adiabatic change. Samples are burnt in presence of pure oxygen. The energy released easily measured by thermo sensor and results (Kcal/gm) displayed in computer.

MATERIALS REQUIRED:

1. Bomb calorimeter unit
2. Bomb
3. Dried sample
4. Measuring cylinder
5. Pellet forming apparatus
6. Tungsten wire
7. Distilled water
8. Pure oxygen

PROCEDURE:

Weigh approximately 1 gm of moisture free sample

↓

Make a pellet in pellet maker

↓

Connect the pellet with a tungsten wire in bomb apparatus

↓

Put 10 ml of distilled water inside the bomb

↓

Place the pellet inside the bomb

↓

Fill the bomb with pure oxygen (20 atm pressure)

↓

Place the bomb inside the insulated jacket of the bomb apparatus

↓

Put the weight of the sample in computer

↓

Fire (ignition) the sample to burn

↓

Wait for 20 minutes

↓

Display of energy value of sample (Kcal/gm)

↓

Note down the gross energy of sample

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Methods of Feed Preparation

Dilip Kumar Chowdhury and Shamna N

1. INTRODUCTION

Diet formulation is a process in which the appropriate feed ingredients are selected and blended to produce a diet with the required quantities of essential nutrients. As the feeding includes 40-70 % of whole operational cost hence greater importance need to be focused on the cost effective feed formulation. Before diet preparation the ingredients identification and selection are very important.

2. IDENTIFICATION OF INGREDIENTS

- Visual observation
- Texture
- Appearance
- Smell

3. COMMON FEED INGREDIENTS

- Soybean meal
- Mustard oil cake
- Rice bran
- Groundnut oil cake
- Wheat flour
- Fish meal

4. BASIC INFORMATION NEED FOR FEED FORMULATION

- Nutrient requirements of the species cultivated
- Feeding habits of the species
- Local availability, cost and nutrient composition of ingredients
- Ability of the cultured organism to utilize nutrients from various ingredients
- Expected feed consumption
- Feed additive needed
- Type of feed processing required

5. FACTORS AFFECT QUALITY OF DIET AND FISH PRODUCTION

- Bioavailability of nutrients
- Palatability
- Feed manufacture
- Storage methods

- Chemical composition of diet

6. TYPES OFFARM MADE FEED

- Mash feed
- Moist ball feed
- Cooked paste
- Cooked ball
- Pelleted Feed

Formulae of farm made feed: Rice bran: 30%, Wheat flour: 30%, Mustard oil cake: 25 % and ground nut oil cake: 25%

Pelleted Feed: This feed is made using the pelletizer in different size range such as 2, 2.5, 3.5 mm as per the size deemed by different fish species

Table1: Ingredient composition of pelleted feed

Ingredients	% inclusion
Soybean meal	34
Mustard oil cake	30
Rice bran	30
Vegetable oil	4
Vitamin premix	1
CMC1	1

7. STEPS OF THE FEED FORMULATION

a) **Ingredients:** Feed formulation is done based on available of ingredients and need to collected and store in ware house to be protected from high moisture, sunlight and insect in order to keep the good quality of feed ingredients.

b) **Grinding:** All ingredients are grinded to make powder form so that the particle size need to be as less as possible. Lower the particle size better the digestibility of feed ingredients.

c) **Weighing:** According to feed formulation, all major and minor ingredients are weight out.

d) Mixing: Major and minor ingredient are mixed separately than mix it with major ingredient. This technique are applicable for farm made feed preparation while in commercial feed industry is automated with mechanical mixer in which all ingredients are mix together at time.

e) Adding water: Water is added to mixture of all ingredients. Amount of water which is needed to be added depend on type of ingredient uses. Usually 70-75% water is mixed with mixture ingredient for farm made feed preparation before cooking. While in commercial feed manufacturing, water is added in steam form and total moisture content is 15-16% in during pellet press machine while in extrusion technique total moisture content need to be 20-25% through addition of steam water.

f) Cooking: All mixture with sufficient moisture content are passed through cooking chamber in commercial feed manufacturing process.

g) Extrusion: Machine is designed to manipulate pressure, temperature through steam (120-130^{o}C) inside the extruded barrel and bulk density is control to produce sinking, slow sinking and floating feed.

h) Pellet press: Temperature is 80-90^{o}C**.** Steam are addedto increase moisture content upto 16%. Mixtures of feed ingredient are pressed through roller and pellet come out.

i) Drying: Farm made feed are dried in sunlight.

j) Coating: Oil coater is used to add additional lipid if require.

k) Packing: Dried feed are kept in dry place in order to avoid increasing moisture content.

