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# Review of SEAFDEC/AQD Fish Nutrition and Feed Development Research

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## Abstract

Research on fish nutrition and feed development at SEAFDEC Aquaculture Department has focused on three major areas: nutrient requirements and their interrelationships, digestive enzymes and digestibility, and practical feed development for important species such as milkfish (*Chanos chanos* Forsskal), sea bass (*Lates calcarifer*), Nile tilapia (*Oreochromis niloticus*), bighead carp (*Ari0stichthys nobilis*), and tiger shrimp (*Penaeus monodon*). Early studies on the nutrient requirements were mainly on protein, lipid and carbohydrate. Studies on essential amino acids and fatty acids, and optimum proteln:energy ratio in the diets for cultured species were conducted later. Likewise, requirements for other essential nutrients in shrimps, like phospholipid and cholesterol, were studied. Dietary calcium and phosphorus required to prevent soft-shelled shrimps were determined. Requirements for water-soluble vitamins and bioavailability of stable forms of vitamin C were evaluated. Little is known of the vitamin and mineral requirements.

The major digestive enzymes in milkfish have been studied. The apparent digestibility of common feedstuffs were determined i*n vivo* and *in vitro* for milkfish and tiger shrimp, and presently, for sea bass. Development of cost-effective practical feed continues to be a major research undertaking at SEAFDEC/AQD. Diet refinement emphasizes on use of inexpensive and indigenous materials in diet formulations. The feasibility of using legumes, leaf meals, and agricultural by-products and wastes as feed components has been demonstrated. Feed and feedstuff quality control and proper processing techniques were found to improve the nutritional value of low-grade raw materials. Improved feeding techniques and practices have been pursued to minimize feeding costs. Studies on the effect of feeds on the environment are being initiated. Economically feasible grow-out diets for semi-intensive culture of milkfish, Nile tilapia, and tiger shrimp, and diets for broodstock and larvae of these species have been developed.

## Introduction

The thrust of the SEAFDEC Aquaculture Department's Feed Development Section is to develop cost-effective fish and shrimp diets for the industry. To achieve this goal, research studies on nutrient requirements and nutrient interrelationships are conducted to provide baseline data for practical diet development and refinement. Considerable effort has been made to determine the nutrient requirements of important aquaculture species: milkfish (*Chanos chanos*), tilapia (*Oreochromis niloticus*), sea bass (*Lates calcarifer*), and shrimp (*Penaeus monodon*). In practical diet development, the primary concern is nutritionally-balanced and low-cost feed that makes use of inexpensive, indigenous feed ingredients. Research efforts are also geared towards a simple and appropriate feeding technology affordable to the small-scale fish farmers.

Highlights of research on nutrition and feed development conducted at SEAFDEC/AQD are reviewed. A similar review was presented earlier (Millamena 1993) during the Aquaculture Workshop for SEAFDEC/AQD Training Alumni held on September 8-11, 1992 and updated to include more recent work for purposes of this seminar-workshop.

## Milkfish

### Nutrient requirements

Milkfish juveniles require 30-40% protein, 7-10% lipid, 25% carbohydrate, and digestible energy of 2,500-3,500 kcal/kg diet. A protein to energy ratio of 44% is optimal for milkfish. The ten essential amino acid requirements of milkfish have been established (Table 1). The requirements correlate well with milkfish tissue levels except lysine, leucine, tryptophan, and valine wherein the levels required are lower than those present in milkfish tissues. In the formulation of a diet for milkfish, the use of tissue amino acid profile as reference may result in disproportionate amounts of dietary lysine, leucine, tryptophan, and valine, and sub-optimal growth rates (Borlongan and Coloso 1993).

Milkfish cultured in seawater require n-3 polyunsaturated fatty acids. Good growth and survival were obtained using either linolenic acid (18:3n-3) or n-3 HUFA as lipid sources (Borlongan 1992). Preliminary studies on mineral requirement of milkfish juveniles are being conducted.

