

DEVELOPMENT OF ARTIFICIAL FEEDS FOR FINFISHES

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INTRODUCTION

Successful fish culture depends upon provision of diets containing an appropriate balance of essential nutrients and adequate level of energy to permit the most efficient growth of fish. Application of modern techniques, with very high production potentials, demands supply of nutritionally balanced complete feeds in intensive systems and supplemental feeds in semi-intensive systems. Nutritionally adequate feeds are not only required for the fish in grow-out systems but also for production of viable brood fish for artificial propagation, as well as for production of healthy larvae, fry and fingerlings. Design of artificial diets calls for information on a variety of nutritional, physiological, biochemical and general biological aspects of candidate species; but to date, only limited information is available on broodfish, larvae and fry of saltwater fin fish species. In spite of this, several empirical feed formulations are in use, which are either improved versions of grow-out diets for the same species or related species. In this paper an overview of the recent developments in artificial feed development for broodfish, larvae and fry is presented. Formulations that have been successfully used for certain species have been given merely as guidelines for feed development for brackishwater finfish found in India. Research needs and priorities in the relevant aspect have also been suggested.

ARTIFICIAL FEEDS FOR BROODFISH

Development of a captive stock of broodfish is essential for a reliable supply of spawners for artificial propagation. For this, the broodfish should be reared in a stress-free environment with proper supply of nutritionally adequate feeds. Researches conducted during the past two decades, world-wide, have revealed the influence of diets on maturation and spawning, fecundity, viability of gametes, fertilization rate, egg quality, hatchability rate, and survival rate of larvae and fry. However, specific nutritional requirements for gonadal maturation of finfish is largely unknown. In an earlier review, Watanabe (1985) examined the role of nutrition on fish broodstock development and stressed the need for more comprehensive studies to define the specific nutritional needs of broodfish.

Effects of dietary nutrient sources:

The few nutritional studies carried out in carp, rainbow trout, ayu, red sea bream and tilapia indicate that the level or the quality of proteins, lipids, vitamins (C, A, D and E), minerals (phosphorus) and carotenoids, and the amount of food offered affect the performance of broodfish.

A variety of protein sources have been evaluated in the diets of broodfish. Shimma *et al.* (1977) examined the effect of fish meal, methanol-grown single cell protein and ethanol-grown single cell protein for common carp broodstock, but did not observe any significant difference in reproductive performance of the fish between diets due to large individual variations in fecundity and hatching rates of eggs produced, although a relationship was evident between the rate of hatching and fatty acid distribution in the eggs. In red sea bream a diet with cuttle fish meal produced

mainly normal buoyant eggs with a high rate of hatching, leading to high productivity of viable larvae (Watanabe et al., 1984c). In rainbow trout Takeuchi et al. (1981) did not find any significant difference in average weight, fecundity, egg diameter, number of eggs reaching the eyed stage and hatchability of eggs between a high protein (crude protein 43-47%) commercial diet and a low protein/high energy (crude protein 33-35%, 390 Kcal./100 g) diet. In Leptobarbus hoevenii diets with high protein levels (32 and 40% crude protein) produced better gonado-somatic index values and fecundity than a low protein (24% crude protein) diet (Pathmasothy, 1986). In another study (Watanabe et al., 1984b) broodstock of rainbow trout given a diet containing 36% crude protein and 18% lipid performed as well as those given a diet with 46% crude protein and 15% lipid suggesting that a diet containing a lower protein content than that normally employed but with a high energy level is as effective for rainbow trout broodstock as the more conventional diets of high protein content.

