ICAR-CIFA Training Manual No: 65

Training Programme on Application and Practices of Fish Feed in Aquaculture (23-28, October, 2017)

Dr. B.N. Paul
Course Director

Dr. S. Adhikari & Dr. R.N. Mandal
Course Co-Directors

Organised by

ICAR-Central Institute of Freshwater Aquaculture
(ISO 9001:2015 Certified Institute)
(Indian Council of Agricultural Research)
RRC,Rahara, 700118,West Bengal

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DirectorDr.J.K.Sundaray

Course Director Dr.B.N.Paul

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Compiled and Edited by

Course DirectorDr.B.N.Paul

Course Co- Directors Dr.S.Adhikari
Dr. R.N.Mandal

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PREFACE

The training on "Application and practices of Fish Feed in Aquaculture" aims to cover the feeds and principles of fish Nutrition. The trainees will be exposed to lectures on different aspects of nutritional requirement of carps and catfishes. Role of natural food a plays very important role vis-à-vis application of formulated feed to be covered. Improvement of Nutritive Value of Plant feed-stuffs for Formulation of aqua feed through the application of Solid State Fermentation will be delivered for better utilization of non-conventional feed stuffs. Feed formulation and application of farm made feed for fish production is one of the key aspects of training programme. In addition to nutritional lectures, the areas of general aquaculture practices, management of water quality parameters, hilsa rearing and fish as health food will be very helpful to the trainees. Practical will be on analysis of proximate composition of fish feed and preparation of pelleted feed through pelletizer. The training will cover both basic and applied aspects of aquaculture nutrition. The present scenario and market trend of aqua feed will also be covered to the trainees so that they get acquainted with fish feed market of the country.We strongly believe that this training programme on application and practices of Fish Feed in Aquaculture will be of great help to the trainees.

B.N. Paul
S. Adhikari
R.N. Mandal

Date:23.10.17

भा.कृ.अनु.प. - केन्द्रीय मीठाजल जीवपालन अनुसंधान संस्थान
(आईएसओ 9001:2008 प्रमाणित संस्थान)
भारतीय कृषि अनुसंधान परिषद
कौशल्यागंग, भुवनेश्वर - 751002, (उड़ीसा), भारत
ICAR - CENTRAL INSTITUTE OF FRESHWATER AQUACULTURE
(An ISO 9001:2008 Certified Institution)
Indian Council of Agricultural Research
Kausalyaganga, Bhubaneswar - 751002, (Odisha), India

Dr. Jitendra Kumar Sundaray
Director (Acting)

Foreword

The training programme on "Application and Practices of Fish Feed in Aquaculture" is a very pertinent topic in fish nutrition. The feed accounts for more than 50-60% input cost in the total cycle of fish production in Aquaculture practices. Nutrient requirement of fish is one of the core area of nutrition. There is acute shortage of fish feed ingredients in the country. Now, it is need of the hour, to identify new feed resources to benefit aquaculture production. Feed and feeding practices are also very much relevant in the Aquaculture practices. Therefore, both are covered in this training programme.

I am glad that the training programme on"Application and Practices of Fish Feed in Aquaculture" is being organized during 23-28, October, 2017 on our regional research centre in an appropriate time. This training programme will help the sector to get qualified technocrats who have been refreshed with the recent advancement of fish nutrition.

I would like to compliment both contributors and editors for their efforts to compile such an important manual. Appreciably, I am sure that the training manual will be highly useful to all beginners, students, fish farmers and other stake holders in the field of fish nutrition and aquaculture

23/10/17
Jiterdra Kumar Sundaray

Date: 23.10.17

Phone: 0674-2465421, 2465446, 2465402 (O), Fax: 0674-2465407

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Application and Practices of Fish Feed in Aquaculture
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B.N.Paul

ICAR-Central Institute of Freshwater Aqua
Regional Research Centre, Rahara, Kolkata-700 118, West Bengal

INTRODUCTION

The fisheries sector occupies an important place in the socio-economic development of the country, which envisages livelihood, nutritional security, employment generation and export earnings. Indian Fisheries occupy the second position in global fish production and second in aquaculture in the world with an annual growth rate of 4.7%, recording 3.2% growth in marine sector and 6.2% in Inland sector, contributing 1.1% to the GDP and 4.5% to the agricultural GDP of the country. About 35% of the Indian population is fish eaters and the per capita assumption is 9.8 kg, whereas recommend intake is 13 kg.

There is huge demand for fish food for human consumption and reduction in capture fishery resources have created a gap between the supply and demand of fish. Under such circumstances increased aquaculture production can only bridge the gap. Indian aquaculture sector comprise of large scale culture of Indian Major carps (IMCs) such as rohu, catla, mrigal with a combination of exotic carps, viz., grass carp, silver carp and common carp in fresh water sector. Carp which contribute for about 87% in total aquaculture production in the country forms mainstay of freshwater aquaculture activities. Leaving aside this, catfish and prawn culture is also an important component.

Fish farming typically involves the enclosure of fish in a secured system under conditions which they can thrive. Interventions in fish life cycles range from exclusion of predators and control of competitors (extensive aquaculture) to enhance of food supply (semi intensive) to the provision of all nutritional requirements (intensive). Intensification implies increasing the density of individuals, which requires greater use and management of inputs, particularly balanced feed.

The use of 1:1 cake-bran traditional feed mixture is still in vogue, which is provided in fertilized pond ecosystem to supplement the nutritional deficiencies. Since the traditional feed mixture is not nutritionally balanced and intensive or super-intensive production is a primary objective of the industry, there is a need to use complete diet which provides the required essential nutrients and energy, necessitating an understanding of the basic requirements in order to formulate cheap and nutritionally balanced diets. Aquaculture is a feed based industry with over 60% of operational cost from feed source alone (Paul and Mohanty, 2002). Feeding of fish with balanced diet is very essential. Feeding excess will not only make financial loss but also disturb the pond water quality. Some information on nutrient requirement of fish are reported elsewhere (ICAR,2013).The nutrient requirement data of fish is largely from the experiment under controlled condition.

Protein

In fish nutrition protein is the most important nutrient promoting growth and is the major component of body tissues (45-75% on DM basis). Since protein acts both as structural component and as most preferred energy source, its requirement for fish is more than the mammals. As protein is the costliest among the various ingredients used for the preparation of fish feeds, it is necessary to ascertain the qualitative and quantitative requirement of dietary protein in order to reduce the cost of feeds.

Fishes are the ammonotelic which excrete ammonia as the end product of protein metabolism. The ammonia is diffused to water through gills. The excretion of ammonia demands lesser amount of energy (4.1 kcal/g), in comparison to ureotelic mammals and uricotelic birds. Hence, fish efficiently utilizes protein in comparison to other terrestrial animals.

The optimum protein requirement for larvae and fry of carp worked out by using purified diet varied from 35–45% (Sen *et al.*, 1978). Mohanty *et al.* (1990) reported that 40% protein requirement was optimum for larvae and fry of this species. The contribution of natural food in the feeding system made the protein requirement less than 40%. Murthy and Varghese (1996a, b) reported that the isoleucine and threonine requirement of *Labeo rohita* fry was 1.2 and 1.71% of diet corresponding to 3 and 4.28% of dietary protein respectively.

The optimum protein requirement for rohufingerlings has also been reported to be 40% (Satpathy *et al.*, 2003). But Gangadhar *et al* (1997) reported that a diet consisting of 30% protein and 6.5 lipid maximised growth in rohu fingerlings. Chakraborty *et al.* (1999) reported that growth was optimum in rohu fingerlings fed on diet having 33% crude protein.

Though under laboratory conditions protein requirement of rohu has been reported to be 40%, but the economically optimal dietary protein content has been reported to be 31% (De Silva and Gunasekera, 1991; De Silva, 1993). Mohanty *et al.*(1992) reported that diet containing 35% casein and 10% gelatin showed the best amino acid balance for rohu, which requires all the 10 essential amino acids like other finfish (Mohanty and Kaushik, 1991). Khan and Jafri (1993) quantified the dietary requirement for some indispensable amino acid in rohu and the requirment of arginine, lysine, methionine and tryptophan was 2.94, 5.88, 2.64 (1% cystine fixed dry diet) and 0.59% of protein respectively. While Murthy and Varghese (1997, 1998) reported that the optimum dietary requirement of lysine, methionine and total sulphur containing amino acid (Meth+Cys) for *Labeo rohita* was 2.24, 2.88 and 2.32% of dietary protein. Khan *et al.* (2005) reported that rohu broodstock require a dietary protein level of 25% for maximum reproductive performance and egg quality. The optimum protein requirement level has been reported to decrease with age and size of fish, 25–30% protein has been reported to be optimum for rohu under pond conditions (Mohanty, 2006).

Protein requirement of *Catla catla* fry was reported to be47% based on casein and gelatin based diet (Singh and Bhanot, 1988). Mohanty *et al.* (1990) reported that growth and nutrient utilization of catla was highest at 45% protein with cake–fish meal based diet.Khan and Jafri (1991) studied the protein requirement of small and large classes of catla (0.134 g and 5.12 g) and the protein levels were 40 and 35%,respectively. Ravi and Devaraj (1991) reported that the fry of catla require 4.80% arginine, 2.45% histidine, 2.35% isoleucine, 3.70 leucine, 6.23% lysine, 3.55% methionine (in absence of cystine), 3.70% phenylalanine (with 1.0% tyrosine), 4.95% threonine, 0.95% tryptophan and 3.55% valine as percent of dietary protein. Seenappa and Devraj(1995) indicated that catla fingerlings perform better on diets of 30 and 35% protein when fat and carbohydrate levels were 4 and 35% respectively. Fingerlings utilized higher levels of carbohydrate when dietary protein levels were low. Higher fat levels resulted in poor growth and a level of 4% was optimum. Lysine is essential amino acid for catla and dietary requirement of lysine is 2.4% diet (Satheesha and Murthy, 1999).

In mrigal fingerlings, Swamy *et al.* (1988) reported that protein requirement is40% with fish meal and groundnut cake based diet. Das and Ray (1991) worked out the protein requirement of mrigal by using semi-purified diet as 35%. Mohanty and Kaushik (1991) did not find any significant difference between the amino acid composition of the three Indian major carp species and they suggested that the amino acid requirement of carps were almost similar. Kalla *et al* (2004) reported that mrigal fry and fingerlings fed with 40% crude protein resulted higher growth with plant based protein. The protein requirements for carp feeds are 35% for spawn and fry; and 25% for growout and Brood stock feed (BIS, 2013).

Lipid and Fatty acid

Lipids are the richest energy component of feed and acts as an insulator and regulates body temperature. Lipids are almost completely digestible by fish and seem to be favoured over carbohydrate as an energy source (Cho and Kaushik,1985). Dietary lipids, besides providing energy, serve as sources of essential fatty acids. Dietary lipids influence flavour and texture of prepared feeds and also flesh quality of fish. Excess dietary lipid suppress *de novo* fatty-acid synthesis and reduces ability of fish to digest and assimilate, resulting in reduced growth. Also, feeding excess lipids is known to produce fatty fish and have deleterious effects of flavour, consistency and storage life of finished products.

Gangadhar *et al.* (1997) worked on varying lipid levels on the growth of rohu and also found that 9% lipid level was optimum. Mishra and Samantaray (2004) reported that the lipid requirement for rohu fingerlings to be 8%. Mohanty (2006) reported a range of 7–9% dietary lipid to be optimum for rohu. Supplementing the feed of juvenile rohu with 1% ω-3 PUFA was reported to enhance immunity against bacterial pathogens (Mishra *et al.*, 2006).

Mukhopadhyay and Rout (1996) reported that a combination of ω-3 and ω-6 fatty acids is important for growth and survivability of catla fry. In the practical diet, while satisfying essential fatty acid the lipid level should be maintained at 9-10% level.Incorporation of soybean oil (2.7%) and fish oil (0.3%) in the diet of brood female catla (3.0 – 5.5 kg) for a period of 93 days results in improved reproductive performance such as advanced maturation, increased fecundity and significantly higher fertilization rates of eggs (Nandi *et al.*, 2001).

Murthy and Naik (2000) reported that optimum protein, lipid and P/E ratio for catla fry were 35%, 12% and 18.51 mg protein/kj/g, respectively. Marimuthu and Sukumaran (2001) reported that the lipid requirement of mrigal fry was 7.5%. The Polyunsaturated fatty acid

(PUFA) (n3+n6) requirement of carp are 0.5% for spawn, fry and grow out and 1% for carp brood stock feed (BIS, 2013).

Rearing of Indian major carp larvae with maximum growth and higher survival rate, a provision of 4% phospholipid at the early feeding stage seems to be optimal (Paul *et al.*, 1998). Dietary phospholipid incorporation study in Indian major carp has shown that tissue phospholipid level can be enhanced in allthe three species to maximize the survival and growth rate. The crude fat requirement of carp feeds are 8% for spawn and fry; and 6% for growout and broodstock carp feed (BIS, 2013).

Carbohydrate

Carbohydrate is an important and less expensive source of energy than any other energy component in diet. Besides, dietary carbohydrate provides carbon chains necessary for the synthesis of physiologically important biochemicals such as steroids, fatty acids and chitin. Distribution of amylolytic activity has been reported in major carps (Dhage, 1968). Sen *et al.* (1978) reported that including 26% carbohydrate (dextrin) attained optimum growth of carp spawn, fry and fingerlings. However, carps and catfish have been reported to tolerate higher levels of carbohydrate in the diet (Mohapatra *et al*., 2003 and Garling and Wilson,1977). Das and Tripathi (1991) showed that fingerlings and adult grass carp, the pattern, distribution and activity of digestive enzymes are related to the type of diet ingested by fish. Erfanullah and Jafri (1995a) studied the protein-sparing effect of dietary carbohydrate in diets for rohu fingerlings. Dextrin was better utilized by *L. rohita* and had a greater protein-sparing effect in the diet than sucrose and glucose.

Erfanullah and Jafri (1995b) studied the response of rohu fingerlings to various sources of dietary carbohydrate and found that sucrose was better source of carbohydrate than glucose, dextrin and potato starch. Satpathy and Ray (1998) indicated that *Labeo rohita* fry can utilize carbohydrate (dextrin) upto 35% in formulated diets.Carbohydrate is an inexpensive and immediate source of energy in fish diets and serves as a precursor for various intermediary metabolic functions. The inclusion of carbohydrate in fish diets improves the pelleting ability of feeds. The source and complexity of carbohydrate and the presence of carbohydrate metabolizing enzymes are known to influence carbohydrate utilization in fish. Carps can utilize complex polysaccharides more efficiently than simple sugars. Erfanullah and Jafri (1998) reported that in catla fry 40% carbohydrate in a 40% protein diet corresponding to an E/P nutrient of 92 mg protein/kcal and 4.36 kcal/g gross energy, produces maximum growth, the best conversion efficiencies and higher nutrient (protein/energy) retention. Rangarcharyulu *et al.* (2000) studied the dietary protein to energy levels on rohu fingerlings and observed that diet having 30% protein and 370 kcal/100g energy yield maximum growth. Mohapatra *et al.* (2003) worked on the utilization of gelatinized carbohydrate (GC) in diets of *Labeo rohita* fry and indicated that a diet containing 45% of carbohydrate and 30% crude protein was efficiently utilized by the *L. rohita.* Ray *et al*. (2012) suggested that fish gut microbiota might have positive effects to the digestive processes of fish, and these studies have isolated and identified the enzyme-producing microbiota. The requirement of crude fibre for carp feeds are 6% for spawn and 8% for fry, growout and broodstock feed (BIS, 2013).

Energy

Protein and lipid are the primary sources of metabolic energy followed by lipid and carbohydrate. The energy level in carp diets are normally maintained at 3.5-4 kcal/g. Under the conditions where energy intake is inadequate, fish derive energy first from protein at the cost of flesh growth. Excess protein is not only wasteful but also causes stress to fish while excess energy is known to induce lipogenesis. These necessitate a balance between protein and energy in diet formulation.

Hassan and Jafri (1996) reported that in mrigal an energy level of 355 kcal/100g, corresponding to an E/P ratio of 8.87 kcal/g protein indicated maximum growth. Rangacharyulu *et al.* (2000) demonstrated that diet having 30% protein and 370 kcal energy for optimum growth in rohu fingerlings. The gross energy (kcal/kg) requirement of carp feeds are 4000 for spawn, 3500 for fry and 3000 for grow out and brood stock feed (BIS, 2013).

In catfish practical feeds prepared by using commonly available ingredients are not likely in extreme site of energy balance. However, very low level of energy in the diet affects the utilization of protein, again excess energy in the diet create nutrient imbalance and gets deposited in the adipose tissues in the form of body fat which is not at all desirable for marketing of fish. It has been suggested that the optimum protein to energy ratio in catfish diet/g of protein digestible energy requirement is 10-11 kcal (90-100 mg protein/kcal DE).

Minerals

Minerals play important role in fish Nutrition as reported elsewhere(Watanabe *et al*.,1997, Paul and Mukihopadhyay,2001 and Paul and Giri,2009).The calcium and phosphorus requirements of mrigal and rohu fingerlings worked out by feeding purified diets containing calcium lactate and sodium phosphate were 0.19 and 0.75% respectively (Paul *et al*., 2004; Paul *et al*.,2006). Availability of P from fish meals has been found to be lower in carp than in the rainbow trout. Sukumaran *et al.*, (2008) reported that dietary phosphorus deficiency causes organ specific induction of HSP70 (Heat Shock Protein) in catla fingerlings. Meena *et al*. (2010) reported that rohu fingerlings require 30mg Zn/kg of feed.

Vitamins

Vitamins are also essential for optimum growth and physiological functions of fish, but required in small quantity as compared to energy and protein (Sau and Paul, 2004). Mukhopadhay *et al*. (1988) reported the ascorbic acid requirement in rohu to be 1000mg/kg; as the species lacked the enzyme ά-gulonolactone oxidase, the terminal enzyme for conversion of glucose to ascorbic acid, thus ascorbic acid is dietary essential in fish. Mahajan and Agarwal (1980) found that in mrigal the ascorbic acid requirement is 700mg/kg. Even marginal deficiencies in some of the macro or trace elements or vitamins lead to severe morphological deformities and other pathological signs in addition of poor growth of carp. Some information is available on the mineral and vitamin requirement for common carp and catfish (NRC, 1993).Mishra and Mukhopadhyay (1996) reported the ascorbic acid requirement of *Clarias batrachus* to be 69.0 mg/kg diet. Gupta and Dodia (1997) worked on dietary thiamin(Vitamin B_1) requirement of *Cirrhinus mrigala* fingerlings for growth and body composition and reported that the species requires 20 mg thiamine/kg diet. Sau *et al.*(2004) worked out the vitamin E requirement of rohu fry to be 131 mg/kg. The vitamin E requirement of mrigal fry was worked to be 99 mg/kg (Paul *et al*., 2004b) and catla fry to be 98 mg/kg (Paul *et al*., 2005).

Feeding practices for carp

The size of the feed particle should commensurate with mouth aperture of fish. Dust or fine particles of feed may often clog gills causing their damage. Feeding of formulated diets as crumbles to fry and early fingerlings is advocated.

Feeding schedule followed in different phases of carp culture practices

a) Spawn to fry (culture period 15 days):4 times of initial body weight during first week and 8 times of initial body weight during second week. Feeding is provided twice a day.
b) Fry to fingerlings (culture period 90 days): 6-8% of biomass during first month, 5-6% of biomass during second month and 3-4% of biomass during third month and feeds are provided twice a day.
c) Grow-out culture (culture period 10-12 months):3-5% of biomass in the first month and 1-3% of the biomass in the subsequent months provided and feeds are provided twice a day.

Finely powdered feed is broadcast in ponds for spawn and fry rearing. The feed is provided in the morning after sunrise and before sunset in the afternoon. In some hatcheries automated feeders are being used for feeding the larvae, which provide feed at desired intervals. Frequently feeding of larvae at hourly intervals in hatcheries resulted in good growth and survival. For feeding fingerlings or growers, feed dough or dry pellets are provided in check-trays, feeding baskets, in perforated bags, which are tied up in bamboo-poles, and these are kept suspended in pond-water at several locations. Each of these bags can hold about 8-10 kg feed. When fish nibbles near the holes, certain amount of feed mixture comes out through the holes, thus acts as a demand feeder. Such feeding practices are common in feeding of carps in Andhra Pradesh. In Punjab farmers use a number of feed baskets tied up in a row in a floating material which is kept floating across water-bodies. There are two types of pelleted feeds viz., sinking and floating feed. The sinking pellet is given in the basket or tray at different corners of the pond. The floating pellet is gaining importance in the recent aquaculture practices. The wastage is minimum on feeding floating pellets thus low FCR. The floating pelletets are provided inside the plastic or bamboo traps or inside the net traps to prevent escape to of pellets.

Conclusion

The natural foods available in the ponds meets the partial nutrient requirement of the animal in aquaculture. Therefore, feed supplementary feeding is important in semi-intensive aquaculture practices. Well balanced formulated feeds are required for the intensive type of aquaculture where availability of natural food is negligible and fish solely depend on the artificial feeds. Nutrient requirement of fish at different life stages give guidelines forfeed formulations. The protein and lipid requirement o carp feed ranges from 25-35% and 6-8%

irrespective of body weight. The protein requirement of catfish and freshwater prawn is about 30-40%, which is higher than that of carps. The aquaculture producers should select appropriate feed to appropriate species in right form to ensure efficient conversion of feed to fish flesh for successful aquaculture.

Table 1: Requirements for Carp feeds

Sl. No.	Characteristic	larval feed	Fry feed	Grow-out feed	Brood feed
1.	Moisture, percent by mass, Max	10	10	10	10
2.	Total crude protein (Nx6.25), percent by mass, Max	35	35	25	25
3.	Crude fat or ether extract, percent by mass, Max	8	8	6	6
4.	Crude fibre, percent by mass, Max	6	8	8	8
5.	Acid insoluble ash, percent by mass, Max	2.5	3	3	3
6.	Gross energy, Kcal/kg, Min	4000	3500	3000	3000
7.	PUFA (n3+n6)	0.5%	0.5%	0.5%	1%

Management of water quality for good aquaculture practice

P.P.Chakrabarti

Principal scientist,ICAR-CIFA,

RRC,Rahara &Kalyani FS

With the advancements of our knowledge in biology, chemistry, physics and allied branches of science, application of scientific knowledge in solving problems in fish culture became apparent in different parts of the world. As fish lives in water, attention was first confined mostly to the study of water of fish ponds.

The more important physical and chemical qualities of water influencing aquatic productivity are temperature, transparency, pH, dissolved oxygen, free carbon dioxide, and total alkalinity and dissolved nutrients like nitrogen, phosphorus, potassium, calcium, magnesium.

Temperature:

Under favorable conditions, the optimum temperature range for many 'coldwater' and 'warm water' fishes is 14-18^{0}C and 24-30^{0}C, respectively. Metabolic activity is nearly doubled for every 10^{0}C rise in temperature and hence fish growth is greatly dependent on water temperature. With the increase in temperature microbial activity is also increased and hence the release of nutrients by the decomposition of organic matter at the pond bottom is more with consequent increase in nutrient status of water.

It is difficult to adjust water temperature in large water bodies. Operation of aerators during calm and warm afternoons helps to break thermal stratification of ponds by mixing warm surface water with cool subsurface water.

Turbidity:

Turbidity is the result of several factors including suspended soil particles, planktonic organisms and humic substances produced through decomposition of organic matter.

Turbidity is measured by Secchi disk visibility. Optimum Secchi disk visibility of fishponds is considered to be 40-60 cm. If you do not have a Secchi disc, you can use your arm instead. Stick your arm vertically underwater. As long as your hand is not visible when your elbow is at the water surface, there is no need for fertilization.

Turbidity resulting from plankton is generally desirable. However, heavy blooms limit heat and light penetration thus reducing the effective volume of the productive zone. Turbidity due to suspended soil particles can be controlled by lime/alum application of 25-50 kg/ha and other ways also.

Secchi disc transparency	***Management/control***
Less than 25 cm	Avoid fertilization: dissolved oxygen is likely to be less Increase water inflow, likely lime application if necessary.
25-40 cm	Fertilization should not be frequent 9inimize quantity.
40-60 cm	Routine fertilization necessary.
More than 60 cm	If pond bottom muck is too high, removal/ bottom drying is better. If it is not possible then inorganic fertilization dose may be increased with higher frequency.

pH:

The pH of pond water indirectly throws light on the availability of different nutrients. pH 7.5-8.5 is desirable for optimum production of fish. The decrease of water pH due to application of heavy dose of organic manure and feeding can be corrected by the application of lime @ 50-200 kg/ha/week in presence of fish till desired pH obtained. Total amount of lime application in a month should not exceed 600 kg/ha. Effect of pH on fish is generalized below:

pH	Effects
4	Acid death point
4-6	Slow growth

6-9	Best for growth
9-11	Slow growth, lethal to fish over long period of time
11	Alkaline death point

Alkalinity and Carbon dioxide (CO_2):

Total alkalinity is a measure of carbonate, bicarbonate and hydroxyl concentration in water and expressed as $CaCO_3$ mg/l equivalent. Most suitable range for fish farming is 80-200 mg/l.. Alkalinity can be improved or raised by the application of lime of any form. Total alkalinity decreases with intensive organic manuring and feeding, which can be improved by liming the pond with 50-200 kg/ha/week maximum for 3 doses in a month till the level of desirable alkalinity achievement, considering the pH of water.

Alkalinity is an index of potential carbon dioxide. When the conversion of carbon dioxide to organic matter by photosynthesis is greater than the available carbon dioxide from biotic respiration and organic matter decomposition, required carbon dioxide is drawn from bicarbonate reserve. Low alkalinity indicates that $CO_2$is not available particularly in latter phases of the day. As a result photosynthesis stops due lack of CO_2.So fishes will not get their food in the afternoon. In general it varies from trace to 30 mg/l in a cultured pond, but care should be taken to keep the level of CO_2 within trace to 16 mg/l for better production.

Total hardness:

Hardness measures the amount of soluble bicarbonate, chloride and sulphate of calcium and magnesium. Most suitable range for fish prawn farming is 50-150 mg/l.

Dissolved oxygen:

Fish cultured pond exhibit a wide fluctuation in the oxygen content from day to night. Although many environmental parameters influences the concentration of the oxygen both in time and space, a single measurement at the end of the dark period would be an important indicator of the risk of fish mortality. Dissolved oxygen is ranged from 2.0-12.0 mg/l throughout the day and night but for fish culture pond water the lower value should not fall below 3.5 mg/l sample. In case oxygen falls below the lower range, pond water may be aerated by a suitable aerator, or bottom pond water suctioned and sprinkled over surface water by diesel pump set. Little liming @ 50kg/ha, a sprinkling of 10kg single S.S.P/ha can save from fish surfacing or mortality to some extent.

Dissolved Nitrogen:

Among the various dissolved nutrients in water, nitrogen and phosphorus are the two nutrients and having major importance. As a constituent of protein, nitrogen occupies a highly important place in aquatic productivity. Soluble nitrogen compounds occur in water in different forms. Organic nitrogen compounds may be converted to inorganic nitrogen compounds as nitrate, nitrite, NH_3 and ammonium salts. Nitrogen in ammonium and nitrate forms is readily absorbed by plants but green algae in their role as a primary producer in the aquatic food chain can use nitrogen in all the forms. Nitrogen and phosphorus in ponds are generally far below the concentration for the optimal growth of plankton. A concentration of 0.2-0.5 mg/L of dissolved nitrogen is considered optimal for productive ponds while below 0.1 mg/L indicative of poor productivity.

The proportion of NH_3 increases with increase in pH and temperature of water. At higher NH_3 concentration in water ammonia excretion by aquatic organisms diminishes which results in an increase of ammonia in blood and tissues. This affects adversely by damaging gills and reducing the ability of blood to transport oxygen. Under normal condition ponds seldom contain such high level of ammonia nitrogen. But high ammonia concentration is most common in ponds with very high feeding rates, high organic matter content and also in sewage-fed ponds. The only feasible mean of reducing ammonia concentration is water exchange.

Nitrite nitrogen is an intermediate compound in the oxidation of ammonia to nitrate. At higher NO_2 concentration the fish may suffer from disease known as brown blood disease. But NO_2 toxicity seldom occurs in natural waters since under normal condition its concentration is generally less than 0.005 mg/L.

Dissolved phosphorus:

Phosphorus is the most important single element responsible for aquatic productivity. In natural freshwater ponds, phosphorus concentration is usually very low and phosphorus occurs in two forms – inorganic and organic but the soluble inorganic from is more important due to its active nature. Dissolved phosphorus below 0.05 mg/L may be considered insufficient while 0.05-0.20 mg/L and above 0.20 mg/L may be indicative of medium to high and highly productive fish ponds. While N/P_2O_5 ratio above 2.6 is strictly indicative of poor production 2.6-1.3 is not strictly optimal.

Ammonia:

Fish are very sensitive to unionized ammonia (NH_3) and the optimum range is 0.02-0.05 mg/l in the pond water. Normally in the case of high dissolved oxygen and high carbon dioxide concentrations, the toxicity of ammonia to fish is reduced. Aeration can reduce ammonia toxicity. Healthy phytoplankton populations remove ammonia from water. The addition of salt @ 1,200-1,800 kg/ha can be used to reduce the toxicity of ammonia in water.

Biological filters may be used to treat water for converting ammonia to nitrite and then to harmless nitrate through nitrification process.

Hydrogen sulphide:

Freshwater fish ponds should be free from hydrogen sulphide. Fish lose their equilibrium and become to sub lethal stress at concentrations of 0.01 mg/l of hydrogen sulphide. Frequent exchange of water is practiced to prevent building up of hydrogen sulphide in the water body. Also, if the pH of water is increased by liming the toxicity of hydrogen sulphide is reduced.

Principles of Nutrient management in composite fish culture

Based on the study of carp prawn culture ponds under different agro-climatic conditions in India, it was observed that pond productivity depends on soil conditions such as soil pH, available nutrient status (nitrogen, phosphorus and potassium) and organic carbon and based on that fish prawn cultured ponds are classified into low, medium and high productivity. The details about liming and fertilizer management in fish ponds are discussed as follows:

Parameters	**Pond category**		
	Low	**Medium**	**high**
Available Nitrogen (mg/100 gm soil)	<25	25-50	>50
Available phosphorus (mg/100gm soil)	<3	3-6	>6
Organic carbon (%)	<0.5	0.5-1.5	>1.5
pH	<5.5	5.5-6.5	6.5-7.5

Liming:

Benefits

Liming is the first step of management of fish pond. Liming materials are not fertilizers, but they will improve the response to fertilization or manuring in acidic ponds. Lime corrects the pH, establishes a good buffering system, rapid decomposition of organic matter and reduces toxic effects of harmful compounds including disinfecting the environment to prevent fish disease, flocculate suspended soil particles.

Cause of acidity:

* Leaching of bases like calcium and magnesium due to prolonged water logging results in the rise in the concentration of H^+ ions in the pond bottom soil and renders the soil acidic in reaction.
* Utilization of bases like calcium and magnesium by the organisms present in the pond environment for their metabolic needs.
* Further removal of bases also occurs by plants like coconut and other high mineral-absorbing plants grown on the bund area also impart acidity in pond soil.
* Accumulation of organic matter due to intensive use of feed materials, manures and some acid forming fertilizers enhances the formation of carbon dioxide and other organic acids which replace the bases from the soil complex with H^+ ions and makes the soil acidic.

Lime materials used:

Lime stone (calcite), $CaCO_{3,}$, dolomite ,basic slags are commonly used as lime materials.

Lime requirement:

* The most common index of lime requirements of a soil is its pH. But it determines only the active acidity or the hydrolysable H^+ ion concentration. But since the reserve or potential acidity of a soil depends upon its cation exchange capacity and buffering this factor also deserves consideration.
* The common and easy method is to follow a ready reckoner for determining the quantity of lime of a pond. According to the soil pH, lime is usually applied in ponds

Rate of lime application in fish ponds

Soil of pH	Soil condition	Dose of Lime (Kg/ha)		
		Sandy soil	**Medium**	**Clavey soil**
4.5 – 5.0	Highly acidic	1000	2000	3000
5.0 – 6.0	Moderately acidic	600	1200	1800
6.0 – 6.5	Slightly acid	500	1000	1500
6.5 – 7.5[@]	Near Neutral	200	400	600

[*]Though this group of soil falls under the near neutral category, yet liming is advised for better utilization of fertilizers and prophylactic measures.

* To determine whether a pond needs to be limed, the levels of total alkalinity in the pond water may be checked. When the total alkalinity of the water sample is less than 40 mg/L, the pond derives greater benefit from liming

* Pond water is also checked for total alkalinity and hardness with water quality test kits and thereafter adjusted to reach a total hardness and alkalinity of desired range and from this value the lime requirement of a pond can be calculated. Since both base saturation and pH of mud are positively and highly correlated with the total hardness of pond water and increase in mud pH, this method provides accurate dose for lime requirement of ponds.

*These methods described may be modified depending on the mechanical composition, organic carbon and free calcium carbonate contents of soil. The dose may either increase or reduce for clayey and sandy soils, respectively.

The pH, texture, cation exchange capacity (CEC), free calcium carbonate, and organic matter contents of the pond soil are mostly considered in deciding the amount of lime to be applied.

Frequency of liming:

*Bulk quantity of about 30% of the total lime requirement of a drainable pond with sandy soils may be applied as first dose before filling water while the balance quantity may be applied in split doses at shorter intervals to achieve higher response of liming. On the other hand, ponds having heavy soils, application of about 50% of the total lime requirement of the pond may be beneficial before filling water and thereafter at split doses of longer intervals.

*In case of non-drainable ponds with sandy soils, bulk application of 20% of the total lime requirement may be done before stocking seed and the balance in split doses at short intervals while for heavy soils, about 40% of the total lime requirement of the pond may be useful before stocking seed and thereafter in split wide intervals. However, the liming frequency may be revised depending on the variations in the environmental conditions of the pond for getting better results.

*The effects of adequate liming usually last for a longer period in ponds with no or little outflow. Ponds, which frequently discharge water, may have to be limed at higher rate.
Frequent light applications are preferred for ponds having sandy soils while in case of heavy soils, larger applications at wider intervals may be beneficial

Method of application of liming materials:

*The best and easiest time to lime a pond is before it is filled with water and should be distributed as evenly as possible over the entire surface.
*Newly constructed ponds should be limed prior to filling with water.