### Digestive enzymes and digestibility

The major digestive enzymes in milkfish are: carbohydrases, proteases, and lipases while cellulase is absent (Chiu and Benitez 1981; Benitez and Tiro 1982; Borlongan 1990). Milkfish reared in ponds rely mostly on filamentous algae and cellulosic plant materials for food. Cellulase activity in milkfish is probably derived from the gut microflora. Milkfish proteases have two well-defined pH optima, one

Table 1. Summary of nutrient requirements of milkfish, sea bass, tilapia, and shrimp.

| Nutrient | Requirement | Reference |
|---|---|---|
| Milkfish, *Chanos chanos* Forsskal | | |
| Protein | 40%, fry | Lim *et al.* 1979 |
| | 30-40%, Juveniles | Pascual 1989 |
| | 44% protein:energy | Coloso et al. 1988 |
| Essential amino acids | | Borlongan and Coloso 1993; |
| Arg | 5.2% | Borlongan 1992 |
| His | 2.0% | |
| Ile | 4.0% | |
| Leu | 5.1% | |
| Lys | 4.0% | Borlongan and Benitez 1990 |
| Met + Cys | 2.5% (Cys, 0.8) | |
| Phe + Tyr | 4.2% (Tyr, 1.0) | |
| Thr | 4.5% | |
| Trp | 0.6% | Coloso *et al.* 1992 |
| Val | 3.6% | |
| Lipid | 7-10% | Pascual 1989 |
| Essential fatty acids | 1-1.5% n-3 PUFA | Borlongan 1992 |
| Carbohydrate | 25% | Pascual 1989 |
| Digestible energy | 2,500-3,500 kcal/kg | Pascual 1989 |
| Sea bass, *Lates calcarifer* Bloch | | |
| Protein | 43%, juveniles | Catacutan and Coloso, in press |
| | 50% protein:energy | |
| Essential amino acids | | Coloso *et al.,* unpubl. |
| Arg | 3.8% | |
| Lys | 4.5% | |
| Met | 2.24% (Cys, 0.7) | |
| Trp | 0.4% | |
| Lipid | 10% | Catacutan and Coloso, in press |
| Essential fatty acids | 0.5% n-3 PUFA | Borlongan and Parazo 1991 |
| | 0.5% n-6 PUFA | |
| Carbohydrate | 20-25% | Catacutan and Coloso, unpubl. |

*Table 1........(con't.)*

| Nutrient | Requirement | Reference |
|---|---|---|
| Tilapia, *Oreochromis niloticus* | | |
| Protein | 35%, fry | Santiago et al. 1982 |
| | 25-30% fingerlings | Santiago et al. 1986 |
| Essential amino acids | | Santiago and Lovell 1988 |
| Arg | 4.2% | |
| His | 1.7% | |
| Ile | 3.1% | |
| Leu | 3.4% | |
| Lys | 5.1% | |
| Met + Cys | 3.2% (Cys, 0.5) | |
| Phe + Tyr | 5.5% (Tyr, 1.8) | |
| Thr | 3.8% | |
| Trp | 1.0% | |
| Val | 2.8% | |
| Tiger Shrimp, *Penaeus monodon* Fabricius | | |
| Protein | 40%, juveniles | Alava and Lim 1983 |
| | 50%, larvae | Bautista *et al.* 1989 |
| | 53%, broodstock | Millamena *et al* 1986 |
| Essential amino acids | | Millamena *et al,* unpubl. |
| Arg | 4.5% | |
| Lys | 5.3% | |
| Met + Cys | 3.3% (Cys, 0.4) | |
| Thr | 3.5% | |
| Val | 3.7% | |
| Lipid | 8-12%, juveniles | Catacutan 1991 |
| | 12-15%, larvae | Bautista *et al.* 1989 |
| | 12-15%, broodstock | Millamena *et al* 1986 |
| Essential fatty acids | 20:4n-6, 20:5n-3<br>22:6n-3 | Millamena and Pascual 1990 |
| | 2.6% n-3 PUFA<br>< 0.5% 18:2n-6 | Catacutan 1991 |
| Cholesterol | 1.0% | Nalzaro 1982 |
| Lecithin | 1-2% | Pascual 1986 |
| Carbohydrate | 20% | Alava and Pascual 1987 |
| Digestible energy | 2,850-3,700 kcal/kg | Bautista 1986 |
| Vitamin C | 100 mg/kg diet<br>(50 mg as ascorbic acid) | Catacutan and Lavilla-Pitogo 1994 |

