Proceedings of the Summer Institute in Recent Advances in Finfish and Shellfish Nutrition

11 TO 30 MAY 1987

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Dr. SALIM ALI ROAD
COCHIN-682 031

PROCEEDINGS OF THE SUMMER INSTITUTE

IN

RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

Recognising the importance of nutrition in aquaculture the Indian Council of Agricultural Research sanctioned a Summer Institute which was held at the CMFRI, Cochin from 11 to 30th May 1987.

Twenty nine candidates sponsored by the Heads of various research, education and development organizations dealing with aquaculture in the country were the participants.

The Institute comprised of lectures, practicals, demonstrations, field visits, group discussions covering the latest developments and recent advances in the field of aquaculture nutrition.

The Summer Institute was inaugurated by Prof.C.A. Abdussalam, Pro-Vice-Chancellor, Cochin University of Science and Technology on the forenoon of 11th May 1987.

A valedictory function was organised on the 30th May 1987, when Dr.M. Sakthivel, Director, Marine Products Export Development Authority delivered the valedictory address and also distributed certificates to all the 29 participants who have successfully completed the course.

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FOREWORD

Aquaculture is recognised as one of the frontier areas for augmenting the fish and shellfish production of the country, to partially meet the demands of animal protein for the increasing population and to mitigate the growing protein-malnutrition to some extent. A traditional extensive type of aquaculture is still practised by the fishermen in the States of West Bengal, Orissa, Kerala and in some parts of the North-Eastern States; but the production from this system rarely exceeded 1000 kg/ha.

Recent studies have shown that substantial increase in production of fish and prawns could be achieved, from an unit area, through judicious use of operational inputs such as feed and fertilisers. Besides, studies in India and elsewhere have shown that survival, growth, maturation, and spawning of finfish and prawns are significantly affected by the quality and quantity of feed supplied. Thus nutrition plays an important role in aquaculture.

In most species, the larvae have been found to require live-food organisms. So, the production of seed of many species depends upon the quality and quantity of live-food organisms supplied to them. Thus, the identification, isolation and mass culture of live-food organisms is an integral part of fish and shellfish hatcheries. While the young and adult molluscs continue

to show preference for live-food, finfish and crustaceans can be cultured on nutritionally adequate, compounded practical feeds in intensive systems and supplementary feeds in semi-intensive systems. For the formulation of feeds, information on the nutritional requirements of the cultured species, the nutritive value of easily available and cheap ingredients, the need for food additives, binders, growth promoters, diet type etc. are required. Besides these, information on effective feed dispensing procedures, frequency of feeding, feeding rates, time of feeding etc. are also required, to obtain maximum efficiency of the feed supplied.

During the past one decade the Central Marine Fisheries Research Institute conducted research on these priority areas of nutrition through mission oriented research by scientists and M.Sc. and Ph.D. students of the UNDP/FAO/ICAR Project "Centre of Advanced Studies in Mariculture". Through these researches a great deal of information, which has relevance to aquaculture has been generated. A few scientists of the Institute were also trained in dvanced nutrition laboratories abroad and a number of exports offerred consultancies at the CAS in Mariculture. Laboratories for advanced research and education were also set up. Thus the Institute has developed an active team of research scientists, students and excellent facilities for research.

Research without extension is of very little use for development. Considering this fact, to disseminate the information gathered by the Institute and to share the expertise developed in the field of nutrition, the Indian Council of Agricultural Research sanctioned a Summer Institute in "Recent Advances in Finfish and Shellfish Nutrition" at the Central Marine Fisheries Research Institute from 11 to 30th May 1987. This volume comprises of the technical papers prepared and presented by the Faculty.

The Director and faculty members express their gratitude to the ICAR for the financial assistance provided. They are also thankful to the Heads of various organisations for sponsoring the candidates for the Summer Institute.

P.S.B.R. James

P.S.B.R. JAMES
Director, Summer Institute and
Director, CMFRI.

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Technical Paper - 1

SUMMER INSTITUTE IN

RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

11-30 May, 1987

FINFISH CULTURE IN INDIA - AN OVERVIEW

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Finfish culture is an ancient occupation in India and it assumed various levels of importance during its development, through many centuries. At present finfish and shellfish culture received considerable national importance in view of the recognition of fish and other aquatic organisms as a source of high quality protein food for the people in many parts of India. Besides, planned development of aquaculture would generate numerous employment opportunities, especially in the rural areas.

Resources available for finfish culture:

India is endowed with large water resources suitable for finfish culture and numerous species of finfish (Table 1) amenable for culture under a variety of environmental situations. Although the total area available for freshwater fish culture in ponds and tanks

is estimated as 1.6 million hectares, only about 0.6 million ha is at present utilized for fish farming (Jhingran, 1982). The area under brackishwater culture (both fish and prawns) is about 50,000 ha (Natarajan, 1985), though an estimated 2 million ha brackishwater area is available along the coastline for development. In addition to these, there are potential areas in rivers, irrigation canals, reservoirs, lagoons, bays where cage and pen culture systems could be developed. According to Natarajan (1985) about 27,300 kms of major river systems, 1.25 lakh km length of irrigation canals, and 30 lakh ha of large and medium reservoirs are available in the freshwater sector; and about 2.4 lakh ha of brackishwater lagoons, estuaries and backwaters are available in the brackishwater sector. In addition to the above, saline lagoons and bays in the islands and salt pan reservoirs are potential areas where cage culture could be developed.

Culture of cold water fish:

India has vast cold water resources such as lakes, streams and rivers and a good number of indigenous and exotic species of fish for development of finfish culture. Most of the cold water resources are in the Himalayas, in the States of Jammu and Kashmir, Himachal Pradesh, Arunachal Pradesh, Uttar Pradesh, West Bengal and North-Eastern Hill States. In the peninsular region Nilgris,

Munnar High Ranges, Kodai Hills have some streams and reservoirs. The most important culturable species are listed in Table 1. Unlike the warm water species, which are exclusively produced for consumption, the cold water fish culture is principally done for development of 'Sport fishery'. Among the cold water species the rainbow trout Salmo gairdnerii gairdnerii is the most important being domesticated both in the Himalayas and Peninsular High Ranges. Brown trout and brook trout are exclusively found in Himalayan region. Recently, indigenous species such as snow-trouts and mahseers are being induced bred and seed production achieved. Commercial farms have also been developed for trouts in the Himalayan region under the State fisheries development programme. A National Research Centre for Cold-water Fisheries has recently been set-up for intensification of research on Cold-water Fisheries.

Culture of warm water fishes:

Warm water fish culture is carried out in freshwater and brackish/coastal waters. The most important species are listed in Table 1. Warm water fish farming has been in vogue for centuries in both freshwater and brackish-water ponds; but the practice until recently has been exclusively of an extensive type of rearing, where the production rarely exceeded 1000 kg/ha. However, with the development of proven technologies and scientific management

strategies productions ranging from 3000-5000 kg/ha have been achieved under semi-intensive carp culture systems. Potential for achieving a production of 10 tonnes per ha for carps in polyculture systems (composite fish culture) and 55 tonnes per ha for live-fishes has been shown by the researches carried out by the Central Inland Fisheries Research Institute.

In brackishwater culture systems, production from the traditional sector varies from 500-700 kg/ha, whereas, the recently developed semi-intensive practices have shown production potential ranging from 2000-2500 kg/ha through polyculture of finfish and prawns. Experiments carried out by the CMFRI, have shown the potential of pen culture of milkfish in coastal saline lagoons, and milkfish and mullets in polythene lined ponds in coastal areas.

Some of the other potential areas for fish culture development are: cage culture of finfish in reservoirs, lakes, irrigation canals, rivers, lagoons and sheltered bays including the lagoons and bays in the islands. These aspects have received very little attention till now.

Another promising area for research and development is culture of ornamental fishes or aquarium fishes of both marine and freshwater origin as these fishes have good export potential.

Thus there is abundant scope for augmenting the fish culture production of the country, by utilising more areas for culture, by adopting new methods such as cage culture, and by intensifying the culture practices in the existing systems.

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Jhingran, V.G., 1982. Fish and Fisheries of India. Hindustan Publishing Corporation, Delhi, 666 pp.

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Table 1. Important culturable finfish species

Freshwater species

Coldwater species:

1. Salmo gairdnerii gairdnerii (rainbow trout) a
2. Salmo trutta fario (brown trout) a
3. Salvelinus fontinalis (brook trout) a
4. Schizothorax plagiostomus (snow trout)
5. Schizothoraichthys esocinus (")
6. Tor putitora
7. Tor tor
8. Tor khudree
9. Acrossocheilus hexagonolepis
10. Cyprinus carpio communis (mirror carp) a
11. Cyprinus carpio specularis (scale carp) a

Warm-water species:

(a) Carps:

1. Catla catla (Catla)
2. Labeo rohita (rohu)
3. Cirrhinus mrigala (mrigal)
4. Labeo calbasu (kalbasu)
5. Labeo fimbriatus (peninsular carp)
6. Ctenopharyngodon idella (grass carp) a
7. Hypophthalmichthys molitrix (silver carp) a
8. Cyprinus carpio (common carp) a

(b) Live-fishes:

1. Clarias batrachus
2. Heteropneustes fossilis
3. Anabas testudineus
4. Channa marulius
5. Channa punctatus
6. Channa striatus

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(c) Miscellaneous sps.

1. Mystus aor
2. Mystus seenghala
3. Wallago attu
4. Pangassius pangassius
5. Tilapia sps.

Brackishwater/coastal species

1. Chanos chanos (milk fish)
2. Mugil cephalus
3. Liza parsia
4. Liza macrolepis
5. Osteomugil cunnesius
6. Etroplus suratensis
7. Etroplus maculatus
8. Lates calcarifer
9. Epinephelus tauvina
10. Sillago sihama
11. Siganus spp.

(a) -Exotic species.

Technical Paper - 2

SUMMER INSTITUTE IN

RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

11-30 May, 1987

PRESENT STATUS OF CRUSTACEAN CULTURE IN INDIA

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INTRODUCTION

The crustaceans that are cultured include the most familiar decapods - the prawns, lobsters and crabs, and the smaller, lower crustaceans such as anostracans, cladocerans and copepods. The former group, due to their greater food value and economical importance, has attracted considerable attention for culture in the confined and manageable water bodies. Among them, the prawns in consideration of their demand, the state of art of culture and the developmental emphasis are most important and occupy the foremost place in the culture fisheries of India. Although the culture of lobsters and crabs has been attempted in the country since the past decade, the total effort involved both in research and development has been limited, and consequently, the technology of their culture is still in an experimental stage. The lower crustaceans cultured at present are mainly used as live food organisms for rearing larval and post-larval stages of finfishes and shellfishes. An attempt is made here to present the information on the status of culture of different crustaceans, constraints encountered and prospects available.

PRAWN CULTURE

The penaeids belonging to the genera Penaeus and Metapenaeus and carideans of the genera Macrobrachium and

Palaemon constitute the important groups of prawns involved in the culture fisheries of India. While the penaeid prawns form the principal component of the production in the aquaculture in the brackish water, Macrobrachium is mainly farmed in the fresh water regimes and in the paddy fields in the rainy season.

Prawn farming in the brackish water practised at present in India can be broadly classified into three categories on the basis of the prevailing farming systems.

1. Paddy cultivation during rainy season (June-September) followed by prawn farming in the fair season (October-April) in the low lying earthern fields adjacent to estuaries and backwaters - this system is principally concentrated in Central Kerala, along the northern coastal waters of Karnataka, Goa and to certain extent in West Bengal.

2. Prawn farming in relatively larger and deeper earthern fields throughout the year as seen in certain areas of Central Kerala and in the large 'Bheries' of West Bengal.

3. Prawn farming in the fields eradicated of undesirable organisms and prepared appropriately before stocking, stocking with species that grow fast and command good price and demand, and growing them to marketable size with supplementary feeding and water supply management as practised by progressive farmers and entrepreneurs in several regions of the country in recent times.

In the former two categories, the basic technology of prawn farming is similar. The stocking of the field is accomplished by the seed brought in by the incoming tide. The seed thus entering the field is allowed to grow for a short period by feeding on the natural food available in

the field and the stock is harvested periodically. The present prawn farming system in the country by and large follows this practice. However, due to the indiscriminate and uncontrolled stocking of seed that come along with the tide, short time allowed to grow the seed before harvesting and since no eradication or control of predatory and competitive species in the field is involved, the quality and quantity of production from these farming systems are found to be low. On the other hand, in the improved system, the yield as well as the quality of prawns harvested are of higher unit value, and consequently, this system is now rapidly spreading and gaining importance in the country.

The precise extent of area involved in each of the above categories of farming systems and the total production realised are not available. The total estuarine and brackish water area, the extent of suitable area available for prawn culture as surveyed at present, the total area under prawn farming in the country and the total prawn production from this source are estimated differently by different agencies. On the basis of the available data, information on these aspects, and the important species of penaeid prawns cultivated in different maritime states are given in Table 1. The total area utilised at present for prawn farming in the country is estimated at 42,653 ha and the total prawn production for this area as 21,119 t.

Following the awareness of the importance of prawn farming as well as the priority assigned for its development in the national and state fisheries programmes, several surveys, investigations, field experiments on the culture of prawns, information on the biological and physical inputs required to improve the system and the production, and hatchery techniques to meet the seed requirements have been endeavoured by different R & D agencies at different regions

of the country during the past 10 years. From the information available, the following observations are made.

1. In all the maritime states, there is an increasing awareness of the role of prawn farming as a definite means for augmenting production both among development promotion agencies and fish farmers.

2. Base-line information on the growth under captivity and on the availability and abundance of seed of candidate species of prawns in different estuaries and backwaters of the states are now available. The data gathered on seed resources have shown that adequate quantities of seed could be collected from the natural source for immediate culture purpose.

3. However, as often fish farmers fail to procure the seed as and when required for culture and since continued collection of seed in large quantities from nature would affect the capture fisheries, the need for establishment of hatcheries has been realised. The technique for hatchery production of seed of penaeid prawns is now available in the country. Following this, two commercial hatcheries, the Regional Shrimp Hatchery at Azhikode near Cochin belonging to the Government of Kerala and the other one at Kovalam, near Madras belonging to M/s. Hindustan Livers Ltd are now producing and supplying the seed. Besides, the commercial hatchery established recently at Asangoan in Thane District in Maharashtra has also started producing the seed. Hatcheries are also being established in Orissa, Andhra Pradesh, Tamil Nadu, Kerala and Karnataka by the Marine Products Export Development Authority and the State agencies.

4. Although there are vast potential brackish water resource, information on the sites suitable for aquaculture in different States is meagre.

5. The rate of production of prawns/fishes in the traditional practice is comparable to those obtained in experimental/semi-commercial monoculture or polyculture of selected species of prawns and fishes. However, in the former, the yield is composed of mainly smaller species of prawns, relatively smaller size of the larger species and consequently, the unit value realised is comparatively less. On the other hand, in the culture of selected species of prawns, the unit production per ha is found to be over 500 kg for culture duration of 3-4 months.

6. Base-line information on the economics of selected species culture of prawns is meagre and those available are found to differ from State to State and from operation to operation. This is due to the type of culture operation followed, farm size and its location, species selected for culture, facilities available and skill of management. Nevertheless, due to the higher unit value realised for the production in the farming of selected species, the rate of net profit is found to be about 3-5 times more over those obtained from traditional culture.

7. In the traditional farming practice followed in the country no supplementary feeding is given to the stocked prawns. Most of the experimental culture carried out have been mainly using ground nut oil cake, rice or wheat bran and fish meal or prawn head powder at the rate of 2 to 10% body weight of the stocked population. There is very little information on the use of pellet feed in the grow-out system.

8. Although increasing information on pond ecology relating to factors such as the physico-chemical parameters of pond water, soil characteristics and biological productivity are being gathered, the effects of fertilisers and manurial treatments in the tide-fed ponds are little understood. Most of the fertilizers used at present are inorganic fertilisers.

9. The techniques of brood stock maintenance and seed production of Macrobrachium rosenbergii have been developed. Although the young prawns grow well in the earthern ponds to reach a size of 200 to 250 mm during an year, the production is found to be influenced by the pond substratum, size of the pond, size-stocking density relationship, water quality, supplementary feeding and managerial skill. With different culture strategies the production is found to vary from 39.5 kg to 1929 kg/ha during a growth period varying from 90 to 150 days. The larval development of M. malcolmsonii and M. idella has been studied. Natural seed grounds of these species have been located at several regions. Field experiments on the culture of M. malcolmsonii have shown a production rate of 285-300 kg/ha/yr.

10. To provide a strong research support for the accelerated development of brackish water culture fisheries including prawn culture in the country, the Indian Council of Agricultural Research has recently established a new Institute, namely, Central Institute of Brackishwater Aquaculture. This is in addition to the active research programmes progressing at the Central Marine Fisheries Research Institute, Central Institute of Fisheries Education and at Agricultural Universities having fisheries Faculty. On the development front, all the maritime states and Union Territories have assigned priority for the development of prawn culture and have drawn up developmental projects for

implementation during the Seventh Five Year Plan period. The Union Ministry of Agriculture during the Seventh Five Year Plan period has proposed to develop 10,000 ha of brackish water area at an estimated cost of Rs. 30 crores. Besides, the Marine Export Development Authority has programmes to develop 2,200 ha during the Seventh Plan under its direct assistance apart from various other assistance to small, medium and large farmers. The Authority has also scheme to set up hatcheries, extending financial assistance and building up of technical manpower. The other Institution involved in the development of the sector are the Central Institute of Coastal Engineering for Fisheries, Indian Institute of Technology, Kharagpur and the regional Bay of Bengal Programme executed by the Food and Agricultural Organisation of the United Nations.

To achieve promising production of prawns through aquaculture it is essential to make available in adequate quantities the inputs such as suitable physical environment, a suitable economic environment, an equitable regulatory environment, incentives, land, water, capital, labour, seed, feed and fertilisers, tools and equipments, trained personnel management, market and information (research, extension and demonstration) at proper time. Thus the choice of suitable location; type of farming system to be taken up including the design of the farm, its type, size and lay-out; species to be taken up for farming; availability of seed; size of seed to be stocked; rate of stocking in the grow-out ponds; availability of suitable feed in adequate quantity and quality; water management; diseases, parasites, predators and competitors affecting the farmed prawns; physical damages caused by storms, cyclones and heavy rain fall; availability of finance to establish farms and corollary infrastructures; availability of trained personnel to execute and manage the culture projects and skilled labourers

to operate the system, and market avenue influence the production front. Besides, the policies, guidelines and priority assigned to the sector, land and water use strategies, economic strength of the society, interest and acceptance of the venture, structure of the organisation and local conventions also limit/promote the production. Nevertheless, given the proper management and a climate, bringing in the resources, technologies, finance and the skill available with us, there is little doubt that this country would be one of the major prawn producing nations in the world through aquaculture.

LOBSTER CULTURE

Although the lobsters are considered as epicurean gourmet, concerted efforts on their culture in India were initiated only ten years ago. Of the six species of the shallow water spiny lobsters available in the country, Panulirus homarus and P. ornatus are the species studied to understand their breeding, larval development and growth in captivity. Isolated experiments carried out prior to 1970 on the breeding of berried P. homarus under uncontrolled conditions and rearing of the phyllosoma larvae gave only limited success. Later, the puerulii that migrate into the coastal waters were collected by special collectors and reared in the laboratory. The results of these experiments showed that the lobsters of 35 mm carapace length grew to 57-58 mm carapace length in about 15 months and attained marketable size in 18 months. Further, during this period of growth, both males and females attained maturity, mated and subsequently, the females spawned releasing the eggs on to their pleopods. The eggs in the pleopods on further development hatched out into free swimming larvae. Although successful breeding of lobsters under controlled conditions is possible, larval rearing through different phyllosoma

stages which number IX stages and require a duration of 4 to 6 months has not so far been achieved.

Following the encouraging results of growth of puerulii in captivity, experiments were carried out to study the growth and breeding of eyestalk ablated lobsters. Fast rate of growth of eyestalk ablated lobsters, ranging from 1.45 to 2.5 g per day as against 0.35 g/day in the normal lobsters was recorded in the experiments. It was also found that the eyestalk ablated lobsters attained 180 to 200 g size during 5-6 months and 400 g in about 9-month period. Further, studies on this aspect are in progress. The main constraints in developing a viable technology of large-scale rearing of phyllosoma larvae are the long duration of larval life, and inadequate knowledge on the appropriate and suitable food on which they could be fed and reared.

CRAB CULTURE

The important species of crabs of India suitable for culture are Scylla serrata, Portunus (Portunus) sanguinolentus, Portunus (Portunus) pelagicus and Charybdis (Charybdis) cruciata. S. serrata grows to a size of 150-200 mm across carapace. It is available in the estuaries and brackish-waters and could withstand wide range of salinity variation. P (P) sanguinolentus grows to a maximum size of 150 mm across carapace and commonly occurs in the inshore sea and brackishwater regions. It breeds during February-April. P. pelagicus occurring all along the coast attains a size of 180 mm across carapace. It breeds from September to March. C. (C) cruciata, like P. (P) sanguinolentus grows to a size of 150 mm across carapace.

Because of larger size and demand, Scylla serrata has attracted more attention to culture than the other

species. Ovigerous crabs have been successfully maintained in the laboratory through the incubation period of eggs and subsequently spawned releasing about 2 million zoea larvae. The mother crabs were maintained in the medium having 32± 2°/₀₀ salinity at 26-30°C. They were fed with bivalves. The incubation period of eggs was found to be varying from 8 to 13 days. The larval development passes through five zoea stages, each of 3 to 4 days duration, and one megalopa stage. The megalopa stage lasts for about 8 to 11 days when it moults to the young crab stage. Thus, the entire larval and megalopa development completes within about 28 to 30 days. The larvae were reared by feeding with Chlorella and rotifers and the later stages with Artemia naupiii.

Experimental field culture of S. serrata has been carried out in cages and in earthern ponds. In the cage culture, basket type of cage made of split cane, box type made out of soft wood and metal framed cages were tried. The results of the experiments show that the crabs grow at relatively faster rate of 11-15 mm across carapace per month till they reach a size of 110 mm and thereafter, the growth rate slows down to 5-6 mm across carapace per month. They attain a size of 145-160 mm (400-500 g) in about 9 months. They are fed with trash fish, crushed bivalves and fish waste. It is also found that metal framed cage is preferred than other types of cages used in the experiment.

In ponds, the crabs are cultured along with milkfish and mullets. The seed crabs of 28 g size is found to grow to 600 g during a period of 8 to 11 months. The production rate is found to be 494 to 690 kg/ha.

Although these preliminary investigations have indicated encouraging results, large-scale culture of crabs requires further perfection of seed production technology,

development of suitable feed and techno-economic information on field culture.

CULTURE OF LOWER CRUSTACEANS AS LIVE FOOD ORGANISMS

Among the live food organisms used for rearing the larval and post-larval stages of finfishes and shellfishes, the brine shrimp Artemia salina is the most important one. The technology of their culture in out door containers have been developed. The preadults and adults cultured in plastic pools are fed with ground nut oil cake soaked in water. Artemia is also successfully reared in out door tanks in open sun light by manuring the medium with pig manure to maintain Chlorella bloom. Besides, the methods for decapsulation of cysts and separation of the hatched nauplii from the hatching debris are also developed.

The cladocerans of the genera Daphnia, Moina and Alona, are also mass cultured for feeding the finfish and shellfish larvae. Moina is reared in 2-ton capacity plastic lined out door pools containing tap water fertilised with ground nut oil cake, urea and superphosphate in various proportion and inoculated with a starter culture of Chlorella. As the Chlorella bloom develops, Moina is introduced. Multiplying rapidly they reach a concentration of 30,000 t - 40,000 units/lit within 7-9 days. Technologies of their culture with direct use of fertilizers, harvesting and storage are also developed.

The culture of Daphnia with brewer's yeast has shown that they multiply to the order of 12,650 to 15,000 units/ lit in seven day period.

Results of the culture experiments on the freshwater copepods and harpacticoid copepods have indicated the feasibility of their large scale culture under controlled conditions, and their utilisation for feeding the fish larvae. The technology of culture of these lower crustaceans as live food has thus greatly helped in the successful rearing of several finfish and shellfish larvae and post-larvae in the country.

Table 1. Estimated estuarine/brackish water area, extent of potential area found suitable for aquaculture as per the survey so far conducted (1985), area found suitable for prawn culture according to the survey so far conducted (1985), average production rate of prawns, estimated total production of prawns and important species cultured in different maritime States of India.

State/ Uts	Estimated estuarine/ brackish water area (million ha)	Potential area suitable for aquaculture (1985) (ha)	Area found suitable for prawn culture (1985)(ha)	Area utilised at present for prawn culture (1985)(ha)	Average production rate (kg/ha/yr)	Estimated total production of prawns (E)	Important species cultured
West Bengal	0.405	INA	INA	25,000	550	13750.0	1,2,4,5,7,8, 10,11
Orissa	0.299	31618	15,333	1,450	400	580.0	1,2,5,11
Andhra Pradesh	0.200	64000	17,000	560	500	280.0	1,2,5,10,11
Tamil Nadu	0.080	56000	16,000	95	300	28.5	1,2,4,5,7
Pondicherry	0.0008	INA	INA	68	250	17.0	1,2
Kerala	0.243	122000	11,473	7,400	600	4440.0	1,2,5,6,7,10
Karnataka	0.008	INA	INA	4,800	300	1440.0	2,3,5,6,7
Goa	0.019	INA	INA	1,300	300	390.0	2,3,5,6,7
Maharashtra	0.081	INA	14,455	1,820	80	145.6	3,5,7
Gujarat	0.376	INA	1,935	160	300	48.0	2,3,5,8,9
Total	1.7118			42,653		21119.1	

INA - Information not available

1. Penaeus monodon; 2. P. indicus; 3. P. merguiensis; 4. P. semisulcatus; 5. Metapenaeus monoceros; 6. M. dobsoni; 7. M. affinis; 8. M. brevicornis; 9. M. kutchensis; 10. Macrobrachium rosenbergii; 11. M. malcolmsonii

Technical Paper-3

SUMMER INSTITUTE IN

RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

11-30 May, 1987

NUTRITION IN AQUACULTURE - AN OVERVIEW

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Aquaculture is gaining more and more importance as a means to augment finfish and shellfish production in both the developed and developing countries of the World, to partially meet the growing demand for fish and shellfish protein. It has been predicted by TAC (1973) that by the year 2000, aquaculture could produce at least 50 million tons of animal protein, if certain research and development measures are undertaken, as against the production of 6 million tons of fish and shellfish through culture in 1975 (Pillay, 1976).

In India, traditionally an extensive type of aquaculture was practiced by the farmers in the states of Kerala, West Bengal, Orissa and some of the North-Eastern States. Production realised from this system of aquaculture was extremely low, being less than 1 ton/ha/yr. These traditional practices being largely governed by local conditions and needs, the farmers seldom felt the need to intensify operations. Low-density culture with minimum inputs and low production per unit area has often been more economical than intensive farming, involving the rearing of dense populations and heavy inputs (Pillay, 1976).

Recent researches have amply proved that by adopting scientific culture procedures and efficient management

practices substantial increase in production could be obtained especially from finfish and prawn culture systems. Production rate as high as 10 tons/ha has been achieved in static earthen freshwater carp culture ponds in India through optimum stocking, fertilization and supplementary feeding.

Feed is often considered as the major operational input in semi-intensive and intensive finfish and prawn culture systems. Feed costs often exceed 50% of the operational costs in intensive culture operations. In view of this practical feeds both supplemental and complete should be carefully formulated, and judiciously supplied considering the specific nutritional needs of cultivated species and the intensity of culture operation. In Semi-intensive systems, the supply of supplementary feeds can be regulated judiciously keeping in view the quality and quantity of the natural food produced in the pond. Natural food production can be increased through systematic and judicious administration of organic and inorganic fertilizers. Thus in this system, the exogenous food supply need to provide only nutrients which may be deficient in the natural food, so that the feeds are effectively utilized.

Among the three major groups of cultivable aquatic organisms, the bivalve molluscs are mainly cultured in open water bodies. Thus the production of most species of molluscs mainly depend upon the availability of plenty of preferred natural food in the system. In contrast to bivalve molluscs, finfish and crustaceans are cultured in ponds, raceways, cages, pens and recirculated systems. In these systems, in order to achieve optimum production, provision of compounded feeds, either supplementary or complete, forms are essential requisite.

Besides the need for feeds for grow-out systems, feeds are also required for hatcheries and nurseries to produce healthy stocking material. The larvae of most of the finfish, crustaceans and bivalve molluscs require microparticulate diets during the early larval phase. In most cases live-food particularly phytoplankton are being fed. While the bivalve molluscs continue to have preference for microparticulate diets, the advanced larvae of crustaceans and the fry of finfish efficiently ingest zooplankton and formulated feeds. In Table 1 the important basic food types ingested by the larvae are presented. Live-food production necessitates additional infrastructure, manpower and operational inputs, thereby the cost of production of stocking material is greatly enhanced.

Table 1: Potential sources of diets for larvae

Viable	Non-viable
a) bacteria	a) detritus
b) motile gametes, spores	b) organic aggregates
c) yeast	c) artificial formulations - microparticulate diets - microencapsulated diets
d) phytoplankton - - diatoms - unicellular algae	d) tissue suspensions
e) zooplankton	

Recent developments in feed technology reveal that microencapsulated diets can be fed to larvae in hatcheries.

Encapsulation is a process by which liquid or particulate materials are enclosed within a specially designed artificial membrane or wall made of natural polymers, as gelatin, gums, waxes or the synthetic polymers of ethyl cellulose or polyvinyl alcohol. The type of capsule required depends on the mode of feeding; for instance, molluscs ingests the food whole and must be provided with a capsule whose walls are stable to sea water but readily soluble in the digestive tract of the animal by the action of digestive juices. Thus for the larvae, development of nutritionally adequate micro-particulate or microencapsulated diets with appropriate size, texture, taste etc. is most important.

Formulated feeds should contain adequate levels of nutrients to meet the physiological needs of the organisms, such as to supply energy, to build and maintain the cells and tissues, and regulate body processes. According to Halver (1976) any balanced formula for fish diets must include an energy source plus sufficient indispensable amino acids, essential fatty acids, specific vitamins and minerals to sustain life and promote growth. Studies with crustaceans show that in addition to the nutrients listed above, a dietary source of sterol and phospholipids are essential for normal growth and metamorphosis (Kanazawa, 1984). All the essential nutrients (Table 2) should be incorporated in adequate levels and in optimum proportions in compounded diets. Any imbalance of these nutrients would affect the efficacy of conversion of food by the animals. Quantitative requirements of specific nutrients vary with species, size, physiological condition, temperature, stress, nutrient balance of the diet and environ-mental factors, thus economical rations must be programmed accordingly.

After determining the nutritional requirements of the species, it is essential to identify feed ingredients which would provide the essential nutrients for formulating practical diets. Thus nutritional and ingredient standards are defined. Finally the diets are prepared as dry pellets, moist-pellets, flakes, pastes, microparticulates, microcapsules etc. keeping in view the specific preferences of various size groups and physiological stages of the species. Binders, antioxidants, mold inhibitors, anabolic agents, colouring and flavouring agents can be added as additives depending upon specific needs.

In the process of feeding aquatic animals, a general understanding of the type of digestive system found in the animal is essential (Mac Grath, 1975). Information about the ability of the animal to chew or break feed particles into smaller units, thus increasing the surface area of feed particles for greater ease of ingestion and digestion, and about type of digestive tract the animal has and its histology are required. In addition, digestion and absorption efficiency are required. These informations would help in evolving suitable diet forms for the species and stage concerned.

Based on the informations on nutritional requirements of the species and availability of nutrients in various feedstuffs and nutritional environmental interactions diets can be formulate keeping the cost of the finished product in view.

For achieving maximum production the feeding strategies employed are very important. Feeding strategies are evolved based on the size and physiological stages of animals, water quality, water temperature, feeding habits of the animal, dietary form, behaviour of the animals etc. Thus nutrition and feed formulation research involves a number of stages, which are summarized in Fig. 1.

Table 2: Essential dietary nutrients for finfish and Shellfish Energy nutrients: Proteins, lipids, carbohydrates
Non-energy nutrients: Vitamins, minerals

Essential amino acids

1. Valine
2. Isoleueine
3. Threonine
4. Tryptophan
5. Arginine
6. Lysine
7. Leucine
8. Phenylalanine Tyrosine
9. Methionine Cystine
10. Histidine

Vitamins

18. Thiamine
19. Riboflavin
20. Pyridoxine
21. Choline
22. Niacin
23. Pantothenic acid
24. Inosital
25. Biotin
26. Folic acid
27. Cyanocobalamin
28. Ascorbic acid
29. Vitamin A
30. Vitamin D
31. Vitamin E
32. Vitamin K

Essential fatty acids

11. Linolenic acid (18:2w6)
12. Linolenic acid (18:3w3)
13. Eicosapentaenoic acid (20:5w3)
14. Docosahexaenoic acid (22:6w3)

Sterol

15. Cholesterol

Phospholipids

16. Phosphotidyl choline
17. Phosphotidyl ethanolamie

Minerals

33. Calcium
34. Phosphorous
35. Copper
36. Magnesium
37. Zinc
38. Cobalt
39. Iron
40. Iodine
41. Manganese
42. Selenium
43. Molybdenum

Fig. 1. NUTRITIONAL RESEARCH

BASIC	APPLIED
Nutritional requirements (size, stage, physiological condition)	Ingredients - potential nutritive value composition-antinutritional factors biological value
Digestive system and digestion	Digestibility of nutrients in diets
Metabolism of nutrients	Feeding rates and factors influencing it
Nitrogen and energy balance	Diet growth
Excretion	Diet form
Metabolic rates	Additives: determining safe levels of antioxidants, mold inhibitors, anabolic agents, binding agents etc.
Nutrition and Environment interaction	
Inter-relationships between nutrients	
Influence of nutrients on body composition	

Nutritional standard

Ingredient standard

Least-cost formulation

Process standards

Process

Finishes feeds

Microparticulate	Pellets
Microcapsules	- hard
Flakes	- soft
Meals	

Storage - Shell-life

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Technical Paper-4

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NUTRITIONAL NEEDS OF FINFISHES AND SHELLFISHES

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INTRODUCTION

Malnutrition, as it adversely affects the human being, impairs the growth, reproduction, health and well-being of the finfishes and shellfishes also. In the farming of these animals in an environment unlike their natural habitat, feeding of the stocked population with nutritionally balanced and quality diets is of critical importance not only to promote their optimal biological and physiological processes, but also to the production. In the different aquaculture systems except that practised on farming the animals feeding on the natural food available in the field, formulated feeds are provided either as supplementary feed or as whole feed. For the preparation of the formulated feed having the optimum dietary nutrient levels, the essential prerequisite is to have an understanding of the nutrient requirements of the species selected for culture.

NUTRITION REQUIREMENTS OF FINFISH

Over the past three decades considerable progress has been made in the study of the dietary nutrient requirements of a number of fishes (Halver, 1972; Cowey and Sargent, 1972, 1979; National Research Council, 1981, 1983; Millikin, 1982; Cowey and Tacon, 1983; Cho, Cowey and Watanabe, 1985).

Although fishes exhibit certain similarities with the terrestrial vertebrates in respect of basic qualitative nutrient requirements, marked difference has been noted from them in the quantitative nutrient needs at the dietary level. This difference is attributed to the carnivorous/ omnivorous feeding habit of fishes, and their preference to use protein over carbohydrates as a dietary energy source. Further, as the fishes live in an ecosystem which supports them and are capable of adjusting to the temperature of the environment; they do not have to expend large amount of energy to maintain the constant body temperature and to develop an elaborate skeletal system as in the case of land-based animals. It is also observed that the fishes expend relatively low energy for reproduction. For these reasons, they are considered to be better feed converters than the other vertebrate groups. Besides, the fish have the advantage of disposing ammonia, the primary end product of nitrogen metabolism, through permeable surface unlike the land-based animals that require conversion of ammonia to urea or uric acid to dispose of the toxic ammonia building up in the tissues. This metabolic characteristic helps the fish to derive relatively more energy for the catabolism of protein than the terrestrial animals.

Fish require 40 or more essential nutrientsl among these the most important ones relate to protein and amino acids, lipids, essential fatty acids, vitamins and minerals.

Protein

Over 20 species of fishes have so far been studied for the dietary protein requirements principally on the basis of feeding experiments on a balanced diet containing gradual levels of quality protein and the recorded optimum growth (weight gain) of the fish. The results of these experiments have shown a high dietary requirement ranging

from 35 to 55% which is equivalent to 45-70% of the gross energy content of the diet in the form of protein. Although such high protein requirement is expected for carnivorous fishes, it is also observed in omnivorous and herbivorous fishes. The use of different dietary protein sources, non-protein energy substitutes, feeding regimes, fish age and methods employed for the determination of dietary energy content and dietary requirement are observed to result partly in the estimation of such high protein requirement. The dietary protein need is also found to be dependent on the size of the fish and environmental factors such as temperature and salinity. Small sized fishes require higher levels of protein for growth than the larger fish. Similarly, increase in dietary protein is recorded in higher environmental temperature. Recent studies and comparisons of results observed in the different feeding experiments to determine the protein requirement have shown that (1) a linear relationship exists between dietary protein requirement (g protein/kg body wt/day) and the specific growth rate, (2) the utilisation of dietary protein for new tissue growth is relatively constant within and between the individual finfishes examined and (3) the dietary protein requirements of fish when expressed relative to feed intake (g protein/kg body weight/day) and live weight gain (g protein/kg live weight gain) are not dissimilar from those of terrestrial animals. It is now recognised that the general protein requirements of fish is the requirement of essential amino acids together with some requirement of non-specific nitrogen.

Amino acids

The fish require ten essential amino acids, namely threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine and tryptophan

in the diet. Generally, quantitative amino acid requirements are determined using dose-response curves. In recent years, the methods based on plasma or serum concentration of free amino acids and carcass deposition have also been employed.

The studies carried out on the amino acid requirements have shown that significant difference in requirement exists within and between individual fish species. The following factors are found to influence the determination of amino acid requirements.

1. Formulation of amino acid test diets,
2. supply of protein in the form of free amino acid and protein bound amino acids,
3. free amino acids are more rapidly assimilated in fish than protein-bound amino acids,
4. the interaction among the essential amino acids themselves and between the essential and non-essential amino acids and between amino acids and other nutrients.

Although, the different individual essential amino acid requirements of several of the fishes have been determined, the dietary requirement of all the ten essential amino acids are established only for four species of fishes, namely, common carp, Japanese eel, channel cat fish and Chinook Salmon (Table 1).

Table 1. Essential amino acid requirements (g/kg dry weight) at stated dietary protein levels of certain fishes

	Chinook Salmon	Japanese eel	Common carp	Channel catfish
Aginine	24	17	16	10.3-17.0
Histidine	7	8	8	3.7
Isoleucine	9	15	9	6.2
Leucine	16	20	13	8.4
Lysine	20	20	22	15.0
Methionine + Cysine	16	19	12	5.6
Phenylalamine + tyrosine	21	22	25	12.0
Threonine	9	15	15	5.3
Tryptophan	2	4	3	1.2
Valine	13	15	14	7.1
Protein in diet	400	377	385	240.0

Lipids

Lipids are important as an energy source in fish diets. Excess or deficiency of lipids affects the growth as well as the body composition of the fish. If the diet is deficient of non-protein energy (Lipids and Carbohydrates), protein is used for energy requirements; if it contains excess, appetite or demand is satisfied before a sufficient quantity of protein is ingested to meet the demand for maximal rate of protein synthesis and growth. Consequently, the experiments to determine the level of dietary lipids are directed to find out the levels which could afford the maximum protein sparing effect and expressed as a function of dietary protein level. Thus, in channel catfish, smaller fishes have shown best growth with diets containing 35% crude protein and 12% lipid,

whereas larger fishes with 25% crude protein with 12% lipid. For rainbow trout, maximum protein sparing is obtained at 15-21% lipid and 35% crude protein. It has also been shown that the protein level could be reduced in marine fish diet, if the energy content is maintained at a high level. Experiments on the use of unsaturated and saturated fatty acids have indicated that the lipids in saturated form could also be used in moderation without affecting the energy requirements of the fishes.

Essential Fatty acids (EFA)

The requirements of EFA of linolenic series have been demonstrated in a number of fishes for achieving better growth rates and food conversion and to avoid certain pathological conditions. However, their requirements differ from species to species as the EFA requirement is found to be far less for channel catfish and carp than those of rainbow trout. Certain fishes such as turbot, red sea bream, black sea bream and yellow tail are found to be not capable of desaturating and chain elongating 18-carbon fatty acids. Consequently, for these fishes, it is essential to supply highly unsaturated fatty acids in the diet.

Carbohydrates

Although carbohydrates form the major source of metabolizable energy in the nutrition of mammals and birds, it is considered to be of relatively little value in fish nutrition. This low efficiency of utilisation of carbohydrates by fishes may be due to insufficient enzymatic break down in the digestive tract, insufficient absorption and inefficient metabolism of monosaccharids. Even if most of the carnivorous fishes are poorly equipped to metabolize sugars and starches, the specific and careful balance of carbohydrate sources would help to spare the protein and furnish

fibre to move other nutrients down the gastrointestinal tract for proper digestion. Recent studies have shown that atleast in certain fishes such as trout, there is no fundamental problem in the utilisation of carbohydrates and sucrose and gelatiminad starch may be of practical value as components of feeds.

Vitamins

Four fat-soluble (Vitamin A, D_3, E and K) and eleven water-soluble vitamins (Thiamine, Riboflavin, pyridoxine, Pantothenic acid, Niacin, Inositol, folic acid, choline, Biotin, B_{12} and Ascorbic acid) are required by the fish. They are required for the metabolism of other nutrients into tissue components. Many of the water-soluble vitamins function either directly or in a modified form as coenzyme. However, fat-soluble vitamins do not function as coenzymes. Specific requirements of vitamins differ from species to species and are affected by the diet composition.

Minerals

Minerals are mainly required for the maintenance of salt and water tissue balance, metabolism of other nutrients and for structural functions. The minerals required by the fish are calcium, chlorine, magnesium, phosphorous, potassium and sodium along with a number of trace elements such as cobalt, copper, iodine, iron, manganese, selenium, zinc, aluminium, chromium and vanadium. Determination of mineral requirements and trace elements in the diet is found to be extremely difficult due to the problem of limiting their concentration and their waterborne characteristics. Between the marine and fresh water fishes, the former require limited supply of minerals as some of the elements are taken from the external environment. For the latter group, mineral supplement in the diet is found to be essential. As

in the case of vitamins, the specific requirements of different minerals are found to vary from species.

NUTRITION REQUIREMENTS OF CRUSTACEANS

Protein

The results of various investigations carried out on the nutrition and nutritional requirements of crustaceans have been reviewed by New (1976, 1980), Castell et al. (1981), Claybrook (1983) and Dall and Moriarty (1983). Dietary protein requirements of cultivable penaeid prawns have been the subject matter of several investigations. These studies have shown that although the protein requirements for penaeid prawns vary from 15 to 80%, generally it is found around 40%. For Penaeus indicus, the optimum protein level is recorded between 35 and 40%; P. mergeriensis, 34-42%; P. monodon, 34-40%; P. japonicus, 52-57%. As in the case of fishes, factors such as protein source used in the diet, environmental factors, effect of other nutrient levels, size and age, amino acid profile of the protein source and that of the animal influence the specific requirement.

Amino acids

Amino acids in crustaceans occur both in free form and bound form as in all organisms. However, the free amino acids in most crustaceans are found to be relatively at higher levels than in vertebrate tissues. Prawns are found to require the same ten amino acids as fish. For several crustaceans such as Cancer, Homarus, Palaemon, Penaeus, Macrobrachium and Uca, these amino acids are found to be essential in the diet. In certain crustaceans, the gut symboints are known to be capable of synthesising certain amino acids. Among the other amino acids, proline appears to enhance the growth.

Lipids

Studies on the quantitative requirements of lipids in the diet of prawns have shown that lipid levels less than 10% have given higher growth increment than that of the lipid levels at 10, 13 and 17%. Generally a lipid level between 5 and 7% in diet is suggested for prawns. The lipid levels show marked difference during the moult cycle of prawns, being low in the post-moult and premoult stages. The de novo synthesis of fatty acids of the linoleic, and linolenic series is found to be extremely limited or non-existent in crustaceans. However, these fatty acids made available in the dietary sources could be chain elongated and further desaturated. Marine crustaceans have shown to have higher levels of linolenic series of fatty acids and higher amounts of C 20 and C 22 polyunsaturated fatty acids than fresh water crustaceans, that have higher levels of linolenic type fatty acids. This indicates that EFA of linolenic series have greater value to marine crustaceans, while the fresh water crustaceans require more linoleic series or a mixture of both. A dietary requirement of 1-2% linolenic acid is indicated in the diet for prawns. Recent studies on the phospholipids requirement of larval P. japonicus have shown that phospholipids containing choline or inositol and linoleic, linolenic, eicosa pentaenoic acid and docosa haxoenic molucules promote growth and survival.

Crustaceans also found to be incapable of synthesising sterols. Cholesterol forms the major sterol in crustaceans and it is synthesised form ergosterol, stigmasterol and -sitosterol but not from non-sterol precurors as in Astacus, Penaeus, Portunus and Panulirus. Growth is found to be better when cholesterol at 0.5% level is added as a dietary component. Cholesterol supplemented with ecdysone and cyasterone in the diet is found to increase the moulting frequency.

Carbohydrates

Carbohydrates although considered not as an essential component of the diet, penaeid prawns are found to utilise disaccharides better than the monosaccharides. In penaeid prawns including P. indicus, increased growth is recorded in the carbohydrate level upto 40%, where starch is used as the nutrient source. The inclusion of carbohydrates in the diet helps to spare more portion for growth than for energy requirement. Cellulose used as roughage and non-nutrient filler in the diet helps better utilisation of other nutrients. The amino sugar glucosamine at 0.5% level in the diet is shown to have a growth promoting effect.

Vitamins

The observations on the dietary requirements of various vitamins for crustaceans by different workers are inconsistent. Gut symboints and bacterial contamination, it is opined, may be involved in the supply of some or all the vitamins. While the vitamins of all prosthetic groups of enzymes are found to be required by Artemia, Moina is shown to require thiamine, nicotinamide, pyridoxine, pantothenic acid, riboflavin and folic acid.

Ascorbic acid, inositol and choline are found to enhance growth and survival in Penaeus. Crustacean appears to be incapable of synthesising carotenes. Panulirus cygnus fed with a low carotene diet became pallid, indicating the requirement of vitamin A.

Minerals

Information on the mineral requirement of crustaceans is scanty. Phosphorus, potassium and trace metals are found to be required in the dietary composition of Penaeus. However, calcium, magnesium and iron are observed to be not essential in the diet. Although calcium is an obvious

requirement for crustacean for exoskeleton build up and needs to be conserved in calcium-deficient environment, utilisation of the calcium from the gastrolithes and from eating of excuviae help to meet their requirement. Further, marine crustaceans absorb directly calcium fron the sea water. Since the magnesium is excreted by most crustaceans and is available to them in the sea water, and since the iron is stored in the mid gut, these minerals may not be a dietary requirement. Copper which is required for haemocyanin synthesis, is derived mainly from the food and the sea water.

Larval nutrition

There has been considerable progress in the studies on larval nutrition, particularly on the penaeid prawns and Macrobrachium rosenbergii that are cultured in several regions of the world. The recent progress made in the development of microencapsulated feed on commercial lines is a noteworthy development in this field. The various aspects of penaeid and palaemonid larval nutrition are reviewed by Rao (1983).

Although there have been several pioneering investigations on crustacean nutrition, comparison of results of studies from different laboratories have rendered difficulties in the evaluation of different diets. In order to permit direct comparison of results among different laboratories, a crab protein diet is formulated as a standard reference diet. The composition of this standard reference diet is as follows:

Crab Protein Reference Diet (%)

Crab protein	..	40
Wheat gluten	..	5
Corn starch	..	15
Dextrin	..	5
Alpha cellulose	..	17.8
Mineral mix	..	4
Vitamin mix	..	2
Dl -α-tocopherol	..	0.2
Codliver oil	..	6
Corn oil	..	3
Choline chloride	..	1
Cholesterol	..	1
Total		100

Crude protein	..	38.1% (dry wt)
Lipid	..	10.5%
Ash	..	6.5%
Gross energy (cal/g)	..	5.0

NUTRITION REQUIREMENTS OF MOLLUSCS

The larval molluscs as well as the majority of the adult bivalves procure their food by filtering plankton or suspended particles from sea water. Adult gastropods graze on algae and detritus, while the cephalopods are active predators feeding on moving live animals such as crustaceans, fishes and other molluscs. In the stomach content of oysters items such as plankton, organic waste, fungi, flagellates, larvae of various invertebrates, sand and mud, have been found. On the basis of the physiology of digestion, it is observed that the animal food material in oysters contribute only 10% or less of the total food.

As the farmed molluscs in the grow-out system derive their food from nature, nutritional aspects for the culture of molluscs assumes importance principally in the rearing of larvae and spat under controlled conditions.

It is now established that a mixed diet containing more than two types of food forms a more balanced diet for shellfishes. Experiments have shown that the shellfish larvae fed with single cell alga raised with vitamins such as B_{12}, thiamine and biotin have given better growth rates.

There have been several attempts to develop artificial diets for marine bivalves. However, these efforts have so far met with only a partial success, as clumping of food particles, leaching losses and development of bacterial population pose difficulties. Further, the food particles must also be digestible and small (2-15 µm). The artificial diet prepared with microgel particles, microcapsules, Kaolin and trace-metal mixture has given good growth in the juveniles of Crassostrea virginica when experimented in a beaker. But the growth rate is found to be poor when the larvae are reared in a flow through system. The bacterial population developing in the flow through system appears to effect the nutrient source of the diet or aid in the breakdown and digestion of the food particles. The artificial diet had protein, soluble starch, rice starch each of 25.7% by weight, soy lecithin 14.4%, DNA and RNA, each 2.6%, phosphorus 1.3%, carboxymethyl cellulose 1.1%, trace-metal mix 1.0%, Menhaden-walled vitamin capsules, (25 capsules/µl, 0.82 mg lipid/ltr), lipid, 97.9%, vitamin mix 1.0%, Ascorbic acid, 1.0% and phenol red 0.1%. Menhaden-walled B_{12} capsules (5 capsules/µl, 0.16 mg lipid/ltr. Egg albumen is found to be the best source of protein.

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RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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PROTEIN AND AMINO-ACID REQUIREMENTS IN FINFISHES AND SHELLFISHES

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Proteins are macromolecules, which are biopolymers made up of many monomers which are known as amino acids. Of the three carbohydrate, lipid and protein, it is only proteins which contain nitrogen. The empherical formula for amino acid is R-CH-NH_2-COOH. Though about 300 or so amino acids are known to occur in nature, only 20 of these are present in proteins and all of them are L-α, amino acids. The sequence in which these amino acids occur in a particular protein follows a precise order, which is genetically controlled. Thus each peptide molecule ie., protein differs from another only by the order of arrangement and in the number of amino acid molecules.

FUNCTIONS OF PROTEINS

Proteins are vital as are the functions they perform. The functions they perform either as pure proteins or as complexes with carbohydrate, lipids, and minerals are many. Growth of the animals is nothing but addition of tissues ie., synthesis of new protein. Thus as structural proteins are responsible for the cellular architecture. The other functions are (2) in the body fluids they transport

substrates; (3) several of the hormones and (4) enzymes which catalyse biochemical reactions are proteins; (5) proteins form component in immunologic molecules; (6) serve as lubricants and protective agents (mucins, mucos); (7) the antifreeze substances in the Antartic fishes are glycoproteins; (8) many of the toxins and venoms of marine organisms are protein complexes; (9) some of the amino acids have been found to be feed attractants; (10) protein molecules also have a high buffering capacity; (11) glucogenic - amino acids (hydroxyproline, serine, cysteine, threonine, glycine; tryptophan, alanine; tyrosine, phenylalanine; isoleucine, methionine, valine; histidine, proline, glutamine, arginine, glutamate; and aspartate) on being deaminated serve as substrates for carbohydrate and fatty acid synthesis (Gluconeogenesis) and (12) thus also yield energy.

CALORIE VERSUS PROTEIN AS UNIT OF MEASUREMENT IN NUTRITIONAL BIOENERGETICS

In the study of energetics energy in terms as calories or joules used to be preferred. But for aquatic organisms partitioning based on protein as nitrogen units is of more suitable over energy units for the following reasons: 1. In comparison with higher animals fin - and shell-fishes being poikilotherms use less energy to regulate their body temperature. 2. For the locomotion and maintenance of position, shellfishes need not spend much energy being mainly bottom dwellers for much of the time. 3. The shellfishes for the purpose of respiration like fishes need not actively maintain ventilation of gills by constant flow of water which in turn compels active swimming, a costly process in terms of energy. 4. In finfishes and in

shellfishes it is an important aspect that protein serves not only as a nutrient for growth but preferred over carbohydrates as dietary energy source. (5) The quantity assimilated over maintenance level in carbohydrate and lipid is stored mostly as fat and as glycogen to a lesser extent; while in the case of proteins goes for meat production. Many consumers do not like fatty aquatic products. (6) The end product of nitrogenous metabolism in the aquatic organisms is mostly ammonia which by passive diffusion can be eleminated into the medium (7) Thus energy need not be spend in converting the toxic ammonia into urea or uric acid, whereby aquatic organisms come to derive more metabolisable energy from catabolism of proteins than terrestrial organisms. To illustrate: for a megajoule of digestible energy in rainbow trout (Salmo gairdneri) 9.6 g body protein is produced which is 2 to 20 times higher for poultry, pig and cattle (Pandian and Vivekanandan, 1985).

ESTIMATION OF PROTEIN

Protein is usually estimated by any one of the following methods. (1) Determination of nitrogen by kjeldahl method; (2) Biuret method and (3) Folin-Lowry method. Of these the first given method is mostly used, where in all nitrogenous matter-both protenous and nonprotenous-is converted into ammonia and calculated in terms nitrogen. (When protein alone need to be determined first protein need to be precipitated out and precipitate is digested for kjeldahl nitrogen). In the conversion of nitrogen into crude protein it is assumed that all nitrogen in the biological material is present as protein and secondly that all crude proteins contain nitrogen 16% by weight and so the conversion factor used is 6.25 (100/16=6.25). This is not always so. Therefore check need to be made for percentage

Soyabean meal: CP 39-41% in dry matter, crude fibre 9%, availability of amino acids is high (CS 82-92%) except for methionine (CS 70%). However heat treatment used to inhibit typsin inhibitor reduces the availability of lysine and cystine, much in the case other amino acids too is reduced. Raw meal causes rachitogenic effect. Therefore higher than normal levels of vitamin D_3 need to be added. It is also suggested to have tocopherol oxidase. Vitamin content too get reduced with heat treatment.

Wheat (Triticum aestivum) : CP 6-22%, average 8-14%, protein (gluten) is of two types. (i) Prolamin (gliadin) and (ii) Glutelin (glutenin). The second contains three times more lysine than the first. Gluten is rich in glutamic acid (33%) and proline (12%).

FORMULAE AND INDICES USED

The indices used in the measurement of protein utilisation are as follows:

1. Assimilability (digestibility) of protein (or assimilation efficiency of protein) A%

$$= \frac{\text{Protein consumed(g) - faecal protein (g)}}{\text{Protein consumed (g)}} \times 100$$

2. Nitrogen balance (NB) = N consumed -(N in faeces+ N excreted through gills and kidney)

NB is measured in terms of mg N/100g body weight/day. Therefore all the 4 parameters need to be in the same unit.

3. Protein efficiency ratio (PER)

$$= \frac{\text{gain live weight (g)}}{\text{protein consumed (g)}}$$

4. Protein conversion ratio (PCR) = $\frac{\text{Protein gained (g)}}{\text{Protein consumed (g)}}$

5. Net protein retention (NPR) = $\frac{\text{Weight gain of TPG(g) + weight loss of PFG (g)}}{\text{Protein consumed (g)}}$

TPG - group fed with test protein

PFG - group fed with protein-free diet

6. Productive protein value (PPV) (%) = $\frac{\text{gain in body protein(g)}}{\text{protein consumed (g)}} \times 100$

7. Meat produced in assimilation (MPA) (%) = $\frac{\text{live weight gain (g)}}{\text{protein assimilated(g)}} \times 100$

8. Protein produced in assimilated protein (PAP)(%) = $\frac{\text{Protein gained (g)}}{\text{Protein assimilated(g)}} \times 100$

9. Gross protein value (GPV) = $\frac{A}{A_o} \times 100$

A = (weight gain of Gr. 2 - that of Gr. 1) $\div$ weight gain of Gr. 2

A_o = (weight gain of Gr. 3 - that of Gr. 1) $\div$ weight gain of Gr. 3

Diet groups:

Group 1:- fed with basal diet

Group 2:- fed with basal diet + Cg of test protein

Group 3:- fed with basal diet + Cg of casein

Basal diet will be having optimal crude protein

10. Apparent biological value (ABV) (%) = $\frac{\text{N consumed - (faecal N + urinary N)}}{\text{N consumed - faecal N}} \times 100$

11. Biological value (BV)(%) $= \dfrac{\text{N consumed} - [(\text{faecal N} - \text{MFN}) + (\text{urinary N} - \text{EUN})]}{\text{N consumed} - (\text{faecal N} - \text{MFN})} \times 100$

MFN - Metabolic faecal nitrogen is that quality of nitrogen excreted in the faeces when the animal is fed with nitrogen free diet.