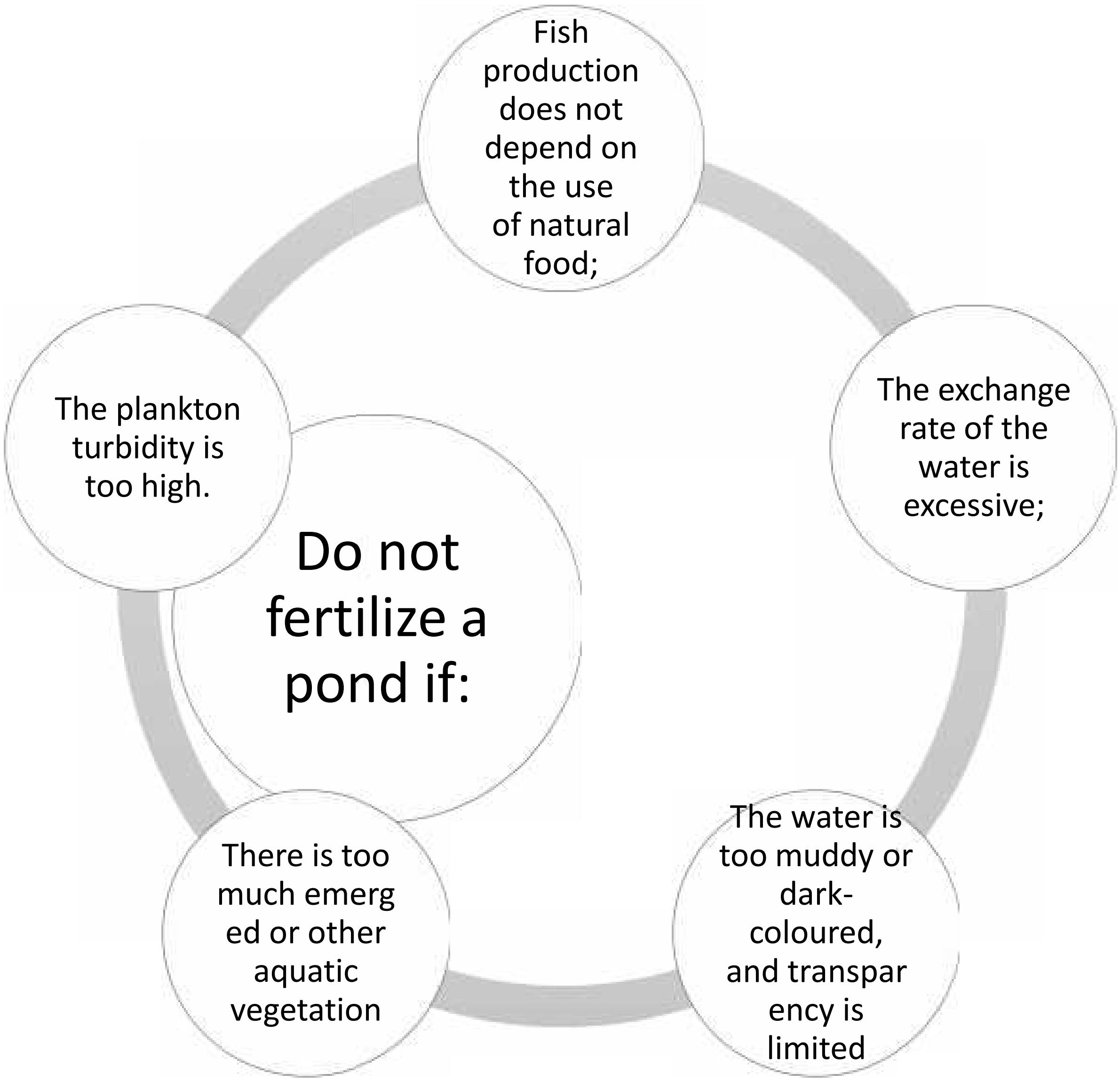
*During culture process, lime can be applied evenly over the entire surface of pond water by loading on a boat and then dissolved, separated uniformly into the pond.

*It is better to lime the pond before water filling for drainable ponds. But for non-drainable ponds phase-wise application is beneficial.
*To get better response of liming during rainy season, about 25% of the calculated dose, should be applied on the inner sides of the bund.
*To be most effective against diseases, powdered quick lime (calcium oxide) or after its hydrolysis (calcium hydroxide) may be used in ponds as both preventive as well as curative measures.

<u>Timing of application</u>:

*The best time to lime a pond is before water filling for drainable ponds. But for non-drainable ponds phase-wise application is beneficial.
*Limestone can be added anytime during the production cycle.
*It is beneficial to apply at higher doses during winter months than other months.
*It is better to apply lime during approach of winter in order to provide effective sanitation in ponds.
*Limestone takes several weeks to complete its impact on the water quality, so application should be at least one month prior to stocking and fertilization in the pond.
*When lime is added as a curative measure to disease like EUS, the best time to apply is in the afternoon hours for achieving better results.
*Lime application during morning hours in such condition often leads to no results or poor results as the applied lime reacts very fast with carbon dioxide-rich pond water during early morning hours to form insoluble $CaCO_3$.

FERTILIZING FISH PONDS



Manuring-when ?

Fish ponds are usually fertilized with animal manure at least ten to 15 days before stocking with fish. In drained ponds, the manure is applied to the pond floor just before refilling with water and mixed by ploughing or other ways. After the first application, the pond should be fertilized at regular intervals throughout the fish production cycle.

One should monitor your pond carefully during fertilization to avoid fish losses. This is especially important if you are using animal manure. Continue manuring a pond only if:

- The water quality remains acceptable
- The behaviour of the fish remains normal.

If I don't have enough animal manure to fertilize all fish ponds. Give priority to those where its effects are especially beneficial:

- Nursery ponds, if frequently drained and/or where limited time is available to boost up natural food production using animal manure safely.
- New ponds, particularly if their bottom soil is sandy and contains little organic matters

Controlling the amount of animal manure to apply:

The amount of animal manure to be applied to a particular pond varies greatly, depending on factors such as climate, water and soil quality, characteristics of the manure and kind of cultural system (type of fish, rearing density, length of rearing period). As for inorganic fertilizers, it is impossible to recommend any treatment valid under all circumstances.

As an approximate guide, in small Indian rural ponds generally from 100 m^2 to 300 m^2 in size, apply once in a week or fortnightly

Name of animal Manure	Application of manure (kg)/100m^2
Poultry droppings	4.5
Ship or Goat dung	3
Pig dung	6
Cattle or Horse dung	5

<u>Checks- while planning mannure</u>

- ✓ It is preferable to use animal manure as fresh as possible.
- ✓ There should be at least a 7-10day interval between liming and manuring.
- ✓ Apply manure preferably in the early hours of the day, about two to three hours after sunrise.
- ✓ Best results are obtained by combining manuring with inorganic fertilization. Additional phosphorus and nitrogen are usually beneficial to maintain a good plankton bloom
- ✓ Always maintain and adjust your fertilization by checking water quality and fish behaviour .
- ✓ **Note**: wastes such as rice hulls, sugar-cane stalks and sawdust are rich in cellulose lingo cellulose fibre, which decomposes very slowly in the pond. Do not use themtoo much, unless you are trying to establish a good pond bottom on sandy soils.

Cassava tubers of the bitter species, which may be soaked in ponds to remove the toxic cyanhydric acid before consumption, form an excellent and cheap way to fertilize your [illegible]

Agro-industrial wastes such as cotton seed, molasses, mahua oilcake and oil-palm sludge (4 to 5 percent nitrogen), breweries sludge etc

Biogas slurry, the digested sludge remaining after biogas production, which contains about 10 percent dry matter;

The details fertilizer measures for grow-out culture of carp ponds are discussed as follows:

Nutrient requirement **(kg/ha/yr)**	Pond category		
	Low	Medium	High
Raw cowdung or Gobar gas slurry	10,000-12,000 or 20,000-30,000	8,000-10,000 or 16,000-20,000	5,000-8,000 or 10,000-16,000
Phosphorus SSP	100-125 625-780	75-100 470-625	50-75 313-470
Nitrogen Urea	200-250 435-545	150-200 322-435	100-150 218-322

While 20-30% of the total amount of organic manures is applied as basal dose, a fortnight before the stocking operation, the remaining amount is applied in equal installments on a monthly basis. Other commonly used organic manures include poultry litter, pig dung, duck droppings, domestic sewage etc. depending on the availability. The annual dose of chemical fertilizer divided by 24 may be applied fortnightly for better utilization. The fertilization programme for particular fortnight must be suspended if any bloom appears on pond water due to nutrient enrichment.

Use of either organic or inorganic or a combination of both for increased production of either natural food or fish from ponds is now a standard practice. The tentative nutrient balance of carbon nitrogen and phosphorus in intensive fish culture ponds with supplementary feeding are of great significance in pond fertilization. However, a proper understanding of the total amount of nutrient involved in production process, quantity available in the system and those required to be enriched through fertilization by monitoring the pond environmental condition, for achieving optimum benefit of fertilizers and manures.

Basic principle of manuring is discussed above; principle may vary from place to place. Type of fish species etc. there are many improvements in fish culture also made by scientists, researchers and farmers but this is a base line of fish culture for the farmers.

Fish as Health Food in respect to its Nutritional value

B.N.Paul

ICAR-Central Institute of Freshwater Aquaculture
Regional Research Centre, Rahara, Kolkata-700118, West Bengal

Fish is an excellent source of protein and accounts for about 17 percent of the global population's intake of animal protein. Fish is truly a unique food which contains all the additional nutrients that it contains in significant amounts. Consumption of fish also provides energy and a range of essential nutrients. Greater attention is focusing on fisheries products as a source of micronutrients such as vitamins and minerals. Fish has got a particular role as a source of the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are important for optimal brain and neural system development in children. Fish consumption also has health benefits for the adult population. In world, at present more than 800 million continue to suffer from chronic malnourishment and the global population is expected to grow by another 2 billion to reach 9.6 billion people by 2050. Therefore, it is a huge challenge of feeding the growing population, and at the same time safeguarding its natural resources for future generations. The micronutrient deficiencies affect hundreds of million people, particularly women and children in the developing world. More than 250 million children worldwide are at risk of vitamin A deficiency, 200 million people have goitre (with 20 million have learning difficulties as a result of iodine deficiency), 2 billion people (more than 30 percent of the world's population) are iron deficient, and 800 000 child deaths per year are attributable to zinc deficiency (FAO, 2014).

Peopleprefer meat as animal protein source in their diet for the past 50 years. But due to wrong food habits peoplefaced health problems like overweight, cardiovascular diseases,Alzheimer's disease, cancer, asthma, diabetes etc.At least 80% of premature heart disease, stroke and type II diabetes and 40% of cancer could be prevented through a healthy diet and regular physical exercise.

But for the last few decades, people have become more aware of fish as a healthier alternative source of protein.Fish accounts for 16.7% of the global population's intake of animal protein and it accounts for 6.5% of total protein intake.A portion of 150g of fish can provide about 50-60 percent of an adult's daily protein requirement (FAO, 2014). Fish makes a vital contribution to the survival and health of a significant portion of the world's population. Awareness regarding importance of diet in human health is increasing day by day. Through research over the years, it is now proved that many of the diseases and health problems of people today are due to wrong lifestyle, characterized by wrong diet. When we think about a balanced diet, fish along with cereals is a good combination. Fish is an important dietary animal protein source in human nutrition, excellent substitute of protein for red meat. So the inclusion of fish in our diet can make a valuable contribution to any diet that contain mainly cereals, starchy roots and sugar for the growth.Information on database of food fishes in India is available elsewhere (Mohanty *et al*.,2015).

Fish provides essential nutrients, especially quality protein, fat, vitamins and minerals (micronutrients). Fish is highly nutritious food and a good source of all the essential amino acid, fatty acid, minerals, vitamin A and D. It is a valuable source of protein to a healthy diet because of its low carbohydrate and polyunsaturated fat, especially ω3 fatty acid (Sargent, 1997). Fish in general is tasty and easily digestible. Eating more fish with main meal is a simple way to improve diet quality.

Proteinis important for growth and development of the body, maintenance and repairing of tissues and for the production of enzymes and hormones required for our body processes. On a fresh-weight basis, fish contains a good quantity of protein, about 12-22% and contains all the 10 essential amino acids viz., isoleucine, leucine, valine, phenylalanine and threonine and provides easily digested protein of high biological value (Mohanty *et al*., 2014).

The fat also contributes to energy supplies and assists in the proper absorption of fat soluble vitamins viz., A, D, E, and K. Fish is rich source of vitamins, particularly vitamin A and D from fatty fish, as well as thiamin, riboflavin and niacin. Vitamin A from fish is more readily available to the body than from plant foods. Vitamin A is required for normal vision and for bone growth. Fatty fish contains more vitamin A than lean species. Studies have shown that mortality is reduced for children under five with a good vitamin A status. As sun drying destroys most of the available Vitamin A better processing methods are required to preserve this vitamin. Vitamin D present in fish liver and oils is crucial for bone growth since it is essential for the absorption and metabolism of calcium (Halver *et al*., 2002).

The minerals present in fish include iron, calcium, zinc, iodine (from marine fish), phosphorus, selenium and fluorine. These minerals are highly ‘bioavailable’ meaning that they are easily absorbed by the body. Iron is important in the synthesis of hemoglobin in red blood cells which is important for transporting oxygen to all parts of the body (Chanda *et al*., 2015).Marimuthu *et al*.(2011) Could not find any significant difference in the mineral content of freshwater fishes after cooking .

Awareness regarding importance of diet in human health is increasing day by day. Through research over the years, it is now proved that many of the diseases and health problems of people today are due to unhealthy lifestyle, characterized by wrong diet. When we think about a balanced diet, fish along with cereals is a good combination. Fish is an important dietary animal protein source in human nutrition, excellent substitute of protein for red meat. So the inclusion of fish in our diet can make a valuable contribution to any diet that contain mainly cereals, starchy roots and sugar for the growth.

It is estimated that around 60% of people in many developing countries depend on fish. The importance of fish in the Indian context is unique in all respect. Fish eating population in India is more than 50% and in some states it is more than 90%. However, the increase is in awareness of health benefits of eating fish as health food.

Lipids

About 50% of the fatty acids in leaned fish (e.g., walleye and yellow perch) and 25% in fattier fish (e.g., channel catfish, herring, salmon and rainbow trout) are polyunsaturated fatty acids. In contrast, the polyunsaturated and saturated fatty acids in beef are 4-10% and 40-45%, respectively, of the total fatty acids present. The fat content of fish ranges from 0.2% to 25%. However, fatty acid composition of freshwater fish is mainly constitute of saturated fatty acid (SFA) (Palmitic and Stearic), monounsaturated fatty acid (MUFA) (Oleic, elaidic and palmitoleic acid) and polyunsaturated fatty acid (PUFA) (Linolenic, EPA and DHA) (Paul et al., 2015). The polyunsaturated fatty acids (PUFAs) namely EPA and DHA, are essential for brain development of new born baby and children and also helps to prevent the occurrence of cardiovascular diseases (CVD). (Das *etal*.2009 and Giri *etal*., 2010). It was reported earlier that EPA and DHA content in fillet of freshwater fishes is more than 3% (Swapna *et al*., 2009).

The fat content of fish varies depending on the species as well as the season, but in general, fish have less fat than red meats. However, fats from fatty fish species contain the polyunsaturated fatty acids (PUFAs) namely EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) which are essential for proper growth of children and are not associated with the occurrence of cardiovascular diseases such as coronary heart disease. In pregnant women, the presence of PUFAs in their diets has been associated with proper fetal brain development. In other studies, omega 3 fatty acids have also been associated with reduced risk of premature delivery and low birth weight.

Essential fatty acids in Fish

Lipids of fish contain high proportion of highly unsaturated long chain fatty acids. Among the saturated fatty acids palmitic and stearic acids are the important ones and in the monounsaturated group, oleic, elaidic and palmitoleic acids are the major constituents. Among the polyunsaturated acids linolenic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major components. In human nutrition, fatty acids such as Linoleic and Linolenic acid and acid are regarded as essential since they cannot be synthesized in the system.

Fish is also valued as a source of omega-3 (n-3) fatty acids, very long chain polyunsaturated fatty acids which is critical for the development of the brain and retina, and which may be protective of some chronic diseases. Fish oil, which is abundant in ω-3 fatty acids, therefore, reduces the risk of heart diseases and heart arrhythmias. It helps to maintain the elasticity of artery walls, prevent blood clotting, reduce blood pressure, stabilize heart rhythm and help combat inflammation. It lowers the levels of LDL cholesterol, which is bad cholesterol, and increases the HDL levels, which is good cholesterol. Fish oil prevents accumulation of triglycerides and further reduces the levels of excess triglycerides. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which account for approximately 90% of the polyunsaturated fatty acids in fish species from the North Atlantic and North Pacific are absent or present in much lower amounts in other foods. The omega-3 fatty acids are essential fatty acids which mean that the body cannot build them from other building materials.

Life style diseases

Associated Chamber of Commerce and Industry (ASSOCHAM), in a study revealed that 68 % of working women (21-52 years) surveyed were found to be suffering from obesity, depression, chronic backache, diabetes and hypertension. As many as 15 % of our country's population are suffering from the crippling disease arthritis. According to the experts arthritis affectsmostly people in their prime, between 20-45 years. India is reeling under a severe burden of chronic respiratory diseases (CRDs). Latest data released by the Indian Council of Medical Research, 13 million people aged 15 years and above suffer from asthma. Another 11 million above the age of 35 suffer from chronic bronchitis. In both categories, men are worse off than women.

Regular fish consumption (1-2 servings per week) is protective against coronary heart disease and ischemic stroke. The serving should provide an equivalent of 200-500 mg of EPA and DHA. ω3 fatty acids have additional therapeutic benefits at higher levels (doses). Fish helps to control disease like Rheumatoid Arthritis, Depression, Asthma, Diabetes, Prostate Cancer, Cognitive function and gestational diabetes, Gestation length etc. Earlier report signifies that freshwater fishes contains a favourable quantity of polyunsaturated fatty acids which are well known for the prevention of those type of diseases. (Jakhar *et al*., 2012).Children can consume fish without any problems and if well cooked they can benefit tremendously from the small fish that are excellent sources of calcium and phosphorus, crucial for the development of strong bones and teeth.

Nutrient Profile of fish

Rohu

The Rohu (*Labeo rohita*),is the most abundant species among the Indian Major Carp (IMC) and consumed in a large proportion. The protein and fat content of rohu ranges from 13.60 to 17.97% and 1.23 to 5.37% respectively. The vitamin A content ranges from 3.22 to 19.63 and vitamin D content ranges from 30.80 to 208.40 (I.U./100g). The zinc content ranges from 1.39 to 2.98 (mg/100g) respectively. It is also a good source of iron, sodium, potassium, calcium and phosphorus. Leucine, valine, threonine and phenylalanine are predominant EAA. The PUFA content in rohu varies from10.62 to 21.72%. The EPA and the DHA in this fishranges from 0.06 to 5.15% and 0.40 to 4.59% respectively which shows that it is also a good source of LC-PUFA.

Catla

The Catla (*Catla catla*) has a deep body with large head and upturned mouth with upper lip absent. The fat content ranges from 1.47 to 6.47% andthe protein content varies from 13.98 -18.09%. The iron ranges from 2.29 to 2.45, phosphorus ranges from 124.37 to 152.36 and calcium varies from 167.16 to 171.23 (mg/100g). The vitamin D ranges from 39.70 to102.40 (I.U/100g). Leucine, isoleucine and valine are predominant essential amino acid (EAA) in catla.It is also a good source of LC-PUFA among which EPA ranges from 2.37-6.82 % and DHA ranges from 0.47-4.74%.

Mrigal

The mrigal has a streamlined body with lower lip indistinct and presence of one pair of rostral barbells. The fat content ranges from2.30 to 2.44% which is lower as compared to rohu and catla and the protein content ranges from13.01-17.68%. The iron content ranges from 1.05 to 3.92 (mg/100gm). Among the essential amino acids leucine, valine, phenylalanine and threonine are mostly found and out of the non essential amino acids glycine, glutamic acid and aspartic acid are prevalent. The vitamin A content ranges from 4.63 to 50.82 and vitamin D ranges from 52.00 to 243.81 (I.U./100g). Among PUFA, arachidonic acid is present in good amount. The EPA ranges from 1.50-6.88% and the DHA ranges from 1.11 to 3.02%.

Magur

The magur is an important species among the catfishes. Its upper jaw is longer than lower jaw and no scales are present in the body. The protein and fat content in magur is 15.33-17.70% and 2.03-6.00% respectively. Magur is a good source of sodium, potassium, calcium and iron. The iron content of magur is 0.84-2.42 (mg/100 g). The vitamin D content ranges from 17.25 to 44.73 (I.U./100g).The predominant EAA are lysine, valine and leucine and among the NEAA are glutamic, glycine and aspartic acid are maximum in magur. Linoleic and linolenic are the predominant PUFA. DHA content ranges from 0.47 to 2.25%.

Singhi

The singhi is an air-breathing catfish whose width of head is shorter than its length. The anal and cordal fins are separated by a distinct notch in singhi. The protein and fat content of singhi are 15.28 to 16.99% and 1.96 to 4.20 % (w/w basis) respectively. Singhi is a good source of sodium, potassium, calcium and iron. The iron content in singhi is 1.65-2.90 (mg/100g). Among the essential amino acids leucine, valine, phenylalanine and threonine are predominant and among the non essential amino acids glycine, glutamic acid and aspartic acid are higher in singhi. The fatty acid profile of singhi constitutes MUFA of about 22.75 to 49.69 % and PUFA 14.18 to 13.80 %. The EPA and DHA content varies from 1.46 to 3.60 and 1.60 to 2.20% respectively.

Koi

The Koi have ctenoid scales on head and operculum and their interopercle are strongly spinous. The fat content varies from 3.98 to 8.69% which is maximum higher compared to other freshwater fish species and protein content ranges from 15.89 to 17.94%. Koi is also rich in sodium, potassium, calcium and iron. The iron content in koi is 1.67 to 4.02(mg/100g). Vitamin A ranges from 85.77 to 94.00 (I.U./100g). Leucine, valine, phenylalanine and isoleucine content are higher among the essential amino acids and glutamic acid, glycine and aspartic acid are higher among the non essential amino acids. Linolenic, linoleic, EPA and DHA are the predominant fatty acid found among the PUFAs in koi. DHA content varies from 1.26 to 2.67%.

Conclusion

Based on these facts, it is suggested that fish should be used as the protein source, instead of meat, several times a week because of the possible benefits of fish for coronary artery disease. Studies have indicated that by consuming more fish and fish oils, cardiac mortality can be reduced and possible underlying coronary atherosclerosis improved.Freshwater fishes have a good oil value and are suitable for applications in pharmaceuticals and food industries.Keeping in view of the health effects, many health agencies worldwide recommended upto 500 mg/day of EPA and DHA for reducing CVD risk or 1 g/day for secondary prevention of CVD patients, with a dietary strategy for achieving 500 mg/day being to consume 2 fish meals per week with at least one oily fish (Aranceta and Perez-Rodrigo, 2012).

Feed and feeding practices of catfish

B. N. Paul

ICAR-Central Institute of Freshwater Aquaculture

Regional Research Centre, Rahara, Kolkata- 700 118, West Bengal

Introduction

The fisheries sector occupies an important place in the socio-economic development of India. With production of 5.30 million tons, India occupies the second position in global map for production of Inland fish that plays a great role in nutritional security, employment potential and the making strong hold of its rural and now urban socio-economic fabric as well. With the annual production of fish and shellfish increasing to about 8.3 million tons in the year 2011, from 0.75 million tons in 1950, there has been over 10 folds increase in the fish production during the period. More than 7.5 million fishermen and fish farmers in the country depend on fisheries and aquaculture for their livelihood.

Nutrient requirement

Protein

Among the nutrients protein is the most important nutrient promoting growth in animals. As protein is the costliest among the various ingredients used for the preparation of fish feeds, it is necessary to ascertain the qualitative and quantitative requirement of dietary protein in order to reduce the cost of feeds. The protein in fish is the major component of body tissue constituting about 45-75% of the total on dry matter basis. The utilisation of dietary protein for new tissue growth is relatively constant in fish. Since protein acts both as structural component and as most preferred energy source, its requirement for fish is more than the mammals.

Total protein requirement for optimum growth in catfish have been reported to vary from 25 to 50% of the diet on dry matter basis. Protein requirement for younger catfish is higher than that of adult ones. Major reasons for these differences in response to varying dietary protein percentages are variation in fish size, daily feed allowance, amount of non-protein energy in the diet, protein quality, water temperature, and amount of natural food available in ponds. Essential amino acid like cystine can spare about 60% of methionine on a molar sulphur basis. Tyrosine can spare about 50% of the total phenylalanine requirement for channel catfish. However, catfish require all the 10 essential airs acids as required for other species of fish. Nutrient requirement of catfish is given in Table 1 (BIS, 2013).

Lipids: Dietary lipids, besides providing energy, serve as sources of essential fatty acids. Dietary lipids influence flavour and texture of prepared feeds and also flesh quality of fish. Excess dietary lipid suppress *de novo* fatty-acid synthesis and reduces ability of fish to digest and assimilate, resulting in reduced growth. Also, feeding excess lipids is known to produce fatty fish and have deleterious effects of flavour, consistency and storage life of finished products.

In the feed of Magur (*Clarias batrachus*) the combination of ω-3 and ω-6 fatty acids elicited the best grow the responses of the species (Mukhopadhyay and Mishra, 1998). In common with other vertebrates catfish cannot synthesize ω-6 or ω-3 fatty acids *de novo*. Hence one or both-fatty acid must be supplied in the diet, depending upon the EFA requirements. Channel catfish do not appear to be as sensitive to fatty acid deficiency as carps, rainbow trout and other fishes. Ithas been observed that high diet concentration of 18: 2 ω-6 fatty acid reduced growth and ω-3 fatty acid increases the growth rate. NRC (1993) has recommended 12% linolenic acid or 0.5-0.75% EPA and DHA are required for chapel catfish. In has been observed that upon 19% fat in the diet of *Clarias batrachus* fingerling diets does not depress the energy digestibility.

Carbohydrate: Carbohydrate is an important and less expensive source of energy than any other energy component in diet. Besides, dietary carbohydrate provides carbon chains necessary for the synthesis of physiologically important biochemicals such as steroids, fatty acids and chitin. Catfishes have been reported to tolerate higher levels of carbohydrate in the diet

Protein and energy ratio: Protein and lipid are the primary sources of metabolic energy followed by lipid and carbohydrate. Under the conditions where energy intake is inadequate, fish derive energy first from protein at the cost of flesh growth. Excess protein is not only wasteful but also causes stress to fish while excess energy is known to induce lipogenesis. These necessitate a balance

In catfish practical feeds prepared with using commonly available ingredients are not likely in extreme site of energy balance. However, very low level of energy in the diet affects the utilization of protein, again excess energy in the diet create nutrient imbalance and gets deposited in the adipose tissues in the form of body fat which is not at all desirable for marketing of fish. It has been suggested that the optimum protein to energy ratio in Catfish diet of protein digestible energy requirement is 10-11 kcal (90-100 mg protein/kcal DE).

Minerals and vitamins: Minerals and vitamins are also essential for optimum growth and physiological functions of fish. Some information is available on the mineral and vitamin requirement for catfish (Dabrowski *et al*., 1988; NRC, 1993). Mishra and Mukhopadhyay (1996) reported the ascorbic acid requirement of *Clarias batrachus* to be 69.0 mg/kg diet. Mohammad *et al*. (2000) reported that biotin deficiencies in *Clarias batrachu*s results in anorexia, dark skin colour and convulsion. The optimum biotin requirement for maximal growth of *C. batrachus* is 2.49 mg/kg diet. In *Heteropneustes fossilis*, fingerlings the optimum dietary biotin requirement is 0.25 mg/kg diet. (Mohammad, 2001). Mohamed and Ibrahim(2001) worked out the dietary niacin requirement of *Heteropneustes fossilis,* and the optimum requirement is 25mg/kg diet.

Phosphorus is of major importance in formulation of practical catfish feeds because of the relatively high physiological requirement, the low amount of available phosphorus in feed stuffs of plant origins and the relatively low level of dissolved phosphorus in natural waters. Calcium requirement is also very high for catfish, however, catfish absorb a significant amount from the water. Minimum requirement for available phosphorus in diets for catfish is approximately 0.45%. Trace minerals are sometimes deficient in plant ingredients produced in mineral deficient areas, thus fish feeds containing low levels of animal byproducts may be deficient in one or more trace minerals. A premix to provide the requirements of zinc, iron, manganese, iodine, copper, and selenium is recommended in commercial feeds.

Feed additives:

In aquaculture nutrition some feed additives have also used for better growth performance.Harpaz (2005) reported that levels of dietary L-canitine supplementation ranged from a few hundred to over 4000 mg/kg of diet for better growth, utilization of high fat level in fish feed through protein sparing effect. Incorporation in small amounts of feed additive in aqua feed helps in aquaculture production.

If antibiotics or other additives are incorporated in to the feeds, these shall be declared on the label. Antibiotics, other Pharmacologically Active Substances, Steroid hormones and any other substance banned by Coastal Aquaculture Authority shall not be used in the feed.

Non-conventional feed resources:

Different non-conventional feed ingredients have been used in carp feeding to reduce the cost of feed.

Phosphorus is of major importance in formulation of practical catfish feeds because of the relatively high physiological requirement, the low amount of available phosphorus in feed stuffs of plant origins and the relatively low level of dissolved phosphorus in natural waters. Calciumrequirement is also very high for catfish, however, catfish absorb a significant amount from the water. Minimum requirement for available phosphorus in diets for catfish is approximately 0.45%. Trace minerals are sometimes deficient in plant ingredients produced in mineral deficient areas, thus fish feeds containing low levels of animal byproducts may be deficient in one or more trace minerals. A premix to provide the requirements of zinc, iron, manganese, iodine, copper, and selenium is recommended in commercial feeds.

Feeding practices

The spawn of magur (5-5.5 mm) are reared for 12-14 days in indoor rearing system and feeding is not required till fourth day. Though period of yolk absorption varies, feeding may be initiated with provision of little quantity of feed and usual meal from fifth day onwards. The feed quantity may be adjusted depending on the density of spawn. Mixed zooplankton, *Artemia nauplii*, molluscan meat, Tubifex and egg custard with 40-65%

proteins are used for larval feeding. Feed particle size or the size of natural fish food organism of 20-30 mm are considered ideal for initial phase of feeding, which is gradually increased to 50-60 mm for feeding 1-week-old magur fry.

The fry are usually reared indoor in tanks for a few weeks before releasing them into ponds, and during indoor rearing; they are fed with high energy and protein diets with main contribution from animal source and having a particle size of 0.3-0.5 mm. The feed should be adequately fortified with water-soluble vitamins to compensate the leaching losses during feeding. Like spawn, fry in tanks must be fed several times a day and feeding at hourly intervals in always preferable.

In ponds, feed is normally dispensed from all sides to provide feeding opportunity to all fish. Feeding should be done twice daily. Acceptability and utilization of feed by catfish in pond is greatly influenced by level of dissolved oxygen and water temperature. With low dissolved oxygen in water, feeding activity of catfish reduces. The feeds, hence, need to have high degree of water stability. Either extruded or pelleted feed is ideal for feeding fish to minimize nutrient leaching and wastage unlike dust or dough feed, which needs to be discouraged.

Supplemental feeding of fry in ponds is done soon after stocking. As size of the fish increases, diameter of pellet feeds needs to be increased. Crumbles of pellets should be used to feed fry and fingerlings whose size should be such that fish can easily ingest, to minimize dissolving of ingredients in water.

Type & size of feed:

Water stable feed is usually preferred by the farmers for catfish culture. Floating, sinking and slow sinking feeds are mostly used for catfishes during their rearing. The size of pellets usually varies as per the age of fishes. Usually dust feed, small crumbles and pellet of 1-2 mm die are being fed to catfish larvae, fry and bigger fish, respectively. Floating as well as sinking feed is used in South-East Asian countries for *Pangasius* and *Clarias* during their rearing. It is always better to provide sinking feed to *Clarias* as it is a bottom feeder. Dust feed made into small compact dough form is fed to *Clarias batrachus* larvae during indoor rearing.

Feed selection:

Farmers usually select the feed on the basis of cost and quality. The cost of catfish feed is always higher compared to carp feed as it contains animal protein. Apart from this, the type of feed to be used during rearing should be selected as per the feeding behaviour of the catfish. Improper feed selection like floating feed for *Clarias batrachus* may not help in success rearing of catfish. Similarly channel catfish accepts well to floating feed.

Ration size:

Usually the ration size during catfish culture is given on the basis of body weight. Small fish eat more compared to their body size and need frequently. It is required to spray more feed in ponds to increase the possibility of getting feed in all areas of the pond while rearing larvae/fry. The ration size is strictly followed during fingerling production till marketable size. The feed ration changes in each day as the fish grows. But it is not easy to monitor daily basis. So monthly sampling is a usual practice followed to manipulate the ration size during catfish culture. Provision of feed @2-3% of body weight is sufficient for bigger fish for their growth. This ration size is generally followed in Asian catfish, African catfish, Channel catfish etc. the ration size in the catfishes is influenced by water temperature and catfishes accept feed less efficiently during winter. So the usual ration size should be judiciously monitored depending on the situation to curtail the feed cost. The decrease of feed ration is also observed due to stress, weather condition, management practice etc.

Feed distribution and frequency of feeding:

Feeding to catfish is undertaken by providing feed in few places of the culture tanks or distributed the feed on the water surface. Fish take few hours for their consumption. Some catfishes like *Horabagrus brachysoma*, *P. pangasius* etc accept quickly as and when the feed is given. The competitive individuals always suppress the feed intake of the less competitive individual resulting size hierarchy. So the catfish culturists always take care of the feed distribution for giving a scope to get feed by all the fishes. The correct feed distribution enables to reduce the size difference among the catfish and also restricts the wastage of feed. Sometimes total ration is divided into few instalments to reduce the competition. The frequency of feeding is usually followed during the seed rearing in early stages of catfishes and also found in highly cannibalistic fishes. It has been seen that the feed conversion efficiency of channel catfish increased when the meal is increased from two to four at a fixed ration of 2% of body weight.

Feeding schedule:

It is a usual practice to feed once in 6-7 days in a week during traditional or intensive culture system. Feeding schedules change with the water temperature and the catfish farmers offer feed minimum twice in a day when the temperature is above 25^0C. The channel catfish is observed 20% higher rate of feed consumption in optimum condition with faster growth rate. So it is always beneficial to work on feeding schedule to stop the feed wastage and maintain good growth of catfish during their rearing.

Daily feed allowances:

The daily feed offered in catfish ponds varies during the culture period. The day to day variation in feed consumption is due to water temperature, size of fish, culture condition, water quality etc. so the usual ration size of 2-3% of body weight may be manipulated as per the response of fish to the feed.

Feeding time:

All the fishes show the rhythmic pattern of feeding and categorized as nocturnal, diurnal and crepuscularfeeders. So suitable time for feeding the catfish as per their nature have a great effect on feed utilization and growth. A positive effect on growth due to feeding during night hours has been confirmed in Indian catfish (*Heteropneustes fossilis*), African catfish (*Clarias lazera*, *Heterobranchus longifilis*) and channel catfish (*Ictalurus punctatus*). So the optimal time for feeding is one of the most important management steps to get better feed efficiency during catfish culture.

Season on feeding:

The suitable temperature required for optimum growth of catfish is approximately 30^{o}C. The decrease in water temperature as well as high temperature reduces the feed consumption. The usual feed consumption in catfish is hampered when the water temperature comes down to 21^{o}C. So normal feeding schedule and amount may lead to wastage and accumulation of feed in the pond. This will lead to water quality problem due to oxidation of unconsumed feed in the pond when the water gets warmer. So the feeding during these periods should be reduced. Sometime catfish farmers stop giving feed to catfish, which lead to weight loss. So it is always beneficial for judicious feeding during these days to keep the fishes healthy.