at slightly acidic (pH 6.2-7.2) and the other at alkaline pH (pH 8-9). The optimum temperature for protease activity is 45-60°C; thus, increased water temperature (30-34° C) during the dry season is favorable for growth of milkfish. Peak enzyme activity occurs at noon time (1230 h) when feeding activity is maximal (Benitez and Tiro 1982).

Apparent protein digestibility of commonly used feed ingredients for milkfish has been determined. Gelatin has high digestibility in milkfish (90-98%) while casein, defatted soybean meal, and fish meal are moderately digestible (50-90%). Ipil-ipil (*Leucaena leucocephala*) leaf meal has low protein digestibility (10-40%). Apparent protein digestibility increases as a function of fish size (Ferraris *et al.* 1986).

### Practical diet

*Grow-out.* Supplementary feeding of milkfish in brackishwater ponds can increase milkfish production (Sumagaysay *et al.* 1991). Diet with 24% protein fed at 4% body weight is the most profitable combination (Sumagaysay and Borlongan, in press). Alternative protein sources such as leaf meals: swamp cabbage (*Ipomea aquatica*), camote (*Ipomea batatas*), cassava (*Manihot esculenta*), and ipil-ipil *Leucaena* (*leucocephala*) have been used to partially replace 15-20% of fish meal in milkfish diets (Borlongan and Coloso 1994).

*Broodstock. A* study on the effect of diets (36% protein) containing high lipid (10%) or low lipid (6%) fed at 2 or 4% body weight showed that milkfish broodstock fed the low lipid diet at 4% body weight spawned the most number of eggs and highest number of eggs per spawning (Emata *et al,* unpubl.)

*Larvae. A* combination of microparticulate diet and rotifer improves survival of milkfish larvae (Borlongan et *al,* unpubl.). Flaked microbound larval diet can be used as supplement to rotifers for feeding larvae starting day 7 and as replacement of brine shrimp starting day 15 up to the fry stage.

## Sea bass

### Nutrient requirements

Sea bass juveniles require 43% protein, 10% lipid, and 20-25% carbohydrate (Table 1). Optimum protein to energy ratio is about 50%. The quantitative essential amino acid and fatty acid requirements of sea bass are being studied. Initial results showed the following requirements for essential amino acids (expressed as percentage of protein): methionine, 2.24%; lysine, 4.5%; arginine, 3.8%; tryptophan, 0.4%. Sea bass juveniles require both n-3 and n-6 polyunsaturated fatty acids (PUFA) at 0.5% in the diet or an n-3/n-6 ratio of 1.0.

### Apparent digestibility

The apparent digestibility of commonly used feedstuffs for sea bass are currently being determined. *In vitro* protein digestibility (IVPD) of unprocessed soybean, white cowpea, green and yellow mungbean were low at 11-24%. Black cowpea and rice bean were indigestible. Boiling for 30 minutes increased the IVPD of raw soybean from 16-34% and green mungbean from 11-30% (Eusebio *et al,* unpubl.)

### Practical diet

Juveniles. A formulated diet for sea bass grow-out has been tested under laboratory conditions and are being verified in ponds and cages. Best weight gain (360%) and feed conversion ratio (FCR= 1.7) were observed in fish given a combination of fish meal, shrimp head meal, scrap squid meal, soybean meal, and swamp cabbage leaf meal as protein sources. Poorest growth rates and FCR were observed in fish given a combination of fish meal, cow's blood meal, scrap squid meal, soybean meal, and mulberry leaf meal (Coloso *et al,* unpubl.).