EUN - Endogenous urinary nitrogen is that quantity of nitrogen excreted by means of gills and as urine when the animal is starved of dietary protein.

12. Daily protein requirement (% live weight/day) = (Optimal dietary protein requirement % x consumption of feed % live wt per day) $\div$ 100

Instead of 100, if divided by 10 will give protein required for kg live weight per day.

13. Protein required for weight gain (g/kg live wt.) = Optimal dietary protein requirement (%) X FCR x 10

Where FCR (food consumption rate) = food consumed $\div$ weight gained (dry weight basis)

14. Chemical score: The quality of protein in a protein source is decided by the quantity of EAA present. Here EAA content of the source is compared with that of a standard protein.

The usual standard used by the nutritionists in hen's egg white. The current trend with the Japanese workers particularly with Ogino group is to use the EAA profile of the fish muscle as standard protein in the fish nutrition. CS is calculated as follows.

Eg: Tryptophan in egg white - 1.7%

Tryptophan in sardine - 1.2%

$$CS = \frac{1.2}{1.7} \times 100 = 70.59\%$$

15. EAA index : Herein the amounts of all the 10 essential amino acids present are taken into consideration. It could be defined as the geometric mean of egg ratios of these acids.

$$EAAI = \sqrt[n]{\frac{100_a}{a_e} \times \frac{100_b}{b_e} \times \frac{100_c}{c_e} \cdots \frac{100_j}{j_e}}$$

a, b, --- j = % EAA in the protein source

a_e, b_e -- j_e = % in the egg albumin

n = number of EAA entering into the calculation

EAAI has the advantage of predicting the effects of supplementation in combination of proteins but proteins of very different EAA composition may come to have a similar index.

OPTIMAL DIETARY PROTEIN REQUIREMENT

Organisms need to be supplied with sufficient quantity of protein in their diet for their metabolic needs and growth. Protein is a costly commodity and so it is protein which is the single major component which decides the price of the feed. When higher levels of protein is available in the feed some portion of it will go waste. Thus protein need to be at an optimal level in the feed. While conducting experiments to arrive at the optimal dietary protein requirement the following points need to be carefully considered. Foremostly the protein which needs to be evaluated should be sufficiently able to meet the requirement for essential amino acids. If one essential amino acid is deficient while all the other amino acids are available in enough quanity, complete spectrum of protein synthesis can not be met. Usually for the purpose purified proteins such as casein, albumin or mixture of proteins are used. Casein is

deficient in argenine and suboptimum in sulphur bearing aminoacids, zein is deficient in tryptophan and lysine. In such cases these essential amino acids need to be supplemented. Usually for the purpose crystalline amino acids are used. It has been found that free crystalline amino acids are not so well absorbed as that of bound amino acids in fishes. Regarding other marine organisms still we do not know how far free amino acids are absorbed (Jacon and Cowey, 1985). It is also to be noted that carnivorous fishess show low palatability of purified proteins.

Second point to be observed is that the level of feeding should not be a constraint for optimal growth. Ad libitum feeding is recommended. Another fact which need to be emphasised is, the feeding strategy should be convenient to the test animal. By feeding strategy, suitability of the feed for the animal's style of foraging, particle size of feed, time, frequency and duration of feeding, form of feed whether pellet, powder or paste etc., and ecophysical conditions of like light, salinity, pH, temperature, vibration and disturbance to the animal etc., are meant.

When the values of indices like K_2, EMP, MPA, PAP, PER, PCR and PPV are plotted against % protein in diet at the optimal protein level the graph will peak, whereby indicating the optimal requirement. At lower levels the assimilation of the feed and protein have been found to be high while low at higher levels. Consumption of the feed has been found to be high in low protein diet and also when the protein is found to be low in one or more EAA, whereby the organism attempts to gather more of the required nutrient. In such cases protein assimilation and consumption rate have been found to be high; while other nutrients available at optimum concentration in the diet are preferentially assimilated less. In crustaceans it has been observed that at

high (about 60%) protein level too feed consumption show a rise. In such cases assimilation for all nutrients is low. Thus the animal used to take to superfluous feeding also known as "gluten effect".

Though weight gain is used by some workers in the interpretation of data, gain in protein (protein retention) or nitrogen balance is preferred. In crustaceans the interpretation of data pose characteristic situation because of moulting. The animals just moulted used to be flaby and high in water content. If such animals happened to be there at the conclusion of the experiment indices in which live weight is taken into calculation can be misleading.

A survey of literature show that optimal dietary protein requirement ranges between 36-50% (Table 1). Though there are a few rare instances where in value as high as 70% has been quoted. The average is around 39% for finfishes and shellfishes. While getting high percentage it is noteworthy to note that EAA deficient protein can elevate dietary protein requirement. Further increase in water temperature above ambient upto an optimal level increases dietary protein requirement. In this connection it need to be pointed out it is well known that increase in water temperature upto an optimum is accompanied by an increase in feed intake coupled with higher metabolic rate and increased growth. Whereby tropical organism have higher feed intake level coupled with faster growth rate.

DAILY PROTEIN REQUIREMENT

Comparatively very few investigations alone have been carried out in this direction. The available data indicate that daily protein requirement does not fall within a narrow

range as do optimum protein requirement discussed above. In fishes it ranges from 0.75 to 5.25 in terms of percentage body weight per day. The interesting fact is that a linear relationship exists between daily protein requirement and the specific growth rate. (Jacon and Cowey, 1985). Thus it is amply clear that optimal dietary protein requirement and daily protein requirement are not related factors. The optimum dietary protein requirement is related to concentration vs activity, ie., quantity required for the optimum rate of digestion and assimilation. While daily requirement is related to the inherent capacity of the animal to grow in other words to the speed of protein synthesis, which is dependent on metabolic activity, age, size, temperature and hormonal control.

REQUIREMENT OF ESSENTIAL AMINO ACIDS

Essential amino acids are those amino acids which cannot be biosynthesised by the organism sufficiently. It is of interest to note that essentiality for 10 amino acids seems to be universal throughout the metazoa, though a few variation from the general pattern is met. The essential amino acids are - threonine, valine, methionine (+ cystine), isoleucine, leucine, phenylalanine (+ tyrosine), tryptophan, lysine, histidine and arginine. Tyrosine, cystine, glycine and serine could not be synthesised by the organism in sufficient level and so need to be supplied in a lesser extent therefore known as semiessential amino acids. Glutamic, aspartic acids, alanine, proline and hydroxyproline are non essential amino acids. Since there can be synthesised in required level. The figure given on the inter-conversion of the major food stuffs amply illustrate the synthetic and interconversion of non essential amino acids. Though cystine from methionine and tyrosine from phenylalanine could be

synthesised, in the absence of cystine and tyrosine the requirement for methionine and phenylalanine is increased.

The synthetic pathways of semi and non-essential aminoacids are as follows. (Fig. 1 & 2).

Alanine	By transamination of pyruvate with glutamate.
Aspartate	By transamination of oxalacetate with glutamate.
Proline	From glutamate via glutamate semialdehyde and pyrroline carboxylate.
Glutamate	By reductive amination of - Ketoglutarate
Arginine	By reactions of urea synthesis
Glycine	By removal of hydroxymethyl group from serine
Serine	By transamination of hydroxypyruvate or phosphohychoxypyruvate with alanine.
Tyrosine	From phenylalanise by hydroxylation
Cystine, Cysteine & Taurine	By transulfuration pathway from methionine

Thus theoretically all non-EAA except the sulphur bearing (Cystine, Cysteine, Taurine) can be synthesised in the organism by feeding sufficient ammonium salts together with glucose to provide the carbon skeleton.

METHODS OF DETERMINATION

The following methods have been used to evaluate EAA requirements.

I. Direct method: Herein as one at a time basis each aminoacid is deleted from the amino acid test diet and a dose-response growth curve is made. Dietary requirement is taken at the 'break - point'.

II. Indirect methods:

2. Nitrogen balance technique: This is a modified method of the first one. Herein quantitative variation in free amino acid levels in specific tissue pools such as, whole blood, plasma, haemolymph or muscle is made with reference to the deleted amino acid on enquiry.

3. Tissue culture method: like dietary deletion herein specific amino acid free media are used.

4. In alternatively starved and fed animals fluctuations in of free amino acids levels are made in tissue pools; wherein EAA fluctuate drastically between feeding and starvation while the levels of non-essential amino acids remain steady.

5. Radioisotopic assay: Animal is fed or injected with one of the radioactivity labelled readily metabolisable metabolite such as (^{14}C) glucose; $^{14}CO_2$, (^{14}C) acetate, (^{14}C) succinate or (^{14}C) pyruvate. Organism (if small) or a part of the tissue is latter assayed after a period of incubation. The non-essential amino acids being able to be synthesised from the precursors take up labelling while EAA remain unlabelled. Since many of the microbes have the capacity to synthesis EAA, in this method microbiological contamination is the chief source of error. It has been found out that molluscs in general have a strong capacity for a rapid biosynthesis of glutamate, alanine, and aspartate and weaker or non-existing capacity for asperagine, glutamine, serine, glycine and proline from glucose moity. Aspartate is most strongly labelled with succinate and CO_2 precursors; while alanine with glucose and pyruvate. Among aspartate, alanine and glutamate, glutamate is generally

most weakly labelled. These indicate a considerable capacity for CO_2 fixation into dicarboxylic acids of TCA cycle and a tendency for many of the molluscs to accumulate alanine under anaerobic conditions (Bishop et al., 1983).

6. Ogino's carcass deposition method (Ogino, 1980a&b): This is the only method devised to determine quantitative requirement for EAA specifically for fishes. Ogino observed similarity in percentage composition existing between dietary EAA requirements of fishes and EAA profile of fish muscle. Since the crustalline amino acids have been found not so ideal as sources for EAA he preferred lipid free fish meal or lipid free fish muscle as dietary protein source ie., standard essential amino acid reference dietary protein. His procedure is to estimate daily nitrogen/protein retention rate, percentage feeding rate for 100 g body weight, percentage digestibility for protein and for each amino acid for the test animal. From these parameters he calculated optimum level for each amino acid required to be present in the dietary protein source and optimum dietary requirement per day for each amino acid. Select list of EAA requirement to some of the cultivable animals is given in the Table 2.

Comments:

The EAA study carried out in Mytilus californianus (Harrison, 1980) show that apart from known ten EAA, proline is also essential. In eel cystine is superior to methionine, while in other animals it is other way. In eel cystine at the rate of 0.05% and methionine at 1.6% in the diet failed to premate growth while at 1.0% and 0.9% levels respectively resulted in enhanced growth.

Many lower marine organisms, especially marine molluscs have ability to absorb all protein amino acids including

taurine from the medium. Only marine molluscs require taurine a non EAA, while freshwater and terrestrial molluscs do not. The uptake of glycine, threonine and glutamin was very fast from the medium while arginine was taken up slowly. The studies show that gills are the main organ of absorbance. The interesting finding is that there are specific transportation site for each group of amino acids, viz., acidic, basic, neutral and imino amino acids. Even dipeptides have been found to be absorbed. Transportation of alanine, glycin and cycloleucine has been found to be either sodium ion and/or energy dependent (Bishop et al., 1983).

In rainbow trout tryptophan deficiency has been known to induce loss of appetite, transient scoliosis and deposition of calcium in bony plates around notochord and kidney. The fish also becomes hyperemic (Cowey and Sargent, 1979).

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Table-1. Optimum dietary protein requirement (from various authors)

Organism	Source	Protein %
Penaeus setiferus	fish meal	28-32
P. japonicus	shrimp meal	40
"	casein	54
"	squid meal	60
"	casein & egg albumin	52-57
P. monodon	casein & fish meal	46
P. indicus	prawn meal	42.8
"	casein, arginine & cystine	39.0
P. merguiensis	Mytilus edulis meal	34-42
Cyprinus carpio	casein	31-38
Ictalurus punctatus	whole egg protein	32-36
Anguila japonica	casein, arginine & cystine	44.5
Ctenopharyngoden idella	casein	50
Fugu rubripes	casein	40-50
Epinephelus salmoides	Tuna muscle meal	40
Chanos chanos	casein	55
Chrysophrys major	casein	40
Tilapia aurea (fry)	casein & egg albumin	56
" (adult)	"	34
T. mosambica	white fish meal	40
T. zellii	casein	35
Microplerus dolomieri	casein & fish protein conc.	45
M. salmoides	"	40

Table-2. Requirement for essential amino acids in diet as percentage of protein and as percentage in diet ( ) - from various sources

	Anguilla japonica	Cyprinus carpio	Ictalurus punctatus	Chinook salmon	Generalised
Arginine	4.5 (1.7)	4.2 (1.6)	4.3 (1.03)	6.0 (2.4)	4.5
Histidine	2.1 (0.8)	2.1 (0.8)	1.5 (0.37)	1.8 (0.7)	1.7
Isoleucine	4.0 (1.5)	2.3 (0.9)	2.6 (0.62)	2.2 (0.9)	2.6
Leucine	5.3 (2.0)	3.4 (1.3)	3.5 (0.84)	3.9 (1.6)	4.0
Methionine (a)	5.0 (1.9)	3.1 (1.2)	2.3 (0.56)	4.0 (1.6) (b)	1.6(b) 2.5(a) 3.1(d)
Phenylalanine (c)	5.8 (2.2)	6.5 (2.5)	5.0 (1.20)	5.1 (2.1)	5.6(c)
Threonine	4.0 (1.5)	3.9 (1.5)	2.0 (0.53)	2.2 (0.9)	3.1
Tryptophan	1.1 (0.4)	0.8 (0.3)	0.5 (0.12)	0.5 (0.2)	0.6
Valine	4.0 (1.5)	3.6 (1.4)	3.0 (0.71)	3.2 (1.3)	3.2
Lysine	5.3 (2.0)	5.7 (2.2)	5.0 (1.50)	5.0 (2.0)	5.4
Protein in diet %	37.7	38.5	24.0	40.0	39

a - In the absence of cystine

b - In the presence of cystine

c - In the absence of tyrosine

d - In the presence of tyrosine

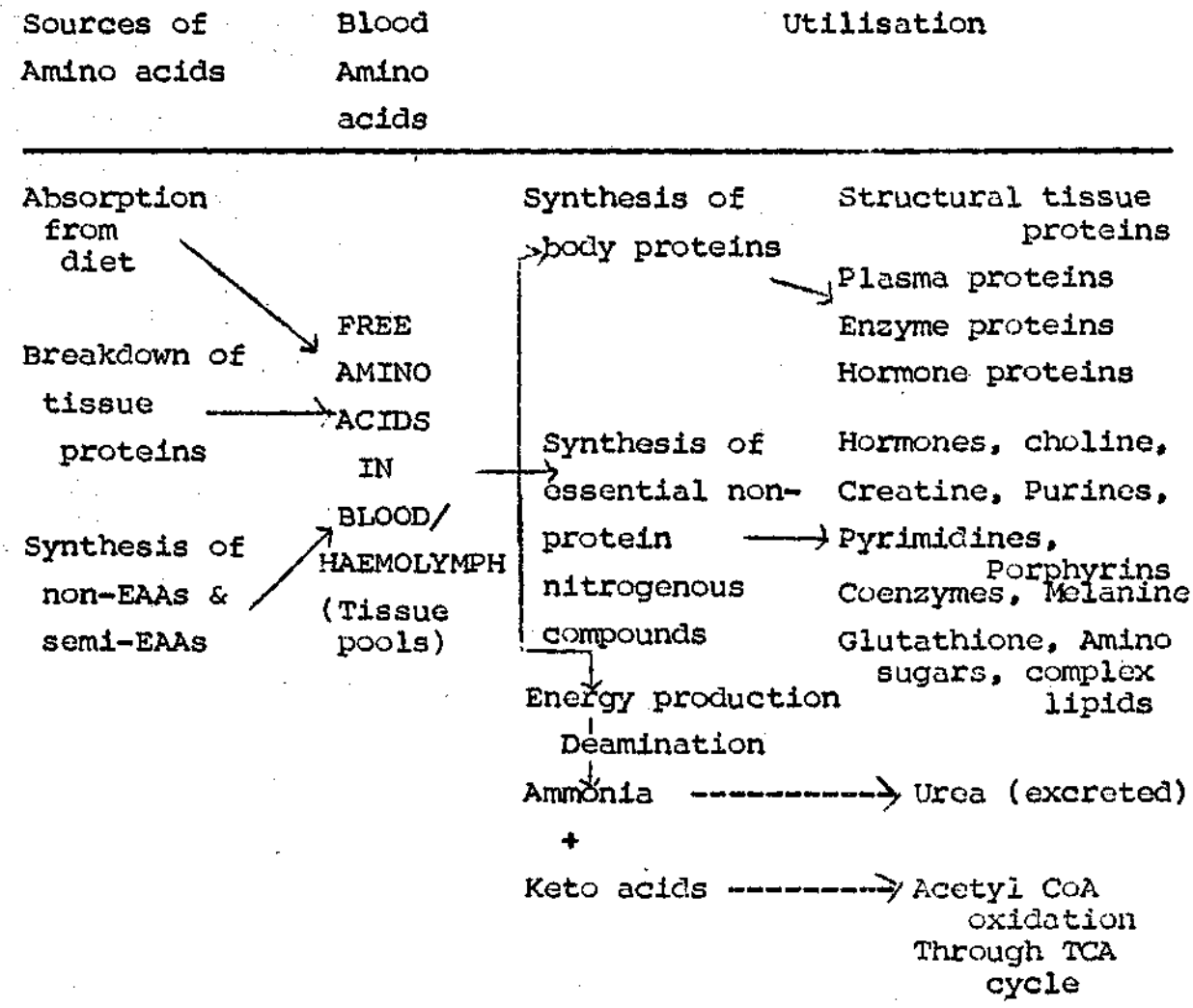

Figure 3. Diagram showing free amino acid pool as related to the metabolism of proteins.

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LIPIDS AND ESSENTIAL FATTY ACID REQUIREMENTS OF FISH AND SHELLFISH

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INTRODUCTION

Lipids, the water insoluble biomolecules were considered as mere sources of energy for animals, until Burr and Burr (1930) for the first time demonstrated the need for a essential fatty acid in the diets of rates. Researches carried out during the past three decades revealed the significance of lipids in the nutrition of fish and shellfish as important sources of energy (8 to 9 Kcal/g ME) and fatty acids essential for normal growth and survival of both finfish and shellfish. Besides these functions, they do have important dietary roles as carriers of certain non-fat nutrients, notably the fat-soluble Vitamins A, D and K. The unsaturated fatty acids play an important role in the transportation of other lipids.

Recent studies indicate that sterols and phospholipids are essential dietary nutrients for crustaceans. Phospholipids and sterol esters, play a vital role in the structure of biomembranes at both the cellular and sub-cellular levels. Lipids are also important in the flavour and textural properties of the feed consumed by finfish and crustaceans.

Lipids are involved in many other aspects of metabolism; for example many of the hormones are steroids. Besides, the long-chain polyunsaturated fatty acids (PUFA) are precursors for prostaglandins in fish and crustaceans. Tanaka and Strickland (1965) have shown that the Na^+ K^+ - activated AT Pase require a phospholipid for its normal function; either phosphatidylserine or phosphatidylglycerol are effective in activating this enzyme (Kimelberg and Papahadjopolus, 1972). Further the work of Nicol et al. (1975) showed the possible role of PUFA of the w3 series in brain and nerve activity. In the case of crustaceans Lester et al. (1975) have shown by in vitro experiments that N- (N-dodecanoyl sarcosyl taurine (DST), the crustacean emulsifier require cis-docec - 5 enoic acid as a constituent.

ENERGY VALUE OF DIETARY LIPIDS

Dietary lipids serve as sources of metabolic energy. In most ingredients they are 85-90% digestible by fish. Experiments with cultured fish have shown that the optimal lipid intake is essentially similar to that for wild fish (Cowey and Sargent, 1979). Lee and Putnam (1973) raised rainbow trout at 12°C on diets containing up to 24% dry weight as herring oil, with excellent feed and energy conversions as well as growth rates. Adron et al. (1976) fed diets containing up to 9% lipid and found that the weight gain and protein utilization of the fish to increase up to the maximum level of lipid used. Channel catfish have been successfully grown on diets containing 10-12% lipid (Stickney and Andrews, 1972). Increasing the energy level of the diet using lipid at constant protein always resulted in improved protein utilization. According to Cowey and Sargent (1979) lipids not less than 10% and not more than approximately 20% can be added to fish diets with excellent results. However, recent

studies conducted at CMFRI, Cochin indicates that the fry and fingerlings of mullets, Liza macrolepis and Liza parsia, as well as the milk fish, Chanos chanos require relatively low lipid levels in the diet for maximum growth. Lipid levels above 6% in these species did not significantly enhance growth or improve the food conversion ratio. In all the species lipid levels above 9% in the diet resulted in growth retardation. The differences observed can be mainly be attributed to the feeding habits of the fish species.

The main protein sparing effect of dietary lipids is to replace protein which could otherwise have been catabolized and used both for energy and to synthesize lipid. The sparing of dietary protein by lipids has been extensively investigated in various species of fish (Lee and Putnam, 1973; Bromely, 1980). Takeuchi et al. (1978) have reported an optimum ratio of protein to lipid in diets of rainbow trout as 35%:15-20%. The protein concentration can be decreased by approximately 15% in rainbow trout diets if high quality lipids, capable of satisfying the EFA requirements of the fish, are added at the level of approximately 18%. Takeda et al. (1975) have reported that the protein level of yellow tail diets can be reduced from 70 to 55% without retardation of growth, if the caloric content was maintained at a high level with pollock liver oil (Takeda et al., 1975). On the other hand, omnivorous fish such as the carp can utilize effectively both carbohydrate and lipids as dietary energy sources. Addition of lipid at levels of 5-15% to diets resulted in no improvement in growth, feed conversion, or the value of NPU, when the dietary protein level remained around 32% (Takeuchi et al., 1979a). Protein to energy ratio requirements of channel catfish fingerlings as a function of protein deposition were found to be 88 mg protein/kcal, between dietary energy concentrations of 275

to 341 kcal/100g (Garling and Wilson, 1976). Striped bass fingerlings reared at 20.5°C and fed 37, 47 or 57% protein with 7, 12 or 17% lipid in a 3 X 3 factorial design showed maximal protein sparing action of lipid for growth when fed 12% lipid combined with 47% dietary protein or 17% lipid combined with 57% protein (Millikin, 1982).

As far as crustaceans are concerned the quantitative dietary lipid requirements have been worked out for very few species. However, several workers used lipids derived from plant products, animal products and their mixtures, according to the availability in local areas. While reviewing the nutritional requirements, Forster (1976) reported that penaeid prawns do not require high levels of dietary lipids and suggested that optimum level may fall between 5 and 10% in the various species. Kanazawa et al. (1970) reported better growth of P. japonicus when fed a purified diet with 8% Soybean oil as the lipid source. In P. merguiensis better growth was observed with 7% codliver oil (Aquacop, 1978). Studies on P. indicus showed that larvae and post-larvae require almost 10% lipid, whereas advanced postlarvae and juveniles require about 10-12% lipid for promoting maximum growth, food and protein conversion, and protein retention (Chandge, MS). Studies with P. indicus (Chandge, MS) and Macrobrachium rosenbergii (Clifford and Brick, 1978) have shown that protein utilization is improved by sufficient amount of fat and carbohydrate.

ESSENTIAL FATTY ACIDS

Fatty acids occur in very large amounts as building block components of saponifiable lipids and only traces occur in free form in cells and tissues. About 100 different kinds of fatty acids have been isolated from lipids of various animals and plants. All possess a long hydrocarbon chain

and a terminal carboxyl group. The hydrocarbon chain may be saturated without any double bond as in palmitic acid or it may have one double bond as in oleic acid then it is called as monounsaturated or monoenoic fatty acid. When two or more double bonds are present in the hydrocarbon chain, it is known as polyunsaturated fatty acid (PUFA), such as linoleic (18:2 w6) and linolenic acid (18:3w3). Sometimes unsaturated hydrocarbon chain may have 20 or more carbon atoms then it is called as highly unsaturated fatty acid (HUFA) such as eicosapentaenoic (20:5w3) and docosahexaenoic acid (22:6w3). Unsaturated fatty acids have lower melting points than saturated fatty acids of the same chain. So they are abundant in marine animals and plants (Sargent, 1976). Certain fatty acids have specific nutritional importance which are not biosynthesized de novo are called as "Essential Fatty Acids" (EFA). These fatty acids have to be included in the diets for normal survival, growth, maintenance and proper functioning of physiological processes (Alfin-Slater and Aftergood, 1968).

In both fresh water and marine fish the w3 acids predominate although substantial amounts of w6 acids are also present. In marine fish, however, the level of w6 PUFA are significantly low, so that the ratio w3/w6 is substantially higher in marine fish than in freshwater fish (Ackman, 1967). Most of the marine penaeid prawns also have high contents of w3 HUFA.

Mead and Kayama (1967) reviewed the fatty acid metabolism in fish. Fish are able to synthesise, de novo from acetate, the even-chain saturated fatty acids, as shown in Fig. Radio tracer studies have shown that fish can convert 16:0 to the w7 monoene and 18:0 to the w9 monoene. The w5, w11 and w13 monoenes are proposed based on the identification

of these isomers in the monoenes of herring oil (Ackman and Castell, 1966). Fish are unable to synthesize any fatty acids of the w6 or w3 series unless a precursor with this 'w' structure is present in the diet. Fish are able to desaturate and elongate fatty acids of the w9, w6 or w3 series as outlined in Fig. 1. The ability to elongate and desaturate fatty acids is not the same in all species of fish. The turbot was able to desaturate and elongate only 1-15% of 18:1w9, 18:2w6 or 18:3w3, while in the rainbow trout, 70% of 18.3 w3 was converted to 22:6w3. Crustaceans also, in general, are unable to synthesize 18:2w6 and 18:3w3 and the rate of conversion of these fatty acids to higher fatty acids seems to be very low (Kanazawa, 1985).

The demonstrated or suggested requirements of a number of fishes and crustaceans of present or possible future aquaculture interest are presented in Table.

Essential fatty acids for finfish:

Rainbow trout, a cold-water fish, requires w3 fatty acids as EFA in the diet and the EFA requirement was met by 1% 18:3w3 in the diet, and no combination of 18:3w3 with 18:2w6 resulted in as fast a growth rate or efficient feed conversion ratio as 1% of 18:3w3 alone in the diet (Castell et al., 1972); Watanabe et al., 1974). Though inclusion of 18:2w6 in the diet did result in some improvement in growth and feed conversion compared with EFA deficient diets, the w6 fatty acids did not prevent some EFA deficiency symptoms such as the "Shock Syndrome" (Castell et al., 1972; Yu and Sinnhuber, 1975). In rainbow trout, dietary 18:2 w6 or 18:3 w3 were readily converted to C-20 and C-22 PUFA of the same series. Takeuchi and Watanabe (1977) found that either 20:5w3 or 22:6w3 or their mixture was superior to 18:3w3 in EFA value for rainbow trout.

In coho salmon (Oncorhynchus kisutch) the optimum level of dietary w3 fatty acids ranged from 1% to 2.5%, and dietary w6 fatty acids higher than 1% depressed the growth of the same salmonid. Chum salmon (Oncorhynchus keta) showed best weight gain and feed efficiency when offered a diet with 3% methyl laurate plus simultaneous supplements of 1% 18:2w6 and 1% 18:3w3 (Takeuchi et al., 1979).

Studies with carp (Cyprinus carpio) have shown that it requires 18:2w6 and 18:3w3 with best weight gain and feed conversion in fish receiving a diet with both 1% 18:2w6 and 1% 18:3w3 (Watanabe et al., 1975b; Takeuchi and Watanabe, 1977a).

The eel Anguilla japonica has also a requirement for both 18:2w6 and 18:3w3. Arai et al. (1971) found that a mixture of corn oil and codliver oil in the ratio 2:1 containing both 18:2w6 and 18:3w3 was the most favourable for growth of eels. Subsequently, Takeuchi et al. (1980) found that the eel required 18:2w6 and 18:3w3 in the same proportion as the carp, but at a level of 0.5% of each.

Takeuchi et al. (1979c and 1980b) investigated the need for EFA of chum salmon (Oncorhynchus keta) held in both freshwater and seawater and found that the requirement of chum salmon for EFA did not change according to their living environment. The best weight gain and feed efficiency were obtained in fish receiving the diet supplemented with both 1% 18:2w6 and 1% 18:3w3.

Kanazawa et al. (1980) examined the EFA requirement of Tilapia zilli, a herbivorous fish, which is able to live in both freshwater and seawater. In this species, the growth promoting effects of 18:2w6 and 20:4w6 were found to be superior to those of 18:3w3 and 20:5w3 indicating that this

fish requires w6 fatty acids rather than w3 fatty acids. The dietary requirement was 1% of either 18:2w6 or 20:4w6.

Significant variations exists among various fish species in their ability to elongate and desaturate dietary 18 carbon fatty acids to 20 or 22 carbon fatty acids. Several marine species appear to have lower enzymatic elongation-desaturation capabilities than freshwater fishes. Yamada et al. (1980) examined the possibility of bio-conversion of 18:3w3 into higher fatty acids by administering 18:3w3 (1-14C) to individuals of red sea-bream, black sea-bream (Mylio macrocephalus) opaleye - (Girella nigricans), striped mullet (Mugil cephalus) and rainbow trout, and found that only rainbow trout exhibited appreciable radioactivity in 22:6w3 of body lipids. Therefore, it was concluded that marine species have limited ability to elongate and de-saturate 18:3w3, resulting in dietary essentiality of eicosapentaenoic (20:5w3) or docosahexaenoic fatty acids (22:6w3). Kanazawa et al. (1979) showed that injections of 1-14C) 18:3w3 into rainbow trout resulted in biconversion to 20:5w3 and 22:6w3, demonstrating the non-essentiality of 20:5w3 and 22:5w3; whereas very low percent bio-conversion of 18:3w3 to 20:5w3 and 22:6w3 occurred in marine fish such as globefish (Fugu rubripes), Japanese eel, red sea bream, rockfish (Sebasticus marmoralis) and ayu (Plecoglossus altivelis.

Cowey et al. (1976) concluded that turbot (Scophthalmus maximus) lack the necessary microsomal desaturates to effectively convert 18:1w9, 18:2w6 or 18:3w3 into polyunsaturated fatty acids for deposition in neutral fats or phospholipids based upon growth and body composition. Yone and Fujii (1975) demonstrated that 18:3w3 is not of much importance per se for the marine fish such as red sea bream, black

sea bream, opal eye and yellow tail (Seriola quinqueradiata). Stickney and Andrews (1971) found that 18:2w6 has a repressive effect in the catfish, Ictalurus punctalis but inclusion of menhaden oil containing 20:5w3 and 22:6w3 had no detrimental effects.

Essential fatty acids for crustaceans:

Crustacean lipids have both saturated and unsaturated fatty acids, particularly greater percentage of w3 HUFA such as 20:5w3 and 22:6w3 (Gopakumar and Nair, 1975; Guary et al., 1976). Although essential fatty acids content of crustaceans is high, they are unable to synthesize these fatty acids from other saturated fatty acids (Kanazawa, 1985). Nutritional studies have demonstrated that crustaceans require essential fatty acids in their diets for normal survival and growth (Kanazawa et al., 1979). Kanazawa and Coworkers through radio active tracer experiments reported the absence of de novo synthesis of linoleic (18:2w6), linolenic acid (18:3w3), eicosapentaenic acid (20:5w3) and decosahexaenoic acid (22:6w3) from acetate or palmitic acid in the prawns, P. japonicus, P. monodon and P. merguiensis (Kanazawa et al., 1979). Similarly, Zandee (1967) reported the inability of the cray fish, Astacus astacus and the lobster, Homarus gammarus to synthesize PUFA. Recent studies have further shown that 18:2w6 and 18:3w3 are poorly converted into HUFA of the same series (Kanazawa et al., 1979). Bottino et al. (1980) reported that P. setiferus, P. aztecus and P. duorarum were unable to biosynthesize C20 and C22 PUFA from C18 fatty acid precursors in adequate levels.

Studies conducted at the Central Marine Fisheries Research Institute also clearly indicated the inability of Penaeus indicus larvae, post-larvae and juveniles to synthe-

size 18:2w6 or 18:3w3. However, slow rate of conversion of 18:2w6 and 18:3w3 to higher fatty acids was observed. Similarly Colvin (1976) suggested limited capacity for bioconversion of EFA to longer chain PUFA in P. indicus juveniles. In Penaeus indicus inclusion of 1% linoleic as well as 1% linolenic acids in the diets resulted in growth improvement over that of the diets deficient in these fatty acids. While more than 1% of these fatty acids had no beneficial effect, fatty acids levels above 3% were detrimental to prawns. Besides, linoleic acid is found to be inferior to that of linolenic acid in efficacy. Among diets tested with various types of lipids, the lipids containing a mixture of 18:2w6, 18:3w3, 20:5w3 and 22:6w3 along provided the best weight gain and food conversion in P. indicus, clearly indicating the essentiality of a blend of unsaturated fatty acids of the w3 and w6 series for proper survival growth, FCR, PER and retention of protein and lipids. Exclusion of polyunsaturated fatty acids from the diets of P. indicus juveniles severely affected the utilization of ingested food and protein, and protein deposition in the body. Besides, diets containing linoleic and linolenic acids alone were poorly accepted by P. indicus larvae and these diets when fed to larvae induced complete mortality. In P. japonicus also similar results have been reported by Kanazawa et al. (1985). In both the above species, it is well proved that lipids containing 20:5w3 and 22:6w3 fatty acids are essential for the normal growth and metamorphosis of larvae.

Hill and Holman (1980) suggested that in chronic malnutrition in which protein intake is usually low, the protein deficiency may increase the EFA requirement and precipitate marginal EFA deficiency. On the other hand Steffens and Albrecht (1973) found that some fatty acids when added to the food accelerated the growth and at the same time reduced the protein amount required for production of unit weight.

TABLE : ESSENTIAL FATTY ACID REQUIREMENTS OF FINFISH AND CRUSTACEANS

Species	Suggested EFA requirement	Reference
Chinook salmon	18:2w6 and 18:3w3	Nicolaides and Woodall (1962)
Rainbow trout	18:3w3- 1% in the diet	Castell et al.(1972)
Rainbow trout	18:3w3- 0.8 to 1.6%	Watanabe et al.(1974)
Rainbow trout	18:3w3 - 1% at a lipid level of 4% and 2% at a lipid level of 14%	Takeuchi and Watanabe (1977b)
Channel catfish (Ictalurus punctatus)	NO. EFA requirement demonstrated. High levels of 18:2w6 or 18:3w3 (10% safflower oil or linseed oil respectively) reduced growth	Stickney and Andrews (1971,72)
Common carp (Cyprinus carpio)	Mixture of 1% 18:2w6 and 1% 18:3w3 superior to either fatty acid fed separately. PUFA 20:5w3 and 22:6w3 more efficient than 18:2w6 and 18:3w3	Takeuchi and Watanabe (1977)
Chum salmon (Oncorhynchus keta)	18:2w6 and 18:3w3 at 1% each in the diet	
Eel (Anguilla japonica	Mixture of 0.5% 18:2w6 plus 0.5% 18:3w3 or 0.5% 20:5w3 and 22:6w3 PUFA	Takeuchi et al. (1980)
Tilapia zilli	1% of 18:2w6 or 20:4w6; both superior to 18:3w3	Kanazawa et al. (1980)

Species	Suggested EFA requirement	Reference
Marine Fish		
Yellow tail (Seriola quinqueradiata)	Fish oils superior to corn oil	Tsukahara et al. (1967)
Ayu (Plecoglossus altivelis)	PUFA - 20:5w3 and 22:6w3 superior to 18:3w3 superior to 18:2w6	Oka et al. (1980)
Turbot (Scophthalmus maximus L.)	0.6% w3 HUFA slightly superior to 3.7% 18:3w3. Both superior to 18:2w6	Leger et al.(1979)
	1% w3 HUFA required	Cowey et al.(1976)
Red sea bream (Chrysophrys major	2% w3 HUFA required	Fujii and Yone (1976)
Milkfish fry Chanos chanos	Require a mixture of marine fish oil and vegetable oil containing 18:2w6, 18:3w3, 20:5w3 and 22:6w3	CMFRI (unpublished data)
Crustaceans		
Lobster (Homarus americanus)	Adults - fish oil high in w3 PUFA superior to corn oil high in 18:2w6	Castell and Covey (1976)
	Juvenile lobsters have similar requirements	D'Abramo et al. (1980)
Shrimp		
Penaeus japonicus	1% w3 PUFA, 20:5w3 or 22:6w3 superior to 18:3w3	Kanazawa et al. (1979a, b)
	Addition of 18:3w3 or 18:2w6 to fish oil high in w3 PUFA resulted in growth enhancement	Kanazawa et al. (1979c)
Penaeus indicus juveniles	Fish oil w3 PUFA superior to 18:2w6 or 20:4w6 supplemented	Read (1981)

Species	Suggested EFA requirement	Reference
	alone. Fish oil plus both 18:3w3 and 18:2w6 superior to fish oil alone	
Penaeus indicus larvae	Incorporation of 18:2w6 or 18:3w3 or their mixture resulted in complete mortality of larvae. Diets containing a mixture of lipids of marine and plant oils containing 18:2w6, 18:3w3, 20:5w3, 22:6w3 essential for survival, growth and metamorphosis	Chandge (MS)
Post-larvae and juveniles	18:3w3 better than 18:2w6. A mixture of both the fatty acids are better than individual fatty acids. Best growth feed efficiency obtained only in diets with a mixture of plant and marine animal lipids	-do-
Penaeus azlicus	Supplementing commercial marine diet with 1% 18:3w3 improved growth	Sheedbart and Mies (1973)
Palaemon serratus	Requirement for both w6 and w3 fatty acids. 18:2w6/18:3w3 in the ratio 2.2 optimal 20:5w3 plus 22:6w3 superior to 18 carbon PUFA	Martin (1980)
Macrobrachium rosenbergii	Diet containing 3% shrimp head oil produced larger shrimp and after 12 weeks the total biomass produced was twice as high as the control. High w3:w6 diets are beneficial to carideans	Sandifer and Joseph (1976)

Species	Suggested EFA requirement	Reference
Crab		
Carcinus maenas	Fish oil superior to vegetable oil high in 18:2w6	Ponat and Adelung (1980)
Oyster		
Crassostrea virginica	Codliver oil with w3 highly unsaturated fatty acids promoted maximum growth	Trides and Castell (1979)

EFA DEFICIENCY SYMPTOMS RECORDED IN FINFISH AND CRUSTACEANS

Species	EFA Deficiency Symptoms	References
Finfish		
Chinook Salmon	Impaired pigmentation	Nicolaides and Woodall (1962)
Rainbow trout	Poor growth and feed conversion; shock syndrome; erosion of caudal fin; heart myopathy, swollen livers; altered permeability of biomembranes as exhibited by increased rate of swelling of isolated liver mitochondria; fatty degeneration of livers; decreased haemoglobin levels and decreased red blood cell volume; increase in *de novo* synthesis of 20:3w9 by rainbow trout	Castell *et al.* (1972 a,b,c)
Yellow tail	Growth, red blood cell counts, hematocrit and haemoglobin levels were affected by EFA deficiency	--
Common carp	Reduced growth and feed efficiency; increased production and incorporation of 20:3w9 into polar lipids	Takeuchi and Watanabe (1977)
Japanese eel	Same as in common carp	Takeuchi *et al.* (1980)
Crustaceans:		
Penaeus japonicus	Poor growth rate, feed efficiency and survival	Kanazawa *et al.* (1979c)

Species	EFA Deficiency Symptoms	References
Penaeus indicus larvae	Delayed larval metamorphosis and mortality	Chandge (MS)
Post-larvae and juveniles	Reduced growth; poor survival; poor food and protein conversion; poor protein retention	Chandge (MS)
American lobster	Reduced growth rate and feed efficiency; reduced blood cell numbers; serum protein levels, and percent edible meat; increased intermolt period; and reduced weight gain per moult	Castell and Covey (1976)

Effect of EFA Deficiency on spawning

The recent studies by Watanabe et al. (1978,1979) have demonstrated that EFA deficiency greatly affected the spawning of rainbow trout and red sea bream and that EFA play as important a role in reproductive physiology as focopherols (Watanabe et al., 1977) in fish as in higher animals.

The adult rainbow trout fed on the EFA deficient casein diet containing methyl laurate as the sole dietary lipid for 3 months before spawning matured, but the eggs produced had a low hatching rate. But addition of 1% ethyl lineleate in place of linolenate in the diet improved the egg condition (Yu et al., 1979). The results obtained in red sea bream fed the EFA deficient purified diet for 6 months before spawning indicated that the total egg production, proportion of eyed eggs and hatchability were significantly influenced by the EFA status in the diet and were quite low in the group given the EFA deficient diet. These low quality eggs showed abnormality in the number of oil globules, the average being seven whereas in normal eggs it is usually one. Almost all the fish larvae obtained from the group also showed various kinds of deformations in the body. Supplementation of cuttlefish liver oil high in w3 HUFA effectively prevented these pathologies.

PHOSPHOLIPIDS

Phospholipids have a transport and structural function in crustaceans (O'Connor and Gilbert, 1968). Phospholipids tend to be more unsaturated than neutral lipids due to their high content of polyunsaturated fatty acids. In view of their importance in the transport of lipids and as structural component of biomembranes, many studies have been

carried out in the phospholipid content and its composition in crustaceans. Gopakumar and Nair (1975) reported that phospholipid constitutes 62% of the total lipids in Penaeus indicus. Several other reports have also shown that phospholipids are the major lipids of crustaceans, such as the lobster Homarus americanus (Bligh and Scott, 1966), the crab Carcinus maenas and the prawn P. japonicus (Teshima and Kanazawa, 1978). In common with other life forms the major phospholipids in crustaceans are phosphatidylcholine and phosphatidylethanolamine which are important from nutritional point of view (Sargent, 1976).

Van Den Oord et al. (1964) and Testima and Kanazawa (1978a and b) suggested that crustacean phospholipids probably play important role in emulsification, absorption and interorgan transport of lipids. Lester et al. (1975) observed that lecithin enhanced cholesberol solubilization when associated with N-(N-dodecanosarcosyl) taurine (DST) a model of the type of detergents synthesized by crustaceans.

Kanazawa et al. (1979) found that inclusion of lecithin from the short-necked clam at 1% level in the purified diet of Penaeus japonicus had a growth promoting effect. Conklin et al. (1980) found that the inclusion of soy lecithin into purified diets fed to juvenile lobsters eliminated mortality associated with a 'moult-death syndrome'. Tridel and Castell (1980) found that survival of juvenile lobsters increased with increasing lecithin level in a casein based diet upto 4-6%.

Studies conducted in Penaeus indicus larvae, post-larvae and juveniles at CMFRI showed that inclusion of phospholipids promote growth, improve and food conversion in juveniles. It was also observed that inclusion of phospholipids at levels greater than 2% in the diet had no

significant beneficial effect. Lecithin deficiency induced high mortality in larvae during the metamorphosis and all the larvae died before reaching the post-larval stage. Inclusion of lecithin in the diet improved the efficiency of protein utilization. It is suggested that lecithin when included in the diet may provide choline, which acts as a methyl donor during trans-methylation reactions, thereby sparing the sulphur amino acid, methionine another methyl donor) for enhancement of protein synthesis. Thus lecithin inclusion in the diet improves protein retention (Chandge, MS).

CHOLESTEROL

The most abundant steroid in animal tissues is cholesterol (Lehninger, 1984). It occurs in the plasma membranes of many animal cells, in the lipoprotein of blood plasma and large quantities occur in the brain and nerve tissues (Lehninger, 1984). Vertebrates including fish are known to biosynthesize cholesterol from precursors such as acetate and mevalonate. But crustaceans do not have the ability to biosynthesize cholesterol (Zandee, 1964, 1966; Kanazawa et al., 1971 a; Teshima et al., 1983; D'Abramo et al., 1984). Therefore cholesterol should be included in their diet.

Functionally cholesterol together with phospholipids helps in transportation of lipids in body of animals. It is also a poor insulator against electrical discharge, especially in the brain, where it acts as insulator against nerve impulses which are electrical in character. Cholesterol is known to decrease the fluidity of artificial lipid bio-molecular leaflets (liposomes) by decreasing the surface area of the membrane (Van Deenen et al., 1972). It has also been demonstrated that diets supplemented with cholesterol can

alter the kinetic properties of enzymes in the erythrocyte membranes (Farias et al., 1975). In crustaceans cholesterol is also the precursor for various physiologically important compounds like steroid hormones, brain and moulting hormones and Vitamin D (Kanazawa et al., 1971; New, 1976). Sterols have also been found to be important components in the cellular and sub-cellular membranes particularly in the hypodermis in Arthropoda (Gilbert, 1969; New, 1976). About 0.5% cholesterol has been found to be sufficient in the diet of most crustaceans. In a series of studies Kanazawa et al. (1971 a, b, c) have shown that P. japonicus utilized cholesterol better than ergo-, stigma and B-sitosterol and that these sterols are transported after ingestion with cholesterol, and also that the spiny lobster is able to convert cholesterol into steroid hormones.

NEGATIVE ASPECTS OF LIPIDS

Rancidity

The requirement by fish for PUFA of the w3 series creates problems with respect to feed storage, since highly unsaturated fatty acids are very labile to oxidation. The products of lipid oxidation may react with other nutrients such as amino acids, vitamins and reduce their biological availability. The effect of oxidized lipids on dietary proteins, enzymes and amino acids have been demonstrated by Andrews et al. (1965) and Crawford et al. (1965) and many others. Fowler and Banks (1969) found that rancid herring and hake meals in fish feeds caused dark coloration, anemia, lethargy brown yellow pigmented livers, abnormal kidneys and some gill clubbing in Chinook Salmon. Addition of alpha-tocopherol rancid fish meals alleviated the symptoms. Sinnhuber et al. (1968) and Watanabe and

Hashimoto (1968) demonstrated the sparing effect of alpha-tocopherol in rancid rainbow trout and carp feeds. Thus, it is clear that when oils with high contents of PUFA are used antioxidants should be added.

Cyclopropenoic fatty acids

Cyclopropenoic fatty acids fond in cotton seed oil and the oil of other plants of the order Malvales, have been suspected to interfere with the fatty acid desaturase enzyme system as well as with normal lipid and protein metabolism. Fatty acid desaturases of fish are affected in the same way by the inhibitor sterculic acid as are free fatty acid desaturases of mammals. Sterculic acid is a cyclopropenoic fatty acid occurring naturally in triacylglycerols from plants of the order Malvales (Cowey and Sargent, 1979). Roehm et al. (1969) fed rainbow trout diets containing salmon oil supplemented with up to 200 ppm of sterculic acid. The inhibitor caused increased ratios of 16:0/16:1 and 18:0/18:1 as well as a decreased level of 22:6w3 in the trout. These changes were accompanied by liver pathology and a decreased growth rate during the initial stages of feeding.

Synthesis of saturated and Monoenoic fatty acids

Acetate

14:0	14:1w5	16:1w5
16:0	16:1w7	18:1w7
18:0	18:1w9	20:1w9
20:0	20:1w11	22:1w11
20:0	22:1w13	

Polyunsaturated fatty acids

18:1w9	18:2w6	18:3w3
20:1w9 18:2w9	20:2w6 18:3w6	20:3w3 18:4w3
20:2w9	20:3w6	20:4w3
20:3w9	22:3w6 20:4w6	22:4w3 20:5w3
	22:4w6	22:5w3
	22:5w6	22:6w3

Fig: 1. Flow diagram for fatty acid synthesis mechanism in fish (adapted from Castell, 1979).

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Technical Paper - 7

SUMMER INSTITUTE IN
RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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CAROTENOIDS AND THEIR IMPORTANCE IN THE NUTRITION OF FISH AND CRUSTACEANS

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Carotenoids are quantitatively very prominent among the substances which render colour - Biochrome. There are an immense array of carotenoids in the animal kingdom and so also in the plants. They are present predominantly in the eye spot of the protozoans, in the body of coelentrates, sponges and other organisms. The only clear cut physiological function they perform is as precursor for vitamin A. Apart from this though many a varied roles ascribed to them in the physiology of the organism are highly speculative in nature. We are at the dawn of revolutionary findings pertaining to the function of carotenoids.

BIOCHROMES

The following group of biochemics are collectively known as biochromes - 1. Carotenoids, 2. Quinones, 3. Flavonoids, 4. Flavins, 5. Tetrapyrroles, 6. Pterins and 7. Indole pigments. The first four are synthesised de novo only by plants of which the first three contain only carbon, hydrogen and oxygen while the four in addition nitrogen. Fifth and sixth also contain nitrogen and could be synthesised by both plants and animals. The last viz., indole pigments are melanins and indigoids which are synthesised by the

animals by catabolic degradation of essential amino acids tyrosine and tryptophan. Of these carotenoids are the most diverse and occur in very high quantity.

CAROTENOIDS

Carotenoids are fat soluble lipochromes and can be divided into two groups. 1. Carotenes, which are carotenoid hydrocarbons lacking in oxygen ie. are made of carbon and hydrogen only. 2. Xanthophylls, which are oxygen containing alcohol soluble, non-acidic, non-saponifiable carotene derivatives. The xanthophylls are classified as follows (Fox, 1976).

1. Carotenoid alcohols - carotenols
2. " ketones - carotenones
3. " alcohol ketones- carotenolones
4. aldehydes - carotenols
5. ethers - caroten - ethers
6. Esters of carotenoid alcohols - carotenol esters
7. Carotenoid acids and their esters.

In Xanthophylls suffix 'Xanthin' is added while for carotenes 'carotene'. Eg: Astaxanthin, β- carotene, tunicarotene.

Carotenoids are present in the organisms in two types of complexes, viz., carotenoproteins - i) pigment is bound to the protein in stoichiometric amounts in non-covalent linkages and ii) dissolved in lipoprotein or lipoglycoprotein component.

The central dogma of carotenoid biochemistry is that animals cannot synthesise the pigments de novo but can only alter the molecules by oxidation, as in the conversion of β-carotene into astaxanthin, or, if the structure is appropriate, by central fission to form vitamin A. Though

the first part of the dogma still holds for the latter part 2 new metabolic activities have come to our knowledge as exceptions. i) Conversion of β-rings into ε-rings in birds and ii) a change of chirality as in the formation of 3' - epilutein from lutein in fishes (Goodwin, 1984).

Though animals lack the capacity to synthesis carotenoids from protozoa to mammalia they contain varieties of carotenoids. It is of interest that both animals and plants put together have more xanthophylls, the marine and freshwater mud contain higher amounts of carotein than xanthophylls. Fox (1979) divides the organisms into the following 5 categories based on their carotenoid nutrition.

1. Carotene selectors: These animals when provided with both forms of carotenoids are able to assimilate hydrocarbon type of carotenes, while defaecate xanthophylls completely. Typical examples are horse and cattle. The marine detritus eating polychaete Euzonus mucronata assimilate and store β-carotene only. The parasitic crustacean Sacculina carcina too assimilate and store in considerable quantity only β- carotene (Fox, 1979).

2. Carotenoid rejectors: There are also animals which assimilate little of carotenoids except for visual pigments from carotenoid rich diets. The examples are sheep, goat, swine and also most strains of rabbits. The hag fish (cyclostome) Eptatretus stoutii, the mako shark Isurus glaucus and the chimaerid Hydrolagus sp. are also carotenoid rejectors.

Xanthophyll accumulators: These organisms store no carotenes but assimilate alcoholic, hydroxylated and ketonic carotenoids. They convert only a little carotene ingested into vitamin A, just sufficient for their needs. Otherwise dispose off all carotenes in faeces. The examples

are domestic fowl, most of the fishes and many invertebrates.

Non-selectors: These organisms readily assimilate and deposit both carotenes and xanthophylls in the body tissues. Examples are man, frog and octopus.

Carotenoid innovators: These assimilate varied kind of carotenoids, oxidises them to yield derivatives. Thus β-carotene could be converted into mono - or dihydroxy-alcohols (Eg: cryptoxanthin, zeaxanthin) or into mono - or dihydroxy - diketo compounds (Eg: astaxanthin).

Many echinoderms, crustaceans and a few fishes come under this category.

Crustaceans:

Artemia salina, the brine shrimp can convert β- carotene into echinenone and canthaxanthin. Penaeus japonicus and Metapenaeus barbata oxidise β-carotene to astaxanthin (Fox, 1979). In most crustaceans free astaxanthin forms the bulk of the pigment in carapace. The pigments are transferred from epidermis during moulting to the carapace (Goodwin, 1984). It is observed that during intermoult stages the carotenoid content in the hepato-pancreas is very high (unpublished data). In hepato-pancreas the conversion of carotenoids take place. Some crustaceans - Penaeus japonicus - can metabolise β-carotene to astaxanthin while still others - Panulirus japonicus, Portunus trituberculatus and Pagurus prideauxi - can meta-bolise upto exhinenone (Fig. 1 and 2). The pigments are transported from hepatopancreas to epidermis through haemolymph while the transfer from gut to hepatopancreas is mediated by a carrier protein, structurally similar to apoprotein of α- crustacyanin (Goodwin, 1984).

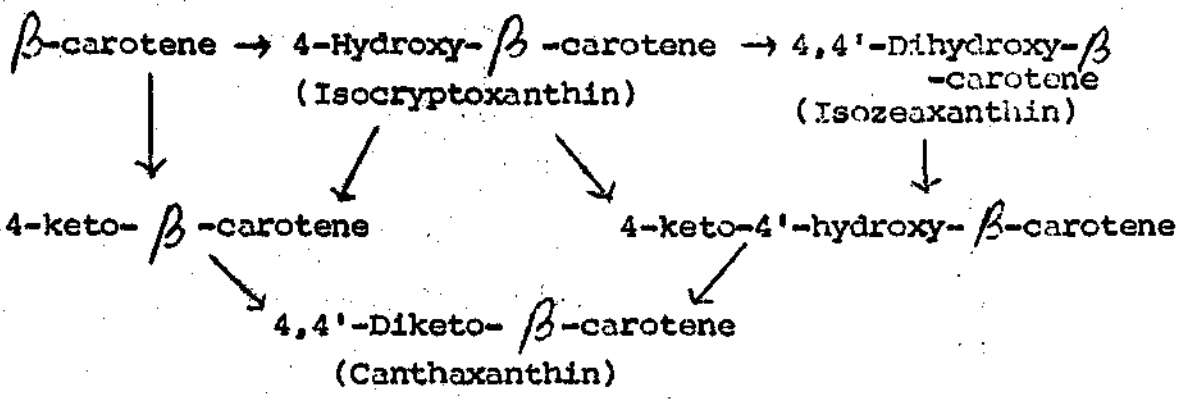

Figure - 1: β carotene to echinone and canthaxanthin synthesis is crustaceans (Goodwin, 1934).

The carotenoid concentrations within the crustacean can vary very much. In Sergestes corniculum carotenoids are very high in the carapace and hypodermis while very little is present in the eyes. In some euphausids about 94% of the total carotenoids occur in the eyes. In Oplophorous spinosus the eggs contain 2447 µg/g carotenoids while the adults only 244 µg/g. In Pandalus borealis and Penaeus japonicus β carotene is high in the hepatopancreas while astaxanthin is abundant in the carapace.

Amino acids: It is of interest that Otazu-Ahrill, et al. (1982) have found that purified amino acids in the diet especially methionine (at 8% level) can elevate carotenoid levels in Palaemon serratus. Methionine and isoleucine pair produced stricking increase in carotenoids. However too high addition of free amino acids is the food reduced the pigment content.