This non-response to feed is not noticed in summer days. The channel catfish does not respond to floating feed during mid-summer days as the surface water gets warm up. Hence, catfish growers prefer to feed slow sinking feed during these days. But it is not problematic to feed bottom dwelling catfishes during these days.

Feeding technique:

Several feeding technique like hand feeding through basket and feeding through automatic feeder are seen in catfish farm. The feeding technique always depends on the level of production and the type of catfish in the culture ponds. Hand feeding is a simple practice in small farm. It helps to know when the fish reach satiation further wastage of feed may be reduced. This type of feeding is always suitable for surface feeding catfishes. Channel catfish are the most suitable catfish variety to be fed by hand feeding. Basket or tray feeding is another device, adopted in catfish pond during feeding the fish. It has also similar advantage like hand feeding. The farmers lift the basket every time while giving the feed and know the feed accessibility status among the fish in pond. If feed remains continuously in the basket, it will indicate several aspects like disease incidence, poor environment or stress in fish. So it is easier for the farmer to monitor the situation to rescue the fish. This basket or tray feeding is noticed while culturing bottom dwelling catfishes. Some catfish in Bagridae and Pangasidae family are also fed by hand feeding as well as basket feeding during their culture. Automatic feeders are most advanced feeding device used during intensive aquaculture. But it is expensive and small farmers cannot afford it. It may be suitable for surfaces and column feeding catfishes, but still doubtful for its suitability for feeding bottom dwelling catfishes.

Conclusion:

Although natural food serves as the feed to certain extent, supply of prepared feed to fish has its own importance. High quality, optimum quantity and cost effective feedsare always desirable for good growth and high production. The aquaculture producers should select appropriate feed to appropriate species in right form to ensure efficient conversion of feed to fish flesh for successful Aquaculture.

Table 1: Requirements for Catfish (*Clarias batrachus*) feeds

Sl. No.	Characteristic	larval feed	Fry feed	Grow-out feed	Brood feed
1.	Moisture, percent by mass, Max	10	10	10	10
2.	Total crude protein (Nx6.25), percent by mass, Max	40	35	35	30
3.	Crude fat or ether extract, percent by mass, Max	10	8	6	6
4.	Crude fibre, percent by mass, Max	4	6	6	6
5.	Acid insoluble ash, percent by mass, Max	2.5	3	3	3
6.	Gross energy, Kcal/kg, Min	4000	3500	3500	3500
7.	PUFA (n3+n6)	0.5%	0.5%	0.5%	1%

Larval Nutrition and Feeding

B.N.Paul

Central Institute of Freshwater Aquauclture

Regional Research Centre, Rahara. Kolkata-700118

The fish larval stage extends from the stage of hatchlings coming out of eggs to the attainment of juvenile stage. During all intervening stages of larval growth, one important challenge concerns larval nutrition.

In the last decade, most of the studies on larval nutrition were related to first stage larval feeding. These studies have contributed to the knowledge needed to estimate the nutrient requirements of larval fish. While it is not easy to quantify the nutritional requirements of larval fish, it is believed that the optimal formulations for the first feeding of larvae should simulate the yolk composition and to some extent reflect the nutrient requirement and metabolic capacities of larval stages of fish.

External characteristics and major organ functions of juveniles match those of adult fish. For practical purposes, larval fish can be divided into three groups according to alimentary tract, and morphology of the gut and enzyme secreted into it. The first group includes fishes such as salmonids and catfish, which appear to have a functional stomach before changing from endogenous to external feed. The second group includes fish, such as striped bass and many marine species, which at the larval stage have a very rudimentary digestive system. The third group of larval fish is of those that develop a functional digestive tract but remain stomach less throughout life, such as carps. Species that, at the time of first feeding, have structurally and functionally differentiated alimentary tracts pose less of problem at initial feeding. Those with immature digestive systems at first feeding are more difficult to feed and usually require live feeds as a part of their diet.

Larval fish undergo different phases of larval metamorphosis and at a certain phase can be weaned to dry, prepared diets. For example, striped bass, which completes metamorphosis in 21 to 42 days, cannot feed on dry diet at day 5, when initial feeding begins, but they can at day 15.common carp can be transferred to commercial dry diets at the size of 15 to 30 mg. The Indian major carps can be transferred to dry diets at the weight of 3-7 mg and length 9-17 mm at day 5, whereas larvae of other white fish must reach size of 50 mg to be weaned to dry diets. This transition from live to dry diet is a gradual process.

The reason for the poor ability of some larval fishes to utilize prepared diets at first feeding may be due to the low affinity of proteolytic enzymes in the immature digestive tract to digest the proteins offered in formulated diets. Several fish species have been shown to be unable to digest the protein from prepared diets at larval stages, larval fish ingest more feed per unit than adult fish, consuming 50 to 300 percent of their body weight daily compared to 2 to 10 percent of body weight for adult fish.

Lipids: Lipids are major sources of metabolite energy throughout the embryonic developmental stages in fish. The amount of lipids as well as the lipid classes catabolized during embryonic development vary among species. Other sources of energy, such as carbohydrates and proteins are also utilized during these stages. After hatching and up to the stage prior to first-feeding, catabolism of phospholipids (PL) is very much pronounced.

In nursery rearing, carp larvae require PL in their diets as they cannot synthesize it *denovo*. Phospholipid incorporation is essential to fulfill the demand for building and renewal of cellular membrane during the early growing stages as well as for improving the efficacy of essential fatty acid utilization. The consumption of PL during embryonic and larval development has also been related to other uses, such as being a source of inorganic phosphate in the synthesis of nucleic acids and of choline in neurotransmission. Work conducted at CIFA suggests that the inclusion of dietary phospholipid at four percent in the larval diet is essential for rapid growth and high survival of Indian major carps(Paul et al., 1997).

The high levels of (n-3) HUFA in the live feed can improve growth and survival of fish larvae in a number of fish species. Increased (n-3) HUFA content in the live feed is probably not the only factor regarding lipids which will enhance the growth and survival of the fish larvae. The DHA:EPA ratio and their individual content in absoolute terms are also important for fish larval nutrition. The specific role of DHA in the development of neural tissues as brain and retina is well established. Thus, the high content of DHA in the developing larva constitutes a significant part of the body mass; and higher dietary content of DHA than EPA during the rotifers stage improved the growth and survival of the larvae of gilt head sea bream.

The type and amount of essential polyunsaturated fatty acids (PUFA) which occur in tissues and cells are of basic importance for the essential structure of living systems and for the regulation of life processes.

Vitamin-C: Vitamin C has an immense role in fish nutrition in general and larval nutrition in particular. Most animals can synthesize ascorbic acid (AA) from glucoronic acid but fish and crustaceans lack the enzyme gulonolactone oxidase necessary for the last step in this biosynthesis. They are dependent on constant supplies of adequate quantities of vitamin C through the feed. Lower dietary AA levels cause typical deficiency symptoms e.g., the broken back syndrome in fish and the black death disease in shrimp. It was observed that relatively low AA levels (20-130 mg AA/kg diet) supplied as a stable and bio-available source allow a normal growth and survival during the nursery phase of both fish and shrimp; on the other hand, a rise in the dietary AA content upto 1500 mg/kg enhances the resistance to stress and diseases, especially in shrimp.

Manufacture of larval diets: The larval diet particle must be very small (5-150 mm for filter feeders, 40-1000 mm for particle feeders), must contain all the nutrients required by the larvae in an available form and must have the physical characteristics such as neutral buoyancy and water stability to remain in tact in the water until being ingested. There are three main categories of larval diets based on manufacturing methods. 1. Macro encapsulated; 2. Micro bound; and 3. Micro coated.

Micro encapsulation: This process involves surrounding a solution, colloid, or suspension of dietary ingredients that have been pulverized into fine particle with a membrane. The membrane must be nontoxic, impermeable and digestible once it has entered the gut of the larvae. Membrane material can be nylon-protein, gelatin-acacia, lipid, chitosan, gylcopeptide and whole egg.

Micro bound diets: This process involves the use of binders to hold micro pulverized complete diets together. Appropriate binders include, gelatin, carageenan, and alginates. The dietary ingredients are mixed together with the binder in a slurry, and the slurry is dried by freeze-drying, over-drying, or drum-drying. After drying, this material in ground and screened to appropriate particle size. Micro coated diets: Micro coated diets are made by coating micro bound diets with cholesterol lecithin or modified com gluten or zein.

Natural Fish Food organisms and its role in Aquaculture

R. N. Mandal

ICAR-Central Institute of Freshwater Aquaculture
Regional Research Centre,Rahara, Kolkata-700118, West Bengal

Which are live foods?

An innumerable number of mostly microscopic organisms, which are part of food web in aquatic ecosystem and support nourishment for fish and prawn species as food resources, are known as 'LIVE FOODS'.

How are they grouped?

All the live food organisms comprise plankton, periphyton, nekton and benthos, with two major groups such as autotrophy and heterotrophy. All the autotrophic organisms,including phytoplankton, periphyton and phyto-benthos, constitute a major plant group known as MICROALGAE. On the other hand, heterotrophic organisms which serve as an important food resourceto fishare known asZOOPLANKTON.

Why are live foods to fish rearing?

Most species of interest particularly juveniles of IMC, catfish, murrels and entire communities of ornamental fishes prefer to accept live food organisms because;

- Instinct behavior most of fish and prawn during their juvenile phase receive to those organismswhich are easily detected and captured while swimming, moving or having any type of motility in the water bodies, since larvae are believed to be visual feeders adapted to capture moving prey in nature.
- The movement of live food is likely to stimulate larval feeding responses.
- Live food comprises various types of organisms: plankton, periphyton, nekton and benthos – a wide spectrum of composition of organisms.
- Live foods facilitate better nourishment as highly digestible to most fish and prawn in their vital phase of life cycle since their lower nutrient concentration suitable for digestion.

Considering their importance in rearing of larvae of most fish and prawn species, live foods are known as **"**LIVING CAPSULES OF NUTRITION**".**

Nutritive value of microalgae

- More than forty different algal species are currently used as live food for aquatic invertebrates and vertebrates.
- Selected microalgae have rich nutritional properties, particularly n-3 highly unsaturated fatty acids (HUFA), which may be helpful for survival of fish larvae juveniles, prawn, and bivalve mollucs.
- Similarly, different microalgae foods have different concentrations of certain polyunsaturated fatty acids (PUFA) which play a very important role in the health status of larvae of many fish.
- The most attractive component of microalgae biomass is crude protein but its utilization by fish larvae depends upon its digestibility.

The nutritional value of some important algae is mentioned below

Component	Spirulina sp.	Chlorella sp.	Scenedesmus sp.
Protein (%)	62-68	40-50	50-55
Lipids (%)	2-3	10-15	12-19
Carbohydrates (%)	15-20	12-16	10-15
Fibre (%)	5-8	6-8	10-12
Ash (%)	10-12	8-10	6-8
Moisture (%)	5-6	5-8	5-7

Why are microalgae preferable to fish larvae?

Important criteria for which microalgae are preferable for larval food may be highlighted as;

- Cell size appropriate to the demands of fish larvae
- Adequate nutritional value
- High digestibility
- Short life cycle
- Tolerance to environmental variations

Role of microalgae

Microalgae(phytoplanktons, Phyto-periphyton and phyto-benthos) are autotrophic primary producers in an aquatic ecosystem. They can absorb CO_2 and other nutritional salts and sunlight for synthesizing organic matters for direct or indirect food of fishes. At the same time O_2 is produced which is essential for aquatic life.

Role of Zooplankton

Zooplanktons are heterotrophic primary consumers and are mainly composed of free living species of Protozoa, Rotefera. Cladocera and Copepod. Zooplanktons feed on detritus, bacteria, phytoplankton and other zooplankton. They are most preferred food of carp larvae until the gill rackers develop and the feeding habits change (Table-1).

Few concepts

- **Planktons** are free floating living organisms, whose intrinsic power of locomotion depends upon the direction of water current. They are basic links in the food web of aquatic ecosystem. The optimum biomass of plankton in fertile water is 20-100 mg/l. Plankton biomass can be divided into phytoplankton, zooplankton, and bacterioplankton.
- **Organic detritus** is one of the basic nutrient sources for aquatic animals and has an important role in trophic process of aquatic ecosystems. Detritus forms 20-80% of total particulate matter and calorific value of detritus is estimated to be 1.5-4.6 kcal/g. The detritus with a calorific value of more than 3kcal/g is a full value of food. The detritus community consumed by carp larvae and thus achieves nutritive substances.

Measurement of Live Foods

Measurement of live foods is a bit of difficult task if anybody maintains its scientific accuracy. Then following requirements are necessary.

Requirements

Plankton net, 1 litre container, Compound microscope, Sedgewick Rafter Counting plankton Cell, cover slip, measuring cylinder/tube, etc.

Table-2. Food organisms with the changing feeding habits of carp fry

Length of fry (mm)	Silver carp	Catla	Grass carp	Rohu	Mrigal	Common carp
7-11	Rotifers, nauplii micro-cladocerans	Rotifer,nauplii, micro-cladoceranas,	Rotifers Nauplii, micro cladocerans, micro green algae	Rotifers Nauplii	Rotifer, micro-cladocerans	Rotifer, micro-cladocerans
12-15	Rotifers, cladocerans, copepods, nauplii, Detritus, phytoplankton	Rotifer,nauplii, micro-cladoceranas, copepod phytoplankton	Cladocerns, Nauplii, green algae	Rotifer,nauplii, micro-cladoceranas, phytoplankton	Cladocerans, Rotifer, phytoplankton, plant origin-Detritus	Cladocerans Rotifer, Animal origin detritus
16-25	Phytoplankton,Rotifers, cladocerans, detritus	Rotifer, cladocerans, Nauplii, copepods, Phytoplankton	Micro cladocerans, benthic animals detritus, green algae	Rotifers, detritus Nauplii, Periphyton, Micro-algae	Phytoplankton, Detritus, Periphyton, Benthic Organisms	Cladoceran, benthic Organisms, Animal origin Detritus
26-30	Phytoplankton (More) Zooplankton (less)	Zooplankton (More), phytoplankton (less)	Micro-cladocerans, Benthic organisms, detritus green algae wolfia	Phytoplankton (More), zooplankton (less) Periphyton	Phytobenthon (More) Phytoplankton (Less) Zoobenthons periphyton	Zoobenthos (more) phytoplankton (Less), Detritus
Above 30	Phytopklankton feeder	Zooplankton feeder	Herbivorous (Grass eater)	Phytoplankton feeder	Phutopenthos feeder	Zoobenthos feeder

Technique

- Get down up to waist level in a pond during sunrise or sunset.
- Collect one liter of water with one liter measuring container from column area of water body and pour it into plankton net.
- One should pour 50-55 liters of water round the pond periphery.
- Collect it into separate measuring tube.
- Keep the measuring tube as such for 2-4 hours.
- Observe the thick level of precipitation.
- If the level measures 2.0-2.5ml, then plankton (mainly zooplankton are measured in this way) population of pond water appears to be suitable for rearing fry and fingerlings.
- In case phytoplankton, one should take one ml of water through dropper and put it into Sedgewick Rafter plankton Counting cell, then count under microscope.
- The standard limit of phytoplankton of any water body should be as 7, 00, 000-10, 00,000/L which is considered to be ideal.

Easy method to measure in field

- One should take a stick, put it down 1.5ft into water
- Observe if the stick is visible clearly down to its end.
- If it is visible clearly, it is assumed that water body does not have ideal amount of live food organisms.
- If the stick is seen obscure, it is assumed thatwater body appears to have ideal amount of live food organisms favourable to fry and fingerlings.

Management of Live Foods

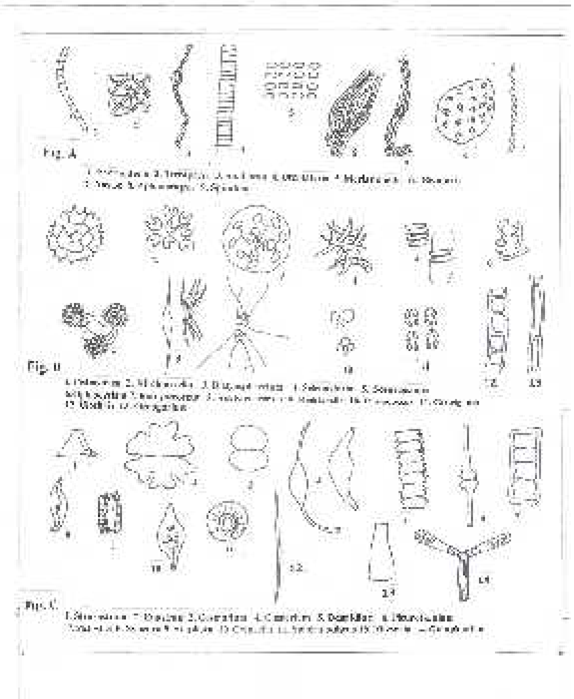
Application of fertilizers is considered to be very important item for management of live food organisms. The followings are mentioned as simple as possible for the farmers to undertake management in aquaculture.

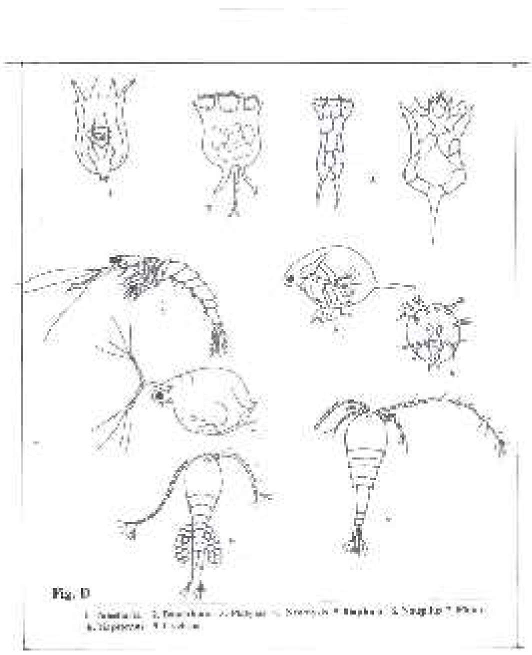
- Calculation: 1 ton=1000kg, 1ha=2.5 acre or 10,000 m^2 or 7.5 bigha (approx.)

Fertilizers	Total dose @	Monthly dose @(as per 10 months calculation)
Cattle dung	**In old pond** 10-15 tons/ha/yr or 10000-15000 kg/ha/year or 1333-2000kg/bigha/year	133-200kg/bigha/month or 65-100kg/ bigha/fortnight or 35-50kg/bigha/week
	In new pond 20-30 tons/ha/yr or 20000-30000kg/bigha/year or 2666-5333kg bigha/year	266-533kg/bigha/month or 130-265kg/ bigha/fortnight or 65-135kg/bigha/week
GNOC/MOC	1.0 tons/ha/yr or 1000kg kg/ha/year or 133 kg bigha/year	13kg/bigha/month or 6.5 kg/ bigha/fortnight or 3.0 kg/bigha/week
Urea	150-200kg/ha/ year or 20-27kg/bigha/ year	2.0-2.7kg/bigha/month
SSP	200-300kg/ha/ year or 27-40kg/bigha/ year	2.7-4.0 kg/bigha/month
Multiplex	1-2kg/ha/ year or 133-260g/bigha/ year	15-25 g/bigha/month
Biogas slurry	25-30ton/ha/ or 3333-4000 kg/bigha/year	333-400 kg/bigha/month Or 165-200kg/ bigha/fortnight

Technique of application of cattle dung

- Raw cattle dung should not be applied directly in the pond.
- Collect raw cattle dung from any source.
- Pput it in large container or in earthen dig
- Mix sufficient water and keep it as such in bright sun light.
- After 2-3 days, stir the entire content with stick and smell if anything odour is emitted till.
- See if brown yellowish colour turns to become black brown colour.
- Collect the entire materials and apply at the edge of the water in pond so that it gradually mix with water body by ripples of surface water.





Importance of Minerals in Aquaculture

B. N. Paul
ICAR-Central institute of Freshwater Aquaculture
Regional Research Centre, Rahara, Kolkata-700118

Mineral elements have a great diversity of uses within the animal body. It comprise the ash of biological materials remaining after the organic substances have been completely burnt or oxidised, are known to be essential for cellular metabolism of all animals including fishThe following macro- mineral elements are recognized as essential for body functions in fish: calcium, phosphorus, magnesium, sodium potassium, chloride etc. Fish, unlike most terrestrial animals, can absorb some minerals (inorganic elements) not only from their diets but also from their external aquatic environment. Part of the nutritional requirement of fish is fulfilled being derived from water.

The prominence of each mineral element in body tissues is closely related to its functional role. As constituents of bones and teeth, minerals provide strength and rigidity to skeletal structures. In their ionic states in body fluids they are indispensable for the maintenance of acid-base equilibrium and osmotic relationship with the aquatic environment, and for integration activities involving the nervous and endocrine systems. As components of blood pigments, enzymes and organic compounds in tissues and organs they are indispensable for essential metabolic processes involving gas exchange and energy transactions.

CALCIUM AND PHOSPHORUS

Calcium is the most abundant mineral in the animal body. It is an important constituent of the skeleton, teeth and scales. Calcium is essential for the activity of a number of enzyme systems including those necessary for the transmission of nerve impulses and for the contractile properties of muscle. It also regulates coagulation of blood

Calcium and phosphorus occur in the body combined with each other for the most part and because an inadequate supply of either limits the nutritive value of both.Calcium and phosphorus. Fish scales are also an important site of calcium metabolism and deposition. Fish can absorb large amount of their calcium requirements from the water; but their phosphorous comes primarily from dietary sources (Nose and Arai, 1979) because of its low rate of absorption from the water (Phillips *et al*., 1954) and its low concentration in natural waters (Boyd, 1971).

Under practical farming conditions, mineral deficiency signs often arise from a dietary imbalance of calcium owing to the antagonistic effect of excess dietary calcium on the absorption of phosphorous (Paul *et al*., 2004). When there is an excess of calcium over phosphorous, the phosphorous is not absorbed by the intestine because it is combined with the calcium to form calcium phosphates that are not biologically available (Cowey and Sargent, 1979). Phosphorus occupies an unique position in biochemical machine and is a major mineral that must be supplied in feed (Chavez-sanchez , 2000).

Almost the entire store of calcium (99 percent) and most of the phosphorus (80 percent) in the fish's body are present in bones, teeth and scales. There appears to be little variation in the composition of bone ash even though bone ash will decrease as a result of dietary deficiency in either calcium or phosphorus. The one percent extra-skeletal calcium is widely distributed throughout the organs and tissues. Calcium in body fluids exists in two distinguishable forms, diffusable and non-diffusible. Non-diffusible calcium is bound to protein whereas the diffusible fraction is present largely as phosphate and bicarbonate compounds. It is this diffusible fraction that is of significance in calcium and phosphorus nutrition. Ionized calcium in the extracellular fluids and in the circulatory system participate importantly in muscle activity and osmoregulation.

Large amounts of extra-skeletal phosphorus are present mostly in combinations with proteins, lipids, sugars, nucleic acids and other organic compounds. These phosphocompounds are vital exchange currencies in life processes and are distributed in different organs and tissues of the fish body. The skin, like the skeleton, also appears to be an important repository for dietary phosphorus as reported in some species.

Deficiency Symptoms

Deficiency symptoms of calcium have not been described in fish although poor growth is observed with diets limited in phosphorus (which leads to reduced accretion of calcium by body tissues). Prolonged feeding of phosphorus-deficient diets to common carps resulted in deformed backs (lordosis) and heads due to abnormal calcification of bone. Bone growth was reduced in the skull and operculum regions. Recent studies with this species and the red sea bream have also shown fatty infiltration of liver and muscle tissues related to dietary phosphorus deficiency.

Calcium and Phosphorus in Feeds

Cereal grains, fish meal and meat meal products containing bone are good sources of phosphorus. Calcium rish feed ingredients are green leafy crops, especially legumes, fish meal, meat and bone meal. Fish meal, a principal ingredient in fish feeds, is rich in both calcium and phosphorus. On the other hand, feed ingredients of plant origin usually lack calcium and, despite a fairly high content of phosphorus the latter is predominantly in the form of phytic acid which is not readily available for absorption by fish. Animal sources of calcium and phosphorus are generally better absorbed, although the stomach less carp cannot utilize bone phosphate present in fish meal as well as fish with functional stomach. Dicalcium phosphate has the highest availability (80 percent). Phosphorus availability of common feedstuffs varies from 33 percent for grains to 50 percent for fish meal and animal by-products. Soybean meal has an intermediate phosphorus availability of 40 percent.

Practical fish meal diets contain appreciable amounts of calcium (Ca) and phosphorous (P) derived from the hard tissues that exist in a very complex crystal structure as hydroxyapatite and for simply tricalcium phosphate (TCP) (Hossain and Furuichi, 1999). Because of that complexity in structure, it has been reported that P from fishmeal was less available (Yone and Toshima, 1979). Not only the individual Ca and P play roles in well being of fish but also they have much relevance in combined effects of Ca and P (Chavez-sanchez, 2000).

Potassium:Potassium plays a very important role , along with sodium, chloride and biocarbonate ions, in the osmotic regulation of the body fluids and in the acid- base balance in fish. Potassium acts principally as the cation of cells. Potassium regulates nerve and muscle excitability and also plays role in carbohydrate metabolism. Deficiency symptoms include retarded growth, weakness and tetany and sometimes result in death.

Sodium: Sodium is the main inorganic cation of extracellular tissue fluids. Most of the sodium of the animal body is present in the soft tissues and body fluids. Sodium also concerned with the acid -base balance and osmotic regulation of the body fluids. Sodium is the chief cation of blood plasma and other extra-cellular fluids. The sodium concentration within the cells is relatively low, and it is replaced mainly by potassium and magnesium when required. It also plays role in the transmission of nerve impulses and in the absorption of sugars and amino acids from the gastrointestinal tract. Most of the sodium is ingested in the form of sodium chloride. Symptoms of sodium deficiency include poor growth and reduced utilization of digested proteins and energy . Meat meal and foods of marine origin are rich sources of sodium. Common salt is the common mineral supplement.

TRACE MINERALS

Minerals, which are present in fairly large quantities, are calcium, phosphorus, potassium, sodium, magnesium, chloride etc. the principal minerals present in microquantities (or trace level) are **iron**, **manganese, zinc, copper, cobalt, selenium, chromium** and **iodine.** The fish can get these minerals both from diet as well as from ambient water. The minerals are responsible for skeletal formation, maintenance of colloidal systems, regulation of acid-base equilibrium and for formation of some physiologically important compounds like hormones and enzymes. Mineral deficiencies can cause biochemical, structural aberrations and functional pathologies, which depend on several factors, including the duration and degree of mineral deprivation.

Information on nutritional requirements of fish for trace elements is scarce particularly because many are needed in extremely small amounts and these pose difficult analytical problems also. If excess amount of the elements are ingested and assimilated, toxicity develops. In this communication importance of some of the trace minerals which play significant role in aquaculture nutrition has been highlighted

Iron

More than 90 per cent of the iron in the body is combined with proteins, the most important being haemoglobin, which contains 3.45 g/kg of the element. Iron plays an important role in oxidation/reduction reactions and electron transport associated with cellular respiration. It binds to proteins such as haem whch serves as carriers of oxygen, in enzymes such as microsomal cytochromes, catalase etc., and in nonhaem compounds such as transferrin, ferritin and flavin iron enzymes. The haem molecule is comprised of ferrous or ferric ions in the centre of a prophyrirn ring. Haemoglobin contains 4 porphyrin group per molecule. Myoglobin a similar type of compound present in skeletal or heart muscle contains one ferrous porphyrin group per molecule.

In fish, the gill membrane absorbs iron to a certain extent, the intestinal mucosa is considered to be the major site of absorption of iron.. Deficiency of iron induces anaemia, as has been reported in trout, yellowtail, red seabream and even in carp. Microcytic, hypochromic anaemia in iron-deficient carp was characterised by a drop in haemoglobin centent and erythrocyte count. Iron deficiency also caused yellowish white liver condition in carp and poor feed utilization and lowering of plasma iron level and transferrin saturation in channel catfish. The hatching rate of rainbow trout eggs was poor due ti iron deficient feed.

It has been demonstrated that fish takes up iron via the gills. Addition of iron (Ferrous sulfate) improved the growth of sword tail, indicating a nutritional benefit from iron dissolved in water. Among the different forms of iron, on comparing the effectiveness in preventing anaemia, it was seen that ferrous and ferric chloride was more effective than ferric citrate. The requirement of iron in fish ranges between 30-170 mg/kg feed .

Feeds of animal origin such as fishmeal, blood and meat meal are rich sources of iron, containing about 400-800 mg/kg,while cereals contain 30-60 mg/ kg. The iron from animal sources may occur as porphyrin, myoglobin and haemoglobin but in cereals in a complex form with phytin in cereals.

Manganese

Manganese is important for fish and is widely distributed in fish and animal tissue and mainly used as co-factor for the enzymes kinase, peptidase, arginase, succinic decarboxylase. Manganese has been implicated in oxidative phosphorylation. The mitochondria have a greater concentration of manganese than cytoplasm. It is responsible for normal functioning of brain and proper lipid and carbohydrate metabolism. It was two roles (1) cofactor for enzymes, which form metal-enzyme complexes and (2) as an integral part of metalloenzymes. Manganese activates leucine aminopeptidase.

Manganese deficient diet resulted in poor growth in rainbow trout and carp. In tilapia deficiency syndrome included reduction in feed intake, loss of equilibrium, poor growth and mortality. Dwarfism linked to disturbances in bone formation and cataract of eye lens was observed in rainbow trout and carp. A reduction in skeletal manganese content has been noted corresponding to insufficient dietary supply of manganese. The manganese content of the diet influences its level and that of other trace elements in gonads. The eggs produced by brood stock of brown trout and rainbow trout fed fishmeal diets lacking manganese contained only low levels of manganese and poor hatchability. Manganese has very important role in brood stock nutrition.

In carp manganous sulfate and manganous chloride and better sources. Good growth was recorded in carp provided with manganous sulfate in the diet and it also increased protein synthesis and prevented fat synthesis in liver. The requirement of manganese in fish is 2-20 mg kg /dry diet. The content of manganese in fishmeal ranged from 4-38mg/kg, depending on the fish. Herring meal contains only 4-12 mg/kg.Among the plant sources, cereals contain 8-50 mg/kgand corn 4-11 mg/kg, rice bran, wheat middling and corn distiller's dried soluble are good source of manganese.

Zinc

Zinc play key role in various metabolic pathways like prostaglandin metabolism and structural role in nucleo proteins. Recent research of zinc-gene interactions has revealed the basic role of zinc in controlling growth.

Deficiency of zinc results in poor growth, lowered digestibility of protein and carbohydrate, probably due to reduced carboxypeptidasc activity. Zinc deficiency also recorded eye lens cataract and erosion of fins and skin, and sign of short-body dwarfism. In catfish, diets low in zinc resulted reduced appetite, low growth, low bone zinc and calcium levels and serum zinc concentration. The mineral composition of common carp gonads was significantly affected by zinc depletion from the mineral supplementation in fishmeal diet. Fortification of brood stock diets of salmon with zinc increased the content of this mineral in the ovaries of female Salmon. In rainbow trout zinc deficiency impairs immunological response. In carps it was found that the availability to zinc was influenced by dietary tracalcium phosphate(TCP), but the ill-effect on mineral utilization was less pronounced than in rainbow trout. TCP was not dissolved in the intestine of carp as it has less gastric juice compared to rainbow trout.

The presence of phytic acid in plant protein sources like soybean meal or cotton seed meal strongly chelates divalent minerals, including zinc to form insoluble phytates in the intestinal lumen, thus lowering zinc availability. Interaction between zinc and phytic acid in soybean meal could be the reason for low bioavailability of zinc. To over come that, heat treatment of soybean meal of extrusion at 150°c improved of the diet having soybean meal. Fish requines zinc to the tune of 15-40 mg kg/dry feed. Fish meal contains about 80-100 mg / kgwhere as the range in vegetable protein concentrates may be 40-80 mg/kg and cereal grains contain15-30 mg /kg.

Copper

Copper plays important role in the activity of enzymes such as cytochrome oxidase, superoxide dismutase, lysyl oxidase, dopamine hydroxylase and tyrosinase. Copper metabolism revealed similarities to mammals in the distribution of copper and copper dependent enzymes. Copper levels are high in eyes, liver, brain and heart.

The copper content of tissue declines when the diets of carp and rainbow trout are devoid of , copper. Copper toxicity cause damage to gills and necrosis to liver and kidney. Higher copper levels depress growth and impaired feed conversion in channel catfish. However, in the same fish copper deficiency affected the activities of cytochrome oxidase in heart and copper zinc superoxide dismutase in liver. The copper requirement in fish ranges from 3-5 mg/ kg dry diet. The requirement for copper depends on physiological state of animal, copper content of the water, and the levels of zinc, iron, calcium and molybdenium,which are metabolic antagonists of copper.Plant and animal protein feed ingredients contain 5-30 mg/kg, whey products and fish soluble, are relatively rich sources of copper, providing over 85mg/kg. Metal contaminations can cause wide variation in the copper content of processed feed ingredients.

Selenium

Selenium is an integral component of glutathione peroxidase. The active level. of this enzyme in liver or plasma is indicative of Selenium supply to the organism. Selenium along with vitamin E is essential to prevent nutritional muscular dystrophy. Selenium is implicated in the metabolism of tocopherol compounds.Both acts as antioxidant substances in fish.Selenium deficiency results growth depression, loss of appetite, lethargy, reduced muscle tone and mortality. Selenium acts as the principal factor in the protective mechanism against oxidative cellular injury. In the liver the glutathione peroxidase activity is lowered, while glutathione transferase activity and pyruvate kinase activity in plasma were increased. Deficiency signs in channel catfish involved interaction between vitamin E and Selenium, but not when either or alone was deficient. High levels of Selenium in the diet have toxic effects, resulting in reduced growth, feed efficiency and increased mortality. Trout when given more than 10mg Se/kgdeveloped renal calcinosis. Renal tubules were degenerated, leading to inflammation.Fish derive Selenium from both diet and water. High levels of Selenium in water are toxic to fish. Selenium is stored in various tissues, except liver, in its iorganic form. Selenium as selenite is effectively taken up through the gills. Selenium and vitamin E are complement to each other activity, and protect biological membranes against lipid oxidation.

Fishmeal and marine by-products as the rich sources of Selenium to fish. Plant materials vary widely in their Selenium content. Among the source of Selenium selenomethionine was the most digestible (92%), and fishmeal (47%) the least digestible sources of Selenium. The glutathione peroxidase Selenium ration indicated that Selenium supplied as selenite or selenocystine was a better source for plasma glutathione peroxidase than was Selenium from selenomethionine. Though the comparative availability of Selenium is poor, still fishmeal-based diets generally provide sufficient selenium to satisfy the nutritional requirement of fish.

