Nutritional requirements of Asian seabass, *Lates calcarifer*

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Introduction

Asian seabass (*Lates calcarifer*) has emerged as an important candidate finfish species for aquaculture in many parts of the world. Availability of seed and appropriate feed are the two important prerequisites for development and propagation of aquaculture of any fish species. After considerable efforts and extensive research, the Central Institute of Brackishwater Aquaculture (CIBA) has succeeded in developing captive brood stock and seed production technology for Asian seabass. Research efforts on nutritional requirements and development of suitable formulated feeds have been in progress simultaneously at CIBA. The nutritional requirements of fish vary with different growth stages and depend upon the feeding habits that change according to the morphology of digesting system. Considerable effort has been made in Australia, Thailand, Philippines and more recently Israel, in defining the nutritional requirements of this species in order to improve production. Understanding the nutritional requirements of the candidate species is the first and essential pre requisite for development of cost effective, efficient and eco friendly feeds.

Nutritional requirements

Investigations on Asian seabass (also known as Bhetki in Bengal, Koduva in Tamil, Kalanchi/ Narimeen in Malayalam) have been mainly concentrated on energy nutrient requirement in the diet. Recently information on micro-nutrient needs such as vitamins has started coming in. The nutrition research undertaken falls clearly into two streams *viz.*, requirements during hatchery and nursery phase and requirement in grow out period.

Requirements during hatchery and nursery phase

The nutritional requirements of larvae that have a body mass less than few milligrams are not very much understood. Based on the composition of the yolk, live prey and larvae themselves it is assumed that the nutritional requirements of larvae were higher than those of the juveniles. The nutritional requirement is not similar for larvae and juveniles. Indeed, a dietary formulation sustaining good growth in juveniles induces poor results in larval growth and survival.

Most of the works conducted on nutritional requirements in fish have focused on lipid requirements. Until a few years ago as there was no compound diets available for larvae, studies on nutritional requirements are limited in general. However, studies on lipid requirements were easier to conduct because total lipid content or fatty acid profile can be modified in live prey, while it is quite impossible to change the amino acid profile of an organism. Nevertheless, growth is essentially protein deposition, and adequate proteins must be supplied to sustain optimal growth.

Lipid requirements

Lipid sources and total lipid

Eggs of marine fish exhibit high lipid content around 20% and reported that fertilized eggs of seabass contain about 27% lipid on DM basis (Syamadayal *et al.*, 2003). Lipids included in microparticulate diets come partly from fish meal or other meals incorporated as protein source and are generally derived from marine sources. Cod liver oil, roe oil, sardine oil or menhaden oil are added as triglycerides in larval diets. In seabass larvae, growth and survival were directly related to the lipid content of the diet. Best results were obtained with the diet containing 30% lipid, as a mixture of cod liver oil and soy bean lecithin (Zambonino Infante and Cahu, 1999).

Phospholipid

Beneficial effects of phospholipid (PL) incorporation in larval diet was reported as early as in 1981 (Kanazawa *et al.*1981) and in 1993, he has reported that fish larvae were incapable of synthesizing PL at a sufficient rate to meet the requirement during a period of high cell multiplication; hence PL is required in larval diets. Studies have been conducted at CIBA to determine optimal level of phospholipids in seabass larvae using soybean lecithin as phospholipid source. Good growth and survival have been obtained by feeding seabass larvae with a diet containing fish oil and lecithin at 5 and 10% respectively. Sargent *et al.* (1999) are of the opinion that the ideal diet for marine fish larvae would include 10% marine fish phospholipid, since egg or yolk sac larvae exhibit 10% phospholipid concentration.

Essential fatty acid

The n-3 highly unsaturated fatty acids (HUFA) have been identified as essential dietary components for marine fish since a long time as marine fish cannot synthesize them. Special attention was paid to eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic acid (DHA= C22:6n-3), which are found in large amount in fish cell membranes. Experiments conducted using live prey (Watanabe and Kiron, 1994) or a compound diet (Zambonino Infante and Cahu, 1999) have shown that the optimal level of EPA+DHA in diet for marine fish larvae is around 3% of dry matter.