Dietary lipid sources such as soybean oil (SBO), cod liver oil (CLO), and coconut oil (CO), singly or in 1:1 combination, have been evaluated for their effects on growth, survival, and fatty acid composition of sea bass fry. Growth and survival of fry fed a diet with 1:1 CLO and SBO are highest, followed by those fed CLO or SBO alone and lowest in those fed the CO diet (Borlongan and Parazo 1991). Feed cost can be reduced if soybean oil partly substitutes fish oil as lipid source.

## Tilapia

### Nutrient requirements

Nile tilapia fingerlings require 25-30% dietary protein. The quantitative requirements of the fry for ten essential amino acids have been established (Table 1).

### Practical diet

*Fingerlings.* Practical diets for Nile tilapia fingerlings containing 20, 25 or 30% protein have been evaluated in feeding trials. Significant differences in weight gains were observed at each protein level and diets with higher protein content do not necessarily produce better growth. In Nile tilapia fingerlings, diets with 18% or more fish meal give higher weight gain than those with 5 or 0% fish meal. Copra meal or ipil-ipil leaf meal as major protein source gives the poorest growth (Santiago *et al.* 1986).

The effects of various inclusion levels of feedstuffs not commonly used in tilapia diets have also been studied. Ipil-ipil leaf meal alone or mixed with rice bran was effective when used as supplemental feed of tilapia fingerlings in cages (Pantastico *et al* 1980).

*Broodstock.* The effect of dietary protein levels on reproductive performance of Nile tilapia broodstock has been determined under laboratory and field conditions. The number of eggs per spawning of broodstock in the laboratory fed to satiation using diets containing 20-50% protein did not differ significantly. Tilapia broodstock in cages and tanks have the best growth and fry production when fed a 40% crude protein diet (Santiago *et al* 1985). Ipil-ipil leaf meal exceeding 40% of the diet is detrimental to the reproductive performance of female broodstock (Santiago *et al.* 1988).

Fry. The influence of feeding rate and diet form on Nile tilapia fry has been studied. Fry fed at the rate of 30% of the fish biomass daily grow fast and have efficient feed conversion ratios. As to diet form, pellet crumbles and non-pelleted form of the same diet were compared. The pellet crumbles slightly enhanced growth and feed conversion ratio and significantly increased survival rates (Santiago *et al.* 1987).

## Tiger shrimp

### Nutrient requirements

*Juveniles.* Tiger shrimp juveniles require 40% protein, 8-12% lipid, 20% carbohydrate, and 2,850-3,700 kcal digestible energy per kg diet (Table 1). A protein to energy ratio of around 56% is required. An amino acid test diet containing casein and gelatin as intact proteins and crystalline amino acid mixture promotes good growth and survival in shrimp juveniles. The formulation has been used to determine arginine, methionine, lysine, threonine, and valine requirements of *P. monodon*

Studies on essential fatty acid requirement show that about 2.6% dietary HUFA enhances growth while levels of 18:2n-6 greater than 5% have negative effect on growth of shrimp juveniles (Catacutan 1991). Phospholipid requirement using soy lecithin as dietary source is 1-2% (Pascual 1986) while cholesterol requirement is 0.5-1.0% (Nalzaro 1982).

The essentiality of some water-soluble vitamins has been tested in *P. monodon* juveniles. Results show that vitamin-free, choline-free, and inositol-free diets significantly suppress growth with severe changes in the histological structure of the hepatopancreas. This indicates that choline and inositol are indispensable in shrimp juvenile diet. Niacin-free and pyridoxine-free diets provide similar growth as a diet with all vitamins present but with slight changes in the histology of the hepatopancreas (Catacutan and de la Cruz 1989). Bioavailability of the phosphated form of vitamin C has also been tested. Results show that *P. monodon* can utilize the phosphated form of vitamin C source. Shrimp without vitamin C supplement develop blackened exoskeleton and have significantly low survival rate (Catacutan and Layilla-Pitogo 1994).

Dietary manipulation of Ca and P is important in the management of soft-shelled shrimps. A dietary Ca to P ratio of 1:1 is effective in hardening the exoskeleton and preventing soft-shelled disease in *P. monodon* (Bautista and Baticados 1990).

