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Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

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NUTRITIONAL REQUIREMENTS OF BROODSTOCK, LARVAE AND JUVENILES OF FINFISH

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Introduction

Finfish culture in India is prominent only in the fresh water aquaculture. In the marine and brackishwater sectors only asian seabass (*Lates calcarifer*) has met with success. Farming of this fish on a commercial scale is still not a reality in India. Technological gaps, and low rate of return when compared with shrimp culture. In this backdrop, the purpose of this article is to review the status of knowledge regarding the nutritional requirements of marine finfish broodstock, larvae and juveniles.

Broodstock nutrition

Broodstock nutrition is one of the least researched areas in fish nutrition because massive facilities required for holding fishes and running costs involved in maintaining such facilities have been the main deterrents. Broadly, it is understood that many of the problems encountered in the early life stages of rapidly growing larval and juvenile fish can be related to the feeding regime including the nutrient level and duration of the broodstock.

Food restriction

Spawning success itself is affected by food restriction. Inhibition of gonadal maturation has been reported in several fish species. Delay in spawning time, sub-normality in eggs as well as newly hatched larvae are common indicators of food restriction when compared with fish fed full rations. Reduction in plasma estradiol levels has been reported by Cerdá *et al*., (1994) in European Seabass.

Nutrition and fecundity

Fecundity if the total number of eggs produced by each fish expressed either in terms of eggs/spawn or eggs/ body weight. Reduced fecundity is caused either by the influence of a nutrient imbalance on the brain-pituitary-gonad endocrine system or by the restriction in the availability of a biochemical component for egg formation.

Rabbit Fish

One of the major nutritional factors that has been found to significantly affect reproductive performance in fish is the dietary fatty acid content. In rabbit fish (*Siganus guttatus*) elevation of dietary lipid levels from 12% to 18% resulted in an increase in fecundity and hatching (Durray *et al*., 1994).

Freshwater fish, Catla

In *Catla*, Nandi *et al*., (2001) reported feeding of five isonitrogenous diets (~33% crude protein) to the brood female carp, *Catla catla* (weighing 3.0 to 5.5 kg), for a period of 93 days in order to observe their breeding performance in earthen ponds. Diet-I (control) contained only basic ingredients like rice bran, groundnut oil cake, roasted soybean meal, fish meal and mineral mixture; diet-II contained added vitamins; diet-III contained added vitamins and vegetable oil (rich in n-6 polyunsaturated fatty acids, PUFA); diet-IV contained added vitamins and fish oil (rich in n-3 PUFA); and diet-V contained added vitamins and a mixture of vegetable and fish oils. The results showed that nutritional quality of the diet considerably influenced breeding performance in the species. The total number of matured females was the highest in the diet-V group and maturity was advanced by 35 days in this group compared to the control. In diet-III and diet-V groups, all the maturated females bred fully and the relative fecundity was increased significantly in diet III, IV

and V. The maximum (73.4%) fertilization rate was observed in the diet-V group, followed by 61.3%, 56.8%, 49% and 22.7% in diet-I, diet-IV, diet-III and diet-II groups respectively. Most of the eggs in the diet-II treatment group remained immature. The various data thus obtained suggest that dietary supplementation of both n-3 and n-6 PUFA, is essential to improve gonadal maturation, breeding performance and spawn recovery in the *Catla* female broodstock.

Fatty acids

Polyunsaturated fatty acids (PUFA) and highly unsaturated fatty acids (HUFA) have very vital roles as in any organism due to which they are known as essential fatty acids (EFA). PUFAs can also regulate eicosanoid production, particularly prostaglandins, which are involved in several reproductive processes (Moore, 1995), including production of steroid hormones and gonadal development such as ovulation. Fish ovaries have a high capacity to generate eicosanoids, among them prostaglandin E (PGE) derived from cyclooxygenase action and leukotrienes LTB_4 and LTB_5 derived from lipoxygenase action (Knight *et al*., 1995) suggest that products derived from lipoxygenase action could also be involved in oocyte maturation. Apart from dietary EFA deficiencies causing detrimental effects in fish, their excess have been also reported to have a negative effect on reproductive performance of fish. For example, high levels of dietary *n* -3 HUFA reduced the total amount of eggs produced by gilthead seabream broodstock despite an increase in egg *n* -3 HUFA concentration (Ferna´ndez-Palacios *et al*., 1995). High dietary *n* - 3 HUFA levels could affect the brain–pituitary–gonad endocrine axis since both EPA and DHA have been found to reduce *in vitro* the steroidogenic action of gonadotropin in the ovary of teleost fish (Mercure and Van Der Kraak, 1995)