Protein requirements

Protein sources

Person Le Ruyet *et al.* (1989) weaned 23-day-old seabass, *Dicentrarchus labrax*, with a compound diet including squid, shrimp and hen eggs. A mixture of fish meal, shrimp meal, squid meal, lactic yeast was used in a diet given to 25-day-old seabass larvae (Zambonino Infante and Cahu, 1994). Protein sources were selected following their amino acid profile and incorporated in microdiet as the only protein source. Fish meal has been used as the main protein source in diet formulated for seabass (Zambonino Infante *et al.*, 1997) up to a level of 65% in the diet used for feeding 20 day post hatch (dph) larvae. The first attempt to determine optimal dietary protein level for seabass at very young stages was conducted by feeding larvae from Day 15 to Day 35 with isoenergetic compound diets incorporating a gradient in protein level (fish meal plus casein hydrolysate @ 30-60%). The best growth was obtained with 50% protein.

Amino acid requirements

No information is available on the amino acids requirement for marine fish larvae and their optimal level in a diet. However, the profiles of essential amino acids of fish body tissue are generally considered as a good indicator of their amino acid requirements.

Molecular form of the protein fraction

The role of free amino acids and short peptides in diet on larval development has been investigated by several authors. As early as 1989, Fyhn (1989) suggested that free amino acids constitute a substrate for energy production in marine fish larvae during early larval stages and the larvae during young stages need an exogenous supply of free amino acids. Watanabe and Kiron (1994) considered that it is not clear if fish larvae have a sufficient ability to digest food protein or whether free amino acid must be provided by diet. In the same way, the incorporation of 10% essential amino acid mixture in fish meal based diet failed to improve growth and survival in seabass larvae compared with larvae fed diet with the same nitrogenous level brought as whole protein (Cahu and Zambonino Infante, 1995). Nevertheless, the dietary incorporation of free amino acids induced an increase in trypsin secretion in early larvae stages suggesting that pancreatic digestion would be improved. Beside their nutritional function, free amino acids play a very important role in first feeding by acting as chemo-attractant. Protein hydrolysate has been since a long time considered as an advantageous protein form for fish larvae and the product was incorporated in most of the larval diets at least for improving microparticle physical properties. Recent experiments have shown evidence of the high nutritional value of protein hydrolysate and its role in larval nutrition. Zambonino Infante *et al.* (1997) showed that a 20% replacement of fish meal by di and tripeptides (obtained from fish meal hydrolysate) in diet resulted in improvement of the main biological parameters in seabass larval rearing: growth, survival and skeletal formation. Incorporating di- and tri-peptides to the diet led to a growth improvement when an amino acid mixture failed to induce the same effect. Hydrolysates are beneficial to larvae, while they do not affect, or in some cases, depress juvenile growth. These results suggest that fish larvae have specific nutritional requirements which can be understood by the analysis of larval digestion.

Nutritional factors affecting larval morphogenesis

Protein hydrolysate enhances larval morphogenesis. The molecular form of the dietary protein supply, native proteins or hydrolyzed into oligopeptides (around 20amino acids), has probably an indirect effect on morphogenesis. Dietary lipids play an essential role in larval growth and survival. Growth and normal morphogenesis increased as the dietary inclusion of phospholipids and vitamins, particularly vitamin A.

Requirements during grow - out phase

Protein and amino acids constitute the key group of essential nutrients required by Seabass for synthesis of protein and subsequently growth. Several studies have been undertaken to define protein requirements, although limited studies have been undertaken to examine specific requirements for key amino acids.