Conclusion

Trace minerals are essential for fish and is involved in the normal metabolism and life processes. The minerals are required in extremely small amounts in the diet but in excess causes toxicity. In deficient condition, growth is retarted in general, and imparis normal metabolism in particular. It is also a matter of concern about the potential causes of conditioned trace element deficiencies such as food processing methods, dietary interactions, disease conditions and genetic disorders. Fish can absorb part of the required minerals directly from the water through gills or even through their entire body surface. The' minerals absorbed from water do not meet the total requirement and a certain supplementation through the diet is required whether in the natural food or supplementary feed. Once this requirement is met, the normal growth, survival and essential cellular metabolism may be ensured.

Farm made Feed in Aquaculture

B. N. Paul
ICAR-Central institute of Freshwater Aquaculture
Regional Research Centre, Rahara, Kolkata-700118

Farm made feed materials are those that can be used without processing them mechanically. These include poultry viscera, fish gills and viscera, kitchen refuse and bread. These feed materials can be directly dumped into ponds upon arrival at the farms. Poultry viscera have been regarded as one of the most effective feeds for grower catfish. On a dry matter basis, this ingredient contains 52.9% protein and 42.4% lipid (New 1987). Besides being nutritious, poultry viscera are highly palatable to catfish and naturally float on the water surface. Catfish fed on poultry viscera are fatty with shiny yellowish-skin, which is in high demand. The demand for poultry viscera by catfish farmers is quite competitive. Larger farms with capital resources gain access to consistent supplies of poultry viscera from chicken slaughterhouses year-round by winning contracts at auction. Small farms, which need smaller quantities of poultry viscera either collect them from poultry stalls in the market or buy from larger farms. The price of poultry viscera ranges from US$ 0.02-0.20/kg, depending upon the bargain struck. The feed conversion ratio for poultry viscera is approximately 4:1 which means that the feed cost could be less than US$ 0.80/kg of catfish produced.

New (1992) defined farm-made feeds as small-scale feed manufacture encompassing everything from simple hand-formed dough balls to small feed production units. Farm-made feed is an alternative to commercial feeds. The majority of farm-made feeds are prepared by catfish farmers who began their operations prior to the availability of commercial feeds in 1980. These farmers have good experience in processing trash fish-rice bran diets. There is no standard composition of current farm-made feeds for catfish. They may be composed of a single or many sources of raw materials processed by cooking or grinding to some extent. The diversity of the composition of farm-made feeds is dictated by the availability of the individual raw materials. The formulation in farm-made feeds may vary, depending on the amount of each raw material delivered to the farm each day.From the farmers' point of view, the quality and proportion of feed is not as important as the amount required to feed the ponds. The success of certain farms in using farm-made feeds is usually determined by the availability and cost of raw materials.The majority of farm-made feeds for Clarias catfish are moist, comprising a variety of unconventional raw materials. Their composition and nutritional value is therefore highly variable from place to place. Although making moist farm-made feeds is practical and cost-effective for certain farms, it may not be so for others since the raw materials used are unconventional.

Rice polishings, though rich both in protein and fat, are available only in small quantities, constituting less than 10% of the total quantity of rice bran. Most often the rice bran is adulterated with husk, mainly contributing fibre. As indicated earlier, rice bran is now extensively used for oil extraction and hence, in several regions, only the defatted rice bran is available for aquaculture use. Even in the case of oil seeds, solvent extraction processes are increasingly used, hence most (for which there is a heavy demand from the aquaculture sector) contain little fat. Among the various types, groundnut cake and mustard oil cake are the most widely used feed ingredients in aquaculture. However, the use of other oil cakes is also gaining popularity due to cost factors as well as the seasonal non-availability of the two types of oil cakes conventionally used. Among those Til oil cake has also made a mark as one of the potential ingredient for farm made feed.Sorghum and maize are used to a smaller extent than rice bran, principally as carbohydrate sources. Tapioca flour, which is rich in carbohydrates, is mainly used as a binder. Spirulina is rich in protein as well as vitamins and minerals. Among the few animal feed ingredients available fish meal is considered the most important. Its composition varies widely, depending on the method of handling and processing. As a result the supply of standard quality fish meal is not assured. Only one factory in Karnataka is reported to produce fish meal with more than 60% protein. In all other places, besides having a low protein content, fish meals contain high amounts of fiber, mainly due to the inclusion of other products such as *Squilla* and shrimp wastes. The high ash content of fish meal is often due to contamination with sand since most is still produced by the traditional method of beach drying, followed by grinding.

Several of the companies producing good quality fish meal export it, making only low grade fish meal available in local markets. Similarly, the proximate composition of shrimp wastes also indicates differences in the proportions of shrimp head and shell. *Squilla* meal, produced largely on the west coast, is frequently contaminated with sand particles. However, these crustacean wastes are good sources of nutrients in shrimp diets (Ahamad Ali and Mohamed 1982).Another upcoming industry in India is sericulture, producing a major by-product, silkworm pupae. Pupae contain significant protein and fat levels and have been found to be an excellent dietary ingredient for freshwater fishes (Jayaram and Shetty 1980). Silkworm pupae are rich in unsaturated fatty acids. Due to an increasing demand for oil from various industries, this ingredient is also extracted. Defatted silkworm pupae, with a high percentage of protein, is presently used in the poultry industry. Other useful animal feed ingredients, like clam meat, squid meat and meat meal, are available only in small quantities, and all are also important sources of human food.Presently there is no organized collection of wastes from slaughterhouses. However, due to the good nutrient content of such wastes and the increasing demand for animal protein, it is anticipated that they will be used in aquaculture. Meat meal and liver meal are only used to a minimal level in shrimp culture due to their high cost and their demand for human food. Earthworm meal, though potentially a good source of animal protein, is not yet available in commercial quantities. Some efforts have been made to promote earthworm cultivation for the production of wormicompost, but these have not yet been successful.

Fish are mainly fed rice bran mixed with a small amount of oil cakes of various origins in an uncooked form. The feeding rate varies from 1-3% and fish are fed by broadcasting once a day, generally in the morning. National average pond production of farms assisted by the Fish Farmers Development Agencies has been reported to be 1,895 kg/ha/yr (Srivastava *et al*. 1990). A survey of these farms has identified seed and feed availability as the major constraints faced by farmers. Poor availability of feed ingredients in adequate quantities and their escalating cost have prevented small-scale farmers adopting regular feeding.

Farm made feed demonstration at Belur Math, Howrah

Under the Outreach Activity on Fish Feeds, two ponds were selected at Rama Krishna Mission, Belur Math, to give on hand training to 30 trainees of Samaj Sevak Sikshan Mandir (SSSM) on farm made feeds. Selected ponds were a) 0.08 h size at SSSM and b) 0.1 h size at Shilpamandira under the Sarada Pith unit of Belur Math. The catla and rohu juveniles were stocked @ 4500 nos./h with rohu and catla at a ratio of 90 : 10 respectively. The stocking size of rohu was 80-90 g and for catla was 220-245 g. The fish seed was procured from local farmers. The objective of the programme was to train the vocational trainees of RKM Samaj Sevak Sikshan Mandir in learning by doing mode.

Locally available low cost feed ingredients were identified and feeds were formulated and prepared at the pond site. Diet was formulated as per NRC (1993).For the demonstration programme two different feeds were formulated by surveying the local oil mills for availability of suitable low cost fish feed ingredients. Two mash feeds were prepared using rice bran, till oil cake, mustard oil cake and linseed sludge in different proportions for feeding the fish in the ponds. Suitable feed dispensing mechanism were also evolved, in which nylon bags were suspended on galvanized wire and connected by a small, pulley so that trainees can feed the fish by standing on the pond site. The sampling of the fish was done at regular intervals. Both the two groups of fish were fed twice @ of 2% of the body weight every day for a period of 8 months. The weight of the feed was increased in relation to the increase in weight of fish so as to maintain the feed @ 2% of the body weight. Monthly sampling was done to assess the growth of the fish. The feed demonstration result indicated that estimated net production (t/h/8months) was 4.9 and 6.8 respectively in Shilpamandira and SSSM ponds. The demonstration programme continued for 235 days and after that the fish grew to 1.5 kg for rohu and 2.2 kg for catla in Samaj Sevak Sikshan Mandir and rohu to 1.07 kg and catla to 1.6 kg in Shilpamandira pond. At the end of the demonstration the trainees of RKMSSSM developed self confidence and skill for taking up aquaculture practice as one of the avenue for self employment for their future livelihood.

There is no doubt that farm-made feeds are economically viable for farms with access to inexpensive feed materials. However, the quality and quantity of moist farm-made feed cannot be easily controlled; this results in unpredictable and unmanageable fish production. Moist feeds disintegrate easily and therefore pollute the aquatic environment. The quality and quantity of farm-made feeds can be improved by processing conventional feed ingredients into a compound form. However, most of the compound feeds made is of the sinking-type, which are not attractive to farmers. However, the cost and palatability of each type of feed, the production management system in use, and the farm-gate value of fish need to be considered prior to feeding the fish.

Figure 1: Preparation of Farm Made Feed

Figure 2: Feed dispensing through bag

Aqua Feed -Market Trend

Amit Tandon, Growel Feeds (P)Ltd

Presently India is the second largest fish producing and second largest aquaculture nation in the world. India is also a major producer of fish through aquaculture and ranks second in the world after China. The total fish production during 2015-16 is at 10.79 million metric tonne (MMT) with a contribution of 7.21 MMT from inland sector and 3.58 MMT from marine sector. The fish production during first three quarters of 201617 has also shown an increasing trend and is estimated at 8.18 Million Tonnes.The fish production has increased from 3.84 MMT in 1990-91 to 10.79 MMT in 2015-16 . The growth in fish production has shown a cyclical pattern with an increasing long-term trend.

Fisheries is a sunrise sector with varied resources and potential, engaging over 14.50 million people at the primary level and many more along the value chain. Transformation of the fisheries sector from traditional to commercial scale has led to an increase in fish production from 7.5 lakh tonne in 1950-51 to 107.95 lakh tonne during 2015-16, while the export earnings from the sector registered at Rs. 30,420.83 crores in 2015-16 . The historical scenario of Indian fisheries reveals a paradigm shift from marine dominated fisheries to a scenario where inland fishery has emerged as a major contributor to the overall fish production in the country.

India is emerging as a significant producer of commercial aquafeeds. The thriving Indian aquaculture industry is the main advantage for aquafeed manufacturers. However, with majority farmers feeding aquatic species with traditional feeds, such as rice/wheat bran, ground nut cake, and other agro products, the transition towards commercial feed will definitely take time. The report anticipates the fish feed market to grow at a CAGR of around 20% during 2012-13 to 2014-15. However, the more advanced shrimp feed market is forecast to post a CAGR growth of around 12% during the same time period. The forecasts are drawn from analysis of past and present market trends, drivers, challenges and recent developments. The report shows the historic, current, and expected future trends of fish and shrimps production across the country, market size of fish and shrimp feeds and key drivers and restraints hampering the aquafeed market. Government initiatives to boost the aquaculture and aquafeed industry have also been included.

India has around 30 aqua feed mills spread across the country. However, their production capacities are only 43.4 per cent with a total production of 1.25 million tonnes. To meet the requirement. With the introduction of vannamei shrimp, the cultured shrimp production is growing at more than 30% percent. The demand from shrimp will drive the growth of the aqua feed industry at five to six per cent annually. The feed demand only from shrimp and fresh water shrimp is around 1 million tonnes. India currently has nearly 20 feed mills that can produce modern feed for the shrimp sector with a production capacity of 1.33 million tonnes. On fish feed, the current installed capacity is 1.55 million tonnes. The total feed production for shrimp and fish stands at 572,000 tonnes and 684,000 tonnes respectively, the report adds. Shrimp production growth has driven consumption growth by 13 per cent between 2007-08 and 2016-17 reaching almost 700,000 tonnes. Disease outbreaks remain one of the major risks affecting the growth of the aquaculture feed industry.

Freshwater fish feed demand has grown rapidly, driven by the growth in the freshwater fish segment in India. Current freshwater fish feed production is estimated at 0.7 million tonnes and significant investment has gone into the creation of capacities. It is expected that the demand for fresh water fish feed is expected to grow at five to six per cent in the near term.

Emphasising on emerging markets for aqua feed, that current market players and feed mill investors are looking for market diversification options to increase feed capacity utilization. Currently, Andhra Pradesh constitutes the major share in the domestic market, accounting for feed sales at almost 80,000 tonnes per month followed by other states with below 8000 tonnes sale a month. After 2008 many aqua feed mills have started manufacturing floating/pellet feeds for the farming of freshwater fish.

Cheaper feed ingredients like deoiled rice bran, wheat bran, cottonseed cake and groundnut cake are extensively used in freshwater aquaculture. Both conventional and non-conventional feed ingredients are used in feed formulation for shrimps.

Feed ingredients like fishmeal, squid meal, lecithin, cereal flour and other essential and propriety additives and fish oil are also used in prawn and shrimp feed formulations.

Freshwater fish are fed with farm made feeds which consists of a mixture of rice bran, wheat bran and oilseed cakes, the report said.

Globally, the aqua feed market has witnessed an exponential growth owing to factors such as growing consumption of seafood and growth of the aquaculture industry as a whole. India along with US, China and Brazil are the leading aqua feed producers across the world.

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Selection of Ingredients and Feed Formulation in Aquaculture

B.N.Paul
ICAR-Central Institute of Freshwater Aquaculture
Regional Research Centre, Rahara, Kolkata-700118

Introduction

Aquaculture has been developing from traditional extensive system to semi-intensive and intensive ones basically by the increase of stocking density to maximiseutilization of culture space. Once this exceeds ‘natural’ carrying capacity, inputs are needed to maintain the growth of cultured species. Feed is considered to be the major constituent of culture inputs. The higher the culture technology applied, more the culture system relies on exogenous feed supply and natural food becomes less significant. Thus the dependence switches away from natural food and a basic understanding of the nutrition and dietary requirements of the cultured species becomes essential to develop a good formulated feed for achieving optimum growth.

Selection of Ingredients

Feed ingredients are selected mainly on the basis of cost, nutrient composition, availability and physical properties. These include principally by-products of agricultural and food processing industry. The main factors determining which sources of feed are best to feed or best to use in a particular location include the fish species to be farmed, the geographic availability of potential feed components, quantities available (including seasonal fluctuation, competition for use in domestic animal feed, price, transport, storage and so on). Indicative data on proximate composition, the primary means of evaluating feed ingredients can now be obtained world-wide for almost any conceivable product from catalogue information on feed ingredients. The system of nomenclature is called international . Feed Vocabulary (IFV) and gives each product an unique International feed number. The system divides feed ingredients into eight classes (Table – 1) indicating their composition and use in formulating feeds.

Table 1. Classification of feed Ingredients

Code No.	Class of ingredients
1.	Dry forages and roughages
2.	Pasture, range plants, forages fed green
3.	Silages
4.	Energy feeds – Less than 20% protein; Less than 18% crude fibre
5.	Protein supplement – more than 20% protein
6.	Mineral supplement
7.	Vitamin supplement
8.	Additives – including colouring matter, hormones, flavours, etc.

Adapted from NRC, 1983

In the compounding of carp feeds (BIS,FAD, 2013) a variety of ingredients are used and they are as follows

1. **Grain and seeds**
   a) Maize
   b) Barley
   c) Bajra
   d) Wheat
   e) Jowar
   f) Oats
   g) Ragi
2. **Grain By-products**
   a) Rice bran or solvent extracted rice bran and polishing
   b) Wheat bran
   c) Maize gluten and maize gluten feed
   d) Grain sieving

3. **Oil Cakes and Meals**
    a) Copra cake, coconut cake (expeller-pressed or solvent extracted)
    b) Cottonseed oil cake (decorticated) (expeller-pressed or solvent extracted)
    c) Groundnut oil cake (expeller-pressed or solvent extracted)
    d) Maize germ cake
    e) Mustard and rape seed cake
    f) Neem seed karnel cake
    g) Sal seed meal, solvent extracted
    h) Safflower *(Canthamus tincotius)* (expeller-pressed or solvent extracted)
    i) Sesame (Sesamum indicum orientale) cake (expeller-pressed or solvent extracted)
    j) Soyabean meal (Solvent extracted)
    k) Sunflower oilcake (decorticated or undecorticated)
    l) Niger seed oilcake
4. **Tubers and Roots**
    a) Tapioca flour
5. **Animal Products**
    a) Blood meal
    b) Fish meal
    c) Liver residue
    d) Meat meal and meat scrap
    e) Meat-cum-bone meal
    f) Fish viscera
    g) Chicken viscera
    h) Silk worm pupae
    i) Shrimp and shrimp head meal
    j) Mollusc meat

6. **Minerals and Vitamins**
    a) Dicalcium phosphate
    b) Common salt
    c) Vitamins (mineral stabilized)

7. **Waste Material and Industrial By-products**
    a) Brewers grain
    b) Dried yeast and yeast sludge
    c) Molasses

Feed Formulation

It is a process which has to address two principal objectives. The first is a problem of economics where feed formulation is performed to achieve optimal production. This refers to high quality animal production in which animal well being is taken into account as well as farmers profit and consumer's preference. The second is the calculation to find the types and amounts of ingredients to be mixed to produce complete feeds. Two basic areas of knowledge are involved i.e. specific nutrient requirements and ingredient selection.

To achieve optimal production most feed formulation fall between two extremes (Hardy. 1989). One extreme is to base the formulation primary on cost and chemical composition producing a feed that is less expensive than other feeds. The other extreme is to bass the formulation primarily on nutritional value thereby producing a more expensive feed that is more productive thus requiring less feed per unit of fish producer. An example of calculation has been given for selecting a best buy ingredient. Further, it is needed to mix selected ingredients to meet specific nutrient requirements.

Table 1. Important Feed formulations for various carp species

Stage	Feed	Ingredients	Composition (%)
Spawn	1	Finely powered soybean meal	10
		Finely powdered groundnut oilcake	32
		Finely powdered fish meal	20
		Finely powdered rice-bran	30
		Vitamin and mineral premix	2
		Phospholipid (as soya lecithin)	4
		Veg. Oil : fish oil (1:1)	2
Fry	2	Groundnut oilcake	28.0
		Soybean meal	20.0
		Rice-bran	30.0
		Meat meal	20.0
		Vitamin mineral mix	2.0
Fingerlings and growers	1	Soybean meal	7.0
		Groundnut oilcake	30.0
		Mustard oilcake	35.0
		Rice-bran	26.0
		Vitamin-mineral premix	2.0
	2	Groundnut oilcake	40.0
		Soybean meal	20.0
		Fish meal	8.0
		Rice-bran	30.0
		Vitamin-mineral premix	1.5
		Vegetable oil	0.5

CIFA has commercialized a carp feed under the brand name CIFACA. The feeds are prepared using ingredients like rice bran, groundnut cake/ mustard cake, soybean meal, fishmeal, mineral-vitamin premix.The selection of ingredients should go for high digestibility and low cost. The feed manufacturers are required to use large quantities of alternative protein sources to replace fish meal partially or completely. Aquaculture feed industry must be granted a status of parity with poultry and cattle feed industries. Feed formulations should be done as per nutrient requirements of the fish species.

Feed Care and Storage

Proteins, lipids and vitamins are especially heat sensitive, and can be readily denatured by high storage temperatures. High moisture stimulates mold growth and feed decomposition. Feeds, when produced in bulk can be stored for some days. Older feed should be used first, and all feed should be regularly inspected for mold prior to feeding. Some of the tips for storing the feed are given bellow.

- Feed pellets are to be used as fresh as possible.
- Polythene or gunny bags are normally used to store the feed pellets.
- Feed storage room should be dry and well ventilated.
- Feed bags are to be stored on wooden platform.
- Feed storage facility should be free from rodents,insects and pests.
- Sun drying of feed pellets are required once in every fortnight to avoid the fungal growth and to maintain better keeping quality.
- Fungal infested feed should not be used for feeding the fish.
- The feed is best used within the three months of its preparation.

Role of Vitamins in Fish Culture

B.N.Paul
ICAR-Central Institute of Freshwater Aquauclture
Regional Research center, Rahara. Kolkata-700118

Vitamins are the organic compounds required in the diet in relatively small quantities for growth, health, proper body metabolism and normal physiological functions of all living beings. Vitamins are classified as water-soluble and fat-soluble forms. Eight of the water-soluble vitamins, which are required in relatively small amounts, have primarily coenzyme functions, and these together are known as the vitamin B complex. Three of the water-soluble vitamins-choline, inositol, and vitamin C are required in larger quantities and have functions other than co-enzymes. Vitamin A, D, E, and K are the fat-soluble vitamins that function independent of role of co-enzymes. In mammals the absence of vitamins leads to characteristic deficiency diseases, but in fish such diseases are less specifically identified. Both qualitative and quantitative vitamin requirements of fish have been determined by feeding chemically defined diets deficient in a specific vitamin.

Each of the fat-soluble vitamin A, D, E and K each occur in different chemical forms having physiological activity. These are absorbed in the intestine of animals along with dietary fats; therefore, conditions favourable for fat absorption also enhance the absorption of fat-soluble vitamins. The animals store these if dietary intake exceeds metabolic needs.

Vitamin A: Vitamin A is derived from certain carotenoids, which are also designated as pro-vitamin A. most of precursors of these carotenoids are alpha, beta and gamma carotene and cryptoxanthin. Vitamin A consists of three biologically active molecules; retinol, retinal and retinoic acid. Each of these compounds is derived from the plant precursor molecule as ß-carotene. Ingested ß-carotene is cleaved in the lumen of the intestine and liver by ß-carotene dioxygenase to yield retinal, which is reduced to retinal by retinal dehyde reductase, as NADPH requiring enzyme within the intestine . transport of retinal from the liver to extrahepatic tissues occurs by binding of hydrolysed retinol to aporetinol binding protein (RBP), the retinol-RBP complex in these transported to the cell surface within the golgi and secreted plasma transport of retinoic acid is accomplished by binding to albumin.

Positive Function: Vitamin A is required to maintain epithelial cells. It stimulates new cell growth and acids in maintaining resistance to infection. It is required in vertebrates for the regeneration of light sensitive rhodopsin in the retina. Vitamin A is required in invertebrates for the regeneration of light sensitive rhodopsin in the retina. Fish have variable abilities to hydrolyze ß -carotene into retinol (Katsugama and Matsumo, 1988).

Syndrome of Deficiency or Excess: Hypovitaminosis A is characterised by poor growth, poor vision, keratinization of epithelial tissue, xerophthalmia, night blindness, haemorrhage in the interior chamber of the eye, haemorrahage at the base of the fins, and abnormal bone formation (Halver, 2002). Nerve degeneration has been reported in fish after long periods of deficiency. Hypervitaminosis A has been described in fish with the enlargement of the liver and spleen, abnormal growth, skin lesions, epithelial keratinization, and abnormal bone formation, resulting in ankylosis and fusion of vertebrae. The serum alkaline phosphates is enhanced due to addition of excess vitamin a in fish feed.

Sources and Requirement: Fish oils are the rich sources of vitamin A. Out of the available sources, black sea bas, sword fish and ling cod oils contain very high amount of vitamin A. Synthetic vitamin A palmitate is available and can be used to supplement fish feed.It has been shown that ß-carotene and canthaxanthin can be biotrasformed in the liver of tilapia into vitamin A_1 and that dihydroxycarotenoids such as astaxanthin, zeaxanthin, lenthin and tunaxanthin are directly bioconverted into vitamin A_2 (Katsuyama and Matsuno, 1988). Some fish species seem to be able to utilize ß-carotene molecule and vitamin A must be added to the diet in the retinol, retinene or retinoic acid form. The vitamin a requirement for carp and Channel catfish is 1000-2000IU (Halver, 2002) and for rohu is 2000 IU (Rangacharyulu et al., 2000).

Vitamin D: Biologically active forms of vitamin D are vitamin D_2 or erogocalciferol and D_3 or activated 7-dehydrocholesterol. Vitamin D (cholecalciferol) is formed in most animal tissue by rupture of one of the ring bonds of 7-dehydrocholesterol when exposed in the skin to ultraviolet radiation.

Positive Functions: Vitamin D functions as a precursor of 1, 25-dihydrocholecalciferol, which stimulates the absorption of calcium from the intestine. Vitamin D is essential for maintaining homeostasis of calcium and inorganic phosphate. Vitamin D regulates alkaline phosphates activity, which promotes intestinal absorption of calcium, and influences the action of parathyroid hormone on bone. Cholecalciferol has been reported to be three times more effective than ergocalciferol in supplying the vitamin D needs of trout and catfish.

Syndrome of Deficiency or Excess: Several workers have described Hypo-vitaminosis D for fish. Rickets and abnormal bone formation have been observed in fish fed on low vitamin D diets in low calcium water. High intake of vitamin D mobilizes Phosphorus and Calcium from the bones and tissues and may result in fragile bones.

Source and Requirement: The vitamin D requirements of many animal can be met by exposure of the skin to sunlight, which activates cholesterol derivatives by ring structure rupture. Since the vitamin is fat soluble and accumulates in lipid stores, fish liver oil is a rich source of the material.

The requirement of vitamin D for channel catfish is 500-1000 IU (Halver, 2002). The actual requirement of vitamin D for maintenance of homeostasis of calcium -phosphorus levels in young growing animals has not been adequately investigated and the true vitamin D requirements of young fish have only been suggested (NRC, 1993).

Vitamin E : The vitamin E is a compound group known as tocopherols and is derivatives of tocol that has a saturated side chain, or of tocotrinol, which contains three unsaturated carbon-carbon bonds in the side chain. There are about eight naturally occurring tocopherols and tocotrienols with vitamin E activity, which have so far been identified. They differ from each other in the number and position of the methyl groups round the ring of the molecule. All have the same physiological properties, although α-tocopherol is the most active and this is the main tocopherol in animal tissue and that is why tocopherol in this form is now synthesized commercially. The pure tocopherols are fat-soluble oils, which are capable of esterification to form crystallilne compounds. The tocopherols are stable to heat and acids in the absence of oxygen but are rapidly oxidized in the presence of oxygen, peroxides, or other oxidizing agents (Dam and Sondergaard, 1964). The tocopherols are sensitive to UV light and are excellent antioxidants. The esters are more stable and are commonly used as dietary supplements, anticipating hydrolysis in the gut and absorption of the free alcohol to act as the and active intra and intercellular antioxidant (Halver, 2002). Vitamin E is added to feeds as DL α-tocopheryl acetate, which is and acetate ester of α-tocopherol.

Function: Vitamin e acts as natural inter and intracellular antioxidants to maintain the homeostasis of labile metabolites in the cell and tissue plasma. It protects the oxidisable vitamins and unsaturated fatty acids, mostly present in all cell wall components. All tocopherols are amphipathic molecules: the hydrophobic phenyl tail associates with membrane lipids and the polar chromanol head groups are exposed to the membrane surface. It appears to be the first line of defence against peroxidation of PUFA contained in cellular and sub-cellular membrane of phospholipids. The phospholipids of mitochondria, endoplasmic reticulum and plasma membranes possess affinity for α-tocopherol and the vitamin e appears to concentrate at these sites. It functions together with selenium and ascorbic acid in the enzymes of glutathione peroxidase and superoxide dismutase to stop the chain reactions of PUFA peroxidation. It acts as free radical traps to stop the chain reactions during peroxide formation and stabilize unsaturated carbon bonds or PUFAs and other long chain labile compounds. It is involved in maintaining the stability of ertythrocytes cell wall, normal permeabililty ofj capillaries and heart muscle. Vitamin E is also involved in embryo membrane permeability land hatchability of fish eggs.

Syndrome of Deficiency or Excess:The deficiency signs for fish fed on normal amounts of polyunsaturated fatty acids is erythrocytes fragility and fragmentation of immature erythrocytes, and variable size erythropoiesis, fragmentation of erythrocytes, marked susceptibility to stress handling, ascites, and exudative diathesis have been reported as deficient syndrome in salmon, carp and trout (NRC, 1993). Muscle dystrophy and xerophthalmia have been described in yellow tail and carp. Hypervitaminosis E involves poor growth, toxic liver reaction and death.

Sources and requirement : Wheat germ and cotton seed cake are the rich sources α-tocopherol. The tocopherol contents of different oils are as follows: coconut oil (8 mg %), soybean oil (130 mg %) and wheat germ oil (260 mg %). Out of total tocopherol 60% is α-tocopherol.

The requirement of vitamin E in carp is 80-100 (mg/kg) (Halver, 2002), rohu is 131.9 (mg/kg) (Sau et al., 2004) ,mrigal 99.0 (mg/kg) (Paul et al., 2004) and Catla 98.0(mg/kg) (Paul et al., 2005)

Vitamin K : Vitamin K exists naturally as K_1 (phylloquinone) in green vegetable and K_2 (menequinonoe) produced by intestinal bacteria and K_3 is synthetic menadione. When administered, vitamin K_3 is alkylated to one of the vitamin K_2 form of menaquinone. These are fat-soluble fairly stable compounds and complexes, which may interfere with physiological activity.

Positive functions: Vitamin K is involved in the synthesis of mRNA in the synthesis of blood clotting proteins – prothrombin, plasma thromboplastin, proconvertin and blood clotting factors II, VII, IX and X substituted forms of vitamin K are strongly bacteriostatic and may serve as an alternate defence mechanism for bacterial infections. Vitamin K is involved with vitamin A, E (Dam and Sondegaad, 1964) and vitamin K may be involved in co-enzyme Q-type compounds, which function between flavor proteins and cytochromes in electron transport mechanisms. The primary role of vitamin K is to maintain a fast normal blood-clotting rate, which is very important to fish, living in a water environment.

Syndrome of Deficiency of Excess: The prothrombin time in salmon fed on diets devoid of vitamin K was increased 3 to 5 times, and during prolonged deficiency states, anaemia and haemorrhage areas appeared in the gills, eyes and vascular tissues. Increased blood clotting time has also been reported for other fish reared on diets with a low vitamin K content. Haemorrhage areas often appear in fragile tissues such as the gills. Trout can tolerate intake of vitamin K at 2000-3000 mg/kg diet but higher levels may cause live toxicity and death.

Sources: Green leaf vegetables, and alfa alfa leaves are the rich sources of vitamin K. Low levels are found in soybeans ,and animal livers.Synthetic menadione is agood supplement for adequate vitamin K intake. The diet should be kept dry prepared with minimum exposure to air oxidation and fed as soon as practicable after manufacture to minimise vitamin K loss through storage interaction, and oxidative destruction. Menadione sodium bisulphite and me'nadione demethyl pyrimidol bisulphite are commonly used as vitamin K supplements in fish diets (Halver,2002).

Water Soluble Vitamins

Thiamin : Thiamin is part of the co-enzyme cocarbxylase (Thiamin pyrophosphate) which participates in the oxidative decarboxylation of a α-ketoacids, especially pyruvic acid, eventually releasing carbon dioxide. Thiamin pyrophorphate, a co-enzyme for the transketolase system, is part of the direct oxidative pathway of glucose.metabolism. It is retained longer in muscle tissue than in brain tissue. It is essential for good appetite, normal digestion, growth, and fertility. It is needed for normal function of nervous tissue and the requirement is determined by the caloric density of the diet.

Riboflavin : Riboflavin functions in the tissues in the form of flavin adenine dinuclotide (FAD) or as flavin mononucleotide (FMN). The flavoproteins function as enzymes of tissue respiration and are involved in hydrogen transport to catalyze the oxidation of reduced pyridine nucleotides (NADH and NADPH). Thus, these function as coenzymes for many oxidases and reductases such as cytochrome-C.-reductase, D-and L-amino acid oxidase, xanthine and aldehyde oxidase; succinic and fumaric dehydrogenase. Riboflavin is involved with pyridoxine in the conversion of tryptophan to nicotinic acid and is most important in respiration of poorly vascularized tissues such as the cornea of the eye. Riboflavin is involved in the retinal pigment during light adaptation.

Pyridoxine: Pyridoxal phosphate is the coenzyme, codecarboxylase also involved in the transaminase systems which have shown to require a distinct enzyme with pyridoxal phosphate as the coenzyme. It is the co-enzyme for decarboxylation of 5-hydorxytryptophan to produce serotonin to cysteine to pyruvic acid by desulfhydrase. It is the cofactor for synthesis of L - amino-levulinic acid, the primary structure of porphyrin. It is involved and essential for tryptophan utilization and metabolism of glutamic acid, lysine, methionine, histidine, cysteine and alanine. It is also involved in fat metabolism, especially of the essential fatty acids. It is also involved in systhesis of mRNA, So, the vitamins B6 play a most important role in protein metabolism and as a result, carvivorous fish have stringent requirements for pyridoxine in the diet and stores are rapidly exhausted.

Pantothenic acid : It is part of acetyl coenzyme A which occurs in many enzymatic processes involving twocarbon compounds. It has been shown to be required by all animal species studied. The acetyl coenzyme A system is involved in the acetylation of aromatic amines and choline, condensation reactions for synthesis of acetate, fatty acids and citrate, the oxidation of pyruvate and acetaldehyde, and is essential for the development of the central nervous system. Patothcnic acid is involved in adrenal function and for the production of cholestrol. Coenzyme A is also involved in many other steps of intermediate metabolism of carbohydrates, fats, and proteins. So it is obviously a key nutrient for normal physiology and metabolism of a growing fish.

Niacin : The major function of niacin in NAD and NADP is the removal of hydrogen from substrates and the transfer of hydrogen or electrons to another coenzyme in the hydrogen transport series. Most of these enzyme systems function by alternating between the oxidized and reduced state of the coenzymes NAD-NADH and NADP-NADPH. These oxidation reduction reactions are anaerobic. The second type of oxidation- reduction reactions is coupled to election transport with subsequent oxidation of reduced NADH or NADPH, and these are aerobic reactions, which function in respiration. Both NAD and NADP are involved in synthesis of high-energy phosphate bonds, which furnish energy for certain steps in glycolysis, in pyruvate metabolism, and in pentose synthesis. It is also involved in lipid metabolism, amino acid and protein metabolism.

Ascorbic acid :L-ascorbic acid as a biological reducing agent for hydrogen transport. It is involved in many enzyme systems for hydroxylation, i.e. hydroxylation of tryptophan, tyrosine or proline. It is involved in detoxification of aromatic drugs and also acts in the production of adrenal steroids. It is necessary for the formation of collagen and normal cartilage as well as normal tooth formation, bone formation, bone repair and wound healing. It acts synergistically with vitamin E and selenuim to maintain activity of glutathione peroxidase and superoxide dismutase. The conversion of folic acid to folanic acid requires vitamin C for the active coenzyme from. It is also involved in the formation of chondroitin sulfate fractions and intercellular ground substance, and is capable of forming sulfate derivatives with very stable chemical characteristics.

Table 1. Vitamin deficiency syndrome

Vitamins	Deficiency signs
Thiamin	Poor appetite, poor growth, muscle atrophy edema, skin congestion and subcutaneous hemorrhage, convulsions, instability, increased sensitivity to shock by physical blow to container or from light flashes, and loss of quilibrium
Riboflavin	Poor appetite and poor feed efficiency, followed by photophobla, uniorbitateral cataracts, and general anemia. Striated constrictions of the abdominal wall and skin atrophy with abnormal pigmentation of both skin and iris has been noted in some fish species. Short body dwarfism has been reported in catfish.
Pyridoxine	Nervous disorders, epileptic fits, hyperirritability, ataxia, anemia, loss of appetite, edema of peritoneal cavity, colour less serous fluid, rapid postmortum rigor mortis, rapid and gasping breathing, flexing of opercles found in almost all the culture species.
Pantothenic acid	Clubbed gills, prostration, loss of appetite, necrosis and scarring, cellular atrophy, gill exudate, sluggishness and poor growth.
Nicotinic acid	Loss of appetite,lesions in colon, jerky movement, weakness, edema of stomach and colon, muscle spasm while resting and poor growth.
Ascorbic acid	Scoliosis, lordosis, impaired collagen formation, altered cartilage, eye lesions, hemorrhagic skin, liver kidney, intestine and muscle.