Other nutrients

Other nutrients proven to have profound effects on broodstock performance are Vitamin E and Vitamin C. Reduced fecundity observed in broodstock fed a diet deficient in á -tocopherol was not associated with reduced vitamin E content of eggs, and only very high dietary vitamin E levels (2020 mg/kg.) were found to increase egg á –tocopherol content. Vitamin C content of rainbow trout eggs reflected the content of this nutrient in the diet and was associated with improved egg quality (Sandnes *et al*., 1984). Changes in the vitamin C content of cod ovaries did not significantly affect hatching rates (Mangor-Jensen *et al*., 1993.) Again these results suggest that the biochemical composition of eggs should not be used as the sole criteria to determine egg quality, despite the fact that several authors (Sandnes *et al*., 1984; Craik, 1985; Harel *et al*., 1994) have suggested that the chemical composition of fish eggs is related to spawning success since nutrients stored in the egg must satisfy nutritional demands for embryonic development and growth.

Broodstock nurtrition and fertilization

Dietary eicosapentaenoic (EPA) and arachidonic acid (AA) levels show a correlation with fertilization rates. Several hypothesis to explain the beneficial effect of EPA and AA on fertilization rates has been proposed by several investigators. Both EPA and AA are involved in cell-mediated functions and are precursors of eicosanoids. EPA is known to be a precursor of prostaglandins (PG) from series III, whereas AA is a precursor of PG from series (Stacey and Goetz, 1982). In vitro AA, but not EPA or DHA, stimulates testicular testosterone in goldfish testis through its conversion to prostaglandin PGE_2 (Wade et al., 1994). On the contrary, EPA or DHA blocked the steroidogenic action of both arachidonic acid and PGE. Both AA and EPA modulate steroidogenesis in the goldfish testis (Wade et al., 1994). Thus, the timing of spermiation may be delayed and subsequently fertilization rates reduced by depressed steroidogenesis caused by a broodstock EFA deficiency or imbalance.

Broodstock nutrition and embryonic development

n – 3 HUFA levels are very important in the normal embryonic development of fish. HUFA as components of phospholipids are very important components of biomembranes. Fatty acids in general are the major source of energy in embryonic development. EFA requirement in broodstock diets are in the range of 1.5 – 2.0 %. Role of carotenoids in broodsotck nutrition is a controversial area in fish with groups in favour and against its inclusion in broodstock diets. Astaxanthin has a positive correlation where as â-carotene is reported to be ineffective. Similarly dietary phospholipid, in general, is reported to improve egg quality.

Valuable feed ingredients in broodstock nutrition

Cuttle fish, squid and krill are considered to be the most valuable ingredients in broodsotck nutrition. The fat insoluble portion of cuttle fish and squid contain proteins which enhance egg production by 40% kg^{-1} female fish. Soybean meal inclusion in broodstock feeds has a detrimental effect mainly because of the imbalance in the fatty acid composition. Raw krill enhances feed intake and contains unidentified spawn quality enhancement factors. As a safe measure in broodstock feeding it is advisable to incorporate *n*-3 HUFA up to 2% and 250 mg kg^{-1} vitamin E.

Larval nutrition

Marine fish can neither biosynthesize 22:6(n-3) *de novo* nor from shorter chain precursors such as 18:3(n-3), therefore 22:6(n-3) and 20:5(n-3) are essential dietary constituents for marine fish. In fish 22:6(n-3) is present in very high concentrations in neural and visual cell membranes and synaptosomal membranes, as in the case of mammals. An insufficiency of 22:6(n-3) in marine larval fish diet is likely to impair neural and visual development with significant if not serious consequences for a whole range of physiological and behavioural processes including those dependent on neuroendocrines. Abnormal pigmentation in cultured marine flatfishes is related to HUFA deficiencies.