Protein

Most of the studies undertaken to examine the requirements for protein in barramundi diets suggest a relatively high protein requirement, consistent with the

Table 1. Summary of protein requirement estimates for barramundi

Crude Protein levels examined (% to %)	Optimal Level (%) (MJ/kg)	Gross Energy level at Optima	Initial Fish Size (g)	Temp (C)	Authors
35 - 55	45 - 55	13.4 – 16.4	n/d	n/d	Cuzon, 1988
45 - 55	50	n/d	7.5	n/d	Sakaras *et al.* 1988
45 - 55	45	n/d	n/d	n/d	Sakaras *et al.* 1989
n/d	40-45	n/d	n/d	n/d	Wong and Chou, 1989
35 - 50	50	50	1.3	29	Catacutan and Coloso, 1995
29 - 55	46 - 55	18.4 – 18.7	76	28	Williams and Barlow, 1999
38 - 52	52	17.8 – 21.0	230	28	Williams *et al.* 2003
44 - 65	60	20.9 – 22.8	80	28	Williams *et al.* 2003

carnivorous/ piscivorous nature of the fish. Seabass being highly carnivorous showed a dietary requirement of 45 – 55% protein. Subsequently Catacutan and Coloso (1995) suggested 42.5% in the diet of the fish. Experiments conducted in CIBA with different level protein feeds on the young-ones of seabass showed a protein requirement of 43 % for this fish. The summary of protein requirement as reported in the literature is given in Table-1.The protein quality in the feed influences the requirement.

The diet energy density and the size of fish used, appear to be the key factors influencing the specific amount of protein required for seabass.

Amino acids

Most of the finfish including seabass show the requirement of the same ten amino acids (arginine, histidine, isoleucine, leucine, lysine, methionin, phenylalanine, threonine tryptophan, tyrosine or valine) as essential. However, determination of quantitative essential amino acid requirement would help in assessing the protein requirement more accurately. There have been several estimates of some specific amino acid requirements for barramundi. Coloso *et al.* (1993) estimated the requirement for tryptophan to be about 0.5% of dietary protein. The requirements for methionine, lysine and arginine have also been determined to be about 2.2%, 4.9% and 3.8% of dietary protein respectively (Millamena *et al.* 1994). It has been reported that excessive dietary tyrosine can cause kidney malfunction in barramundi (Boonyaratpalin, 1997).

A series of experiments by Australian researchers examined the capacity of barramundi to utilise crystalline and protein-bound amino acids. One study, based on the addition of crystalline lysine to a wheat gluten based, high-protein diet, compared its utilisation to complementary diets modified to have an equivalent level of lysine enrichment, but with protein-bound amino acids. A second study examined the similar addition of crystalline amino acids into a lower protein diet. Both studies showed that the utilisation of the crystalline amino acids was as effective as that of protein-bound amino acids, but only at the low inclusion levels in the high-protein diet. Estimations of essential amino acid requirements have also been made based on the composition of the body tissues relative ratios of key amino acids to lysine, usually regarded as the first limiting amino acid in most formulated diets.

Lipid

Lipids comprise an important dietary energy source for seabass and are also a source of essential fatty acids. Much work has been devoted to exploring the inclusion of lipids in barramundi diets to increase their energy density. At protein levels of 45% to 50% Sakaras *et al.* (1988; 1989) observed best growth from barramundi fed diets with 15% to 18% lipid content. Studies also showed a similar growth from barramundi fed diets with either 9% or 13% lipids, but noted that feed conversion ratio was significantly lower with the higher lipid levels. Studies by Catacutan and Coloso (1995) examined inclusion levels of 5%, 10% and 15% lipids with three protein levels (35%, 42.5% and 50%). Growth rate was highest at the 15% lipid level, provided protein was also at the highest levels (50%). Similar growth was also observed of fish fed diets with 10% lipids and 42.5% protein. Somatic deposition of fat was observed to increase with dietary fat levels. In a study of some extruded commercial diets, Glencross *et al.* (2003) found that two diets of similar protein levels, but differing substantially in lipid levels (16% vs. 22%) sustained equivalent growth of 555 g fish, but that the higher lipid levels resulted in a significantly lower feed conversion ratio. These authors suggested that this was primarily a response by the fish to the energy density of the diets.

Essential fatty acids

Long-chain polyunsaturated fatty acids have been shown to provide some essential fatty acid (EFA) value to barramundi (Boonyaratpalin, 1997). Boonyaratpalin (1997), suggested that n-3 EFA levels (primarily as a mix of 20:5n-3 and 22:6n-3) of 1.0% to 1.7% of the diet were adequate to support growth. Catacutan and Coloso (1995) examined the total lipid levels and observed signs of EFA deficiency (fin erosion) at 5% dietary lipid levels.