Dietary lipids and fatty acids, and fatty acids profile of eggs seem to have significant influence on the performance of broodfish. Shimma et al. (1977) who compared the fatty acid distribution in the eggs of ayu broodstock fed diets containing single cell proteins with those from wild ayu found a higher proportion of 18:3^{w3} and 20:5^{w3} in the wild fish eggs and suggested that if eggs from the wild fish are superior to those of cultured fish then lipids rich in these fatty acids should be supplied in broodstock diets. In common carp a relationship was observed between the rate of hatching and the proportion of 22:6^{w3} in eggs. Hatchability was low in eggs containing less than 10% by weight of 22:6^{w3} and the estimated requirement for 22:6:3 was about 20% by weight (Shimma et al., 1977). These results suggest that the fatty acid distribution in egg lipids, especially the highly unsaturated fatty acids (HUFA) such

as 20:5w3 and 22:6w3 may possibly be used as one criterion of egg quality.

Diets deficient in essential fatty acid(18:3w3) when fed to rainbow trout broodstock resulted in low total egg production, percentage of fertilized-eggs produced and total hatch(Watanabe et al., 1984b). Interestingly, addition of linoleic acid (18:2w6) to the essential fatty acid (EFA) deficient diet led to a marked improvement in percentage fertilization and hatch compared to the diets deficient in essential fatty acid. Besides, there was no accumulation of the abnormal polyenoic acid, 20:3w9, in the eggs produced. Linoleic acid is known to be inferior to linolenic acid(18:3w3) as an EFA for rainbow trout fingerlings. However, the fatty acid distribution in the sperm was found to be affected by feeding the EFA deficient diet, with high level of 20:3w9 in sperm, quite different from that of eggs. Watanabe(1985) suggests that there is a small, but absolute requirement for w6 fatty acids in rainbow trout broodstock. Conversely, in red sea bream a diet with corn oil, rich in 18:2w6 as a fatty acid source produced the lowest number of buoyant eggs(Watanabe et al., 1984a).

The importance of dietary carotenoids on the performance of salmonid and red sea bream broodstock has been reported (Deufel, 1965; Watanabe et al., 1984b). Deufel (1965) reported that dietary canthaxanthin improved egg production and rate of fertilization in rainbow trout. Salmonids are known to mobilise their carotenoid pigments, astaxanthin and canthaxanthin from flesh and deposit them in eggs and skin during maturation. Feeding a red sea bream broodstock with Krill, mysids, shrimp and crab wastes resulted in pigmentation of eggs within a matter of hours (Watanabe, 1985). Supplementation of diets with one or the other of carotenoid pigments (β -carotene 0.1%; canthaxanthin 0.3%) and Krill oil extract (9%) containing astaxanthin mono

and diesters and fed to red sea bream broodstock shortly before spawning resulted in increased percentage of buoyant eggs and normal larvae (Watanabe et al., 1984d). Egg astaxanthin in Salmo gairdneri is known to enhance chemotaxis of spermatozoa and carotenoids may have a role in cellular respiration under severe oxygen conditions (Tacon, 1981). Watanabe (1985) assigns an antioxidant function to carotenoids in fish eggs rather than their being an absolute need for the pigment itself, since no carotenoids were detected in the high quality eggs of red sea bream broodstock fed on cuttlefish meal diet (Watanabe et al., 1985).

A possible role of ascorbic acid in reproduction has been suggested by Lutwak-Mann (1958), who revealed that the ascorbic acid content of mammalian ovaries varied with the different stages of ovarian cycle. This observation is confirmed by the studies of Seymour (1981) in crucian carp (Carassius carassius) and Sandnes and Braekkan (1981) in cod (Gadus morhua). Besides, chemical analyses have consistently revealed high concentrations of ascorbic acid in fish roe (Ikeda et al., 1963 ; Hilton et al., 1979). Sandnes et al., (1984) established the essentiality of dietary ascorbic acid in the reproduction of rainbow trout (Salmo gairdneri) and suggested that the dietary level of ascorbic acid should be sufficient to give 20 mg total ascorbate/g wet weight of eggs for normal fry development. Soliman et al. (1986) showed that ascorbic acid supplementation of tilapia broodstock diet (125 mg ascorbic acid per 100 g dry diet) improved hatchability of eggs and fry condition. The above authors suggest that ascorbic acid supplementation of broodstock diets results in some transfer of the vitamin via the eggs to newly hatched fry resulting in some amelioration of ascorbic acid deficiency during the early stages of life. Most recently Waagb et al. (1989) reported the role of ascorbic acid in vitellogenesis

in rainbow trout. Adult rainbow trout fed a diet devoid of ascorbic acid for 21 months, including the stages of gonadal development, has lower serum levels of estradiol-17 β and vitellogenin and became anaemic at the end of feeding.