Detailed studies examining the appropriate ratios of fatty acids, mainly 22:6(n-3), 20:5(n-3) and 20:4(n-6) have revealed that given a sufficiency of 22:6(n-3), excess of 20:5(n-3) is not deleterious, where as 20:4(n-6) is, because of a generalized biochemically-induced stress in the fish through excess eicosanoid production. In commercially available fish oils, 20:4(n-6) are found to be consistently at low levels (< 1% of the total fatty acids). Neither is an excess of dietary 22:5(n-6) a practical problem. But the major limiting fatty acid in commercial fish oils would be 22:6(n-3). The availability of the fatty acid through enriched *artemia* is also problematic because brine shrimp nauplii retro-converts 22:6(n-6) to 20:5(n-3). Thus, oils particularly rich in 22:6(n-3) are essential for the supplementation process and other than commercial (n-3) HUFA concentrates Tuna orbital oil (TOO) is the only identified natural oil, which has the levels and ratios of 22:6(n-3), 22:5(n-3) and 20:4(n-3) that lead to satisfactory, though not optimal survival growth and metamorphosis of turbot larvae was noticed which may not be applicable to all marine fishes. There is evidence that seabass larvae require more of 20:4(n-6) and TOO with ca. 2% proved to be satisfactory. Commercial fish oils with less than 1% arachidonic acid has to be blended with oils rich in arachidonate to achieve this objective.

Presentation

Nearly all mariculture production systems rely heavily on live feeds viz., rotifers, *atremia* nauplii and copepods of *Tisbe, Acartia, Eurytemora. Artemia* and *Brachionus plicatilis* are naturally deficient in 20:5(n-3) and no known strains of *artemia* contain significant levels of 22:6(n-3) making (n-3) HUFA enrichment necessary. Procedures for enrichment with emulsions of marine fish oils are well developed. Commercial products are readily available to achieve this objective. However, up gradation of current procedures in the light of recent knowledge of PUFA requirements is essential. Current problems in enrichment of live feed are – 1. 22:6(n-3) content is very small in triacylglycerol micelles generated in enrichment procedures and are prone to autooxidation, especially under vigorous aeration. 2. Natural antioxidants such as á-tocopheryl acetate and ascorbyl palmitate are not effective especially until hydrolysed in the intestinal tract and absorbed. Ethoxyquin and Butylated hydroxy anisole minimizes peroxidation. However, the level of these in enrichment emulsions is an area where there is no information.

Lecithin can be used to considerable advantage in enriching the nauplii with 22:6(n-3) rich fish oils, because lecithin acts as a natural emulsifying agent and a natural protectant against autooxidation. Thus the ideal enrichment mixture tested is a combination of 90% 22:6(n-3) rich fish oil + 10% lecithin from fish eggs. Lecithin derived from fish eggs is superior to soy lecithin because fish egg lecithin contains readily assimilable 22:6(n-3) and 20:5(n-3) in the ratio of 2:1. Soy lecithin contains only 18:2(n-6) linoleic acid. Commercial availability of (n-3) PUFA rich phospholipids is limited. This limitation has to be overcome by exploring fishery products other than fish roe and milt.

Alternatives to fish oil fractions rich in 22:6(n-3) are (1) a heterotrophic dinoflagellate *Crytothecodenium cohnii* which is mass produced commercially to produce triacyl glycerol rich in 22:6(n-3) - commercial product by MARTEK®;

frozen thawed cells are used to supplement *artemia*. (3) Spray dried *Schizochytrium* spp. rich in PUFA is a single celled heterotrophic marine protist of the group labyrinthulomycota - commercial product KELCO®. (4) Copepods cultures have to be developed because they have a preponderance of phospholipids rather than triacylglycerols in their body. Levels and ratios of 22:6(n-3): 20:5(n-3): 20:4(n-6) more closely resemble larval natural diets and the probability of natural protection of PUFA by natural antioxidants and delivery to larvae is always advantageous. Copepods enriched with freeze thawed cells of *C. cohni* or *Schizotricodinium* spp. is another possibility ensuring the appropriate HUFA ratio delivery to larval marine fishes, which is not popular.