Eyestalk secretions: Ablation of eye-stalks in Rithoropanopeus harrisii during development of ovaries resulted in increase in ovarian weight with accumulation of carotenoids, while if ablated during the resting period, [illegible] ovarian weight increased but not the carotenoid content [illegible]. Removal of eyestalk also seems to have a bearing on chromatophores

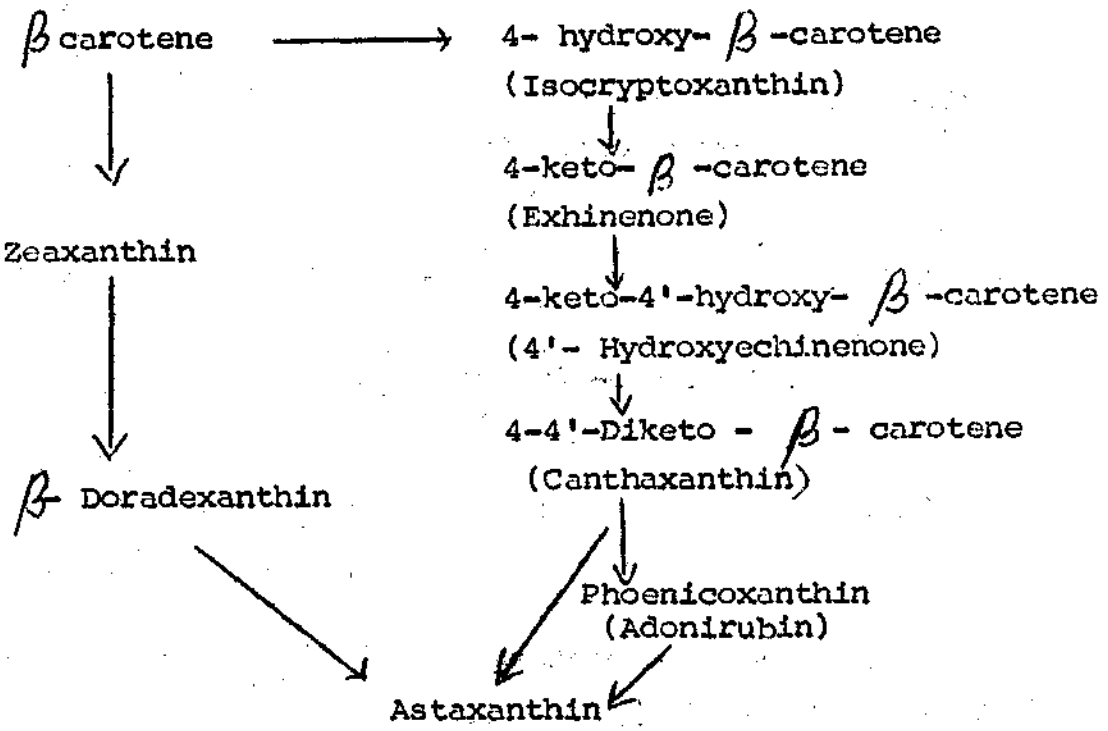

Figure- 2: Metabolic pathway from β -carotene to Astaxanthin in crustaceans (Goodwinn, 1984)

and colour change. In Palaemon serratus pigment granules are reduced with the loss of xanthophyll metabolism. In Uca pugilator bilateral removal results in loss of mobilisation of carotenoids from the hepatopancreas. Bilateral removal lowered the amounts of esterified axtaxanthin in Dardanus arrosor while in Macrobrachium rosenbergii increase in both free and esterified astaxanthin was observed.

FISHES

Carotenoids have been located in skin, muscle, liver, egg, sperm and in buccal mucosa of fishes. It is difficult to give authoritative values for carotenoids in fishes, since carotenoid content is influenced by dietary availability. In salmon Oncorhynchus nerka is the male during spawning 75% of the carotenoids are in the skin while during pre-spawning 98% is concentrated in the muscle. In the testis

it is nil. In the female during spawning 85% are in the ovaries, 14% in the skin and 5% in the muscle. During prespawning 90% are in the muscle, 1% in the skin and little in the ovaries. Thus mobilisation of carotenoids vary ... between sexes and with reproductive cycle. In fishes too as given in the figures 1 & 2 both metabolic pathways are evident. Much ... of the information on this is based on reasonable evidences, yet intermediary links and mediating enzymes are to be studied (Goodwin, 1984).

FUNCTIONS ASCRIBED

The only major function known is the role of carotenoids in the synthesis of vitamin A. β-carotene by breaking at 15-carbon can give rise to two molecules of vitamin A. α-carotene, β-cryptoxanthin and β-apocarotene - 8'-al are active retinol precursors. Thus the two essentials to be a precursor for vitamin A seems i) an unsubstituted β-ring and ii) a side chain of conjugated double bonds at least as long as that in retinol. Zeaxanthin, lutein and β-carotene are all inactive (Goodwin, 1984). In freshwater fishes desaturation at C-3 does not destroy activity which is the characteristic of vitamin A_2 (dehydroretinol). But saturation of a double bond in the side chain or removal of a methyl group (eg. at C-13) entirely eliminated the vitamin activity. The usual form of natural carotenoids is all - transform, though some Cis-isomers do exist. The pro-γ-carotene though being a cis-isomer has potency equal to all-trans-γ-carotene, because one half contain all the structural requirements of vitamin A precursor (Goodwin, 1984).

All other functions proposed are just hypothesis only. Goodwin (1984) catalogues the following as the supposed functions of carotenoids in crustaceans, perception

of light, electron acceptor, protective-chromatic adaptation, protect eggs from solar radiation, high temperature, reducing reflectivity, masking luminescence of prey in stomach, and protecting gut wall against digestive enzymes - stabilisation of proteins, stabilisation of chitin, transfer of carotenoprotein pigments, reproduction, and chemo-reception in antennae.

Tacon (1981) speculate the following as the possible function of carotenoids in fishes. 1. Antioxidant - β carotene is 50 times faster as oxygen quencher than α- tocopherol. 2. Fishes are capable of storing and modifying carotenoids to suit their requirements, whereby indicating some vital necessity for carotenoids other than vitamin-A precursor. 3. Egg astaxanthin in Salmo gairdneri enhances chemotaxis of spermatozoa. 4. In Cyclopterus lumpus there is intensive expenditure of carotenoids in yolk sac also in Salmo trutta. 5. When canthaxanthin is supplemented in the diet of Salmo gairdneri resulted in enhancement of growth and maturation rate. 6. Degree of pigmentation is related to tolerance to various stringent environmental conditions - low oxygen level, elevated temperature and ammonia levels. 7. Carotenoids may be respiratory in cellular level under adverse oxygen conditions. Eggs deposited in places of low oxygen and animals which live in low oxygen localities are more highly pigmented. 8. Though the role of carotene in gametogenesis is not clearly known in animals, in certain filamentous fungi (phycomycetes) β-carotene serves as a precursor to sex hormone trisporic acid which controls gametogenesis. 9. In fishes it is most likely they are mainly antioxidents since their association with high lipid content site make one to speculate so.

QUINONES

Though the carotenoids are the subject of discussion it is better that a very brief mention is made of other biochromes. Of the quinones, ubiquinones (Q - coenzymes) belonging to benzoquinone series serve as biocatalysts in cellular respiration. Eventhough they occur almost in all the animals, always present in very low quantities.

In the skeleton of the echinoderms naphthoquinones pigments are present but their physiological role is not established. Vitamins K_1 and K_2, prothrombin and anti-haemorrhagic factors are also related to naphthoquinones.

Anthraquinones are predominently present in the scale insects. (Fox, 1979)

FLAVONOIDS

Flavonoides are less common than the former. These could have a role similar to that of vitamin P. Rutin has been found to accelerate the healing from severe X-ray burns and prevents capillary fragility. In the animals subjects to irradiation calcium flavonoids decreased haemorrhagic lessions and mortality (Fox, 1979).

FLAVINS

Riboflavin is the most stricking example under this group of biochromes. Riboflavin could be sunthesised by the plants and specially young and tender parts are rich. Riboflavin is greenish - fluorescing, water soluble, yellow (when oxidised), heterocyclic compound and serves as a reversible hydrogen acceptor in the redox systems. (Fox, 1979)

TETRAPYRROLES

Porphyrins and Bilichromes belong to this group. Chlorophylls and haem are porphyrins. Cyanonocobalamin (B_{12}) is a tetrapyrrole. Bilirubin and the allied bile salts and the blood pigment biliverdin are the examples of bilichromes. (Fox, 1979)

PTERINS

These are polycylic nitrogenous compounds. Fluorescyanin present in fish scales are found to have physiological properties like accelerating oxygen consumption like those of vitamin B_1. Xanthopterin is found to stimulate normal cell division but suppresses tumorous growth and also functions as anti-anaemic. (Fox, 1979)

INDOLE

The melanins, which are black or brown coloured substances common on the skin, fur, scale and feathers. The ink of cephalopods are melanins. Melanins are synthesised by the organism from the amino acid tyrosine while indigoids from oxidation of tryptophan. (Fox, 1979)

In aquaculture, by tactful addition of specific biochromes the shell/skin/meat colour of the cultured organism can be modified to suit the aesthetic taste of the consumer market.

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Technical paper - 8

SUMMER INSTITUTE IN
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CARBOHYDRATE REQUIREMENTS OF FINFISH AND CRUSTACEANS

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INTRODUCTION

Nutrition supplies the raw materials for the maintenance of life. Some materials are used for the formation of body tissues (anabolism) and some for the production of energy (catabolism). Foods may be classified as energy and growing foods carbohydrates (CHO), fats and proteins and non energy foods (Minerals, vitamins, water and oxygen). Nutrition of fish has received attention for many years. The nutritional value of a diet is measured by the presence of necessary elements and catalysts an abundant supply of the anxillary foods and a proper balance between the energy and growing foods. A proper balance between the energy and growth foods assures an adequate supply of both energy and raw materials for optimum anabolic activity which in addition to growth includes tissue repair, reproduction and the formation of essential body products.

Phillips et al. (1948a) reported for trout the digestion of carbohydrate that of glucose 99%, maltose 92%, Sucrose 73%, Lactose 60% and Starch 57% and Raw starch 38%. They also reported that the levels in the diet under their experimental conditions was limited to 12% digestible carbohydrate because the additional amounts caused a deposition of excess of liver glycogen. It was suggested that higher

levels of carbohydrates was unable to utilise by the troup physiologically. Buhler and Halver (1961) found that chinook salmon tolerated relatively high levels of dietary carbohydrates without the development of abnormal conditions. Schaeperclaus (1933) suggested carbohydrate as a source of energy for carp and reported digestibility of from 30 to 92%. Philips et al. (1966, 1967) showed that carbohydrates were utilized for energy by trout and thus spared protein for protein purpose in the body. Kitamikado et al. (1965b) found that large amounts of starch in the diet of rainbow trout decrease the digestion of protein and therefore decreased the amount available for metabolism. The carbohydrates are stored as immediate reserve energy by showing increased liver and muscle glycogen. However the reported studies shows that there was increased body fat after feeding surplus carbohydrate. The carbohydrate absorbed by trout assumed that the digestive enzymes sucrase, maltase, lactase and amylase are present in the tract. The maltase activity was higher than sucrase and lactases.

McGeachin and Debnam (1960) demonstrated a relatively high amylase activity in the digestive trace of number of fresh water fishes. Kitamikado and Tachino (1961a) found an amylase activity in the digestive tract of rainbow trout that was less than that of carp but more than that of eel, reflecting differences in the feeding habits between fish species. Kenyon (1925) found a similar relationship in an abundance of the starch-digesting enzyme amylase was present in the intestinal mucosa of carp but almost none in Pickerel. Fish (1962) correlated the digestive enzymes with normal diet. In predominantly herbivorous Tilapia, amylase activity was distributed throughout the gastrointestinal tract, but carnivorous perch the pancrease was the only source of amylase. The inability of fish to mobilise liver glycogen

rapidly under circumstances such as starvation suggests either a paucity of phosphorylase or that metabolic and hormonal factors restrict the activity of the enzyme. Nagai and Ikeda (1972) experimented carp subjected to either regain oxidized glutamate rapidly and glucose slowly, deduced that aminoacids are a superior energy sources to glucose for carp and that energy utilization in the fish resembles that in a diabetic mammal.

Singh and Nose (1967) reported the carbohydrate digestibility was measured in young rainbow trout using the indirect method i.e. reference to the concentration of carbohydrate in diet. Glucose, sucrose, lactose were easily digested and absorbed and their digestibility remained nearly constant regardless of the level in diet. Dextrin and potato a starch were far inferior to the above mentioned sugars in their digestibility, the rate being 77 and 69% respectively at 20% carbohydrate levels in diet and their digestibility had a negative correlation to the concentration of starch in diet.

Nagai and Ikeda (1971) have shown for the carps that under starvation the conversion of lipid to glycogen in hepatopancreas and temporary increase of the blood, glucose levels were found at the same time. During breeding the carbohydrate content in diet increased or protein decreased, glycogen in hepatopancreas increased while lipid in hepatopancreas and blood glucose level decreased. This suggests that carp unlike in mammals that carbohydrate is hardly converted to lipid but that protein is principally converted to lipid where as carbohydrate does not precede either protein or lipid as energy sources but certain amount of it is necessary for the activity. They also showed by conducting experiments by using lablend carbon (^{14}C) and found that Glucose 6 - ^{14}C was incorporated in to glycogen in all

groups but oxidised to $^{14}CO2$. When more than 50% of protein was contained in diet, oxidation of glucose 6.^{14}C decreased remarkably and at the same time blood glucose and extent of randomization of ^{14}C in glucose was increased. From these observations it was suggested that carp possesses the active and reversible Embdon-Meyerhof path way but that glycogen is not a principal storage depot of energy.

Carbohydrate levels of about 25% in the diet is as effective as fat for the survival of channel catfish, rainbow trout and Plaice Singh and Nose (1967) explained high levels of starch in a sharp fall in digestibility for the thus species. Chion and Ogino (1957) has demonstrated 85% ingested starch was digested by the species 18-48% only. Cellulose activity was demonstrated by Sticknery and Shunaway (1974) in the digestive tracts of several finfish species of estuarine forms. These cellulose activity was associated with the micro-flora in the alimentary tract. The hepatic hexokinase isoenzymes of six species of fin were studied by Nagayama and Ohshima (1974) but no isoenzyme which resembled mammalian glucokinase was detected. Glucose dehydrogenase activity in liver of carnivorous fish is said to be four to seven times that in mammalian livers, whereas the activity in herbivorous fishes is similar to that of mammals (Nagayama et al., 1973). Hexokinase activity in different organs from various species of fish has been measured by several workers. Hexokinase (+ glucokinase) activity in rat liver is about 10 fold higher than in the liver of fish that in rate kidney about 3 fold higher than in fish kidney. This appears one of the prime reasons for the inability of fish to metabolize glucose rapidly.

Hexokinase activity in tissues of fish and rat

	Liver	Heart	Kidney	Muscle
Grass carp	0.28	3.58	0.99	0.19
Carp	0.29	1.99	0.88	0.29
Rainbow trout	0.52	1.45	0.99	0.19
Eel	0.25	3.14	0.94	0.17
Rat	2.5	6.1	2.8	2.0

Nagayama et al. (1972) have attempted to assess the relative ability (Latest activity) of four species of fish to metabolise glucose on the basis of the total hepatic activity of certain enzymes in fish of unit weight. On this criterian species rated in the order grass carp, eels, carp and rainbow trout. Fish do not mobilize liver glycogen rapidly when they are starved Nagai and Ikeda (1971a) found that blood glucose and liver glycogen levels of carp which have been starved for 22 days were not significantly different from those of carp which had been given diet varying markedly in gross composition. Even after 100 days without food appreciable amount of glycogen (1.5%) remained in carp liver. This phenomenon is common to other species such as European and Japanese eels.

The carbohydrate and protein interaction was found to be 45-40% respectively (Kandasami et al., unpublished) for mullets (Liza macrolepis).

CARBOHYDRATE REQUIREMENTS OF CRUSTACEA

Carbohydrates digestion in crustacea has been demonstrated by Kooiman (1964) and presence of carbohydrates including amylase, maltase, saccharase, chitinase and cellulase shown later may be from the bacteria in the

intestine. Metabolically carbohydrates are important in Kerbs cycle, in glycogen storage in chitin synthesis and in the formation of steroids and fatty acid. Wheat starch, dextrin and oyster glycogen are completely assimilated by Palaemon serratus (Forster and Gabboh, 1971) potato starch whether cooked or uncooked is less digested. Addition of 20% glucose to a menhaden based diet for Penaeus oztecus reduced growth rate (Andrews et al., 1972). It was postulated that dietary glucose was rapidly but inefficiently utilised for energy metabolism, whereas the glucose from digested poly saccharides was absorbed more slowly and effectively. Inclusion of 40% corn starch in casein based diet for Penaeus duorarum produced faster growth than 10 or 0% starch (Sick and Andrews, 1973). Deshimaru and Kurski (1974 a) obtained increased growth rate with P. japonicus though 6% dextrin inclusion in a casein-based diet. 6 and 12% addition of glycogen had a proportionately similar effect whether in the presence of dietary lipids or not.

While shrimp can utilize CHO efficiency varies according to sources. Partial digestion of cellulose takes place and extracellular chitinase enable digestion of chitin from dietary sources or from cast exuvial. The ability to digest specific sources of CHO varies between species. Starch seems to be more efficiently utilised than glucose.

The role of CHO and of its sources will become more important as dietary protein levels are lowered. Rahman, Kanazawa and Teshima (1979) have studied the effect of dietary CHO on the body weight gain, survival, PER and the hepatopancreatic glycogen and serum glucose levels of the prawn P. japonicus. The highest weight gain was obtained in the diet contained 19.5% maltose as CHO source. Growth of prawn was poor in high levels of monosaccharids, glucose or

galactose as compared i.e. maltose and the polysaccharids soluble starch, potato starch, dextrin and glycogen. Feeding with diet containing glucose or galactose for 30 days resulted in high glycogen level in hepatopancreas. Also the serum glucose level increase quickly after oral administration of glucose and remained at high after 24 hours whereas after the administration of di and poly saccharids it increased maximum after 3 h and then decreased to the pre-test levels.

Deshimaru and Yone (1978) have studied the carbohydrate requirements of the prawns P. japonicus and observed that the poorest feed efficiency and high mortality were found with the group fed on diet containing glucose. Also the diets containing starch and dextrin produced high mortality. On the other hand the two groups maintained on the diets with glycogen and sucrose had a low mortality. From this it was concluded that sucrose and glycogen are desirable sources of dietary CHO for the prawn while glucose is unsuitable.

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MINERAL REQUIREMENTS OF FINFISH AND SHELLFISH

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INTRODUCTION

In animal nutrition, while protein, lipid and carbohydrate are required in major quantities, vitamins and minerals are required in small quantities in the diet. Though minor in nature, the mineral nutrition is no less important. Minerals are very essential because animals are not capable of synthesising them and should be supplied through external source. Many of the minerals are vital for healthy growth of the animals and their prolonged non-availability, either through diet or through environment, may cause irrecoverable deficiency disease. On one hand mineral elements are basic units of skeletal structures of the animals on the other hand mineral ions are important co-factors of enzymes and other biological chemicals involved in life process. Minerals are of paramount importance for aquatic animals like fish and shellfish since these animals need to keep their osmotic balance through mineral ions. Mineral elements required by fish may be classified as bulk elements such as calcium, phosphorous, potassium, chlorine, sodium and magnesium (these are required comparitively in large amounts) and trace elements which are copper, cobalt, iron, iodine, manganese, selenium, zinc, aluminum, chromium and vanadium (these are required in very small quantities).

MINERAL REQUIREMENT STUDIES

Experiments to study mineral requirements are rather difficult to conduct, mainly because it is not easy to prepare diets with limiting concentrations of each mineral. Secondly feeding experiments with the animals should be of very long durations, in order to observe the manifestations of mineral requirements, deficiency and excesses. It is much more complicated in the case of aquatic animals since water is the largest contributor of a variety of mineral elements. Further there is possibility of interactions between minerals which may create complications in assessing the dietary requirements. The interactions may be either antagonism or synergism. For example large amounts of calcium in the diet may lower the availability of zinc present in the diet. Calcium deposition occurs in fish which is deficient in magnesium. Minerals have a role in many facets of metabolism such as hormones, respiratory pigments, structural elements, high-energy bonds and enzyme co-factors. Thus studies on mineral requirements, assume greater importance in fish and shellfish nutrition.

MINERAL REQUIREMENTS OF FINFISH

Among the aquatic animals, the mineral nutrition of finfish has been studied in greater detail. Fresh water fish show higher requirement of most of the minerals compared to the marine fish. The later group are capable of absorbing some of the mineral elements present in salt water. After detailed experiments, it was shown that red sea bream need only iron, potassium and phosphorous in their diet, the remainder of the elements coming from the external environment. Many of the mineral requirement studies are therefore directed on the fresh water fish species. The mineral

elements calcium, phosphorous, sodium molybdenum, chlorine, magnesium, iron, selemium, iodine, manganese, copper, cobalt and zinc are recognised as essential for body functions in fish. Fluorine and chromium have also been added of late to the list of essential elements.

Calcium and phosphorous

Calcium and phosphorous are closely related in metabolism and are discussed together in fish nutrition. Major portion of calcium (99%) and phosphorous (80%) are found in bones, teeth and scales. The ratio of calcium and phosphorous in bone ash is found to be approximately 2:1.

The extra skeletal calcium is widely distributed throughout the organs and tissues and exists in diffusable and non-diffusable form. Non-diffusable form is bound to proteins and the diffusable form which is generally found as phosphate, plays a significant role in the nutrition of animal. Ionised calcium in the extracellular fluids and in the circulatory system participate in muscular activity and osmoregulation.

Extra skeletal phosphorous is present mostly in combination with proteins, lipids, sugars, nucleic acids and other organic compounds. In some species the skin also appears to be an important repository for dietary phosphorous.

As stated earlier, fish are capable of extracting calcium directly from the surrounding water through gills. On the other hand the absorption of phosphorous is negligible from the environment, and the fish mainly depend upon the dietary sources of phosphorous.

Calcium and Phosphorous are absorbed in fish in the upper gastro-intestinal tract. While calcium is rapidly deposited as calcium salts in the skeleton, phosphorous is

distributed to all the major tissues. Water temperature influences the absorption of phosphorous and increases with increase in temperature. Higher content of glucose in the diet was found increase the absorption of dietary phosphorous by the fish. Absorption and retention of calcium are not influenced by any such external factors. The level of phosphorous in the diet influences the calcium retention in the body. Higher levels of dietary phosphorous enhances the retention of calcium to maintain the ratio between the two elements in the body.

The dietary phosphorous requirement of fish are as follows:-

Fish	Requirement
Atlantic salmon	0.7%
Channel catfish	0.4 - 0.47%
Common carp	0.6 - 0.7%
Red sea bream	0.68%
Rainbow trout	0.7 - 0.8%

Thus in general the phosphorous requirement in the diet of fresh water and marine finfish is almost same. Dietary calcium levels for fish are not recorded due to the absorption of this element from the environment by most of the fish species.

Many ingredients used for feed making are rich in calcium and phosphorous. Fish meal contains rich amounts of both the minerals. Calcium is generally deficient in plant ingredients and the phosphorous present in them is not available to the fish especially when the plant materials contain phytin or phytic acid. Phosphorous is readily available to the animals if potassium or sodium hydrogen phosphate or mono calcium phosphate is used in the diet. On the other hand phosphorous from tricalcium phosphate is not readily

available. Fish meal is rich in tri-calcium phosphate. Due to its poor availability to fish, it is released into to the water through faeces. This can combine with ammonia in water and lead to extensive eutrophication in the ponds. Deficiency symptoms of calcium are not described in fish. Poor growth, reduced feed efficiency, low bone ash and low haematocrit levels were observed in channel catfish fed phosphorous deficient diet. Prolonged feeding of phosphorous deficient diet has resulted in lordosis and abnormal calcification of bones (brittle structure) in common carp. The symptoms were similar in red sea bream.

Magnesium

About 60% of the body magnesium is found in skeletal structure and the remaining is distributed throughout the organic and muscle tissues. It is an important enzyme co-factor and component of cell membranes.

Marine fish are capable of extracting magnesium from the environment. Since this element is very limited in fresh water, the fresh water species seem to depend upon dietary source of magnesium. The dietary requirement of magnesium for rainbow trout is 0.06-0.07% and for carp it is 0.04-0.05%. Most of the compounded feeds prepared with ingredients of animal or plant origin have adequate levels of magnesium.

Apart from the general symptoms (reduced growth and poor food conversion efficiency) magnesium deficiency in rainbow trout leads to renial calcinosis and a flaccidity of the muscle due to increase in the extra cellular fluid volume. Loss of appetite, sluggishness and convulsion followed by tetany were also observed in common carp and rainbow trout fed magnesium deficient diets.

Zinc

Zinc is an important co-factor of many enzymes like carboxy peptidase, dismutase and superoxide in the animals. Many metabolic functions are effected by its deficiency. In rain bow trout, zinc requirements are normally met by dietary levels of 15-30 mg/kg. Large amounts of calcium present in the diet appears to lower the availability of zinc to the animal due to antagonism. Calcium and zinc perhaps compete for the same binding sites of protein or have same metabolic pathway and absorption mechanism. Zinc sulphate ($ZnSO_4$) seems to be the best source of zinc in the diet.

Deficiency of zinc was found to cause 'dwarfism' and cataract of the eye in rain bow trout. However several hundred mg of Zn per kg of diet do not appear to be injurious to rainbow trout.

Copper

Different dietary levels of copper have no influence on the growth of rainbow trout. Copper when included in the diet of common carp fingerlings at 0.7 mg/kg diet, the growth was low. The growth was better with the diet containing 3.0 mg copper per kg diet. Dietary copper level for channel catfish does not appear to be more than 1.5 mg/kg dry diet. Copper concentrations of 20-30 mg/kg diet, very much reduced the growth of catfish.

Iron

Iron deficiency in the diet of red sea bream resulted in a form of microcytic, hypochromic anaemia similar to iron deficiency in land animals. Common carp fed a semi purified diet with iron grew normally but exhibited sub-clinical symptoms of hypochromic and microcytic anaemia. Iron

concentration of 150 mg/kg diet was found to be required at minimum level, to prevent anaemia in red sea bream and common carp.

Manganese

Manganese deficiency in rainbow trout gives rise to abnormal curvature of the back bone and mal formation of the tail. Manganese content of 12-13 mg/kg diet, produced higher growth rates in rainbow trout and carp. Manganese sulphate ($MnSO_4$) and Manganese chloride ($MnCl_2$) are found to be good sources of manganese in fish diets.

Selenium

Selenium is a component of metallo-enzymes glutathione peroxidase. It plays an important role in the antioxidant defence mechanisms of the fish. It functions synergestically with Vitamin E. Deficiency of selenium leads to rapid onset of muscular dystrophy and exerdative diathesis. Maximum glutathione peroxidase activity was observed at dietary selenium levels of 0.15 and 0.38 mg/kg diet. At 13 mg/kg diet it was found to be toxic, causing uncoordinated spiral swimming behaviour leading to mortality in fish.

Iodine

Iodine deficiency produces goitrous condition in trout. A dietary requirement of 1.1 mg iodide/kg is recommended in the diet of chinook salmon.

The role of other trace elements in fish are not clarified. The requirements may be similar to those described for land animals.

A summary of the mineral requirements of different fish and their deficiency symptoms are given in Annexure - I.

Mineral mixtures used in the standard fish diet are given below.

I. Mineral Mixture (USP XII No. 2) (Halver 440)

Calcium phosphate (g)	13.58
Calcium lactate	32.70
Ferric citrate	2.97
Magnesium sulphate	13.20
Potassium phosphate (dibasic)	23.98
Sodium biphosphate	8.72
Sodium chloride	4.35
Aluminum chloride	0.015
Zinc sulphate	0.300
Copper chloride	0.010
Manganese sulphate	0.080
Potassium iodide	0.015
Cobalt chloride	0.100

Ogino salt Mixture for Fish

NaCl (g)	1.0
MgSO4 "	15.0
NaH2 PO4	25.0
KH2 PO4	32.0
Ca(H2 PO4)2	20.0
Ferric citrate	2.5
Calcium lactate	3.5
Trace elements*	3.5
Trace elements	1.0
	100.0

* composition of trace elements

ZnSO4	7H2o (g)	35.3
MnSO4	4H2o (g)	16.2
CuSO4	"	3.1
COCl2	"	0.1
KIO3	"	0.3
Cellulose	"	45.0
		100.0

MINERAL REQUIREMENTS OF SHELLFISH

Mineral requirements of cultivable species of prawns are studied to some extent among the shellfish. Penaeid prawns were found to require calcium and phosphorous in their diets. Best growth was obtained when the diets of the prawn Penaeus japonicus diets were supplemented with 1.04% of phosphorous and 1.24% of calcium. It was indicated that the calcium and phosphorous ratio in the diet of prawn should be 1.2:1. When this ratio was increased to 2:1 growth was inhibited and pigmentation decreased. Through studies with radio activity labelled calcium (45 Ca), it was demonstrated that prawns could absorb calcium from the surrounding water. It was estimated in P. japonicus that approximately 0.83 mg of calcium is absorbed per day per gram of body weight from the environment, when the sea water contained 0.44 mg/ml of calcium. This had lead to the conclusion that the calcium requirement could be satisfied by the calcium in surrounding seawater.

Supplementation of the diet with calcium (2%), magnesium (0.3%) and iron (0.02%) did not improve the nutritive value of the diet P. japonicus. Iron in the diet rather reduced the growth. Phosphorous at 2% level in the diet effectively improved the performance of the diet.

Potassium at 1% level in the diet showed higher growth and feed efficiency.

The requirement of individual trace elements was not studied. However when a mixture of trace elements, consisting of aluminum chloride (5 mg), Zinc sulphate (90 mg), manganese sulphate (20 mg), copper chloride (5 mg), potassium iodide (5 mg) and cobalt chloride was added to the diet at 0.2% level, enhanced the efficiency of the feed; higher level of this mixture above 0.2% in diet lowered the growth.

Studies in detail, on the requirement of each individual mineral element are needed to understand thoroughly the deficiency and excess symptoms of different minerals which are not available at present. However nutritionists have been adding mineral mixtures to their diet formulations. The mineral mixture used in the diet of P. japonicus is given below.

Mineral mixture used in prawn diet per 100 g.

	(g)
K2HPO4	2.0
Ca3 (PO@)2	2.72
NaH2 PO4 2.H2O	0.79
Mg SO4	3.02
FeSO4. 7H2O	0.015
MnSO4. 5H2O	0.004
	8.549

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Technical Paper-9.

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MINERAL REQUIREMENTS OF FINFISH AND SHELLFISH

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INTRODUCTION

In animal nutrition, while protein, lipid and carbohydrate are required in major quantities, vitamins and minerals are required in small quantities in the diet. Though minor in nature, the mineral nutrition is no less important. Minerals are very essential because animals are not capable of synthesising them and should be supplied through external source. Many of the minerals are vital for healthy growth of the animals and their prolonged non-availability, either through diet or through environment, may cause irrecoverable deficiency disease. On one hand mineral elements are basic units of skeletal structures of the animals on the other hand mineral ions are important co-factors of enzymes and other biological chemicals involved in life process. Minerals are of paramount importance for aquatic animals like fish and shellfish since these animals need to keep their osmotic balance through mineral ions. Mineral elements required by fish may be classified as bulk elements such as calcium, phosphorous, potassium, chlorine, sodium and magnesium (these are required comparitively in large amounts) and trace elements which are copper, cobalt, iron, iodine, manganese, selenium, zinc, aluminum, chromium and vanadium (these are required in very small quantities).

MINERAL REQUIREMENT STUDIES

Experiments to study mineral requirements are rather difficult to conduct, mainly because it is not easy to prepare diets with limiting concentrations of each mineral. Secondly feeding experiments with the animals should be of very long durations, in order to observe the manifestations of mineral requirements, deficiency and excesses. It is much more complicated in the case of aquatic animals since water is the largest contributor of a variety of mineral elements. Further there is possibility of interactions between minerals which may create complications in assessing the dietary requirements. The interactions may be either antagonism or synergism. For example large amounts of calcium in the diet may lower the availability of zinc present in the diet. Calcium deposition occurs in fish which is deficient in magnesium. Minerals have a role in many facets of metabolism such as hormones, respiratory pigments, structural elements, high-energy bonds and enzyme co-factors. Thus studies on mineral requirements, assume greater importance in fish and shellfish nutrition.

MINERAL REQUIREMENTS OF FINFISH

Among the aquatic animals, the mineral nutrition of finfish has been studied in greater detail. Fresh water fish show higher requirement of most of the minerals compared to the marine fish. The later group are capable of absorbing some of the mineral elements present in salt water. After detailed experiments, it was shown that red sea bream need only iron, potassium and phosphorous in their diet, the remainder of the elements coming from the external environment. Many of the mineral requirement studies are therefore directed on the fresh water fish species. The mineral

elements calcium, phosphorous, sodium molybdenum, chlorine, magnesium, iron, selemium, iodine, manganese, copper, cobalt and zinc are recognised as essential for body functions in fish. Fluorine and chromium have also been added of late to the list of essential elements.

Calcium and phosphorous

Calcium and phosphorous are closely related in metabolism and are discussed together in fish nutrition. Major portion of calcium (99%) and phosphorous (80%) are found in bones, teeth and scales. The ratio of calcium and phosphorous in bone ash is found to be approximately 2:1.

The extra skeletal calcium is widely distributed throughout the organs and tissues and exists in diffusable and non-diffusable form. Non-diffusable form is bound to proteins and the diffusable form which is generally found as phosphate, plays a significant role in the nutrition of animal. Ionised calcium in the extracellular fluids and in the circulatory system participate in muscular activity and osmoregulation.

Extra skeletal phosphorous is present mostly in combination with proteins, lipids, sugars, nucleic acids and other organic compounds. In some species the skin also appears to be an important repository for dietary phosphorous.

As stated earlier, fish are capable of extracting calcium directly from the surrounding water through gills. On the other hand the absorption of phosphorous is negligible from the environment, and the fish mainly depend upon the dietary sources of phosphorous.

Calcium and Phosphorous are absorbed in fish in the upper gastro-intestinal tract. While calcium is rapidly deposited as calcium salts in the skeleton, phosphorous is

distributed to all the major tissues. Water temperature influences the absorption of phosphorous and increases with increase in temperature. Higher content of glucose in the diet was found increase the absorption of dietary phosphorous by the fish. Absorption and retention of calcium are not influenced by any such external factors. The level of phosphorous in the diet influences the calcium retention in the body. Higher levels of dietary phosphorous enhances the retention of calcium to maintain the ratio between the two elements in the body.

The dietary phosphorous requirement of fish are as follows:-

Fish	Requirement
Atlantic salmon	0.7%
Channel catfish	0.4 - 0.47%
Common carp	0.6 - 0.7%
Red sea bream	0.68%
Rainbow trout	0.7 - 0.8%

Thus in general the phosphorous requirement in the diet of fresh water and marine finfish is almost same. Dietary calcium levels for fish are not recorded due to the absorption of this element from the environment by most of the fish species.

Many ingredients used for feed making are rich in calcium and phosphorous. Fish meal contains rich amounts of both the minerals. Calcium is generally deficient in plant ingredients and the phosphorous present in them is not available to the fish especially when the plant materials contain phytin or phytic acid. Phosphorous is readily available to the animals if potassium or sodium hydrogen phosphate or mono calcium phosphate is used in the diet. On the other hand phosphorous from tricalcium phosphate is not readily

available. Fish meal is rich in tri-calcium phosphate. Due to its poor availability to fish, it is released into to the water through faeces. This can combine with ammonia in water and lead to extensive eutrophication in the ponds. Deficiency symptoms of calcium are not described in fish. Poor growth, reduced feed efficiency, low bone ash and low haematocrit levels were observed in channel catfish fed phosphorous deficient diet. Prolonged feeding of phosphorous deficient diet has resulted in lordosis and abnormal calcification of bones (brittle structure) in common carp. The symptoms were similar in red sea bream.

Magnesium

About 60% of the body magnesium is found in skeletal structure and the remaining is distributed throughout the organic and muscle tissues. It is an important enzyme co-factor and component of cell membranes.

Marine fish are capable of extracting magnesium from the environment. Since this element is very limited in fresh water, the fresh water species seem to depend upon dietary source of magnesium. The dietary requirement of magnesium for rainbow trout is 0.06-0.07% and for carp it is 0.04-0.05%. Most of the compounded feeds prepared with ingredients of animal or plant origin have adequate levels of magnesium.

Apart from the general symptoms (reduced growth and poor food conversion efficiency) magnesium deficiency in rainbow trout leads to renial calcinosis and a flaccidity of the muscle due to increase in the extra cellular fluid volume. Loss of appetite, sluggishness and convulsion followed by tetany were also observed in common carp and rainbow trout fed magnesium deficient diets.

Mineral mixtures used in the standard fish diet are given below.

I. Mineral Mixture (USP XII No. 2) (Halver 440)

Calcium phosphate (g)	13.58
Calcium lactate	32.70
Ferric citrate	2.97
Magnesium sulphate	13.20
Potassium phosphate (dibasic)	23.98
Sodium biphosphate	8.72
Sodium chloride	4.35
Aluminum chloride	0.015
Zinc sulphate	0.300
Copper chloride	0.010
Manganese sulphate	0.080
Potassium iodide	0.015
Cobalt chloride	0.100

Ogino salt Mixture for Fish

NaCl (g)	1.0
MgSO4 "	15.0
NaH2 PO4	25.0
KH2 PO4	32.0
Ca(H2 PO4)2	20.0
Ferric citrate	2.5
Calcium lactate	3.5
Trace elements*	3.5
Trace elements	1.0
	100.0

* composition of trace elements

ZnSO4	7H2o (g)	35.3
MnSO4	4H2o (g)	16.2
CuSO4	"	3.1
COCl2	"	0.1
KIO3	"	0.3
Cellulose	"	45.0
		100.0

MINERAL REQUIREMENTS OF SHELLFISH

Mineral requirements of cultivable species of prawns are studied to some extent among the shellfish. Penaeid prawns were found to require calcium and phosphorous in their diets. Best growth was obtained when the diets of the prawn Penaeus japonicus diets were supplemented with 1.04% of phosphorous and 1.24% of calcium. It was indicated that the calcium and phosphorous ratio in the diet of prawn should be 1.2:1. When this ratio was increased to 2:1 growth was inhibited and pigmentation decreased. Through studies with radio activity labelled calcium (45 Ca), it was demonstrated that prawns could absorb calcium from the surrounding water. It was estimated in P. japonicus that approximately 0.83 mg of calcium is absorbed per day per gram of body weight from the environment, when the sea water contained 0.44 mg/ml of calcium. This had lead to the conclusion that the calcium requirement could be satisfied by the calcium in surrounding seawater.

Supplementation of the diet with calcium (2%), magnesium (0.3%) and iron (0.02%) did not improve the nutritive value of the diet P. japonicus. Iron in the diet rather reduced the growth. Phosphorous at 2% level in the diet effectively improved the performance of the diet.

Potassium at 1% level in the diet showed higher growth and feed efficiency.

The requirement of individual trace elements was not studied. However when a mixture of trace elements, consisting of aluminum chloride (5 mg), Zinc sulphate (90 mg), manganese sulphate (20 mg), copper chloride (5 mg), potassium iodide (5 mg) and cobalt chloride was added to the diet at 0.2% level, enhanced the efficiency of the feed; higher level of this mixture above 0.2% in diet lowered the growth.

Studies in detail, on the requirement of each individual mineral element are needed to understand thoroughly the deficiency and excess symptoms of different minerals which are not available at present. However nutritionists have been adding mineral mixtures to their diet formulations. The mineral mixture used in the diet of P. japonicus is given below.

Mineral mixture used in prawn diet per 100 g.

	(g)
K2HPO4	2.0
Ca3 (PO@)2	2.72
NaH2 PO4 2.H2O	0.79
Mg SO4	3.02
FeSO4. 7H2O	0.015
MnSO4. 5H2O	0.004
	8.549

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VITAMIN REQUIREMENTS OF FINFISH AND PRAWNS

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Vitamins are complex organic substances, usually of comparatively small molecular size (molecular weight usually less than 1000). They are distributed in feedstuffs in small quantities and form a distinct entity from other major and minor food components (Cho et al., 1985). The importance of vitamins as essential constituents in the diets of animals came to light in the early part of this century and during the past five decades active and rapid progress in vitamin research was made almost in all the commercially important species. While contributions to vitamin nutrition of mammals and poultry are numerous (Mitchell, 1964), contributions from aquatic species are relatively less, and mostly came from studies with finfish (Cowey and Sargent, 1972; Halver, 1972, 1980; Mahajan and Agarwal, 1979; Millikin, 1982). In recent years, active research is in progress on the vitamin requirements of crustaceans, because of their growing commercial importance (Deshimaru and Kuroki, 1979; Guary et al., 1976; D' Abramo and Baum, 1981; Heinen, 1984).

The slow progress in nutrition research on aquatic organisms was partly due to the inherent problems posed by the aquatic medium. Leaching of vitamins from diets when introduced into the water is one of the major constraints. Another factor is the contribution from gut microbial flora

in certain species, which mask the actual requirements. It has also been observed that vitamins and their precursors, since already present in the raw materials, 'Blanket Applications' of vitamin premixes in multi-ingredient diets may result in some excesses (New, 1976). Conversely, as the vitamin requirements remain unknown, formulated diets may still be deficient in certain vitamins even after supplementation.

VITAMIN TEST DIET

McLaren et al. (1947) developed a vitamin test diet for fish containing crystalline vitamins, casein, dextrin and oils with crab meal or dried liver as the source for the antianaemic factor. Thus these pioneer fish nutritionists paved the way for vitamin requirement studies in aquatic species. Subsequently, Halver (1957) developed the vitamin-free casein purified diet, which is widely used as the standard diet for determining the qualitative and quantitative requirements of a number of species of finfish. The composition and preparation procedure for Halver's H-440 diet, which is recommended by the EIFAC of FAO/IUNS/ICES task force as the standard diet, is given in Table 1.

So far, four fat-soluble and 11 water soluble vitamins are known to be required by fish and crustaceans. Many of the water-soluble vitamins function either directly or in a modified form as coenzymes for one or more of enzymes. None of the fat-soluble vitamins is known to function as coenzyme. In contract to higher vertebrates, vitamin deficiency symptoms, reported in finfish are non-specific (Table 2, Cho et al., 1985).

WATER-SOLUBLE VITAMIN REQUIREMENTS

VITAMIN C

Vitamin C, the antiscurvy agent participates actively in the metabolism of all species. But fishes and crustaceans are unable to biosynthesize ascorbic acid (Halver, 1972, 1980; Magarelli and Colvin, 1978; Magarelli et al., 1979). This inability in these groups of animals is attributed to the genetic failure of enzyme synthesis or lack of expression of the same (Levin, 1976). An excellent review by Knox and Goswami (1961) highlights its specific role in intermediary metabolism. Some of the important functions of this vitamin are: as protector of enzymes and hormones from oxidation and inhibition; involvement in the functioning of neural stimuli; transmitter; in RNA synthesis; mild detergent action responsible for the dissolution of fat-cholesterol and cholesterol-phospholipid - calcium complexes; as endogenous protectors; growth regulators, reactants in enzyme systems such as in the hydroxylation of epinephrine and tryptophan and in the oxidation of tyrosine in collagen synthesis. In crustaceans, vitamin C has also been reported to influence the alkaline phsophatase activity during the synthesis of chitin and sclerotization of the epicuticle (Conklin, 1983). One of the important functions of ascorbic acid is in the collagen synthesis (Stone and Meister, 1957), which is an important aspect in muscle development of all animals including fish and crustaceans (Harper et al., 1967). Stone and Meister (1957) demonstrated the necessity of vitamin C for the hydroxylation of proline and lysine to form hydroxyproline an unusual amino acid that exclusively occur in collagen.

L-ascorbic acid is a biological reducing agent in hydrogen transport. It is involved in the detoxification of aromatic drugs and also acts in the production of adrenal steroids. Ascorbic acid is also involved in erythrocyte maturation.

Deficiency symptoms:

Deficiency of ascorbic acid molecules in the diets of animals results in metabolic disorders leading to diseases. Deficiency symptoms could be clearly delineated on deletion of the vitamin from diet. Deficiency leads to spinal deformities, e.g., scoliosis and lordosis in fish. X-rays of spinal deformities in the affected fish showed extreme dislocation of vertebrae, and atrophy of the spinal cord in the area of acute deformity. Gill cartilage was found to be distended and twisted; filamentous cartilage occurred in ascorbic acid deficient fish. In channel catfish a broken back syndrome appeared in those fed diets containing suboptimal levels of vitamin C. Internal and external haemorrhage, fin erosion, dark-skin color and reduced formation of collagen are important symptoms observed in channel catfish. Agrawal et al. (1978) reported a protective effect of high dietary levels of ascorbic acid for snake-head fed an organochlorine insecticide.

Channel catfish had increased susceptibility to pathogenic bacterial infestation (Aeromonas liquefaciens) and occasional formation of hemivertebrae (Lovell, 1973). Lim and Lovell (1978) reported the following ascorbic acid deficiency symptoms in smaller channel catfish fingerlings (initial mean weight = 2.3 g): anaemia after 9 weeks, scoliosis, lordosis and dark pigmentation after 10 weeks; lower hematocrit values after 18 weeks.

Snake-heads fed with ascorbic acid deficient diets had elevated liver cholesterol content after 150 days, in addition to the occurrence of scoliosis, lordosis and decreased ascorbic acid concentrations in blood and kidney (Mahajan and Agrawal, 1979). Mahajan and Agrawal (1980) reported that snake-heads had reduced absorption of calcium

from-surrounding water by gills and skin, and lower muscle and bone calcium content when fed an ascorbic acid deficient-diet for 210 days. They attribute this to distortion of gill filaments from cartilage malformation.

In fish hyperplasia of jaw and snout have been reported. In Coho salmon, hypertrophy of the adrenal tissue and haemorrhage at the bases of fins have been observed. However on replacement of ascorbic acid in the ration growth becomes normal. Anaemia eventually develops in extremely deficient fish and scoliosis and lordosis do not repair but are walled off by new growth around the afflected areas of the spine when ascorbic acid is once again added to the ration (Halver, 1980).

In fish examination of fragile support cartilage in the gill filaments under low magnification will detect early hypovitaminosis before clinically acute symptoms become noticeable. However, the best tissue for routine clinical analysis in fish tissue is anterior kidney of the fish.

Recently in Clarias betrachus ascorbic acid was reported to be essential. Deficiency resulted in scoliosis, external haemorrhaging, fin erosion, and dark skin colour at 12 weeks (Butlhep et al., 1985).

In crustaceans, very few studies have been carried out on ascorbic acid and the information available is restricted. Conklin (1983) reported that under vitamin C deficiency alkaline phosphatase activity was inhibited resulting in poor chitin synthesis and sclerotization of the epicuticle. In the prawns, Penaeus californiensis and P. stylirostris, malformation of collagen tissue culminating in the melanization of hemocytic lesions leading to death and designated as "Black Death Disease" occur (Lightner et al., 1979; Lightner, 1983). Studies conducted at the

CMFRI have shown that deficiency of ascorbic acid in the diet of P. indicus results in reduced food intake, poor conversion of food and protein, high incidence of post-molt deaths, dystrophy of muscle and hepatopancreas, blackening of gills etc.

In early juveniles of prawns (P. indicus) ascorbic acid between 0.4 and 0.8 g/kg diet has been suggested. While Guary et al. (1976) reported high survival rates with 2 g ascorbic acid/100 g diet, in the present study 0.4 g ascorbic acid/kg gave highest survival. Magarelli et al. (1979) reported maximum survival in P. californiensis at 1.2 g/100 g diet and in P. stylirostris 2.2 g/100 g diet. In P. indicus a concentration of 2 g and more ascorbic acid depressed growth. Ascorbic acid deficiency led to increased post-moult deaths. In these prawns, the calcium and phosphorous contents were relatively low and since ascorbic acid is essential for the uptake of calcium, it is suspected that ascorbic acid deficiency would have affected the calcium absorption and metabolism of the prawns.

Dietary requirements:

According to Halver (1980) an intake of 100 mg of vitamin C/kg of dry ration was sufficient for rainbow trout under normal conditions. However, the ascorbic requirements doubled or tripled by stress when severe abdominal or intramuscular wounds were inflicted. Young fish needed at least 500 mg of active ascorbate for tissue repair comparable with control fish receiving 1 g or more of ascorbate in the diet/kg of dry diet. Coho salmon, however required about half of these requirement for maximum severe wound repair. The requirement for ascorbic acid is related to stress, growth rate and size of the animal as well as to the other nutrients present in the diet. Halver (1980) suggests a compromise

value of 200 mg of ascorbic acid/kg diet for trout and salmon raised in freshwater systems between 10-15°C.

Large common carp can synthesize some ascorbate and the requirement for this species may be dependent on fish size and the environment in which they are reared. For carp and channel catfish 30-50 mg/kg diet.

Studies conducted at CMFRI has shown the essentiality of ascorbic acid for the fry of milkfish, Chanos chanos and the mullet Liza parsia fry for survival, proper growth and food intake.

The recommended levels of ascorbic acid for finfish and crustaceans are shown in Table. The requirement is in the range of 100-150 mg/kg diet for salmonids and 30-50 mg/kg diet for carp and channel catfish.

Sources and stability:

Ascorbic acid is widely distributed in nature with citrus fruits cabbage, liver, and kidney tissues are good sources for the vitamin. Fresh insects and fish tissues contain reasonable amounts of the vitamin. Synthetic ascorbic acid is also readily available. Ascorbic acid is added to feed as a dry dilution. Ethycellulose or fat-coated products improve ascorbic acid stability in feeds. Ethylcellulose coated ascorbic acid show higher retention in crumbles (84%) than crystalline ascorbic acid (48%). Room temperature for 3 months resulted in 40% loss in crumbles for the ethyl-cellulose-coated product (Frye, 1978). Ascorbate - 2 - sulfate is considered as a good source of ascorbic acid. Fish food should be protected from oxidising agents and kept sealed or frozen to prevent loss of the vitamins. Dipotassium ascorbic - 2 - sulfate (DAS) and dipotassium L-ascorbate - 2 - sulphate hydrate (AS) were found to prevent

deficiency symptoms. Rainbow trout require 160 mg DAS/kg dry diet to achieve normal growth (Halver et al., 1975) and 80 mg to avoid deficiency symptoms. Channel catfish fingerlings require about 25 mg as to avoid deficiency symptoms.

CHOLINE

It serves as a source of methyl groups; involved in a number of trans-methylations; as-phosphatidyl choline it has an important structural role in biomembranes; in methylated state as acetylcholine, functions as an important neuro-transmitter; also functions as a lipotropic and anti-haemorrhagic factor.

Deficiency symptoms:

Poor growth, and food conversion and impaired fat metabolism. Halver (1957) reported increased gastric emptying time in salmons fed on choline deficient diets. In rainbow trout anaemia and kidney degeneration reported. In channel catfish haemorrhagic kidneys and intestine and enlarged livers have been reported. Japanese eels became anorexic after 4 weeks on a deficient diet (Arai et al., 1972).

In prawns, choline deficiency led to poor growth in P. japonicus (Kanazawa et al., 1976). In P. indicus poor growth and survival, poor food intake, aversion towards feed, hyposensitivity to shock, passive activity, dystrophy of muscle and hepatopancreas, postmolt deaths were observed. Lecithin (phospholipid) has a partial choline sparing action in P. indicus. If adequate levels of lecithin is included in the diets, choline can offset the requirements of choline (Gopal, 1986).

Sources and stability:

Rich sources of choline are what germ, beans, brain and heart tissue. Choline hydrochloride, the commercially available form, may inactivate -tocopherol and vitamin K when in direct contact with these vitamins. Choline is added to feeds as a 70 per cent choline chloride solution or as 25 to 60 per cent dry powder (Adams, 1978). Choline chloride is stable in multivitamin premixes but can decrease the stability of other vitamins in the premix (Frye, 1978). Choline is stable during processing and storage in pressure-pelleted extruded diets. Loss during water immersion of pellets is less than 10 per cent after 60 minutes (Goldblatt et al., 1979). Choline is hygroscopic, very soluble in water, and is stable to heat in acid but decomposes in alkaline solutions.

INOSITOL

Inositol is a water-soluble growth factor for which no co-enzyme function is known. The only known function of inositol is as component of the inositol phosphoglycerides that are found in many cells. It has lipotropic action by preventing accumulation of cholesterol in one type of fatty liver disease and is involved with choline in maintaining normal lipid metabolism. It is a growth promoting substance for micro-organisms.

Deficiency symptoms:

Poor growth, increased gastric emptying time, oedema, dark colour and distended stomach are symptoms observed in salmon, trout, carp and catfish held for long period. The major deficiency sign is inefficiency in digestion and food utilization and concomitant poor growth leading to a population of fish with distended abdomens. Inositol is not

normally required by channel catfish.- In Japanese eels and in red sea bream reduced growth was observed. In prawns, P. japonicus (Kanazawa et al., 1976) and P. indicus (Gopal, 1986) also reduced growth was observed.

Dietary requirements:

Inositol is added to feeds as a dry dilution (Adams, 1978). Rainbow trout 200-300 mg/kg, Chinook and Coho salmon 300-400 mg/kg; carp 200-300; sea break 300-500.

THIAMINE

Thiamine functions in all cells as the coenzyme co-carboxylase, thiamine pyrophosphate, which participates in the oxidative decarboxylation of pyruvic acid to acetate for entry into tricarboxylic acid (TCA) cycle. Thiamine pyrophosphate is also a co-enzyme of erythrocyte transketolase system by which direct oxidation of glucose occurs in the cytoplasm of cells (via) the pentose-phosphate-pathway. Erythrocyte levels of metabolites of this system have been used to indicate thiamine status in experimental animals, including fish. Thiamine is essential for good appetite, normal digestion, growth and fertility, normal functioning of the nervous tissue and the requirement is determined by the caloric density of the diet.

Deficiency symptoms:

Deficiency signs in salmonids include impaired carbohydrate metabolism, nervous disorders, poor appetite, poor growth and increased sensitivity to shock. A trunk-winding symptom in eels has been reported, together with haemorrhage at the base of the fins. Skin congestion and subcutaneous haemorrhage occur in carp and eels fed thiamine deficient diets. Deficiency signs are evident in 6 to 8 weeks in

and about 8 weeks
channel catfish; 8 to 10 weeks in Japanese eels/in common carp fed a high carbohydrate diet. In prawns P. japonicus poor growth was reported after 10 weeks (Deshimaru and Kuroki, 1979). In P. indicus poor growth, survival, poor food intake and hypersensitivity were observed (Gopal, 1986).

Dietary requirements:

-William and Spies (1938) based on the information available till that time reported that all species of animals require thiamine in their diets. Recent studies have shown that thiamine requirement of aquatic species is much higher than that of domesticated land animals (Hasting and Cowey, 1977), mainly due to leaching of the vitamin, from diets (New 1976; Infanger et al., 1980) into the surrounding water. Composition of dietary ingredients should be considered while determining thiamine requirements. In common carp the dietary requirement for thiamin has been correlated with the carbohydrate level of the diet (Aoe et al., 1969). Omnivorous fishes might be expected to have a higher dietary requirement for thiamine than carnivorous fishes, but this has not been found to be true (NRC, 1981).

Studies with crustaceans have shown that thiamine is essential in the diet of Kuruma prawn, P. japonicus (Deshimaru and Kuroki, 1979), the cladoceran, Moina macrocopa (Conklin and Provasoli, 1977), the lobster Homarus americanus (Conklin, 1980) the giant tiger prawn, Macrobrachium rosenbergii (Heinen, 1984) and the penaeid prawn Penaeus indicus (Gopal, 1986).

Infanger et al. (1980) observed that thiamine loss is maximum (68-100% in 2 hrs time) amongst all the vitamins from the diet, thus necessitating higher concentrations of vitamins in the diet of prawns.

Fat content of diet may affect not only calorie intake but also the thiamine requirement because cocarboxylase participates in the oxidation of that through -ketoglutarate. Therefore, fish on a high fat diet and low thiamine intake might take longer to develop deficiencies and will give an erroneous requirement (Halver, 1980).

Sources and stability:

Common sources of thiamine are dried-peas, beans, cereal bran. Thiamine is easily lost by holding diet ingredients too long in storage or by preparing the diet under slightly alkaline conditions or in-the presence of sulphide. Wet or frozen diet pose a problem because moisture content increases the chance of enzymatic hydrolysis's and subsequent destruction of thiamine. Wet or moist diet preparations containing any fresh fish or shellfish tissue must be used immediately.

Thiamine is added to feeds as thiamine mononitrate or thiamine hydrochloride. Thiamine mononitrate is stable in vitamin premixes that do not contain trace minerals and choline chloride. Thiamine mononitrate premixes containing any of those substances and stored at room temperature can lose as much as 80 to 90 percent of their thiamine activity in 3 months (Frye, 1978). Thiamine losses in the pelleting or extrusion process range from 0 to 10% and during storage of feeds 11 to 12 percent (Slinger et al., 1979).

Thiaminases occurring in fresh water fish tissues and tissues of certain-shrimps and mussels render thiamine biologically unavailable. Thiaminase is inactivated by heating or pasteurization.

RIBOFLAVIN

Riboflavin was the second water soluble vitamin to be discovered. It is synthesized by all plants and many micro-organisms but not by animals. Lactoflavin, hepatoflavin and oveflavin were also shown to be identifical with the pure riboflavin. Riboflavin is found in the tissue coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes function as prosthetic groups of oxidation-reduction enzymes that are needed for the degradation of pyruvate, fatty acids, and amino acids. Thus they function as coenzymes for many oxidases and reductases such as cytochrome c oxidase, D and L amino acid oxidases, Xanthine and aldehyde oxidase, SDH, glucose oxidase and fumaric dehydrogenase. Involved with pyridoxine in the conversion of tryptophan to nicotinic acid; and in the retinal pigment during light adaptation.