The importance of vitamin E in broodstock diets has been established for ayu (Takeuchi et al., 1981), carp (Watanabe and Takashima, 1977), rainbow trout (Kinumaki et al., 1972) and red sea bream (Watanabe et al., 1984d). In the carp, Cyprinus carpio deficiency of α -tocopherol in the diet resulted in lower gonad weight, gonado-somatic index, retarded oocyte development, muscular dystrophy characterised by a marked loss of flesh from the back, higher moisture levels, and lower levels of protein, lipids and phospholipids in the gonads than the control fish (Watanabe and Takashima, 1977). In rainbow trout vitamins A, D and E are transferred from broodfish to the eggs as well as to fry (Kinamuki et al., 1972).

The mineral requirements of broodfish are not clearly established, but for phosphorus. Feeding a diet without supplemental phosphorus resulted in lowest growth of ayu broodstock with the lowest egg production. In red sea bream a diet low in phosphorus produced large number of abnormal eggs with 2 oil globules as against one oil globule in the normal eggs (Watanabe et al., 1984a). In rainbow trout manganese concentration in the eggs was affected by trace element deficiency (Watanabe, 1985).

Effects of food levels on maturation and fecundity:

Diet restriction on a long-term basis has been shown to cause a reduction in number of fish maturing and in number of eggs produced in rainbow trout (Scott, 1962) and reduction in fecundity and delayed maturation in brown trout (Bagenal, 1969). In laboratory maintained haddock egg production and

feed levels were positively correlated (Hilsop et al., 1978). In stickleback high food levels increased both the percentage of fish maturing and their spawning frequency, but the food level had no effect on egg size (Wootton, 1977). Poynter (1976) clearly demonstrated that the fecundity of hatchery reared lake-trout is 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 fertilization rate than those fed at a rate of 0.5% of their body weight. Springate et al. (1985) examined the effects of feeding a half-ration (0.35% of body weight) or a full ration (0.7% of body weight) of a dry commercial pellet with 47% protein, 22% carbohydrate, 7% fat, 13% ash, 2% fibre and 9% moisture) to rainbow trout. Fish fed on the full ration produced significantly more and larger eggs per fish than those on half-ration. It has been suggested that food deprivation reduces oocyte numbers by inducing atresia or by modifying the recruitment of oocytes into vitellogenesis (Robb, 1982).

Feed formulations used for broodstock of brackishwater finfish species relevant to India:

A variety of supplemental feeds, relatively high in protein level (about 45% crude protein) and vitamin additives have been prepared and used successfully for Mugil cephalus broodstock (Nash and Shehadeh, 1980). The ingredients composition of two of the formulations successfully used are given in Table 1. The feeds are prepared as follows: milled and sieved (0.5 mm size) ingredients are mixed and stored dry at ambient temperature; freshwater is added just before feeding, sufficient to make the dry feed mix stick together. The feeds are offered at a rate of 1% of body weight per day for fish in outdoor ponds with natural food and 5% of body weight per day for fish in indoor tanks. In the Philippines captive broodstock of the milkfish, Chanos chanos, are initially fed on a commercial

pellet feed (20% protein) at 1.5%-2.0% body weight until they are three years old, after which they are given a commercial pelleted crustacean feed (42% protein) at 2-3% of the body weight and in the fifth year the feeding rate is increased to 5% of body weight (Lopez et al., 1986).

Broodstock of carnivorous species like the sea bass (Lates calcarifer) and grouper (Epinephelus tauvina) are mainly fed on trash fish in Southeast Asian countries. Very recently experimental artificial diets have been developed for evaluating their performance in sea bass and grouper (Meyers, 1987b). The ingredient composition of one of such diets is given in Table 2.