Table 2. **Vitamin requirements for growth**

Vitamin (mg/kg dry diet)	Common carp	Catfish	Prawn
Thiamin	2-3	1-3	50-100
Riboflavin	7-10	9	30-50
Pyridoxine .	5-10	3	30-50
Pantothenic acid	30-40	25-50	50-100
Niacin	30-50	14	100-150
Folic acid	N R	R	5-10
V itamin B 12	NR	R	0.02-1.00
Myo-inositol	200-300		2.00-300
Choline	1500-2000	R	400-2000
Biotin	1-1.5	R	0.5-1.00
Ascorbic acid	30-50	60	200-400

Conclusion: Fat-soluble vitamins are essential in the diet of fish (Halver,1995) and Prawn (Reddy, *et al.,* 1999).They aretherefore added in feed in-minute quantities for optimum growth, health, cellular metabolism and normal physiological functions.The fat soluble vitamins differ from the water soluble vitamins in their accumulative action. Hypervitaminosis is a problem in fishandother animals when large quantities of any one of fat soluble vitamins are ingested. Fish feed often, includes large quantities of fishmeal or fish viscera and are often enriched with fish oils to increase thecaloric density or content of polyunsaturated fatty acids of the ration.In such cases, excessive intake of fat-soluble vitamins is often'encountered (Halver,2002). Therefore, fat-soluble vitamins should be used in proper dose to avoid hypervitaminosis aswell as hypovitaminosis.

Solid State Fermentation as a Function to Improve Nutritive Value of Plant Feed-Stuffs: Prospect in Aquafeed Formulation

Dr. Koushik Ghosh
Associate Professor
Aquaculture Laboratory, Department of Zoology, The University of Burdwan, West Bengal,
E-mail: kghoshbu@gmail.com; kghosh@buruniv.ac.in

Introduction

Improving feed efficiency has become the major stay in intensive aquaculture systems to maximize production. Quality of nutrients is determined by its source, i.e., the ingredients used in diet formulation that in turn determines the cost of the feed. Therefore, it is a great challenge to the aquaculture nutritionists to achieve both, increasing feed efficiency and decreasing feed cost simultaneously. Protein is the essential component in any animal tissue and constitutes about 65-75% of the dry matter in fish tissues (Wilson, 2002). Therefore, protein is considered as an indispensable source of nutrient, providing a group of essential and nonessential amino acids required for both, maintenance and growth. Feed proteins are expensive, and their inclusion in aquaculture diets has a significant role on overall feed costs. In aquaculture production, fishmeal is regarded as the main protein source in formulated diets due to its high level of protein, excellent amino acid profile, low carbohydrate level and high digestibility (Zhou et al., 2004). Demand of fish meal as the chief protein source has been increased to a great extent with intensification and expansion of diverse food production sectors, viz., farmed animals, poultry and aquaculture. Therefore, sky-high rise in price, high protein requirement in the diet and supply fluctuations of fishmeal during the last few decades has emphasized the need to look for alternative protein sources in aquafeeds (Kumar et al., 2010a). The growing demand to replace fishmeal in aquafeed has emphasized to search for unconventional, less expensive and protein rich sources. Plant proteins are deemed to be the most practicable option in this respect for reducing feed cost and environmental impact in most of the developing countries (Hardy, 2010). The use of plant-derived materials (e.g., aquatic weeds, legume seeds, different types of oilseeds and cakes, leaf meals, leaf protein concentrates, and root tuber meals) as fish feed ingredients is limited by deficiencies in essential amino acids and minerals, and the presence of diverse endogenous secondary metabolites better known as antinutritional factors (ANFs), viz., lectin, phytate, saponin, trypsin inhibitors, tannins and other polyphenols (Francis et al., 2001; Ghosh & Mandal, 2015). Even though, possibilities for uses of plant ingredients have been investigated and achieved experimental success (Kalita et al., 2007; Ghosh & Mandal, 2015). It is, therefore, important to realize the need to include different processing techniques to reduce endogenous ANFs to boost bioavailability of the nutrients so that the non-conventional plant feedstuffs might play a more significant role in formulated feeds.

Formulation of aquafeed: from history to future perspectives

Although fish have been produced in captivity for several centuries, mostly fish farming has been carried out extensively in ponds relying on the natural food organisms (Nash, 2011). Therefore, instead of detailed knowledge on the nutritional requirements of the fish, the fish farmer needed to have skills in pond management to improve fish production (McLarney, 2013). Starting from the backyard culture fish farming has emerged out as an industry since the middle of the twentieth century with the impetus to shift towards intensive farming systems. Conventionally, growth and production was the major area of interest among the fish culturists during earlier days. Keeping the focus unchanged, at present the practitioners are concerned with a broader out-look and involved in solving the basic issues that controls the growth and well being of fish, i.e., nutrition and disease. Therefore, quantitative requirements for several essential nutrients were assessed and incorporated in feed formulations to optimise growth in fish (Jobling, 2015). In addition to the experiments based on direct observation of growth at several incorporation levels for nutrients, statistical methods that rely on regression analyses and numerous non-linear models helped to work out the accurate estimation of requirement levels of the nutrients (NRC, 2011; Jobling, 2015). Although information about the nutritional requirements of farmed fish at different life history stages is far from complete, there is growing interest to formulate feed to address specific need. Development of brood-stock or larval feed to influence reproductive development or survivability are important to be recognized. There have been several reports on the influence of specific essential amino acids, vitamins and certain trace elements, although lipid and fatty acid nutrition seem to be the widely studied area (Holt, 2011; Merrifield & Ringø, 2014). Development of feeds based on fishmeal and fish oils promoted appreciable growth in most of the fish species similar to the terrestrial animal production sectors. However, expenses involved in feed formulation have directed to attract attention to formulate practical feed incorporating non-conventional and least-cost ingredients. Paradigm shift towards intensive culture also demands product evaluation, not only feed evaluation. In addition to growth, nutrient accumulation in the farmed fish should be ensured for its likely transfer to the consumers. In the coming years, aquaculture productions including fish will be viewed as not only the meagre source of nutrients, but also as the neutraceuticals. For example, o*mega-3 fatty acids* are found to occur all through the aquatic food chain and therefore, *fish* are considered as the natural sources of *omega-3 fatty acids that reduce the risk of cardiovascular diseases in human (*Mohebi-Nejad & Bikdeli, 2014*). Consequently, enrichment of the farm raised fish with vitamins, minerals or essential fatty acids might be the area of interest in the days to come. Nutritionally balanced and value-added feed will possibly hold the key to achieve success in this regard.*

Plant feedstuffs in aqua-feed formulation: impediments

Inclusion of plant protein at elevated levels or complete replacement of animal proteins by plant proteins have resulted in poor growth and feed efficiency (Lim, 1992), which are chiefly attributed to the presence of anti-nutritional factors (ANFs) and improper amino acid balance (Francis et al., 2001). Antinutrients can be defined as the substances that by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Mohanta, 2012). To reduce the damage caused by herbivory, plants have evolved a broad array of defences, majority of which are plant secondary metabolites (PSM). In fact, PSM represent a diverse group of natural products (Wink, 2004), of which some may be nutritionally valuable but mostly they have no nutritional value or with anti-nutritional properties. Herbivores prefer to avoid ingesting tannin if alternate foods are available (Wynne-Edwards, 2001). As plants have evolved defence mechanisms for their protection, many animals, microorganisms and parasites have also evolved parallel mechanisms to overcome these defences (Hartmann & Witte, 1995). Thus, defences and counter defences lay down the step for the co-evolution of plants and herbivores (Mandal & Ghosh, 2010a). The bio-activity of PSM has been extensively discussed in literature (Acamovic & Brooker, 2005). The structures of many PSM have been shaped to interact with different molecular and cellular targets that include enzymes, hormone receptors, neurotransmitter receptors and trans-membrane transporters. Thus, being ingested by the herbivores, farmed animals or fish, some of the PSM may exert considerable anti-nutritive effects that in turn affect the growth and production of the animals.

The endogenous plant-derived ANFs are reported to hinder bio-availability and digestibility of the nutrients. Important among them are tannin, phytic acid, trypsin inhibitor, lectins, saponins, glucosinolates, non-starch polysaccharides, phytoestrogens, alkaloids, antigenic compounds, gossypols, cyanogens, mimosine, anti-vitamins and phorbol-esters (Francis et al., 2001; Mohanta, 2012). Plant ingredients occurring in nature usually contain more than one ANF in different parts of the plants. As for example, soybean meal contains some ANFs, such as protease (trypsin) inhibitors, phytohaemagglutinin (lectins), anti-vitamins, phytic acid, saponins, and phytoestrogens (Francis et al., 2001). The major ANFs in canola include fibre, oligosaccharides, phenolic compounds, phytic acid and glucosinolates (Bell, 1993). *Leucaena* leaf contains a toxic non-protein amino acid, mimosine. The Indian major carps and exotic carps cultured in India are mostly herbivorous or omnivorous. Therefore, they depend on phyto- and zooplankton or other plant materials for their feeding in natural habitats. In addition, the scope for low cost feed formulation by substituting fish meal in aquafeed with the less expensive and protein rich plant ingredients has been further expanded in the tropical countries because of the availability of agro based materials not consumed by human beings. As aquaculture in the tropical countries largely depends on the culture of herbivore / omnivore carp species and tilapia, the use of plant based fish feed could be justified in the countries like India. However, before using them as feedstuffs, we should be well aware about the impediments so that the ways to overcome them would be ensured .

Solid State Fermentation: an approach for processing of plant feedstuffs

ANF-degrading capacity of the diverse microorganisms can be judiciously utilized for bio-processing of the plant derived feed resources through Solid state fermentation (SSF). SSF is a bioprocess in which microorganisms are grown on solid substances with low or minimum water level (Pandey, 1994). SSF has several advantages over submerged fermentation, like higher product titers, lower waste water output, reduced energy requirements, simplicity, simpler fermentation media, lesser space requirement, easier aeration, reduced bacterial contamination, high reproducibility, absence of rigorous control of fermentation parameters etc. (Pandey, 1994). Substrate selection in SSF depends on several factors mainly related to cost and availability, and thus may involve screening of several agro residues. Research on the selection of a suitable substrate has mainly focused on tropical agro-industrial crops and residues (Sabu et al., 2002); however, terrestrial macrophytes, seed meals, oil-cakes and aquatic weeds are less studied in this respect (Bairagi et al., 2002b; Ramachandran et al., 2005). Despite low cost, appreciable nutrient contents and abundant in the tropical countries, their use as feed ingredients (Hassan et al., 1994; Ray & Das, 1996; Bairagi et al., 2002b; Kalita et al., 2007), or, fermentation substrate for production of microbial bio-products (Bairagi et al., 2002b; Saha & Ray, 2011; Banerjee & Ghosh, 2016) have been restricted by the presence of a large number of ANFs as mentioned previously. SSF by exo-enzyme producing autochthonous fish gut microorganisms have been shown to reduce the contents of tannin or phytic acid in the plant ingredients because of the action of tannase or phytases produced by these microorganisms (Bairagi et al., 2002b, 2004; Ramachandran et al., 2005; Ramachandran & Ray, 2007; Khan & Ghosh, 2013b). The major advantages for utilization of oil cakes and terrestrial or aquatic macrophytes as substrates in SSF might be its immense economic viability and also the scope that it provides for nutrient recycling in an eco-friendly way (Khan & Ghosh, 2013b). Sustainability of the aquaculture industry depends on the availability of low-cost and high-quality feeds. The expansion of microbial biotechnology might offer a scope for preparation of high quality feeds in course of bioconversion of low-cost feed ingredients through SSF (Esakkiraj et al., 2009).

Apart from de-activation of the ANFs like tannins and phytic acid, increase in the nutrient level through microbial synthesis of essential bio-molecules might be expected during the SSF (Banerjee & Ghosh, 2016). In contrast, possible inclusion of harmful metabolites in the fermentation-product cannot be excluded during the SSF process. During the last few decades there has been an improved understanding on occurrence, diversity and likely function of the fish gut-microorganisms (Ringø et al., 2010). Fish gut-microbiota has been emphasized to play beneficial roles pertaining to either nutrition of the host fish through supplementation of extracellular enzymes or disease resistance by acting as probiotic bio-control agents (Ray et al., 2012). Phytate, tannin, protease inhibitor, cellulose and other non-starch polysaccharide degrading capacity of the fish gut-microbiota might indicate their involvement in increasing the nutritional efficiency of the host fish (for review, see Ray et al., 2012). Although, fermentative nutrition in aquatic animals is less understood (Esakkiraj et al., 2009), strategically *in vitro* processing of plant ingredients by SSF with the autochthonus ANF-degrading organisms could be suggested to decrease the level of ANFs and increase nutrient availability (Ramachandran & Ray, 2007). Moreover, the organism itself and their metabolites would not cause harm to the fish providing the basis for mutual relationship (Khan & Ghosh, 2013b; Mandal & Ghosh, 2013b, Banerjee & Ghosh, 2016). SSF is an age old practice that has gained interest at the present time due to its potential in solid waste treatment, production of secondary metabolites, novel foods and feed ingredients. It is a cheap and simple technique for value addition of agricultural wastes that is adaptable for use in small scale processes. In this process microorganisms utilize soluble sugars and organic acids, and biosynthesize essential amino acids, fatty acids and vitamins thus enhance the nutrient content of ingredients used as substrates. SSF is expected to enhance digestibility by improving bioavailability of nutrients and reducing ANFs. The basic steps involved in scaling up SSF as a bio-processing strategy are represented in **Figure 1** (Ghosh & Ray, 2017) . The key steps are as follows:

(1) Selection of suitable substrates (low cost, nutrient rich) and microorganisms
(2) Optimization of the process parameters (duration, pH, moisture etc.) for fermentation using different strains of microbes and different substrates
(3) Determination of the changes in nutritional profile of raw materials used for SSF
(4) Evaluation of the potential of fermented product as aquafeed ingredients (either as partial substitute for fishmeal or as a minor / major ingredient, or as an additive) through feeding trials

Waste utilization through the generation of value added by-products is the major benefit for using agro-industrial residues in SSF (Immanuel et al., 2010). As indicated, due to breakdown of complex molecules and bio-synthesis of several bio-active compounds by the microorganisms ameliorating the nutrient level through microbial synthesis might be expected during fermentation (Ghosh & Mandal, 2015). For example, analyses of proximate composition in the substrate (linseed oil-cake) following SSF at optimal conditions by the fish gut isolates *B. pumilus* LRF1X and *B. tequilensis* HMF6X revealed that there was minor improvement in the contents of crude protein, lipid, ash, total free amino acids and fatty acids as compared to the raw substrate (Banerjee & Ghosh, 2016). Moreover, the study revealed that SSF of the oil-cake was effective in significantly ($P<0.05$) reducing the contents of NSPs (both, cellulose and hemicellulose), crude fibre and other ANFs, e.g., tannins, phytic acid and trypsin inhibitor. Amino acid profile revealed significant increment in the levels of several amino acids(arginine, histidine, isoleusine, methyonine, phenylalanine, threonine, tryptophan,valine and cystine)in the SSF-processed oil-cake, although, leusine and lysine contents were decreased as a result of SSF. In another study, improvement of amino acid balance in the groundnut oil-cake has been reported through SSF (Ghosh & Mandal, 2015). Plant feedstuffs are usually deficient in the sulphur containing amino acids, viz., lysine, cystine and methionine. Therefore, increase in the contents of methionine and cystine in consequence of SSF seems worth to mention (Banerjee & Ghosh, 2016). Thus, as a consequence, SSF presumably assists in processing of the substrate that in turn leads to improvement of its nutritive value.

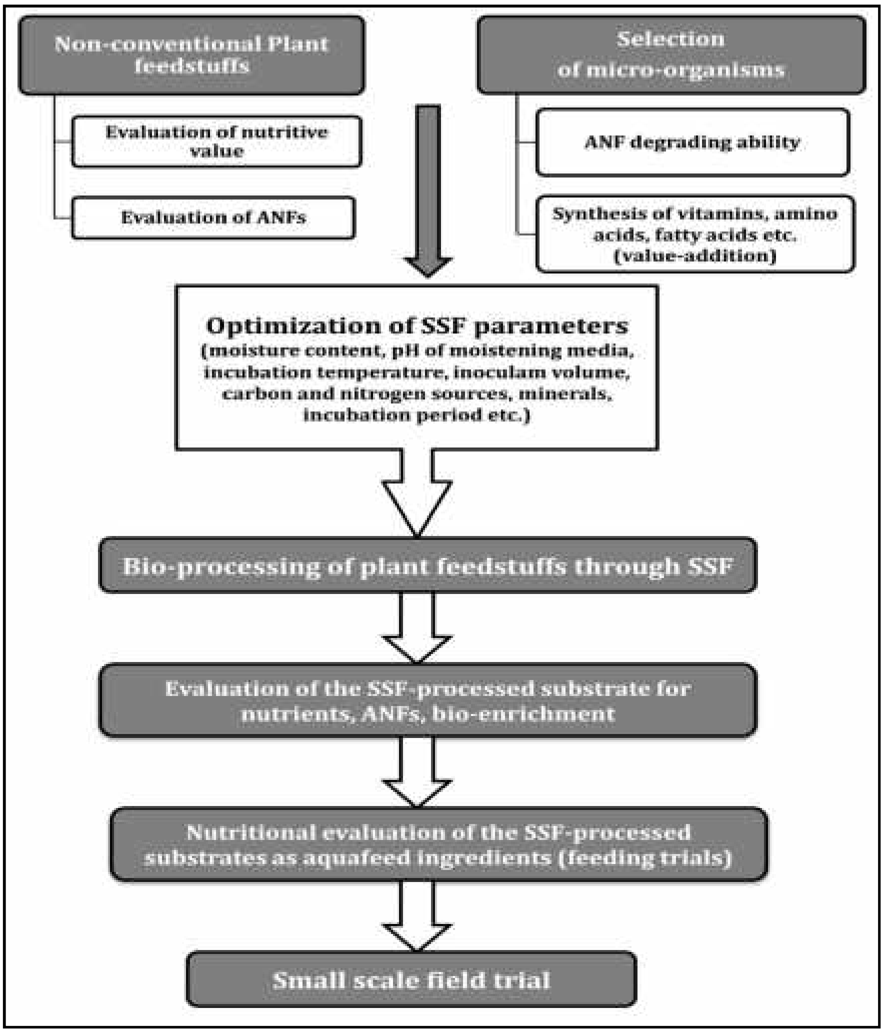


Figure 1.*Basic steps for processing of plant feedstuffs through solid state fermentation*

Amelioration of ANFs in plant feedstuffs: conventional methods *vs.* SSF

The common detoxification methods to overcome most of the ANF are inactivation by heat and/or soaking in water (Egounlety & Aworh, 2003; Ramachandran & Ray, 2008). In addition, cconventional processing methods such as dehulling and germination were also employed by several workers (Alonso et al., 2000; Idris et al., 2006). Roasting or heat treatment (cooking, autoclaving) might not only inactivate ANFs, but also increase nutrient digestibility. Extrusion is another means of processing to improve the nutritive value of legumes primarily. This method is meant for reducing the level of heat-labile ANFs. Thermal processing by extrusion and micronising (infra red heat) has been positively correlated to digestibility. With reference to the processing of specific ANFs through physical methods, moist-heat treatment (pressure cooking) for trypsin inhibitors, heat treatment and pressure cooking for glucosinolates, supplementation of phytase for phytate containing ingredients, and de-hulling of seeds and/or aqueous extraction for tannins and saponins are some of the ameliorative or preventive measures (Mohanta, 2012). However, these physical processes are not conclusive in removingthe deleterious effects of ANFs. In contrary, heat damage affects the usefulness of plant ingredients when fed to fish (Glencross et al., 2004a,b). Nutritional loss of some amino acids through heat treatment and the influence of protein, carbohydrate and moisture on Malliard reactions is well known. Soaking in water may be effective in removing some polyphenols like tannins, however, water soluble nutrients may leach out by this process.

Advancement of microbial biotechnology may offer a solution to these problems.Bioinactivation processes using exogenous enzyme producing microorganisms has been suggested as an alternate approach as indicated in some of the contemporary literatures (Ghosh & Mukhopadhyay 2006).Bacterial cellulase supplementation in the digestive tract of fish and pretreatment of plant feedstuffs with cellulase producing bacteria has been indicated in some of the previous studies (Bairagi et al., 2002b; Ghosh et al., 2004). Ramachandran & Ray (2007) reported that fermentation of black gram seed meal with a *Bacillus* sp. was effective in reducing crude fibre, tannin and phytic acid. However, except the use of cellulase producing bacteria, none of them attempted to inactivate other ANFs using specific exoenzyme producer autochthonus microorganisms or exoenzyme from such organisms. Apart from the cellulose degrading microbiota, tannin and phytate degrading gut microbiota in fish have been documented (Mandal & Ghosh, 2013a; Khan & Ghosh, 2012). The treatment of fish feed with phytase has been reported to result in improvement of protein digestibility and retention in fish (Cheng & Hardy, 2002; Debnath et al., 2005; Baruah et al., 2005). Roy et al. (2014) used two phytase-producing bacterial strains (*Bacillus licheniformis* LF1 and *B. licheniformis* LH1) isolated from the gut of adult *L. rohita* for fermentation of sesame oilseed meal and diets incorporated with fermented oilseed meal significantly ($P<0.05$) improved the apparent digestibility of protein, lipid, ash and minerals (phosphorus, calcium, manganese, copper and iron). Bio-processing of Sesame oil cake (SSC) through SSF under optimized conditions by another phytase-producing fish gut bacterium, *Bacillus subtilis* subsp. *subtilis* (JX292128) resulted in significant reduction of anti-nutritional factors (e.g., phytic acid, tannins, trypsin inhibitor) and crude fibre; while levels of free amino acids, fatty acids and different minerals were enhanced (Das & Ghosh, 2015). Growth and feed utilization efficiencies in fish fed diets containing SSF-processed SSC were superior to the groups fed diets containing raw SSC. SSF-processing by a tannase producing yeast, *Pichia kudriavzevii*(GU939629) improved nutritive value of the de-oiled groundnut oil cake (GOC) and when used in diets, SSF-processed GOC produced better performance in *L. rohita* fingerlings (than the raw GOC) in terms of growth, feed utilization, nutrient digestibility, carcass composition and digestive enzyme activity (Ghosh & Mandal, 2015).Otherwise, autochthonous microbial strains seemed to be good candidate for supplementation, since microorganisms from the same fish species will ensure their colonization and enzyme supplementation within the intestine.

Conclusion

It is really a challenge to formulate aquafeed by reducing the use of fishmeal and replacing it with the alternative plant-derived protein sources. Challenges could be associated with the product quality, environmental impacts or the economics of aquaculture production.As evident from the research findings and economic predictions, fishmeal will no longer be available as the primary protein source in aquafeeds in the near future, rather it will be a subject of value-addition to enhance palatability, balance dietary amino acids, supply biologically active compounds and other essential nutrients or enhance product quality (Hardy, 2010). Thus, the need for alternative protein sources to replace fishmeal in aqua-feeds is obvious and incorporation of plant ingredients rich in ANFs is unavoidable. Since it is not realistic go away with the use of plant ingredients in fish feed formulations, it is better to address this issue in such a way so that their harmful effects may be avoided. Therefore, research on processing techniques needs to be organized to minimize nutrient loss and also to enrich the products, if possible. Further, genetic engineering in plants (e.g., deletions, gene knockout etc.) could lead to the production of plants with reduced concentrations of ANFs making them suitable for use as feedstuffs. Deletion of certain fatty acid desaturase enzymes might allow accumulation of oils that would contain more of monounsaturated fatty acids than the polyunsaturates (Ronald, 2014; Voytas & Gao, 2014), which in turn might substitute the use of fish oil in feed formulation.

Note: References may be available from the author upon request.

Feed Additives in Aquaculture Nutrition

B. N. Paul
ICAR-Central institute of Freshwater Aquaculture
Regional Research Centre, Rahara, Kolkata-700118

Anadditive is a substance that is added to a basic feed, usually in small quantities for the purpose of fortifying it with certain nutrients, stimulants or medicines other than as a direct source of nutrients.

The term "**feed additive**" refers to a non nutritive product that affects utilization of feed or productive performances of the animal. These are added with feed to avoid deterioration during storage, or to improve fish health, nutritional value of feeds or otherwise increase feed efficiency or fish production. Feed additives can mainly classify into two categories viz., additives that affect feed quality and additives that affect fish performance and quality.

Feed additives are substances which are added in trace amounts to a diet or feed ingredient for certain purposes:

1. To preserve its nutritional characteristics prior to feeding (antioxidants and mould inhibitors),
2. To facilitate ingredient distribution or feed pelleting (emulsifiers, stabilisers and binders),
3. To facilitate feed ingestion and consumer acceptance of the product (feeding stimulants and food colorants).
4. To supply essential nutrients in purified form (ie. vitamins, minerals, amino acids, cholesterol and phospholipids).
5. Act as a growth promoters.

Preservatives, binders, feeding stimulants, and food colorants are the major feed additives. Commonly used feed antioxidant and antimicrobial preservatives are Octyl gallate, Dodecyl gallate, N-propyl gallate, BHA (Mixture of 3-and 2-tert butyl 4-hydroxyanisole), BHT (2,6-di (tert butyl)-4-methylphenol), propionic acid, sorbic acid etc. Binders are substances which are used within aquaculture feeds to improve the efficiency of the feed manufacturing process, to reduce feed wastage, and to produce a water-stable diet. For example, binders such as bentonites, lignosulphonates, hemicellulose and carboxymethylcellulose. The use of dietary feeding stimulants is therefore essential to elicit an acceptable and rapid feeding response. Two types of feeding stimulants may be considered for use within aquaculture feeds. Natural ingredient sources which exhibit attractant or feeding stimulant properties or the use of the purified or synthetic chemical derivatives which are responsible for the attractant property of natural ingredient sources. Food colorants are substances which are added in trace amounts to a diet or feed mixture to facilitate its ingestion.

The validation behind the use of feed additive is to improve dietary feed intake at a faster rate so as to minimize the leaching of water soluble nutrients, and wastage of feed. Literature is scanty on the use of feed additives in fish nutrition and on trace minerals requirement. Therefore, evaluation of some selected feed additives and study of selected trace mineral requirement is proposed for research work.

In aquaculture nutrition feed additives are used for better growth performance. Major feed additives are preservatives, binders, feeding stimulants, and food colorants. Some information is available for role and requirements of different feed additives to increase voluntary feed intake. Nanjundappa and Varghese (1988) used 3 mg/kg of diethylstilbestrol on growth performance of rohu fry without any adverse effect. Keshavanath *et al*., (1991) studied the influence of Virginiamycin in fish; the highest weight gain of rohu and common carp was recorded under 80mg/kg and 20mg/kg treatments respectively. Mohanty *et a*l., (1993, 1996) observed fast growth rate and 100% survival in spawn of rohu and catla in indoor rearing when they were fed a larval diet containing 30% bioboost forte (a probiotic supplement source of live yeast culture of *Saccharomyces cerevisiae* and *Lactobacillus coagulants*). Swain *et al*., (1996) studied the effect of 0.15% probiotic supplement on growth of mrigal fry which demonstrated good growth performance and nutrient utilization. Mukhopadhyay and Paul (1995) reported that addition of trimethyl ammonium hydrochloride (TMAH) and addition of amino acids viz., glutamic acid, aspartic acid, serine, alanine and lysine are probable feeding stimulants in fish. Paul *et al*., (1996) worked on aspects of feed colourant in rohu; and results of the experiment indicated that blue colour at 1% inclusion in practical diet increased significant growth. The effect of dietary inclusion of the beta-adrenergic agonist, salbutamol @ 3mg/kg in the diet of rohu fingerling had a beneficial effect on growth (Satpathy *et al*., 2001).Paul *et.al* .(2004)reported the use of plant based attractant in the feed of *Labeo rohita* fry at 1% level of inclusion. Incorporation of Livol (Herbal growth promoter, Indian Herbs) in fish improves growth (Gireesha *et.al.*, 2002 and Maheshappa, 1994). Paul *et.al* (2004) reported the use of plant based attractant in the feed of *Labeo rohita* fry at 1% level of inclusion. Harpaz (2005) reported that levels of dietary L-canitine supplementation ranged from a few hundred to over 4000 mg/kg of diet for better growth, utilization of high fat level in fish feed through protein sparing effect. Incorporation in small amounts of feed additive in aqua feed helps in Aquaculture production. Fish feeding pattern, whereby preferred foods are searched for and ingested, is one of the most important behavior pattern exhibited by fish (Venkateshwarlu et al., 2009). Ekangi, tambul, kharboj, chotokakla and latakasturi are the plant materials that have been used for alluring fish during harvest and angling by traditional fish farmers (Paul *et al*., 2004) (Figure 1-5 and Table 1)

Fig1: Rhizomes of Ekangi

Fig 2: Seeds of Tambul

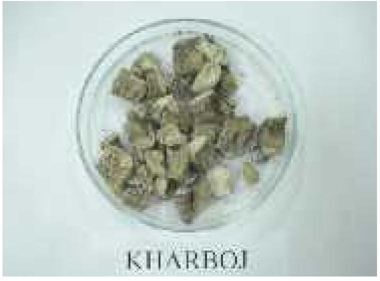


Fig 3: Roots of Kharboj

Fig4: Fruits of Chotokakla

Fig 5: Latakasturi

Table 1: Herbs and their plants used for attraction activity

Local name	Plant name	Parts used
Ekangi	*Kaempferia galanga*	Rhizomes
Tambul	*Zanthoxylum acanthopodium*	Seeds
Kharboj	*Cucumis melo*	Roots
Chotokakla	*Piper cubeba*	Fruits
Latakasturi	*Hibisscus abelmoschus*	Seeds

Awbel at 1% level can be incorporated in the diet of mrigal fingerlings for better feed intake and growth performance (Paul *et al.,* 2012). Paul *et al* (2012) incorporated ekangi, a plant based attractant at 1% level in the feed of *Ompok pabda* fry.

Series of work has been carried out in other countries also; Johnsen and Adams (1986) reported that *Tilapia zilli* strongly get attracted to glutamic acid, aspartic acid, alanine, serine and lysine. Addition of attractants in feed have a positive effect on prawn (Harpaz, 1997),eel (Kamstra and Heinsbroek, 1991),black abalone and yellowtail (Harada et.al, 1996), *Cyprinus carpio* (Nakajima et al., 1989). Supplementary feeding of stimulants enhance the growth performance of young fish in terms of protein efficiency ratio and retention of nutrients(Takeda and Takii,1992) Addition of betaine as a feed attractant, resulted 17%

increase in growth in freshwater prawns, *Macrobrachium rosenbergii* (Harpaz,1997). He also reported that addition of an attractant to the water leads to an additional thirst in food searching activities. So, food consumption increases with increasing in food searching behaviour. The possible mechanism responsible could be the activation of olfactory nerve associated with taste or feeding behaviour (Nakajima et al., 1989). Addition of betaine as a feed attractant, 17% increase in growth occurs in juvenile freshwater prawns, *Macrobrachium rosenbergii* (Harpaz, 1997).

ANTIMICROBIAL COMPOUNDS:

Microorganisms like mold, yeast and bacteria grow on feeds containing more than 125 unbound water unless the feed is frozen or the water activity is reduced by the addition of salts, sugar, glycerol or propylene glycol. Pellets are generally dried to 8-9% moisture, but exposure to humid conditions or rain can increase the pellet moisture levelssufficiently to support mold growth. Mold growth is visible within 3 days in semimoist feeds stored at room temperature (22^0C), while at refrigerated temperature; it may not be visible for 10-20 days. During frozen storage, microbial growth is completely stopped. Many molds produce compounds that are toxic to fish (Hendricks and Bailey, 1989) and at the very least, will decrease feed consumption. Over 20 compounds are used in the feed industry to inhibit fugal or microbial growth (Table-2). The benzoates and parabens are wide spectrum antimicrobials, which are effective against bacteria, fungi and yeast. Propionates are used primarily to inhibit yeast and molds but are also effective against bacteria, fungi, and yeast.

Control of spoilage in intermediate moisture products like semi moist feed (17-25%) and Oregon moist pellet (28-32%) can be obtained by using combination of approaches. Oregon moist pellet must be stored frozen to prevent spoilage. The first element of microbial control is to begin with a feed mixture that has a low microbial load. Pasteurizing wet fish ingredients accomplishes this. A second means of controlling microbial spoilage in feeds is by the use of special packaging that permits the maintenance of a controlled atmosphere after manufacture until feed is used. The purpose is to reduce O_2 tension in the feed package and there by preventing the growth of aerobic microorganisms. A third approach is by controlling the water activity as explaioned earlier. A fourth element of microbial control is to add antimicrobial compounds to the feed. The antimicrobial compo0unds should not lower feed palatability or otherwise affect fish health or performance.

ANTIOXIDANTS:

Antioxidants are chemical compounds that are added to feed ingredients to control oxidation of lipids. Autooxidation or atmospheric oxidation of moderately unsaturated fatty acids results in products that produce off flavours and off odours and the process is accelerated by a variety of environmental factors including increased temperature and exposure to prooxidants e.g., copper and iron, light, UV radiation and Oxygen (Hrdy, 1989). Antioxidants protect the feed from autooxidation, but abusive feed storage condition will eventually result in autooxidation, regardless of the antioxidant content of feeds.

Autooxidation of lipid involves following three steps:

1. Initiation : formation of free radiacals. Factors enhancing initiation reaction include light, heat, UV radiation, presence of copper and iron (known as prooxidants).
2. Prooxidation : It involves the reaction of free radicals formed in the initiation step with more free double bonds on fatty acids forming a number of secondary products and radicals.
3. Termination : here free radical production slows and finally stops. Various secondary products of fatty acids oxidation react in various ways to form stable end products.

Antioxidants work by chelating prooxidant divalent cations, by acting as free radical acceptors or by donati9ng hydrogen. Antioxidants which act as free radical acceptors or donate hydrogen atoms are called **'sacrificial antioxidants'** because they no longer possess antioxidant properties. Sacrificial antioxidants commonly used in aquatic feeds include ethoxyquin (santoquin), buitylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Jauncy, 1989). BHA and BHT are added to feeds at a level of 0.1% while ethoxyquin is added at 0.015%. Other antioxidants of this type include thiodipropionate, propyl gallate, and dilauryl thiodipropionate. Antioxidants that prevent oxidation by chelating metallic prooxidants include ascorbic acid, oxalic acid, and ethylene diamine tetra acetic acid (EDTA). A synergistic effect has also been observed when sacrificial antioxidants are combined with antioxidants that chelate prooxidants. Some nutrients commonly found in feeds also have antioxidant property like ascorbic acid, phytic acid, lecithin, and Selenium (Jauncy, 1989).