Deficiency symptoms:

In salmonids poor appetite and poer diet efficiency are the first signs followed by photophobia mono- or bilateral cataracts; corneal vascularization, eye haemorrhage, incoordination, and general anaemia (Halver, 1980). Channel catfish fed riboflavin deficient diets for 8 weeks developed deficiency signs including anorexia, poor growth, short-body dwarfism (Murai and Andrews, 1978) and cataracts (Dupree, 1966).

In common carp Aoe et al. (1967) reported nervousness, photophobia and haemorrhages. Rainbow trout fed riboflavin-deficient diets developed bilateral corneal and lenticular lesions (Poston et al., 1977). Fin necrosis, snout erosion and spinal deformation (Poston et al., 1977). In P. indicus poor food intake, poor survival, incoordinated movements and

sensitive to shock have been observed.

Dietary requirements:

Riboflavin requirements of carp fingerlings seems to decline with increasing fish size. Common carp fingerlings with an initial mean weight 1.5 g required 20 mg riboflavin. But slightly larger ones-required 10 mg, and those weighing 3.4 g required 5.7 mg riboflavin.

Rainbow trout of mean weight 7 g require 12.2 mg. Whereas larger rainbow trout require 3 mg riboflavin. Channel catfish fingerlings require 9 mg for maximum growth and 3 mg to prevent occurrence of short-body dwarfism (Murai and Andrews, 1978 b).

Sources and stability:

Milk, liver, kidney, heart, yeast, germinated grains, peanuts, soyabeans and eggs are good sources. Riboflavin is added to feed as a spray dried powder or dry-dilution product. It is stable in multivitamin premixes. However, processing loss is about 26 percent. Storage losses in pelleted feeds are slight. About 40% of the riboflavin may be lost when pellets are introduced in water (Goldblatt et al., 1979).

PYRIDOXINE

Pyridoxine (B6) is an essential vitamin, required by all animal species so far studied. This vitamin is widely distributed in almost all the natural products principally as complexes of proteins, such as pyridoxal phosphate (PLP) which is an active coenzyme participating in amino acid decarboxylation, transmination, racemization, desulphydration of methionine and cysteine, deamination of hydroxy amino acids and in a variety of miscellaneous transformations.

PLP also functions as a coenzyme for 22 or more transaminases, occurring in the body and in the decarboxylation of 5 hydroxytryptophan to produce seratonin. It is also involved in the synthesis of porphyrin, -amino levulinic acid and messenger RNA, which determines amino acids sequence in polypetide synthesis. It is also essential for the metabolism of essential fatty acids and protein.

Deficiency symptoms:

Deficiency has been shown to affect the amino acids and protein metabolism in higher animals. A number of genetic diseases involving pyridoxine dependent enzyme systems have been reported. In fish epileptic type fits, general nervous dis-orders, hyperirritability, alteration in the control of melanopheres and edema in the peritoreal cavity, rapid and gasping breathing with flexing of the opercles, and rapid occurrence of post-mortem rigor mortis, have been reported.

Channel catfish fed a pyridoxine-deficient diet for 6 to 8 weeks exhibited signs of deficiency, including anorexia, tetany, nervous disorders and greenish blue colouration (Andrews and Murai, 1979).

In rainbow trout normocytic normochromic anemia was reported indicating that pyridoxine has a function in the maintenance of normal erythropoiesis. Also lower aspartate amino-transferase activity in white muscle and reduced aspartate and alanine amino-transferase activity were reported.

Dietary requirements:

In fishes, the requirement of pyridoxine ranges from 2 mg to 20 mg/kg (Table). Though in crustaceans few works have been done, yet most nutritionists supplement their diets with pyridoxine ranging from 60-100 mg/kg dry diet.

The intimate association of pyridoxine with various phases of amino acids metabolism implies that the pyridoxine requirement of an animal should be greater on a high protein than on a low protein diet. In carnivorous fish, the pyridoxine requirement has been shown to depend upon dietary protein level (Hardy et al., 1979).

Among crustaceans, its essentiality and requirements have been reported for Artemia (Provasoli and Shiraishi, 1959), Moina (Conklin and Provasoli, 1977) M. rosenbergii (Heinen, 1984) and P. indicus (Gopal, 1986).

Sources and stability:

Good sources are yeast, whole cereals, egg yolk, liver and glandular tissues. Pyridoxine is added to animal feeds as pyridoxine hydrochloride in a dry dilution. It is stable in vitamin premixes. The loss of pyridoxine from feeds after 10 months of storage was 7-10 percent (Slinger et al., 1979).

PANTOTHENIC ACID

Pantothenic acid plays a stellar role in general metabolic pathways. It is a dipeptide derivative and its name (Panto-Greek Everywhere) implies its almost ubiquitous distribution. Pantothenic acid acts metabolically as a part of coenzyme (Co A) to transfer acyl groups in enzymatic reactions. It has significant role in fatty acid oxidation and synthesis, synthesis of cholesterol and phospholipid, in phosphate energy transfer and in the acetylation of organic compounds, acylation of acetate, succinate, benzoate, propionate and butyrate. Acetyl - Co A is also required in reactions in which the carbon skeletons of amino acids enter into energy yielding metabolic pathways. Since pantothenic acid is a part of acetyl Co A, it has been shown to be

required by all animal species studied so far, including the microorganisms (West et al., 1965).

Deficiency symptoms:

Symptoms associated with pantothenic acid deficiency are mostly non-specific and vary from species to species. Deficiency studies in higher vertebrates have shown retardation of growth, impairment of reproduction imbalance of salt-water metabolism and reduction of Co A content in tissues leading to poor utilization of pyruvate (Chow, 1964).

Aquatic species, especially fishes like salmon and trout, reared at 10-19°C water temperature, fed with pantothenic acid deficient diets were found to exhause the vitamin stores rapidly in 8-12 weeks. The fish stop feeding and the gill filaments show proliferatien of epithelial surface in addition to swelling and clubbing together of the filaments and lamellae (Phillips et al., 1945). The opercles become distended and the surface of the gills is often covered with an exudate. Fish become prostrate or sluggish. Necrosis, scarring and cellular atrophy of the tender gill elements occur and anaemia develops after prolonged deficiency. The same type of symptom has been observed in salmon, trout, eel, carp and catfish. In channel catfish erosion of skin, lower jaws, fins and barbels reported.

In Macrobrachium rosenbergii (Heinen, 1984) and Penaeus indicus (Gopal, 1986) unusual partial moulting of prawns has been reported.

Dietary requirements:

The dietary pantothenic acid requirements are shown in Table. Murai and Andrews (1975) suggested that the relatively high dietary pantothenic acid requirements of

channel cat fish fry might be partially due to higher rates of micronutrient losses in small feed crumbles fed to fry compared with larger feed particles fed to fingerlings.

Heinen (1984) reported relatively higher growth in prawns (M. rosenbergii) fed with pantothenic acid deficient diet, than their control counter parts having 0.06% pantothenic acid and he presumed that the poor growth is due to the detrimental effect of excess vitamin dosage. In Penaeus indicus a dietary level of 50 to 75 mg/100 g diet seems to be required for proper growth, survival and utilization of the food and protein.

Sources and stability:

Good sources are cereal bran, yeast, liver, kidney, heart, spleen and lung. Fish flesh is a relatively rich source pantothenic acid is added to feed as either calcium-d-pantothenate (92 percent activity) or calcium dl-pantothenate (46% activity). Pure pantothenic acid is unstable hydroscopic and viscous. Calcium pantothenate is relatively stable in moist and dry diets. Processing losses during pelleting or extrusion can be as high as 10%.

NIACIN

The vitamin exists in its amide form nicotinamide, under its physiological active state, serving as co-enzyme for a variety of metabolic enzymes. The major function of niacin in NAD and NADP is hydrogen transport in intermediary metabolism. These co-enzymes serve as hydrogen acceptors from metabolic reactions activated by certain anaerobic dehydrogenases passing H-molecule to flavoproteins in glycolysis, Kreb's cycle and other metabolic cycles. Both NAD and NADP are involved in the synthesis of high energy phosphate bonds which furnish energy for certain steps in

glycolysis, in pyruvate metabolism, and in amino acid and protein metabolism.

In many animals the amino acid tryptophan can be converted to nicotinic acid, so deficiency signs can only be induced by restricting dietary tryptophan.

Deficiency symptoms:

Stores of niacin are more slowly exhausted under experimental conditions than are some of the other vitamins resulting in less defined and more slowly developing symptoms. Deficiency of niacin reduces the concentration of coenzymes in liver and muscle (Goldsmith, 1964). It has also been observed that niacin in the diet, increases the secretion of both free and total acids in the gastric juice (Goldsmith, 1964). Niacin deficiency symptoms are developed much slower in invertebrates and fishes than in higher vertebrates; the reason being that niacin is replenished through microbial population present in the intestinal region in many species, which produce the vitamin quantum just sufficient to meet the animal requirements (Halver, 1972). Niacin demands are also met through the conversion of the amino acid tryptophan present in the diet.

Loss of appetite, skin and fin lesions, deformed jaws, anaemia, exophthalmia, high mortality rates, poor feed conversion, appearance of lesions in colon, motion, oedema of the stomach and colon reported. In common-carp skin haemorrhages have been reported. In M. rosenbergii food efficiency was not markedly affected by niacin deficiency. In P. indicus poor survival and growth, poor food intake, black lesions in the body and gills were reported.

Dietary requirements:

The recommended levels of vitamin the diets are given in Table. Niacin requirement varies with the protein content in the diet. Besides, the type of carbohydrate, amount of dietary micro-nutrients like steroids, trace elements like chromium, zinc and molybdenum and a number of B vitamins significantly influence niacin requirements (Halver, 1980).

Sources and stability:

Niacin is found in most animal and plant tissues. Rich sources are yeast, liver, kidney, heart, legumes etc. In feeds it is added as either nicotinic acid or niacinamide as a dry dilution. Processing losses of niacin in extruded diets is about 20 percent. In aquatic systems, dietary losses are widely encountered due to leaching of the vitamin from diets. About 50% of the vitamin is lost from purified diets in 24 hrs due to leaching (Infanger et al., 1980). To compensate losses during diet preparation and due to leaching higher dosages are incorporated.

BIOTIN

Biotin is a monocarbexylic acid slightly soluble in water and alcohol and insoluble in organic solvents. It is required as an intermediate carrier of CO_2 in several specific carboxylation and decarboxylation reactions, including the carboxylation of pyruvic acid to form oxaloacetic acid. Acetyl Ce A carboxylase, pyruvate carboxylase and propionyl Co A carboxylase are enzymes requiring biotin. Biotin is also required for the metabolic pathways associated with the biosynthesis of long-chain fatty acids, purine, and the metabolism of odd-carbon fatty acids. It is also involved in the conversion of unsaturated fatty acids to the stable cis

form in the synthesis of biologically active fatty acids.

Deficiency symptoms:

In salmonids skin disorders, muscle atrophy, lesions in the colon, loss of appetite, spastic convulsions and fragmentation of erythroeytes have been reported (Halver, 1980). In brook-trout "blue slime patch disease" is caused specifically by biotin deficiency. Fish reared in 10° to 15°C water exhaust biotin stores in 8-12 weeks and the first signs are anorexia, poor food conversion-depressed liver acetyl Co A carboxylase and pyruvate carboxylase (Poston-and Page, 1982). In channel catfish depigmentation has been noted (Robinson and Lovell, 1978). In brook trout abnormal synthesis of liver fatty acids and high liver glycogen content reported (Poston and McCartney, 1974). In Japanese eels reduced growth, abnormal swimming behaviour, and dark coloration have been reported after 8 weeks (Arai et al., 1972).

Dietary requirements:

Biotin is synthesized in channel catfish by intestinal microflora, but it has a role in blood glucose regulation and improves cell membrane function.

Deficiency symptoms:

Macrocytic normochromic anaemia; poor growth, anorexia, general anaemia, lethargy, fragile fins, dark skin pigmentation and infaretion of spleen (Halver, 1980). In Coho salmon reduction in number of erythrocytes also reported (Smith and Halver, 1969). Dupree (1966) failed to demonstrate any major deficiency symptoms in channel catfish.

Common carp did not showed any major deficiency symptoms (Aoe et al., 1967). Folic acid is synthesized by the intestinal bacteria of common carp. John and Mahajan (1979), however, found a reduction in growth rate and

erythrocyte number in the Indian major carp, rohu, after 15 weeks on a folic acid deficient diet.

Dietary requirements:

Salmonids have been shown to require about 6-10 mg/kg dry feed (Table). Lake trout fingerlings required a minimum of 0.1 mg biotin/kg dry diet for optimal growth rate. Dietary biotin concentrations of 8 mg/kg dry diet enhanced liver pyruvate decarboxylase activity in channel catfish fingerlings (Robinson and Lovell, 1978), whereas 6 mg biotin/kg dry diet increased acetyl Co A carboxylase in brook trout fingerlings. Common carp fingerlings require 1 mg biotin/kg dry diet for maximal weight gain and biotin content in liver (Ogino et al., 1970).

Sources and stability:

Yeast, green vegetables, liver, kidney, glandular tissues, fish tissues and viscera. Insects contain xanthopterin which has folic acid activity. Folic acid is added to feeds as a dry dilution. Storage losses are as high as 43% in three months. Supplemental biotin in the diet may be required for maximum growth (Robinson and Lovell, 1978). The diets should be protected from strong oxidizing agents or conditions which promote oxidation of ingredients. In feeds it is added as d-biotin in a dry dilution. It is stable in multivitamin premixes. Processing losses account for about 15 percent.

FOLIC ACID

Folic acid is required for normal blood cell formation. It is involved as a coenzyme in one-carbon transfer mechanisms, particularly in the interconversion of serine and glycine, methionine - homocysteine synthesis, histidine synthesis and pyrimidine synthesis. The erythrocytes are the most

sensitive to folic acid deficieney. It is also involved in the conversion of megaloblastic bone marrow to normoblastic type. It has a role in blood glucose regulation and improves cell membrane function.

Deficiency symptoms:

Macrocytic normochromic anaemia, poor growth, anorexia, general anaemia, lethargy, fragile fins, dark skin pigmentation and infaretion of spleen (Halver, 1980). In Coho salmon reduction in number of erythrocytes also reported (Smith and Halver, 1969). Dupree (1966) failed to demonstrate any major deficiency symptoms in channel catfish.

Common carp did not show any major deficiency symptoms (Aoe et al., 1967). Kashiwada et al. (1971) showed that folic acid is synthesized by the intestinal bacteria of common carp. John and Mahajan (1979), however, fond a reduction in growth rate and erythrocyte numbers in the Indian major carp, rohu, after 15 weeks on a folic acid - deficient diet.

Dietary requirements:

Salmonids have been shown to require about 6-10 mg/kg dry feed. In channel catfish and common carp no requirement has been demonstrated.

Sources and stability:

Yeast, green vegetables, liver, kidney, glandular tissues, fish tissues and viscera. Insects contain xanthopterin which has folic acid activity. Folic acid is added to feed as a dry dilution. Storage losses are as high as 43% in three months.

CYANOCOBALAMIN

Cyanocobalamin is a large molecule containing cobalt. Neither plants nor animals can synthesize this vitamin; but both depend upon certain microorganisms for the trace amounts required. Cyanocobalamin is required for normal maturation and development of erythrocytes, for the metabolism of odd carbon fatty acids and for the methylation of homocysteine to form methionine. A coenzyme incorporating the vitamin is involved in the reversible isomerization of methylmalonyl coenzyme A to succinyl coenzyme A and in the isomerization of methylaspartate to glutamate. It is also essential for normal cholesterol metabolism and in purine and pyrimidine biosynthesis.

Deficiency symptoms:

In salmon pernicious anaemia characterised by fragmented erythrocytes has been reported. Poor appetite, poor growth and food conversion precedes anaemia in salmon (Halver, 1980). In channel catfish growth retardation occurred after 36 weeks (Dupree, 1966). Limsuwan and Lovell (1981) demonstrated that intestinal microorganisms synthesized about 1.4 ng of cyanocobalamin/g body weight per day in channel catfish. John and Mahajan (1979) reported occurrence of megaloblastic anaemia in the Indian major carp rohu fed diet deficient in folic acid cyanocobalamin or both the vitamins.

Salmonids require about 0.015 to 0.02 mg/kg dry diet.

Sources and stability:

Fish meal, fish viscera, liver kidney, glandular tissues and slaughter house wastes. It is affected by storage temperature and in mild acid solutions easily destroyed by heating.

FAT SOLUBLE VITAMINS

VITAMIN A

Vitamin A occurs in two forms, vitamin A1 (retinal) found in marine fishes, and vitamin A2 (retinol 2) found in fresh water fishes (Lehninger, 1975). Interconversion of the two forms in living fish tissue has been demonstrated.

Vitamin A is essential for maintaining epithelial cells, preventing atrophy and keratinization of epithelial tissues. It promotes growth of new cells and aids in maintaining resistance to infection. It is also involved in calcium transport across some membranes, in reproduction and embryonic development, and in cellular and sub-cellular membrane integrity.

Coldwater fish can utilize -carotene at 12.4°C to 14°C, but not at 9°C (Poston et al., 1977). Dupree (1970) reported that channel catfish could utilize -carotene as a vitamin A source if it was provided in the diet at over 2000 IU/kg of feed.

Deficiency and hypervitaminosis symptoms:

Hypovitaminosis A is characterized by poor growth, poor vision, keratinization of epithelial tissue, xerophthalmia, night blindness, haemorrhage in the anterior chamber of the eye, haemorrhage of the base of the fins and abnormal bone formation.

Conversion efficiency of -carotene to vitamin A has been examined for channel catfish and brook trout. Dupree (1966) found inefficient conversion of -carotene to vitamin A in channel catfish. Poston et al. (1977) demonstrated indirectly that brook trout can convert

dietary -carotene into vitamin A with conversion efficiency being greater at 12.4°C than at 9°C.

In rainbow trout, thickening of the corneal epithelium and degeneration of the retina have been reported. Channel catfish fed 0.4 ppm -carotene developed signs of deficiency that included exophthalmia, edema and kidney haemorrhage (Dupree, 1966). Common carp showed anorexia, faded body colour, fin and skin haemorrhages, abnormal gill opercula etc. (Aoe et al., 1968).

Hypervitaminosis A results in enlargement of liver and spleen, abnormal growth, skin lesions, epithelial keratinization, hyperplasia of head cartilage and fusion of vertebrae. Very high liver oil vitamin A content and elevated serum alkaline phosphatase also reported.

Dietary requirement:

Rainbow trout require 2000-2500 I.U. Carp 100-2000 I.U.

Sources and stability:

Codliver oil is the best source, though many other fish oils contain relatively high levels of the vitamin. Whale liver oil contains kitol, which has no biological activity until heated to 200°C, when one molecule of kitol yields one molecule of vitamin A.

Vitamin A is added to animal feeds as acetate, palmitate or propionate esters to enhance vitamin A stability (Adams, 1978). Approximately 20% of vitamin is lost during extrusion of foods. At room temperature, storage loses is about 53%. Some fish species seem able to utilize -carotene as a vitamin A source; whereas others are unable to split the -carotene molecule and vitamin A must be added to the diet.

VITAMIN D

Vitamin D2 or ergocalciferol and vitamin D3 cholecalciferol have vitamin D activity. Vitamin D3 which is also called 7-dehydrocholesterol has the chemical formula C27 H44 O. It is formed in most animal tissues by the rupture of one of the ring bonds of 7-dehydrocholesterol by ultraviolet radiation.

Vitamin D is essential for maintaining calcium and inorganic phosphate homeostasis. It is also involved in alkaline phosphatase activity. Although fish can sequester calcium from water through the gill membrane, a need for dietary vitamin D has been demonstrated for fish (Barnett et al., 1979).

Symptoms of deficiency or excess:

Qualitative requirements for cholecalciferol have been determined for channel catfish and rainbow trout. Lovell and Li (1978) demonstrated the essentiality of dietary cholecalciferol for channel catfish fingerlings for greater weight gain and bone mineralization. Vitamin D deficiency in diet induced reduced weight gain; lower body ash, lower body phosphorus and lower body calcium compared with controls. Barnett et al. (1979) established the essentiality of cholecalciferol for rainbow trout fingerlings using two dietary concentrations. Symptoms of cholecalciferol deficiency included decreased weight gain and feed efficiency, marked increase in plasma triiodothyronine (T3) levels, lethargy, anorexia, increased lipid content of white muscle and liver and clinical signs of tetany. In rainbow trout tetany of the white muscle fibers has been observed (George et al., 1979).

Hypervitaminosis in brook trout showed impaired growth, lethargy and dark colouration. High intake of vitamin D mobilizes phosphorous and calcium from the bone and tissue and may result in fragile bones.

Dietary requirements

Relative efficacy of dietary ergocalciferol compared with dietary cholecalciferol was examined in channel catfish fingerlings (Andrews et al., 1980) and rainbow trout fingerlings (Barnett et al., 1979). Based upon weight gain channel catfish fingerlings (2.3 g) were reported to require dietary cholecalciferol at greater concentrations than 1000 I.U./Kg dry diet; but less than 4000 I.U./Kg dry diet. But slightly larger channel catfish require dietary cholecalciferol at greater concentrations than 1000 IU/KG dry diet but less than 2000 IU/KG dry diet. In channel catfish hypervitaminosis occurred at 50,000 IU/Kg of ergo or cholecalciferol as evidenced by reduced weight gain and feed efficiency.

Leatherland et al. (1980) reported an inverse relationship between T3, A growth stimulating hormone and dietary vitamin-D concentration (cholecalciferol or ergocaleiferol) in rainbow trout fingerlings. Barnett et al. (1979b) reported that rainbow trout fingerlings require between 1600 and 2400 IU of cholecalciferol/Kg dry diet and that cholecalciferol is three times more effective than ergocalciferol in promoting weight gain.

Sources and stability:

Fish liver oil is a rich source of vitamin D. Shark liver oil contains about 25 I.U./g of vitamin D; Codliver oil 100-500 I.U./g and albacore tuna liver oil 200000 I.U./g. One international unit (I.U.) is equal to 0.025 mg of crystalline vitamin D. In feeds it is added as a spray dried powder. Processing and storage losses are not clearly defined.

reported (Murai and Andrews, 1979). Addition of 25 mg/kg tocopherol plus 125 mg/kg ethoxyquin or 100 mg/kg -tocopherol prevented symptoms associated with oxidized menhaden oil. In common carp feeds with oxidized silk-work caused a disease "Sekoke disease" which is characterized by a marked loss of flesh on the back of the fish. This was prevented by supplementing diet with 500 mg/kg dl- -tocopherol acetate (Hashimoto et al., 1966).

Adult female common carp (100 g) displayed reduced weight gain, lower gonadosomatic index, apparent muscular dystrophy, higher muscle water content, lower muscle protein content and lower concentrations of yolk granules (Watanabe and Takashima, 1977).

Dietary requirements:

Channel catfish fingerlings - 25 to 100 mg/kg Dl- -tocopherol. Common carp adults 700 mg -tocopherol/ kg dry diet. Common carp fingerlings (6.4 g) require about 300 mg/kg-dry diet. Larger rainbow trout fingerlings (10 g) require about 20 to 30 mg where as small fingerlings (0.9g) require 50 mg/kg.

Quantitative requirements depend upon interaction of several factors (1) Dietary concentration of polyunsaturated fatty acids (2) dietary selenium concentration (3) dietary concentrations of proxidants and antioxidants (4) diet storage temperature and (5) length of diet storage.

Sources and stability:

Vegetable oils are rich sources. Synthetic -tocopherol in esterified acetate or phosphate form is commonly used as a diet supplement. Considerable losses of vitamin E occur during processing and storage particularly

VITAMIN E

Vitamin E is composed of a class of compounds known as tocopherols. One of the most important tocopherols is - -tocopherol. Tocopherols are stable to heat and acids, but are rapidly oxidized in the presence of nascent oxygen, peroxides or other oxidizing agents and ultraviolet light.

The tocopherols act as extracellular and intracellular antioxidants to maintain homeostasis of labile metabolites in the cell and tissue plasma. As physiological antioxidants, these protect oxidizable vitamins and labile unsaturated fatty acids. Tocopherols along with selenium and vitamin C provide normal reproductive activity and in the prevention of muscular dystrophy in yellowtail and carp. They also act as free radical traps to stop the chain reaction during peroxide formation and stabilize unsaturated carbon bonds of polyunsaturated fatty acids. This vitamin has been shown to be important in reproductive physiology of fishes.

Deficiency and excess symptoms:

In chinook salmon, poor growth, exophthalmia, ascites, anaemia, clubbed gills, epicarditis and ceroid deposition in the spleen reported (Woodall et al., 1964). In brook front fingerlings reduced growth rates, increased mortality and lower microhematocrit values have been reported (Poston, 1965). In Atlantic salmon anemia, pale gills, anisocytosis, poikilocytosis, exudative diathesis, dermal depigmentation, muscular dystrophy and increased carcass fat and water content.

In channel catfish poor growth, reduced food conversion, exudative diathesis, muscular dystrophy, depigmentation, fatty livers, anemia and atrophy of pancreatic tissue

in hot climates (Adams, 1978). Vitamin E acetate is however, stable during feed preparation and storage.

VITAMIN K

Vitamin K is required for normal blood clotting mechanisms. Menadione or vitamin K3 is a synthetic-product used to supplement animal diets. Though fairly stable, it can be destroyed upon oxidation and exposure to ultraviolet radiation.

Vitamin K is involved in hepatic synthesis of blood clotting proteins - prothrombin and proconvertin.

Dupree (1966) reported haemorrhages in channel catfish fed a vitamin K-deficient diet. However, Murai and Andrews (1977) were unable to demonstrate any requirement for 30 weeks. In salmon prothrombin time, was increased five times and prolonged deficiency states lead to anaemia and haemorrhagic areas in gills, eyes and vascular tissues (Halver, 1980).

Sources and stability:

Green and leafy vegetables, alfalfa leaves soybeans and animal liver. Vitamin K found in alfalfa is fairly stable. Synthetic components added to feeds are either menadione sodium bisulfite (50% K3), menadione sodium bisulfite complex (33% K3) (Adoms, 1978). Basic pH, heat, moisture, and trace minerals increase the rate of destruction of menadione salts in pelleted feeds.

TABLE 1: Halver's Water-soluble vitamin test diet H-440 for fish

Complete test diet	(g)
Vitamin-free casein	38
Gelatin	12
Corn oil	6
Cod liver oil	3
White dextrin	28
-cellulose mixture	9
-cellulose 8	
vitamins 1	
9	
Mineral mix	4
Water	200
	300

Vitamin mix	(mg)
Thiamine HCl	5
Riboflavin	20
Pyridoxine HCl	5
Choline chloride	500
Nicotinic acid	75
Calcium pantothenate	50
Inositol	200
Biotin	0.5
Folic acid	1.5
L-Ascorbic acid	100
Vitamin B_{12}	0.01
Menadione (K)	4
-Tocopherol acetate	40

Dissolve -tocopherol in oil mix.

Add vitamin B_{12} in water during final mixing

Mineral mix (mg)		
USP XII	No.2	: 100 g
Al C13	15	
Zn SO4	300	
Cu Ci	10	
Mn SO4	80	
KI	15	
CoC12	100	

per 100 g of salt mixture

USP XII No. 2	(g)
Calcium biphosphate	13.58
Calcium lactate	32.70
Ferric citrate	2.97
Magnesium sulphate	13.20
Potassium phosphate (dibasic)	23.98
Sodium biphosphate	8.72
Sodium chloride	4.35
	99.50

Contd..

Diet preparation:

- Dissolve gelatin in cold water
- Heat with stirring on water bath to 80°C.
- Remove from heat
- Add with stirring - dextrin, casein, minerals, oil and vitamins as temperature decreases. Mix well to 40°C. Pour into containers. Move to refrigerator to harden.
- Remove from trays and store in sealed containers in refrigerator until used.
- Consistency of diet adjusted by amount of water in final mix and length and strength of heating.

Technical Paper-11

SUMMER INSTITUTE IN

RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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NUTRITIONAL BIOENERGETICS IN FISHES AND SHELLFISHES

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Matter or energy can neither be created nor destroyed but can be converted thus says the second law of thermodynamics, which is the cardinal concept in bioenergetics. In nutritional bioenergetics also known as physiological bioenergetics we are concerned about energy entering into the organism as food and its partitioning into various forms of work. The quality of food consumed is also known as ration (C). It is possible to draw up a balance sheet or energy budget, which in a very simple form can be given as

$$C = P + R + U + F.$$

where C stands for consumption, P for growth, R for metabolism, U for non-faecal excretion, F for excreta as faeces. The partitioning can be well be explained in the form of a energy flow chart.

FORMULAE AND INDICES USED

Since we are not concerned at present with ecological bioenergetics let me summerise briefly indices useful in nutritional bioenergetics. For fuller list of formulae also take into consideration these given in the chapter on proteins.

1. Gross conversion efficiency (k_1) (%) $= \frac{P}{C} \times 100$

Herein we take the parameters in dry weight for the sake of uniformity. In the field this parameters is difficult to obtain. Therein we use FCR.

2. Food conversion ratio (FCR) $= \frac{\text{Food (fed) offered}}{\text{weight gain}}$

FCR is used in a highly arbitrary manner. Some use both in dry weight, while some one of the parameters in dry and the other in wet weight and still some authors use both in wet weight.

3. Assimilation efficiency $= \frac{C-F \times 100}{C} = \frac{A}{C} . 100$

or

digestibility (%)

4. Net conversion efficiency $= \frac{P}{A} \times 100$

K_2 (%)

where A = C-F

5. True digestibility (%) $= \frac{C-(F-MFN)}{C} . 100$

While MFN - Metabolic faecal nutrient (endogenous nutrient)

6. Digestibility of a nutrient % $= 100 - \left\{\frac{\text{100\% indicator in food}}{\text{\% indicator in faces}} X \frac{\text{\% nutrient in faeces}}{\text{\% nutrient in food}}\right\}$

7. Total digestibility Assimilation $= 100 - \left\{\frac{\text{100\% indicator in food}}{\text{\% indicator in faeces}}\right\}$

8. Trophic coefficient $= \frac{C}{P}$

9. Partial growth efficiency (%) $= \frac{P}{C-m} - 100$

m = maintenance ration

10. Body weight gain (%) $= \frac{Wt - Wo}{Wo} \times 100$

Wo and Wt are live weight at the time of starting the experient and at the end of the experient for that duration of days for the size used.

11. Nutrient retention (%) $= \frac{\text{Nutrient gained}}{\text{Nutrient consumed}} \times 100$

12. Optimum ratio of dietary energy (DE) to protein (P)

$$(\text{DE/P ratio}) = \frac{\text{(Kcal/Kg dry diet)}}{\text{\% dietary protein}}$$

13. Metabolizable energy (Kcal/g of diet) $= \frac{Ae\ E - Ue\ (Ap\ P - Gp)}{C}$

Where

Ae = apparent digestibility for energy (%)

E = Total energy for the quantity of consumed food (Kcal)

Ue = Energy loss of nitrogenous products per gram of protein deaminated (Kcal/g protein)

Ap = apparent digestibility for protein (%)

P = quantity of protein in the consumed diet (g)
(% protein diet X feeding rate in % live weight ÷ 100)

Gp = quantity of protein retained as growth (g)
(protein balance ie. assimilated protein -
(non-faecal (exogenously excreted nitrogen) X 6.25)

C = weight of food consumed (g)

14. Specific growth rate (SGR) (% body weight per day) $= \frac{\ln Wt - \ln Wo}{t} \times 100$

where t is duration in days.

15 In grown up organisms (Eg. large fishes) growth rate (k) is constent (log Wt - log Wo/t = k)
in such cases mean daily growth per day in percentage body weight ($\bar{P}$) is calculated by the following formula

$$\bar{P} = \frac{2(Wt - Wo)}{t\ (Wt + Wo)} \times 100$$

15 b. When the growth changes in short intervals

$$\bar{P} = \left\{ 10^{\frac{1}{t}\ (\log Wt - \log Wo)} - 1 \right\} \times 100$$

16. Average food consumption per day in percentage body weight $\bar{C} = \frac{2C}{(Wt + Wo)\ t} \times 100$

17. In case of mortality occurring to the experimental organisms during the course of the experient food consumption ratio (r) is useful

$$r = \frac{C}{(\bar{W}t + D) - \bar{W}o}$$

Where D is the total weight (wet weight) of dead animals (g), C is the total quantity of food consumed (g) and $\bar{W}o$, $\bar{W}t$ are average initial and final weight of fishes (g).

CONSUMPTION

The forms of feed used in nutritional studies can be classified roughly under the following heads.

i. Live feed
   - i. Algal) Plain or enriched
   - ii. Animal)
   - iii. Algae & Animal mixed

ii. Test diets
   - i. Purely plant origin (dried algae, single cell protein)
   - ii. Animal tissue meal (silk worm, pupae, etc.)

iii. Compounded (formulated) diet

a. Dry - pelleted diet
b. Semi - dry diet (crumbles)
c. Moist diet (dough)
d. Encapsulated diet
e. Particulated (bound) diet

Consumption is the quantity of food eaten by the experimental organism during the unit time, usually per day. Though experimenters use weight,units in terms of calories or gram protein is preferred. Fin fishes and shell fishes use protein and lipid as the major energy sources rather than carbohydrate and lipids. Thus protein is used by the organism as a source of energy on being deaminated. Therefore by many in the budget protein units alone are used either as gram protein or as nitrogen units. Much of blood glucose is derived from gluconeogenesis rather than from carbohydrates. Optimum protein/calorie ratio for salmons has been worked out to be 100 mg protein/digestible Kcal in food, while in mammals it is only about 70 mg. For both carnivorous and omnivorous fish lipids are the principal non-pool energy source. In the chapter on protein-requirements a critical discussion was made on the importance of using protein over calories as a sole unit of measurement. Calorie is the amount of heat required to heat 1 g water by 1°C at 15°C. 1000 calories (also known as gram - calorie or small calorie) make one Kcal (large calorie). In older literatures small and large calories used to be written as calorie and Calorie. Now a days kilojoules is preferred over kilo calories. One kilojoule (KJ) is 4.184 Kcal, and similarly 1 calorie = 4.184 joules. Various methods of determination of calorie values is being dealt with else where in this manual. Calorie or the heat unit is the only unit which can be used as the single unit for protein, carbohydrate and fat put together and

independently.

Methods:

Consumption can be measured directly only for a few organisms, for example in the large carnivorous fishes and in the carnivorous squids from the number and weight of the whole fish eaten per day, consumption can be easily estimated. But for the organisms of other feeding types it is not so easy. The method being followed in fishes to estimate total consumption is by gastric evacuation, herein the average rate at which a fish passes food out of its fore gut is taken as the rate of consumption. For this purpose either the serial sacrifice method or radiographic method is followed. In the former from a batch of fishes after feeding, at time interval one fish is killed and the movement of food within the alimatory canal is watched. This involves killing of many test fishes. In the radiographic method either the fish is X'ray photographed at time intervals after feeding with plain food or feeding with food mixed with a radio opaque substance like iron powder of 100-200 µm at the rate of 4% W/W with 20% water or with barium sulphate at 20% W/W level.

In the third method the organism is fed with a radioactivity substance; either the loss of radio activity from the medium or percentage activity of the whole organism over that of the food offered gives the clue for the quantity of food consumed.

The most popular method is by the use food marker. The important criteria of a substance to be used as the marker is that it must be thoroughly resistant to digestive enzymes, non toxic, easily determinatable, should not overtake or undertake the feed through the passage and well acceptable by the organism. Usually acid and distilled

water washed fine power of $Cr_2 O_3$, a green substance is used. The marker is well mixed with the diet at about 2% level or less and the organism is feed at libitum level to satation. The faecal matter alone is carefully collected. The quantity of $Cr_2 O_3$ is determined usually by calorimetric method. The determination is as follows.

Eg: $Cr_2 O_3$ in the feed - 2%

$Cr_2 O_3$ in the faeces - 180 mg

$$\therefore \quad \frac{100 \times 0.180}{2} = 9 \text{ g}$$

Food consumed = 9 g

Other makers used are ammonium molybdate, hydrolysis resistant organic matter (HROM), hydrolysis resistant ash (HRA), crude fibre (CF), titanium (IV)-oxide, mineral elements like iron powder, polyethylene, magnesium ferrite, $^{144}CeCl$, lignin and colourents.

Many times digestibility of the organisms in nature can be calculated by picking out an indicator substance present already in the diet. For example the ratio of silica and organic matter in the diet and in the faecal matter can be used in the determination of digestibility and even for the estimation of consumption if one can calculate the ratio in the surrounding, say in the surface film for a detritivore. The Silica : organic matter ratio in the consumed diet can be calculated by collecting a few well fed animals at random from the field and chemically estimating the stomach contents. Shorter the duration between feeding and removal from the stomach is ideal. To collect the stomach contents from the stomach free from contamination by the gut tissues, the fresh animals can be kept in the deep freezen for a duration allowing the gut contents to become solid (ice) and by

opening the stomach without thawing. Thus the stomach contents can be secured as a solid mass. Latter it can be dried for the sake of consistency of the value and the ratio of silica:organic matter expressed in terms of dry weight. Similarly the faecal matter is also collected, concentrated by centrifuging, dried and analysed.

Fifthly consumption can be calculated from the balanced equation where one has estimated all other parameters. This method is also known as physiological method, where,

$$P + R + U + F = C$$

For the filter feeders consumption can be calculated from the rate of filtration.

Pandian and Vivekanandan (1985) have worked out that in fishes, feeding rate in percentage body weight has a direct bearing on the latitude of the habitat. Thus fishes which live between 70°-27°N (temperate region) consume about 1.8 to 17.3% body weight per day with a mean of 5.9%, while tropical (21° - 7°N) fishes consume 4.1 to 36 % with a mean of 16.7% body weight per day. Thus the feeding rate of tropical fishes over that of the temperate is about 180% greater. It is been calculated that tropical fishes .. .. incur an energy expenditure of 2.1 KJ/kg/hr while temperate spend only 1.2 KJ/kg/hr.

Fisher et al. (1973) have calculated consumption for the grass carp in the form of a regression formula under laboratory conditions as follows:

For animal and vegetable - mixed diet

$$C = 1.06.W^{0.81}$$

For exclusive vegetable food

$$C = 0.30.W^{0.81}$$

Where weight is in grams and C in calories per day.

The time taken for an organism in foregoing and to stop its feeding is known as the time taken for the animal to get satiated or satation time. Filter feeders usually have a prolonged satation time, while the carnivorous a short one. In the bivalves it has been found that the feed particles need to be at an optimum concentration known as critical cell density (CCD). At higher concentration they take very little into the oesophages and more of the food particulars are encased in the muces and sent out as pseudofaeces.

The critical cell density (CCD) at which the ingestion system is saturated for bivalves varies with particle size. Thus for food particles of sizes of 5 µm; 5-75 µm, 7.2-9.4 µm; 7.5-10 µm, 40-50 µm and 60 µm CCD in terms of X 10^{-6}/litre works out to be in the order 450, 60, 35-40, 50, 20-30 and 2. Thus CCD decreases with larger food particles (Newell, 1979).

Crustaceans, esp., in copepods when food is supplied at above optimum levels they resort to superfluous feeding. The algae taken into the gut passes out unaltered.

The average ingestion rate per day for Crassostrea virgenica works out to be 3.8% body weight per day while for Penaeus setifuous 9.3% and for Mugil cephalus 3.2%. The benthic molluscs have found to process the sediments at the rate of 0.7-200 g/m^2/yr. While for Clymenella torquata it is 3288 g/m^2/yr (Newell, 1979).

In Metapenaeus monoceros juveniles of about 27 mm consumption is about 12 percentage body weight per day (Qasim and Easterson, 1974) for natural detritus. Consumption in % body weight is high in fast growing animals and in those whose metabolic rate is high. Thus juveniles have higher rate over

that of the adult. Consumption rate also is bound to increase with increase in temperature upto an optimum. Consumption is also high in animals feeding on low nutritive substances. Thus largest values are met with detritus feeders and animals which live on ooze. These organisms practically feed continuously.

ASSIMILATION (A)

That portion of the nutrient not excreted as faeces out of the quantity consumed is known as assimilation. Other equivalent terms used are absorption and digestibility. In the earlier part of this chapter the formulae need to be used in estimating total digestibility and digestibility of a specific nutrient is given when an indicator is used in the diet. When the quantity of metabolic nutrient excreted along with the faeces is deducted from the faecal nutrient in the calculation the coefficient obtained is known as true digestibility. When no correction is made for release metabolic nutrient into the faeces the coefficient is called as apparent digestibility. Though $Cr_2 O_3$ (Chromium oxide) is used commonly as the indicator substance the use of titanium oxide has the advantage in the protein studies, wherein TiO_2 can be determined directly in the kjeldahl digest (Njaa, 1961).

Removal of faecal matter is the subject of many authors. Still date there is no foot proof method available. The use of resin to trap liquid metabolic wastes is not useful while working in a saline medium as in the case of brackish and sea water animals.

The Practice followed in our studies for the collection of faeces is as follows. In order not to agitate the water

always the experimental tanks are not aerated heavily. When the water used is well aerated and in the room too where enough of forced air is available during day time aeration can even be dispensed with. Aeration is critical at night usually after midnight. Usually around 3 A.M. it is at this time it has been found that oxygen exchange is minimum between air and water. The faecal matter is very carefully siphoned out using wide mouthed, pipettes having fine polished tip. Thus removed faecal matter is transfered on to a very fine meshed bolting silk which has been retained over the mouth of a wide mouth beaker using a rubber band. The sea water drops into the beaker. The faecal matter is always kept at the centre of the netting. After the collection is over little quantity of distilled water is poured drop-wise at the periphery of the beaker mouth. Thus droped distilled water used to slide to the centre and after removing the salt from the adhering sea water drops into the beaker. Latter the netting is removed turned upside down keeping the central portion containing faeces over the mouth of the preweighed wide mouthed clean container. Just a drop or two of distilled water dropped from a little distance over is sufficient to remove the entire lot from the netting into the container. Then the faeces along with the container is dried on a hot air over at 55°C. Thus pooled faeces is used for analysis.

It is also possible that a little of liquid matter also excreted along with the faeces. Elliott (1976) has found out this quantity of organic matter to be of the order of 4% or less to the total faecal matter. Therefore the error arising out of this is small (Brafield, 1985).

Winberg (1956) is of the opinion that total assimilation efficiency is around 80% (P+R). According to Phillips (1972) it is 90% for protein, 85% for lipid and

40% for starch. All for a normal diet. Carbohydrates of cellulose type are not digested well, except in some herbivorous fishes, wherein the cellulolytic microorganisms present in the gut does the job. Schaeperclaus (1933) is of the opinion that carbohydrates are digested 30-90% by the omnivorous carp. For grass carp total assimilation is less than 13%. For typical carnivorous organisms it can be over 70%. Usually animal proteins are assimilated well; beef heart 96%, white fish meal 92%, casein 99%, and fish protein concentrate over 90%. Assimilation of protein of vegetable origin is slightly lower, in silver carp (Hypophthalmichthys molitrix) total assimilation efficiency is 73-93%, while for gold fish (Carassius auratus) 54-63%. At some special situations and for feeds, value over 90% is possible. At optimal level assimilation efficiencies of 57-80% are highly likely. The assimilation efficiency for a nutrient various with the concentration of the nutrient in the diet and feeding level. Physiologically also depends on the physiological status of the organism, feeding habit, temperature of the medium and special situation in the life history like, maturation, spawning, incubation period of the egg etc., when the particular nutrient is available at low level assimilation for that has been observed to be high. So also when limited feeding regimen is used assimilation is high. Therefore at ad libitum feeding only assimilation efficiencies should be calculated. When assimilation is generalised assimilation efficiency for test and experimental diets are to be taken into account separately with that of the organism's natural diet. Since A% is either bound to be high or low with test diets, which may or may not be of the organism's liking.

ENERGY - TOTAL, DIGESTIBLE AND METABOLIC

Total energy:

The total energy content of the biological matter (ash free) is measured by total combustion in the bomb caloriemeter. Thus bomb caloriemetric value for the total substance gives the total energy in it, when we need to know how much calorie out of the total is from protein, carbohydrate and fat we need to estimate using conversion factors for the available quantity of nutrients. The generalised conversion factors for carbohydrate, fat and protein for one gram of substance are 4.10 Kcal (17.2 KJ); 9.45 Kcal (39.5 KJ); and 5.65 Kcal (23.6 KJ) respectively. These values are biased towards mammalian tissue nutrients. The mammalian lipid is mainly saturated in nature, while that of marine/aquatic organisms are highly unsaturated. Therefore for the lipids of finfishes and shellfishes 8.50 Kcal (35.56 KJ) per gram is preferred, while specifically working with fish lipid 8.65 Kcal (36.2 KJ) per gram is more appropriate (Brafield, 1985).

The above given value for protein is correct only when protein, free from non-protein nitrogen is used. Jobling (1983 b) quotes that non-protein nitrogen in the salmonid muscle tissue can be about 2% of total nitrogen while for elasmobranchs it is as high as 38%. Thus conversion of total kjeldahl nitrogen into protein will give erraneous caloric value.

Though between different purified carbohydrates the combustion values differ significantly -3.74 Kcal/g for glucose and 4.23 Kcal/g for starch - the values given above is generally acceptable while using for tissue carbohydrates.

Digestible energy:

The terminology used for the part of energy digested from the energy nutrients varies between different authors. Brett and Groves use the term physiological energy while others use the term metabolisable energy. I am the one who prefer the term digestible energy, since it is self-explanatory. Digestible calorie = heat of combustion value X % digestibility ÷ 100. Since digestibility is highly variably it is better that actual digestibility for each nutrient is used. The mean digestibility values for carbohydrate, fat and protein are 40%, 85% and 90% respectively (Philips, 1972). This is a highly generalised opinion.

Metabolic energy (ME):

Often ME is also termed physiologic energy. ME is calculated based on respiratory (oxygen consumption) studies - viz., Respiratory Quotient (RQ = CO_2 liberated/O_2 utilised) and Oxycaloric (oxycalorific quotient) values (Q_{ox} = caloric liberated/unit O_2 utilised). The type of physiological fuel used as energy source can be estimated from RQ and the quantity of non-faecal excretion.

Under total aerobic conditions RQ for carbohydrate and fat are 1.0 and 0.7 in the order. For ureotelic organisms RQ for protein is around 0.82 and about 0.90 for ammonotelic organisms. Nitrogenous wastes are excreted either as ammonia, or as urea or as uric acid, alone or a mixture of the above and in many along with creatinine and amino acids. Marine teleosts excrete along with a little quantity of trimethylamine oxide (Brett and Groves, 1979). Further proteins contain sulphur at about 1% level. Since Q_{ox} varies with the end products of catabolism, all these varied excretory products of protein make

Table 1 - Energy equivalents used - bioenergetics (Brutt & Groves, 1979)

Nutrient		Energy of food and body resources: Food: Heat of combustion (kcal/g)	Food: Digestible energy kcal/g	body: Metabolic energy kcal/g	Respiratory energy equivalents Oxycalorific values: Qox kcal/lit. O_2	Qox cal/ mg O_2	Respiratory quotient CO_2/O_2 Ratio
Carbohydrate	Mammal	4.10	4.0-3.2	4.10	5.04	3.53	1.0
	Fish	4.10	3.3-1.6	4.10	5.04	3.53	1.0
Fat	Mammal	9.45	9.0	9.45	4.69	3.28	0.71
	Fish	8.66	8.0	9.45-8.66	4.69	3.28	0.70
Protein	Mammal	5.65	4.2-3.9	4.70	4.82	3.37	0.81
	Fish	5.65	4.5-3.9	4.80	4.58	3.20	0.90
Mixed	Mammal	5.95	-	-	4.86	3.40	0.83
	Fish	5.89	-	-	4.63	3.25	0.90

calculation of RQ and Q_{ox} for protein complicated. Brafield and Llewellyn (1982) present the production of ammonia from 100 g of protein as follows: (4.42 C, 7.00 H, 1.44 O, 1.14 N) + 4.6 $O_2 \longrightarrow$ 1.14 NH_3 + 4.42 CO_2 + 1.79 H_2O.

RQ values of above 1.0 is obtainable during active fat synthesis and RQ 1-2 is possible when anaerobic respiration is resorted to (Kutty and Mohammed, 1975).

1 mole of glucose on complete combustion will liberate 677.1 Kcal (2833 KJ) of energy utilising 6 mole of oxygen (ie 192 g). Therefore Q_{ox} for glucose (carbohydrate) is 14.76 KJ/g or 3.53 Kcal/g oxygen consumed. Lipids differ in the order of saturation and the generally accepted Q_{ox} value is 4.63 Kcal/liter O_2.

For mixed diet Brett and Groves (1979) suggest 4.63 Kcal/liter of O_2 while others are of the opinion that it is 4.8-5.0 Kcal/liter O_2 as Q_{ox}. (Table - 1 & 2).

Jobling (1983 b) suggest the following semi-direct method for the measurement of 'potential' metabolisable energy based on values of total energy, digestibility and incorporating the assumption that nitrogenous excretion is in the form of ammonia, wherein 0.95 Kcal is lost per gram of protein catabolised.

Thus ME (Kcal/g) = Digestibility coefficient of energy X total energy content of the diet (Kcal/g) - 0.95 X digestibility coefficient for protein x dietary protein content on a relative weight basis (g/g).

He also is of the opinion that digestible energy for carbohydrates and fats are as good as ME. Therefore 'potential' ME for protein, carbohydrate and fat are as follows. Following Brody (1945) he has taken for protein energy lost in nitrogenous excretion as urea 1.3 Kcal/g.

ME of protein (Kcal/g)	= (5.65 - 1.3) X 0.90	=	3.9
ME of lipid (Kcal/g)	= 9.45 X 0.85	=	8.0
ME of carbohydrate (Kcal/g)	= 4.10 x 0.40	=	1.6

METABOLISM (R)

Metabolic rate in fishes and crustaceans is distinguishable into 5 major categories. Basal metabolism that is the metabolic rate at physiological rest at optimum oxygen saturation is difficult to measure in aquatic organisms. The available dissolved oxygen in aquatic and terrestrial animals is in the order of 10 : 10,00,000 by weight (Priede, 1985). Therefore the term standard metabolism is used.

1. Standard metabolism (R_s) - metabolic rate at minimal maintenance or resting metabolism of an unfed fish below which physiological function would be affected someway. This value is arrived at from nos. 2 & 3 by curve fitting as intercept.

2. Routine metabolism or ordinary metabolism (R_r) - metabolic rate of the animal during its normal spontaneous activity.

3. Maximum sustained metabolism (R_{max}) - maximum metabolic rate for the animal for a sustained maximal activity. Usually obtained at maximum exercise speed.

4. Active metabolism (R_a) - metabolism related to swimming and to stress.

5. Internal heat increment (R_f) - use of energy for SDA. (Feeding metabolism)

Scope for activity or metabolic scope is the difference between $R_{max} - R_s$; and varies with stage of development, environmental factors such as temperature and species. Always metabolic rate is calculated to a standard temperature and pressure, since it involves, physiology of the animal and gases viz., O_2 and CO_2.

During short burst of muscular activity (glycolysis) fish can greatly exceed R_{max}. During this short burst of glycolysis, the glycogen reserve in the skeletal muscle is depleted and the level of lactate increases resulting in oxygen debt. Similarly R_s is not the absolute lowest limit of metabolism. During low oxygen tension in the medium metabolic rate may be depressed and tissues could function anaerobically for a short duration, which also results in oxygen debt.

As given above the aquatic cultivable organisms need to meet their metabolic demands over R_s within the scope for metabolism. Though the enhanced metabolism due to increased locomotor activity connected with chasing of prey, escape from enemies is ecological one. Physiological metabolic demands are specific dynamic action-(specific dynamic effect) also known as heat increment-and physiological stresses (for eg. osmotic stress). As in the beginning of this chapter it is pointed out energy is partitioned. When the organism spends energy in metabolism over that of routine metabolism, it needs to spend it from the metabolised energy. When this excess is not spent, this part of energy too would have gone for growth. Thus metabolic demand

and growth are competitive. The organism many times need to compromise growth for its metabolic energy needs.

SDA: Specific dynamic effect or heat increment is increase in heat production observed following a meal. This includes metabolism associated with gut motility, and general post feeding activity. It is difficult to measure by direct calorimetry and so is known as apparent SDA by Beamish (1974). SDA has been found to be greater if the diet is rich in protein. Therefore it is also thought SDA is energy of deamination. Estimates for SDA are reported to be 4-45% of the total energy of ingested food. Some are of the opinion it is 5-20% and most authorities feel it is 9-15% of the consumed energy (Brett and Groves, 1979). Jobling (1983 a) suggest that a close relationship exists between thyroid hormone secretion, protein synthesis and metabolic rates. Thus SDA is energy used in protein synthesis and directly associated with the activity of thyroid hormone, in otherwords it is the inescapable cost of growth.

SDA is usually calculated thus $SDA = M - (M_s + M_e)$ where M is metabolic rate of just fed ones, M_s - metabolic rate of starved animals and M_e - the elevated metabolic rate due to feeding.

Energy spent on activity

Oxygen consumption is always related to the body weight of the organism. In the regression equation $R = a . W^b$, R is oxygen consumption per unit time at optimum oxygen tension, W is the live weight of the organism, 'a' is intercept which is equal to R of an organism of unit weight (ie. 1 g), and 'b' is the constant that indicate at which speed and in which direction R changes with increase in weight. As explained earlier in the respirometers routine

metabolism is measured rather than standard metabolism. In order to compare the regression formula oxygen consumed is standardised to standard temperature and pressure. Further 1 liter of O_2 consumed is equivalent to 4.63 Kcal. Thus R (where $R = R_r$) can be calculated for 24 Hrs. In nature ie. in the farm active animals like fishes are never in any one of the stage always. That is, they are neither at rest (R_s) nor at (R_r) nor very active (R_{max}) throughout. They are at all these rates at many times for different durations. Now our problem is how to estimate this actual metabolism per day. Winberg (1956) after pooling up all the available data on respiratory studies of his time proposed the following equation for routine metabolism.

$R = 0.56 \cdot W^{0.81}$ - Fish and crustaceans

$R = 0.297 \cdot W^{0.81}$ - Freshwater fishes

$R = 0.266 \cdot W^{0.87}$ - Marine fishes

$R = 0.3 \cdot W^{0.8}$ - for freshwater & marine fishes

These are also known as Winberg's basic equation. The interesting factor is that value for b is more static. According to Fry b varies between 0.5 - 1.0 and Kleiber is of the opinion it is around 0.75. Thus Winberg's basic equation stands for the average position. Winberg then proposed that the actual metabolism for fish per day is twice that of routine metabolism. Mann (1965) confirmed this preposition to be practically good in this studies on the fishes of Thames.

In this connection the following facts need to be pointed out. The metabolic rate of young ones are higher and that of the starved fishes is decreased. It is of interest to note that eels on starving for 3 months did keep respiratory (metabolic) level at the same level that

of fed ones (Inui and Ohshima, 1966). Fisher (1977) observed the similar fact in eel after starving for 400 days. It is proposed that they derive energy by gluconeogenesis.

URINARY LOSS (U)

Of all the parameters of the energy budget this is the only parameter which is least measured. The main reason is that quantitatively percentage loss of nutrients/ energy on consumption by non-faecal loss is very little and ~~the~~ due to the difficulty in the estimation of ammonia, the main (60 to over 90%) excretory product. The estimation of ammonia-a highly volatile substance-accurately over a period is difficult. Ogino et al. (1973) devised a flow through system in which outgoing water was passed through a column of Amberlite IR-120 H to absorb liquid nitrogenous wastes and latter to retrieve them quantitatively. This system is not suitable for saline media and further urea and taurine are not retained by Hagel (Cowey and Sargent, 1979).

Gerking (1965) and Iwata (1970) have calculated that endogenous excretion of nitrogen is approximately $W^{0.54}$ and $W^{0.9}$ (where W is live weight in gram) for fish - for blue gill sunfish and crussian carp. The few studies available on exogenous (dietary origin) nitrogen indicate that about 22 - 11.4% of the consumption (N) is lost as U. At times about 27% of nitrogen consumed is excreted as non-faecal nitrogen (Brett and Groves, 1979). From Brafield (1985) it is calculated that 5.2 to 9.4% of energy intake is excreted as U. Thus it is evident that U is about 7% of C.