Sources

Traditional commercial fish oils especially byproducts of industrial pelagic fisheries are the richest sources of fats and fatty acids. Basically fish oils are rich in 20:5(n-3) and the ratio of 22:6(n-3):20:5(n-3) is found to be < 1:2. MAXEPA™ type oils available commercially contain 12% 22:6(n-3) and 18% 20:5(n-3) which are sourced from southern hemisphere low latitude fisheries, mainly, plichards, anchovies, sardines and menhaden. Northern hemisphere high altitude fisheries yield oils with decreased (n-3) PUFA mainly from capelin, sand eels, herring, sprat and mackerel. 20:5(n-3):22:6(n-3) ratios do not differ from the former with an increased % of 20:1 (n-9) and 22:1(n-11) serving as metabolic source of energy. Cod liver oil has a higher PUFA (n-3) content and a lower% of 20:1(n-9) and 22:1(n-11). Commercially fish oils are enriched with 22:6(n-3) and 20:5(n-3) by fractional distillation, solvent extraction or by urea adduction or by a combination of all these methods. (n-3) PUFA's are available as ethyl ester, free fatty acids and rarely as triacylglycerols among which ethyl esters are already used to enrich *artemia*. Commercial fish oils can meet enrichment requirements because saturated and monounsaturated fatty acids in fish oils are as important as energy yielding molecules and (n-3) PUFA are useful for structural purposes. Eventhough (n-3) PUFA can be catabolized for energy, they are more difficult to catabolize than saturated or monounsaturated fatty acids. Thus over enrichment with PUFA could conceivably result in insufficient energy content in the diet. The only 22:6(n-3) rich natural fish oil known so far is tuna orbital oil (TOO), which contains 30% 22:6(n-3), 7% 20:5(n-3) and 2% 20:4(n-6). It has been proven that blending of 90% TOO with 10% lecithin from fish roe produces the most ideal enrichment emulsion known to date. However, maintenance of the levels of DHA: EPA: AA in *artemia* till the larval fish feeds on it has not been successful because all these fatty acids especially DHA is metabolized by *artemia* after bioencapsulation leading to lowering of its content in the enriched organism. Surprisingly, a strain of *artemia* from China designated as *Artemia sinica* is found to retain the levels of DHA up to 24 h post-enrichment. The future direction of PUFA nutrition in mariculture is to blend the range of products available to us to achieve either economical larval survival or brood stock maturation and spawning. The clues have naturally come from the nutrient profiles of mature fish eggs.

To conclude, the nutrient requirements of popularly cultured finfish in Asia is appended below.

Nutrient requirements of common fish cultured in Asia

Summary of nutrient requirement of seabass

Nutrient	Requirement	References
Protein (% diet)	40-50	Cuzon, 1988
DE/P (kcalg^{-1})	6.8-7.33	Tucker *et al.,* 1988 Sakaras *et al.,* 1988
Lipid (% diet)	13-16	Cuzon et al., 1990, Tucker *et al.,* 1988
n-3 HUFA (% diet)	l-l.7	Buranapanidgit *et al.,* 1989
Pyridoxine (mg kg- ' diet)	5[a]-10 [b]	Wanakowat *et al.,* 1989
Pantothenic acid (mg kg^{-1} diet)	15[a]-90 [b]	Boonyaratpalin *et al.,* 1993b
Ascorbic acid (mg kg^{-1})	700 [c]-13 [d]	Boonyaratpalin *et al.,* 1989b, 1992
Phosphorus (% diet)	0.65	Boonyaratpalin and Phongmaneerat, 1990

[a] For growth, [b] For maximum tissue storage, [c]When crystalline vitamin C is used.

[d] When L-ascorbyl-2-monophosphate-Mg or ascorbic acid glucose is used.