ARTIFICIAL FEEDS FOR LARVAE

Most species of fish pass through a larval stage before assuming the adult form. Sometimes the newly hatched fish is called a 'prolarva' until the yolk is absorbed and then a postlarva or fry. During the prolarval stage yolk provides the essential nutrients for meeting the energy demand. But after yolk absorption (Postlarval stage) the larvae need food to satisfy their nutritional needs. Thus, feeding of the larvae after yolk absorption, with suitable feeds is an important aspect of fish seed production.

At present, selected species of live food organisms are mass cultured and used for feeding the larvae of marine fish. Live food provides the essential nutrients in adequate levels and balanced proportions to the larvae. Besides, the presence of attractants, feeding stimulants and digestive enzymes in the live food and the soft texture are considered advantageous to the larvae which invariably have poorly developed digestive organs and digestive enzymes. But separate facilities, raw materials, energy input and man-power are required for production of live food. Thus the prolonged use of live food can be costly and variations

in quality can adversely affect survival and growth of larvae and fry. So development of weaning diets received priority in several countries, and at present, some species are exclusively grown on artificial diets.

Unlike natural foods, artificial diets are not subject to seasonal variations in supply or nutritional composition and can be thoroughly quality-controlled during fabrication (Meyers, 1979). The feedstuffs they are made from can be easily stored and the ingredient composition can be adjusted to the requirements of the larvae and fry; However, there are certain inherent problems in the development of complete artificial feeds, as live food replacements, for the first feeding stages of marine fish larvae, as most of the larvae after hatching are very small in size (2.5 mm to 4.5 mm).

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

1. Minced diets: A feed paste is prepared by homogenising wet or wet and dry ingredients with addition of mineral and vitamin premix and binders and as such fed.
2. Wet microparticulate diets: Preparing a custard diet with chicken eggs, clams, fish solubles, vitamins, minerals, feeding stimulants and flour and homogenising to get fine particles. The desired particle size can be obtained by sieving.
3. Dry microparticulate diets: Preparation of a water-stable matrix of dry ingredients or a mixture of dry, moist and wet ingredients followed by suitable drying (freeze-drying, vacuum drying, oven-drying), grinding and sieving to get desired particles. This is the most widely used type of artificial diet for larval rearing.

4. Spray-dried diets: Well mixed, finely ground materials are sprayed into hot air and then dried. Particles ranging in size 50-100 microns are produced and used for rearing marine fish larvae in Japan (Kuronuma and Fukusho, 1984). Ingredient composition of a spray-dried larval feed is given in Table 3.
5. Microbound diets: Powdered diets with a binder. Carrageenan, agar, zein, alginic acid and gelatin microbound diets have been proposed (Kanazawa, 1986). An outline of the procedure for microbound diet preparation is given in Fig.1.
6. Microcoated diets: Prepared by coating microbound diets with some materials such as zein and cholesterol-lecithin.
7. Microencapsulated diets: The concept of a miniature packaging assemblage (a microcapsule) in which liquids or particulate dietary components are enclosed in a carefully engineered wall, with release under specific macro or microenvironmental conditions has broad applications in fish culture (Meyers, et al., 1975). Release of the internal nutrient components at active sites can be accomplished by rupture (enzymatic action, pH change or bacterial action) of the capsule wall. A major advantage of capsulated diets is that there is minimal loss of nutrients within the aqueous environment, thus minimizing organic load in the system and alterations in oxygen and pH levels. The capsules can be produced in a range of sizes (5 mm to 700 mm capsules are produced by Frippak) thereby, suitable sizes can be offered to the fish larvae and fry as they grow. Depending upon the capsule wall material Kanazawa (1986) proposed the following categories: Nylon-protein microencapsulated diet (MED), Gelatin-gum acacia MED, egg albumin MED, Glycopeptide MED, Chitosan MED. An outline of the procedure for nylon-protein MED preparation is shown in Fig.2.