Table 2 - Qox values as quoted by Brafield (1985)

	After Brafield & Llwellyn (1982)	After Elliott & Davidson (1975)	After Gnaiger (1983)
Carbohydrate	3.53 cal 14.76 J	3.53 cal 14.77 J	3.52 cal 14.72 J
Fat	3.28 cal 13.72 J	3.28 cal 13.72 J	3.29 cal 13.75 J
Protein to ammonia	3.19 cal 13.36 J	3.20 cal 13.39 J	3.34 cal 13.97 J
Protein to urea	3.25 cal 13.60 J	3.25 cal 13.60 J	3.27 cal 13.69 J

Generalisation of K_1 and K_2

Winberg (1956) generalises that fishes in nature assimilate 85% of ration and 80% of ration is available as (net) physiologically useful energy. He considered urinary loss is negligible and metabolic loss is 3.7 of C. Therefore $P + T = A = 0.8\ C$

$$\therefore\ C = 1.25\ (P + T) \qquad \text{where } K_1 = \frac{P \times 100}{C}$$

$$K_2 = 1.25\ K_1$$

The energy the growing embryo utilises for development/growth is considered to be the maximum net conversion (k_2) efficiency possible for total nutrients. This works out to 65% at maximum and never over 70% (Vijayaraghavan et al.,). Whereby K_1 can be theoretically maximum of 44-55%.

GROWTH AND OVERALL BUDGET

Moulting is the feature of growth in crustaceans. Whereby their growth in terms of length are in the form of stanzas. There is little work on the quantity of energy lost by moulting. The study conducted by Thomas et al., (1984) show that 0.60% of ingested nitrogen is utilised as moult.

When the organism neither shows increase nor decrease in weight on feeding, then the quantity of nutrient uptake is said to be at maintenance level (C_m). At levels lower than C_m the organism will show reduction in weight and the meat becomes high in water content. Then it is said to be in degrowth. At degrowth it will be utilising the synthesised body nutrients for its metabolic needs by the process of autolysis. Thus in Penaeus indicus at 25-30 mm size, below 10-12% dietary protein level at the

consumption rate of 11.4% body weight per day, though carbohydrate, lipid and other nutrients being at optimum show degrowth (unpub. data). Below this level the dietary protein is not sufficient for metabolic needs and such requirement need to be met by degradation of already formed tissues. Protein cannot be stored. The only little storage possible is by way of free amino acids. Thus when starved of protein the only way left for the fishes and shellfishes is to use its own tissue protein.

With the increase in ration the organism shows increased growth and with further increase in ration the growth does not show any increase. It becomes plateaued. From such a graph it is possible to identify as shown in the figure optimum and maximum ration size (Fig. 2).

Brett and Groves (1979) have come to the following energy budget for young fishes.

Carnivorous: $100\ C = 29\ P + 44\ R + 7\ U + 20\ F$

Herbivorous: $100\ C = 20\ P + 37\ R + 2\ U + 41\ F$

In the omnivorous shrimps the budget appears as follows for nitrogen.

Omnivore $100\ C = 1432\ P + 83\ (R+U) + 2.2\ F + 0.5$ moult

Concluding it should be emphasised that as shown above the fish need to budget its energy. When metabolic demands are too high it should meet it at the cost of growth. At times it need to meet even at the cost of feeding whenever it becomes the question of high SDA and metabolism (Priede, 1985).

Table 2 - Qox values as quoted by Brafield (1985)

	After Brafield & Llwellyn (1982)	After Elliott & Davidson (1975)	After Gnaiger (1983)
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Fat	3.28 cal 13.72 J	3.28 cal 13.72 J	3.29 cal 13.75 J
Protein to ammonia	3.19 cal 13.36 J	3.20 cal 13.39 J	3.34 cal 13.97 J
Protein to urea	3.25 cal 13.60 J	3.25 cal 13.60 J	3.27 cal 13.69 J

When growth response to diet is low one or a few of the following could be the cause.

1. Nutritional
    a. Food not enough
    b. Food not assimilable
    c. Food not palatable
    d. Low in essential factors
    e. Leeching out of nutrients

2. Physiological
    a. Rapid gonadial development
    b. Infection, Disease

3. Management stress
    a. Heavy stocking, competition
    b. Feeding schedule and strategy not suitable
    c. Improper form of diet and particle size
    d. Improper positioning of feed pellets
    e. Type and form of feed not suitable

4. Environmental stress
    a. O_2, temperature, pH, S °/₀₀ and pollutants.
    b. Engineering - system & design

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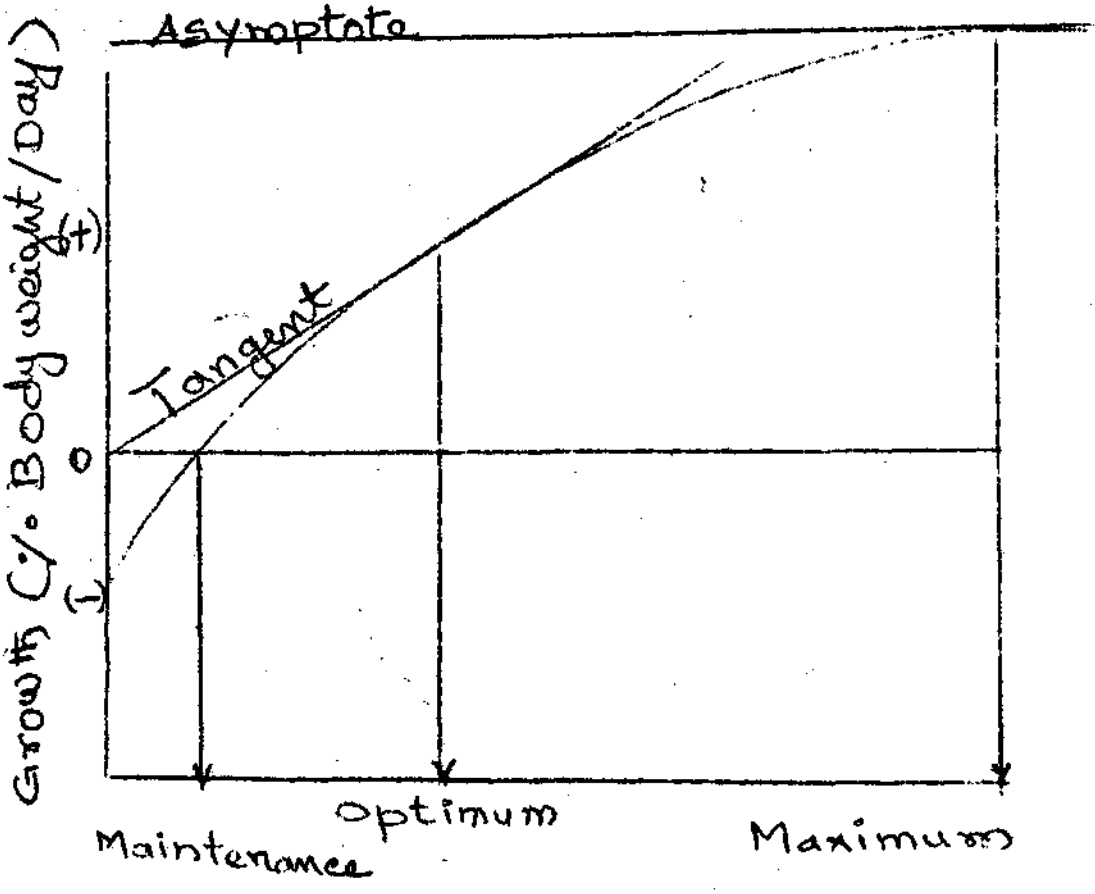

No food	C = 0	P = negative
At maintenance level	C = small	P = zero
At optimum level	C = optimum	P = good
	K_1 = maximum	
At maximum level	C & P are maximum	

Figure- 2. Showing the graphical method of finding out the levels of ration and meat production economics.

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Technical Paper-12

SUMMER INSTITUTE IN
RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION
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DIGESTIVE SYSTEM AND DIGESTION OF FOOD IN CULTIVABLE FINFISH AND CRUSTACEANS

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The digestive system in fish and shellfish shows much variation in structure and function and is specialised and adapted to suit specific diets. Variation in crustacean digestive system is mainly in the foregut which may be a simple passage way or highly complex chambered structure provided with triturating, straining and filtering mechanisms. In fish the length of the intestine is highly variable depending on the diet and the intestine-body length ratio is low in carnivores and very high in herbivores and detritivores. In cyprinids a true stomach is completely absent.

DIGESTIVE SYSTEM OF FISH

The alimentary tract of a fish consists of the mouth, oesophagus, stomach - if it is present -, intestine, rectum and associated glands like liver and pancreas.

There is no chewing or predigestion of food in fish mouth which serves for selection, seisure and orientation of food towards stomach. Dentition is developed to suit the feeding habits of particular fish and is highly variable. The buccal cavity has stratified mucoid epithelium which produce mucus to lubricate the food.

Oesophagus in fish is usually a short straight muscular tube leading from mouth to the cardiac stomach. The epithelium of oesophagus is folded and can distant to swallow large prey. Oesophageal sacs or oesophageal teeth are present in some fishes.

Usually the stomach is a sigmoid, highly distensible sac with numerous folds in its lining. Size of the stomach varies considerably and is related to the nature of food. Numerous blind diverticulae - the pyloric caecae - are present at the junction of pyloric stomachand anterior intestine in teleosts and these structures aids in digestion and absorption. The stomach is highly muscular and its wall is modified to deal with a particular diet in teleosts. The gastric mucosa is very mucoid with numerous glands at the bases of the folds. Detritivores like mullet and chanos stomach have a gizzard like that of a fowl and in cyprinids no true stomach is present, probably for quick passage of indigestible matter consumed in large quantity by these fishes.

The intestine is a very simple tube which is usually long and coiled in herbivores. The gut length-body length ratio is 0.6-0.8 in carnivores and 1.3-4 in herbivores, with silver carp and Labio sp. (Africa) having a ratio of 13 and 15-17 respectively. The intestine has a simple mucoid columnar epithelium overlying a submucosa with oesinophilic granule cells and limited by dense muscularis mucosa and fibroelastic layer.

Rectum in fish has a thicker muscle wall than that of the intestine and its lining is highly mucigenic and capable of considerable distension.

Liver is the most important digestive gland in fishes and its colour is reddish brown in carnivores and light brown in herbivores. In some fishes liver forms a compound organ with pancreas and is termed hepatopancreas. Hepatocytes are polygonal with distinctive central nucleus and a nucleolus and are usually swollen with glycogen and neutral fats in normally fed fishes. Under starvation the cells may shrink and the liver will be loaded with yellow ceroid pigments. Gall bladder is associated with liver and contains greenish yellow bile which is transported to the intestine via the common bile duct.

Pancreas is more variable in location even with in a single species, most common cites of which are among the fat cells in the mesentary of pyloric caeca and around spleen and hepatic portal vein. Pancreatic juice contain protease and the endocrine component of pancrease - the "Islets of Langerhans" - produces insulin.

Digestive tract of Malacostraca

The digestive system of malacostracans consists of the gut divisible into three distinct regions, its accompanying glands, caecae and diverticulae. The fore and hind guts are derived from the embryonic ectogerm and lined with chitin while midgut is endodermal in origin and has the midgut gland, caecae and diverticulae.

The fore gut or proventriculus has an anterior distensible part, the posterior end of which constricts towards a gastric mill. There is a complex system of muscular attachments, especially around the gastric mill. The gastric mill has a median dorsal and two lateral ossicles with many subsidiary ossicles. Gastric mill leads to the posterior part of the proventriculus which possess a filter press on its ventral chamber. Mixing of food with digestive fluids

and triturition occurs in the anterior part and the fluids then pass into the ventral grooves of the anterior chamber which carries it to the filter press which excludes particles above 1 µm and leads the contents to the entrance of the digestive gland. Further digestion and absorption of nutrients are carried out inside the tubules of the digestive gland. The anterior diverticula could also be contributing essential components of digestive enzymes.

There is a difference of opinion about the naming of the midgut gland which is variously described as hepato-pancreas, digestive gland and midgut gland. The digestive gland is a pair of bilobed glands lying on either side of the gut and opens at or near the foregut. In penaeids only a median sac like bilobed caecum is present in the midgut. The digestive gland secretes digestive enzymes and is the major site of absorption of nutrients. The epithelium of digestive gland has two cell types the 'R' cells which stire nutrients and the 'B' cells which is generally believed to contain digestive enzymes. The digestive gland also serves as an organ for accumulation and metabolism of calcium and copper and inactivation of other potentially toxic metals.

Midgut is lined by a dense columnar epithelium with light and dark cells that have absorptive and secretory functions. A cylindrical peritrophic membrane is secreted by a ring of cells behind the digestive gland opening. Faeces are enclosed in this membrane to form dry pellets and it protects the gut from abrasive particles and aids in defecation.

Digestion

Digestion involves a series of processes in the digestive tract by which the complex food particles are broken down into their simple forms that can be readily

absorbed into the body. This is accomplished by a combination of mechanical and enzymatic processes.

In malacostraca mechanical triturition is done in the proventriculus aided by gastric mill while in some fishes oesophagial teeth aids in this process. Oral teeth in many fishes are used only to seize and hold the prey. Chemical process of digestion is carried out by enzymes secreted by digestive glands and in fishes this process is aided by secretion of gastric fluids that contain hydrochloric acid and bile which emulsify the fats. Rates of enzymatic activity depends on variable amounts of enzymes, substrates, enzyme activators and inhibitors, and biproducts reacting under the modifying influence of pH and temperature.

Digestion in fish

Digestion is initiated in the stomach in fishes that possess a true stomach. Hydrochloric secreted by the gastric glands leads to activation of pepsin, whose pH optima is 2-2.2. In tilapia pH of the gastric fluid is reported to be as low as 1. Common carp which does not possess a true stomach maintain a digestive tract pH that is slightly alkaline.

Carbohydrates are digested in the first and second quarters of the intestine while protein digestion is completed in the first quarter. Amino acid absorption, however, continues the length of the intestine.

Di and polysaccharides are broken down to monosaccharides which are readily absorbed into the blood. Cellulose, hemicellulose and lignins are not digested at all by fish but microbial cellulase activity has been reported in tilapia and channel catfish. The carbohydrate digesting enzymes in fish are Amylase, maltase, chitinase, cellobiase, oligo-1,6 glucosidase lactase and sucrase.

Protein is the most important component of the fish diet and its requirement is 2-4 times more than that of terrestrial animals. Proteins are broken down to amino acids which are absorbed in the intestine. Dipeptides and tripeptides are also rarely absorbed. The peptade uptake theory states, however, that small peptides with 2-6 aminoacids are absorbed more readily by the intestinal mucosa than individual amino acids.

Proteins are acted on by proteinases like pepsin and trypsin which breaks them into small peptides which are then broken down to constituent amino acids by peptidases like carboxypeptidase, aminopeptidase, dipeptidase and there are various nucleases that digest the nucleic acids.

Fish can completely oxidise fats and release all energy. Fish lipases can act on all the three fatty acids in a triglyceride while mammalian lipases can act only on the first and the third fatty acids. Fats are absorbed as fatty acids, mainly in the hepatocytes of the liver. Marine fishes have enormous surface area and extensive pyloric caecae that permits longer time for emulsification of waxes and wax esters which are abundant in zooplankton especially copepods. Bile emulsifies fats which are then digested by lipase and other esterases in fish.

Digestion in Malacostraca

Storage, triturition and digestion of food takes place in the proventriculus and the digestion and absorption are completed in the midgut. Secretion of enzyme is limited to midgut in crustacea. Enzyme secretion increases immediately after feeding and digestion aided by gastric mill would then begin almost immediately.

There is a second peak in enzyme secretion after few hours which may represent final phase of digestion in the tubules of the digestive gland.

About 80-90% of nutrients are absorbed in the digestive gland and midgut while only a fraction is absorbed in the hingut. Lysine and glycine are actively transported in penaeids in the midgut and glucogen is also absorbed in a similar way. The digestive gland in crustacea is considered to be under endocrine control.

Digestive enzymes in crustacea

A large number of proteinases and peptidases are present in crustacea. Trypsin of penaeids is similar to the mammalian trypsin with pH optima of 7-9 but differ in that they attack undenatured proteins like collagen and synthetic substrates. Other proteinases with light molecular weights and specific activities are detected in several crustaceans. Among the peptidases, carboxypeptidase, arylamidase and a dipeptidase have been reported in cray fish Astacus astacus. No zymogens (inactive precursers) of crustacean proteolytic enzymes have been detected so far.

Lipid and esterase activity have been demonstrated in many crustaceans. Fats are probably absorbed as a mixture of free fatty acids, mono and diglycerides.

Amylase activity has been demonstrated in all the crustaceans investigated. Maltase and oligo 1,6, glucosidase is also suspected to be present in crustacea, since these are necessary for complete oxidation of starch and crustaceans are capable of digesting starch completely. Cellulolytic activity has been demonstrated in many crustacea, but whether it is synthesized or secreted by micro organisms is yet to be ascertained. 1,3 gluconase

(Laminarinase) is also believed to be present in all crustaceans. All crustaceans are capable of digesting chitin which demonstrates the presence of chitinase. Glucosidases that hydrolyse less well characterised polymers present in algae and micro-organisms are believed to be present in those crustaceans that feed on these items. These may degrade glycolipids and glycoproteins in addition to mucopolysaccharides and polysaccharides.

pH optima

Foregut fluid of most crustaceans is reported to be slightly acidic. Some enzymes such as those related to trypsin have alkaline optima.

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Technical Paper-I

SUMMER INSTITUTE IN
RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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FEED INGREDIENTS AVAILABLE IN INDIA AND THEIR POTENTIAL NUTRITIVE VALUE

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Development of practical feed formulations depend upon information on two major aspects; the nutritional requirements of the animals and the nutritive value of the potential feed ingredients. Once information on these aspects along with other essential parameters, become available for a specific species and size, it should be possible to develop low-cost practical feeds using linear programming. During the past two decades there has been a phenomenal increase in research activities relating to identification of raw materials for formulating feed ingredients both in the developed and developing nations of the World.

As against the highly nutritious practical feeds developed in the developing countries, which can make use of large quantities of feed ingredients of high quality, developing countries have to rely mostly on relatively nutritionally poor quality raw materials.

Information on the raw materials available in different parts of the country and their nutritive value are important for identifying ingredients for incorporation into practical diets of aquatic organisms. While a great deal of information has accumulated on the potential nutritive

value of feed ingredients, information on the biological value for finfish and crustaceans is relatively limited. Based on their composition the raw materials, used for feed compounding are grouped into eight classes (Table 1). Since, protein is the most expensive and most important nutrient in the diet extensive surveys have been made to identify natural protein rich raw materials for practical feed formulations. So far, a large number of conventional ingredients have been identified and their potential nutritive value have been worked out. In view of the shortage of these conventional feed ingredients, as a result of increased demand competition with animal husbandry, in recent years. Considerable emphasis has been laid on identifying new sources.

PROTEIN RICH INGREDIENT SOURCES

The most important protein rich ingredient sources are oil cakes, fish meals, crustacean meals, blood meal, slaughter house waste and poultry wastes, certain unicellular algae etc.

Oil cakes

Soyabean oil cake: Of all the major plant protein sources soybean is considered as the best protein source. Since 1972, soyabean production has risen primarily owing to the high demand for edible oils. In some countries like USA soyabean meal ranks as the most widely used source of supplemental protein for livestock. In recent years soybean oil cake is successfully used as an ingredient in the feeding of finfish. Soybean oil cake has the highest crude protein and energy contents among oil cakes. The energy content will vary with the level of residual oil and percentage fibre in the meal. Lysine, threonine and methionine are the limiting amino acids, with tryptophan and valine limiting under

certain circumstances. With the exception of methionine, the biological availability of amino acids is quite high. But, heat treatment required to inactivate protease inhibitors results in reduced biological availability of both lysine and cystine, and partial destruction of arginine, tryptophan, histidine and serine. About 50 to 70 per cent of the phosphorus in Soyabean cake is present in the form of phytic acid, which is biologically not available. Besides, during processing phytate-protein-mineral complexes form, resulting in decreased availability of Ca, Zn, Cu, Mn, Mo and Fe. Among the vitamins, choline is found in relatively high levels; but niacin, riboflavin pantothenic acid and thiamine are significantly reduced (losses of 10-75%) during heat treatment.

Cotton seed oil cake:

The protein content of cotton seed oil cake varies between 29 and 37 per cent, depending on the amount of hull removed. The content as well as biological availability of lysine, threonine, tryptophan and methionine may be lower than that of soybean oil cake. The energy value is inversely related to its fibre content. It is deficient in calcium, but is a richer source of Mg than is soybean cake. It is a good source of thiamin and of vitamin E. The presence of the polyphenolic pigment gossypol and cyclopropenoic fatty acids adversely affects its nutritional value. However, glandless variety of cotton seed is almost free from gossypol.

Groundnut oil cake: It is most commonly made from the peanut kernels, husks of pods being removed by the process of decortication. Although the crude protein content is almost equal to that of soybean cake, it is lower in lysine, tryptophan, threonine and methionine. It is a good source of Mg, S and K. Vitamins niacin, pantothenic acid and

thiamine are abundant, while choline and vitamin E tend to be deficient.

Sunflower oil cake: The protein quality of sunflower oil cake is regarded to be lower than that of soybean cake, with lysine being especially deficient. Heat treatment during processing severely depresses the availability of aspartic acid, arginine, threonine, leucine, lysine and tryptophan while the content of glutamic acid, serine and ammonia increase (Smith, 1969; Christison, 1980). It is higher in crude fibre than in soybean cake. The fibre content varies depending on the proportion of hulls removed prior to processing the meal. Sunflower oil cake contains relatively higher levels of available calcium. It is a poor source of trace minerals, but is high in sodium and sulphur. B-complex vitamins and carotene are found in relatively greater levels.

Rapeseed oil cake: The composition has been shown to vary depending on the growing conditions. The presence of crucic acid and glucosinolates adversely affects its nutritional value. However, development of rapeseed varieites with lower levels of crucic acid and glucosinolates has been a major breakthrough which allows their inclusion at much higher levels in animal feeds (Clandinin et al., 1978). The available lysine and threonine content is approximately 10 per cent lower than that in Soyabean oil cake (Saver et al., 1981). But it has more methionine and cystine than soybean cake. Crude fibre levels can be as high as 16 per cent. By removing the hulls crude fibre content can be reduced. Phytic acid and fibre reduce the availability of P, Ca, Mg, Zn, Cu, and Mn. In spite of this it is a better source of available Ca, Fe, Mn, P, Mg. and Se than soyabean oil cake. It also contains higher levels of cholins, niacin, riboflavin, folic acid and thiamine but lower levels of pantothenic acid than soyabean cake.

Safflower oil cake: Safflower oil cake is relatively high in crude fibre. The protein is lower in amino acid content than soybean oil cake. It is a good source of Ca, P, Fe. Vitamin content is somewhat superior to soybean cake, but it contains very little vitamin B_6.

Gingelly oil cake: The hull of the gingelly seeds accounts for 15-20 per cent of the whole seed, which contains high levels of oxalic and phytic acids. These acids impart a bitter taste to the oil cake and complexes with calcium and other minerals, rendering them nutritionally unavailable. Dehulled, expeller processed oil cake is high in methionine, cystine and tryptophan, but low in lysine. The presence of phytic acids reduces the availability of Zn, Ca, Mg and Fe. Has high levels of niacin and pyridoxine.

Linseed oil cake: Is relatively high in fibre content due to the mucilage coating in the hull. The mucilage contains a water dispersible carbohydrate which has low digestibility. Besides, linseed oil cake has lower protein and an inferior amino acid profile compared with soybean cake. Lysine and methionine levels are very low. The presence of pyridoxine antagonist linatine leads to pyridoxine deficiency.

Coconut oil cake: This has relatively low protein (average 24.6%) and high crude fibre (average 14.5%) contents. Deficient in methionine and cystine. Rich in potassium and Iron. Niacin and choline are found in good levels.

PROTEIN SOURCES OF ANIMAL ORIGIN

Blood meal: One of the richest source of protein, containing 75-85% crude protein. It is a very good source of essential amino acids, histidine, lysine, phenylalaine and valine but poor source of arginine, methionine and cystine. The

amino acid leucine is found in very high levels compared with isoleucine. It is a poor source of calcium and phosphorus and most of the minerals. But is a rich source of Iron containing as high as 2784 mg/kg dry matter. Niacin and cyanocobalamin are the two vitamins found in relatively good levels.

Chicken eggs (without shells): Has about 46% crude protein and 43% ether extract and 4% ash. Iron and zinc are found in good quantities. Good source of all the essential amino acids pantothenic acid, cyanocobalamin and riboflavin levels are high.

Crab meal: Contains about 30 to 40% protein depending upon the size and species. It is a rich source of chitin. Ash content is very high. Arginine is found in high levels. Very rich source of choline, niacin, pantothenic acid, and cyanocobalamin. High in calcium, iron and manganese levels.

Fish meal: Depending upon the species crude protein varies from 50 to 75%. Ash content from 17 to 30%. Calcium content varies from 2.2 to 7%; phosphorus from 1.9 to 3.8%; rich source of iron, copper and zinc. Rich source of choline, biotin, pantothenic acid, niacin, cyanocobalamin. Good source of all the essential amino acids.

Poultry byproduct meal (with viscera, afeet and heads): Crude protein levels range from 50 to 60%. Good source of all the essential amino acids, calcium, phosphorus, iron and zinc, choline, niacin, pantothenic acid, riboflavin and cyanocobalamin.

Poultry feather meal (hydrolysed): Has high protein content crude protein levels range from 78 to 85%. It is a rich source of sulphur (1.5-1.6) but poor source of most of the minerals, though phosphorus content is higher than calcium.

Niacin and cyanocobalamin are found in relatively good levels. Contains low levels of histidine, lysine and tryptophan.

Shrimp wastes: Crude protein varies from 30 to 40%. Chitinous material is found to be in levels as high as 16%. Ash content ranges from 25% to 40%. Is a rich source of calcium. Has a very high content of choline.

NUTRITIVE VALUE OF OTHER INGREDIENTS

Alfalfa: Crude protein 13-17%; crude fibre 25-30%. Good source of calcium, potassium, iron, manganese and zinc; choline, biotin, niacin, pantothenic acid, riboflavin and vitamin E contents are high.

Spirulina (New source of protein): Contains 55 to 65% protein with good levels of most of the essential amino acids.

Corn gluten: Contains about 25 to 30% protein. Contains low levels of arginine but high levels of leucine. Good source of iron and zinc, niacin and vitamin E.

Molasses (dehydrated): Crude protein 8 to 10%, ash 10-16% and fiber 6-10%. Contains high levels of potassium, copper, iron and manganese.

Rice bran: Crude protein 10-12%; crude fiber 12 to 18% or more depending on the level of husk; ether extract 7 to 12% and ash 8 to 12%. Rich source of energy, phosphorus, potassium, magnesium, iron, manganese, biotin, niacin, pantothenic acid thiamin and vitamin E.

Sorghum: Energy feed; crude protein 8 to 12% poor profile of minerals. Rich source of niacin and pantothenic acid.

Wheat bran: Energy feed; crude protein 10 to 14%; crude fibre 12 to 18%; ash 6-8%; good source of phosphorus; potassium, manganese and zinc; niacin, pantothenic acid and biotin contents are high.

Yeast brewers: Crude protein 40-45; ash 6-9%. Good source of phosphorus; potassium and iron. Richest source of biotin, choline, niacin, folic acid, pantothenic acid, pyridoxine, riboflavin and thiamin.

Tapioca chips: Rich source of carbohydrate. Presence of hydrocyanic acid should be monitored.

NON-CONVENTIONAL INGREDIENTS

Silkworm pupae
Insect larvae
Fish silage
Zooplankton
Molluscs

Recycled wastes to produce:

- yeast
- phytoplankton
- bacteria
- algae
- higher plants.

Single cell proteins
- bacteria
- yeast
- algae

leaf protein
vegetable silage
Aquatic plants
Marshland plants
Sea grasses

The utilization of the above products needs extensive research in terms of nutritive value, cost of production etc.

Technical Paper - 14

SUMMER INSTITUTE IN
RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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ANTINUTRITIONAL FACTORS IN FEED INGREDIENTS AND THEIR EFFECTS IN FINFISH

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One of the important criteria in selecting feed ingredients for manufacturing complete and supplemental feeds relate to the presence of antinutritional factors, which can significantly reduce the nutritional value of the feeds. These anti-nutritive substances are often referred to as 'toxic factors' because of the deleterious effects they produce when eaten by animals. However, most of these produce sub-lethal effects such as reduced growth, poor feed conversion, hormonal changes and occasional organ damage.

Antinutritive substances can be broadly grouped into (1) those which are generated in the natural feedstuffs by the normal metabolism of the species from which the material originates; (2) the artificial antagonists such as preservatives, chemical additives, toxic compounds introduced as a result of different manufacturing processes, pesticides, herbicides and heavy metals and (3) natural contaminants such as mould, fungus and bacteria leading to the production of microbial toxins.

In the first group there are three main classes of substances: (1) Substances depressing digestion or metabolic utilization of proteins - protease inhibitors, lectins

(haemagglutinins), saponins and certain polyphenolic compounds; (2) Substances reducing the solubility or interfering with the utilization of mineral elements - phytic acid, oxalic acid, glucosinolates and gossypol; (3) Substances inactivating or increasing the requirements of certain vitamins - Anti-vitamins A, D, E and K and anti-B vitamins - thiamine, nicotinic acid, pyridoxine and cyanocobalamin.

GROUP I

SUBSTANCES DEPRESSING DIGESTION OR METABOLIC UTILIZATION OF PROTEINS

(a) Proteast inhibitors:

They are distributed widely in plants especially among the legumes. In soyabeans two main groups are found, each of which is a mixture of proteins - the Kunitz and Bowman - Birk inhibitors. The Kunitz inhibitors have specificity towards trypsin, whereas, Bowman - Birk inhibitors inhibit both trypsin and chymotrypsin. Growth depressing effect was due to the slow release of methionine by proteolytic enzymes in the presence of trypsin inhibitors. In some species the release of all the amino acids have been affected by the inhibitors.

Growth inhibition has been reported for fingerling channel catfish (Robinson et al., 1981) and rainbow trout (Sandholm et al., 1976). Raw soyabeans and defatted soyaflour besides, inhibiting growth and reduce protein digestibility, depress metabolizable energy and fat absorption, cause pancreatic hypertrophy, stimulate hyper and hyposecretion of pancreatic enzymes, and reduce amino acid, vitamin and mineral availability (Rackis, 1974).

Autoclaving or heat processing of raw soyabeans destroys the trypsin inhibitors (Ham and Sandstedt, 1944). Extrusion cooking and infra-red cooking or micronization also is effective.

(b) Haemagglutinins (lectins):

These are found in both plant and animal tissues (Stockert et al., 1974). Mostly found in legumes. They are protein in nature and have high affinity for certain sugar molecules. Soyabean haemagglutinin is readily inactivated by pepsin and thus in animals with true stomach, the haemagglutinating fraction appears to be inactivated before feed enters the intestine. For stomachless fish and species which have low peptic activity haemagglutinin containing feedstuffs should be pretreated before usage.

Although very resistant to dry heat, they can be destroyed by cooking, autoclaving and micronization.

(c) Saponins:

They occur in a wide variety of plants and have three important characteristics; a bitter taste, foaming in aqueous solutions and haemolysis of red blood cells. On hydrolysis they yield sapogenins which are either steroids or triterpenoids and sugars. Their primary biological effect is an interaction with cellular and membranal compounds. They form complexes with cholesterol and has a blood-lowering effect in chicks. Dietary cholesterol has also been shown to reduce the growth - depressing effect of saponins. Cheeke and Oldfield (1970) showed that alfalfa saponins inhibit in vitro succinate oxidation by rat liver enzymes and also inhibit digestive enzyme secretion (Ishayya and Birk, 1965).

Their presence in feeds have been shown to affect food intake in chicks due to the bitter taste and affect the egg production. Their haemolysing effect on red blood cells is due to an interaction with cholesterol in the erythrocyte membrane.

(d) Polyphenolic compounds:

The most important are the tannins, which are polyphenolic substances with a molecular weight greater than 500. All cereals contain tannins; certain strains of sorghum contain upto 5%. Sal seed cake contains as high as 20% tannin and rapeseed meal is another rich source. Growth depression has been reported in rats and chicks due to the reduced protein and dry matter digestibility. Tannins seems to interfere with activity of trypsin and -amylase.

Chlorogenic acid is a polyphenolic compound which occurs in sunflower seed meal (about 1.2%). Has growth depressing effect and affect the feed utilization due to its inhibiting effect on the activity of proteinase, amylase and lipase. However, these effects can be counteracted by compounds with methyl donors - for example, methionine and choline (Singeleton and Kratzer, 1969).

SUBSTANCES REDUCING THE SOLUBILITY OR INTERFERING WITH THE UTILIZATION OF MINERAL ELEMENTS

(a) Phytic acid (phytate):

Plant phosphorus is found in the form of phytic acid, which is a cyclic compound containing six phosphate groups. Phytin forms a protein - phytic complex with zinc, manganese, copper, molybdenum, calcium, magnesium and lowers the dietary availability of these essential elements (Rackis, 1974). The addition of 0.5 percent phytic acid to purified diets

fed to rainbow trout resulted in a 10 per cent reduction in growth and feed conversion (Spinelli et al., 1982). Protein digestibility and zinc availability has been significantly affected by phytin. High levels of vitamin D has been shown to improve the utilization of phytate phosphorus.

(b) Oxalic acid:

Certain plants like beet, spinach and seeds of sesame contain oxalic acid, and its main antinutritional effect is through complexing with calcium. Growth depression and reduction in calcium retention have been reported in animals.

(c) Glucosinolates (thioglucosides):

These are responsible for the pungent flavours found in condiments, horse-raddish, mustard and rapeseed meal. Their main biological effect is to depress the synthesis of thyroid hormones. The thyroid depressing effect is due to their reducing the incorporation of iodine into the precursors of thyroxine as well as interfering with its secretion. In young chicken 0.15 percent causes depression of growth. Hyperplasia and hypertrophy of the thyroid. Liver haemorrhage and enlargement of the liver and kidneys also reported in some mammals.

(d) Gossypol pigments:

These are found exclusively in the pigment glands of cotton seed. They are present in both oil and meal and exist in the free form or as a gossypol - protein complex. Whole seeds contain about 1.09-1.53 g/100 g, of which 0.19 g/100 g exists in free form. Decorticated seeds contain 0.15 g/100 g in free form. Dietary gossypol causes depressed growth and decreased utilization of feed for weight gain.

In fish anorexia and lipid deposition in the liver have been reported. (Wood and Yasutke, 1956). Roem et al. (1967) reported adverse effects on the growth of rainbow trout fed levels of 1000 ppm or higher but 250 ppm had little detrimental effect. Sinnhuber et al. (1968) found that dietary gossypol along with aflatoxin B to act as cocarcinogen in rainbow trout. In channel catfish fingerlings growth inhibition occurred when cotton seed meal greater than 17.4 percent was included in the diet (Dorsa et al., 1982). However, in glandless cotton seed gossypol is absent.

In higher animals reduction in haemoglobin, has been reported. Other physiological effects include reduced appetite and loss of body weight, reduced oxygen-carrying capacity of blood and adverse effect on certain liver enzymes (Chubb, 1982).

SUBSTANCES INACTIVATING OR INCREASING THE REQUIREMENT OF CERTAIN VITAMINS

This group consists of natural organic compounds which can either destroy certain vitamins or combine with them to form unabsorbable complexes or interfere with their digestive or metabolic utilization.

Anti-thiamine - has been reported in rice bran, mustard seed, cotton seed and the greatest source is raw fish. Most of the freshwater fish mussels and clams contain high levels of thiaminase, whereas a saltwater fish contain relatively less quantities. Thiaminase can be inactivated by heat.

Anti-nicotinic acid - Niacinogen present in maize has been shown to combine with nicotinic acid thereby making it resistant to enzyme digestion, leading to pellegra

dermatitis in higher vertebrates.

Pyridoxine antagonist - Linseed meal contains 0.002 to 0.005% of a pyridoxine antagonist, 1 - amino - D - proline, which occurs naturally in combination with glutamic acid as a peptide called linatine. This substance can be extracted with water and can be destroyed by autoclaving.

Anti-vitamin B_{12} - Raw soyabeans by autoclaving shown to contain a heat-labile substance which accentuates the requirement for vitamin B_{12} in rats, but the causal factor is not yet identified.

Anti-Vitamin A - Raw soybeans contain the enzyme lipoxygenase which destroys carotene and lowers the levels of vitamin A. This enzyme is destroyed by heating for 15 min. with steam under atmospheric pressure.

Anti-vitamin D - Protein isolated from unheated soyabean has been shown to produce rickets in turkeys. Autoclaving this protein eliminated this rachitogenic effect.

Anti-vitamin E - Found in kidney beans and cause muscular dystrophy in chicks and lambs. It can be destroyed partially by autoclaving.

Alkaloids: Pyrrolizidine alkaloids are toxins found in many plants. At 100 ppm in the diet caused severe growth depression and mortality in rainbow trout (Hendricks et al., 1981). When fed at 2 ppm these toxins caused severe hepatic lesions.

Cyanogens: Cyanide in trace amounts is fairly widespread in the form of glucosides, and relatively high levels can be found in certain grasses, pulses and root-crops. Tapioca contains relatively high levels (53 mg/100 g to

while collecting plankton from the wild and fed to larvae of fish and shellfish care must be taken to exclude toxin containing algae.

Toxic algae, such as Gonyaulax sp. and Gymnodinium breve are avidly eaten by some molluscs that store toxins in their tissues (Sparks, 1972). Incorporating the contaminated molluscs into feeds for finfishes or crustaceans could result in toxicity.

In addition to algae, metabolites or streptomycetes and actinomycetes have been shown to cause muddy off-flavour in channel catfish in the U.S. (Lovell, 1979).

GROUP II

ANTINUTRIENTS RESULTING FROM NATURAL CONTAMINATION OF FEEDSTUFFS (MAINLY MICROBIAL IN NATURE

Animal by-products and fish meals are often contaminated with Salmonella. There are approximately 1200 serotypes of Salmonella of which 80 serotypes have been associated with diseases in animals. Their effect on fish and crustaceans need elucidation.

Raw materials and to a lesser extent finished feeds contain considerable number of bacteria, yeasts and moulds. Whenever these have a moisture content greater than the

accepted 'dry level' the potential for bacteria, yeast and mould to propagate exists and can result in poor performance of the animals. This can arise from three distinctly different causes.

(i) The microbes may be pathogenic and cause an infection ex:bacterium - Pathogenic serotypes of Salmonella, the yeast Candida spp. and fungus Aspergillus sp.

(ii) Microbial action can alter the nutritional status of the product. Mould growth can cause fustiness which results in reduced palatability. Microbial action can destroy or make unavailable to the animal certain vitamins-- for example the B complex vitamins or the fat-soluble vitamins A, E and D.

(iii) Mould growth can result in the production of mycotoxins, which can cause a wide range of pathological and physiological effects in fish.

Mycotoxins can be produced from any stage from growing crop to the formulated feed as indicated in Fig. A list of feed ingredients with demonstrated natural contamination by aflatoxin are given in Table . If proper production and storage conditions are maintained mycotoxins will not be produced in the formulated feed.

In order to control the mycotoxin accumulation, removal of damaged and discoloured particles is suggested to reduce the overall concentration in finished feeds. Another way of reducing the animals exposure is to reduce the percentage incorporation of the raw materials into the formulated feed with reference to safe-concentrations. In oilcakes aflatoxin can be destroyed by ammoniation and monomethylamine treatment. The best way to prevent problems with aflatoxins and other mold - produced mycotoxins is to store

feedstuffs under conditions of low humidity and temperature.

Symptoms of mycotoxin depend upon the amount of toxin in the feed, the period for which the feed is ingested, the nutritional status of the feed and the susceptibility of the animal. There are four important groups of mycotoxins, aflatoxins, ochratoxin A, zearalenone and the tricothecenes.

Aflatoxins:

These are the best documented mycotoxins. There are mainly four compounds named B_1, B_2, G_1 and G_2. Aflatoxin is a potent liver toxin and a carcinogen with aflatoxin B_1 being the most toxic compound. In trouts high incidence of hepatoma was reported by Wolf and Jackson (1963) when fed in a cotton seed meal diet and the causative agent was found to be aflatoxin B_1 (Halver, 1967). In trout hepatoma occurred in a dosage as low as 0.5 ppb of aflatoxin (Ashley et al., 1965; Sinnhuber et al., 1965). Rainbow trout are extremely sensitive. But brook trout and Coho salmon are less sensitive to aflatoxin ingestion (Wolf and Jackson, 1967; Halver et al., 1969). Warmwater fishes also seem to be less sensitive. Ashley (1966) and Friedman and Shibko (1972) reported that channel catfish fingerlings showed a relatively low response when fed aflatoxin in doses up to 100 mg/kg body weight. Thus there seems to be significant differences in susceptibility of fish to aflatoxin. This calls for intensive research on the effect of aflatoxins on cultivated finfish and crustaceans of India.

The carcinogenicity of aflatoxins has been affected by several other dietary factors. Cyclopropenoic fatty acids and gossypol present in cotton seed meals have been shown to serve as cocarcinogens with aflatoxin in rainbow trout (Lee et al., 1968; Sinnhuber et al., 1968). The presence of dieldrin, a pesticide, in a diet with 6 ppb aflatoxin B,

increased the incidence of hepatocellular carcinomas (Hendricks et al., 1979).

Ochratoxin:

Produced by species of the genera Aspergillus and Penicillium. Ochratoxin A affects the proximal kidney tubules causing nephropatty. It is teratogenic to many animals. This is mostly reported from temperate countries. Recently rice bran and soyabean meal from tropics also have been found to contain this toxin. Decreased growth and egg production and diarrhaea have been reported as symptoms in poultry.

Zearalenone:

In an oestrogenic mycotoxin produced by several Fusarium species and occurs in many agricultural commodities, affect the reproductive system.

Tricothecenes:

Also produced by Fusarium fungi and four compounds have been known to be associated with animal disease, deoxynivalenol, nivalenol, T-2 toxin and diacetoxy-scirpinol.

Wu and Sonekha (1978) reported mycotoxin - producing fungi associated with dry shrimp. Friedman and Shibko (1972) identified 27 mycotoxin - producing fungi in dry shrimp. T-2 toxin proved lethal to rainbow trout at levels of 6 mg/kg body weight (Marasas et al., 1967). Feed refusal and vomiting syridromes have been observed in fish.

GROUP III

CHEMICAL CONTAMINANTS

The most common contaminants are organo-chloride pesticides, DDT, DDE, dieldrin, endrin and industrial chemicals such as polychlorinated biphenyls (PCB), phthalate esters and hexachlorobenzene (HCB) and heavy metals.

Heavy metals:

The toxicity of dietary heavy metals will depend upon the level of those metals in the water as well as the hardness of the water (Spear and Pierce, 1978; Carrol et al., 1979) and its pH (Reed et al., 1980). The levels of metal chelators in the diet also affect the heavy metal toxicity.

The U.S. Food and Drug Administration recommend a safe level of 0.5 ppm of dietary mercury. Selenium has been found to have protective effect against the toxicity of methyl mercury (Friedman et al., 1978). However, selenium itself has been found to be toxic to rainbow trout at a dosage of 13 µg/g of food (Hilton et al., 1980). Chronic poisoning in animals occur in dosages ranging 2 to 20 mg/g (Lo and Sindi, 1980).

Cadmium is a potentially toxic metal for fish and shellfish (Smith et al., 1976). Seaweeds and molluscs have been found to accumulate cadmium in levels as high as 20,000 to 1,000,000 times to that of seawater (McLeese, 1980). The inclusion of these items in feeds should be based on prior knowledge of their cadmium levels.

Ashley (1972) noted that Coho Salmon tolerated copper at 1 mg/g dry diet but with retarded growth and impaired pigmentation. Low levels of dietary silver (0.5 mg/kg dry weight) has been shown to protect juvenile lobsters (Homarus

americanus) from growth depression and mortality associated with high levels (greater than 16 mg/kg dry weight) of copper (Chou et al., 1982).

Polychlorinated biphenyls (PCBs)

These organic chemicals are widely used as plasticizers and in hydraulic fluids. PCBs are poorly biodegraded, tend to accumulate in lipids and bioconcentrated. A PCB dosage of 14.5 mg/kg body weight resulted in 100 percent mortality of coho salmon after 260 days (Mayer et al., 1977). Sublethal effects of PCB in fish include liver enlargement, alterations in liver structure, induction of hepatic microsomal enzymes, increased thyroid activity (NRC, 1981). The maximum permitted concentration in finished feeds is 0.2 ppm.

Pesticides and Herbicides:

Pesticides and herbicides tend to bioaccumulate or bioconcentrate (NRC, 1981). Their toxicity is usually greatest in young fish, causing dysplasia or sterility of gonads, weakness, nervous disorders, loss of appetite and death (Ashley, 1972). Crustaceans are particularly highly sensitive to most pesticides.

Technical Paper - 15

SUMMER INSTITUTE IN
RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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FORMULATION OF COMPOUNDED FEEDS FOR PRAWNS

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INTRODUCTION

Compounded feeds have been one of the essential requisites in the development of aquaculture. Rearing of larvae in the hatchery, postal larval rearing in the nursery until they become stockable size and their subsequent culture in grow-out ponds require appropriate and nutritionally balanced compounded feeds. Compounded feeds have many advantages. By understanding the nutritional requirements of the candidate species of culture, nutritionally well balanced feeds could be formulated using low-cost feedstuffs available in the concerned region. These feeds could be prepared in large quantities with good shelf life and can be used off the shelf wherever and whenver required. Preparation of compounded feed does not require extensive area and highly skilled man power. High efficiency of the feed could be achieved by judicious manipulation of feed ingredients and can be made economically feasible. Dispensing of compounded feeds is quite convenient over large farm areas and automatic feed dispensing devices could be successfully employed.

Compounded feeds are two types. (1) Purified diets for studying nutritional requirements of animals and are generally formulated using purified ingredients, and (2) practical feeds which are formulated using natural ingredients.

In order to achieve the desired performance of a compounded feed, there are many a factors to be taken into consideration. The strategies to be employed in formulating compounded feeds for prawns are discussed below.

SELECTION AND EVALUATION OF RAW MATERIALS

Before formulating a compounded feed for a candidate species of prawn, the feed ingredients which may go into the formula have to be identified. A survey has to be conducted in the concerned region for identifying the materials. Since practical feeds are meant for large scale commercial production, the ingredients selected should be available in large quantities during the required season, the material should have consistant quality and should not be expensive. As far as possible, feedstuffs which are used for human consumption should not be selected for making feeds for prawns. This may create competition for the same material and its cost may go up very high. The selected raw materials should be analysed for their bio-chemical composition of protein, lipid, carbohydrate, minerals (ash) and crude fibre. If the ingredient is a protein source, the quality of the protein can be determined by analysing its amino acid composition. Next the biological evaluation of the feed stuff should be carried out. This could be done by feeding the material in appropriate form to the test animals and measuring the digestibility, growth of the animals and the feed conversion efficiency.

Feed materials may be classified as (1) roughages (2) energy feeds and (3) protein supplements. Roughages are materials with more than 18% crude fibre. Energy feeds are ingredients with less than 20% protein and less than 18% crude fibre. And the protein supplements are feed stuffs with more 20% crude protein.

Using this classification, the selected raw materials can be grouped into energy feeds and protein supplements. Each feed ingredient that is selected for inclusion in the feed formula should have a definite purpose. It should be either a protein source or energy supplement. It may be a good source of lipid, vitamins of minerals. Some times the material may be a growth promoter, an appetizer or may have any other specific function.

Materials of both plant and animal origin are suitable for formulating feeds. These may be agricultural, marine and industrial bi-products or waste materials. Some of the common feed materials are given below:

Energy feeds: Rice bram, wheat bram, tapioca, barley, corn sorghum and maize, fish oil, vegetable oil and animal fats.

Protein supplements:

(i) Plant origin : Oil cakes like, ground nut, cotton seed, linseed gingelly, coconut, rape seed, sunflower and soybean cakes.

(ii) Animal origin : Meat meal, blood meal, fish meal, slaughter - house waste, clam meat, prawn waste, mantis shrimp and silkworm pupa.

Non convertional feed stuffs	:	Poultry bi-products like feather meal, single cell protein like Spirulina, krill and yeast.

BINDING AGENTS AND THEIR SELECTION

The characteristic difference between the feed of homothermic land animals and that of the aquatic animals is that the later has to be provided feed in/under the water column. If the feed, disintegrates and dissolves away in the water, it is not available to the animals. It may cause large scale pollution of the rearing medium. To prevent this, substances known as binders have to be used which provide the feed the required water stability and prevent disintegration. Selection of a suitable binding material is essential and important. A poor binder would result in poor growth and conversion efficiency leading to economic loss. Selection of binder should be done carefully to control the cost of the final feed, at the same time it is effective in binding action.

Agar agar, carboxy methyl cellulose, gelatin, guar gum, poly vinyl alcohol (PVA) sodium alginate and starch are some of the chemical substances which can be used as binders. Wheat flour, rice flour and tapioca are good natural binders which can be used for binding prawn feeds. The criteria for selecting a binder is that a feed prepared using that binder should have good water stability (minimum 3 hrs.), it should not impair the assimilation of the feed and should not unduly enhance the cost of the feed. Tapioca powder is one of the good natural binders in prawn feeds. When added to the feed (upto 40% level) it provides good source of carbohydrate and at the same time acts as the binder. The feed pellets, prepared with tapioca as binder, have good water stability. The pellets when added to water, absorb water quickly and become soft and retain the shape of pellets at least for six

hours. The binding quality of tapioca was comparable to that of agar agar, PVA and sodium alginate. Tapioca is a low cost material available in large quantities. Thus tapioca is a double action material in prawn feeds.

FORMULATION OF FEEDS

Feed formulation is essentially making of a recipe according to the requirements. The selected raw materials have to be fitted into a formula to meet the requirements of the candidate species. Each feed material has to be adjusted to provide a particular nutrient and evolve a formula which can meet the requirement of protein, lipid, carbohydrate and the energy. If necessary additional amounts of vitamin and mineral mixtures should be added. Finally the binder at the required level should be incorporated to make the formula complete in all respects. The simplified flow sheet of feed formulation is given below:

Feed Formulation

NUTRITIONAL REQUIREMENTS OF THE ANIMAL — ENERGY, PROTEIN, LIPID, CARBOHYDRATE — BALANCE — SELECTED FEED INGREDIENTS

+

VITAMINS AND MINERALS

+

BINDER

BALANCED FEED FORMULA

No single feed material is a complete feed by itself. It is always advisable to have multi-ingredient feed formula. One ingredient may be rich in a particular nutrient and deficient in others. A balance of the required nutrients could be obtained only by including more than one ingredient in the formula. Generally animal proteins are more superior to plant proteins for prawns. A mixture of 60% animal protein and 40% plant protein is a good combination in prawn feeds. There is a simple method to balance the requirements (protein or energy, only one at a time) using feed ingredients. This is called the 'Square Method' and is described below.

Example 1: To formulate a feed with 30% protein using two ingredients prawn head (35% protein) and ground nut cake (protein 45%). First a square is constructed and the names of the feed ingredients are written on the two left corners along with the protein content of each.

The required protein level is written in the middle of the square. Next, the protein level of the feed is

Prawn Head (35.0)

Groundnut cake (45.0)

substracted from that of the feed stuffs and the answer is placed in the corner opposite to the corresponding feed stuff, ignoring the positive or negative sign as shown in figure. Now add the figures on the right hand side of the square 5 + 10 = 15. To make the feed with 30% protein we must mix

$$\text{Prawn head } \frac{10}{15} \times 100 = 66.67\%$$

$$\text{Groundnut cake } \frac{5}{15} \times 100 = 33.33\%$$

Example - II

To prepare a feed with 28% protein using five ingredients prawn head (35% protein) mantis shrimp (45% protein) fish meal (60% protein) groundnut cake (45% protein) and tapioca (2% protein), the ingredients are grouped into basal feeds with less than 20% crude protein and protein supplements with more than 20% crude protein. The protein content in each group is averaged and plugged into the square as follows:

Basal feeds : Tapioca 2.0%

Protein supplements: Prawn head = 35.0
Mantis shrimp = 45.0
Fish meal = 60.0
Groundnut cake = 45.0
Total 185.0

Average = 46.25%

Basal feeds 2.0 18.25

Protein supplements 46.25 26.0

Total = 18.25 + 26.0 = 44.25

Now the composition will be

Basal feeds = $\frac{18.25}{44.25}$ X 100 = 41.24%

Protein supplements = $\frac{26.0}{44.25}$ X 100 = 58.76

The final feed formula is:

Prawn head	=	$\frac{58.76}{4}$	= 14.69%
Mantis shrimp	=	$\frac{58.76}{4}$	= 14.69%
Fish meal	=	$\frac{58.76}{4}$	= 14.69%
Groundnut cake	=	$\frac{58.76}{4}$	= 14.69%
Tapioca			= 41.24%
			100.00

Thus the feeds can be formulated using the selected ingredients by balancing the nutrients like protein. This method can be used to balance energy content of the feed. In the place of protein values, energy values have to be used.

FEED FORMULATION BY LEAST-COST APPROACH

Least-cost feed formulation or Linear programming (LP) was first introduced to the animal feed compounding in mid fiftees. Recently it has been introduced in fish feed formulation also. LP is a mathematical procedure by which limited resources are allocated to achieve an optimal solution to a particular objective. These operations are completely computerized and a detailed information regarding the ingredients is needed before attempting it.

In formulating feeds by LP, the nutritionist, first lays down a set of constraints, and then lists all available raw materials which he wishes to be considered for selection by the computer to achieve the objectives. The objective is least-cost feed that will satisfy all the constraints.

The digestibility coefficients and other nutrient composition of ingredients along with constraints are supplied to the computer in its proper form. The computer will analyse and would be able to give the formula with the desired objectives. This could be achieved only when the nutritional requirements of the candidate species are known in total and the nutrient composition of the ingredients is thoroughly analysed.

The least-cost approach will become relevant and useful only when the operation of feed production is very larger. But with limited knowledge in prawn nutrition, over emphasis on the least-cost formulation of feeds could be premature. However feed cost is the single largest expense (35-50%) in prawn production. The unit price of prawn feed is relatively higher. Any saving in the feed cost by least-cost approach for LP will help the prawn culture industry.

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Technical Paper - 16

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LINEAR PROGRAMMING TECHNIQUE IN FISH FEED FORMULATION

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Nutrition plays a vital role in improving animal productivity. Good deal of work has been done in India on nutritional requirements of livestock and poultry. But studies on fish nutrition especially on the marine species are comparatively of recent origin. The Central Marine Fisheries Research Institute has been undertaking experimental work to estimate the digestibility coefficients of different feed stuffs and the nutritional requirements of selected fin and shell fishes. Experiments are also conducted to test the efficiency of different feed mixtures and to study their economics. In this paper a versatile tool called 'linear programming technique' has been discussed in relation to fish feed formulation.

Linear Programming

Today the word 'programming' is almost synonimous with computer programming which is purely an aid to computation such as in solving a set of equations or evaluating an expression. By itself a computer programme does not directly contribute anything to the development of the formulations leading to the set of equations or the derivation of the expressions. On the other hand linear programming is essentially a mathematical formulation for the determination of optimal solutions which do not violate certain specifications imposed. When several variables and specifications

are involved the computations in getting optimal solutions are very heavy and an electronic computer facilitates quick and efficient execution of the computation scheme.

A simple example will give an insight into the linear programming model. Consider two feed ingredients to be combined in such a way that the mixture satisfies certain vitamin requirements and at the same time involves minimum cost.

Let x_1 and x_2 be the quantities required from the ingredients '1' and '2' respectively. It is stipulated that the mixture contains at least 'a' units of vitamin A, and 'c' units of vitamin C. Let ingredient '1' contains a_1 units of vitamin A per kg and c_1 units of vitamin C and the ingredient '2' contains a_2 units of vitamin A and c_2 units of vitamin C. If p_1 and p_2 be the respective prices per kg, the linear programming model can be written as

Minimise

$$p_1 x_1 + p_2 x_2$$

subject to the condition that

$$a_1 x_1 + a_2 x_2 \geq a$$

$$c_1 x_1 + c_2 x_2 \geq c$$

Also for meaningful solution

$$x_1 \geq 0 \text{ and } x_2 \geq 0$$

This is the standard form of a linear programming problem.

It consists of 3 parts namely (i) the function whose value is to be minimised (or maximised if it is a profit function) called the objective function (ii) structural constraints to take care of the minimum requirements and (iii) the non-negativity condition.

The formulation is called 'linear' because the expression to be minimised and the inequalities involve

only variables multiplied by constants and added together. There are no x^2 term, 10 g x or any non-linear form of the variables.

Historically, the first problem in linear programming was formulated in 1941 by the Russian mathematician L. Kantorovich and the American economist F.L. Hitcheock, both working independently. A systematic way for arriving at optimal solution is provided by the 'simplex method' developed by the mathematician George Dantzig who published it in 1947. Charnes et al. (1953) Heady and Candler (1960), Hadley (1963), Gass (1964) and Loomba (1978) gave a full account of the principles of linear programming and the step-by-step calculations involved.