Growth, feed conversion and survival of seabass fed trash fish with and without vitamins supplementation (Phromkhuntong *et al.*, 1987)

Feed	Weight		Feed conversion	Survival
	Initial	Final		
Trash fish	1.48	9.36	7.44	95.00
Trash Fish + vitamin mix	1.34	23.48	3.82	100.00
+ folic-free vitamin mix	1.26	20.49	3.91	99.50
+ niacin-free vitamin mix	1.25	20.39	4.02	99.50
+ vitamin C-free vitamin mix[a]	1.37	16.5	4.81	99.50

Example of practical diet for seabass (Boonyaratpalin, 1988)

Ingredient	Percentage
Fish meal	70
Rice bran	12.40
Vitamins	1
Minerals	2
Gelatinized starch	10
Ascorbic acid	0.10
Marine fish oil	1.50
Vegetable oil	3
Water	40-50

Suggested feeding rates for small seabass (Boonyaratpalin, 1988)

Fish size (g)	Feeding frequency (times per day)	Feeding rate (% body weight per day)
1.8-5.4	2-3	7.18
5.5-11.5	2-3	5.70
11.6-19.2	2	4.59
19.3-27.9	2	3.90
28.0-45.0	2	3.50
> 74	1	

Dietary protein requirements of milkfish

Fish size (8)	Protein requirement (% of diet)	References
0.01-0.035	52-60	Camacho and Bien (1983)
0.04	40	Lim et al. (1979)
0.5-0.8	30-40	Pascual(1984)
2.8	42.8	Coloso et al. (1988)

Model formula of a practical pond (26% protein) feed for milkfish (Lim, 1991)

Ingredient	% in diet
Fish meal, anchovy or menhaden	8.0
Soybean meal 48% protein	31.5

Grains or grain by product	56.0
Pellet binder [a]	2.0
Dicalcium phosphate	1.5
Vitamin premix [b]	0.5
Mineral premix [c]	0.5

[a] Pellet binder may be hemicellulose or lignin sulfonate.

[b] Vitamin mix for supplemental diets for warm-water fish.

[c]Mineral mix for practical diets for warm-water fish.

Composition of a formulated diet for grouper and seabass (Kanazawa, 1984)

Ingredient	Percentage
Fish meal	34
Meat and bone meal	10
Soybean meal	15
Sesame cake meal, expellar	5
Groundnut meal, expellar	5
Ricebran, solvent extracted	10
Leaf meal	5
Tapioca	8
Vitamin and mineral mixture	1
Soybean or corn oil	4
Squid or pollack liver oil	3
BHT	0.02
Ethoxquin	0.015

Composition of formulated test diets for grouper (Tacon and Rausin, 1989)

Ingredient Formulation	1	2
Brown fish-meal	75	66
Shrimp head meal	-	5
Squid liver powder	-	5
Suehiro UCF	-	5
Wheat middlings	8.6	3
Wheat flour	10	10
Zeolite	5	0.75
Fish oil	4.2	3.8
Soy lecithin	0.75	0.75
Choline chloride (50%)	0.40	0.40
Vitamin premix AGJT/Fl [a]	0.33	0.33
Mineral premix AGJT/Fl [b]	0.037	0.037
Total	100.067	100.067

[a] Vitamin premix AGJT/Fl supplies per kilogram of dry diet: vitamin A, 4000 IU; vitamin D,, 2000 IU; vitamin E, 200mg; vitamin K,, 8 mg; thiamin, 32 mg; riboflavin, 40mg; pyridoxine, 32 mg; pantothenic acid, 120mg; nicotinic acid, 160mg; biotin, 0.4mg; folic acid, 8mg; vitamin B,,, 0.04 mg; inositol, 300 mg; vitamin C, 8OOmg.

[b] Mineral premix AGJT/Fl supplies per kilogram of dry diet: iron, 30mg; zinc, 50mg; manganese, 25mg; copper, 3 mg; cobalt, 0.5 mg; iodine, 3 mg; trivalent chromium, 0.25 mg; selenium, O.lOmg.

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