Growth was significantly affected by the replacement of fish oil with either canola or linseed oils, but not with soybean oil. This observation may be due to the altered n-3 to n-6 ratios. Soybean oil is about 60% linoleic acid (18:2n-6) and therefore would have substantially altered the ratios of the diets more so than either canola or linseed oils, both of which have substantially higher levels of n-3 fatty acids than soybean oil. An optimal n-3 to n-6 fatty acid ratio of 1.5-1.8:1 reported for seabass with an increase in demand at higher water temperatures. A "shock-like" or "fainting" response was observed in some barramundi from treatments where there were low levels of n-3 EFA.

Carbohydrates

Asian Seabass have no specific requirement for dietary carbohydrates. It can derive dietary energy from some carbohydrate sources. Research findings infer that carbohydrate as gelatinised bread flour had some capacity to provide dietary energy to barramundi. Fish fed diets that were iso-lipidic and iso-proteic with 20% carbohydrate performed better than those with only 15% carbohydrates.

Vitamins

The quantitative requirements of vitamins and their deficiency signs in the fish are presented in Table. 2

Table 2. Summary of vitamin requirements (mg/kg of diet) for barramundi

Vitamin	Requirement (mg/kg diet)	Deficiency Signs
Thiamine	R	Poor growth, High mortality, Stress susceptible
Riboflavin	R	Erratic swimming, Cataracts
Pyridoxine	5 – 10	Erratic swimming, High mortality, Convulsions
Pantothenic acid	15 – 90	High mortality
Nicotinic acid	n/a	Fin hemorrhaging and erosion, Clubbed gills, High mortality
Biotin	n/a	
Inositol	R	Poor growth, Abnormal bone formation
Choline	n/a	
Folic acid	n/a	
Ascorbic acid (Vitamin C)	25 – 30a (700b)	Gill hemorrhages, Exophthalmia, Scoliosis, Lordosis, Broken back syndrome, Fatty liver, Muscle degeneration, Poor gill development, Bone deformations
Vitamin A	n/a	
Vitamin D	n/a	
Vitamin E	R	Muscular atrophy, Increased disease susceptibility
Vitamin K	n/a	

Summary of nutrient requirements for seabass:

Nutrient	Requirement in diet
Protein	45 – 55%
Lipid	6 - 18%
Fatty acids (n-3 HUFA essential)	1.72%
Carbohydrate	10 – 20%
Protein : Energy ratio	128mg protein/kcal
Vitamin C	700 mg/kg

References

Boonyaratpalin, M. 1997. Nutrient requirements of marine food fish cultured in Southeast Asia. Aquaculture 151, 283-313.

Cahu, C. L., Zambonino Infante, J. L. & Barbosa, V. (2003). Effect of dietary phospholipid level and phospholipid:neutral lipid value on the development of seabass (Dicentrarchus labrax) larvae fed a compound diet. Br. J. Nutr. 90, 21-8.

Cahu, C.L. and Zambonino Infante, J.L. 1995. Maturation of the pancreatic and intestinal digestive functions in seabass (Dicentrarchus labrax): effect of weaning with different protein sources. Fish Physiol. Biochem., 14: 431-437.

Catacuttan,M.R. and Coloso, R.M. 1995. Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian sea bass, *Lates calcarifer*. *Aquaculture* **131**, pp. 125–133

Coloso, R.M., Murillo, D.P., Borlongan, I.G. Catacutan, M.K., 1993. Requirement of juvenile seabass *Lates calcarifer* Bloch, for tryptophan. In: Program and Abstracts of the VI International Symposium on Fish Nutrition and Feeding, 4-7 October 1993, Hobart, Australia.

Fyhn, H.J., 1989. First feeding of marine fish larvae: are free amino acids the source of energy? Aquaculture, 80: 111-120.

Glencross, B.D., Rutherford, N., Hawkins, W.E. 2003. Determining waste excretion parameters from barramundi aquaculture. Fisheries Contract Report Series No. 4. Department of Fisheries, Perth, Western Australia. pp 48.