One of the early studies following a systematic approach in arriving at least-expensive feed mixtures was made by Waugh (1951). In India too linear programming techniques for evolving feed mixtures for livestock were attempted in the sixties and seventies (Jacob, 1972). However in the fishery field very little work has been done in the country on least-cost feeds meeting nutrient requirements. One reason was the lack of information on requirements of nutrients like protein and minerals for fish. With the work done in this direction at C.M.F.R.I. the information base has widened and it is felt that attempts could be made now to use the technique for formulation of fish feeds (Chandge, 1987; Gopal, 1986; Kalyanaraman and Paul Raj, 1984; Paul Raj, 1983; Paul Raj and Ali, 1982; Paul Raj and Thirunavukkarasu, 1987). The present note is more for introducing the technique to the nutritionists, some of whom may not be familiar with it, and for illustrating the procedure through case studies.

Illustration 1

A nutritionist proposes to mix two available ingredients such that the mixture contains at least 12 units of vitamin A and 15 units of vitamin C. Ingredient '1' contains 1 unit of vitamin A per kg and 2.5 units of vitamin C. Ingredient '2' contains 2 units of vitamin A and 1 unit of vitamin C. It costs 3.0 Rs. per kg for ingredient '1' and Rs. 4.0 per kg for ingredient '2'. Determine the minimum-cost feed mixture.

Let the mixture contains x_1 kg of ingredient '1' and x_2 kg of ingredient '2'. The linear programming model can be written as

Minimise

$3.0(x_1) + 4.0\ (x_2)$ (cost function)

Subject to the constraints

$1.0(x_1) + 2.0(x_2) \geq 12.0$ (vitamin A constraint)

$2.5(x_1) + 1.0(x_2) \geq 15.0$ (vitamin C constraint)

and the non-negativity conditions.

$x_1 \geq 0$ and $x_2 \geq 0$.

This being a two-variate case can be solved graphically. For this, consider the limiting cases of the constraints namely,

$$1.0(x_1) + 2.0(x_2) = 12$$
$$\text{and} \quad 2.5(x_1) + 1.0(x_2) = 15$$

By suitable substitution, the graphs of these two straight lines can be drawn (see Fig. 1). The area common to these lines away from the origin is called the feasible region because any point in that region satisfies the specifications imposed. Now consider the cost function. Give the total cost a zero value and also a convenient value,

say, Rs. 20. The lines can then be drawn as in the previous case on the same graph. For different values of the cost a set of parallel lines result, called iso-cost lines. An iso-cost line is the locus of all points (combinations of x_1 and x_2) which result in the same cost. It may be noted that as the iso-cost lines move away from the origin the cost also increases. We need to concern only that point of the iso-cost line which just touches the feasible region. The co-ordinates of that point gives the optimum solution as it is a point in the feasible region and at the same time involves only the minimum cost. If the cost line is moved up, the cost increases and if it is moved down it will not be in the feasible region. Thus the co-ordinates of the point 'A' namely 4.5 kg of ingredient '1' and 3.7 kg of ingredient '2' provide the optimum combination of the inputs (Fig. 1). Substituting in the cost equation the minimum cost works out to Rs. 28.7.

In the above case as there are only two constraints (apart from the non-negativity conditions) the optimum point is obvious from Fig. 1, namely 'A' the intersection point of the two constraint lines. (The intersection points with the axes are not considered here for simplicity). If there are three constraints there would be two intersection points (A_1 and A_2 in Fig. 2) and unless the iso-cost lines are drawn and shifted away from the origin towards the feasible region it would not be possible to decide on the optimal point (A_1 in the present case). It can be proved that the optimal point of a linear programming problem will always lie on the boundary of the feasible region.

With three variables the graphic solution becomes cumbursome. With more than three variables one may follow the 'simplex' method involving a systematic and step-by-step procedure to arrive at a feasible and at the same time optimal solutions (please see references).

Illustration 2

Consider formulation of a feed mixture for P. indicus with ingredients shown in the following table and subject to minimum nutrient contents. The quantity of the mixture to be prepared is 100 kg.

Nutrients \ Ingredients	Ground nut cake	Fish meal	Shrimp head meal	Rice bran	Minimum nutrient contents specified
Protein (%)	38	55	40	11	35
Gross Energy (Mcal/kg)	3.8	4.1	3.0	3.2	3.2
Calcium (%)	0.25	4.50	10.00	0.06	1.0
Phosphorus (%)	0.65	2.50	2.20	1.50	1.5
Price (Rs/kg)	3.00	6.00	1.00	1.50	Minimise cost

Let x_1, x_2, x_3 and x_4 be the respective quantities in kg of groundnut cake, fish meal, shrimp head meal and rice bran required for the mixture. The minimum-cost linear programming model can be written as,

Minimise

$$3.00(x_1) + 6.00(x_2) + 1.00(x_3) + 1.50(x_4) \quad \text{(cost function)}$$

Under the constraints

$$0.38(x_1) + 0.55(x_2) + 0.40(x_3) * 0.11\ (x_4) \geq 35.0$$

(Protein requirement)

$$3.8(x_1) + 4.1(x_2) + 3.0(x_3) + 3.2(x_4) \geq 320.0$$

(Energy requirement)

$$0.0025(x_1) + 0.045(x_2) + 0.10(x_3) + 0.0006(x_4) \geq 1.0$$

(Calcium requirement)

$$0.0065(x_1) + 0.025(x_2) + 0.022(x_3) + 0.015(x_4) \geq 1.5$$
(Phosphorus requirement)

$$1.0(x_1) + 1.0(x_2) + 1.0(x_3) + 1.0(x_4) = 100$$
(Quantity requirement

$$x_1 \geq 0, \quad x_2 \geq 0, \quad x_3 \geq 0 \quad \text{and} \quad x_4 \geq 0$$
(Non-negativity requirement)

The computations involved in solving the above problem is quite heavy. But computer programme packages are available and with the aid of an electronic computer the solution can be obtained in a few minutes. The solution is not given in this note. Here the stress has been for the procedures for building up of the linear programming scheme utilising the relevant information base.

Concluding Remarks

Application of the linear programming technique in the field of fish feed formulation envisages the computation of a minimum-cost feed mixture meeting several specifications. The main tasks are to quantify the nutritional and other specifications, to fix the values of the coefficients for conversion of feeds into their nutrient equivalents and to ascertain the availability and price of feed ingredients.

In the examples given 'greater than' restriction alone has been used. But the scheme is highly flexible. One would like to specify the requirements in the form of a range with upper and lower limits rather than a single limit. Sometimes the feed ingredient can be given an upper or lower limit, for example, an upper limit can be fixed for an ingredient, say, fish meal, which may be available only in limited quantities. Similarly changes in the price regime can be introduced into the problem and solved without much additional computation. The technique is thus highly

manoeuvrable and the nutritionist should take full advantage to investigate the varied types of alternatives for deciding on the best scheme for implementation.

One of the important assumptions made in the formulation of the linear programming problem is that of linearity. The expression to be optimised and the inequalities are assumed to be linear functions of the variables. The linear model employs an assumption of fixed prices and constant returns to scale. Under the linear model the total protein content for example, of the feed mixture is assumed to be the sum of the protein contents of the individual ingredients whatever be the proportion of the ingredients in the mixture. Thus no interaction is envisaged. Another assumption is that the coefficients such as the ones used for conversion of feed ingredients into their nutrient equivalents and also the prices of ingredients are known with certainty. These assumptions may be unrealistic for some situations and more sophisticated techniques like non-linear and stochastic programmings could be thought of. But within certain limits, for the problem of getting optimum feed mixtures the assumptions can be taken to be fairly reasonable.

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Technical Paper - 17

SUMMER INSTITUTE IN
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MANUFACTURE OF PELLETISED FEEDS, THEIR STORAGE AND QUALITY CONTROL

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INTRODUCTION

Presentation of feed in its most suitable physical form is key to the successful performance of the feed. The physical design of a feed should be in accordance with the feeding habits of the candidate animal and should not cause any impediment to its feeding activity. It is also important to consider that the design of the feed should have the practicability of inexpensive storage and easy way of dispensing. Involvement of sophisticated processing enhances the cost of production. Finfish generally graze the feed. The convenient way of presenting the feed to them is in the form of wet dough, certain fish can also freely feed upon floating pellets and flaked feeds. In the case of prawns, the larvae are filter feeders and require micro-particle feeds with good suspension quality in the water column. On the other hand, post larvae, juveniles and adult prawns can conveniently hold the feed and nibble on pellets, with suitable diameter, are more suitable form of the feed for prawns. Investigations have shown that moist (with 30-40% moisture) pellets are preferable. But preparation of moist pellets needs cold storage facility which is very

expensive and makes the process cumbursome, especially when large quantities of the feed is required. On the other hand preparation of dry pelletized feed appears to be more practical. The technical procedure involved in the manufacture of pelletized feeds is detailed below.

PROCESSING OF RAW MATERIALS

The raw materials involved in the feed manufacture should be received individually in proper condition. It is convenient to obtain the solid feed materials in dry form. The liquid ingredients such as oils may be procured in proper containers and stored carefully. The quality of the feed materials should be checked before processing.

Grinding: To obtain a homogeneous mixture of the feed, the raw materials should be powdered individually to a specified particle size. Some times it is possible to grind the ingredients together after mixing them according the feed formula. Grinding of ingredients generally improves the digestibility and pelletability of the feed. Grinding of the raw materials to a particle size of about 200 microns, gave the highest digestibility coefficient of the feed, best growth, food conversion ratio and good water stability of the pellets. The feed consisted of prawn heads, mantis shrimp, groundnut cake, fish meal and tapioca powder.

There are different types of machines available for grinding a variety of ingredients. These are pulverizers, hammer mills, atrition mills, roller mills and cutters. The selection of the grinder should be according to nature of raw materials involved.

MIXING AND HOMOGENISING

The powdered ingredients are weighed and mixed according to the formula of the feed. The feed mix should be thoroughly homogenised. This is important to avoid selective feeding of a particular ingredient in the feed, and also to achieve good pelletability. If liquid ingredients are to be added they are also included at this stage and mixed well. It is also possible to spray the liquids like fish oil after the pellets are prepared and dried. Vitamins like Vitamins C, which are heat sensitive can be incorporated after the heat treatment step is completed. When binders like tapioca is used, it can be mixed along with the other ingredients. If chemical binders are used, they must be dissolved/melted in cold or hot water and the solution is then added to the feed mixture.

For mixing of feed in large scale, rotating ribbon type, horizontal or vertical mixers can be employed. Both batch type or continuous mechanical blenders of varying capacity are available for this purpose.

STEAMING

After mixing the feed, it is steamed for a short time of about 15 minutes. Steaming of the feed improves its digestibility and kills the bacteria present and renders it inoccuous. The starch content present in the feed gets gelatinized and improves the binding quality. If starch based binders like tapioca are used, steaming is an important step and cannot be avoided. However, cooking at higher temperature and for a longer period should be avoided as it may destroy many important nutrients.

Steaming can also be done after extrusion of the feed. The pellets can be directly steamed and dried immediately.

PELLETING

Feed is pelletised by pressing the material through dies with different size holes. Pellets with 1 mm, 3 mm and 5 mm diameters are suitable for post larvae, juveniles and adult prawns respectively. Pelletization can be accomplished by compression, extrusion and adhesion. Depending upon the procure used, the feed pellets can be hard, non-compact and floating pellets.

Hard pellets: To prepare hard pellets, the feed mixture (dry), is subjected to steaming and the water content is increased only by 4 to 6%. The temperature is increased to 80-90°C and the material is quickly compressed through holes of the die. The friction further increases the temperature to 92°C. The pellets coming out of the machine are air-cooled quickly (within 10 minutes) and further dried to a moisture content of below 10%. The pellets thus prepared are hard and compact. The hardness of the pellet depends upon the nature of feed ingredients and the initial moisture in the feed. Feed mixtures containing large amounts of fibrous ingredients often result in too hard pellets. On the other hand feeds with high fat and excess moisture (water content) result in very soft pellets, which may be called as poor quality pellets.

Feeding hard pellets is hazardous, especially for finfish. It may lead to over feeding resulting in inefficient digestion. Undigested food may cause gastric disturbance in the stomach and some times fish may float upside down with mortality.

Non-compact pellets

Non-compact pellets are light and not compressed as in the case of hard pellets. The advantage of these pellets are that they do not sink rapidly in water.

Non-compact pellets are prepared by spraying water mist on dry feed mix, on an oscillating table. The feed comes out as ball shaped pellets. This technique is used for preparing fertilizers and chemicals into pellets and the methods is called the DRAVO process. This process is directly applicable for making non-compact feed pellets.

The Dravo-pelletizing process consists of a disc that rotates approximately at an angle of 45°. As the feed is tossed about on the disc, a fine spray mist causes the feed to form a ball shape of finely controlled particle sizes. Three metal projections aid in separating the particle sizes. The size of the particle can be decreased or increased by increasing or decreasing the feeding rate of the feed mixture to the disc and increasing or decreasing the speed and angle of the disc. The pellets obtained can be dried to the required moisture (below 10%).

The advantages of this method of pelletization is that the production cost if comparitively less and the problems of like clogging of die is eliminated. Another advantage is that it is possible to prepare the feed in the required particle size directly. However the ingredients should be ground finely to a uniform particle size. Otherwise the denser particles of individual ingredients may separate out during the process and disturb the homogenity of the feed.

Floating pellets:

Floating pellets are useful only for finfish which come to the water surface, grab the pellets and feed. This will help to control the rate of feeding and also observe the fish stock without sampling.

Floating pellets are prepared by the 'extrusion' process in which the resulting pellet is expanded instead of compression.

The extrusion process could be accomplished by adding 25-30% water to the dry feed mix. The wet feed mixture is placed in a pressure-sealed cylinder and steam is injected. This feed at high pressure is extruded through a die to ordinary atmospheric pressure. Feed almost explodes through the holes of the die and comes out uncompressed. The feed is thus collected in appropriate containers and dried. The digestibility of floating pellets is found to be higher than the compact pellets.

The process of floating pellet preparation is expensive, compared to the other methods of feed manufacture, in terms of equipment, steaming and drying cost.

DRYING

The feed pellets should be dried to a moisture content below 10%, otherwise the shelf-life of the feed will be very poor. The problems of storage are discussed separately. Generally the temperature imparted to pellets in the process of their manufacture will help to remove the moisture by air-drying process. The pellets are spread in thin layers in trays or on the floor of drying chambers and air is blown over them. If the pellets have high moisture, as in the case of floating pellets, hot air is blown to remove

the excess moisture. Both vertical and horizontal cooler-dryers and hot air dryers can be used for this purpose.

STORAGE

Proper storage of feeds and feed ingredients is essential to maintain the quality of the feeds and prevent health hazards to fish. During storage the feed can be subjected to chemical deterioration, infestation by insects and micro-organisms, mould growth and attack by rodents. All these result in loss of nutritive value of the feeds, economic loss and health risks.

Insect and mould growth

Initial moisture content of the feed, temperature, humidity and method of storage determine its shelf life. Moisture content in the feed should be below 10%. The feeds should be stored in polythene lined bags or bins and prevent absorption of moisture from atmosphere. Air tight containers should be used in places where the relative humidity is very high. Steps should be taken to keep the storage facility clean and hygenic to prevent insect infestation.

When the relative humidity goes beyond 70%, mould growth occurs. Some of the fungi like Aspergillus spp can produce mycotoxins. Mycotoxins are metabolites produced by the fungi and are highly toxic and some times corcinogenic. The most effective method of controling mould growth is to check the rise in the moisture content of feed. Chemicals like propionic acid and its salts upto 1000 ppm, and gentian violet can be used as preservatives to prevent mould growth.

Lipid oxidation

Lipids, especially those which are rich in poly unsaturated fatty acids (PUFA) are highly unstable compounds. These can be easily hydrolysed and oxidised leading to rancidity. Due to atmospheric oxygen, lipids under go auto-oxidation producing hydropronides. This is followed by secondary reactions yielding diperoxides and ketoglycerides. The carbonyl compounds produced during the oxidation react with the amino-group (epsilon amino-group) of the amino acid lysine in the protein and render it unavailable to the animals. Light, higher temperature and metal ions catalyse lipid oxidation.

Lipid oxidation can be inhibited by adding anti-oxidants. The common anti oxidants are ethoxy quin, butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT). Tocopherols (Vitamin E) are the natural antioxidants.

QUALITY CONTROL

Control of quality of both the raw materials and the prepared feeds is of paramount importance to achieve the expected efficiency of the feed. There are Government regulations and standard specifications for the production of animal feeds. However no such specific standards have been laid down so far for aquatic feeds.

Before processing the raw materials for compounding the feed, the quality of the ingredients should be checked. The feed stuffs should be free from insect infestation, mould growth and extraneous matter such as sand, stones and other impurities. By adopting proper sampling method, the materials should be tested for moisture content, crude protein, fat, crude fibre and ash (minerals). These must also be checked for toxins like gossypol, isothiocyanates and aflotoxin and for urease activity.

During compounding the feed, strict adherence to the ingredient composition of the formula should be made. This will ensure the specified nutrient levels and calorific value of the final feed. During the process of manufacture of the feed, the factors which can affect the quality of the feed or destroy nutrients should be controlled by adopting appropriate methods. Strict hygenic conditions should be maintained throughout the process to preserve the quality of the feed during its storage. Finally the proximate composition of the feed should be analysed by determining moisture, protein, lipid, carbohydrate, crude fibre and minerals using standard methods and tally with the nutritional specifications of the feed. The feed should be properly packed and stored to preserve its quality. Poor quality feed should not be fed to fish because loss of feed is much less costly than loss of fish.

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Technical Paper - 18

SUMMER INSTITUTE IN
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MICROPARTICULATED AND MICROENCAPSULATED DIETS FOR FEEDING PRAWN AND BIVALVE LARVAE

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Food is normally the largest single item in the running expenditure in fish and shellfish farming. Hence, the suitability and cost effectiveness of the ration is of paramount importance to commercial success. Many different types of feed may require development to meet the varied needs of different species and size of larvae. Palatability and physical structure of shrimp and bivalve ration are inter-related. Both factors alone and in conjucation affect ingestion and have an important impact on prawn and bivalve nutrition. Microencapsulated diet for larval and post-larval diets have been advocated by Meyers (1973). Gelatin microencapsulated diets suitable in sea water. Until the development of microparticulated diets the recent years the study of larval nutrition was impossible. This was mainly because the larvae were semi microscopic whereas the feeds need to be microscopic and water resistant. The development of med made offering of liquid and solid nutrients in pure form made a possibility. This serve as a tool in nutritional research but also an effective way of feeding the larvae with nutrient rich food. Knowledge of the preparation of diets in this form is sure to help in our efforts in developing prawn and bivalve hatcheries in India.

Teshima et al. (1981) have attempted to culture the Rotifers with microencapsulated diets and found to be suitable using the Nylon protein diets. However a prolongered culture of rotifers with microencapsulated diets resulted in a decreased population. Kanazawa et al. (1982) succeeded in rearing of prawn larvae (P. japonicus) using microparticulated diets. Kandasami et al. (1987, in Press) have studied the use of gelatin coated microencapsulated diet for the rearing of bivalve larvae successfully.

I. MICROENCAPSULATED DIETS

(1) Procedure for the preparation of Nylon-Protein microencapsulated diet:

1. To the suspension of the artificial diet (2.5 ml), 1.5 ml of diamine solution (0.92g/10 ml) was added and mixed with the hand.
2. The mixture of diamine solution and artificial diet suspension was added dropwise to the mixed solution of 25 ml of cyclohexane and 0.5 ml of Span 85, homogenizing continuously.
3. Homogenizing continuously, the mixture of 10 ml of cyclohexane and 0.2 ml of sebacoyl chloride was added at a time and thus further homogenized for 15 minutes.

Reaction: Sebacoyl chloride + Diamine

Nylon + Hcl

$$ClCO\ (CH_2)_8\ COCl + NH_2\ (CH_2)\ NH_2$$

$$- CO\ (CH_2)_8\ CO - NH - (CH_2)_6 - + HCl$$

4. After that 30 ml. of cyclohexane was added to the reaction product and allowed to stand for 30 - 60 min.

5. Washing of the microencapsulated diets.

   i) Remove the supernatant by decantation. Repeat with 100 ml of cyclohexan.

   ii) Add Sucrose monolawrate (5-7 ml) and agitate by using a magnetic stirrer for 3 hours to expell the cyclohexane.

   iii) Pour into 2 lit. water and agitate to overnight and then the capsules were collected by centrifugation (at 3700 rpm for 10 minutes).

6. Storage:

   The microencapsulated diet can be stored in 1.0 mol Nacl in a refrigerator (4-5°C).

(2) Procedure for the preparation of Gelatin/acacia capsulated diets (Green and Schleicher, 1957).

1. To one ml of lipid solution add 40 ml of 2% (W/v) at 40°C kept under nitrogen atmosphere in dark.
2. Homogenize for 2 minutes at maximum speed (14,000 rpm) and transferred to 500 ml flask of 3 necked.
3. Reduce the speed to 500 rpm for 1 minute and adjust the pH to 3.9 by the addition of 0.01 M.HCl dropwise which cause the coacervation to the walls of the capsules.
4. Reduce the speed to 100 rpm and allowed to stand for 40 minutes at 40°C.
5. Rise to pH to 9.3 by the slow addition of 1. M.NaOH and transferred to 300 ml water at 5°C. The content was stored in the fridge for one hour to harden the capsules.

6. Centrifuged in a refrigerated centrifuge at 3700 rpm at 10°C for 10 minutes washed repeatedly to remove the excess gelatin solution.
7. Autoclaved at 115°C for 15 minutes and stored in Nitrogen for further use.

(3) Procedure for the preparation of Ethyl-cellulose capsules (V. Rancken and Claeys, 1970).

1. To 20 ml of 5% (W/V) ethyl cellulose in a diet solution. (degree of substitution 2.42 - 2.53) in dichloromethane to a 500 ml round bottom flask at 0-4°C.
2. 13 ml of 20% (W/V) aquous solution of dextrin slowly added with constant stirring at 1000 rpm in a paddle shaped stirrer.
3. After 2 min reduce the speed to 250 rpm and stirr further for 13 minutes.
4. Gradually poured in to a flask containing 100 ml polyvinyl alcohol at 5°C and stirred to 1000 rpm for 1 minute.
5. Reduce the speed to 250 rpm for 10 minutes.
6. Dichloromethane is removed under vacuum at 35°C for 3 hours.
7. Polyvinyl alcohol removed and washed with distilled water and stored in the fridge for further use.

II. MICROPARTICULATED DIETS

(1). Procedure for the preparation of Carregeenan microbinding diet:

Diet ingredients were weighed and mixed well, with 25 ml of water (10 gms diet). The whole mixture was placed in a water bath at 80°C, carregeenan (5 gm. for 100 gm. diet) was added slowly with constant

mixing. Then Potassium chloride was added (5 gm. for 100 gm) slowly with constant mixing. The whole diet was cooled in a refrigerator for 30 mts. The solid diet was cut into small pieces and a bit was tested for the binding effect. The diet was freeze dried and made into powder. The powder was sieved to required sized particles and the same stored in refrigerator.

(2) Preparation of agar-gelatin microbounded diet:

To 10.0 gm of diet ingredients 7% water mix, 0.3 gm agar and 1.2 gm gelatin were added. The whole mixture was mixed well and heated over a water bath at 80°C for 5 mts. cooled to room temperature and freeze dried. The dried diet was made into powder and sieved to adequate size. The particles were stored until further use.

III. MICRO-COATED DIETS

(1) Preparation of Zein microcoated diet:

10 gm of the diet ingredient was taken and 25 ml zein solution was added. (1.0 gm zein dissolved in 25 ml of 60% EtoH). The whole mixture was mixed well and the diet freeze dried. The dried diet was made into powder and sieved to adequate size and stored in a refrigerator for further use.

(2) Preparation of Cholesterol-lecithin microcoated diet:

The diet ingredients were weighed out into a beaker and mixed well. The wet mixture was freeze dried and sieved to adequate size. The particles were coated with Cholesterol-lecithin using 10 ml cyclohexane mixture (0.08 gm. cholesterol, 0.16 gm soybean lecithin and 10.0 ml cyclohexane) for 10 g diet. The cyclo-hexane was dried over nitrogen gas and further dried in

a vacuum desiccator for 8 to 12 hours. The particles were stored in a freezer for further use.

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Technical Paper - 19

SUMMER INSTITUTE IN
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IMPORTANCE OF ANABOLIC AGENTS, BINDERS, ANTIOXIDANTS AND MOULD INHIBITORS IN FISH AND PRAWN FEEDS

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Binders, antioxidants, mould inhibitors and growth promoters are non-nutritional additives added in the feeds. The chemical compound choosen of the purpose should be neither toxic, nor antimetabolic, nor mutagenic, nor carcinogenic, nor teratogenic and nor bioaccumulative. Secondly must be effective at minimal concentration. Thirdly should neither retard growth, nor reduce palatability, appetite and assimilation of dietary nutrients. Fourthly should not react with the feed ingredients chemically in a way that would alter the nutritional quality of the feed adversely. Should be economical . And lastly should not reduce the quality of the meat produced by way of affecting taste, appearance, flavour and texture.

A. Binders

Binders are the substances as the name itself signifies used to bind the various feed ingredients into a compact mass and prevent the feed pellet from disintegrating

on being added to the water. This have two fold function. The popular binding substances used in aquatic feeds are as follows.

1.	Carboxymethyl cellulose (CMC)	- organic compound
2.	Polyvenyl alcohol (PVA)	"
3.	Sodium polyacrylate	"
4.	Propylene glycol alginate	- polysaccharide
5.	Algenic acid	"
6.	Na/K/Ca - alginate	"
7.	Agar	"
8.	Carrageenan (Kappa & iota)	"
9.	Natural gums - guar gum, gum arabic etc.	"
10.	Starches - corn, potatto, tapioca, rice etc.	"
11.	Wheat glutten	- protein
12.	Zein	"
13.	Gelatin	"
14.	Collagen	"
15.	Chitosan	- Amino-sugar

Items 2-3 are used 0.2 to 1.0% w/w concentration in the diet, while all others are used at 1-2 at times as high as 5% w/w levels. Algenic acid salts are the strongest among the list given. CMC is not stable in saline water and is also report to retard growth. The corn starch on boiling form hard wax-like gel contrary to that of potatto starch. Among lambda, iota and kappa varieties of carrageenan, kappa forms rigid gel, while iota forms flexible gel and lambda do not form gel. Proteinaceous binders also serve as protein sources, similarly many of the polysaccharides on digestion to varying extent serve as glucose sources. Heiner (1981) studied water stability of various binders and the finding are as given in the Table 1.

Table- 1: Evaluation of some binding agents for water stability (Heiner, 1981)

Binder	Concentration (% dry wt.)	Rank	Maximum stable time (Hrs.) Moist pellets	Dry pellets
None	-	1	6	6
None (hot water)	-	2	6	6
Corn starch	3	2	6	6
CMC	3	2	6	6
Guar gum	3	3	12	6
Collagen	3	4	12	12
Chitosan	3	3-5	18-24	9-12
Carrageenan	3	8	24	24
Agar	3	8	24	24
Sodium alginate	2	13	24	24

B. Mould Inhibitors

The formulated diet and the feed ingredients (carbohydrate and protein) on being highly nutritive under the conditions of storage and moisture come to have growth of moulds such as Penicillium spp., Aspergillus spp., Fusarium spp. etc., among fungus; Candida albicans and the like among yeasts; and bacteria like Salmonella agona. These by themselves are pathogenic, alter nutritional status, cause bad flavour and taste. Their exocrines also have been found to be toxic (mycotoxins). The most common and lethal among the mycotoxins are Aflatoxin (12 varieties), Tricothecenes (100 varieties), Ochratoxin and Zearalenone. To inhibit the growth of such above said organisms mould inhibitors need to be added to the feeds. Following is a select list of mould

inhibitors used in aquarium feeds - Table 2.

Table-2. Recommended mould inhibitors

Chemical	Recommended concentration in diet
Sorbic acid	No limit
Ca - sorbate	"
K - sorbate	"
Na - sorbate	"
Propionic acid	"
Na - propionate	"
Ca - propionate	"
Menadione	"
Sodium benzoate	less than 0.1%
Propyl-p-hydroxybenzoate	"
Methyl-p-hydroxybenzoate	"

In storing cattle feed copper sulphate at the rate of 120 mg copper per kg of feed (maximum of 240 mg Cu/kg diet) is being used. Among the two gentian violet is better and could be effective for a period upto 12 weeks (Jensen, 1977). Copper could be bioaccumulated and the toxic effects of copper could be reduced by the application of high supplementation (4 g/kg of diet) of sulphur amino acid methionine (Jensen, 1977).

C. Antioxidants

Lipids especially unsaturated fatty acids on exposure to air, light, heat and moisture go bad and come to acquire bad taste and odour. This process is known as rancidity. Polyunsaturated fatty acids are highly prove for oxidation and whereby come to loose its essentiality. On oxidation

they become saturated and yield organic acid by the following reaction.

unsaturated fatty acid + O_2 Peroxide + H_2O

Unsaturated fatty acid + Peracid Aldehyde

Antioxidation

Ordinary organic acid (Eg: Butyric acid) Cu, Fe, EDTA, cyanide and chlorophyll enhance the said process of oxidation and are known as prooxidants. Antioxidants on the otherside absorb oxygen available for oxidation and whereby prevent oxidation. Their role can be illustrated by way of the below given equations, where A - oxidant, AO_2 - peroxide and B - antioxidant.

AO_2 + B AO+BO A + B + O_2

The antioxidants are capable of functioning at minimum levels. Thus one molecule of hydroquinone can protect 40,000 molecules of acrolein from oxidation. Apart from the antioxidants given in Table 3, gallic acid and phosphoric acid are some of the important antioxidants.

The quantity of unsaturated fatty acids in a lipid is measured in terms of iodine number, saponification value, Acetyl number and Reichert-Meissel number.

Table 3 : Antioxidants used in diets

Antioxidant	Recommended concentration in the diet in terms of lipid content
Citric acid	No limit
Ascorbic acid	"
Lecithin	"
S and -tocopherol	"
Butylated hydroxyanisole (BHA)	Less than 0.2%
Butylated hydroxytoluene (BHT)	"
Nordihydroquaiaretic acid	"
Propylgallate	"
Thiodipropionic acid	"
Resin guaiac	"
Ethoxyquin	Less than 0.015%
Santoquin	"

D. Anabolic Agents

The use of growth promoting substances in the rearing of cattle, sheep, pig and chicken is a common practice. The growth promoting agents ie anabolic substances can be grouped under the following categories: 1. Hormones 2. Antibiotics and 3. Organics. Anabolic substances should not be confused with essential nutrients. When certain essential nutrients on being deficient, supplementation of them would result in higher growth rate. These essentials are not called as anabolic agents. In this case growth retardation is due to deficiency of such essential nutrient. Anabolic agents are, chemical substances which on trace quantities capable of enhancing protein synthesis well above the normal.

The use of anabolic agents is beneficial (1) by way of fast growth reduction in growth out period is made possible. Thus either a hatchery or in a grow out system with the available facility production turn over could be increased. (2) Production of oversized ('giant') aquacultural product is possible (3) By way of increased utilisation higher food conversion efficiency is obtained, whereby better utilization of offered diet is possible.

I. Hormones

The anabolic hormones can be grouped under 4 categories:

i. Growth hormones (natural source - pituitary)
ii. Anabolic steroid hormones (Nat. sour. - Gonad)
iii. Thyroid hormones
iv. Insulins

i. Growth Hormones: Donaldson et al. (1979) have made a very detailed review on the subject. The usual methods of administration of growth hormone (GH) is by way of intramuscular injection (im), intraperitonial (ip) injection and by implanting the pellet in the muscle whereby permitting slow release of the hormone over a period. Administration of GH through the medium was not successful while, bathing the gills with GH marginally enhanced growth. With the higher dosage (200 µg/g) and increasing the frequency of injection the growth increase was elevated but the increase was not proportional. Thus at about 3.5 µg/g in one per week seems to give optimal growth in Oncorhynchus kisutch (Cocho salmon).

Among the use of human, ovine and bovine GH, bovine gave better result and the human least. Slight elevation of temperature along with improved the growth.

GH increased the growth by way of increasing the appetite and conversion efficiency. Mobilisation of fat and oxiation of lipids was increased. Fat come to be used as energy source and proteins conserved for growth. An increase in protein/g the body weight too was observed. Further synthesis and release of insulin too was elevated.

ii. Steroid hormones:

The following steroids have been tested (Donaldson et al., 1979) in fishes.

(a) Androgens (Nat. source - Testis)

Testosterone
17 -Methyltestosterone
11 - Ketotestosterone
4 - chloro-testosterone acetate
Ethylestrenol
Methenolone acetate
Dimethazine
Oxymetholone
Testosterone propionate
Methylandrostenediol
Stanozolol
1-Dehydrotytosterone
17 -Ethyenyltestosterone

(b) Estrogens (Nat. Source - ovary)

Estradiol
Estrone
Estradiol
Diethylstilbestol
Estradiol benzoate
Estradiol monopalmitate

(c) Progestogens (Nat. source corpus lutium of ovary)

Progestrone
Pregnanediol
Melengestrol acetate

(d) Corticosteroids (Nat. source - Interrenal gland in fishes, Adranal cortex in mammals)

Cortisol

Deoxycorticosterone acetate

a. Andogens: The usual route of administration is by way of moist diet. Im and Ip injections need handling of fishes and in the process of handling the fish by all possibility could get injured. Mixing of hormone in the watery medium has proved unsuccessful and retarded growth. The successful results have been obtained in the order of lesser value are - 17 methyltestosterone; ethylestrenol; oxymetholone, stanzolol and 1 - dihydrotestosterone acetate. Androgens supressed the growth in females and also resulted in impotent males.

b. Estrogens: Diethylstilbestol only shows slight improvent in growth in fishes even here it is only marginal. Further the results are not consistant with different species.

c & d. Progestrons and corticosteroids: Here too the results are not promising in fishes. It is of interest that many of the above give hormones have enhanced the growth in mammals and in aves but have proved unpromising in fishes. The study in crustaceans is very scarce.

iii. Thyroid hormones: 3,5,3' - triiodothyroxine (T_3) and 3,5,3',5' - tetraiodothyroxin (T_4) are the most common mammalin a hormone used. The addition of the hormone to the watery medium has resulted in growth enhancement in more cases than when either injected or give through diet. Often growth

abnormalities and physiological syndromes have been observed to result on the use of TH. In the water the following concentrations were beneficial $1:1 \times 10^7$; and $1:5 \times 10^5$. The administration of thyroxin resulted in the elevation of SDA and food uptake.

iv. Insulin: Evidences show that insulin play a specific role in the incorporation of plasma amino acids into muscle protein. But the administration insulin to fishes has produced contradicting results.

II. Organics

In the higher vertebrates arsenicals and tranquilisers have been found to increase growth but not much work have been conducted in aquatic organisms. Further arsenic is a cumulatory to poison and so caution need to be observed in the actual application in aquaculture. The common chemical used are as follows:

i. Arsenical

3-nitro-4-hydroxyphenylarsonic acid
p-amino-phenylarsonic acid
Na-arsanilate (Na salt of the second above given acid)

ii. Tranquillisers

Chlor promazine
Reserpine
Natural alkaloid Ranwolfa

How far tranquillisers are anabolic is not clear. It is supposed that by way of their action of reducing excitability and environmental stress the organism is able to conserve energy from metabolism and use them for body building (McDonald et al., 1977).

III. Antibiotics

The antibiotics permitted to be used in diet (0.001 to 0.005%) in USA are as follows: Bacitracin and derivatives, Chlortetracyclin, Lincomycin, Erythromycin thiocyanate, Oxytetracycline, Oleandomycin, Procaine penicillin, and Tylosin. In the rearing of birds, swine, cattle, sheep and rabbits the results have been encouraging while it is negative in case of hamster, guinea pig, cockroack, bugs, aphids and protozoans. The known study is aquatic organisms are as given below.

Animal	Dose	Result
Lebistes reticulatus (fish, guppy)	Chlor. Strep., 1 mg/ml water	Death
Salmo gairdneri (fish, rainbow trout)	Chlortet 1:10,000-1:6,000	Inhibition of metamorphosis and death.
Venus mercenaria (Bivalve)	Chloram 0.1-0.2 ppm	Increased larval length
Astralorbis glabratus	Pleocidin, Nystatin, Patulin	growth retardation

In case of animals in which growth promotion has been effected the reasons given are as follows.

1. Change of intestinal microflora to increase the number (of vitamin synthesisers) useful symbiots, ie preferential anihilation of microflora.

2. Inhibition of gut micro-organisms which utilise or destroy nutrients (better conservation of nutrients)

3. Inhibition of microorganisms that produce toxins (reduction of pathogens)

4. Prevention of thickening of gut walls whereby facilitating increased absorption of nutrients, especially lysine, lipids and calcium (Mc Donald, et al. 1977).

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Technical Paper - 20

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DETERMINATION OF ENERGY CONTENT OF FEEDS

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INTRODUCTION

In nutrition studies it is often necessary to know the calorific value of the feed, faeces and flesh. These values are necessary to compute the energy budget and to determine the efficiencies of absorption and conversion. In nutrition requirement studies it is essential that the test diets be isocalorific. Therefore determining the energy content of feeds and its components is one of the basic techniques used in nutrition studies.

UNIT OF MEASUREMENTS

Generally the energy content is expressed in terms of calorie (cal) or kilogram calorie (Kcal). The calorie is defined in terms of heat units as the heat required to raise the temperature of one gram of water at 15°C by 1°C. The usefulness of calorie is due to the fact that all forms of energy can be converted into calories, while they cannot be wholly transferred into any other forms of energy. However, in the international unit system SI (System International) which has been adopted by many countries, instead of calorie, the joule (j) a unit of mechanical energy has been introduced. The direct link between mechanical energy and heat energy is expressed in joule's law, namely that the equivalent of

one calorie in units of mechanical energy is 4.187×10^7 ergs or 4.187 joules (Grodwinski et al., 1975). A list of energetic equivalents and conversion factors are given below.

1 kilo calorie (Kcal, C)	= 1000 gram calorie or calorie (cal, C)
1 Kcal	= 3.968 British thermal units (Btu) at 60°F
1 Kcal	= 4187 joules
1 joule	= 2.388×10^{-4} cal
1 Btu	= 0.252 Kcal at 15°C

It is best to express the energy content of the sample in terms of ash free dry weight, for ash is often responsible for 10-15% of the dry weight. In general, calorie value (ash free) should occur between a lower limit-set by glucose (3.74 Kcal/g) and an upper limit determined by the value of oils and fatty acids (9.37 Kcal/g). Not all substances fall within this range for example glycine (2.08 Kcal/g), urie acid (0.91 Kcal/g) etc., (Paine, 1971).

METHODS OF DETERMINING ENERGY CONTENT

There are four common methods for determining the energy content of materials.

I. Component analysis: Lipid, protein, and carbohydrate are the energy nutrients. If their quantitative values are known, calorific value can be calculated by applying appropriate caloric conversion factors. Most of the conversion factors (Table 1) given in literature closely agree with each other.

Table 1 - Calorific value (KJ/g)

Protein	Lipid	Carbohydrate	
23.7 (5.65)	39.6 (9.41)	17.2 (4.1)	Brody (1945)*
23.4	39.2	17.2	Cho (1973)
23.6	39.5	17.2	Jobling (1983)

* (The values given within brackets are in Kcal/g from which the values in KJ/g have been calculated on the basis of

$$1 \text{ joule} = 2.388 \times 10^{-4} \text{ Kcal}$$

Usually the energy content of pellets comprising a mixture of the three basic nutrients is usually 20-25 KJ/g.

The draw back in component analysis is that it needs results out of three analytical procedures before computing the energy value in contrast with the less time consuming bomb calorimeter method. The errors in estimating each of the components is reflected in the final energy value. The conversion factor for each component has been based on Brody's (1945) work on mammals. Brett and Groves (1979) have pointed out that though 39.5 KJ/g is appropriate for the saturated fats of mammals, a more appropriate figure for the highly unsaturated fats associated with fish may be 36.2 KJ/g.

The advantage of component analysis is that unlike other studies involving energetics, in nutrition the proximents composition of the feed is always worked out and therefore it is easier to calculate the energy value. Also, in laboratories where a bomb calorimeter is not available, the component analysis is the next best method to get an accurate value of energy content.

II. Wet oxidation:

The principle of this method is that the sample material is heated with an oxidising agent so that the sample is oxidised. By titration, the remaining oxidising agent is determined, from which the amount of oxygen consumed by the sample material could be determined. The sample calorie value is calculated by multiplying the oxygen consumed (mg) x 3.38 cal. Various oxidising agents like dichromate and potassium iodate have been used. The spectrophotometric method of determining organic oxidisable matter as carbon can also be used.

The draw backs in this method are that it is still not certain whether the wet oxidation technique for all the oxidisable carbon (Paine, 1971). The method assumes that all the carbon present is as carbohydrate. Therefore, the amount of nitrogen in the sample, greater is the deviation of, the wet oxidation value from that of bomb calorimetry.

III. Thermo chemical method:

Theoretically it is possible to calculate the enthalpy of any given substance from known heats of formation. If the percentage composition of constituent elements are known, then a balanced empirical equation could be developed representing the oxidation reaction of a bomb.

The above method is more of theoretical interest than of practical utility.

IV. Bomb calorimetry:

The principle of all bomb calorimetry is that a known weight of sample is ignited electrically and burned in an excess of oxygen in the bomb. The oxidation is explosive and complete so that the whole heat output is instantaneous.

The heat output is estimated by directly measuring the rise in temperature of water surrounding the bomb or by means of a thermocouple and a potentiometer. Comparing this rise with that obtained when sample of known calorific value is burnt, the calorific value of the sample material can be determined. There are different types of calorimeters and they can be grouped into two categories - (1) whether the heat loss from the calorimeter during test is eliminated or not and (2) based on the sample weight that need to be used.

Adiabatic and non-adiabatic calorimeters: In adiabatic bomb calorimeters the bomb is placed in a calorimeter vessel containing a fixed quantity of water. The calorimeter vessel is surrounded by an outer jacket and there are provisions to add hot or cold water to keep the jacket temperature equivalent to the calorimeter. In non-adiabatic calorimeter this provision is lacking and therefore the temperature rise in the calorimeter vessel has to be corrected for radiation.

Micro, Semi micro and Macro calorimeter: Based on the range of sample size that can be used the bomb calorimeter can be grouped into micro with a sample range 1-10 mg, semi-micro with 10-100 mg and macro with 200 mg to 1.5 g. The minimum and maximum sample size in eaeh of these categories varies depending on the make of the bomb calorimeter. Phillipson micro-bomb calorimeter, the semi micro Parr calorimeter and the macro Gallenkamp calorimeter are the commonly used bomb calorimeters.

Basic features of a bomb calorimeter: All types of bomb calorimeters have the following features namely (i) Bomb (ii) Oxygen supply (iii) firing assembly (iv) temperature sensing and recording device.

(i) Bomb: This is made of thick stainless steel and the capacity depends on the type of bomb calorimeter. It consists of two halves, both of which can be screwed together with a teflon or rubber 'O' ring washer in between them. One part of the bomb is hollow and to the other part two ignition circuit terminals and sample holder are attached. Of the two terminals one is insulated with which sample holder is attached, and the other connected to the bomb. At the time of loading the sample a platinum fuse wire is attached connecting the terminals. The platinum fuse wire is bent in such a way it is in intimate contact with the sample. In some instruments instead of pt fuse wire a cotton wig used. There is an inlet for oxygen which can be closed and an outlet controlled by a needle valve. The oxygen pressure inside the bomb is read through a gauge. The sample is placed in a platinum cricible and kept over the sample holder of the bomb.

(ii) Oxygen supply: The oxygen is supplied to the bomb from an oxygen cylinder by means of reducer and two gauges measure the oxygen pressure inside the bomb and the oxygen cylinder.

(iii) Firing assembly: It consists of batteries, condensor, resisters, charging and firing circuit, timer and operating switches. The whole thing is mounted on a control board to which are attached other electrical connections.

(iv) Temperature sensing and recording device: This consists of either a sensitive thermometer (with an accuracy of 0.0002°C) or a thermocouple. In bomb calorimeters with thermometer the bomb is immersed in a fixed weighted amount of water which is constantly stirred during the test. The temperature rise in the bomb is passed to the water and its rise in water is recorded. In those calorimeters with the thermocouple, it is attached directly to the body of the bomb.

It is advantageous over the thermometer in that it takes lesser time and a permanent record can be made by attaching the thermocouple to a potentiometric strip chart recorder. High precision spot galvanometer or a potentiometer too can also be used.

Sample preparation

The sample for the sake of uniformity need to be dried in a hot air oven-(55-80°C) or a freeze drier. Drying rapidly as far as possible is essential because of the progressive decomposition of fats to fatty acids with different calorific values. The next step is grinding the sample to a fine powder and an aliquote of the powdered material is-made into a pellet in a pellet press. It is also advisable to dry the pellet until a constant weight is attained. The criteria governing sample size is that the total heat released by the sample should fall-within the limited temperature range over which each-bomb calorimeter is linear. For example in Gallenkamp bomb calorimeter, the recommended heat release is 4.0 Kcal. This gives a sample range of about 0.4 g for fat to about 1.5 g for a material such as urea. If the sample is difficult to ignite or not adequate enough, a known amount of standard material usually benzoic acid has to be mixed to give the recommended total heat release. All materials which have low bulk density and high surface area must first be compacted in order to reduce their rate of combustion and to prevent incomplete combustion. If needed few drops of water with a wetting agent can be added and allowed to soak in before igniting the sample.

Calibration: Since 1934 benzyoic acid - 6.32 Kcal/g (C_6H_5 COOH) has been the-international standard used in thermochemistry and all bomb calorimeters are calibrated against it. It is obtainable from the National Bureau of Standards, Washington DC 20234 as standard sample 39i. The

purpose of calibration is to check whether there is direct dependences between the amount of substances burned and the thermometer recorded readings and to obtain calorific equivalent for one recorder division. The first step involves burning benzoic acid samples of different weight to find the range over which the measurement is linear. The second step involves burning 10 or more benzoic acid samples falling within the linear range of the calorimeter. From these recordings calorific equivalents for one recorder divisions is calculated.

Precautions

1. Weighing and temperature measurement errors should be avoided especially when handling low sample weights.
2. The sample material should be homogenous and this can be achieved by grinding and mixing. If the aliquote taken for making the pellet is not representative of the sample, it can cause serious errors especially in microbomb calorimeter.
3. A correction for the constant heat gain due to the firing current and firing cotton or fuse wire should be carried out.
4. In those calorimeters with thermometer, it is advisable to tap the thermometer gently before and during each reading so that adhesion between the mercury column and surrounding glass is minimised. After each run a stem correction to correct for the error due to differential expansion of thermometer's mercury column which is exposed to both water and air need to be carried out.
5. A radiation correction for the heat loss or gain during the temperature rise due to combustion of sample has to be carried out.

6. Another source of error is the formation of acids like nitric and sulphuric following combustion. An acid correction can be estimated by assuming that all the acid is HNO_3 and titrating with 0.0725 N sodium carbonate. (At this normality 1 ml titrate is equivalent to 1 cal).

7. Incomplete combustion can result in an underestimate of calorific value. This happens mainly due to rapid admission of oxygen to the bomb which can blow part of the sample away. Other reasons are loose or too densely compacted pellet or low oxygen pressure.

8. Material with high ash content due to endothermic reactions within the bomb do not give an accurate value.

9. A violent combustion due to not compacted pellet or higher oxygen pressure or oxygen leak in the bomb during firing can lead to hot gases escaping past the sealing ring and cause irreparable damage to the bomb.

Ballistic bomb calorimeter: In ascertaining the calorific value of feeds and other non homogenous materials it is difficult to obtain a truly representative single sample. In such cases, the average of a number of less accurate results is superior to a single very accurately determined one. In Ballistic bomb calorimeter which is a macro-nonadiabatic type, replicate testing can be done very fast (upto 10 results per hour). Therefore, for estimating energy content of feeds, it is the ideal bomb calorimeter.

Procedure:

1. The crucible with the weighed sample is kept on the support piller in the base of the bomb. One end of a 50 mm length of firing cotton is inserted between the coils of the firing wire and the other end dropped into centre of sample in the crucible.

2. After checking that the bomb sealing ring is in position in the groove, raise the knurled locking ring and engage the thread-of-the bomb body with that of the ring. Lower the bomb body and grasping it firmly with one hand screw the sealing ring.

3. Plug the thermo couple into the hole in the top of bomb body.

4. Close the pressure release valve in the bomb and open the valve in the front panel of the control box about ¼ turn. Allow the pressure to rise to 25 atmo. and close the valve.

5. By means of galvo zero knob on the control box, bring the light spot index on the galvanometer to zero and check for 30 sec. for any drift due to temperature variation.

6. Stand back from bomb and press the firing button. After 10-15 sec. increasing deflections on the pressure gauge and then in the galvanometer will indicate that the firing has been successful.

7. Note the maximum deflection of the galvanometer.

8. Release the gases from the bomb through the pressure release valve.

9. Carry out the above procedure for estimating the correction for constant heat gain and for calibration with 0.7 g of benzoic acid.

Calculation of results:

Mass of benzoic acid	=	W1 gram
Calorific value of benzoic acid	=	6.32 Kcal/g
Heat release from benzoic acid	=	W1 x 6.32
Galvanometer deflection without sample	=	X1

Deflection with benzoic-acid = X2

Deflection only due to benzoic acid = X2 - X1 = X3

Calibration constant = $\frac{6.32 \times W1}{X3}$

= Y1

The mean of 10 calibration constants is calculated

= Y2

Mass of sample = W2 gm

Galvanometer deflection with sample = X4

Deflection only due to sample = X4 - X1

Heat release from sample = X5 x Y2

Calorific value of sample = $\frac{X5 \times Y2}{W2}$

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Technical Paper -1

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DIGESTIBILITY OF FEEDS IN FISH AND SHELLFISH AND METHODS TO DETERMINE DIGESTIBILITY

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Digestibility of a feedstuff can be described as the amount of feed that can be digested and absorbed by the animal in relation to consumption. Digestibility is measured as digestibility coefficient when it is expressed as percentage and as digestible energy which is most often used in warm water fishes. Knowledge of nutrient availability is necessory for effective substitution of ingredients for formulating low cost diets.

Factors affecting digestibility

Digestive coefficients are influenced by several factors and do not remain constant for a given feedstuff or species. Some of the factors affecting digestibility are:

- Nutrient composition of diet. This is a very important factor. Protein digestibility decreases with increase in carbohydrate in the diet in channel catfish. Similar observation made in some other fishes also.
- Gastric evacuation time. Lack of time for complete digestion and absorption which may result from increased food intake may affect digestibility. Amount of food consumed does not affect protein

digestion in fish but may have influence on digestion of lipids and carbohydrates.

- Increase in meal size reduces absorption in many fishes probably by reducing the surface area of the meal to be digested in comparison with small food particles.
- Crude fibre in diet. It has protective value for other nutrients and also help in easy passage of food through the alimentary canal.
- Microorganism in the diet.
- Temperature. Increase in temperature increases digestibility in some fish (channel cat fish). In carp, however, no change in protein digestibility over a range of temperature that supports growth, but digestibility reduces when temperature is reduced below this range.
- Species diversity

Nutrient digestibility in fish and shellfish

In general the digestible energy of fishes is 75-80% of the energy consumed and 96-98% of this digestible energy is metabolisable. Digestibility of proteins is affected by the presence of carbohydrates, both in quality and quantity, in fish and shellfish. In Penaeus japonicus 93% of digestion and absorption of glucose and galactose are completed in midgut but only 76-80% of sucrose and glycogen are digested and absorbed in this region. The process is completed in the hindgut. Starch digestibility is very good in warmwater fish and prawns. In common carp 85% of starch is absorbed at a dietary level of 10-60%.

Most animal proteins appears to be highly digestible in fish and prawns, as much as 99% of protein can be digested in some fishes.

Lipid digestibility in fishes varies from 70-94% depending on the nature of fat and water temperature. Oxidised lipids reduces digestibility of diets but oxidised lipids supplemented to the diets do not reduce digestibility of proteins in carp. When dietary fat is low, yearling carp secretes endogenous fats into the lumen of the intestine and this may affect estimation of apparent digestibility. In P. japonicus fatty acids are easily digested when esterified.

Differential absorption has also been observed for minerals. In common carp calcium absorption was more when its level in the diet was increased from 0.09 to 1.24% while phosphorus was held constant. Mineral absorption is also affected by the mineral base used in the diet formulation.

Determination of digestibility

Determination of digestibility involves measuring total quantity of nutrients ingested and the amount of corresponding nutrients egested.

$$\text{Digestibility} = \frac{\text{Amount of nutrients injested} - \text{Amount of nutrients egested}}{\text{Amount of nutrients ingested}} \times 100$$

$$\text{True digestibility} = \frac{I - (F - F_k)}{I}$$

where I = Intake, F = Faeces egested and F_k = Endogenous metabolic faeces.

Endogenous materials such as secretion from within the intestinal tract, sloughed epithelial cells and other materials of metabolic origin also may occur in faeces. It is very difficult to measure such endogenous material and hence apparent digestibility is usually measured rather than true digestibility.

Apparent digestibility can be measured by direct faecal collection method or by indirect method using inert marker or indicator which can be external (added to the diet) or internal (integral part of the diet). Ideally, an indicator should be totally indigestible and excreted at the same time as the other gut contents. It should not reduce palatability of food and should be readily determined. Digestibility is determined indirectly by calculating the rate of indicator concentration in dry food and the same rate form the test food.

$$\text{Apparent digestibility of nutrients} = 100 - 100 \frac{\%\text{ indicator in food}}{\%\text{ indicator in faeces}} \times \frac{\%\text{ indicator in faeces}}{\%\text{ indicator in food}}$$

The most popularly used indicator is chromic oxide, Cr_2O_3. Other indicators used are Polythene markers, radioactive phosphorus (P_{32}), silica, Hydrolysis Resistant Organic Matter (HROM), Hydrolysis Resistant Ash (HRA), crude fibre, titanium (IV) oxide and metalic iron particles.

Faeces collection is very important in digestibility estimations. Faeces should represent quantitatively the ingested residue of the food consumed. Main problem in faeces collection is the leaching of nutrients in water. Faeces can be collected by sacrificing the animal, by stripping the intestine and from the rearing tank after egestion.

1. Direct faeces collection method

Materials needed:

- Specially designed aquaria for faeces collection. Troughs (closed system) can be used in its absence but faeces should be collected frequently if the latter is ned.
- Reagents and equipments required for protein, lipid and carbohydrate determination.
- Hot air oven
- Feeding trays, polythene tubes for siphoning out the faeces, aerators etc.

Procedure

The feeding trials should be conducted for 10-15 days. First the animals are to be acclimatised to the rearing conditions and to the test diet. Before the start of the experiment the animal has to be starved for one or two days for complete gut evacuation. Feeding with test diets should last at least 10 days and faeces should be collected at regular intervals, washed with little quantity of distilled water if the fish is reared in saline water, and dried in an hot air oven at 60°C. After the completion of the experiment weigh the total faeces collected and determine the nutrients in dry faeces.

Calculation

$$\text{Apparent digestibility} = \frac{I - F}{I} \times 100$$

where I = Nutrient intake and

F = Faecal nutrient

2. Chromic oxide indicator method

The experimental design is same as in direct faeces collection method. The test animals should be fed with the test diet without chromic oxide for about a week before start of the experiment.

Determination of chromic oxide content

The method involves oxidation of organic material in the sample using concentrated nitric acid followed by oxidation of insoluble (green) chromium III in chromic oxide to soluble (yellow) chromium IV, which is then determined spectrophotometrically.

Reagents

- Conc. Nitric acid and Perchloric acid

Apparatus

- Kjeldahl digestion tubes
- Heating mantles
- Gloves and Goggles for protection against acid fumes
- 5 ml graduated pippette with filling bulbs
- 100 ml volumetric flasks
- Spectrophotometer
- Cuvettes.

Procedure

1. Weigh accurately 50-100 mg of sample into a piece of foil
2. Transfer sample carefully into a numbered Kjeldahl flask and reweigh the foil.
3. Add 5 ml conc. HNO_3 washing the sample down from the sides of the flask.

4. Place the flask on a heating mantle and boil gently for 20 minutes. Do not allow to boil it dry, if it is add more HNO_3.
5. Turn off the mantle and allow to cool.
6. **Add three ml of perchloric acid to flask with care.**
7. Boil in the mantle until colour changes from green to yellow orange or brown and continue to boil for a further 10 minutes.
8. Allow the flasks to cool - if the colour reverts to green it must be reboiled.
9. Cool the flask to room temperature and then carefully wash the contents into a 100 ml volumetric flask with distilled water and make upto 100 ml.
10. Transfer an aliquote to a spectrophotometer cuvette and read optical density at 350 nm with distilled water as blank.

Calculation - 1

Weight of sample = A

$$\text{Chromic oxide in the sample} = \frac{\text{OD of sample}}{\text{OD of standard}} \times \text{Concentration of standard}^* = X$$

$$\% \text{ Chromic oxide in sample} = \frac{X}{A} \times 100$$

* Known concentration of chromic oxide should be treated in the same way as the sample for standardisation.