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

Considerations in artificial larval food 'design' include those of colour, size, attractability, rehydration characteristics and degree of buoyancy in the aqueous systems (Meyers, 1979). Also, the feed manufacturing process should not adversely affect the nutritional integrity of the final product. Excessive and prolonged heating, inclusion of chemicals that may denature dietary components or reduce the biological value of heat-labile components may pose real problems in the fabrication operation.

Artificial diets used for mullet larvae:

Several artificial diets, compounded from natural or synthetic materials have been fed to larvae and fry of mullets. Nash and Shehadeh (1980) suggest use of artificial preparations for Mugil cephalus after 35-40 days of hatching. Artificial diets, composition given in Table 1, have been successfully used after milling and sieving to obtain adequate particle sizes of about 100 microns for the post-larvae of mullets. Other prepared diets that have been tested for the larvae of mullets at one time or other include codliver oil, powdered oil cakes, bean cream, fish ovaries, egg albumin, boiled egg yolk, liver juice and enzymes, amino acids, brewers yeast, fish meal, urea, rice bran and flour, milk powder, powdered oats, and commercially available tropical feeds (Nash and Shehadeh, 1980).

Artificial diets used for milkfish larvae:

A fish meal based diet containing 30% protein produced better growth than lower protein levels and corn-gluten meal was found to be a poor protein source (Seneriches and Chiu, 1988) for milkfish fry. In another study, Alava and Lim (1988) successfully used several artificial diets containing 40.8% mean crude protein for milkfish fry (100 mg body weight and 10 mm total length) collected from the nursery grounds. Composition of the diet that gave the best response is give in Table 4. The diets were prepared by mixing presieved dry ingredients (250 microns particles), oil and gelatinised wheat flour (binder) and extruding the dough through a 2 mm hole pelleting machine, dried in an oven at 60°C until the moisture was 10% or less, ground and sieved through 60 mesh sieve (250 microns) and 40 mesh sieve (425 microns). The 250 microns feed was fed for the first 14 days followed by the 425 microns feed for the next 14 days of rearing. Survival rates ranging from 92-98% have been obtained with this feed. Santiago et al. (1988) obtained the best growth and survival of milkfish fry with a formulated diet containing 41.5% crude protein and 11.9% crude fat (Ingredients composition: fish meal 56.6%, soyabean meal 11.4%, shrimp meal 9.0%, rice bran 12.7%, codliver oil 2.7%, corn oil 2.5%, starch 1%, vitamin mix 0.7% and mineral mix 3.6%) as compared to a diet of rice bran, and a diet with Spirulina powder and formulated feed in combination. Larvae fed to satiation metamorphosed earlier than those subjected to a restricted feeding regime.

Weaning diets for sea bass (*Lates calcarifer*) larvae:

In Thailand, sea bass fry (10 mm total length and 150 mg weight) have been successfully reared on small clean particles of fish meat passed through a 3-4 mm mesh screen. Egg yolk and yolk mixed with instant milk were found to be poor quality feeds (Manee Wong, 1986). In Queensland, sea bass was weaned on a commercial salmon starter feed fine

granules containing 52% protein and 16% fat, from the size of 13-15 mm (Mackinnon, 1986). Tubongbanu (1986) evaluated the efficacy of formulated diets containing 40% protein and 3700 ME to sea bass fry (Weight 0.18-0.57 g and total length 2.3-3.7 cm). Moist feeds were prepared using trash fish, soyabean meal, copra meal, wheat flour, rice bran, salt and vitamin premix as ingredients, preserved by lactic acid fermentation and fed to the fish in a dough form. The ingredients composition of a processed moist diet for fry and fingerlings of sea bass and grouper (ADCP, 1983) is given in Table 5.