ENZYMES:

Enzymes are added to feeds to enhance the digestion of feed components that the fish either cannot digest or cannot digest efficiently and contribute to increased growth, improved nutrient utilization and pollution reduction. Enzyme supplements are either single, purified enzymes or crude enzyme preparation containing multiple enzymes. Phytase is an enzyme that releases phosphorous from phytate, thus increasing its availability to monogastric animals like poultry, swine, and fish. The growth rate of *Marsupenaeus japonicus* larvae was improved with the addition of microencapsulated bovine trypsin to a formulated feed (Maugle *et. al*., 1983). Microencapsulated amylase, a starch digesting enzyme, also increased growth of shrimp larvae (Maugle *et. al.*, 1983). Enzymes are typically denatured at temperatures above 65^0C, so they are typically sprayed on feeds after pelleting.

HORMONES:

Hormones have been used to influence fish growth rates, sexual development and osmoregulation. Hormonal control of sexual development is advantageous in species where it is desirous to grow monosex in culture system to prevent reproduction or increase growth rate. Incorporating androgenic steroids (ethyltestosterone and methyltestosterone) in diets (30-60 mg/kg) fed as first food to tilapia fry *(Tilapia mossambica, O. nilotica or O. aureus)* and continued for 14-21 days resulted in 90% to 100% development of male fish. Fish meal produced from mature fish contains biologically significant quantities of testosterone compounds that stimulate muscle growth (Sower and Iwamoto, 1985). Some feed ingredients contain phytoestrogens, however, their effect on fish growth or maturation is not well known. Recently, Schelling et. al. (1999) demonstrated that injections of recombinant bovine somatotrophin (bST) greatly accelerated growth rate of sturgeon. In the future, it may be feasible to add growth hormone to feed to influence growth and reduce the time required to raise long lived fish, such as sturgeon, to maturation.

PROBIOTICS:

Probiotics may be defined as live organisms that beneficially affect the microbial balance of the host. A variety of mechanisms have been proposed to explain the actions of probiotics, including their production of antimicrobial substances, competition for adhesion receptors and the provision of nutrients and direct immunostimulatory effects. Probiotics may be a single species of microorganisms or a mixture of species. Probiotics are of two types, viz., Feed probiotics- they are administered through artificially prepared pelleted feed and Water probiotics/water additives- they are applied directly to the water surface as a part of water quality management.

Probiotics used in aquaculture increase the growth rate; help to maintain the water quality; increase the survival rate during the hatchery operation; stimulate non-specific immunity by promoting production of vitamins, thereby increasing the host's resistance by to various diseases and suppress the growth of harmful bacteria such as *Vibrio sp.*

Water probiotics is a new concept that can be applied to the concept of probiotics, in a broad sense and comprises living microbial preparations used to treat the aquaculture ponds. When these additives are added directly to the culture water, they modify the microbial composition of water or pond sediments and eliminate or minimise the pathogens. The probiotics used in aquaculture are: *Lactobacillus thermophilus, L. halveticus,L. bulgaricus, Streptococcus lactis* and *Vibrio alginolyticus.* The strategy often followed for administering probiotics includes isolating bacteria from mature fish and including these bacteria in the feed of juvenile fish of the same species (Gildberg et. al., 1997). Lactic acid bacteria such as *Carnobacterium sp*., which produces bactericins, are often used in this manner (Gatesoupe, 1994). One concern of including probiotics into the food chain or environment of larval fish is that the probiotics colonization and proliferation within the host must remain under control. Gatesoupe (1994) observed a negative correlation between high concentrations lactic acid bacteria and survival in turbot larvae fed greater than 2 X 10^7 colony forming units, indicating the need for careful determination of inclusion levels of lactic acid bacteria in order to obtain beneficial, not detrimental, effects of probiotics.

Soil Carbon Sequestration in Fish Ponds

S. Adhikari

ICAR-Central Institute of Freshwater Aquaculture,

Regional Research Centre, Rahara, Kolkata- 700 118, West Bengal

Introduction:

With the increase of atmospheric carbon dioxide (CO_2) concentration, considerable attention has been devoted to terrestrial carbon (C) storage in soils, forests, and grasslands. The depositional environments (i.e., lakes and impoundments) represent short to long term scale storage of atmospheric CO_2. Globally, this storage of organic C (OC) in the sediments of natural lakes ranges from 30 to 70 Tg C/yr (Tg= teragram=10^{12}g =1 million metric ton). The burial in impoundments is much larger ranging from 150 to 220 Tg C/yr (4).These rates of C burials are comparable to the OC storage in the sediments of the global oceans estimated at 120-240 Tg C/yr. The annual magnitude of OC stored in lakes and reservoirs is also comparable to the delivery of OC by rivers to the ocean, about 400 Tg C/yr. However, these rates of OC storage in lakes and agricultural impoundments are modest compared to the current total storage of OC terrestrially which ranges from 1000 to 4000 Tg C/yr. Wetlands also accumulate significant amounts of C in their soils, compared to adjacent upland sites. Strategies to reduce C and other greenhouse gas (GHG) emissions from agriculture and enhance C sinks on farms/wetlands have also been identified, but options for different aquaculture farming systems have not been widely assessed.

There is 11.1 million hectare (Mha) of aquaculture ponds globally. Manures, fertilizers, feed and other agricultural wastes are applied to ponds for higher production, and these inputs stimulate OC production by phytoplankton photosynthesis in ponds. Aquaculture ponds do not have large external sediment loads like reservoirs or watershed ponds in agricultural or other rural areas. Further, sediments in aquaculture ponds are eroded by rain, waves and water currents generated by mechanical aerators, activities of different culture species, and harvesting operations. Thus, coarse soil particles suspended by internal erosion settle near edge of ponds while smaller particles tend to settle in deeper areas of the ponds. Uneaten feed, organic fertilizers, organic matter (OM) from dead plankton, and excreta from different species settle at pond bottoms, and gradually mix with soil particles. When ponds are drained for harvest, organic detritus is discharged from the ponds and after draining, pond bottoms are exposed to sunlight for drying which enhance soil aeration and accelerate decomposition of labile OM. Despite these practices in aquaculture ponds, a layer of sediments with a higher OC concentration than that in the original soil develops at the pond bottom. Aquaculture ponds store as much as 0.21% of the annual global C emissions of about 10 Pg/yr, and represent a small sink for C which is an important ancillary benefit in considerations of the global C budget. However, detailed data on C storage by aquaculture ponds are scanty globally. Thus, the present topic will be dealt with the potentialities of the soil carbon sequestration in fish ponds.

Materials and methods:

Inputs in aquaculture activities: Aquaculture activities involve a variety of inputs for fish production including manures, fertilizers, feed and a combination of all these things. In addition to this, lime is applied, if necessary. With high fertilization rate, the nutrients assimilated in fish biomass were estimated to be less than 20-30 % for nitrogen (N) and 10-15 % for phosphorus (P) and 10-20 % OC. Most of those lost nutrients are distributed in water, fish biomass and sediments of the pond systems. It is generally believed that a large proportion of nutrients received in ponds end up in pond sediments and discharged effluents. The organic fertilizer (cow dung) contained 40-42% C (on dry weight basis) while feed contained 46-48% C (on dry weight basis).The carps, freshwater prawn (scampi), shrimp, tilapia contained 11.0 to 13.5 % C (on live weight basis), respectively.

Sediment core analysis: The average depth of fish ponds vary between 1.0 to 2.5 m. Sediment cores could be collected manually from the ponds. The OC sequestration rate could be estimated by multiplying the annual rate of accumulation of dry sediment by the sediment dry bulk density and the OC concentration in the sediment.

Results and Discussion:

(a) *Rate of Sediment Accumulation*: The rate of sediment accumulation ranges from 0.9 to 1.20 cm/yr in shrimp ponds; 1.10 to 1.54 cm/yr in freshwater prawn ponds; 1.10 to 2.33 cm/yr in tilapia ponds; 188 cm/yr in polyculture ponds; 1.00 to 3.70 cm/yr in carp ponds; 1.20 cm/yr in channel catfish ponds; 2.50 cm/yr in Clarias catfish ponds; 0.45 cm/yr in yellow perch ponds; and 0.80 cm/yr in bait minnow ponds, respectively (Table 1). Overall, the sediment rate accumulation ranged from 0.45 to 3.70 cm/yr in these ponds. When ponds are drained, considerable amount of sediments are lost from the bottoms of the ponds through outflow or runoff. Ponds are usually drained once in every three years till the upper layer cracks to reduce the concentration of toxic gases in the pond bottom. The dry bulk density (g/cm^3) of sediment in these ponds ranged from 0.49 to 0.63 in shrimp, 0.18 to 0.46 in prawn, 0.34 to 0.56 in tilapia, 0.58 in polyculture, 0.28 to 1.10 in carps, 0.38 in channel catfish, 0.18 in Clarias catfish, 0.84 in yellow perch, and 0.47 in bait minnow ponds (Table 1). Overall, the bulk density of sediments of these ponds ranged from 0.18 to 1.10 g/cm^3.

(b) *Organic Carbon Concentration in Sediment*: The OC concentration (%, g/g) in sediment was 1.22 to 3.66 in shrimp, 1.20 to 1.38 in freshwater prawn, 3.00 to 4.17 in tilapia, 1.37 in polyculture, 1.10 to 3.02 in carps, 2.92 in channel catfish, 1.46 in Clarias catfish, 2.80 in yellow perch, and 1.58 in bait minnow ponds (Table 1).

Overall, the OC in sediment varied from 1.10 to 4.17 % in different ponds of the world. There were no clear trends in OC concentration related to culture species or intensity of culture. Significant quantities of OM can accumulate in bottom sediments in these aquaculture ponds. Feeds applied to these ponds to increase fish/prawn/shrimp settle at the bottom where it is mostly eaten by aquatic species. Inorganic nutrients released into the water from microbial decomposition of uneaten feed and feces of these species stimulate heavy plankton blooms. Phytoplankton cells have a short life span and continually die and settle to the bottom. In some cases, water supplies contain solids of appreciable OM content which may deposit in ponds. High rates of water exchange may result in large sediment inputs. Mechanical erosion may often erode bottom particles where water currents are higher and sedimentation occurs where currents are slower. Such a current flow pattern alters the shape of the bottom of the pond, reduces pond volume, and provides organic substrate for microorganisms.

(c) *Rate of Carbon sequestration*: The annual C accretion rate estimated from sediment accumulation rate, dry bulk density, and percentage OC in sediment ranges from 0.95 to 2.76 Mg/ha/yr in shrimp ponds; 0.28 to 0.86 Mg/ha/yr in prawn ponds; 1.15 to 4.37 Mg/ha/yr in tilapia ponds; 1.43 Mg/ha/yr in polyculture ponds; 1.21 to 3.12 Mg/ha/yr in carp ponds; 1.33 Mg/ha/yr in channel catfish ponds; 0.66 Mg/ha/yr in Clarias catfish ponds; 1.06 Mg/ha/yr in yellow perch ponds; and 0.59 Mg/ha/yr in bait minnow ponds (Table 1). Overall, The C sequestration rate in these ponds ranges from 0.28 to 4.37 Mg/ha/yr with an average of 1.56 Mg/ha/yr. Tilapia and carps culture recorded the highest C storage while freshwater prawn recorded the lowest. The freshwater prawn prefers clear bottom (sandy) for their growth. In general, tilapia and carp stir pond sediment in search of food organisms, and this action possibly incorporates recently deposited OM into sediment to minimize its loss during pond drainage for harvest.

The applied nutrients from different sources in the culture systems and also the nutrients entering from the terrestrial into aquatic ecosystems through run-off have an important role in carbon sequestration by the sediment in fish ponds. Fertilizers and feeds generally are the largest inputs of N and P to fish ponds. Feeds also supply C to fish ponds. Organic fertilizers (fermented rice straw, cow dung etc.) often are added to ponds to boost fish yields by increasing primary productivity through released inorganic nutrients, or by providing OC through heterotrophic pathways. Depending on the species, fish feed directly on attached or planktonic algae, detritus / fungal flocs, or smaller animals such as zooplankton and snails which feed on algae and detritus.

The carbon sequestration capacity of sediments, algae, and zooplankton from fresh water aquaculture ponds in Odisha has also been documented (Table 2). The percent organic carbon in the pond sediments ranged from 0.39 to 1.31 with an average value of 0.912 ± 0.321 whereas the carbon sequestration capacity ranged from 0.442 to 1.882 Mg

C/ha (1 Mg = 10^6 g) with an average value of 1.018 ± 0.447 Mg C/ha. In the case of zooplankton and algae from pond, the percent organic carbon was 7.688 ± 0.196 and 2.354 ± 0.047, respectively, whereas the total estimated carbon burial rate was 0.009 ± 0.005 and 0.150 ± 0.003 Mg C/ha, respectively.

The estimated, average annual C sequestration rate for aquaculture ponds monitored in this study is lower than that of agricultural impoundments and large river reservoirs, but higher than that of natural lakes and inland seas (Table 3). Aquaculture ponds can sequester 17.2 Tg/yr of C globally. Aquaculture ponds store less C than that by large reservoirs [160 to 280 Tg/yr] and agricultural impoundments [163 Tg/yr]. The aquaculture ponds store C at a lower rate than agricultural impoundments and large reservoirs because of lower input of external sediment and associated OM to aquaculture ponds than in other impoundments.

Conclusion: Aquaculture pond management minimizes OM accumulation. For example, ponds are dried at least once in three years to reduce the gaseous emissions from the bottom and also to flush out sediments by using pressurized water. This intervention is needed because a thick layer of the sediment reduces the productivity of the pond. Though aquaculture ponds sequester comparatively small amount of C, globally pond farming systems under different management practices can sequester a large amount over long term.

Table 1. Carbon sequestration from the data of sediment core analysis for different aquaculture systems*

Species	Country	Sediment accumulation (cm/yr)	Sediment dry bulk density (g/cm^3)	Organic carbon (%)	Carbon sequestration (Mg/ha/yr)
Shrimp	Honduras	0.90	0.50	2.10	0.95
	India	1.62	0.49	1.22	0.97
	Thailand	1.20	0.63	3.66	2.76
Freshwater prawn	India	1.54	0.46	1.20	0.86
	Thailand	1.10	0.18	1.38	0.28
Tilapia	Brazil	1.25	0.51	4.17	2.65
	South Africa	2.33	0.56	3.35	4.37
	Thailand	1.10	0.35	3.00	1.15
India major carps with prawn (polyculture)	India	1.88	0.58	1.37	1.43
Carps	India	1.00	1.10	1.10	1.21
	Thailand	3.70	0.28	3.02	3.12
Channel catfish	Alabama, USA	1.20	0.38	2.92	1.33
***Clarias* catfish**	Thailand	2.50	0.18	1.46	0.66
Yellow perch	Piketon, USA	0.45	0.84	2.80	1.06
Bait minnow	Arkansas, USA	0.80	0.47	1.58	0.59

Table 2. Estimated organic carbon content and carbon burial rates of different components in aquaculture ponds*

Component	Organic carbon (%)	Carbon burial rate (Mg C/ha/year)
Pond sediments **(n = 9)**	0.912 ± 0.321	1.018 ± 0.447
Zooplankton **(n = 3)**	7.688 ± 0.196	0.009 ± 0.005
Algae **(n = 3)**	2.354 ± 0.047	0.150 0.003

Source**: Anikuttan, K.K., Adhikari,S.**,** Kavitha, M., and Jayasankar, P. (2016). Carbon sequestration capacity of sediments, algae, and zooplankton from fresh water aquaculture ponds. *Environmental Monitoring and Assessment,188**: 422-26.

Table 3. Areas of global inland water bodies and annual rates and amounts of organic carbon storage in these systems*

Inland water body	Global area (Mha)	Carbon sequestration rate (Mg/ha/yr)	Global carbon storage (Tg/yr)
Aquaculture ponds			
Freshwater	8.75	1.56	13.6
Brackishwater	2.33	1.56	3.6
Large lakes	118	0.05	6.0
Small lakes	32	0.72	23.0
Inland seas	100	0.05	5.0
Large river reservoirs	40	4.00	160
Agricultural impoundments	7.7	21.20	163

* **Source**: Adhikari, S., and Lal, R. (2017). Carbon Sequestration: Fish Ponds. In: Encyclopedia of Soil Science (Rattan Lal, ed.), Taylor & Francis, Third Edition DOI: 10.1081/E-ESS3-120052896, pp. 274-278.

Nutritional Disorders in Fish

Farhana Hoque
ICAR-Central Institute of Freshwater Aquaculture
Regional Research Canter, Rahara, Kolkata-700118, West Bengal

Introduction

Non-infectious diseases of fish are caused by adverse environmental conditions, nutritional disorders, or genetic defects. The continuous expansion and improvement in efficiency of aqua-cultural production requires continuous improvement in nutritional formulation and feed technology. Nutritional status is considered one of the important factors that determine the ability of fish to resist diseases. Outbreaks of fish diseases commonly occur when fish are stressed due to a variety of factors including poor nutrition. The primary cause of many diseases may be only due to defective nutrition. The need for proper diets to improve health and prevent diseases of farmed aquatic animals is widely recognized. Nutritional and physical characteristics of diets can modulate susceptibility of fish to infectious diseases. In the most severe cases, diets that are inadequate with respect to essential nutrients (protein, amino acid, essential fatty acids, vitamins and minerals) lead to gross malnutrition and high disease susceptibility.

Nutritional diseases

Nutritional diseases of fish may develop as a result of deficiency (under-nutrition), excess (over nutrition), or imbalance (malnutrition) of nutrients present in their food. The disease usually develops gradually because animals have body reserves that make up for nutritional deficiency up to a certain extent. Disease signs develop only when supply of any diet component falls below critical level. When there is too much food, the excess that is converted to fat and deposited in fish tissues and organs, may severely affect physiological functions of the fish.

Nutritional disorders: Reasons and prevention

1. Nutritional imbalance in commercial food: Fishes can be either plant eaters (herbivores), meat eaters (carnivores), or both (omnivores). And although commercial food is available for fishes, a nutritional disorder can still occur because each species of fish has a different nutritional requirement, which is not always fulfilled by the commercial food. Therefore, fishes will need more than one type of commercial food to meet their dietary requirement.
2. Incorrectly stored food: Improperly stored food is another reason fishes acquire nutritional disorders. Dry food should be stored in a cool, dry place and replaced after two months.
3. Vitamin deficiency: Nutritional disorders in fishes can also be due to a vitamin deficiency. Vitamin C or ascorbic acid deficiency leads to Broken back disease –

where the backbone of the affected fishes get bent (deformed). Vitamin B-complex (thiamin, biotin, niacin, and pyridoxine) deficiency can cause brain, spinal cord and nerve disorders in fishes. Unfortunately, vitamin deficiency is diagnosed only after the fish's death. Therefore, it is important you give your fish a vitamin-rich diet.

4. Infected live food: Food that is alive and infected with bacteria, viruses, fungi and parasites can lead cause problem in your fishes. To prevent such infectious diseases, buy live food only from reputable sources.
5. Feed toxicity: Nutritional disorders caused by toxins found in food occur frequently in aquarium fishes. The most common of these is the aflatoxin produced by the growth of the mold, *Aspergillus flavus*, in the stored food. Aflatoxin causes tumors and is fatal in fishes. Store your fish food hygienically and replace it every two months, or when there appears to be mold in it.

The deficiency and imbalance diseases

Deficiency diseases are of two types,

1. Deficiency or imbalance of the macronutrients in the diet—the protein, carbohydrate, lipid, and fiber
2. Deficiency of the micronutrients—the vitamins and minerals.

Among macronutrients it is usually in the lipid component of the macronutrients that cause serious problems. Among the micronutrients, any of a wide range of components can exert an effect, especially in fast-growing, younger fish.

Protein

All fish require relatively high levels of protein as a source of amino acids for protein synthesis and, generally, for gluconeogenesis as well. Thus fish diets must contain high levels of high-quality protein. Since protein is one of the most expensive components of the diet, feed manufacturers have to optimize diet formulae to allow economies while still maintaining an adequate complement of protein. The principal feature determining protein quality for fish nutrition is the level and availability of the essential amino acids (EAA). Deficiency of one or more of these leads to deficiency disease.

Reported essential amino acid (EAA) deficiency signs in fish		
EAA	Fish species	Deficiency signs
Lysine	*Oncorhynchus mykiss*	Dorsal/caudal fin erosion, increased mortality
	Cyprinus carpio	Increased mortality
Methionine	*O. mykiss*	Cataract
	Salmo salar	Cataract
Tryptophan	*O. mykiss*	Scoliosis, lordosis, renal calcinosis, cataract, caudal fin erosion, decreased carcass lipid content; elevated Ca, Mg, Na and K carcass concentration
	Oncorhynchus nerka	Scoliosis, cataract
	Oncorhynchus keta *Oncorhynchus kisutch*	Scoliosis
Miscellaneous	*C. carpio*	Increased mortality and incidence of lordosis observed with dietary deficiencies of leucine, isoleucine, lysine, arginine and histidine

Carbohydrate

Since fish have a much more limited capacity for carbohydrate metabolism than higher vertebrates, there is little chance in increasing carbohydrate levels, and only limited information is available on the effects of high carbohydrate levels. Excessive carbohydrate intake will result in excessive glycogen deposits in the liver, and continued intake results in extensive lipid deposited in the viscera.

Fats

Dietary disease problems associated with the lipid component of the diets appear to be among the most serious and prevalent of all nutritional problems in fish. Deficiency syndromes result when sufficient levels of ω-3 or ω-6 or longer-chain members of the series are unavailable.

Report essential fatty acid (EFA) deficiency signs in fish	
Fish species	Deficiency sign
O. mykiss	Increased mortality, elevated muscle water content, increased susceptibility to caudal fin erosion by *Flexebacterium* sp., fainting or shock syndrome, decreased haemoglobin and red blood cell volume, fatty infiltration and degeneration of liver, swollen pale liver, reduced spawning efficiency
Oncorhynchus kisutch	Swollen pale liver, increased hepatosamtic index (fatty liver), high mortality
C. carpio	Increased mortality, fatty liver

Fiber

The role of dietary fiber in animal nutrition has become a major area of investigation. There does not appear to be any pathological effect associated with excessive or low levels of fiber, although it may affect the growth rate. Some species, namely, catfish, need substantial amounts of fiber in the diet to move nutrients along the absorptive pathways slowly.

Micronutrient deficiencies and imbalances

The micronutrients are, as the name suggests, those components of the diet which, although essential, are required in only relatively small quantities. If these are absent, or insufficient, they give rise to specific deficiency diseases which often have particular clinical and histopathological features. Trace elements such as zinc, iron, copper and selenium are required as coenzymes for metalloenzymes and are vital for maintenance of cellular functions in the immune system of higher vertebrates. In fish little is known about the effects of trace elements on immune function. Although almost all necessary minerals are available in most practical fish diets and fish can also absorb minerals from the surrounding water, mineral deficiencies do, on occasion, arise in farmed fish.

Reported essential mineral deficiency signs in fish	
Element/fish sp.	**Deficiency signs**
Phosphorus (P)	
C. carpio	Reduced growth, poor feed efficiency, bone demineralization, skeletal deformity, abnormal calcification of ribs and the soft rays of the pectoral fin, cranial deformity, increased visceral fat
O.mykiss	Reduced growth, poor feed efficiency, bone demineralization
Calcium (Ca)	
I. punctatus	Reduced growth, low carcass ash, Ca and P content (fed vitamin D deficient diet, 6)
O. mykiss	Anorexia, poor growth and feed efficiency
Potassium (K)	
O. tshawytscha	Reduced growth and feed efficiency, anorexia, convulsions, tetany, death
Magnesium (Mg)	
C. carpio	Reduced growth, sluggishness, anorexia, convulsions, high mortality, reduced bone magnesium content, cataracts
O. mykiss	Reduced growth, anorexia, cataract, sluggishness, calcinosis of kidney, increased mortality, vertebral curvature, degeneration of muscle fibres and epithelial cells of pyloric caeca and gill filaments, reduced bone ash, Mg and elevated Ca content
Iron (Fe)	
General	Hypochromic microcytic anaemia
Element/fish sp.	Deficiency signs
Zinc (Zn)	
C. carpio	Reduced growth, cataracts, loss of appetite, high mortality, erosion of fins and skin, elevated tissue concentration of Fe and Cu in intestine and hepatopancreas
O. mykiss	Reduced growth, increased mortality, cataracts, short body dwarfism, fin erosion
Manganese (Mn)	
C. carpio	Reduced growth, short body dwarfism, cataracts
O. mykiss	Cataracts, reduced growth, short body dwarfism, abnormal tail growth
Copper (Cu)	
C. carpio	Reduced growth, cataracts
Selenium (Se)	
S. salar	Increased mortality, muscular dystrophy, depressed glutathione peroxidase (enzyme) activity, reduced growth

C. carpio	Reduced growth, cataracts, anaemia
Iodine	
Salmonids	Thyroid hyperplasia (goitre)

Vitamins

Vitamin deficiency leads to serious biochemical dysfunctions and consequent cellular and organ dysfunction (clinical symptoms). Vitamin deficiencies may be caused by their low content in feeds, environmental or physiological stresses and by diseases especially those, which occur in the early stages of development. Vitamin deficiencies result in depressed immune function and slow or no recovery from disease.

Nutritional components	Deficiency Symptoms
Vitamins (water soluble)	1.Thiamine (vit-B1) deficiency resulted in poor appetite, muscle atrophy, loss of equilibrium similar to that of whirling disease symptoms in trout, odema and poor growth. 2.Riboflavin (vit-B2) corneal vascularisation, cloudy lens, hemorrhagic eye, photophobia, dim vision, incardination, discoloration, poor growth and anemia. 3.Pyridoxine ((vit-B6) Nervous disorders hyper irritability, aemia serous fluid, rapid gasping and breathing. 4.Panthothenic acid. Loss of appetite, necrosis and scarring, cellular atrophy, exudates on gills, sluggishness, cubbed gills, poor growth 5.Inositol. Fin necrosis anaemia, distended stomach, skin lesions and poor growth. 6. Biotin.Blue slime patch on body, loss of appetite, muscle atrophy, fragmentation of erythrocytes, skin lesion and poor growth. 7.Folic acid. Poor growth, lethargy, fragility of caudal fin, Dark colouration, macrocytic anaemia, decreased appetite. 8.Choline. Anaemia, hemorrhagic kidney and intestine, poor growth. 9.Nicotinic acid. Loss of appetite, photophobia, swollen gills, reduced cooridation, lethargy 10.Vitamin (B12) cobalamin derivative. Erratic haemoglobin level, erythrocyte counts and cell fragmentation.

	11.Ascorbic acid. Lordosis and scoliosis eroded caudal fin, deformed gill operculum, impaired collagen formation.
Fat soluble vitamins	Vit-A - Causes expthalmos, ascite, odema, hemmorhagic kidney. Hypervitaminosis (A) cause necrotic caudal fin Vit-D - Necrotic appearance in the kidney Vit-K - Mild cutaneous hemorrhages due to ineffectiveness of blood clotting Vit-E - Exophthalmia, distended abdomen, anemia with reduced RBC numbers and haemoglobin content. Accumulation of ceroid in fish liver.

Anti-nutritional factors

The presence of endogenous anti-nutritional factors within plant feedstuffs is believed to be the largest single factor limiting their use within compounded animal and fish feeds at high dietary levels.

The endogenous toxic factors occurring in plant foodstuffs of agricultural importance	
Proteins	Protease inhibitors / haemagglutinins
Glycosides	Goitrogens, cyanogens, saponins, estrogens
Phenols	Gossypol, tannins
Miscellaneous	Anti-minerals, anti-vitamins anti-enzymes, food allergens, microbial/plant carcinogens, toxic amino acids

Toxic algae

Algal blooms in the water kill fish by removing oxygen from the water.

Anthropogenic chemicals

The widespread use of pesticides on agricultural crops has meant that residues of substances such as the organochlorines and the organophosphates have become available in feedstuffs or, in the case of wild fish, within the food web. Normally the levels are unlikely to cause pathology, in properly controlled systems, but occasionally accidental overdosing or dietary contamination can result in acute toxicity.

Binders

High levels of polycellulose binders in the diet have been associated with a chronic degenerative condition known as hepatorenal syndrome. Affected fish grow slowly, show poor appetite, become darkened, and eventually succumb.

Photosensitizers

A number of chemical compounds which may be incorporated into, or contaminate, fish diets. The most commonly implicated compound associated with this condition in fish culture is phenothiazine, a drug used for the control of the intestinal parasite *Octomitus*.

Sekoke disease

In Japan, spontaneous diabetes in carp has been described in association with the incorporation of significant levels of silkworm pupae in the diet. The pathological picture is typical of diabetes in higher animals and degenerative changes in extrinsic muscle and retina of eye.

Antibiotic and chemotherapeutic toxicity

Antibiotics and chemotherapeutics are frequently incorporated in fish diets and normally serious pathology is induced only if excessive dosage or prolonged incorporation occurs. Erythromycin and sulfonamides are most commonly associated with pathological effects. Prolonged sulfonamide therapy in salmonids has been reported to induce retardation of growth, and renal tubular casts, focal hepatic necrosis, and visceral arterial sclerosis.

Mycotoxins

Probably the most important of the natural contaminants are the mycotoxins, metabolic products of fungal contaminants of feed components. The most significant of these toxic metabolites, in terms of fish diets, are the aflatoxins, substances produced by toxic mutants of the blue–green mold *Aspergillus flavus*, which is a common contaminant of oil seeds such as cotton and peanut. The aflatoxins are powerful carcinogens responsible for the widespread occurrence of hepatocarcinoma in cultured trout.

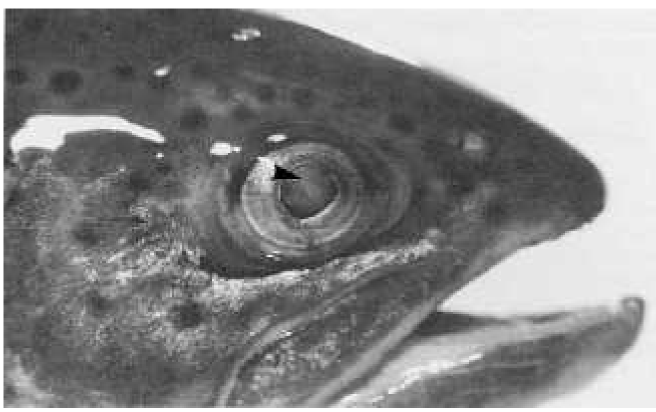


Fig.1. Cataract in Salmon due to zinc deficiency

(Picture courtsey: Ronald J. Robert, Nutritional Pathology)

(Picture courtsey: Ronald J. Robert, Nutritional Pathology) f

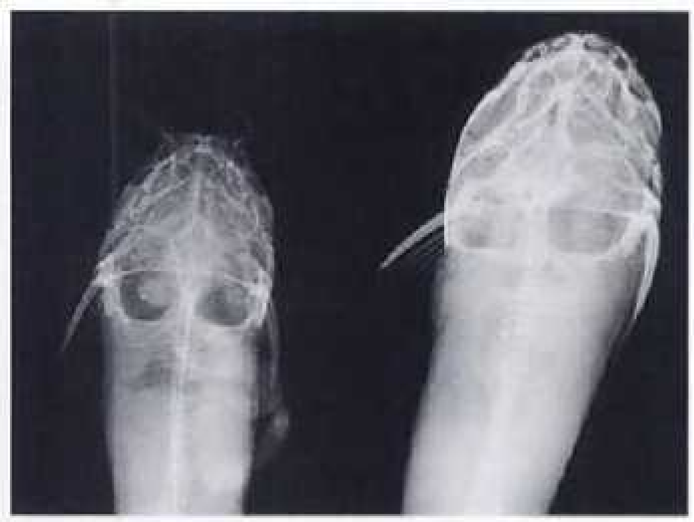


Fig.2. X-ray photograph of normal (left) and ascorbate- deficient Asian catfish (crackhead) showing distinct loss of radio opacity (Picture courtsey: Ronald J. Robert, Nutritional Pathology)

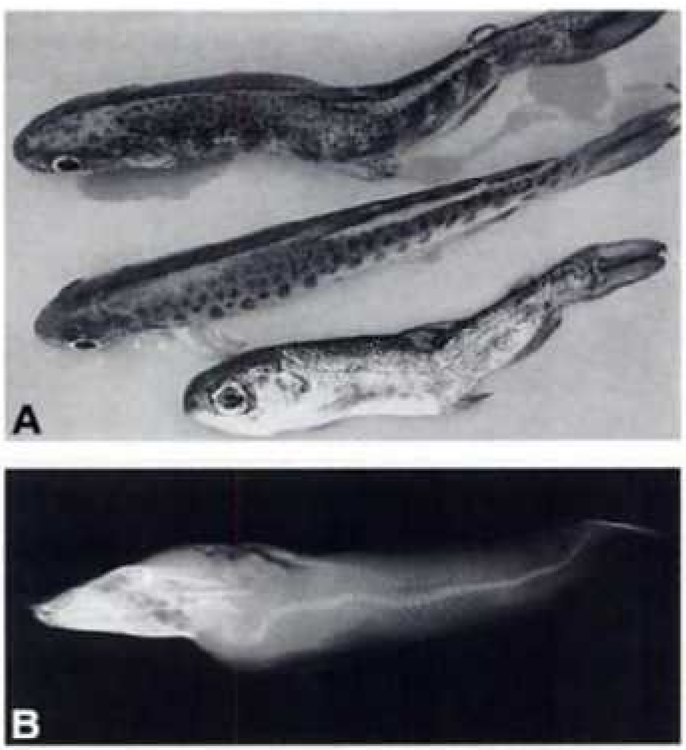


Fig. 3 A.Spinal deformity in young coho salmon fed diet deficient in ascorbic acid for 22 weeks. Scoliosis in upper fish, lordosis in the bottom fish and middle fish : normal

B. X-ray of Asian catfish fed on ascorbic acid deficient diet. Severe spinal deformity is evident at the level of cervical, thoracic and lambar vertebrae

(Picture courtsey: Ronald J. Robert, Nutritional Pathology)

Breeding and larval rearing of Hilsa (*Tenualosa ilisha*)

D.N.Chattopadhyay, A. Chakrabarty and P.K.Ray

ICAR-Central institute of Freshwater Aquaculture
Regional Research Centre, Rahara and Kalyani Field Station8

Introduction

Hilsa (*Tenualosa ilisha*) belongs to the order Clupeiformes, family Clupeidae and sub family Alosinae. The fish is migratory, euryhaline and anadromous in nature. It is widely distributed in Bay of Bengal, Indian Ocean, Persian Gulf and Arabian Sea and coastal areas, estuaries and freshwater rivers of India, Bangladesh, Pakistan, Indonesia, Sumatra, Myanmar (Burma), Kuwait, Iraq, Iran, Sri Lanka and Vietnam. In India the fish is found in the rivers such as Hooghly, Godavari, Narmada, Tapti, Rupnarayan (near Kolaghat) and also in Ukai reservoir (Vallab-sagar) of Gujrat. In Bangladesh, the major rivers are Padma, Meghna, Jamuna and Rupsa where hilsa is available. The species is fast swimmer and travels up to 71 km in one day. They can ascend river up to 1200 km.