Calculation - 2

Weight of sample = Amg

Optical density = Y

$$\text{Weight of chromic oxide in sample (mg)} = \frac{Y-0.0032}{0.2089} = X$$

$$\% \text{ chromic oxide in sample} = \frac{X}{A} \times 100$$

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Technical Paper - 22

SUMMER INSTITUTE IN
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DETERMINATION OF METABOLIC RATES AND QUOTIENTS IN FISH

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Before any growth can be achieved by an organism there must be sufficient energy in the food to provide for the metabolic demands of maintenance and any activity associated with food intake. In culture practices it is possible to reduce these energy costs to a minimum and to optimize food conversion. This can be done through an understanding of the environmental and biological factors affecting metabolic rate with a view to reduce the demands on the system and hence increase the production. It is therefore desirable to examine the broad picture of energy exchange within an animal, then proceed to more specific considerations in fish. Since it is the principles and prospects which are of major interest here, no attempt is made to make this a major review of the subject.

A general balance sheet of the biological uses (profits) and losses associated with the conversion and utilization of energy by fish can be prepared by using the available sources of information. Starting with a given intake of 100 calories, an average of 80 calories remaining from assimilation as net energy available for biological use. If the metabolic demand is low, nearly 50 calories may be converted into growth. If the metabolic demand from activity is high, all 80 calories may be involved in total metabolism. The sources of energy loss (20 calories) are

feces, urine+NH_3 and heat increment.

Metabolic rate/power, or the rate of expending energy, has usually been measured by determining the rate of oxygen consumption of fish at various levels of activity. It should be stressed that the assumption implicit in all such measurements is that a state of equilibrium exists between oxygen demand and supply.

In practice, basal metabolism is defined as the minimum energy cost when the animal is at rest in a temperature acclimated environment in the post-absorptive condition. The possibility of obtaining absolute rest has been questioned frequently for animals subject to excitement from handling. This has led to the use of standard metabolism which is usually measured after 24 hours of fasting, during the slump of any diurnal metabolic cycle.

Active metabolism is the maximum rate consistent with the highest continued level of activity.

Routine metabolism has been used to express the average oxygen consumption of fish which are moderately active. It forms a closer approximation of normal demand because fish are neither continuously resting nor continuously moving.

Many factors influence the metabolic rate. The abiotic factors are temperature, salinity, oxygen, carbon dioxide, ammonia, pH, photoperiod, season and pressure, and the biotic factors are activity, weight, sex, age, group (schooling), O_2 debt, condition, starvation and diet. To illustrate the multiplicity of involvement between metabolic rate and environment it is apparent that within the bounds of knowledge the factors of temperature, oxygen and activity exert the greatest effect on metabolism--in unpolluted waters.

Even oxygen at 100% air saturation may act as a limiting factor to sustained performance when the combined factors of temperature and activity are high. It is perhaps surprising that salinity does not appear to have more effect on reducing metabolic rate since it might be expected that the lack of ions in freshwater would impose a fair energy demand to maintain osmotic balance. On this basis it may be predicted that except in the estuary any advantage which the marine environment might confer on growth, through the energy-saving mechanism of reduced osmoregulation, would be small.

Activity has a tremendous effect on metabolism, frequently elevating the oxygen uptake by a factor of 4 times at optimum temperatures and reaching a maximum of 8 times in some fish species. In the case of active metabolism, undernourished, unexercised, disease-inhibited, sluggish or lazy fish could reduce the potential level. However, excitation and oxygen debt replacement (unless depressed by excessive waste products) make large demands on metabolism. Since the ventilation of the gill chamber could be the limiting factor, and free swimming rather than just opercular movement can facilitate ventilation, the circumstances within the respirometer must not be overlooked.

The gill system of fish is remarkably efficient, working on the counter-current principle and capable of removing 80% of the dissolved oxygen. When there is excess oxygen (respiratory independence) no limitation to activity is imposed. Below this level of excess, active metabolism is dependent on oxygen availability (respiratory dependence). Where standard metabolism becomes dependent, any continued activity will result in death from irreplaceable oxygen debt (level of no excess activity).

More recent manifestations of interest in fish metabolism studies are apparent in the various tests on stamina, using water tunnels, flumes, or rotating circular troughs - the nearest equivalent of the step-test or bicycle ergometer for man. The step that was necessary to relate metabolism and performance was to conduct experiments where swimming speed could be accurately documented while determining the accompanying the stable respiratory demand for oxygen. In recent years, the apparatus commonly used for metabolism studies are Fry's respirometer and Blazka's respirometer.

The Fry's respirometer is a modification of the annular respirometer of Fry and Hart (1948) and of Smit (1965). The earlier respirometer (Smit, 1965) had a concave transparent plastic lid covering the annulus of the chamber and did not provide an effective seal against diffusion of gases. The present one is modified in that its top is covered by a transparent plastic sheet with only two wells opening above. Fitting these wells are two plastic cups with a hole at the bottom in each. These wells alone offer open surface to the exterior. The design of the respirometer is such that the diffusion of gases into and out of the respirometer is minimized. The activity of the fish is recorded by the electronic counter which is a part of the respirometer assembly. When the fish moves in the annular chamber it interrupts the light beams focussed on the photocells, and these events are recorded in a counter. An interlocking system is provided so that, when the fish obstructs the same light consecutively, the second and the following events will not be recorded for counting, unless otherwise the other light is interrupted in between the two events to actuate the counter. Thus mal-operation due to minor events such as the flick of tail or head, is avoided. More details of the apparatus are given in Kutty et al.(1971).

Blazka's respirometer (Blazka et al., 1960) consists mainly of two concentric transparent (Perspex) cylinders, one fitted inside the other. A propeller is fixed on one side and a shocker grid on the other side. The propeller is fixed to rotate just inside the inner cylinder. The rotation of the propeller induces a water current inside the inner cylinder. The water circulates back to the propeller-end through the clearance between the outer and inner cylinders. Water currents in required velocities could be obtained with the help of a voltage regulator connected to the motor. The apparatus is so designed that water can flow in and out of the respirometer (open system) or can be left to stagnate (closed system) when the fish is being exercised depending on the experimental needs. The shocker grid serves two purposes, viz., one of closing the inner cylinder without letting the fish escape to the outer cylinder and the other of preventing the fish from resting on it. When a fish rests on the grid the exposed wires become connected and a circuit is established resulting in a shock to the fish. Unless a fish is unable to swim due to low ambient oxygen or is really fatigued it will not rest on the grid.

Metabolic rates can be estimated by simultaneous measurements of rate of O_2 consumption, CO_2 output and NH_3-N excretion. Anaerobic metabolism and its link with protein utilization can also be estimated from concurrent measurements of R.Q. and A.Q.

$$\text{R.Q. (Respiratory Quotient)} = \frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ consumed}}$$

$$\text{A.Q. (Ammonia Quotient)} = \frac{\text{Volume of } NH_3\text{-N excreted}}{\text{Volume of } O_2 \text{ consumed}}$$

Under high ambient oxygen concentrations R.Q. near unity will be maintained by the fish, which indicates that the fish was drawing energy aerobically or maintaining aerobic metabolism. If the R.Q. goes above '1' which indicates the anaerobic energy utilization. There are instances (during hypoxia) where the increased carbon dioxide production is combined with increased ammonia excretion. This may be of advantage to the fish in acid-base regulation (preventing acidosis) and iono-osmotic regulation (conservation of Na^+). It is possible that non-ionic ammonia is excreted by passive diffusion down a concentration gradient across the gill surface from blood to water, and the activities of glutaminase and glutamic acid dehydrogenase are high in fish gills. However, the ammonia source could be peripheral or otherwise.

The discussions made at present although provides some insight into the importance of physiological aspects especially metabolic rates and quotients in fish, more information is necessary to elucidate the metabolic and behavioural responses of more species in view of their importance as cultivable warm-water fishes. However, the involvement of environmental interaction is not a deterrent to progress but a measure of the complexity to be met.

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Technical Paper-~~22~~ 23

SUMMER INSTITUTE IN
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FEEDING STRATEGIES IN THE LARVAL REARING OF PRAWNS

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INTRODUCTION

In the early life history of extremely fecund fishes and shellfishes food and feeding is the most important factor. Availability adequate right kind of food in the growing environment is essential for the survival and growth of the larvae. Because of the complex life history of prawns with different larval stages and sub-stages and the process of metamorphosis requires lot of energy, identification of suitable feed and feeding techniques becomes very important in the larval rearing of prawns. In recent years significant achievements have been made in the development suitable diets and evolving feeding strategies in the prawn hatchery techniques worldwide. Though a general agreement could be seen in the type of feed, experiences gained indicate that it is not easy to evolve a fixed strategy in feeding the larvae as it varies according to the techniques followed various other physico-chemical environmental and biological factors. However, the following general outline may be considered in adopting the feeding strategies in the penaeid prawn larval rearing.

FOOD AND FEEDING OF THE LARVAE

Different type of feed such as live feed (phyto and zooplankton) and artificial feed are being used in the larval rearing of penaeid prawns. The phytoplankton generally include the species of Chaetoceros, Skeletonema, Thalassiosira, Tetrasulmis, Isochrisis etc. in the early stages of the larvae. Zooplankters such as the brine shrimp Artemia nauplii, Rotifers (Brachionus sp.) and cladoceran (Moina sp.) are used as live diets from mysis stages. Microencapsulated and microparticulated compounded feed are also used in recent years.

Penaeid prawn egg hatches out into Nauplius stage which undergo six substages before metamorphosing into the next protozoea stage in 2 days period. Nauplius does not have move or alimentary canal and cannot feed and subsists on yolk material present in the body.

Protozoea stage has three substages and lasts for 3-4 days before metamorphosing into mysis stage. The larvae in this stage have an alimentary canal, mouth and feeding appendages and starts feeding on the unicellular algae. It has an efficient mechanism to seive the algal cells from the water.

Mysis stage also has three substages lasting for 3-4 days period after which it metamorphosis into postlarval stage. In mysis stage also the larvae retain the filtering mechanism for feeding on algal cells. The clams in the first three walking legs are not functional and cannot be used effectively for capturing fast moving prey.

In the post larval stage, the food and feeding habit changes drastically. The larvae becomes a particulate feeder, capable of capturing and ingesting zooplankton and other particles. It is essential for evolving a suitable

feeding strategy this food and feeding behaviour of the larval stages should be duly considered.

LARVAL REARING SYSTEMS

The feeding strategy mainly depends upon the methods followed and the following are the important rearing systems practiced worldwide in penaeid prawn larval rearing.

The Japanese system:

In this system large concrete tanks of 60-200 tonne capacity are used. Tanks filled with filtered seawater in low level (about 0.40 m) and spawners are kept in net cages for spawning. After spawning the spawners are removed. The tank is fertilized with nutrients and the water level is gradually increased. The desirable algal blooms are allowed to develop *in situ* . The cell concentration is maintained @ 5,000 to 20,000 cells/ml in the rearing tanks. From mysis stage onwards larvae are fed with *Artemia* Nauplii or washed clam meat to postlarval stage. The rearing techniques have been well documented by various authors (Hudinaga and Kittaka, 1966, 1967; Shigueno, 1975; Yang, 1975). In Taiwan also this technique has been successfully used (Liao and Huang, 1973). In Philippines and some of the private hatcheries in other parts of the world also this system is being practiced.

The Galvaston system:

A close cycle system for the larval rearing of penaeid prawns was developed in Galvaston Laboratory, USA (Cook, 1969; Mick and Murphy, 1973; Mick and Neal, 1974). This involves the independent process of larval rearing and live feed cultures and suitable integrating the two with proper water management. Desirable species of pure culture of diatoms especially unicellular algae are used as feed

for protozoea stages, maintaining a concentration of 10,000 to 15,000 cells/ml in the rearing medium. From mysis stage onwards Artemia nauplii are fed and a concentration of 3-5 nos/ml is maintained as food concentration in the rearing container.

Methods followed in India:

In India the CMFRI has developed a viable hatchery technique for the large scale seed production of penaeid prawn seed under Indian conditions. The technique includes feeding the larvae from protozoea stage onwards with mixed culture of diatom, dominated by Chaetoceros sp. upto post-larval stage after which the larvae are fed with compounded microparticulated feed. This system is found to be successful in rearing almost all the cultivable penaeid prawn species under Indian conditions. The water quality is maintained by suitably changing the water. Food concentration of diatom is maintained @ 20,000 to 30,000 cells/ml (Silas et al., 1985).

Addition of dry formula feed developed at the MPHL, Narakkal, in the larval rearing tanks also gave encouraging results by developing suitable ecosystem in the larval rearing tank. The particle size of the compounded feed and the quantity is adjusted to the required level. (Mohamed et al., 1983).

In the regular commercial hatchery operations at Azhikode, in Kerala ground tissue of mantis shrimp is used as feed in suspension form. This feed addition in outdoor rearing tanks serves the purpose partly as feed and helping adequate algal blooms in the rearing tank (Alikunhi et al., 1980).

Quality of the Feed

The feed supplied should meet the nutritional requirements of the larvae and should be available in the rearing medium adequately continuously.

In the larval rearing procedures where the larvae are fed with pure cultures or mixed cultures of diatom the culture should be in the developing phase. Exponential phase or declining phase cultures often deteriorates the water quality in the rearing container by immediate breakdown of cells and thereby accumulating the organic load in the tanks. The larvae could not feed. Under this circumstances, especially during night hrs. mass mortality is encountered mainly due to oxygen depletion.

In the case of compounded feed freshly prepared are desirable. Long stored feed should be tested before used for nutritive value and toxins before feeding.

Quantity of the feed

Normally in quantifying the feed to be supplied in the aquaculture systems the biomass is considered. But in the larval rearing the larval concentration, the general conditions of the larvae the quantities of food in the rearing medium, the food concentration of the source, water quality, other environmental conditions such as light intensity, photoperiod, temperature are to be considered in determining the quantity of the feed to be supplied.

In the feeding of phyto cultures under Indian conditions normally 150-200 litres of phytoplankton/day (of the concn. of 2-3 lakhs cells/ml) is recommended for 2 tonne capacity rearing tank with 150-200 thousand larvae. However, this quantity has to be adjusted according to the conditions already mentioned (Silas et al., 1985).

Artemia nauplii feed is given at the rate sufficient enough to maintain a concentration of 3-5 nos/ml in the rearing tank (Murphy, 1969). Rotifer is fed at the rate of 100 nos/postlarvae/day. Frozen cladoceran Moina is provided as food for the postlarvae @ 20 nos/larva per day.

In the outdoor tanks 12-25 gm/day for a 2 tonne capacity rearing tank is suggested dosage for post-larval feeding (Silas et al., 1985).

Size of the feed

Considering the feeding habits of the larvae, the appropriate size of the feed supplied is important for successful rearing. Over the years much efforts were put in this field. The feed should be a reasonably appropriate size so that the larvae can ingest. Diatom of 5-10 micron (Chaetoceros sp.) is desirable especially in the early stages. Rotifers used in the mysis stage or postlarval stage range from 150-250 microns in size. Mysis/postlarval diet Artemia nauplii is about 400 micron. Postlarval feed like cladoceran Moina is in the size range of 0.70 to 1.2 mm.

The microencapsulated and microparticulated diet developed for various stages of penaeid prawn also of this size. Hence in any feed development and feeding strategy the size of the feed also to be considered.

Feeding time

Supply of quality of feed in optimal concentration is important step. Unlike the adult where feeding periodicity is exhibited in most of the cases, the larvae are continuous feeder and hence fall for continuous food abundance in the rearing medium for avoiding starvation.

Dispensing the required food and desired concentration continuously is most desirable but practice not possible. Hence, the rationing can be adjusted 5-6 hourly

intervals in the feeding schedule.

Water quality

The feeding strategy should give due consideration for the ecological requirement of the larvae. It has been observed that in the rearing containers the permissible range are as follows (Silas et al., 1985).

Salinity	..	29-34 ppt
Temperature	..	27-32°C
pH	..	8 - 8.5
Dissolved oxygen	:	4.0 to 8.0ml O_2/l
Ammonia	..	< 0.1 ppm
Nitrite	..	< 0.05 ppm
Light intensity (during day time)	..	2000-1,25,000 lux.

Since, the feeding quantity especially in outdoor tanks by the interaction of environmental factors tends to create unforeseen developments care should be given to maintain the above said water conditions in the rearing container.

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Technical Paper - 24

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PHYTOPLANKTON CULTURE AS FEED - ISOLATION, IDENTIFICATION AND MAINTENANCE

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INTRODUCTION

It is an established fact that the phytoplankton forms the primary or basic food of almost all the larval organisms of molluscs and fishes. In the natural environment, the larvae feeds on the phytoplankton available in the environment. In a hatchery, we have to isolate these organisms after identification and test them whether the feed is acceptable to the rearing larvae. Moreover, these organisms should have high protein content and they have to be maintained in good condition as stock cultures using various culture media and then supplied to the hatchery in required quantities.

CULTIVABLE SPECIES

Realising the importance of diatoms (Skeletonema costatum and Chaetoceros spp.) as the essential food of the prawn larvae and phytoflagellates as the food of the bivalve larvae, the process of isolation, identification, maintenance of stock culture and mass culture is imperative in the hatchery system throughout the world. The bivalve larvae

can ingest nothing larger than 10 microne and appear to rely for food on nannoplankton flagellates belonging to the algal Classes Haptophyceae and Chrysophyceae. Isochrysis galbana, a member of Haptophyceae has proved to be the ideal flagellate food for the rearing operations of both pearl and edible oyster larvae. Apart from the different strains of Isochrysis, species of Pavlova, Dicrateria and Chromulina were also found to be ideal food to the bivalve larvae during rearing operations.

ISOLATION

Isolation of the required organisms can be done by the following methods:

1. Pipette method: Large organisms can be pipetted out using a micro-pipette and transferred to culture tubes having suitable culture media.

2. Centrifuge or washing method: By repeated centrifuging of the sample and by inoculating the deposit, we may get different organisms.

3. By exploiting the phototactic movements: By this method, most of the phytoflagellates can be isolated. The water sample in a beaker is kept inside a dark chamber having a hole at one corner. A candle is placed near the hole. Since the flagellates have a tendency to move towards light, they will come to the surface near to the candle. By pipetting, we can separate these organisms and by tube culture method, they can be raised to a pure culture.

4. By using agar or agar plating method: For preparing the agar medium, 1.5 gm of agar is added to 1 litre of suitable medium or even natural seawater. This agar solution is sterilized in an auto-clave for 15 minutes under 15 lbs pressure and 120°C temperature. This

medium is poured in sterilized petri-dishes and keep for 24 hours.

The required species to be isolated, can be picked up by a platinum needle or platinum loop, under microscope and streaked on the surface of agar plate. After inoculation, these petri-dishes are placed in an incubation chamber for 7-8 days providing constant light (1 k lux) and temperature (25°C). Within this period if the required species, has grown into a colony, it is removed by a platinum loop and transferred to culture tubes. Further, from the culture tubes to small culture flasks and larger conical flasks, the algae can be cultured on a mass scale.

5. Dilution culture method: For the isolation of required species of phytoflagellates, the serial dilution culture technique is employed. In this method, mainly 5 dilution steps (the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3} & 10^{-4}) or 4 steps 1, 10^{-1}, 10^{-2} & 10^{-3}) are required for the isolation of the flagellates (Sournia, 1971).

After filtering the seawater through 10 microne seive, the filtrate is inoculated to 4 series of culture tubes in various concentrations and kept under constant light (1 k lux) and temperature conditions (25°C). After 15 days, one can observe some discolouration of the culture tubes. On examination, the growth of a unialgal species could be noted in these tubes. Further purification of these organisms can be done by sub-culturing the same in 250 ml, 500 ml and 1 litre conical flasks. Finally if the culture is fully purified, it is transferred into 3 litre or 4 litre Haufkin culture flasks as stock culture.

CULTURE MEDIUM

For the successful culturing of micro-algae various chemical solutions have been recognised depending on the organisms, class and genera. Although all micro-algae are photo-autotrophic and can grow in purely inorganic media, many require organic compounds, the requirements of which may be either absolute or stimulatory. Usually, for culturing the micro-algae, Schreiber's medium or Miquel's medium were used in earlier days. However, for culturing the flagellates, Conway or Walne's medium is used for the maintenance of stock culture as well as for mass culture. Since this culture medium contains the chemicals, trace metals and vitamins (B_1, B_{12}) required for the micro-algae, the flagellates are being cultured by using this media alone (Gopinathan, 1982).

IDENTIFICATION

For maintaining the nannoplankters and flagellates as stock culture, first of all these organisms have to be identified and isolated. By noting the cell structure, shape of the cell, nature of cell walls, number of flagellae and movement of the organisms, the favourable ones can be identified. Most of the Haptophycean and Chrysophycean flagellates are having golden yellow colour, measuring less than 10 microns, round or oval shaped, with 1-3 flagellae and having wavy or rotatory movements. Silicified cell walls are the characteristic feature of diatoms while the flagellates are having cellulose cell walls. Further, a preliminary trial by feeding in a 10 litre beaker should be conducted to test whether the isolated organisms are acceptable to the rearing larvae.

STOCK CULTURE MAINTENANCE

Stock cultures of all the micro-algae are maintained in a special room having uniform temperature and light conditions. The cultures are kept in 3 or 4 litre Haufkin culture flasks. The autoclaved or heated seawater after cooling is poured to the culture flasks and required nutrients are added. Walne's medium enriched with vitamins is the ideal one suitable to maintain the stock of all the flagellates. About 10 ml of the inoculum in the growing phase of the culture is transferred to the culture flask and placed in front of two tube lights. For about 5-6 days, these flasks were kept exposed to two tube lights and when the maximum exponential phase has reached, only one tube light is needed for further growth. The time required to reach the maximum cell density will vary depending on the species. However, it was noticed that all the Haptophycean flagellates required 2 weeks for the completion of growth phase before entering into the stationary phase. In the stationary phase, the flagellates can be kept for a period of 2 months in the stock culture room under controlled condition of light and temperature, with or without aeration. A maximum of 5 Haufkin culture flasks required for keeping the stock culture of selected species. Periodical check-up is necessary to verify that the culture has not been contaminated. At least once in a month, all the species should be re-cultured to ensure viability and for better survival.

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SUMMER INSTITUTE IN
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PHYTOPLANKTON CULTURE AS FEED - ISOLATION, IDENTIFICATION AND MAINTENANCE

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INTRODUCTION

It is an established fact that the phytoplankton forms the primary or basic food of almost all the larval organisms of molluscs and fishes. In the natural environment, the larvae feeds on the phytoplankton available in the environment. In a hatchery, we have to isolate these organisms after identification and test them whether the feed is acceptable to the rearing larvae. Moreover, these organisms should have high protein content and they have to be maintained in good condition as stock cultures using various culture media and then supplied to the hatchery in required quantities.

CULTIVABLE SPECIES

Realising the importance of diatoms (Skeletonema costatum and Chaetoceros spp.) as the essential food of the prawn larvae and phytoflagellates as the food of the bivalve larvae, the process of isolation, identification, maintenance of stock culture and mass culture is imperative in the hatchery system throughout the world. The bivalve larvae

can ingest nothing larger than 10 microne and appear to rely for food on nannoplankton flagellates belonging to the algal Classes Haptophyceae and Chrysophyceae. Isochrysis galbana, a member of Haptophyceae has proved to be the ideal flagellate food for the rearing operations of both pearl and edible oyster larvae. Apart from the different strains of Isochrysis, species of Pavlova, Dicrateria and Chromulina were also found to be ideal food to the bivalve larvae during rearing operations.

ISOLATION

Isolation of the required organisms can be done by the following methods:

1. Pipette method: Large organisms can be pipetted out using a micro-pipette and transferred to culture tubes having suitable culture media.

2. Centrifuge or washing method: By repeated centrifuging of the sample and by inoculating the deposit, we may get different organisms.

3. By exploiting the phototactic movements: By this method, most of the phytoflagellates can be isolated. The water sample in a beaker is kept inside a dark chamber having a hole at one corner. A candle is placed near the hole. Since the flagellates have a tendency to move towards light, they will come to the surface near to the candle. By pipetting, we can separate these organisms and by tube culture method, they can be raised to a pure culture.

4. By using agar or agar plating method: For preparing the agar medium, 1.5 gm of agar is added to 1 litre of suitable medium or even natural seawater. This agar solution is sterilized in an auto-clave for 15 minutes under 150 lbs pressure and 120°C temperature. This

medium is poured in sterilized petri-dishes and keep for 24 hours.

The required species to be isolated, can be picked up by a platinum needle or platinum loop, under microscope and streaked on the surface of agar plate. After inoculation, these petri-dishes are placed in an incubation chamber for 7-8 days providing constant light (1 k lux) and temperature (25°C). Within this period if the required species, has grown into a colony, it is removed by a platinum loop and transferred to culture tubes. Further, from the culture tubes to small culture flasks and larger conical flasks, the algae can be cultured on a mass scale.

5. Dilution culture method: For the isolation of required species of phytoflagellates, the serial dilution culture technique is employed. In this method, mainly 5 dilution steps (the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3} & 10^{-4}) or 4 steps 1, 10^{-1}, 10^{-2} & 10^{-3}) are required for the isolation of the flagellates (Sournia, 1971).

After filtering the seawater through 10 microne seive, the filtrate is inoculated to 4 series of culture tubes in various concentrations and kept under constant light (1 k lux) and temperature conditions (25°C). After 15 days, one can observe some discolouration of the culture tubes. On examination, the growth of a unialgal species could be noted in these tubes. Further purification of these organisms can be done by sub-culturing the same in 250 ml, 500 ml and 1 litre conical flasks. Finally if the culture is fully purified, it is transferred into 3 litre or 4 litre Haufkin culture flasks as stock culture.

CULTURE MEDIUM

For the successful culturing of micro-algae various chemical solutions have been recognised depending on the organisms, class and genera. Although all micro-algae are photo-autotrophic and can grow in purely inorganic media, many require organic compounds, the requirements of which may be either absolute or stimulatory. Usually, for culturing the micro-algae, Schreiber's medium or Miquel's medium were used in earlier days. However, for culturing the flagellates, Conway or Walne's medium is used for the maintenance of stock culture as well as for mass culture. Since this culture medium contains the chemicals, trace metals and vitamins (B_1, B_{12}) required for the micro-algae, the flagellates are being cultured by using this media alone (Gopinathan, 1982).

IDENTIFICATION

For maintaining the nannoplankters and flagellates as stock culture, first of all these organisms have to be identified and isolated. By noting the cell structure, shape of the cell, nature of cell walls, number of flagellae and movement of the organisms, the favourable ones can be identified. Most of the Haptophycean and Chrysophycean flagellates are having golden yellow colour, measuring less than 10 microns, round or oval shaped, with 1-3 flagellae and having wavy or rotatory movements. Silicified cell walls are the characteristic feature of diatoms while the flagellates are having cellulose cell walls. Further, a preliminary trial by feeding in a 10 litre beaker should be conducted to test whether the isolated organisms are acceptable to the rearing larvae.

STOCK CULTURE MAINTENANCE

Stock cultures of all the micro-algae are maintained in a special room having uniform temperature and light conditions. The cultures are kept in 3 or 4 litre Haufkin culture flasks. The autoclaved or heated seawater after cooling is poured to the culture flasks and required nutrients are added. Walne's medium enriched with vitamins is the ideal one suitable to maintain the stock of all the flagellates. About 10 ml of the inoculum in the growing phase of the culture is transferred to the culture flask and placed in front of two tube lights. For about 5-6 days, these flasks were kept exposed to two tube lights and when the maximum exponential phase has reached, only one tube light is needed for further growth. The time required to reach the maximum cell density will vary depending on the species. However, it was noticed that all the Haptophycean flagellates required 2 weeks for the completion of growth phase before entering into the stationary phase. In the stationary phase, the flagellates can be kept for a period of 2 months in the stock culture room under controlled condition of light and temperature, with or without aeration. A maximum of 5 Haufkin culture flasks required for keeping the stock culture of selected species. Periodical check-up is necessary to verify that the culture has not been contaminated. At least once in a month, all the species should be re-cultured to ensure viability and for better survival.

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Technical Paper - 25

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MASS CULTURE OF PHYTOPLANKTON

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INTRODUCTION

Large-scale culture of micro-algae especially nanno-plankton flagellates and selected species of diatoms are necessary for feeding the larval forms of molluscs or fishes, in a hatchery system. Since the bivalve larvae can feed on organisms measuring less than 10 microns, these forms have to be isolated from the seawater and maintained as stock culture. Utilizing the inoculum, mass culture can be carried out in the laboratory conditions as well as in the open system.

Culture containers

The containers that can be used for the laboratory mass culture of the micro-algae are 10 litre polythene bags, 20 litre glass-carbuoys as well as 100 litre persepex tanks. These containers are kept in wooden racks provided with aeration and light facilities. Fully grown culture from the stock culture is used as inoculum for the mass culture in these containers. About 200 ml of the culture is used for 10 l polythene bags, 500 ml for the 20 l glass carbuoys while 2 litre of the culture is used for the 100 litre persepex tanks. After the inoculation, on 5-6th day, the culture

will reach in the optimal growth phase and ready for supply to the hatchery. Leaving one litre of the same culture in the 20 l glass carbuoy, fresh enriched water can be added for the further mass culture in the same container.

CULTURE MEDIUM

Conway or Walne's medium (Walne, 1974), PM and TMRL medium are used for the mass culture of flagellates and other nannoplankters in a hatchery system (Gopinathan, 1982). For the mass culture of flagellates, the vitamin mixture is not necessary.

During the course of the larval rearing of oysters or any other bivalves, the flagellates forms the basic food upto the stage of spat. However, for the better growth and survival of the spat, the food has to be changed from flagellates to a mixture of diatoms and other nannoplankters. For making a culture of the mixed phytoplankton in the open area using direct sun-light, the following medium could be used in a one ton capacity fibre-glass (white colour) tank.

Potassium nitrate	..	12.0 gm
EDTA (Na)	..	6.0 "
Sodium ortho phosphate	..	6.0 "
Sodium silicate	..	6.0 "

(Sodium silicate dissolve separately in 50 ml of dist. water)

To 100 litre of fresh filtered seawater (fresh seawater can be filtered through No. 3 organdy cloth to remove zooplankton) 1/10 of this medium is added. The water is poured in 2 or 3 white-lined basins or fibre-glass tanks and kept in bright sun-light. Within 36 hours, a slight discolouration can be noted on the sides of the basin. On

examination, we can notice a mixture of planktonic diatoms and other nannoplankters. If the temperature is very high and sunlight is very bright, only the blooming of Chaetoceros spp. could be observed.

Besides the above mentioned chemicals which serve as nutrients, commercial fertilizers can be used for the mass culture of nannoplankters (eg. Chlorella) in open tanks on a economical basis. The media used for the open culture of Chlorella are:

Urea 46	...	10 mg/l
16-20-0 (NPK)	...	10 mg/l
20-0-0	...	100 mg/l

GROWTH PHASES OF ALGAL CULTURE

The usual way of the laboratory culture of the micro-algae is one in which a limited volume of medium containing the necessary inorganic and organic nutrients is inoculated with arelatively small number of cells and these exposed to suitable conditions of light, temperature and aeration. Increase in cell numbers in such a culture follows characteristic pattern in which the following phases of growth may usually be recognised:

1. Lag or induction phase: The cells taken from the stock culture and inoculated to a new flask have to acclimatise to the new medium. So there is no cell division for a few hours and this phase is known as lag or induction phase.

2. Exponential or growing phase: Once the cells are acclimatised to multiply and grow rapidly. It is assumed that within 12-24 hours, one cell will divide into 2 and

further these cells carry the growth till the culture reaches its maximum concentration. This growing phase is known as exponential phase.

3. Declining phase: Once the cells reach maximum concentration, the growth and multiplication of the cells will be arrested and slowly the cells show the symptoms of decline. This arrested growth of the cells in the culture is known as declining phase.

4. Stationary phase: After the arrested growth for a few days the culture will be stationary without any further growth and multiplication of the cells. Actually, stationary phase is prolonged in the case of flagellates. They may develop some cover or cyst around its body for thriving the unfavourable environment. During the stationary phase, if the cells get a new environment, they may start further growth and reproduction.

5. Death phase: After a long period in the stationary phase, the cells may lose its viability and start to die and thus culture will become useless either for re-culturing or for feeding.

DETERMINATION OF ALGAL DENSITIES

Regular count of the algal cells must be made in order to schedule inoculation of the various culture flasks and mass culture containers, to monitor growth of the algal cultures and to determine the quantity of algae to be fed to the rearing larval organisms.

Since the nannoplankton organisms measure less than 10 μ, we have to use a Haemocytometer for counting. Initially, the sample has to be treated with a drop of eosin or formalin to kill the cells and after stirring well, one drop is taken with a sterilized pipette. After placing the cover-slip on

the haemocytometer, the pipette is brought to the edge of the slide and touched. The sample runs inside and thus we will get a thin film of the culture in which the cells are equally distributed. Since the haemocytometer has got 9 chambers, 4 sides having 16 divisions and 5 chambers with 25 division, we have to restrict the counting to at least 3 chambers. The average number of cells in one ml is calculated by using the equation,

Average counts per chamber x 10^4 = Total number of cells/ml.

HARVEST AND PRESERVATION

The maintenance of the culture and constant supply to meet the requirements of the hatchery is a problem especially during adverse weather conditions. The preservation of the algae either by freezing or by sun-drying is advantageous in that during scarcity of food, the rearing operations could be successfully continued. For freezing, the culture has to be flocculated either by adding alum or lime or by adjusting the pH using NaOH. After determining the quantity of culture to be flocculated, measure the volume of NaOH solution needed to flocculate and add to the solution. Vigorous stirring should be done and the culture is left for one hour. After one hour, we can see the algal mass deposited in the bottom. The clear water is decanted and the sediment collected. The pH is brought down to its original value by adding slowly dilute HCl. Now the algae is ready for freezing or drying. Drying of the algae can be done by pouring the same in white enamel trays and keeping it in the bright sun-light. If the algae has dried up thoroughly, it can be scraped from the enamel trays and kept in glass bottles. Before freezing, the algal mass has to be treated with a few drops some protective reagents like Dimethyl sulphoxide or glycerol. The frozen algae can be kept for

3 months. Whenever adverse condition arises, the frozen feed can be used for rearing the larvae, though the protein content may be little less when compared to the live feed.

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Technical Paper-26

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RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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LIVE FOOD ORGANISMS - ROTIFER BRACHIONUS PLICATILIS AND CLADOCERAN MOINA Sp.

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Fish fry and crustacean larvae are apparently specific to type and size of the feed for their survival and growth. At present successful rearing of fish and crustacean larvae depends upon the availability of suitable live feed organisms. Existing literature reveals that only very few species have been used as live feed organisms of which rotifers and cladocerans are of prime importance in aquaculture industry.

ROTIFER BRACHIONUS PLICATILIS

For early larval stages, rotifers are found to be suitable, initial live-feed which are intensely utilized in hatcheries throughout the world. In Japan, rotifers are given as initial diet for freshly hatched fish fry (of above 2-3 mm in body length) and feeding with rotifer is continued for about 30 days after hatching. For the culture of fish larvae, which are vulnerable to starvation, rotifers are used as initial food in United States, Russia and United Kingdom. The rotifer Brachionus plicatilis is found to be an ideal food for larvae of the blue crab, Callinectes sapidus. At present, without the mass culture of rotifers, larval rearing of marine fishes is virtually impossible in Japan although

artificial microdiets are being gradually developed to replace live-feed. The species Brachionus plicatilis is one of the most commonly used rotifer in aquaculture, serving as an important food source for predatory larvae.

Biology of rotifer Brachionus plicatilis:

B. plicatilis shows important features of a live-food organism such as size suitability, high rate of multiplication within a short period of time, utilization of minute phyto (primary) food as feed and the ability to withstand the adverse condition by producing cyst.

Size of B. plicatilis suits with the requirement of early predatory larvae and it varies with reference to the sex of the species. The length of the (lorica of) female is 165-192 microns while the width is 118-140 microns and the size of the female is bigger when compared to the male which is about 150 microns in length and 55 microns in width. In addition to the suitability of the size, B. plicatilis is having a very high multiplication rate. Under favourable conditions, the above-mentioned rotifers reproduce by parthenogenesis. The average life span of B. plicatilis is observed to be 5.25 days at 28°-30.5°C during which period a parthenogenetic female produces 19 eggs. The doubling time of the population is found to be 0.45 days and theoretically it is calculated that totally 3079 rotifers can be obtained from a single parthenogenetic female during its short life span (Muthu, 1983 a). Rotifer B. plicatilis goes for sexual reproduction during unfavourable conditions. It produces males and subsequently resting eggs to assure the survival of the species during adverse situations. The males, which are diminutive, do not resemble the females and they are without alimentary canal as their purpose of creation appears to be

only for reproduction.

In the parthenogenetic mode of reproduction diploid amictic females produce diploid eggs by mitosis. These eggs normally develop into amictic parthenogenetic females. However, these diploid eggs, under unfavourable conditions, develop into mictic (sexual) females. These mictic females meiotically produce haploid eggs which if unfertilized develop into haploid males and if fertilized develop into thick-shelled diploid resting eggs. These resting eggs hatch into a new generation of amictic females (Fig. 1) (King and Snell, 1977).

Brachionus sp. are filter feeders which consume feed-particles of less than 5 microns in size. They have been cultured by different authors by utilizing a variety of diets such as live Duaniella, live Chlorella, live Scenedesmus costato-granulatus, live Tetraselmis suecica, live Tetraselmis and powdered Spirulina, yeast, "Torulose" yeast, dry Chlorella powder, powdered commercial Spirulina, Chlorella and methanol-grown yeast and mahua oil cake (Muthu, 1983 a). The algal-bacterial-biomass, developed in ponds which are employed for treatment of piggery waste, has also been used for growing Brachionus rubens. Rotifers are also grown on the algal-bacterial-biomass, produced by fertilizing the water with a combination of fertilizers such as groundnut oil cake, cowdung, urea and superphosphate. When Chlorella or other nanoplankters, which are less than 5 microns in size, are offered as feed, B. plicatilis multiply very fast. The growth is observed to retard when diatoms or green algae which are more than 10 microns in size, are given as feed. Thus size is appeared to be the prime determing factor for the selection of the particular phytoplankton as feed for rotifer. Further fertility is considerably high with algal diet while it is

very low when groundnut oil cake is provided as a direct-feed.

Mass-culture of rotifer B. plicatilis

B. plicatilis is mass-cultured in two ways. In the first method, the feed for rotifer is added to the rotifer tank whenever it is required. In other words, feed preparation and rotifer rearing are separate aspects of the culture programme. Trotta (1980) and SEAFDEC follow this method. In the second method, both rotifer and its feed are cultured together in one and the same container and this method is followed at Narakkal Prawn Culture Laboratory.

Trotta (1980) has made use of a continuous system, in which Chlorella is grown with sterile culture medium in one large bag. This Chlorella-bag has separate interconnections to a reservoir, containing nutritive medium (for phyto-growth) and a rotifer-bag, which is twice the size of the Chlorella-bag. As soon as Chlorella has attained optimum concentration, it is allowed to flow from the Chlorella-bag into the bottom of the rotifer-bag, which expels a portion of rotifer medium through the outlet at the top of the rotifer-bag. Rotifer will be harvested from the medium, thus expelled. Subsequently, the Chlorella-bag will be refilled with fresh enriched medium, obtained from the reservoir, thereby making the above culture process as a continuous one. In SEAFDEC, 350 ton capacity concrete tanks are separately used for rotifer and Chlorella culture. Chlorella is cultured by fertilizing filtered seawater with inorganic fertilizers such as urea, superphosphate and ammonia. This Chlorella water is siphoned into the adjacent rotifer tank which is already inoculated with a stock culture of B. plicatilis. When the rotifer population attains a density of 200-300 Nos/ml, part of the rotifer medium is

siphoned off through a fine-meshed phytoplankton net to harvest the rotifer. Water level in rotifer tank is restored by replenishing with Chlorella water from the Chlorella tank. After a few days, rotifer will again attain the harvestable density and once again the same process has to be repeated. Thus the whole process goes on continuously until the culture becomes contaminated by unwanted organisms either in the Chlorella tank or in the rotifer tank.

At Narakkal Prawn Culture Laboratory, outdoor containers of 2 ton, 10 ton and 40 ton capacity are used for the mass-culture of B. plicatilis. Initially, feed for rotifer is developed prior to rotifer inoculation in these tanks by fertilizing the filtered (through 50 micron net) seawater with groundnut oil cake (juice), urea and super-phosphate at the rate of 200 gm, 2 gm and 2 gm respectively per ton of water. Profound aeration is necessary and a starter culture of Chlorella is inoculated. After taking these measures to develop the rotifer feed, a starter culture of B. plicatilis is added to the same container when pH of the medium increases above 7. The rotifer starter should be obtained from a healthy population and it is preferable to be with egg-bearing parthenogenetic females. It must be devoid of either males or cyst-bearing females. Inoculation is preferable to be at the rate of females. Inoculation is preferable to be at the rate of 5-10 rotifers per ml of the medium. A Chlorella bloom develops within 2-3 days and the rotifers multiply rapidly, attaining a population density of about 250 Nos/ml within 4-6 days after which harvesting is done everyday in the morning when rotifers swam at the surface. pH and oxygen content of the medium play an important role in rotifer multiplication. Schluter (1980) has observed that at pH above 9.5 and below

4.5 no rotifer is survived and high rotifer densities are associated with a pH value of 6-8. If dissolved oxygen content is above 1.15 mg 02/1, the reproduction rate is not inhibited while rotifers ceased to reproduce and die within a few days at 0.72 mg 02/1. The presence of a male or a cyst bearing female indicates the existence of adverse conditions in the culture tank. Hence routine observation is essential in order to understand the biological and physical conditions prevailing in the culture tank and the growth pattern of the population.

When Chlorella bloom declines, half the volume of water is replaced with fresh seawater and refertilized with the abovementioned fertilizers at half of the initial dose. The culture process is repeated until the culture gets contaminated with filamentous blue-green algae or ciliates. If contamination occurs, the present culture operation has to be closed and the whole process has to be restarted from the beginning. When this culture method is followed, population may reach upto a high density of 560 rotifers per ml.

Utilization of harvested rotifers

The harvested rotifers, have to be washed with clean seawater before giving them as live-feed. However, they can be stored by freezing them into frozen blocks in a deep freezer using 10% glycerin as cryoprotectant.

Nutritional quality of the rotifer, as a live-feed, depends on the feed, used for rotifer culture. The rotifers, cultured with yeast, are quite low in W^3 highly unsaturated fattyacids such as 20:5w^3 and high in monoenoic fatty acids such as 16:1 and 18:1. Those cultured with marine Chlorella contain high amount 20:5w^3 which is one of the required essential fatty acids for marine fish. Hence rotifers

cultured with yeast are always inferior to those cultured with marine Chlorella in their nutritional quality as a live-food. The reason for this difference in nutritional quality is that the baker's yeast, used for mass culture of rotifer, contains no w^3 highly unsaturated fatty acids while the rotifer-feed, Chlorella on the other hand, contains a high level of 20:5w^3. Nutritionally poor rotifer, obtained as a result of feeding with substances such as yeast, can be enriched and made as better ones at the time of offering them as feed to predator. For this purpose, the rotifers which are cultured with nutritionally poor diet, have to be fed with enriched diet such as marine Chlorella, microencapsulated diets, w-yeast and emulsified lipids rich in w^3 highly unsaturated fatty acids, for a period of 3-6 hours (Watanabe et al., 1978).

Rotifer resting eggs

One of the possible ways of ensuring good supply of rotifers will be building up of reserve stocks and making use of them when the demand rises. Lubzens et al. (1980) are of the view that this goal can possibly be achieved by finding methods for inducing rotifers to produce resting eggs and finding ways for preserving and hatching resting eggs. It has been reported that changes in environmental conditions such as increase in crowding, cold shock, decrease in food quantity and changes in photoperiod may induce production of males and subsequent formation of resting eggs in rotifers (Gilbert, 1974). Ito (1960) reported that B. plicatilis can be induced to produce resting eggs when transferred from 18°/₀₀ chlorinity culture media to lower chlorinity media. Lubzens et al. (1980) have produced males and resting eggs in B. plicatilis by transferring them from 100% seawater (38 ppt) to 25% seawater.

Resting eggs may be preserved for at least 12 weeks by freezing at -14°C without significant loss of viability. Eggs, dried and kept desiccated at room temperature, retained their viability for upto 3 weeks (Lubzens et al., 1980). Little is known about the stimulus which initiates development of rotifer resting eggs. Pourriot et al. (1980) have observed no or very little hatching of the resting eggs in B. rubens, during the first month after laying, at whatever light and temperature conditions and they are of the opinion that most of the resting eggs have to undergo an obligatory dormancy. Further they observed that hatchability in resting eggs of B. rubens is increased by a dormant period in darkness at low temperature (similar to conditions in winter) followed by illumination and an increase in temperature (similar to spring and summer conditions). When the conditions are favourable, resting eggs hatch out into parthenogenetic females and hatching is achieved by keeping them in well-aerated fresh seawater for 24-48 hours at 29-30°C.

CLADOCERAN MOINA Sp.

Cladoceran Moina Sp. are characteristic inhabitants of temporary freshwater pools. Although cladocerans of the genera Daphnia and Moina are freshwater organisms, they have been successfully used in the frozen condition to feed marine animals also. They are utilized as suitable feed for the culture of fish fry and postlarval crustaceans.

Biology

Cladoceran Moina is bigger in size when compared to rotifer Brachionus plicatilis. The size of Moina fulfils the feed-size requirement of slightly grown-up fish fry and

postlarval crustaceans while the small sized rotifers form an ideal sized feed to early fish fry and crustacean larval stages. The female Moina with embryos, measures 0.78-1.02 mm in length and 0.43-0.74 mm in width (Muthu, 1983 b). Like rotifer B. plicatilis, cladoceran Moina also possesses desirable features like high reproduction capacity, filter feeding habit, ability to produce dormant cysts during adverse conditions and ready acceptability. Moina also multiplies parthenogenetically when the conditions are favourable and changes over to sexual reproduction at the onset of adverse conditions. The males which appear when conditions become unfavourable, are smaller in size and are 0.54-0.72 mm in length and 0.23-0.37 mm in width. These males mate with the females to result in the formation of dormant cysts and cyst production ensures future survival of the species. The average life-span of Moina is found to be 11 days within which one parthenogenetic female produces about 85 eggs. Forty eight hours after birth, the female releases the first batch of 8-12 youngones, which become as parthenogenetic females. For the rest of its life, the female releases similar batch of youngones at every 24 hours and a total of 42765 animals have been resulted from a single parthenogenetic female within its life period of 11 days. The population doubling time is found to be 0.32 day (Muthu, 1983 b).

Culture

The culture technique for cladoceran is basically similar to that of rotifer. Cladocerans have been reared in small scale under sterile conditions as monoxenic cultures using Chlamydomonos reinhardii (Murphy, 1970) and as dixenic cultures with C. reinhardii and Scenedesmus obliquans (D'Agostino and Provasoli, 1970). However, only few generations of cladoceran can be achieved when algae, grown under

sterile conditions in inorganic media, are used as feed. To overcome this problem, nutritional quality of the algae has to be raised by adding vitamin mixture and liver extract to the algal culture medium. A synthetic biphase medium, consisting of a liquid phase to supply micronutrients and a particulate phase to provide macronutrients, has been used to rear Moina macrocopa by Conklin and Provasoli (1977). For large scale cladoceran production, culturing in sterile media or biphase media, is not practicable because of high cost of maintaining such cultures. So simplified and economically viable technology to mass-culture cladoceran gains importance.

By making use of ordinary water, many scientists have mass-cultured cladocerans either by feeding with particular diets or by developing the feed in the medium by enriching with certain fertilizers. Micronized ricebran of less than 60 microns in size and 1% brewer's yeast solution are utilized as prepared feed while fertilization is carried out with swine manure or chicken manure or groundnut oil cake or a combination of groundnut oil cake, urea and superphosphate as done at Narakkal Prawn Culture Laboratory (Muthu, 1983 b). In SEAFDEC, cowdung is used as a manure while in Jepara, a mixture of coconut oil cake and chicken dung is made use of. When the medium is fertilized, algal-bacterial-biomass develops forming as food to the cladoceran. This fertilization-method is basically same for culturing any filter-feeding live feed organism and it is normally carried out in outdoor containers. Any freshwater source, which is free from pollution can be made use of for Moina culture.

At Narakkal Prawn Culture Laboratory, tap water, which is aerated for 2 days to get rid off chlorine, is

initially fertilized with groundnut oil cake, urea and superphosphate at the rate of 250 gm, 2 gm and 2gm respectively per ton of water. Subsequently it is inoculated with a starter Chlorella culture. When the medium gets slightly greenish in colour due to the growth of Chlorella, Moina is to be stocked at the rate of 1 animal per litre of the medium. The groundnut oil cake first stimulates bacterial growth and then induces Chlorella bloom. The cladocerans seem to feed on both the bacteria and the Chlorella and also on the finely divided groundnut oil cake particles, suspended in the water. The cladoceran multiplies very fast and when it reaches a density of 30000-40000 animals per litre in 7-9 days at 29-30°C, it is harvested. In both rotifer and Moina cultures, same procedures are followed for harvesting and utilizing the harvested product. As a result of explosion of Moina population, Chlorella concentration declines and to maintain the optimum Chlorella concentration, partial water change and refertilization with 100 gm groundnut oil cake per ton of water are to be done at at interval of 4-5 days. Frequent harvesting and partial change of water increase the yield and prolong the life of the culture.

If the culture-container is not covered with a net, chances are there for the development of mosquito larvae in the culture medium. Sometimes the culture gets contaminated with ciliates or undesirable star-like Pediastrum and filamentous blue green algae, which are unsuitable as feed to cladoceran. Such contaminated cultures are better to be discarded. Fresh cultures can be initiated from the stock of dormant cysts. The viability of these cysts, if kept in dry test tube with cotton plug, is about 2 months and these dormant cysts hatch out into parthenogenetic females in 24-28 hours at 28-30°C when kept for hatching in well aerated freshwater.

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Technical Paper-2

SUMMER INSTITUTE IN
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11-30 May, 1987

LIVE FOOD ORGANISMS - *ARTEMIA*

PART I - *ARTEMIA* CYSTS AND THEIR UTILIZATION

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INTRODUCTION

Extensive literature reveals that only very few organisms have been utilized as live feed of which the brine shrimp *Artemia*, is the most important one. In the growing aquaculture industry, a great demand exists for *Artemia* cysts which form as dried inert food source. In these cysts embryos, after development upto gastrula stage, are kept in diapause. The cysts are brownish in colour and measure about 0.3 mm in diameter. When dehydrated, they are biconcave in shape.

CYST HARVEST AND CYST PROCESSING

Artemia cysts can be collected from natural saline habitats which are located in coastal as well as inland areas in both temperate and tropical countries. Literature reveals that a good *Artemia* biotope produces 10-20 kg cysts per hectare per season (Persoone and Sorgeloos, 1980). In *Artemia* biotopes, cysts float in saline waters (except that of *Artemia monica*) and are blown to shore by wind action, where they accumulate. Cysts should be harvested as soon as

possible after accumulation. Cysts should preferably be harvested from the water surface rather than from the shores thus assuring less contamination with impurities. The cysts, collected, may be contaminated with sand particles, dirt material, dead Artemia, algae, debris, empty shells, broken shell bits etc. The hatching efficiency of the collected cysts is largely determined by the effective removal of from the cysts and keeping the cyst water content less than 10%. Hence, the cysts have to be cleaned first and then adequately dehydrated.

Cleaning can be done by washing the cysts with freshwater using different mesh-sized screens so that corresponding sized dirt materials can be removed. Generally 400 microns and 100 microns sieves are used for this purpose. When the raw cysts are allowed to pass through 400 microns sieve and collected on 100 microns sieve in the process of washing, debris of above 400 microns and of lesser than 100 microns are removed. Cleaning in freshwater must be very quickly done as prolonged cleaning in freshwater will initiate hydration and subsequent embryological development resulting in energy loss. The dirt materials which are equal in size with the cysts can be removed by the biphase floatation method, in which initially the raw cysts have to be suspended in brine for a period of 24 hours. Cysts and light debris will float while heavy particles such as sand will sink to the bottom. Secondly the floating cysts have to be removed from brine and introduced in freshwater for a short period of 15 minutes. The viable cysts will sink to the bottom whereas the empty shells, non-viable cysts etc float at the surface. The cleaned cysts are to be siphoned off. They have to be spread in uniform thickness over a drying surface and kept for drying in the shade or in a hot air oven at 30-40°C until cyst water content has reached to the level

of 2-9%. Now cysts attain biconcave shape. They have to be packed under vacuum or nitrogen atmosphere and stored in dry place. It is reported that the cysts, thus processed and stored under ideal conditions, retain their viability for at least 12-13 years.

CYST HATCHING

At least five conditions are essential for restarting the arrested embryological development in cysts leading to hatching (Sorgeloos and Kulasekarapandian, 1984). They are hydration of the cysts, oxygenation of the medium, illumination of the hydrated cysts, temperature of 26-35°C and pH above 8.0. Hatching can be carried out in salinities ranging from 5 to 75 ppt. Artemia cysts never hatch at high salinities because they cannot hydrate enough which is one of the pre-requisite for the onset of hatching mechanism. For practical convenience, seawater (enriched with 2 gm $NaHCO_3$ per litre) is used for hatching. Continuous moderate aeration, which keeps the cysts in suspension, is beneficial in hatching. Hatching efficiency decreases when dissolved oxygen content goes below 2 ppm. It is completely inhibited at 0.6 to 0.8 ppm in California strain. The light triggers the "biological clock" to start again in the hydrated cysts. Illumination for 10 minutes at an intensity of 1000 lux is sufficient in California strain. Eventhough several types of hatching containers have been used by different workers (Shelbourne et al., 1963; Jones, 1972), funnel shaped glass or plastic containers are better for hatching. Because of transparency, adequate illumination is ensured and as the bottom is funnel-shaped moderate aeration is sufficient to aerate the medium and simultaneously keep the cysts in adequate suspension. In the cylindrical containers, 7-10 gm cysts per litre of medium, can be subjected for hatching.

SEPARATION OF HATCHED NAUPLII FROM THE HATCHING DEBRIS

Consumption of empty shells block the gurt of the predator when an uncleaned hatching mixture is fed. Moreover, unhatched and empty cysts have a very high bacterial load and their introduction will spoil the predatory medium. Hence hatched nauplii alone have to be collected from the hatching debris and utilized as feed.

The positive phototactic behaviour of the nauplii is exploited for separating the nauplii from the empty and unhatched cysts. Directing a light beam on the transparent hatching device results in the larvae swimming towards the light as soon as the aeration has been turned off. They can be siphoned off from that particular place. However, this rough separation technique, although commonly used, requires skill to remove the nauplii without siphoning off the debris, accumulated in the bottom and surface. These problems can be solved by using a separator box which has a central dark compartment and an outer bright compartment both being separated by a partition. When the mixture of nauplii and debris is introduced in the central dark compartment of the box, the nauplii swim through holes or slits from the dark compartment to the brighter side. Once separation is completed, the partition can be closed and the larvae can be siphoned off from the brighter outer compartment, while unhatched cysts and empty shells being retained in the dark, central compartment. Shelbourne et al. (1963) have used rectangular separator boxes in which separation is poor because the light stimulus is not uniform in all directions. However, Persoone and Sorgeloos (1972) have used cylindrical separator boxes in which the light stimulus is uniform for all the nauplii from any direction.

CYST DECAPSULATION

The hard shell or chorion of the cyst can be removed without affecting the viability of the embryo by short exposure of the hydrated cysts to a hypochlorite solution and this process is known as decapsulation, which eliminates the cumbersome process of separating nauplii from the hatching debris. Treatment with hypochlorite solution disinfects the cysts and the decapsulated cysts can be directly used as food to predators. For example, the larvae of Lebistes, Macrobrachium, Penaeus, Portunus, Scylla and Xiphonophorus have been successfully reared by giving a diet of decapsulated cysts.