Weaning diets tried with other species:

Various types of weaning foods tried for turbot, sole and cod larvae include fish powder, mussel powder, starter feeds, nylon-protein coated capsules (Bromely and Sykes, 1985). Of these, microcapsules and fish powder gave poor results; the microcapsules appeared to pass through the gut without the coating being ruptured. Kanazawa et al. (1984) reported on the mass production of ayu and carp seed with a microbound diet containing lecithin (Table 6). Microcapsules are not digested by the newly hatched larvae of ayu and red sea bream until about 11 days after hatching (Kanazawa et al., 1982).

IMPORTANCE OF FEEDING-STIMULANTS IN ARTIFICIAL FEEDS

Inclusion of a feeding-stimulant to artificial feeds of fish particularly for fastidious feeders and for larval and fry stages may improve the palatability of the diets leading to increased intake. Feeding stimulants that have been found effective for a few species are summarised in Table 7. Mackie and Mitchell (1985) termed a mixture of L-amino acids, glycine-betaine and inosine or inosine 5' monophosphate as "universal feeding stimulants" for fish.

During spoilage of fish incosine will get decomposed to the inactive hypoxanthine. If spoiled fish are used for artificial feed development for fish, a feed stimulant supplement (either synthetic or natural product) should invariably be included for improving its palatability. Among natural ingredients squid and shrimp extracts, are known to be good sources of feeding stimulants, especially for the carnivorous finfish such as sea bass, eels, and red sea bream. Artemia powder has been found to be of attractant value to european sea bass (Dicentrarchus labrax) and mussel meat for Lates calcarifer.

CONCLUSIONS AND RECOMMENDATIONS

Nutritional studies so far conducted with finfish broodstock indicate that the level and/or the quality of proteins, lipids, certain vitamins, carotencids and minerals in the diets, and the amount of food offered significantly affect the performance of brood fish. However, quantitative requirements of essential nutrients have not been established so far for any of the culturable brackish-water species relevant to India, viz., mullets, milkfish, sea bass (Lates calcarifer), grouper and pearl spot (Etroplus suratensis). In spite of this, a variety of artificial diets have been used for the captive broodstock of most of the said species in other countries. Similar feed formulas could be used for rearing the broodstock of brackishwater species found in India, and feeds can be prepared using indigencus raw materials. The potential nutritive value of a number of raw materials found in India and the antinutritional factors present in them have been discussed by Paulraj (1987a,b). However, to develop nutritionally balanced practical feeds, a great deal of nutritional information is required, and thus broodfish nutrition research should receive top priority.

It is also evident from some of the studies that the level of certain nutrients (essential fatty acids, phospholipids and trace elements) in the eggs may reflect the nutritional status of the female fish as well as its performance. Detailed planned investigations are suggested on the biochemical changes occurring in the gonads, blood, liver and muscle of a stock of wild and captive broodfish by sequential sampling of the tissues to ascertain the rate of synthesis and/or mobilisation of nutrients.

Performance of female fish alone is considered in most of the diet-evaluation studies with broodstock. Performance of the male fish also should be evaluated while examining the effects of diets on broodstock. New biotechnological approaches, especially the genetic improvement programmes, require high quality sperms besides the eggs.

Systematic experimental studies are necessary to define the essential nutrient levels in diets, optimum protein-lipid-carbohydrate ratios, optimum rations, and to identify suitable natural ingredient sources, feeding stimulants, diet from etc for broodfish. A well-designed data collection programme is very essential to evaluate the performance of broodfish.

Suitable artificial diets (complete and supplemental) should be developed for weaning the larvae. Experimental studies should be taken up, as and when larvae become available, to determine desired particle size and form of diet, and also to identify effective natural feeding-stimulants, attractants and binders. Information on digestive system, digestive enzymes, feeding behaviour, feeding frequency, rations required must be obtained.

Microparticulate and microencapsulated diets can be tried for larvae and fry keeping in view their utility and cost.

The economic advantages of live food versus artificial feeds require proper assessment.

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Dr. P.S.B.R. James, Director, Central Marine Fisheries Research Institute, Cochin, for the encouragement given and facilities offered in preparing this paper.

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