Kanazawa, A., 1993. Essential phospholipid of fish and crustaceans. In: Kaushik, S.J. and Luquet, P. (Eds), Fish Nutrition in Practice, Edition INRA, Paris, Les Colloques n°61: 519-530.

Kanazawa, A., Teshima, S. Inamori, S., Iwashita, T. and Nagao, A. 1981. Effect of phospholipids on growth, survival rate and incidence of malformation in larval ayu. Mem. Fac. Fish., Kagoshima Univ., 30: 301-309.

Millamena. O.M. 1994. Review of SEAFDEC/AQD fish nutrition and feed development research. In: Feeds for Small-Scale Aquaculture, Proceedings of the National Seminar-Workshop on Fish Nutrition and Feeds (C.B. Santiago, R.M. Coloso, O.M. Millamena, I.G., Borlongan). SEAFDEC Aquaculture Department, Iloilo, Philippines., pp. 52-63.

Phromkunthong, W., Boonyaratpalin, M., Storch, V. 1997. Different concentrations of ascorbyl-2-monophosphate-magnesium as dietary sources of vitamin C for seabass, *Lates calcarifer*. Aquaculture 151, 225-243.

Ronnestad, I., Thorsen, A. and Finn, R.N. 1999. Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture, 177: 201-216.

Sakaras, W., Boonyaratpalin, M., Unpraser, N., Kumpang, P. 1988. Optimum dietary protein energy ratio in seabass feed I. Technical Paper No. 7. Rayong Brackishwater Fisheries Station, Thailand, 20 pp.

Sakaras, W., Boonyaratpalin, M., Unpraser, N., Kumpang, P. 1989. Optimum dietary protein energy ratio in seabass feed II. Technical Paper No. 8. Rayong Brackishwater Fisheries Station, Thailand, 22 pp.

Sargent, J., Bell, J.G., Bell, M.V., Henderson, R. J. and Tocher, D.R. 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. In: Lalhou, B. and Vitiello, P. (Eds), Aquaculture: Fundamental and Applied Research. Coastal and Estuarine Studies, American Geophysical Union, Washington D.C., 43, pp 103-124.

Sargent, J., Mc Evoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J. and Tocher, D. 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture, 179: 217-229.

SyamDayal, J. Ali, S.A., Thirunavukkarasu, A.R., Kailasam, M. and Subburaj, R.2003. Nutrient and amino acid profiles of egg and larvae of Asian seabass,*Lates calcarifer* (Bloch). *Fish Physiol. Biochem.* 29: 141–147

Wanakowat, J., Boonyaratpalin, M., Pimolindja, T, Assavaaree, M. 1989. Vitamin B6 requirement of juvenile seabass *Lates calcarifer*. In: The Current Status of Fish Nutrition in Aquaculture (M. Takeda and T. Watanabe Eds.), Tokyo University of Fisheries, Tokyo, Japan, pp. 141-147.

Watanabe, T. and Kiron, V. 1994. Prospects in larval fish dietetics. Aquaculture 124: 223-251.

Williams, K.C., Barlow, C.G. 1999. Dietary requirement and optimal feeding practices for barramundi (*Lates calcarifer*). Project 92/63, Final Report to Fisheries R&D Corporation, Canberra, Australia. pp 95.

Williams, K.C., Barlow, C.G., Rodgers, L., Hockings, I., Agcopra, C., Ruscoe, I. 2003. Asian seabass *Lates calcarifer* perform well when fed pellet diets high in protein and lipid. *Aquaculture* 225, 191-206.

Zambonino Infante, J.L. and Cahu, C.L. 1994. Influence of diet on pepsin and some pancreatic enzymes in sea bass (*Dicentrarchus labrax*) larvae. Comp. *Biochem. Physiol.*, 109: 209-212.

Zambonino Infante, J.L. and Cahu, C.L. 1999. High dietary lipid levels enhance digestive tract maturation and improve *Dicentrarchus labrax* larval development. *J. Nutr.*, 129: 1195-1200.

Zambonino Infante, J.L., Cahu, C.L. and Péres, A. 1997. Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. *J. Nutr.* 127: 608-614.