In recent years, the natural population of hilsa has been declined due to several factors viz., over-fishing, indiscriminate catch by advanced crafts and gears, construction of dams and barrages across the rivers, reduction of river flow, reduction of river depth due to siltation and bar formation, industrial and domestic pollution, disturbances of their breeding ground, low rain fall and climatic change. Hence there is an urgent need for its conservation. Apart from several methods of conservation like closed season, sanctuary, net mesh size regulation, river dredging, banning of the use of certain specific nets (bag net, scoop net, lift net etc.) during specific period of the year, the most important strategy for their conservation is controlled breeding, larval rearing and culture in confined freshwater system.

The adult hilsa both male and female live in marine environment and migrate to freshwater rivers for breeding (spawning migration) twice in a year during their breeding season (Sept-Oct-Nov and Feb-March).Breeding takes place in freshwater river where the eggs hatch and the larvae grow to fry and fingerling. The advanced fingerlings migrate from freshwater river through estuaries to sea where they grow up to adult stage. The spent and unspent brood returns to sea.

Breeding

Till now induced breeding of Hilsa has not developed possibly because of non-availability of sufficient number of suitable ripe brood and constraints of keeping the brood in live condition for long time for hormonal injection as the fish are very much susceptible to handling stress. Besides, Hilsa are exclusively caught by nylon monofilament gill net and therefore, they mostly died either during netting or immediately after catch. Moreover, till recent no mature brood stock has been developed anywhere under pond condition.

However, breeding can be done through artificial fecundation using stripping method. In this process fully ripe brood fish both male and female are caught from Hooghly river and they are artificially bred. Males with freely oozing milt and females with freely oozing eggs are selected. Females are hand stripped and eggs are spread over a dry steel tray immediately followed by stripping of males. Miltis spread over the collected eggs. After that, milt and eggs are mixed with bird's feather followed by sprinkling of water. As a result, the sperm becomes activated and fertilize the eggs. The tray is gently moved sidewise for thorough mixing so that all the eggs and sperms come in contact with each other for proper fertilization. More water is given in tray and kept for few minutes undisturbed. Then the eggs are thoroughly washed with water several times and temporarily kept in large tubs/aluminum hundi with aeration and water exchange at regular interval of 15-30 minutes. Justafter sprinkling and adding water on the mixture of egg and milt, the eggs starts swelling. The fully swelled fertilized eggs (2.1 mm in diameter) are observed after 45 minutes of stripping.All the eggs are found to be separated from each other. The rate fertilization can be up to 98%. The fertilized eggs are spherical and transparent having yellowish yolk with numerous oil globules.The unfertilized eggs (1.8 mm in diameter) contain whitish yolk which can be easily differentiated from the crystal like yolk of fertilized eggs.Egg membrane is single layered.In undisturbed water the eggs settle at the bottom. However, the eggs float even in feeble water movement. The fertilized eggs are then transferred to different holding system viz., trays, aquaria and hatching pool for incubation, hatching and production of larva or spawn.The most important aspects of egg incubation are handling, water management and aeration. Hatching occurs after 19-22 hours at 23-28 ^{0}C. Hatching rate is observed up to 98%. The newly hatched larvae also called hatchlings are transparent and tiny (2.16 mm in length) with very little swimming ability. The larval mouth appears when they are 4 day old and at this stage they are called as spawn (5 mm/0.5 mg) that are ready for stocking for further rearing to produce fry.

Brood collection from river

Stripping

Hilsa larvae

Larval rearing

The method of larval rearing has been developed by ICAR-CIFA for the first time in hilsa. The 4-day old larvae are reared for 30-45 days in circular FRP/cemented tanks to grow up to fry (30 mm/0.3 g). The tanks are maintained with a soil base, 1 m water depth, underground water and continuous aeration. The 4-day old larvae are stocked at 300 / m^3. Hilsa is a filter feeder. The first-feeding larvae with very small mouth gape (139 micron) are fed with Chlorella which is very much appropriate in respect to small size (normally 3-5 micron) and circular shape. As the larvae grow in size co-feeding is done with Brachionus, mixed phytoplanktons and mixed zooplanktons at different ages of their rearing. The fry survival reaches up to 60% through the provision of strict feeding schedule and management of rearing tanks. It is suggested that before conducting the breeding operation, all the required facilities for larval rearing should be in operational well ahead including preparation of rearing system and production of live food organisms in sufficient quantities for strict adherence to the rearing protocol to harvest the optimum output with regard to larval survival of the species which are very much sensitive to stress.

Larval rearing tank

Fry harvested

Feed Quality and Feed Management related problems in Aquaculture

Ajmal Hussan

ICAR-Central institute of Freshwater Aquaculture
Regional Research Centre, Rahara, Kolkata-700118

1. Introduction:

Fish need to be provided enough nutritious food in order to attain marketable sizes in a short period of time under culture conditions. Two broad type of fish food include:

A) ***Natural food*** mainly tiny plants (phytoplankton) and animals (zooplankton), can be stimulated to develop in a pond through pond fertilisation.

B) ***Artificial feeds***are those feeds prepared and given to fish. The main objective of feeding fish artificially is to provide the nutritional requirements for good health, optimum growth, optimum yield and minimum waste within reasonable cost so as to optimize profits (Schmittou et al., 1998). The nutrients that should be included in fish feeds include:

- Protein for body building
- Fat for normal functioning of the body and for energy
- Carbohydrates for energy
- Mineral salts for bone structure and body functions
- Vitamins for good health.

But sufficient nutrient availability in a feed alone can't ensure optimum production performance. Better results can be obtained when fish are fed correctly using the right techniques that ensure all fish have access to the feed, the fish's nutritional needs are being met and that no excess feed is fed. Feeding fish correctly means:

i. giving feed of the correct nutritional quality for the specified age of fish,
ii. feeding the right feed size for easy consumption,
iii. feeding the correct amounts,
iv. feeding at the right time(s) each day.

So, every farmer should be particular about the quality and quantity of feed fed to the fish. The main categories of problems with feed and feeding are:

(i) problems with the fish feeds themselves and
(ii) problems related to the fish feeding techniques that the farmers employ.

2. Problems related to the fish feeds quality:

The quality of feed refers to the nutritional as well as the physical characteristics of the feed that allow it to be consumed and digested by the fish. The feed should contain all the nutrients required by the fish, in the right proportions for good performance (growth and health). The specific nutrient requirements for fish vary with the fish's size and reproductive state. The nutrients within the feed should also be easily accessible to the fish and be digestible.When the feed is of poor quality and lacks or contains certain important nutrients (especially protein for body building) only in small quantities, fish does not grow well. It has to eat a lot more feed in order to make little growth. Even if the price of such a feed is low, the farmer spends more money on the feed for each gain of 1 kg.

2.1. Specific Feed ingredients related problems:

Quality problems of fish feed ingredients used in mash feed is of concern to many farmers. Poor ingredient quality can results in suppressed growth rates, extended culture periods, and higher production costs. Quality problems of de-oiled rice bran include:

- unacceptably high moisture content, resulting in fungal growth;
- high fibre levels;
- lower crude protein levels than specified and paid for;
- adulteration of the product with a high percentage of rice husk or saw dust; and
- adulteration with urea to indicate a higher crude protein level than was the case (urea adulteration is usually suspected if the protein content of the rice bran exceeds 16 %).

The quality problems of groundnut cake include:

- contamination with fungal growth;
- high fibre contents due to excessive levels of shell debris;
- adulteration with powdered tamarind seeds and fine sand to increase the weight; and

Quality problems of cottonseed cake include:

- unacceptably high fibre contents
- high levels of anti-nutrition factors such as gossypol and
- sometimes cottonseed cake form hard balls which could not be used as feed.

2.2.Quality problems related to industrially manufactured pellets:

2.2.1. ***High percentage of mouldy feeds:*** Presence of fungal growth or mouldy feed found in certain feed batches can be result of:

- high moisture content in fish feed pellets resulted of inadequate drying,
- prolonged storage of the fish feed batches under sub-optimal environmental conditions.

2.2.2. ***High levels of dust:*** This problem may be due to many reasons, including problems during the manufacturing process as well as physical breakage of a portion of the feed pellets as a consequence of poor transporting and unloading methods. In certain occasions the percentage of the observed (and later weighted) dust inside the bags of certain batches exceeded 20% of the total weight.

2.2.3. ***Big differences in the size of the pellets:*** In some occasions grain sizes supplied to the farmers are completely out of the specifications concerning the dimensions of the pellets. This is particularly problematic when the fish are relevantly uniform in size due to recent grading. This may be caused again by mistakes at some stage of the feed production procedure in a fish feed plant.

2.2.4. ***Feed floating related problems:*** Some of the extruded feeds that were designed to float sank in the water, and *vice versa* that those that were designed to sink sometimes floated.

3. Importance and attributes of quality Feed:

Use of well-balanced feeds covering the nutritional and energy requirements of the species is must. Specifically, the optimum dietary protein and energy levels, which are crucial parameters for effective feed formulation, need to be determined and evaluated on the farm. The cost-efficient use of diets with formulations targeting the specific seasonal and developmental needs of the fish will effectively improve production FCR and have a significant economic benefit for aquaculturists. **Few attributes of good quality feed are:**

i. The ingredients used in the feed should be finely ground. The pellets will have uniform colour and you should not be able to distinguish morsels of maize for example.
ii. The pelleted feed must be without fines or dust. If too many fines are in the feed, too much will be wasted in the form of a powder that floats on the water surface.
iii. The pellet should be firm with a water stability of at least 30 minutes. The pellet's water stability refers to the time it takes for the pellet to completely fall apart in water.
iv. The pellets should be of uniform size and of correct size so the fish can swallow them. A size of about ¼ the gape of the mouth is advised.
v. The feed should be palatable to the fish with a good taste, smell and feel. Fish will spit out or only slowly consume feed that is not palatable.

4. Feeding related problem and feeding management:

Feed costs account for up to 40% to 60% of total production costs, and inappropriate feeding and feed management can therefore be detrimental to the profits of farmers. Any feed that is not eaten by the fish drops into the pond water, which may be a result of two things. Either the farmer gives too much feed for the fish, or the feed is of poor quality and the fish only eat a small proportion. Either way, the uneaten feed drops into the pond and starts rotting (decomposing). Under such conditions, the fish gets stressed, stops eating and in extreme cases the fish dies.

Aquacultural feed management strategies control how a farmer feeds his fish. In addition to influencing key performance indicators, such as weight gain and feeding efficiency, each of these components can also have a profound effect upon fish behaviour and welfare. A primary concern among aquaculturists is to deliver a ration size that optimizes both growth and feeding efficiency; many aquaculturists still rely upon experience or feed tables to establish the daily ration sizes for fish. Although these recommended rations are based upon extensive research into fish nutrition, they assume that fish will consume food whenever it is offered, irrespective of time of day or feed regime. However, farmed fish show marked variations in appetite both within and between days (Noble *et al.*, 2007), and farmers need to understand this appetite variability in order to prevent episodes of underfeeding or overfeeding. Underfeeding reduces feeding efficiency and growth, and increases competition.
Overfeeding also reduces feeding and increases feed wastage, which in turn can increase environmental impacts and environmental degradation (Cho and Bureau, 1998).

Optimal feed management includes the use of well-balanced feeds covering the nutritional and energy requirements of the species and cost efficient feeding regimes. The accurate determination of the basic nutritional requirements throughout the production cycle and best practices in feeding regimes and technology is required in order to achieve a significant reduction in production FCR values.. In addition, better knowledge is required for the main influencing biotic and abiotic factors, which are fish size, diet composition, feeding level and frequency, and water temperature and oxygen levels. Optimizing feed utilization efficiency, fish growth, health and welfare, besides promoting production efficiency and economy, will also have a significantly positive environmental impact. Overfeeding results in excess nutrients entering the environment that need to be assimilated or they will accumulate.So, better results can be obtained when fish are fed correctly using the right techniques that ensure all fish have access to the feed, the fish’s nutritional needs are being met and that no excess feed is fed. Feeding fish correctly means:

- giving feed of the correct nutritional quality for the specified age of fish,
- feeding the right feed size for easy consumption,
- feeding the correct amounts,
- feeding at the right time(s) each day.

4.1.Estimating the Correct Amount to Feed.

In order to avoid over or under feeding the fish, the right amount of feed must be given each time. The amount of feed to be provided to the fish per day, the feeding rate (*ration*), is dependent on the fish’s body weight. Fish adjust their food consumption rates to meet their metabolic energy requirements. Therefore, the required ration varies with time during the production cycle depending on:

- the fish' size (i.e. its average weight)
- the pond water quality - notably in terms of water
- temperature, dissolved oxygen and pollutant levels.

4.2. Adjusting the Ration

Feeding rations should be adjusted either weekly or fortnightly depending on the fish's size. Smaller fish have a much higher metabolic rate and grow at a much faster rate so their rations need to be adjusted more frequently (preferably weekly). Feeding rations can be adjusted with the aid of feeding charts and occasional sampling (at least monthly) to ascertain actual fish sizes and growth rates. At sampling, adjust the ration based on the average weight of the fish obtained.

Fish do not feed at the same intensity every day. The amount of food they take in each day depends on the water quality on that day, notably the temperature and any stressors (low DO, high pH, high ammonia, disease, etc.) to which the fish are exposed. Feed rations should, therefore, also be adjusted on a daily basis. Therefore, on rainy cold days one needs not to feed if fish show no interest in feeding as a result of lower water temperatures.

4.3. Feeding Frequency

The *feeding frequency* is the number of times fish in a pond are fed in a day. The feeding frequency affects the efficiency of feed utilisation (i.e. the FCR) so it is important to establish the optimal frequency of feeding so as to attain the best possible (optimal) FCR and uniform sizes of fish.

4.4.Feeding Response

It is extremely important to feed fish in ponds by response, because:

- It enables the farmer to feed the fish based on their actual needs at each meal. Therefore, the likelihood of overfeeding or underfeeding is reduced to a minimum.
- It enables the farmer visually assess the number of fish in the pond, and their growth on a daily basis without actually having to physically handle the fish. The only time a farmer can see most of the fish in the pond in one mass, is during the course of feeding.
- When water quality conditions in the pond are poor, or fish are sick, their first response is to go off feed. When fish are fed by response, it becomes easy to detect when they have lost their appetite. Therefore problems can be detected sooner, and remedial measures effected promptly before it is too late. The fishes feeding response, is therefore, the first indicator of the fishes well being.

The fish's feeding response depends on the:

- ✓ **Suitability of the Feed.** The feed's appearance, smell, texture/feel and taste also influence the fish's appetite. The more palatable the feed is, the better the feed response should be.
- ✓ **Culture (Water) Environment**. The most important water quality parameters that affect feeding response in ponds are water temperature and dissolved oxygen. The warmer the water and more dissolved oxygen it has, the more active fish will be and the better their feed consumption and FCR.
- ✓ **Other Stressors,** such as pollutants in water, other water quality variables (notably of ammonia and pH), handling and social interactions also affect the fish's appetite. When fish are stressed, their *appetite* drops quickly.

Judging Feeding Response

The following is a description of the criteria used to judge the fishes feeding response:

- ❖ **E – Excellent –** Fish are very active and come to feed immediately. The feed administered is all consumed by the fish within 5 to at most 10 minutes of feeding.
- ❖ **G – Good –** Fish are less active and come to feed over a longer duration. Feed gets consumed in about 15 to 20 minutes.
- ❖ **F – Fair –** Fish are sluggish but do consume about three quarters of the feed. However, they do so in over more than 30 minutes.
- ❖ **P – Poor –** When feed is applied, fish do not come to feed. More than three quarters of the feed administered is left over.

Training Fish to Feed by Response

Fish should be trained to come up, and get their feed at the water surface. In order to do this, the following steps should be followed when fish are fed by the slow broadcasting technique:

- Administer the feed at the **same place in the pond and at about the same time every day.** This gets the fish into the habit of being in a certain area of the pond at feeding time. If the fish do not come to the area to feed initially, do not add any more feed until they learn to come to the assigned feeding area. It may take up to a week to train fish to come and feed from the same area and learn their feeding times. Do not worry if in the mean time they do not get much. One may stomp at the edge of the pond, to call the fish at feeding time before administering the feed to them.

4.5. When Not to Feed Fish

- **The Feeding Response is Poor.** When the fish show a poor feeding response, it is normally for a reason. The water quality may have changed. For example, on a cold wet day, the pond water temperature may have dropped. Therefore, do not add more food than the fish are interested in consuming. Rather, find out the cause of the poor response and if it is due to something you can address, then correct it.

- **They are Feeling Unwell.** When fish are sick, they go off feed. If you insist on feeding them, they still will not eat. The feed administered will instead accumulate at the bottom of the pond, and cause the water quality to drop. No positive returns accrue from wasted feed. Instead losses accrue due to reduced water quality, higher FCRs and the lost income from the wasted feed.
- **At least Two Days before Harvest and Transportation.** This is to allow them to empty their guts before harvest and transportation. In so doing, water quality in transport containers can be better maintained and stress levels during transportation reduced. The other objective is to improve quality of the harvested product for the market.
- **When Treatments are applied to the Pond.** When some treatments like formalin are applied to the pond, the fish get stressed because the water quality within the pond will have temporarily been altered. Their appetite subsequently drops. It is best to allow the water quality to improve and when it does, so will the fishes feeding response.
- **When Water Temperatures are Low on Rainy Days.** After a series of rainy days if the water temperatures drop below 22 oC, the fish are unlikely to be interested in feeding. Therefore, do not feed.

5. Summary:

- ✓ Cheaper fish feeds are not necessarily cost effective.
- ✓ The *optimum ration* is the one that gives best growth rates, uniform growth and the optimum FCR. This is because at this level of feeding, there is minimum feed wastage and minimum deterioration of water quality. This is often achieved when fish are maintained at a feeding level just below that of '*satiation*'.
- ✓ It is important to supply exact amount of feed needed by the fish and avoid wastage. A good quality feed that is poorly administrated is not better than a poor quality one.
- ✓ **DO NOT** overfeed fish because it results in feed wastage, deterioration of water quality and subsequently poor growth. Overfeeding only serves to reduce your profit margin. Likewise, substantial underfeeding results in poor growth and production.

Fish Feed Processing Technology for Entrepreneurship Development

K.C. Das
Fish Nutrition and Physiology Division
ICAR-CIFA, Kausalyaganga, Bhubaneswar, Odisha, India

Introduction

Feed comprises the biggest cost in intensive fish farming and the objective of feeding fish is to provide the nutritional requirements for good health, optimum growth, optimum yield and minimum waste within reasonable cost so as to optimize profits (Schmittou et al., 1998). Even when the natural feed forms the main source of nutrition, supplemental feeding is necessary to obtain increased production in ponds. India produces enormous quantities of feed materials derived from crops. These include a wide variety of oil cakes, pulses, mill byproducts of seeds and grains. Also available are smaller quantities of byproducts from the meat, fish and dairy processing industries. These types of feed ingredients can form good quality supplemental feed for fish.

Unlike natural foods which normally are present as discreet living forms and therefore biologically stable until consumed, artificial feeds undergo rapid nutrient loss through normal deteriorative processes and leaching by water unless quickly consumed. Moreover, when feed aggregates tend to disintegrate and separate into their ingredients components, thus losing their original nutritional properties. So a successful artificial feed apart from meeting nutritional requirements should have good water stability and acceptance by the fish. To produce feed with the desired physical characteristics, feed processing and technology has a major role for quality feed preparation.

Feeding practices in India

Multi species carp farming in India rely mostly on fertilizers and low-cost feed ingredients like rice bran and ground nut oil cake for a long time. The productivity of such a system is complex to understand and manage. Commercial feed is used by very few advanced farmers and others do not use it because of high cost in the market. Farmers/Entrepreneur does not have sufficient technology using the conventional and non-conventional feed resources for floating/sinking feed production resulting increased cost of the feed in the market.

In traditional feeding, a part of the supplementary feed in sinking form goes waste as it sinks to bottom and fish cannot consume it. Floating feed floats in water and so wastage is very less. The farmer can also directly observe the feeding requirements and adjust feeding rates accordingly. It is important in maximizing fish growth and feed efficiency by determining whether feeding rates are too low or too high. The advantages of feeding floating feed are less waste, higher feed intake, improved feed efficiency, lower handling costs, easier storage, and less bacterial contamination. It has been reported that, aquatic animals cannot digest starch effectively resulting in excessive excrement which causes physiological problems such as excessive gas, bloating diarrhoea and these apart from affecting the growth of the fish also lead to water pollution. So starch in the feed are effectively utilised by extrusion for preparation of floating feed.

Fish Feed Technology in India

The concept of feed technology in India started about 30 years ago. In 1967, the compounded livestock Feed Manufacturers Association of India (CLFMAI) was set up to represent the interest of manufacturers in the government, cooperative and private sectors with an estimated designed capacity of 1.73 million tones/ annum. However, the production of compounded feed was for livestock and poultry. There was no commercial production of fish feed. Few mills gradually produced pelleted feed by using old pelleting technique suitable for producing large size pellets for livestock and Poultry; it was inadequate for making small sized pellets of sufficient hardness and compactness suitable for fish feeding. Small size pellets are required for fish feeding that not only possess hardness but also remain water stable when fed to fish. A laboratory size feed pelleting machine manufactured by California Pellet Mills Co. in USA was installed long back at CIFA, Bhubaneswar, for production of laboratory size feed pellets producing about 100 kg product per hour. However, it was having nos of limitations and feed manufacturers started producing fish feed on commercial scale. Now, many states have feed mills producing fish feed on commercial scale, both sinking and floating type. CIFA installed the extrusion machine under the Division of Fish Nutrition and Physiology and started producing both sinking and floating pellets for carp and Prawn feeding.

Basic steps of Feed Manufacturing and Technology

Raw material collection

The 1st operation for feed manufacturing is the collection of raw materials. Initially the raw materials are checked by physical observation and chemical analysis to ascertain the quality. Good quality locally available feed ingredients like Maize, soyabean meal, ground nut cake, til oil cake, rice bran, rice polish, fish meal, mustard oil cake are procured from the market that arrive in bags or small containers are stored in dry location preferably in specially designed feed store. Proper storage temperature and humidity are maintained during storage, so the nutrient losses are minimized. Liquid ingredients like oil and molasses are generally stored in bulk tanks.

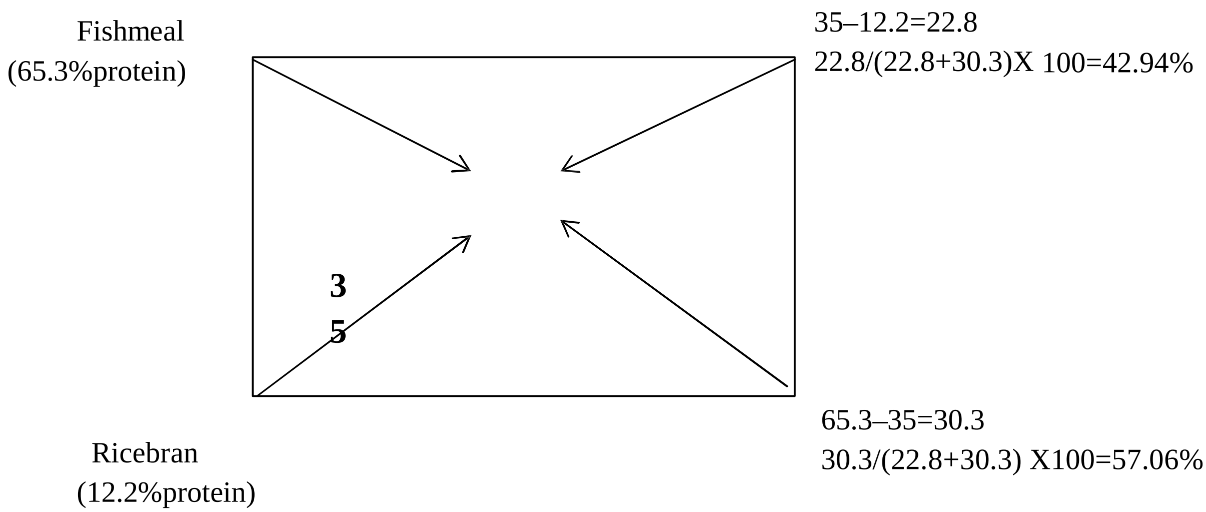
FeedFormulation

Itisaprocesswhichhastoaddresstwoprincipalobjectives.Thefirstisaproblemof economicswherefeedformulation isperformedtoachieveoptimalproduction.The second is thecalculationto findthetypesand amountsof ingredientsto bemixedto producecompletefeeds.

A. Handformulation

Asimplemethodinhandformulationis‘Pearsonsquarewhichcanbesimplyapplied formixingtwo,ortwo groups ofingredients consideringtherequirement(usuallyprotein). Fig1.showsasimple applicationof Person’ssquaretomix meal(65.3%protein)and rice bran (12.2%

protein) to formulate 35% protein supplementary feed. Application becomes more complex for more ingredients and requirement considerations for which worksheetisneeded.



The solution is42.94%fishmeal+57.06%ricebran

Fig: PearsonSquaremodel

Photo: Harvesting of freshwater IMC in ICAR-CIFA pond

B. Linearprogrammingcomputerizedformulation

Linear programming is a class of mathematical programming models concerned with the efficient allocation of limited resources to known activities with the objective of desired goals such as maximizing profit or minimizing cost when there are alternate use for the resources.Basically linearprogramming works by solving of a number of linear equations. A series of equations which describe in mathematical terms the conditions of requirements of the formula is established. These requirements are to be measured in numerical terms.These are fairly easy to accomplish for some items like protein, fat Calciumetc. but difficult for other factor like palatability. Such a programme would allow a number of constraints, maximum or minimum levels of nutrient requirements and ingredient inclusion to be set based upon cost and nutritive values. The primary advantage of using linear programming(LP) in the formulation of feed is that it can rapidly and accurately determine combination of feedstuffs that will meet specific nutritional and physical requirements at lowest possible cost and not have any bias towards any ingredient. But there arelimitations also.

C. Quadraticprogrammingformulation

Nutrient requirements use dinlinear programming feed formulation are for fixed usually maximum rate of growth. This may not be the best decision from economic point of view. Nutrient constraints may be relaxed to bring downfeedcostwhile still achieving acceptable lower growth. Quadratic programming takes into account the growth response within a range of nutrient constraint. A good understanding of biological response functions from actual feeding trials is therefore, essential in the use of quadratic programming.

Grinding

Grinding or particle size reduction is a major function of feed processing. Grinding generally improves feed digestibility, acceptability and increases the bulk density. Different types of grinders are used for grinding the feed ingredients like Hammer mills, attrition mills, Roller mills, cutters etc. Hammer mills are mostly impact grinders with swinging or stationary steel bars forcing ingredients against a circular screen or solid serrated section designed as a striking plate. The material is held in the grinding chamber until it is reduced in size of the opening in the screen. Attrition mills use the hammermill principle to a certain extent. Grinding is done between two discs equipped with replaceable wearing surfaces. One or both of these discs is rotated, if both they rotate in opposite directions. In roller mill, a combination of cutting, attrition and crushing occurs. These are smooth or corrugated rolls rotating at the same speed set at a predetermined distance apart with material passing between the two.

Mixing

This is a process in which each small unit of the whole is the same proportion as the original formula. Only when all ingredients as per the formula are thoroughly mixed, the feed will prove worthy. Various types of mixers are available in the market like vertical and horizontal type. Feed mixing include all possible combinations of solids and liquids.

Extrusion cooking

Extrusion processing has become the primary technique used for fish feed production, mainly because of the high physical and nutritional quality of the feed (Hilton et al. 1981). Basically, *an extruder* is a long barrel with one or two rotating screws (single – or twin screw extruder) which is specially designed to subject feed mixtures to high heat and steam pressure. When feed exits the die at the end of the barrel, trapped steam blows off rapidly, the soft warm pellets expand, and a low density floating pellet is produced. The system is also equipped with a preconditioner as well as an accompanying achine control system. The preconditioner is a high speed mixing unit designed for the purpose of mixing water and steam into the blend of dry ingredients. The overall goal with preconditioning is to supply the extruder barrel with an evenly moistened and preheated mix. Preconditioning allows more efficient transfer of heat through friction in the extruder barrel, and also reduces the extruder barrel wear and energy. Most materials require milling prior to extrusion, especially large granular ingredients like Maize or Soya. After extrusion, cooling is required to remove excess moisture. A moisture content of 12-14% should be achieved to prevent fungal activity.

Moreover, aquatic animals cannot digest starch effectively resulting in excessive excrement which causes physiological problems such as excessive gas, bloating diarrhoea and these apart from affecting the growth of the fish also lead to water pollution. So starch in the feed are effectively utilised by extrusion for preparation of floating feed. Extrusion processing has become the primary technique used for fish feed production, mainly because of the high physical and nutritional quality of the feed (Hilton et al. 1981).

Extruded feed may be of sinking type or floating type. A part of the supplementary feed for fish in sinking form goes waste as it sinks to bottom and fish cannot consume it. This wastage is less in floating feed and farmer can directly observe the feeding requirements and adjust feeding rates accordingly. The advantages of feeding floating feed are less waste, higher feed intake, improved feed efficiency, lower handling costs, easier storage, and less bacterial contamination. Floating feed for fish has become very common for feeding of fish including fresh water fish.

Benefits of extrusion

1. In extrusion, raw material is expanded, starch is gelatinised and oil cells are ruptured. So the digestibility of nutrients is increased. Denaturation exposes sites for enzyme to attack and may thus make the protein more digestible. So it increases the nutritional value of protein-containing ingredients.
2. Extrusion can destroy harmful organisms like salmonella.Heat labile proteinaceous anti-nutritive factors such as trypsin inhibitors and lectins may be destroyed.
3. The heat and pressure deactivate destructive enzymes such as those that cause rancidity.
4. Increase availability of carbohydrates.
5. It neutralises growth inhibitors.
6. There is increase availability of sulphur containing amino acids.
7. There is also improvement of palatability.

Cooling and drying

The temperature imparted to pellets after extrusion cooking assists the removal of moisture by the air drying process. Generally, within ten minutes after extrusion, hard pellets are cooled to ambient temperatures and brought to moisture content slightly above that of the entering soft feed. This may be done by spreading pellets in a thin layer on the floor and blowing air over them. Commercially, it is done by passing the hot pellets through a vertical or horizontal chamber designed to bring air at ambient temperature into intimate contact with the outer surface of the pellets. The cooling and drying operation are of vertical type or horizontal type. Heated air may be used in the cooling and drying process. Pellets after preparation may also be dried by using dryer fitted with electrical heaters.

Packing, storage and distribution

Most feed are sacked. The sacking operation includes weighing, sacking, taping, coding and sewing. The sacked bags are then sent for distribution. The bulk products are stored in large bins.

Photo: Carp feed in the CIFA Feed Mill before packaging

Effects of extrusion processing condition on nutrient quality

A. Temperature

Effects of extrusion temperature on digestibility and utilization of the diets by rainbow trout were examined. In one experiment, a fishmeal-based diet was extruded with a twin-screw extruder at three temperatures (100, 125 and 150^{o}C) and in another experiment; fishmeal-based diet was extruded using a single screw extruder at two temperatures (100 and 140^{o}C). In two experiments, it showed that extrusion processing with temperatures in the range from 100 to 150^{o}C did not affect digestibility of protein, individual amino acids or energy. Similarly, feed conversion and net accumulation efficiency (retention) of protein and energy were not significantly different in trout fed diets extruded at 100 and 140 ^{o}C. In line with these results, Barrows et al. (2007) found no significant effect of extrusion temperature on apparent digestibility coefficients of protein, organic matter, lipid, energy or carbohydrate in diets containing soybean meal for rainbow trout. The high temperature (127 ^{o}C) resulted in improved FCR compared to the low temperature of 93 ^{o}C (Barrows et al., 2007). This indicates that feed extruded at the highest temperature of this experiment was better utilized either due to improved availability or utilisation of the nutrients or favourable feed structure. Effect of extrusion temperature on physical quality was investigated by Aarseth et al. (2006). Feed produced at the lowest temperature, 100^{o}C, showed a significantly higher durability and hardness compared to feed produced at 140 ^{o}C.

In our experiment in CIFA, the effects of the quality of floating feed on different extrusion temperatures were studied. There was no significant difference in floating time, sinking time and proximate composition of feed when the temperature of extrusion varied from 130-150 ^{o}C where as higher or lower temperatures were not favourable

B. Moisture

In general, low moisture content, especially in combination with severe heating; have shown to cause reduced digestibility of nearly all amino acids in fish meal, especially cysteine (Andorsdòttir, 1985). Reduced digestibility of cysteine was also shown in rainbow trout when water addition to the extruder was restricted, compared to when the diet was produced at elevated moisture conditions (Sørensen et al., 2002). Cysteine reacts readily during heat treatment to form disulphide bonds between cysteine units. The reduction in cysteine digestibility in heat treated proteins has been explained by introduction of SS bonds, assumed to be resistant to proteolytic cleavage. The improved performance of shrimps fed diets extruded at elevated moisture contents also emphasizes the significance of moisture during processing (Obaldo et al., 2000). From this it can be concluded that "dry" extrusion conditions should be avoided. In order to prevent losses of essential nutrients, a moisture content of 25-30% during wet extrusion of diets for fish and pets has been recommended (Rokey, 1994). In our feed mill, moisture content of 20-25 percentages were maintained for preparation of floating feed for carp.

C. Protein source and starch level in ingredients

Various protein-containing ingredients in combination with starch affect expansion of the extrudate differently. It has been reported an increase in expansion when soy protein isolate was added to pure starch, whereas gluten protein caused reduced expansion (Faubion et al., 1982). The results also showed that expansion increased linearly as the starch level was increased. Increasing the starch content from 0 to10% did not result in any significant increase in expansion, but the expansion increased significantly at higher starch inclusion levels. Starch gelatinization increased significantly with increasing amount of starch in the SBM diet, whereas the FM-diet showed decreased starch gelatinization with increased starch level. There was a significant difference in expansion due to protein source. The highest expansion was observed for the fish meal diet. As starch level increased, the differences in expansion among protein sources became more pronounced. In the Feed Mill at CIFA, it was observed that, increasing the maize level in the diet produced better quality floating feed compared to low level of maize.

D. Quality of feed ingredients

The qualities of feed ingredients affect the quality of floating feed. High physical quality of the feed pellet is necessary in order to minimize feed wastage and thereby maximizing feed intake and feed conversion. The pellet must be durable and remain in one piece until eaten by the fish, since dust and small fractures of the feed are not ingested and will result in poor feed conversion ratio. Fish do not physically disrupt the feed in the oral cavity but gulp the pray whole (Steffens, 1989). If the feed is too hard, the pellet may stay intact during passage through the gastrointestinal tract, and the nutrients may be unavailable for enzymatic degradation. The ingredient compositions affect physical quality of the feed. Moreover, physico-chemical properties of different ingredients seem to affect extrusion processing parameters, and hereby pellet quality.

E. Other conditions

Other conditions like screw speed and rate of flow of ingredients to the extruder affect the quality of floating feed.

Photo: Floating carp feed produced in the Feed Mill of ICAR-CIFA, Bhubaneswar

Evaluation of floating Pellet

The floating feed must be evaluated in order to ascertain quality. The evaluation may be of physical or chemical.