In the decapsulation process, complete removal of the chorion will be possible when the cysts are spherical in shape and to obtain this desirable stage, the cysts, as an initial step, have to be hydrated by keeping them for required period (1 to 2 hours for most of the strains) either in freshwater or seawater at about 25°C. Prolonged hydration has to be avoided as it will induce embryological development and consequent loss of energy. Subsequent to hydration, cysts have to be transferred to decapsulation solution which can be prepared with either liquid bleach (Sodium hypochlorite), NaOCl or bleaching powder, $Ca(OCl)_2$ and NaOH. 0.5 gm active product and 14 ml of decapsulation solution are required to decapsulate one gram of cyst. Decapsulation solution has to be made up with 35 ppt seawater. When the cysts are in the decapsulation solution, a gradual colour change in the cysts will be observed from dark brown to grey and then to orange. During decapsulation the temperature should not be allowed to raise above 40°C. Prolonged immersion in decapsulation solution will kill the embryo and hence cysts have to be removed from the solution as soon as

the completion of the process which can be judged by periodical observation oc systs under microscope. Cysts, after treatment in decapsulation solution, have to be washed with tap water and afterwards dipped a couple of times in 0.1 N Hcl. Subsequently, the cysts have to be washed again with tap water or seawater. The hydrated, decapsulated cysts can be offered directly as food to the predator. If needed they can be stored for a few days in the refrigerator at 0-4°C. When used directly as food, it is essential to keep the cysts in suspension, by sufficient aeration and circulation, for better utilization by predators since the hydrated decapsulated cysts sink in seawater or freshwater. Decapsulated cysts can be stored for a short period of six months by keeping them for dehydration in NaCl saturated brine (± 330 g/l). The decapsulated cysts sink in brine and become coffee-bean shaped as a result of dehydration. Brine will not help for long term storage for which the hydrated decapsulated cysts have to be dried and packed in dry and oxygen free containers.

NUTRITIVE VALUE AND QUALITY IMPROVEMENT IN ARTEMIA NAUPLII

Quality of Artemia cysts as naupliar food source, is evaluated on the basis of its nutritive value. It is profitable to use decapsulated cysts as feed instead of freshly hatched nauplii as the former contain 30-40% more energy when compared to the latter. In other words, hatching mechanism consumes 30-40% energy which can otherwise be utilized by directly giving decapsulated cysts as feed. The energy contents and individual dry weights decrease with 22-37% and 16-34% respectively while the freshly hatched nauplii (instar I larvae) develop into instar II larvae. Hence it must be taken into account that Artemia nauplii have to be fed as soon as the hatching is over to avoid the energy loss.

Marine predators need high levels of the fatty acids $20:5w^3$ and $22:6w^3$, the latter being seldom present in detectable levels in Artemia nauplii. The fatty acid pattern; more particularly the content in polyunsaturated fatty acids (PUFA), appears to vary in the Artemia as a function of the source of the cysts and even within a particular strain from cyst harvest to harvest. With PUFA poor Artemia nauplii, low survival is reported in marine fish and shrimp hatcheries. The reason for the poor PUFA-profile in some Artemia cyst sources, is related to the biochemical composition (i.e. PUFA content) of natural food of Artemia. In fact, in the high salinity environment where Artemia live, those species of diatoms and flagellates known to be rich in PUFA, do not or only seldom occur. Correspondingly the cysts produced by the Artemia of these types of biotopes, are also poor in PUFA content.

These nutritively poor 1-2 days old Artemia nauplii can be enriched with PUFA via their diet. Different particulate products can be used for enrichment of the instar II Artemia nauplii through bioencapsulation and metabolisation (accumulation). Microencapsulated diets and micronized particles respectively coated with PUFA-rich oils such as cod liver oil and PUFA-rich emulsions or micro algae rich in PUFA such as Isochrysis, Chaetoceros, Chlorella can be used as enrichment diets for instar II Artemia nauplii (Sorgeloos and Kulasekarapandian, 1984). Optimum enrichment can be achieved by feeding the Artemia nauplii, which are stocked at the rate of 25000-50000 nauplii per litre during 24 to 48 hours post-hatching with dense concentration of PUFA-rich algae or particle suspensions (100000-500000 cells/ml).

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SUMMER INSTITUTE IN
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LIVE FOOD ORGANISMS - ARTEMIA
PART II - ARTEMIA CYST AND BIOMASS PRODUCTION

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INTRODUCTION

The brine shrimp, Artemia, a crustacean which is naturally found in hypersaline waters throughout most of the world, has become the most important source of live-feed for the last twenty years. All its life stages such as cysts after decapsulation, freshly hatched nauplii, juveniles, preadults and adults, are used as live-feed according to the feed-size requirement of the predator. Artemia decapsulated cysts and freshly hatched nauplii form as ideal food for the larvae of cultured fish and crustaceans in their hatchery phase while juveniles, preadults and adults form as suitable diet for prawn/fish juveniles in their nursery phase. Artemia grows from nauplius stage to adult stage in about two weeks. During this short period, nauplius with 0.4 mm in length and 0.002 mg in weight grows into an adult with about 8 mm in length and 1 mg in weight thereby ensuring 20 fold increase in dimension and 500 fold increase in biomass. After attaining adult stage, the brine shrimp produces nauplii/cysts (according to the prevailing environmental conditions) at the rate of upto 300 numbers per batch and goes on producing at the interval of about five days throughout its life period of six months. For cysts and biomass production,

Artemia can be cultured in large scale in salt water ponds where environmental parameters cannot be fully controlled. They can also be produced in hatcheries and laboratories under controlled conditions.

PRODUCTION IN SALT WATER PONDS

In India, a vast area of salt ponds, all along the coastline, are used in salt production during dry season and these man-made solar saltpans offer excellent scope for carrying out Artemia culture. Many of these saltpans have most of the infrastructures required for this purpose. The area, selected for Artemia culture, should have suitable climatic conditions such as moderate temperature (ranging from 25°C to 35°C) and salinity (with a range of 30 ppt to 200 ppt). It should have a high evaporation rate with little rainfall and be closer to the sea from where water can be easily drawn to the reservoir either by pumping or through tidal influence. It is desirable to drawn water to the reservoir from mangroove area which has a very high productivity. The water source should be free from pollution and the pond should maintain the water level without having any seepage or leakage.

In a classic typic of solar saltpan, water enters the first evaporation pond from the reservoir. After increasing slightly in salinity by solar evaporation, water flows from the first evaporation pond to the next of the series and this continues until the water becomes brine i.e., saturated with sodium chloride. The brine is then introduced to the crystalizing ponds where sodium chloride crystallises. Artemia can be intensely cultured in evaporation ponds with minimum inputs.

Subsequent to initial fertilization, freshly hatched Artemia nauplii have to be inoculated at the rate of 1 to 10 numbers per litre of pond water (Sorgeloos and Kulasekarapandian, 1984). Nauplii stocking has to be done at night or early hours of the day to minimise the temperature stress. Growth of the population has to be monitored by collecting data on the population composition which can be carried out by analysing the population samples after grouping them as nauplii, juveniles, preadults and adults. Changes in the population composition can be correlated with the overall production status of the population. For example, presence of only adults reflects the status of no recruitment. Reproductive status of the adults, which can be found out by observing the presence of nauplii/cysts and shell glands in the brood sac, will also indicate whether the population is in growth phase or in stationary phase. (Determination of population density through sampling procedure will not help in view of strong heterogenic distribution of Artemia). Data on the parameters such as minimum-maximum water temperature, rainfall, salinity levels and water turbidity have to be daily collected. These informations are necessary to ensure continuous recruitment through ovoviviparity by adjusting the intensity of fertilization, harvest and water management.

When the population is mostly of adults, they can be manually harvested with a dipnet. Large net, with a cod-end having less than 100 microns mesh size, is to be installed in the canal/gate that connect two evaporation ponds. Artemia will be retained when the water flows from one evaporation pond to another. The net should be emptied at about 1 hour intervals to avoid the death of the accumulated Artemia in the cod end.

If the intention is cyst-harvest, Artemia have to be exposed to higher salinity levels (above 150 ppt) thereby subjected to stress. This can be achieved by careful water management as at very high salinities (250 ppt and above), Artemia become weak and finally die. The salinity stress, created, will result in the induction of oviparity (Fig. 1). The liberated cysts will float and due to wind action, they will accumulate on the shore from where they can be harvested.

Eventhough it is reported that in Thailand, a production of 15 g live weight/m^3/day and 60 kg dry cysts/ha/5 months is achieved, one can expect an average production of 10 to 20 kg dry cysts/ha/year and a few metric tons of live biomass in saltpans.

PRODUCTION UNDER CONTROLLED CONDITIONS

Biomass production

Biomass production under controlled conditions can be carried out either in batch or in flow-through culture systems.

In both culture systems, provisions are made to maximise oxygenation of the medium and to ensure food availability to all the larvae, while culturing at high density.

Biomass production by batch culture system

In batch culture system, nauplii were reared upto adult stage, without any water renewal, in air-water-life (AWL) operated raceway, which provides continuous aeration, almost homogenous circulation of the medium and uniform distribution of the added feed within a short time. Raceway system further keeps all particulate matter in suspension thereby minimising sediment accumulation.

Construction of air-water-lift raceway

An Artemia raceway essentially consists of a rectangular tank with a central partitioning (Fig. 2). The distance between the central partitioning and the lengthy side of the tank is called the channel width. In order to obtain an optimal water circulation, the distance between central partitioning and the small side of the tank is to be about 1 to 2/3 of the channel width. The partitioning should also be kept 2 to 5 cm off the bottom of the tank either by suspending it from two or more wooden bars resting on the sidewalls of the raceway or by keeping it in its central position on top of small blocks. The water depth should not exceed 1 metre to ensure optimal water circulation with the help of axial blowers. Various materials such as concrete, marine plywood and fibreglass, can be used to construct raceway tanks.

PVC pipes and elbows are used to construct air-water-lifts, which have to be fixed to the central partitioning by rings with screws to keep them in a well defined position in raceway. For optimal water circulation, the elbow outflows should make an angle of 30-45° with the central partitioning (Fig. 3). The interval between successive air-water-lifts should be 25 to 40 cm. The diameter of the air-water-lift is related to the water depth and if the water level is 40 cm, the inner diameter of air-water-lift should be 40 mm which will provide 6.6 litre/minute/AWL of air to displace 12.5 litre/minute/AWL of water. (If the water level is 20 cm, the inner diameter of the AWL should be 25 mm to provide 2.7 litre/minute/AWL of air which will displace 4 ,itre/minute/AWL of water). 3-6 mm diameter polythene tube can serve as aeration line and it can be mounted in the AWL through a hole at the top of the PVC elbow. To assure the best water-life-effect, the aeration lines should extend as deep as

possible in the AWL. All aeration lines will be drawn from a central air distributing container so that each aeration line need not have a separate regulating valve system.

Culture procedure

It is convenient to carry out batch culture in raceway system in 50-100 ppt salinity because contamination with ciliates and other competitiors and predators, can be avoided in high saline media. Stocking has to be done with freshly hatched nauplii and rate of stocking depends upon the feed availability and water management. 10,000 instar I nauplii per litre can be stocked if feeding is maintained at 15-20 cm transparency with rice bran. As Artemia is a non-selective filter feeder, it can be cultured by feeding with a wide range of feed both live and inert materials. However, the particle size of the feed should be less than 50 microns. Hence, the feed should be squeezed (if inert feed) or passed (if algal feed) through 50 microns sieve. Soluble products are not taken up by Artemia and hence the feed if it is an inert one, should be properly prepared to get rid off the dissolved matter. This can be achieved by aerating the feed solution for 1-2 hours and allowing the feed particles to settle by cutting off the aeration for ½ hour. Dissolved matter will be in solution and it will be discarded, while only settled product will be used as feed. As Artemia is a continuous filter feeder, medium must contain adequate food at all times and hence food distribution is very important. Transparency of the culture medium found to be a very useful parameter for determining the food level present in the medium. During rearing, particulate wastes, such as faecal pellets and exuviae, will form and they have to be continuously removed from the culture medium from 4th culture day onwards, as they affect the water quality and hamper the food uptake by Artemia. This can be achieved by pumping the water

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achieve this, air supply system is to be connected with the nitrogen supply unit, by a timer and a magnetic valve. At every three hours, the timer activates the magnetic valve which closes the air supply but allows the nitrogen to pass through the medium for a period of five minutes. After the supply of nitrogen for 5 minutes, the oxygen drastically decreases from 60% to 8% saturation thereby creating a sudden and effective stress. Subsequent aeration will keep the animal in living condition. Only after 1-1½ hours, the dissolved oxygen content will reach to the original level of 60% saturation, thereby indicating that the stress will fairly be a prolonged one. Artemia will produce haemoglobin in oxygen stress and hence the animals will become red-coloured. Within a week, eggs will be formed in the ovaries and coated with the secretion of the shell gland in the ovisac. The cysts, liberated will be collected in a 110 microns filter bag.

STORAGE OF HARVESTED BIOMASS AND CYSTS

Adult Artemia, harvested from saline (more than 100 ppt) waters, will remain alive for 3 to 5 hours even-though they are provided as feed directly after collection and subsequent washing with freshwater/seawater. If the biomass, harvested, has to be stored, it has to be frozen for which the live biomass has to be spread out in thin layers in plastic bags/ice trays and be transferred to -25°C in a quick freezer.

The harvested cysts have to be cleaned, processed and stored in closed containers in saturated brine or under vacuum/nitrogen atmosphere.

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Technical Paper - 28

SUMMER INSTITUTE IN
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ROLE OF NUTRITION IN BROODSTOCK MANAGEMENT - PRAWNS

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For successful hatchery production of prawn seed a steady supply of spawners of desired species of prawns at the proper time is a necessary prerequisite. The uncertainity of procuring spawners from the wild has stimulated worldwide interest in the efforts to induce prawns to mature under controlled conditions. A successful technology for inducing maturity and spawning of penaeid prawns has been developed at the Marine Prawn Hatchery Laboratory (MPHL) of Central Marine Fisheries Research Institute by employing the method of unilateral eyestalk ablation. Using this technique many commercially important species of penaeid prawns (Penaeus monodon, Penaeus indicus, Penaeus semisulcatus, Metapenaeus dobsoni, Metapenaeus monoceros and Parapenaeopsis stylifera) have been induced to mature and spawn in captivity (Muthu and Laxminarayana, 1977, 1979, 1982; Laxminarayana and Sasidharan, 1983). A system for sustained production and maintenance of broodstock has been established.

One of the important factors in the development and management of broodstock is the availability of an appropriate feed. Dietary nutrition plays an important role in the maturation, spawning and the quality of eggs spawned. However, precise information on the nutritional requirements

of broodstock prawns is scanty. Of late, considerable interest has been generated in the nutritional studies of prawns. The major dietary nutrients like proteins, lipids, carbohydrates and minor nutrients such as vitamins and minerals play a vital role in the maturation of prawns. Recent studies on the nutritional requirements of prawns have focussed on lipids which provide energy as well as essential nutrients such as fatty acids and steroids. Research work on the requirements of other nutrients for broodstock prawns is very much needed to establish the missing links. Based on the knowledge of the nutritional requirements, a comprehensive feed for broodstock has to be evolved. The information available on the nutritional studies of broodstock prawns is presented in this paper.

NUTRITIONAL REQUIREMENTS

Earlier studies have shown that penaeid prawns have a very high lipid content which is maintained by dietary intake. Of the lipid components, steroids and fatty acids are most important. Steroids are needed by shrimp as moulting hormones, sex hormones and membrane components, yet these animals are unable to biosynthesize the steroid nucleus. Fatty acids are also membrane components and fulfil a significant role in energy storage. A range of these components can be biosynthesized, but others ("essential fatty acids") are needed in the diet of prawns.

Middleditch *et al.* (1979) studied the fatty acid profiles of gonad, digestive gland and tail muscle samples of male and female penaeid shrimp (*Penaeus setiferus*, *P. stylirostris* and *P. vannamei*) obtained from the sea. The major fatty acids of the lipids from mature ovaries were C_{20} and C_{22} polyunsaturated fatty acids (PUFA). They obtained ovarian maturation and spawning in *P. setiferus*

by feeding the prawns with an annelid (Glycera dibranchiata) which is rich in C_{20} and C_{22} polyunsaturated fatty acids. Based on their studies Middleditch et al. (1979) suggested that the reproduction of prawns is mediated by prostaglandins derived from these fatty acids.

In whild Penaeus japonicus, Teshima and Kanazawa (1983) observed an increase in the ovarian lipid concentration from slightly mature to yellow ovarian stages reaching constant levels in mature ovaries and declining after spawning. In contrast, lipid levels in the hepatopancreas declined in mature ovaries after reaching a maximum in the yellow ovaries, suggesting a possible movement of lipids from the hepatopancreas to ovaries during maturation. Ovarian lipid concentration in wild Penaeus aztecus showed an increase from early developing to ripe stages and a decline in spent stages (Chamberlain and Lawrence, 1983). There was also an increase in ovarian carbohydrate levels from nearly ripe to ripe stages but no changes in the protein concentration for all maturation stages. Ovarian lipid concentration in immature Penaeus monodon increased upon reaching full maturity from 5.8 to 17.0% in wild (unablated) females (Willamena et al., 1984) and from 7.5 to 21.9% in wild ablated females. The fatty acid profile showed 12.14 - 24.87% and 11.81 - 24.50% for total fatty acids in wild (unablated) and wild ablated females respectively, to consist of 20:4W6 (arachidonic acid) 20:5W3 (eicosapentaenoic acid) and 22:6W3 (docasahexaenoic acid). The same plyunsaturated fatty acids were reflected in spawned eggs, indicating their importance in the reproductive process.

FOOD AND FEEDING

Broodstock prawns have generally been fed on fresh or frozen mussel, clam, oyster or squid meat. Other food items used are fresh or frozen marine worms, mysids shrimp and fish and dried pellets. These various items may be given alone or in combination. The broodstock are fed ad libitum or according to a daily feeding rate of approximately 3-5% for dry feed (pellets) and 10-30% for wet (fresh or frozen) feed. Feed is given once upto 4 times a day and daily ration divided accordingly. Best results were obtained when the broodstock of prawns (Penaeus indicus, P. monodon, P. semisulcatus, Metapenaeus dobsoni, M. monoceros and Parapenaeopsis stylifera) were fed ad libitum on clam (Sunneta scripta) meat (Muthu and Laxminarayana, 1977, 1979, 1982; Laxminarayana and Sasidharan, 1983). The clam fed to broodstock prawns usually have mature gonads which may be supplying the essential fatty acids and carotenoids needed for the ovarian development of prawns. Middleditch et al. (1980 b) have found that bivalves are rich in C20:4, C20:5 and C22:6 fatty acids.

A mussel-pellet and all-mussel feeding combination gave better maturation and hatching rates than a squid pellet or all-pellet feeding for ablated Penaeus monodon (Primavera et al., 1979). The composition of the pellet used by Primavera et al. (1979) is given below.

Ingredients	% in diet
Fish meal	20
Shrimp head meal	20
Squid meal	25
Rice bran	10
Wheat flour	10

Agar	4
Sago palm starch (Landing)	4
Soybean oil	5
V22 (Vitamin mix)	1.9
Ascorbic acid	0.1

Aquacop (1979) found that among the different compounded pellets tested, the best ones were high protein diets (60%) containing squid meal. They also reported that if females are isolated and allowed to complete the ovarian development in separate tanks where a supplement of fresh troca (Trochus niloticus) flesh is given, the quality of eggs spawned is much better.

Chamberlain et al. (1981) fed four single diets (clams, shrimp, squid and worms) and one composite diet consisting of all four foods to the broodstock of Penaeus vannemei. They found that the composite diet was the best overall diet while squid was the best single-food diet, followed by shrimp, worms and clams. (Caillouet (1973) fed unablated Penaeus duorarum with diets to which additives such as beta carotene, phosphatidylcholine, cholesterol, DL alpha tocopherol, calciferol and 17 beta estradiol were added, but the prawns did not attain maturity.

Lawrence et al. (1980) fed the broodstock of Penaeus setiferus on oyster (Crassostrea sp.), squid (Loligo sp.), sandworm (Neries viridens) and a prepared dried feed. They have used three feeding times: 0800 hrs, oyster or prepared dried feed; 1130 hrs, squid; and 1500 hrs, sandworm. Maturation and repeated-spawning and successful hatching was achieved when the above feed combination was used. The composition and percent protein, carbohydrate and lipid content of the prepared feed is given below:

Component of feed	Percent
Shrimp Meal (sun dried)	35.0
Squid meal	25.0
Manhaden meal	15.0
Rice bran	12.5
Vitamin mix (AIN 76)	2.0
Mineral mix (AIN 76)	1.0
Fish soluble	2.0
Menhaden oil	2.5
Cholesterol	0.5
Lecithin	1.0
Sodium Hexametaphosphate	1.0
Sodium Alginate	2.5

The percent protein, carbohydrate and lipid content of the above feed was 40.8, 28.2 and 12.4 respectively.

REMARKS

For feeding the broodstock prawns definite time schedules and feeding rates should be established so that the quantity of food would not be limiting. These schedules will also ensure that a variety of nutrients will be supplied during different time of day. Care should be taken to see that the excess food and faecal matter in the maturation pools and tanks are removed daily. If this is not done, the water quality will deteriorate rapidly as decay of these substances, will increase the biological oxygen demand of water. Under such circumstances the intake of food by the prawns declines markedly (Muthu and Laxminarayana, 1982).

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SUMMER INSTITUTE IN

RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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NUTRITION OF BROODSTOCK - FINFISH

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One of the requisites for development of finfish culture is an assured supply of the fish seed throughout the year. The production of healthy larvae, fry and fingerlings of finfish depend on the nutritional and physiological status of the broodstock. Though nutrition is known to have considerable effect upon gonadal growth and fecundity, precise information on the nutritional requirements for gonadal maturation in finfish broodstock is lacking.

Poynter (1976) clearly demonstrated that the fecundity of hatchery reared lake trout appears to be directly related to food availability. Fish fed at a daily rate of 0.75% of their body weight produced more and larger eggs with higher fertility than those fed at a rate of 0.5% of their body weight. Scott (1962) found that variations in egg number are related to the size of the fish, size of the eggs, and adequacy of the diet. Hester (1964), working with Lebister reticulatus, determined that reduced numbers of offspring and reduced large - middle - sized oocyte with reduced ration. Bagenal (1969) also observed that the number of eggs produced by the brown trout Salmo trutta was higher in the better fed fish. Dahlgren (1980), who studied the effect of three different protein levels on the fecundity of the guppy Poecilia reticulata reported that the average gonadosomatic

index (GSI) was highest in groups fed the highest protein diet, but the fecundity of the fish was not affected by diet.

Watanabe et al. (1984 a, b, c) have carried out a series of nutritional experiments on broodstock of both rainbow trout and red sea bream.

Rainbow trout:

Studies with rainbow trout have shown that there was no significant difference in the egg production (3000 egg diameter (5.2 mm), the proportion of eggs reaching the eyed egg stage (90%), and the proportion of hatching (87%) between treatments fed with low-protein high energy diets (33-35% crude protein and 390 Kcal/100 g) or a high protein diet (43-47% crude protein). However a diet lacking supplementary minerals produced relatively less number of eggs (2000 eggs/female), less number of eggs reaching the eyed state (3.7%) and hatching (0.4%). These results demonstrate that diets with low protein and high energy can be successfully used for broodstock. But, a trace metal supplement was indispensable for the reproduction of rainbow trout. Subsequent trace metals analyses revealed that manganese concentration was significantly low (4.1 $\pm$ 0.7 µg/g) in eggs of females given the diet with minerals, when compared to diet lacking in trace metal supplement (1.6 $\pm$ 0.1 µg/g).

In a subsequent experiment, it was confirmed that broodstock diets with 36% crude protein and 18% lipid performed as well as those given a diet with 46% crude protein and 15% lipid. Besides, beef tallow when used at a level of 7% as on energy source had no adverse effect on the reproduction of rainbow trout. However diets deficient in essential fatty acids provided the lowest egg numbers, eyed eggs and total hatchlings. One salient finding is that addition of linoleic acid, 18:2W6 to the EFA deficient broodstock diet

led to marked improvement in percentage fertilization, percentage of eyed eggs, and total hatch compared with broodstock given diets lacking essential fatty acids.

This is a very interesting finding since linolenic acid (18:3W3) is found to be the EFA for rainbow trout fingerlings. Subsequent research has shown that the eggs produced by rainbow trout contain 20:4W6 (arachidonic acid and a possible dietary importance of W6 fatty acids in rainbow trout has been suggested.

Red Sea bream

In red sea bream, when krill, Mysis, shrimp and crab wastes are fed to broodstock, pigmentation of the eggs has been noticed within a matter of hours, suggesting that the nutritional value of the diet given to broodstock shortly before spawning may affect the results of spawning. It is suggested that the quality of eggs may be improved by feeding the broodstock with some fat-soluble nutrients such as essential fatty acids and vitamins. Subsequent studies by Watanabe et al. (1984 a, b, c) showed that supplementation of diets with -carotene and canthaxanthin or krill oil extract led to a slight decrease in the total number of eggs produced. But the percentage of buoyant eggs increased from 49.1% to 56.4% and 69.6% respectively in the above diets with pigments. Frozen raw krill led to marked improvements in both the total number of eggs produced and percentage of buoyant eggs. In eggs from broodstock fed the diet containing corn oil, abnormalities in the number of oil globules increased to 94%. The number of normal larvae obtained were highest for the krill diet (91.2%) but lowest (24%) for the corn oil (10%) based diet. These results suggest the important of proper nutrition for fish broodstock.

Leptobarbus hoevenii:

Studies carried out on this species (Pathmasothy 1985) showed that relatively high protein levels (32 and 40%)

are required for optimum performance compared to a low protein diet (24% crude protein). The GSI values and fecundity were also significantly higher with the 32 and 40% diets than with the 24% diet. However, there were no significant differences in the individual egg weight.

Common carp:

Vitamin E has been shown to be important in the reproductive physiology of fishes. Adult female common carp (100 g) fed a vitamin E deficient diet for 17 months displayed reduced weight gain, lower gonadosomatic index, apparent muscular dystrophy, higher muscle water content, lower muscle protein content, lower concentrations of yolk granule in oocytes compared with individuals fed 700 mg -tocopherol/kg dry diet (Watanabe and Takashimo, 1977).

Assessment of nutritional status of broodstock based upon the biochemical composition of fully mature ova and fertilized eggs.

One of the best clues about the nutrient requirements of a fish broodstock can be gained from the biochemical composition of the ova as well as fertilized eggs. However, thus for work on this aspect has been very limited and incomplete. By determining the protein, lipids, carbohydrate, amino acids pattern, fatty acids profile, composition of minerals and vitamins, hormones, prostaglandins etc., guidelines can be evolved in providing nutritionally adequate diets. An important area of research requiring greater emphasis is the nutritional bioenergetics of the brood fish, to provide information as to how the nutrients are partitioned in maturing fish. Information on somatic growth would provide additional clues about the requirement.

Ackman (1964) concluded that the fatty acid composition of fish egg lipids is distinctive for each species and did not necessarily related to the diet or depot fat of the adult. Ackman (1964) found that cod roe contained increased levels of 16:0, 20:4W6, 20:5W3 and 22:6W3 compared with the liver lipids of the same female fish.

Shimma et al. (1977) found that the hatchability of eggs from carp fed several different formulated feeds was greatly reduced when the 22:6w3 of the egg lipid was less than 10%. They also observed that the muscle, plasma, and erythrocyte fatty acid compositions were more affected by dietary lipid than that of the eggs.

Lasker and Theilacker (1962) found elevated levels of 16:0, 20:5w3 and 22:6w3 and reduced 18:1 in the ovary compared to mesenteric fact of Pacific sardines fed a natural copepod diet. Ovary of sardines retained high levels of 20:5w3 and 22:6w3.

Thus, there is an urgent need to study the biochemical changes occurring in the gonad and muscle during maturation with reference to nutrients intake for evolving practical feeds for broodstock.

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STATISTICAL DESIGNING OF FISH NUTRITION EXPERIMENTS

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It is the pioneering work of two renowned statisticians Prof. R.A. Fisher and Dr. F. Yates in the nineteen thirtees which laid the foundation for the development of the subject 'design of experiments'. The subject had a phenomenal growth to cope up with the fast increasing needs of experimental sciences. Today it is a very well established branch of applied statistics and there are several text books and a large number of theoretical and applied papers in literature. The present note discusses briefly the need for statistical designing and presents some simple designs useful for fish nutrition experiments.

1. Need for Statistical Designing

The validity of the findings of scientific experiments depends on the type of data collected and their amenability to statistical analysis. Considering the importance, statistical methods are now taught as compulsory subjects in all the professional courses. Even then, many a research worker feels hesitant to avail of the appropriate statistical back-up for his experimental programmes. No doubt a statistical design has an underlying theoretical model and assumptions. But the applicational part is relatively simple and easy to implement. A little experience would convince

that designing ensures objectivity in the procedures and adds confidence to the ensuing results.

Statistical designing involves the formulation of a scheme or a lay-out plan where the placements of treatments in experimental units are specified keeping in view the objectives of the programme and the statistical requirements. Here the word 'treatments' is used in a general sense and may mean factors like levels of feeding, doses of stimulae and stocking densities.

Consider 4 feeds denoted by A, B, C and D in a fish culture experiment for comparison of growth rates. Let there be 20 ponds/sub-ponds which can be taken to be homogeneous. Under the simplest design, namely, 'Completely Randomised Design' the feeds are randomly allotted to the different ponds and a lay-out is as follows.

D	B	D	C	A
A	C	A	B	C
A	B	C	B	D
C	D	D	A	B

Each feed is replicated five times and they are randomly allotted. Do you need these replications and the randomisation process bringing in constraints and affecting the freedom of the experimenter? Here comes the basic question, namely, why statistical designing?

Variability in experimental material is an inevitable feature in any field of research. Consider for example two fish culture ponds kept under conditions as similar as possible with the same area, species, stocking density etc.

At the time of harvesting one would find that the yield of one pond is different from the other. This may be attributed to the uncontrolled variation inherent in the production process. Consider another two ponds kept under almost identical conditions except that in one pond supplementary feed is given. Here again, at the time of harvesting the yields would be found to be different. Can we attribute the difference to the effect of levels of feeding? We cannot. May be the supplementary feed did not contribute anything to the difference in yield and the difference could be purely due to the inherent uncontrolled variation. Differences are expected even when similarity is maintained in the two ponds.

Thus variation introduces a degree of uncertainty into the conclusions that are drawn from the results. The observed variation between treatments may be partly due to real treatment differences if there are any and partly to the uncontrolled factors (commonly called experimental error) which influence yield even in the absence of any real treatment differences. It is therefore necessary to evaluate the magnitude of variation due to experimental error and compare with it the observed variation between treatments through an appropriate test of significance to conclude whether the experiment indicates any real differences in the effects of treatments. Only a statistically designed experiment can result in the estimation of the different components of variation and permit valid test of significance involving probability statements whether a particular difference is due to chance causes or can be attributed to the real difference between treatments.

2. Principles of Designing

Two primary requisites in designing experiments are replication and randomisation. Replication or repetition of treatments provide stability to the mean but more than that makes it possible to estimate the experimental error by say, considering the differences between units under the same treatments in different replications as in a completely randomed design. It also increases the precision of the estimates of both the treatment mean and the experimental error.

Randomisation which means random allocation of treatments to various experimental units, insures that a treatment will not be unduly favoured or handicapped in successive replications. It ensures unbissedness of the estimates of experimental error and provide for valid treatment comparisons against the experimental error (Fisher, 1954). When treatments are replicated and allocated randomly to the various units we are in a position to test the significance of observed treatment differences by the use of test of significance procedures. Thus it is essential to provide for adequate number of replications and ensure proper randomisation at the planning stage (Panse, et al., 1964).

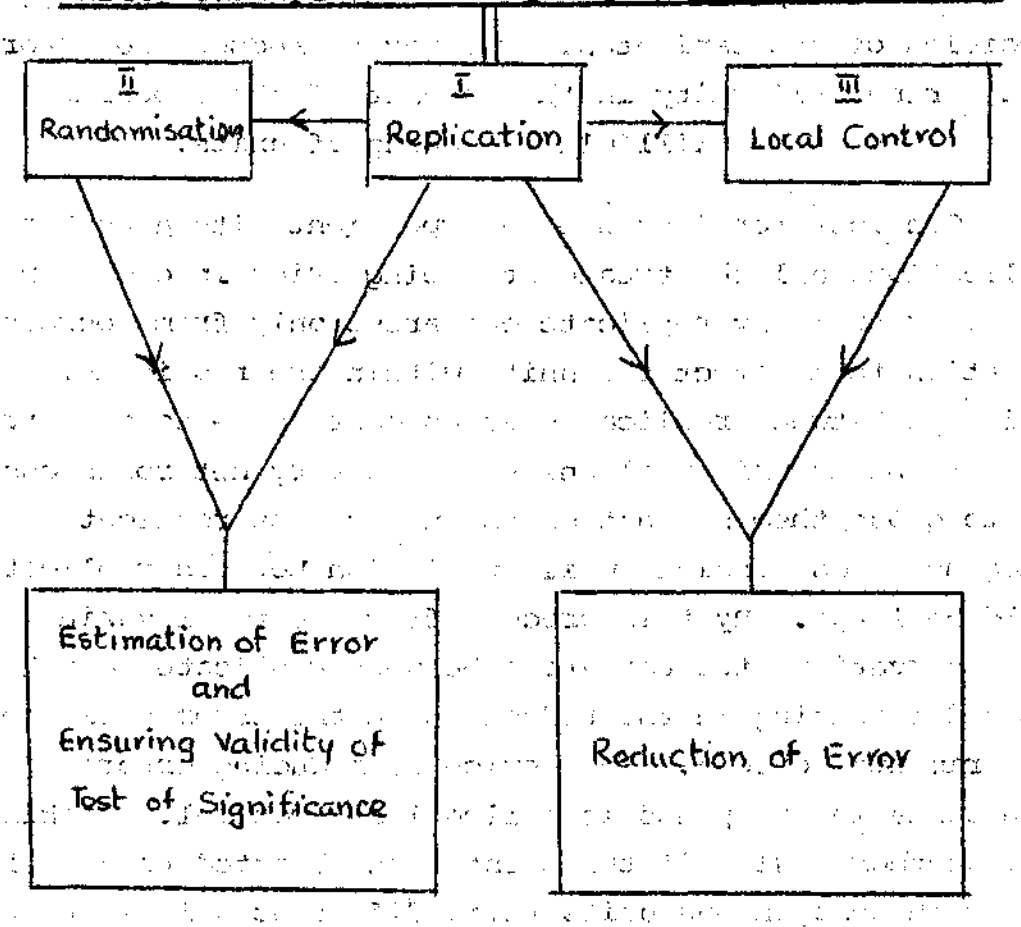
As indicated earlier the results of an experiment are affected not only by the action of treatments but also extraneous variation which tend to mask the effects of treatments. This extraneous variation is conventionally termed as experimental error (or sometimes called 'error'), where the word 'error' is not synonimous with mistakes but indicates all types of extraneous variation (Cochran, et al., 1973). There are two types of experimental errors, one refers to the inherent variability in the experimental material or units to which the treatments are applied and the other type

refers to the failure to standardise the experimental technique. It is desirable that the experimental error is kept as minimum as possible as otherwise a large difference in the treatment means will only be detected as significant. Reduction of experimental error automatically increases the precision of the estimates. One way to reduce the error is by ensuring uniformity in the conduct of the experiment. Another way is by skillfully grouping of units.

Consider for instance an experiment with a number of replications all the treatments being tried in each replicate. The error from any replicate can arise only from sources of variation that affect the units within the replicate. Variation between replicates do no contribute to the error. Thus if the experimental units form a very heterogeneous set, try to group them so that units in the same replicate is as homogeneous as possible while variation between replicates could be large. By this process from the total variation in the observations the variation between replicates can be removed resulting in the reduction in the error variance (experimental error). The device of reducing errors through such suitable groupings is called local control. Looking from another angle, if treatments are allotted to a replication with homogeneous units their differences indicate the real variations between the treatments. The principle of local control is the basis for experimental designs such as 'randomised blocks' and 'latin squares'. Where the number of treatments to be accommodated in a replicate becomes large, the homogeneity within a replicate tends to be lost and can be restored by dividing the replication into smaller blocks which is the basis of confounding in factorial experiments and also various incomplete block designs.

A flow - chart indicating the three principles of designing and their functions is shown below:

Three Principles of Designing and their Functions



3. Some Designs Useful For Nutrition Experiments

(i) Randomised block

One of the most commonly used plans is the randomised block design where experimental material is divided into block each of which constitute a single replicate in such a way that the units within a block is as homogeneous as possible. The treatments are now randomly allotted to the experimental units within a block. This increases the comparability of treatment effects

as they act under conditions which are similar except for the treatments. For instance in an experiment to select an economic supplementary feed mixture from among 4 prepared mixtures for prawn culture, 4 ponds all located by the side of the main water body like the backwater or estuary could be grouped as one block or replication and allot treatments at random. The next 4 could be ponds running paralled to the first set but more inside the land so that within a block salinity and associated features are likely to be similar. This arrangement takes care to a good extent salinity gradient likely to be reducing when moved away from the main water body. In the experiments if there are 5 replications there will be total 20 ponds. If all the 20 ponds are more or less similar no blocking or stratification is required and the treatments could be randomly allotted over the entire range of the 20 ponds. Such a design is called completely randomised design. However if heterogeneity in the features in the ponds is suspected it is desirable to provide blocks which may help in reducing the experimental error. A lay-out plan for randomised block design is given below

Block

I	II	III	IV	V
B	A	B	C	D
D	C	C	D	A
C	B	D	A	B
A	D	A	B	C

(ii) Latin square

In randomised blocks one-way restriction is imposed. If heterogeneity is suspected in two directions the experimental area can be divided into rows and columns and treatments are applied in such a way that a treatment appears only once in a row and once in a column. Such an arrangement is called a latin square design. Through elimination of row and column effects the residual error variance may be very much reduced. With two-way stratifications the latin square controls more variation than randomised block design resulting often in smaller error mean square. However the number of treatments is limited to the number of rows or columns and for large number of treatments it is no preferred.

(iii) Factorial experiments in complete and incomplete blocks

Consider an experiment to study the effect of different levels of protein and energy on weight of fish in culture ponds. If there are say 2 levels for each factor there will be in all 4 (2^2) treatment combinations. A group of treatments which contains two or more levels of two or more factors in all combinations is known as the factoral arrangement. The different combinations could be allotted as in a randomised block design. The experimenter could try a one-factor-at-a-time approach. The advantage in factorial experiments is that not only the main effects but also the interactions between factors can be studied and tested for statistical significance.

If the number of factors and levels are large, say, 3 factors salinity, temperature and oxygen content at 3 levels each, the number of treatment combinations will be 27 (3^3). It may be difficult to get 27 experi-

mental ponds, which are more or less homogeneous with regard to factors other than being tested so that the principle of stratification to reduce experimental error cannot be implemented. An ingenious device to overcome this situation is called confounding where a homogeneous block will not accommodate the full replication. One replication is divided into say, 3 compact blocks such that the units in the smaller blocks are homogeneous. The 27 treatment combinations can be divided into 3 groups of 9 each and allotted to the 3 compact blocks (Cochran and Cox, 1973).

(iv) Switch-over

There are occasions in which treatments are applied in sequence over several periods on a group of individuals. Consider an experiment to study the effect of mineral supplementation of two types on lobsters kept in artificial tanks. If there are say, 12 groups of lobsters separated and kept in tanks with sub-partitioning, then the two types of supplementations are given such that half the groups received say, type A and the other half type B in period 1. The lobsters receiving type A in period 1 will get type B in period 2 and vice versa. Such a design is called switch-over or change-over design (Federer, 1973). On the other hand if a time trend is expected in the character under study a switch-back or a double reversal design will have to be used. In these procedures a rest period is to be provided between two treatment periods so that there is no carry-over effect or residual effect influencing the treatment during the second period. However if a reasonably long rest period is not feasible or the residual-effect is itself a topic of interest the procedure is to be modified so that direct and residual effects of treatments can also be measured.

4. Statistical Analysis

Once the data become available it is essential to follow appropriate statistical procedures for analysis. The type of analysis basically depends on the design used. Computational details for forming the analysis of variance table and for performing test of significance are available in several publications (Cochran and Cox, 1973).

Some experimenters do not bother to follow a design but try to analysis the data statistically. Some others follow a design but do not care to follow the appropriate procedure of analysis. It is essential in scientific experimentation to follow a suitable design and analyse the data through appropriate procedures.

5. Number of Replications

One aspect need to be stressed here, namely, the provision of enough number of replications in an experiment. Consider the earlier example of 4 feed mixtures which are tried for economic evaluation in prawn culture. If the mixtures are allotted only one each in four ponds without replication we will get only a single figure on, say, cost of production of a unit weight, for one mixture. Thus with four treatments the character under study will have only four values, a single value for each, and no statistical analysis is possible (Jacob et al., 1978). One way is to partition the ponds into 4 sub-ponds which may provide 20 values, 5 each for one treatment for analysis. It may be stressed that apart from reducing experimental error replication of treatments alone can provide an estimate of the experimental error essentially needed for treatment comparisons.

The question of minimum number of replications required is of great importance in aquaculture experiments because of the cost involved and the inherent special problems compared to experiments on land. An important consideration in determining the minimum number of replications is that the test of significance should be sufficiently sensitive to detect real difference between treatments as distinguished from variation due to chance causes. The sensitiveness of the test will depend primarily on the magnitude of variations in the experimental units with regard to the character under study. If the magnitude is known the number of replications required for detecting a particular difference with certain confidence can be worked out (Fedever, 1967, Panse et al., 1964). In the absence of any knowledge regarding the magnitude of variability the number of replications could be decided in such a way that at least 12 degrees of freedom are ensured for error. This is inferred from the fact that the tabulated value of 'F' at the conventional level of significance of 5 per cent ceases to fall off rapidly for degrees of freedom beyond 12. On this basis the minimum number of replications can be worked out for a particular design.

6. Concluding Remarks

The need for statistical designing and some guidelines for planning experiments have been dealt with in the preceeding pages. Some of the simple designs which can be used in fish nutrition experiments have been presented. The references given at the end would provide a wide range of useful designs. However, it may be observed that considering the resources available and the special nature of certain problems some amount of tailoring may have to be resorted to, to suit particular situations. Once the data are acquired, statistical analysis appropriate to the design employed has to be carried out so as to arrive at conclusions relating to the hypothesis under test.

To sum up, statistical designing of experiments attempts to minimise the effects of heterogenity in experimental units from treatment comparisons, reduce experimental error, provide unbiassed estimates and ensure validity in test procedures. The test of significance emanating from the design exerts a sobering influence on the type of experimenter who jumps to exciting conclusions that can as well be ascribed to the natural variation inherent in the experiment (Cochran, et al., 1973).

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HISTOLOGICAL EXAMINATION OF TISSUES OF EXPERIMENTAL FISH

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Nutrition and pathology are related disciplines. Gounelle (1961) observed that "Every disease has a nutritional aspect. Pathology is concerned with specific disease process and resultant tissue changes in the individual what ever its systemic position. The study of disease process involves at a greater extent the histological examination of various organs and tissues. The histological examination of tissue in a nutritional experiment provide an opportunity to observe the resultant changes occurring at cellular level. Many times a particular treatment may not produce any physiological or reproductive effect immediately; however histological examination may reveal considerable changes. It is also important before advocating any feed or a combination of feed for field use we have to ascertain whether the said combination contain any type of toxic factor. This can be settled only by conducting an experiment in which histopathological examination forms the major component.

General histological picture in fishes

A knowledge of general histology is essential for interpreting changes due to experimental manipulation. The basic histologic pattern is similar in all vertebrates and a knowledge of mammalian histology is helpful. However it

should be noted that fishes are not primitive vertebrates and they have undergone considerable adaptation to suit their aquatic environment. Even among fishes there is considerable variation between various species. It is not possible to discuss each individual separately. We will confine to a general discussion on histology.

Integumentory

The skin of fish has got following layers (1) cuticle (2) epidermis (3) basement membrane (4) dermis (5) hypodermis. Cuticle is a muco poly saccharide layer secreted by epidermis and may contain sloughed cells, immunoglobulines, lysozyme and fatty acids. Epidermis is a startified squamous epithelial layer formed from Malpighian cells. Many of the cells are mucoid (goblet cells). In addition club cells (Shreckstoffzellen) which produce alarm substances, granule cells and cysts are found in epidermis. Dermis consist of stratum spongiosum containing chromatophores (melano phores Lipophores erythrophores), mast cells, scale buds and the stratum compactum which provides structural strength.

Hypodermis is a lose tissue containing fat cells and is highly vascular.

Structure of Bone: Bone tissue is a cellular.

Muscles are organised as distinct zones in many species. There are two or three types.

(i) Muscularis lateralis superficialis (red muscles) rich vascular supply.

(ii) Muscularis lateralis profoundus (white) poor vascular supply.

(iii) Pink muscles - contain both type of fibres. In red muscles nerve ending are engrappe (middle), in white muscle they are en platte or terminal.

Respiratory system

- Gills are made up of four holobranchs and each holobranch is divided into two hemibranch. Each hemibranch has a row of filament projecting like the teeth of a comb. They are the primary lamellae. The surface of primary lamellae has numerous semilunar projections called secondary lamellae. The gillarch is osseous structure from which radiate the bony support of primary lamellae. The arches contain afferent arteries and efferent arteries. The gillarch and lamellae are covered by epidermal tissue. At primary lamellae the epidermis thicker and contains numerous mucus cells. Below the epidermal layer lymphoid cells eosinophilic granule cell and phagocytic cells are seen in loose connective tissue.

Secondary lamellae consist of an envelope of epithelial cells one layer thick separated by contractile pillar cells which are arranged in rows 9-10 microns apart. The pillar cells spread on basement membranes in the form flanges and coalesce with the neighbouring pillar cells forming the lining of blood sinuses which connect afferent and efferent vessels. The pillar cells can regulate blood flow and blood pressure through gills.

Circulatory system

Heart: Muscle fibres are approximately 6 micron in diameter and are similar to mammalian one with intercalated discs. In atrium muscles are arranged in the form trabeculae with a lining of endothelium which is phagocytic. Sinus venosus is mainly collagenous connective tissue.

Ventricle had two layers of muscles. Outerlayer is compact and inner layer spongy and in the form of trabeculae. Outer layer receives nutrition from coronary vessel where as inner spongy layer takes it directly from luminal blood. Bulbus arteriosus is formed of elastic tissue. Pedicardium is similar to other vertebrates.

Arteries and veins: The basic pattern is same as in mammals.

Haemopoetic tissue: Haemopoetic tissue is located in stroma of spleen, interstitum of kidney and periportal areas of liver.

Renal haemopoetic tissue

Anterior kidney is exclusively haemopoetic and the support matrix of posterior kidney also contribute to a greater extent. It consist of a stroma of reticulo endothelial cells with numerous blood sinuses and blast cells.

Spleen

It is a lymphoid organ. Splenic capsule is fibrous without any trabeculae. The main elements in spleen are ellipsoids, spleenic pulp and melanomacrophage centres. The ellipsoids are thick walled filter capillaries derived from spleenic arterioles. Each consist of thick walled basement membrane bound tube in which artery is usually placed ecentrically sheathed with phagocytes and erythrocytes. Spleenic pulp consist of phagocytic tissue supported by argyrophilic fibres and with numerous blood sinuses.

Melano macrophage centres

Found in kidney, liver and spleen. These are foci containing numerous pigmented cells/phagocytes containing cercid, haemosiderin and melanin.

Thymus

Located at dorsal commissure of operculam as paired organ. It is an aggregate of small lymphocyte covered with fibrous capsule and stroma formed by fine argyrophilic cells and fibres. Epithelial cords are seen occasionally.

Reticulo endothelial system (RES)

RES in fish consist of promonocyte of haemopoetic tissues, monocytes of blood and lymph, macrophage of connective tissue, kidney and endothelial cells (Phogocyte) of atrial lining of heart. Melanomacrophage centres are also part of this system. There are no lymphnodes.

Excretory kidney

Kidney in fishes is a complex organ having haemopoetic, reticulo endothelial, endocrine and excretory functions. Anterior kidney is haemopoetic and posterior portion only had the nuphrons. Nephron structure varies between marine and freshwater species. In fresh water forms nephron comprises vascular glomerulus, ciliated neck, two proximal segments, one with brush border other without brush border, a narrow ciliated intermediate segment and a distal segment which joins collecting duct system. In marine forms nephron consist of glomerulus neck segments, two or three proximal segment occasionally inter-mediate segment found between 1 and 2 proximal segment and the collecting system. In euryhaline species nephron is similar to marine except it may have a distal segment.

Digestive system

1) Mouth is lined with stratified squamous mucoid epithelium on a thick basement membrane and condensed dermis attached to bony structure. Mouth and lips contains the taste buds also.

Oesophagus

Epithelial lining is stratified and rich in mucus cells. Muscularis is straited.

Stomach

Mucosa is lined with mucoid columnar epithelium. Mucosa is thrown into folds and pits. Submucosa contains eosinophilic granule cells. Muscularis comprise of several layers smooth muscle fibres.

Pybric caeca

Histological features are similar to intestine.

Intestine has a simple mucoid columnar epithelium, overlaying a submucosa containing large number of Eosinophilic granule cells and limited by a dense muscularis mucosa and fibroelastic layer. Rodlet cells are frequently seen (oval cells).

Liver: Histology of fish liver differs from mammalian in that the hepatocytes are arranged not in typical cords or lobules. Sinusoids are irregularly distributed and their number is much less compared to mammals. Sinusoids are lined by endothelial cells. Kuppfer cell are not present. The endothelial cells are fenestrated. Number of fat storage cells (cells of Iato) are seen in-space of disse. Billary system orginate as intracellular bile canaliculi which by anastomosis form the bile duct. The gall bladder is lined by transitional epithelium which contain rodlet cells.

Pancreas: It varies in its location. It may be found among the fat cells in mesentery of pyloric caecum; Sub capsular investment of spleen or around the hepatic portal vein. The acinar structure of exocrine pancreatic tissue is very

similar to that of the mammals. Pancreatic duct usually joins the common bile duct.

Reproductive system

Testis: Comprised of series of tubules or blind sacs, the seminiferous tubules which are lined with spermatogenic epithelium which also has tall pyriform sertolicells. Leydig cells are (Polygonal) seen in between tubules interstitial.

Ovary: Structure varies from species to species.

Nervous system: It has two components (NS) (central nervous system) and PNS (Peripheral nervous system).

CNS: Brain and spinal cord are invested by single menigeal layer, the menix primitiva enclosing cerebro spinal fluid produced by choroid plexuses. CNS tissue is divided into grey and white matter. Other histological elements are same except Mauthenerian group of cells - they are two large neurons found in the medulla. The parts of brain are Telencephalon, Diencephalon, the mesencephalon and medulla oblangata. Cells constituting nervous system are neurons and supporting cells, the neuroglia (astrocytes Oligo dendroglia and microglia).

Special sense organs

Eye - basic structure is similar to that of mammals with species variation.

Labyrinth - It consist of semicircular and ottolith organs.

Lateral line system: Paired lateral line canal and in some head canals also. The mechano receptors are situated basally forming the neuromost which comprise pyriform cells with hair like structures.

Olfactory and gustutory senses

Olfactory tissue consist of focal groups of receptor cells, surrounded by mucoid and ciliated columnar epithelium. Sub epithelial loose connective tissue contains large number of eosinophilic granule cells. Axon of olfactory bulbs collect from bases of receptor cells.

Gustatory organs or taste buds are situated on outer surface of lips, head, barbels, fins, gillrackers, gill arches and mouth. Buds are formed by elongated cells forming a sphere the receptor the basal and supporting cells.

Endocrine system

Pituitary - situated in cella tursica of skull. Consist of neurohyophysis and adenohypophysis. Neurohypophysis consist of a stalk of axons whose neuro secretory neurons are situated in hypothalmic nuclei.

Adenohypophysis divided antomically into pars inter media and pars distalis composed of basophils, acidophils and chromophobes.

Thyroid gland - Consist of various follicles, lined with cuboidal cells distributed diffusely throughout connective tissue of pharyngeal area, around eyes, ventral aorta, hepatic veins, adrenal haemopoetic tissue.

Adrenals - Cortical tissue is located in anterior kidney as strands of lightly staining cuboidal eosinophilic cells. Medullary tissue or chromaffin tissue is found accompanying the sympathetic ganglia, in between anterior kidney and spine or even in anterior kidney.

Ultimobranchial gland - Serum calcium regulating gland. Appear as cords of polygonal cells lying ventral to oesophagus within the septum separating sinus venosus from the abdomen.

Corpuscles of stannius - Paired whitish cluster of tissue consisting large clear endocrine cells secreting into centre of the cluster are located retroperitonially on the surface of kidney.

Islet of Langerhans

Scattered throughout the pancreas are small islets which are poorly staining structures comprising of small fusiform alpha, Beta and Delta cells. In some teleost there is a major islet called Brockman body.

Urophysis - It is a whitish ventral expansion of spinal cord at the caudal end. It is composed of neurosecretory axons extending from cord.

Pseudobranch and Chroid body

Red gill like structure attached to internal surface of operculum derived from first gill arch. It consist of parallel blood capillaries supported by cartilage rods and have direct connection with choroid of eye.

Choroid rete:- consist arrays of capillaries alternating with rows of slender fibroblast like cells.

HISTOLOGICAL LESIONS DUE TO NUTRITIONAL FACTORS

Starvation: Marked reduction in sacroplasmal content of myofibrils with vacuolation and central migration of nuclei. In digestive tract reduction in goblet cells in muscosa. In submuscosa increase in fibro blast and collagen.

Pancreatic acini become shrunken. Increase in the size of melano-macrophage centres.

Proteins: Information on proteins is very little. Growth reduction and various abnormalities of bone may result from deficiency.

Carbohydrates: Excessive carbohydrates may lead to liver cell degeneration and excessive deposition of glycogene in liver. Blood sugar level increase was noticed in trout with degranulation and hypertrophy of beta cells of islet of langerhans.

Lipids: Excessive fat may lead to fatty liver syndrome. Essential fatty acid deficiency may lead to depigmentation, fin erosion cardiac myopathy and fatty infiliteration of hepatic cells; ceroid in liver and thickening of cell membranes of fatty tissue. Rancid fat or fish fed with trash feed may develop lipoid liver condition in which extensive lipid infiliteration of hepatocytes with distortion of hepatic muralia. Haemopoetic tissue undergo degeneration with high levels of pale staining pigment in melano macrophage centres. Extra haemopoesis in portal triad and epicardium.

Vitamins

Fat soluble vitamins

Vitamin A: Hypo vitaminosis A in fish will result in Kerato malacia, blindness, and haemorrhages at the base of fins. Hyper vitaminosis result in wide spread epithelial squamous metaphasia and osteopathic conditions.

Calciferol (Vit. D).

This is an area which has not been explored properly.

Tocopherol (Vit. E)

Deficiency in the diet result in degeneration and necrosis of striated muscle fibres, steatitis lipoid degeneration and hepato renal syndrome. (Fatty degeneration of liver and nephrosis withnephro calcinosis).

Vitamin K

Deficiency result in prolonged clotting time. There will be extensive capillary haemorrhages in muscles and viscara coupled with anaemia.

Water soluble vitamins

Thiamin (Vitamin B

The histological changes due to thiamin deficiency can be seen in brain as haemorrhages and degeneration of neurons of periventricular nuclei.

Riboflavin

Deficiency of this vitamin result in vascularisation of cornea of eye leading to cataract. Haemorrhages in eyes and opercula have also been reported.

Pyrodoxine

No detailed study on histological lesion has not been done however symptoms of deficiency suggest nervous system involvement.

Biotin

Deficiency result in cuticular thickening.

Folic acid

Deficiency leads to suppression of haemopoeisis in kidney and spleen. Absence of blast cells are the main feature.

Cynocobalamin (Vitamin B_{12})

Not much information is available.

Choline

Deficiency result in fatty infiliteration of liver and haemorrhages.

Ascorbic acid

Deficiency of Vitamin C results in poor wound healing, failure of granulation tissue to fibrose and abberant development of cartilages and bone.

Minerals

Very little is known about mineral deficiency in fishes except goitre caused by iodine deficiency. The hepato renal syndrome and renal calcinosis are suspected to be due to mineral imbalance.

Hepatorenal syndrome and renal calcinosis

The liver showed peribiliary cirrhosis and in kidney biliary hyperplasia, extensive tubular necrosis or fibrosis. In a number of species hyaline droplet deposition occurs within the cells of proximal convoluted tubules. Extensive cast formation and urolithiasis may result in later stages after considerable tubular necrosis and fibrosis.

Nephrocalcinosis or urolithiasis is characterised by deposition of calcium or magnesium salts within renal tubules.

Toxic components

Aflatoxin at the level 1 PPB can induce neoplastic changes in liver other metabolites inducing neoplasia are dimethyl nutrosamine and carbontetra chloride.

Mercury, cadmium and other heavy metals may cause degeneration and necrosis in proximal convoluted tabules of

Antibiotics and chemothesapeutics

Continuous therapy may induce toxic changes in tissues such as depression of haeomopoesis and especially with sulphonamides tubular necrosis and cast formation.

Binders

Chemically substituted cellulose binders in artificial feeds may cause hepato renal syndrome.

Gossypol

An ingradient of cotton seed which accumulate in liver and kidney causing severe liver degeneration and glomerulonephritis in kidney.

Diabetes mellities - May be produced in fishes due to protein/carbohydrate ratio change or feeding silkworm pupae. In these cases hypertrophy of islets of langerhans with beta cell degranulation and mesengeal scapillary wall thickening in glomeruli of kidney are the main histological features.

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