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Estimation of Water Stability of the Feed

Dilip Kumar Singh, Arunadevi and Shamna N

Water stability is an important physical parameter for fish feed and same can be enhanced through addition of different binders such- carboxy methyl cellulose, guar gum, gelatin, starch, sodium alginate, tapioca flour, rice, wheat flour etc.

Methods

The water stability of the feed pellet can be determined by dipping the certain amount of pellet (around 5 g) in a glass beaker containing tap water.

- Take 5 g pellet sample
- Dip the 5 g feed in a glass beaker containing at least around 800 ml tap water.
- Observe the immersion for 30 min, 1 hr., 2hr., and 4 hr.
- Filter the un-dissolved solid and water through filter paper
- Dry the filtrate in the hot air oven (105 ^{0}C for 30 min)
- Further drying at 65^{0}C till a constant weight
- Cool in a desiccator.

The difference in the weight of the beaker containing feed before immersion and after drying is used to calculate the percentage dry matter loss, which is a measure of the water stability of the pellet for the corresponding time intervals.

$$\text{Leaching rate} = \frac{A \times (1-r) - R}{A \times (1-r)} \times 100$$

Where,

A: Weight of pellets before immersion;

r: Moisture content of pellets

R: Dry weight of the remaining solid.

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List of participants of Skilled Development Programme on "Aqua feed preparation and feeding management for inland saline aquaculture" during 11-15 February, 2020 at ICAR-CIFE Kolkata Centre under National Agriculture Higher Education Project (NAHEP)

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17	SUBHASH KUMAR (M) Add-JuranChhapra, Road No.6, Ward No.4, P.S. Bramphura, P.O. M.I.T. Muzzaffarpur		IFAF, R.D.S. College	OBC
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ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION
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Time Schedule for Skill Development Training Programme on "Aquafeed Preparation & Feeding Management for Inland Saline Aquaculture" (11th– 15thFebruary 2020)

Programme Leader: Dr. Gopal krishna, Director, ICAR-CIFE, Mumbai
Course Director: Dr. N. P. Sahu, HoD, FNBPD & Dr. G. H. Pailan, OIC, Kolkata Centre
Training Coordinators: Dr. Parimal Sardar, Dr. Shamna N, Mr. Dilip Kumar and Dr. Manish Jayant

No. of trainees: 31

Date	Time	Lecture/Practical/Visit	Faculty/Facilitator
11.02.2020 Tuesday	10:00 am	Registration of the trainees	Mrs. G. Aruna Devi &Mr. R. Chowdhary
	11:00 am	Pre-training evaluation	Mrs. G. Aruna Devi
	11.30 am	Nutrient Requirements and Potential feed ingredients	Dr. G. H. Pailan
	1:00 pm	Lunch Break	
	2:00 pm	Nutrigenomics application in fish nutrition	Dr. Sujata Sahoo
	3:30 pm	Demonstration of proximate analysis of feed	Mr. D. K. Singh & Mrs. G. Aruna Devi
12.02.2020 Wednesday	10:00 am	Aquafeeds, digestion and absorption of nutrients	Dr. Shamna N
	11.30 am	Disease in inland saline water and their remedies	Dr. A. Biswas
	1:00 pm	Lunch Break	
	2:00 pm	Importance of live food in inland saline aquaculture	Dr. S. Munilkumar
	3:30 pm	Demonstration on feed formulation and preparation	Mr. D. K. Singh Mrs. G. Aruna Devi & Dr. Shamna N
13.02.2020 Thursday	10.00am	Visit to ICAR-CIBA, Kadwip	Mr. P.K. Behera
14.02.2020 Friday	10.00 am	Feed and feeding strategies in brackish water aquaculture	Researcher from Kakdwip Centre, ICAR-CIBA
	11.30 am	Analytical technique of different water parameters in aquaculture	Mr. D.K. Singh&Mr. P.K. Behera