Physical evaluation

Strength of rupture

Hardness or strength at rupture, defined as the maximum force needed to crush a pellet, is commonly determined using a texture analyser. This method assesses resistance to breaking when pellets are exposed to external pressure, and can be used to mimic the force on pellets during storage in bins or silos, crushing of pellets in a screw conveyor, and crushing of feed pellets between animal teeth (Kaliyan & Morey 2009). Despite the reliability of the test, there is a lack of standardization for analyzing the texture of feed pellets. The result of the test may also vary with the various probes and attachments used. Hardness may be reported as shear force if a knife is used or pellet hardness if a flat ended probe is used. Hardness of the pellet varies with degree of expansion, ingredients and processing conditions.

Water stability:

Water stability of feed is an important quality trait for slow eating aquatic animals. Feed has to be soaked in water for hours with minimum leaching of nutrients.The Water stability was calculated as the difference in DM weight before and after incubation in water divided by DM weight of the feed before incubation. A procedure to determine water stability over time was described by Baeverfjord et al. (2006). In general, the feed having better water stability is preferred even for fast eating carp.

Bulk density

Bulk density is an important property that determines floatability or sinking velocity of pellets and is directly related to the degree of expansion during extrusion. A floatingpellet is more expanded and has a lower bulk density compared to a sinking pellet. Bulk density of pellet needs to beadjusted according to feeding management practices and feeding habits of the target species, and usually a bulk density greater than 525 g L _1 is needed for sinking pellets in seawater 35 g L _1. Bulk density is analyzed by filling up a measuring cylinder of known volume. Pellets are carefully poured into a tared cylinder until a pile of feed has developed on top. A scraper is used to remove the excess feed by pulling once gently over the edge of the cylinder. The content of the full cylinder is then weighed on a balance. In order to standardize the procedure, pellets should be poured from a funnel, preloaded with feed, and the cylinder should not be tapped prior to weighing. Each measurement should be carried out in triplicate, and bulk density for each replicate is calculated as mass of the sample to the unit volume of the sample (g L_1). Volumetric displacement methods can also be used to measure specific density of the pellets with improved accuracy.

Durability

Durability is the amount of fines produced from a sample of pellets after being subjected to mechanical or pneumaticagitation. Pellet durability simulates forces on pellets takingplace during filling of bins, during transportation from the feed factory to the farm, and during distribution in thefeeding system at farms. Pellets with high durability formfewer small particles and fines during bagging and storage and finally, show low degradation in pneumatic feedingdevices when fed to fish. Different devices have beendeveloped to assess durability of pellets; however, most of these cannot be used on oil-coated high energy extrudedfeed.

Fat absorption

Oil absorption is determined as weight increase of sample (g) divided by initial weight of sample (g) times 100. Oil leaking can be determined as the loss of oil from pellets. Leaking of oil from high energy diets is a problem because of lowered energy content and different nutritionalprofile. Oil leaking from the pellet should be avoided. Research has shown that oil leaking is affected by choice of ingredients and processing conditions. Sørensen et al. (2010) found that oil leaking was not associated with oil level in the feeds, but was related to feeds with low absorption capacity. Neither was oil leaking correlated with expansion of the pellet or other physical quality parameters. Most likely oil leaking is related to microstructure of the pellet. Expansion of the pellets is correlated to oil absorption capacity.

Chemical Evaluation:

The floating pellets can also be evaluated by chemical evaluation. The chemical evaluation may be of different types.

Proximate composition

It is based on the separation of feed components into groups or fractions in accordance with their feeding value. These nutrients are known as proximate principles of feed or proximate analysis of feed. The various fractions are water, Crude Protein, Crude fat or ether extract, crude fibre, nitrogen free extract and mineral matter or ash. Proximate composition is very common practice for feed evaluation.

Amino acid and Fatty acid composition:

The primary function of protein is to supply the amino acids. Therefore, the requirement of protein is essentially the requirement for amino acids. The quality of protein depends upon the amino acids makeup of the feed. All the amino acids can be analysed by using amino acid analyser. The ingredients are initially hydrolyzed by 10N HCl after which the amino acid composition is characterized by HPLC. In practical condition, the most limiting amino acids like lysine and methionine are also analysed by indirect method.

The dietary sources of lipid in feed are estimated through gas Chromatography for qualitative fatty acid analysis. The feed materials are initially cold extracted with organic solvents i.e. chloroform and methanol and the extracted lipid were esterified and then analyzed by using gas chromatography where it is compared with standard fatty acid mixture.

Mineral and vitamin composition:

Minerals are analysed in the laboratory both for major or macro minerals and minor or micro minerals. Atomic absorption spectrophotometer is routinely used for estimation of mineral in feed stuffs. However, by using near Infrared spectroscopy, all the minerals can be analyzed in very short period of time. The fat soluble vitamins namely Vit A, Vit D, Vit E and Vit K are analysed by the saponification process.

Bioavailability/digestibility

The potential value of feed for supplying a particular nutrient can be determined by chemical analysis, but the actual value of the feed can be arrived only after making allowances for the inevitable losses that occur during digestion, absorption and metabolism. These losses can be very correctly measured in nutrient digestibility laboratory to know the digestibility of feed stuffs. In the Fish Nutrition and Physiology Division of CIFA, nutrient digestibility laboratory was set up and the digestibilities of newer and nonconventional feed stuffs are measured in this laboratory. In this laboratory, the culture practice is conducted in a re-circulatory aquaculture mode where cylindro-conical tanks of 200 litre water capacity are used for collection of faecal matter of fish in order to calculate the digestibility of nutrients.

Conclusion

Lots of technologies have been developed in this area, some technologies are meant for farmers and some are for academic point of view. Extruded feeds have many advantages like less waste, higher feed intake, improved feed efficiency, lower handling costs, easier storage and less bacterial contamination. The feeding of extruded feed for fish need to be popularized among the farmers of this region.

Photo: Different types of fish feed produced in the ICAR-CIFA Feed Mill

Importance of Bioenergetics in Aquaculture Nutrition

Arabinda Das

ICAR-Central institute of Freshwater Aquaculture

Regional Research Centre, Rahara, Kolkata-700118

INTRODUCTION

Bioenergetics is the study of the flow and transformation of energy in and between living organisms and between living organisms and their environment (The American Heritage® Medical Dictionary, 2007). It is a branch of biophysics, a multi-disciplinary field that studies the effects of thermodynamics on biological systems (Segen's Medical Dictionary, 2011). Nutritional bioenergetics describes energy requirements and flow of energy and nutrients within a biological system. Energy is very important and required for the maintenance of all living beings. Animals including fish cannot utilize energy directly from the sun unlike plants. They have to get their needed energy from oxidation of the complex molecules which are eaten by the animal.The feed, that is consumed, is transformed in the body, complex chemical compounds are broken down into simpler components - protein into amino acids, carbohydrates into glucose, lipids into fatty acids and with this process energy is released - which is used for maintenance, for renewing worn out tissue and building new tissue – for growth. The major organic compounds in feeds such as lipid, protein and carbohydrates are the sources of energy but they also supply the building material for growth (Lupatsch, 2013).

In aquaculture, feed is considered as the most critical input in aquaculture constituting about 60 - 70% of total recurring cost. It is important to provide supplementary feeding because the natural food produced through pond fertilization is not sufficient enough to sustain the standing crop of cultured species in semi-intensive or intensive farming. Therefore, the use of artificial feeds balanced with all nutrients and energy is required for optimum growth and reproduction in fish. It's well known that feed wastes and faecal matter contributes to nutrient loadings in aquatic environment which in turn results in poor water quality and algal bloom formation, whereas the reduction of these wastes through underfeeding affects the growth rate. Therefore optimal feeding levels under practical conditions are required to reduce waste output.In this write-up explanation is given for nutritional bioenergetics approach in general and the need of engineering of nutritional bioenergetics to develop feeding charts to meet nutritional requirements precisely and make aquaculture more sustainable is highlighted.

TYPES OF ENERGY AND TRANSFORMATION EFFICIENCY

There are different types of energy: chemical, mechanical, electrical and heat. These different forms of energy can be transformed into each other but only at a cost, the transformation is not 100 percent efficient (Lupatsch, 2013). The transformation loss of energy is mostly in the form of heat. Heat is also the only form of energy, into which all the others can be transformed and measured. Heat energy is usually expressed in kilocalories (kcal) or kilojoule (kJ). One kcal is the energy needed to raise the temperature of one kg of water by one degree Celsius (^oC). One kcal is equal to 4.184 joule. The chemical energy stored in feed and animal tissue is measured using a bomb calorimeter. The amount of heat produced by complete oxidation of feed or tissue is known as the heat of combustion or gross energy (GE) (Lupatsch, 2013).

For the bio-energetic model, the two laws of thermodynamics can be applied, 1) Energy cannot be created or destroyed within a system but may be changed into different forms (what goes in must go out); 2) In a system where energy is transformed (from feed to flesh) there is a degradation and loss of energy in the form of heat (nothing is 100 percent efficient).

ENERGY METABOLISM IN FISH

Energy metabolism in fish is different from that of terrestrial farm animals in two important respects. *Firstly*, in contrast to warm-blooded animals, fish are aquatic ectotherms (cold blooded) and so do not have to expend energy in maintaining a body temperature well above ambient at 37°C. Fish therefore have much lower maintenance energy requirements than terrestrial farm animals (Cho and Kaushik, 1985). *Secondly*, fish are able to obtain 10–20% more energy from the catabolism of proteins than terrestrial farm animals, as they do not have to convert ammonia (the end product of protein catabolism) into less toxic substances (i.e. urea or uric acid) prior to excretion (Brett and Groves, 1979). The excretion of waste nitrogen therefore requires less energy in these aquatic animals than it does in homeothermic terrestrial animals (FAO, 1980, 1987).

The flow of energy from feed to growth in an animal is illustrated in Figure 1. Not all the energy from the feed is digested, substances such as cellulose and fibre from plant ingredients pass through the digestive system without being available to the fish. The consumed GE minus faecal energy losses (FE) is called the digestible energy (DE) which is then available for the metabolic processes of an animal. The next major losses occur, when energy containing compounds (on DE basis) are transformed by the fish, broken down to smaller units and then used to build its own energy reserves or to deposit protein as growth. Metabolizable energy (ME) is the DE minus energy losses from the urine and gills in fish. ME is a more precise measure of available energy for metabolism but it is difficult to measure in fish. As mentioned above, this process of transformation is never 100 percent, there are always losses and they are mostly in the form of heat. In poikilotherms such as fish this heat is lost to the surrounding water, in homeotherms it is partly used to keep the body temperature constant. Only the net energy (NE) is now available for maintenance and for growth. Maintenance requirement represents energy needed for movements, osmo-regulation, blood circulation, first this energy has to be supplied before the remainder can be channelled into growth - the main product in fish culture (Lupatsch, 2013).

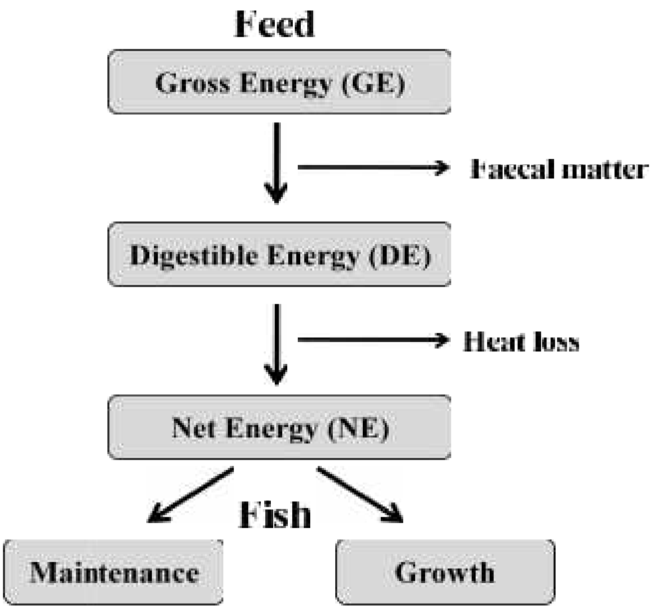


Figure 1: Schematic presentation of the energy flow in a fish

By quantifying the energy budget – the energy input on one hand and the various energy losses on the other hand, valuable information can be gained in order to optimise feeds and guarantee optimal fish growth. By defining demands for maintenance and growth (Figure 1) and anticipating certain losses beforehand, feeds can be formulated and feeding tables established (Lupatsch, 2013).

ENERGY LOSS

Energy is lost from the body of a fish mainly through gill excretions, in the faeces and urine, and as heat. The energy lost as heat that comes from three sources is difficult to measure separately. These are:

(a) Standard metabolism (SM)

It is the energy required to keep the fish alive. It is similar to basal metabolism measured in humans but the definition is not applicable to fish, because it is difficult to obtain a "motionless" animal. When a fish is restrained to a motionless condition it struggles to free itself and uses more energy than if allowed to swim freely in still water. SM is the minimum heat production of an undisturbed fish in the "post absorptive" state in still water (FAO, 1980). It is affected by body size, oxygen availability, ambient temperature, osmoregulation, stress and cycles.

(b) Voluntary physical activity

It is the energy expended by a fish moving about, seeking food, maintaining position, etc (FAO, 1980). Thus, locomotion and gonadal growth are important factors affecting non-standard metabolic rate;

(c) Heat of nutrient metabolism or specific dynamic action (SDA)

It is the heat released by the many chemical reactions associated with the processing of ingested feed. It includes the energy expended in digestion, absorption, transportation, and anabolic activities. It also includes the cost of excretion of waste products (FAO, 1980).

ENERGY SOURCES

The energy needs of fish are supplied by fats, carbohydrates, and proteins. Fats are the principal form of energy storage in plants and in animals. Fat provides metabolizable energy (ME) value of about 8.5 kcal /g. The inclusion of fat usually increases the palatability of a feed. Generally fats are well digested and utilized by fish (FAO, 1980).Carbohydrates are the cheapest, instant and most abundant source of energy for animals. The ME values of carbohydrates in fish range from near zero for cellulose to about 3.8 kcal/g for easily digested sugars (FAO, 1980). Heat and moisture associated with the pelleting process improves the digestibility of starchy feed materials. Gelatinization of starch following cooking is digested in a better way and also improves immune competence. Fish have a very efficient system for excretion of waste nitrogen from protein. Therefore, protein is used very efficiently by fish as a source of energy. Protein has a ME value of about 4.5 kcal/g for fish, which is higher than that for mammals and birds. The low energy cost of excreting waste nitrogen in fish is primarily responsible for this (FAO, 1980). As protein represents the most expensive macronutrient in fish feed, for economic reasons it should be kept to a minimum, consistent with good growth and feed conversion and cheaper carbohydrate and fat should be used to supply most of the energy. The most recent approach to reduce the feed cost is to reduce the protein level as much as possible without compromising growth and health of fish.If too much protein is supplied in the diet, only part of it will be used to make new proteins and the remainder will be converted to energy. Moreover, incorporation of dietary protein beyond the optimum level results in high level of ammonia production, which affects the voluntary feed intake and growth of fish.

ENERGY REQUIREMENTS OF FISH

It should be kept in mind that energy needs for maintenance and voluntary activity must be satisfied before energy is available for growth. Also during times of low food intake fat and protein are withdrawn from the animal body to provide the energy needs for maintenance and the animal loses weight. Therefore, feeding levels must be high enough to supply maintenance needs and still have energy remaining for growth. Digestion efficiency in fish decreases as feeding level is increased (FAO, 1980).

Energy is released during metabolic oxidation of proteins, lipids and carbohydrates. Fish do have a low energy requirement because no energy expenditure is involved for maintenance of body temperature. Moreover they expend less energy for excretion of ammonia, mainly through gills into surrounding water. Physical activities like swimming, escaping from predators and stress, temperature, size, growth rate, species and food are some of the factors that affect energy requirements of fish. Proteins, lipid and carbohydrates contain 5.6, 9.4 and 4.1 kcal of GE/g respectively.

Providing the optimum energy level in diets for fish is important because an excess or deficiency of useful energy can result in reduced growth rates (NRC, 1983). For example, excess dietary energy may result in decreased nutrient intake by the fish or excessive fat deposition in the fish, whereas a low dietary energy density will result in the animal utilizing protein as an energy source rather than for tissue synthesis as growth (Robinson and Wilson, 1985). Incorporation of appropriate levels of non-protein energy sources in the diet determines the efficiency of protein utilization and hence the growth of fish. Carbohydrate and lipid are the major non-protein sources in fish diet. At present there is very little useful information on the practical dietary energy requirements of fish; this has been due primarily to difficulties encountered with the quantitative measurement of the energy losses within the energy budget equation (Brafield, 1985).

FACTORS THAT INFLUENCE THE ENERGY NEEDS

There are several factors which can alter the energy requirements of fish such as temperature, water flow, body size, level of feeding, composition of diet, fish species, age of fish, etc. Feeding rates should be adjusted to compensate for these factors to avoid overfeeding, but still providing sufficient energy for optimum growth.

(a) Temperature. Most freshwater fish do not attempt to maintain a body temperature which is different from the environment. As water temperature declines, body temperature of the fish declines and metabolic rate is reduced. The low metabolic rate at low temperatures enables fish to survive for long periods where little food is available. Usually at optimum temperature which is species dependent, the difference between maintenance requirement and voluntary food intake is greatest and optimum efficiency of growth occurs.

(b) Water Flow. Energy which is used for physical activity is not available for growth. Fish which are forced to swim against a strong current are expending energy which would otherwise be used for growth. However, still water allows stratification and the accumulation of waste products. Fish rearing facilities should be designed to obtain maximum use of water without undue stress on the fish.

(c) Body Size. Small animals produce more heat per unit weight due to higher metabolic rate than do large animals. Small fish should be fed a higher percentage of body weight than large fish.

(d) Level of Feeding. The level of feeding also has an effect on the energy expenditure of fish. Dissolved oxygen is usually the first limiting factor in fish rearing. The oxygen consumption increases shortly after feeding due to the physical activity of feeding and the heat of nutrient metabolism. Facilities must be designed with adequate safety margins.

e) **Physiological status.** Energy requirement in fish increases during periods of gonad production and reproductive activity such as spawning migration.

(e) Other Factors. Several other factors can contribute to high energy requirements. Anything which makes the fish uncomfortable increases physical activity and reduces growth. Crowding, low oxygen and waste accumulation are some of these factors.

ENERGY BUDGETING FOR CATFISH FEED

Correct balance of dietary energy is an important consideration when formulating catfish feeds because too much energy can result in a reduction in food intake and thus reduce nutrient intake. In addition, excess dietary energy may result in an increased deposition of body fat. If the dietary energy level is too low, protein will be used for energy instead of tissue synthesis. Energy requirements reported for catfish, which have generally been expressed as a ratio of DE to crude protein (DE/P), range from 7.4–12 kcal/gram. These values are considerably lower than the DE/P ratios of 16–25 kcal/gram reported for swine and poultry. Based on current knowledge, a DE/P ratio of 8.5–10 kcal/gram is adequate for use in commercial catfish feeds. Increasing the DE/P ratios of catfish diets above this range may increase fat deposition, and if the energy value is too low, the fish will grow slowly (Robinson *et. al*, 2001). Catfish can use amino acids, lipids, and carbohydrates for energy. Although lipids and amino acids are more highly digestible by catfish than are carbohydrates, the major source of energy in commercial catfish diets is from carbohydrates contained in grains and grain milling by-products. Carbohydrates, which are the least expensive source of energy, are used to spare protein for growth. Lipids, which are the most concentrated and most highly digestible sources of energy that can be used in catfish feeds, are used sparingly because of several negative aspects of using high levels in catfish diets. The use of high level of lipid as dietary energy source may create problem in pelleting and keeping quality of feed in addition to adversely affecting the fish whole body composition.

CONCLUSION

Normally to optimize feeding levels, fish farmers in India do not follow feeding charts, but they do predict/expect growth and assume feed efficiency based on farm performance history and derive their own feeding schedule. But they hardly apply nutritional bioenergetics approach to devise feeding charts that consisting of growth prediction, accurate digestible energy (DE) value in feeding formulations. Using bioenergetics approach, one can calculate the feed required to avoid waste and pollution. To enable fish farmer to have specific growth rate for his fish, energy content of the fish has to be predicted based on energy retained. But, one needs to have data on maintenance energy needs (standard metabolism) and data on energy partitioning. Thus feed required in kg can be calculated by dividing DE need by dietary DE content per kg feed. Thus it very much necessary to know precise nutrients and energy requirements of variety of fish species of this country under variety of culture conditions for efficient nutrient utilization, reduced waste output at source and for making aquaculture more sustainable.

Effect of broodstock nutrition on reproductive performances of fish

Arabinda Das

ICAR-Central institute of Freshwater Aquaculture

Regional Research Centre, Rahara, Kolkata-700118

Proper nutrition is one of the most important factors influencing the ability of cultured organisms to attain the genetic potential for growth and reproduction. It's well known that the fecundity, fertilization rate, egg quality, embryo development, and larval quality are affected directly by broodstock nutrition, and thus influence the success of hatchery operations. In fishes, the gonadal development and fecundity can be manipulated by giving diets shortly before or during spawning. However, broodstock nutrition is still not properly understood due to difficulties in conducting studies in different species involving proper feeding and reproduction of broodstocks. Growth, gonad development, and reproduction in fish are influenced by many factors. These factors are protein and amino acids, lipid and phospholipids, essential fatty acid (n-3 fatty acids), minerals- P, Ca, Zn, Mn, etc., vitamins- Vit. A, Vit. E and Vit. C, carotinoids: astaxanthin,*Chlorella vulgaris and Spirulina*, feed types: live or artificial diets, ration size and frequency and environmental factors (temperature, photoperiod, light intensity).

Protein and amino acids

Adequate protein is essential for egg development, spawning, formation of follicles, ovarian tissues, growth and development of embryo (Shim *et al.*, 1989). The effect of dietary crude protein level on fecundity varies between fish species (Luquet and Watanabe, 1986; Watanabe, 1984). Dahlgren (1980) found that guppies fed a high (47%) protein diet had higher gonado-somatic indexes than those maintained on lower (31 and 15%) protein diets, however no difference in fecundity was recorded. Shim and Chua (1986) found that fish fed a 40% protein diet exhibited both the highest gonado-somatic index and significantly higher numbers of oocytes in the ovaries of guppy. Role of dietary protein on several reproduction parameters of tilapia have been well documented (De Silva and Radampola, 1990; Ganasekera *at al*. 1995; 1996; 1997; Fontainhas-Femandes *et al.*, 2000; EI-Sayed *et al.*, 2003).

Lipid

Lipids are the major components stored in egg yolk and play a major role in many vital reproductive processes such as oocyte development, egg production and embryo development (Mazorra *et al*., 2003). During fish maturation and reproduction, lipids are transported into oocytes from maternal reserves, stored and accumulated in yolk and utilized by developing embryos subsequently (Brooks *et al*., 1997). Lipids play a major role in membrane structure and function, as well as comprising the most important source of energy reserve in the developing fish embryo (Sargent *et al., 1989).* The muscle lipid content acts as a source of lipid in the ovary, making it a useful indicator of reproductive performance. The ability of these fish to build up somatic lipid stores during the rest of the reproductive cycle varies between species, and there is some evidence of a reproductive lipid (energy) limitation when body lipid content is low (Meffe and Snelson, 1993). It is thus important to ensure an adequate dietary energy supply throughout the cycle. To optimize protein utilization approx. 10% lipid is required in feed, but excess lipid will reduce intake of other important nutrients.

Fatty acids

Many studies have shown that the fatty acid profile of lipids in a broodstock diet has a direct effect on the fatty acid composition of the eggs and fry (Xu *et a/.*, 1994 and Mourente and Odriozola, 1990; 1990). Essential fatty acid deficiencies have been shown to be detrimental to oogenesis in fish (Luquet and Watanabe, 1986; Watanabe *et aI.*, 1984; Watanabe, 1985). In general, warm water fishes require polyunsaturated n-6 fatty acids or a mixture of n-3 and n-6 fatty acids, while coldwater species require n-3 forms (Webster and Lim 2002). For the n-6 family, the metabolically active form is primarily arachidonic acid; for the n-3 family, the active forms are EPA (eicosaoentaenoic acid) and DHA (decosahexaenoic acid). In freshwater fishes, EFA requirements can usually be met by supplying the shorter-chain precursors: linolenic acid, linoleic acid or both, although better growth performance can be often achieved by supplying the ''bioactive'' HUFA forms preformed in the diet (Kanazawa 1985). About 1% of linolenic acid is required in the diet of carp to keep lipogenesis low and to prevent overproduction of oleic acid (Farkas *et al*., 1978). Marine fish generally lack, or have low activity of the desaturase necessary to synthesize n-3 HUFAs from C18 fatty acids. Therefore, n-3 HUFAs are considered essential fatty acids (EFA) in the diets of marine fish as they are required for normal growth and survival. High dietary levels of HUFA, however, can negatively affect fish growth as HUFAs are readily oxidized by reactive oxygen species to lipid peroxides (Porter *et al* 1995) and HUFA tend to be less available for energy (Murata 1983). Therefore, formulated feed enriched with vitamin E showed best reproductive performance.

Minerals

Phosphorus is commonly supplemented as its concentration in water is low. It is essential in growth, bone mineralization and lipid and carbohydrate metabolism. Zinc should be an essential component in broodstock diets as observed in rainbow trout (Takeuchi *et al.*, 1981). Satoh *et al.* (1987) reported that the mineral composition of common carp gonads was significantly affected by zinc deletion from the mineral supplement in a fish meal diet. The importance of manganese has been recognized in broodstock nutrition. The manganese content of the diet influences its level and that of other trace elements in gonads (Satoh *et al.*, 1987). The absence of manganese in a fish meal diet significantly influenced the mineral composition of common carp gonads. The hatching rate of rainbow trout eggs was poor when the iron content was low (Hirao *et al.*, 1955).

Vitamins

Vitamins E and C are considered antioxidants owing to their ability to reduce the stress response in fish. Dietary fat soluble vitamins, in particular vitamins E and A, are very efficiently transferred to developing eggs (Luquet and Watanabe, 1986). Vitamin C is essential in many metabolic processes including collagen synthesis (tissue repair), protection of cell membranes, metal absorption and detoxification of xenobiotics. In addition, vitamin C is considered an intra- and intercellular reducing agent. A number of studies have demonstrated the beneficial effects of adequate vitamin C provision in broodstock diets in fish (Soliman *et al.*, 1986; Sandnes *et al.*, 1984; Eskelinen, 1989). The activity of L-ascorbic acid is time-dependent and oxidation labile, therefore it is necessary to make a fresh diet. In fish feed, L-ascorbic-2-phosphate is incorporated which is most stable (ascorbic acid phosphate). Other sources of vitamin C which are more stable and resistant to oxidation include ascorbic- 2-glycoside, L-ascorbic-2-sulphate, and ascorbate-6-palmitate.

Carotinoids

The carotenoid has a variety of functions including antioxidant, inducer of provitamin A activity, enhancing immune response, reproduction, growth, maturation and photoprotection.Carotenoid pigment accumulation in gonads and eggs of many fish species is a well-documented phenomenon. These pigments are obtained primarily from dietary sources, while in salmonids, muscle reserves are mobilized and transferred to the eggs. Suggested fimctions of carotenoid pigments in reproduction include a role in spermatazoid motility, a respiratory function in ova (Mikulin and Soin, 1975), and a possible antioxidant role in eggs. Since fish, like other animals, do not biosynthesize carotenoids *de novo,* it is essential to provide them with dietary supplements to allow the storage in tissue and teguments. Carotenoids are only synthesized by plants, phytoplankton (microalgae), zooplankton and crustaceans; therefore, the primary productivity in ponds directly affects the skin pigmentation due to the availability of natural or live food to synthesize these compounds. The most common carotenoids in freshwater include astaxanthin, zeaxanthin, xanthophylls, lutein, α- and β-carotene, taraxanthin and tunaxanthin.

Feed types

Tubifex worms, mosquito larvae, blood worms,earthworms and bovine liver and heart are some alternative foods offered frequently in some freshwater ornamental fish with the aim to improve the reproductive maturation and spawning. Linoleic acid and arachidonic acid are the most common fatty acids in alternative diets which are involved in the reproductive physiology. These fatty acids are prostaglandin precursors which are important in the ovulation process.

Environmental factors

Many environmental factors, particularly photoperiod and ambient temperature play a role in determining the reproductive success of broodstock fish (Wootton, 1982).Temperature is the primary controlling factor determining food consumption in fishes. Within the normal physiological temperature range of a species, the difference between maximum ration consumed and the maintenance ration increases with temperature, and thus the quantity of energy available for reproduction after somatic requirements have been fulfilled also increases (Wootton, 1982). Induction of early maturity in fish is associated with changes in environmental cues such as photoperiod.In carps, catfish and other tropical and subtropical fish, high temperatures and long photoperiods appear to be important for the final maturation of the oocytes and ovulation (Rocha, 2008).

REGIONAL RESEARCH CENTRE
ICAR-Central Institute of Freshwater aquaculture
(ISO 9001:2008 Certified)
RRC, Rahara, Kolkata-700118 West Bengal
E-mail: **rahara_cifa@rediffmail.com**

Training Programme on
"Application and Practices of Fish Feed in Aquaculture"
Duration: 23.10.17 to 28.10.17 (6 days), Venue: RRC, Rahara of ICAR-CIFA

PART I – INAUGURATION PROGRAMME

Date: 23.10.17

1.	Registration	9:30-10:00 am	Shri Sukhendu Biswas
2.	Training Class	10.00-01.00pm	
	Inaugural Programme		
2.	ICAR song	03:00-03:15 pm	
3.	Welcome Address	03:15-03:25 pm	Dr. P.P.Chakrabarti
4.	Self introduction by participants	03:25-03:45 pm	
5.	Overview of the training programme	03:45-04:00 pm	Dr. B.N. Paul
6.	Speech by Chief Guest	04:00-04:30 pm	**Dr. Purnendu Biswas V.C. , WBUAFS**
7.	Vote of Thanks	04:30-04:40 pm	Dr. S. Adhikari
	Group Photograph & Tea Break	04:40-05:00pm	

PART II – TECHNICAL PROGRAMME

Date	Time	Topic	Th./Pr.	Resource Person
23.10.17	10:00-11:00 am	**Nutritional requirement & feeding practices of Carps**	Theory	Dr. B.N. Paul
	11:00-12:00 pm	**Management of good water quality for good aquaculture practices**	Theory	Dr. P.P.Chakrabarti
	12:00-01:00 pm	**Importance of bioenergetics in aquaculture nutrition**	Theory	Shri A. Das
	01:00-02:00 pm	***Lunch Break***		
	02:00-03:00 pm	**Fish as Health food in respect to its nutritional value**	Theory	Dr. B.N. Paul
	03:00-05:00 pm	**Inauguration programme….**		

Date	Time	Topic	Th./Pr.	Resource Person
24.10.17	10:00-11:00 am	**Feed and feeding practices of catfish**	Theory	Dr. B.N. Paul
	11:00-12:00 pm	**Field Visit**	Practical	Dr. B.K. Pandey & Shri J.Ghosh
	12:00-01:00 pm	**Larval nutrition and feeding**	Theory	Dr. B.N. Paul
	01:00-02:00 pm	***Lunch Break***		
	02:00-03:00 pm	**Fish Feed Processing Technology for Entrepreneurship Development**	Theory	Dr. K C Das
	03:00-04:00 pm	**Soil carbon sequestration in fish ponds**	Theory	Dr. S.Adhikari
	04:00-05:00 pm	**Importance of minerals in Aquaculture**	Theory	Dr. B.N. Paul
25.10.17	10:00-11:00 am	**Farm made feed in Aquaculture**	Theory	Dr. B.N. Paul
	11:00-01:00 pm	**Aqua feed- Market Trend**	Theory	Mr.Amit Tandon Growel Feeds (P)Ltd
	01:00-02:00 pm	***Lunch Break***		
	02:00-03:00 pm	**Selection of ingredients and feed formulation in Aquaculture**	Theory	Dr. B.N. Paul
	03:00-05:00 pm	**Demonstration of feed preparation**	Practical	Dr. B.N. Paul Ms Sadrupa Bhowmick Mr. Durbadal Mahanty & Mr. Asit Pal
26.10.17	10:00-11:00 am	**Nutritional disorders in Aquaculture**	Theory	Miss. Farhana Hoque
	11:00-01:00 pm	**Improvement of Nutritive Value of Plant feed-stuffs for Formulation of Aqua feed: Application of Solid State Fermentation**	Theory	Dr. Kaushik Ghosh Associate professor University of Burdwan.
	01:00-02:00 pm	***Lunch Break***		
	02:00-05:00 pm	**Analysis of Proximate composition of Fish feed**	Practical	Dr. B.N. Paul Ms Sadrupa Bhowmick & Shri Durbadal Mahanty

27.10.17	10:00-11:00 am	**Effect of Broodstock nutrition on reproductive performances of fish**	Theory	Shri. A. Das
	11:00-12:00 pm	**Feed Wastage and Feed Quality related problems in Aquaculture**	Theory	Shri. A. Hussain
	12:00-01:00 pm	**Feed Additive in aquaculture**	Theory	Dr. B.N. Paul
	01:00-02:00 pm	***Lunch Break***		
	02:00-05:00 pm	**Field Visit: Kalyani Feed Mill**	Practical	Dr. Kalyan Poddar Kalyani Feed Mill
28.10.17	10:00-11:00 am	**Natural Fish Food organismsand its role in Aquauclture**	Theory	Dr.R.N.Mandal
	11:00-12:00 pm	**Breeding and larval rearing of Hilsa (*Tenualosa ilisha*)**	Theory	Dr. D N Chattopadhyay
	12:00-01:00 pm	**Role of Vitamins in Fish culture**	Theory	Dr. B.N. Paul
	01:00-02:00 pm	***Lunch Break***		
	02:00-03:30 pm	**Evaluation of Trainees**		Dr. B.N. Paul Dr. S. Adhikari Dr. RN. Mandal
	03:30-05:00 pm	**Valedictory Programme**		

Note:

Lecture Hall and Audiovisual Management	Ms Sadrupa Bhowmick, Shri Durbadal Mihanty and Mr.Asit Pal
Accommodation of Trainees, Preparation of Training material and others	Dr.B.N.Paul, DR.S.Adhikari, Dr.R.N.Mandal, Shri Sukhendu Biswas and Shri Durbadal Mohanty
Contact person on emergency	Dr.B.N.Paul, 9432334390, Dr.S.Adhikari,8282951825 Dr.R.N.Mandal, 8420780650, Mr. Asit Pal, 9836159658

The Programme may alter depending on the field condition.

Advisor : Dr. J.K. Sundaray, Director, ICAR-CIFA
Officer-in-charge : Dr. P.P. Chakrabarti
Course director : Dr. B.N. Paul
Co- Course director : Dr. S.Adhikari
Dr. R.N. Mandal

20/X/17

Director, ICAR-CIFA

ICAR-Central Institute of Freshwater Aquaculture
(ISO 9001:2015 Certified Institute)
(Indian Council of Agricultural Research)
RRC,Rahara, 700118,West Bengal