	1:00 pm	Lunch Break	
	2:00 pm	Breeding and culture of ornamental fishes	Dr. B.K. Mahapatra
	3:30 pm	Effect of anti-nutritional factor and its mitigation	Mr. D. K. Singh
15.02.2020 Saturday	10:00 am	Quality Control and storage of ingredients and finished feeds	Mr. D. K. Singh/ Dr. G. H. Pailan
	11.30 am	Demonstration on culture and enrichment of live food in aquaculture	Dr. S. Munilkumar& Mrs. G. Aruna Devi
	1:00 pm	Lunch Break	
	2:00 pm	Physiological homeostasis in Fish reared in ISW: Problems and mitigation	Dr. S. Dasgupta
	3.00 pm	Post-Training Evaluation of trainees	Mrs. Aruna Devi and Mr. P.K. Behera
	3:30 pm	Feedback from trainees	Mrs. Aruna Devi and Mr. P.K. Behera
	4:00 pm	Valedictory Programme	

List of Resource Persons

Sl. No.	Name & Designation	Office Address	Phone/Fax/e-mail
1.	Dr. G. H. Pailan Principal Scientist & Officer-in-Charge	Central Institute of Fisheries Education, Kolkata Centre, Salt Lake, Kolkata-700 091	033-23575269/Extn-12 09674719372 033-23573469 ghpailan@cife.edu.in
2	Dr. B. K. Mahapatra Principal Scientist	Central Institute of Fisheries Education, Kolkata Centre, Salt Lake, Kolkata-700 091	033-23573893/Estn.23 09836849332 033-23573469 bkmahapatra@cife.edu.in
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6	Dr. S. Sahoo Scientist	Central Institute of Fisheries Education, Kolkata Centre, Salt Lake, Kolkata-700 091	033-23573893/Extn.31 08820467721 033-23573469 sujatasahoo@cife.edu.in
7.	Dr. Shamna N, (Scientist)	FNBP Division of ICAR-CIFE, Mumbai	081086900937 shamna@cife.edu.in
8	Mr. D. K. Singh Scientist	Central Institute of Fisheries Education, Kolkata Centre, Salt Lake, Kolkata-700 091	033-23573893/Extn.27 09836740344 dilipkumarsingh@cife.edu.in
9.	Dr. A. Biswas Chief Technical Officer	Central Institute of Fisheries Education, Kolkata Centre, Salt Lake, Kolkata-700 091	033-23573893/Extn.20 09836359543 ashokbiswas@cife.edu.in
10.	Dr. Debasis De Principal Scientist & Officer-in-Charge	Kakdwip Research Centre, ICAR-Central Institute of Brackishwater Aquaculture	Phone: +913210 255072, Fax:+91 3210 257030 debasiskrc@yahoo.com dedebasis47@gmail.com

Technical Assistance:

1. Mrs. G. Aruna Devi, Sr. Tech Asst., ICAR-CIFE, Kolkata Centre
2. Mr. Prakash Behera Sr. Tech Asst., ICAR-CIFE, Kolkata Centre

NAHEP PEDAGOGY TEAM

Project PI	:	*Dr. Gopal Krishna*
Pedagogy Team Leader	:	*Dr. N. P. Sahu*
SDP Co-ordinator	:	*Dr. Shamna N.*
Team Members	:	*Dr. Rupam Sharma*
	:	*Dr. Shashi Bhushan*
	:	*Dr. Saurav Kumar*
	:	*Dr. Shamna N*
	:	*Mr. Desari Bhoomiah*
	:	*Mr. Vishnu R Nair*
	:	*Ms. Nuzaiba P.M*

Skilled Development Programme on
"Aquafeed Preparation and Feeding Management for Inland Saline Aquaculture"
Organised by ICAR-CIFE, Kolkata Centre & Fish Nutrition, Biochemistry and Physiology Division, ICAR-CIFE, Mumbai
11- 15 February, 2020

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