*Broodstock.* Tissues (hepatopancreas, muscle, gonad) of wild *P. monodon* broodstock have been analyzed for lipid and fatty acid composition. Profiles of fatty acid consistently show the presence of the following highly unsaturated fatty acids (HUFA): arachidonic (20:40n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic (22:6n-3) acids. High levels of HUFA in the phospholipid fraction of maturing ovaries suggest that HUFA plays a significant role in ovarian maturation (Millamena and Pascual 1990)

## Apparent digestibility

The protein digestibility coefficients of commonly used feedstuffs for *P. monodon* juveniles have been determined. Purified proteins (casein and gelatin) are highly digestible (98-99%). Soybean meal is more digestible (88%) than Peruvian fish meal (61%). Dietary carbohydrate levels of 5-35% do not affect protein digestibility (Catacutan 1991). Dehulling process significantly increases the apparent protein digestibility of rice bean but not of cowpea. This is attributed to the removal of anti-nutritional factors like tannin and other poly-phenols present in rice bean (Eusebio 1991).

## Practical diet

A low-cost grow-out diet (P 17/kg) for *P. monodon* can effectively support production of shrimp in brackishwater ponds. Results after 135 days of feeding showed that weight gain was highest (30.24 g) at stocking density of $5/m^2$ and almost similar at $10/m^2$ (19.13 g) and $20/m^2$ (19.20 g). Survival was significantly low at $20/m^2$ with 3% soft-shelled shrimps. Net production were 0.98, 1.52, and 2.08 tons/ha at stocking densities of 5, 10, and $20/m^2$, respectively. The formulation is recommended for semi-intensive systems with stocking densities of 5-10 shrimp/$m^2$ (Feed Development Section Manual 1994).

Practical diets (53% protein) supplemented with various sources of lipids (cod liver oil, soybean lecithin, or their 1:1 combination) have been compared based on their effects on reproductive performance of pond-reared *P. monodon.* Reproductive performance (number of spawnings, eggs and nauplii production, larval quality) is best with cod liver oil-supplemented followed by lecithin-supplemented diet. The combination of both lipids gives the lowest response but is better than the all-natural food diet (Millamena *et al.* 1986)

The performance of *P. monodon* larvae fed kappa-carageenan microbound larval diet (C-MBD) has been assessed. Feeding C-MBD in combination with natural food results in highest survival of the larvae but this is not significantly different from those obtained with natural food alone or C-MBD alone (Bautista *et al.* 1991). Large-scale hatchery production of *P. monodon* larvae using C-MBD in combination with natural food is feasible.

*Alternative ingredient sources.* Dehulled cowpea and ricebean can be used as partial replacement (15.6%) for animal protein in diets for *P. monodon* juveniles. Growth rates of shrimp given dehulled cowpea and ricebean are comparable with those given defatted soybean meal (Eusebio 1991).

Animal lipids (cod liver oil, pork lard, and beef tallow) and plant lipids (soybean oil, coconut oil, and corn oil) were used as lipid sources for shrimpjuveniles. Weight gain and specific growth rate of shrimp fed 12% cod liver oil were significantly higher compared with those of other treatments. Pork lard, beef tallow, and coconut oil are poor lipid sources for *P. monodon.*

*A* feeding experiment using unsoaked papaya leaf as partial replacement of fish meal in diets for juvenile *Penaeus monodon* was conducted. Shrimp were fed diets with 0, 16, 19, and 22% papaya leaf meal. After 56 days, the highest weight gain (2034%) was obtained in the 0% group (control) and lowest (1535%) in the 22% group. Weight gain (1846%) of those in the 16% was not significantly different from that of the control but those at 19% were significantly lower. Survival rate, however, was similar in all treatments regardless of leaf meal level.

*Feed/feedstuff evaluation.* Some local fish meals derived from herring, slipmouth, and tuna are comparable with imported ones based on their essential amino acid index (EAAI). The EAAI of local fishmeals are 0.92-0.95 while those of white and Peruvian fish meals are 0.96 and 0.92, respectively (Peñaflorida 1989).

Shrimp diets containing locally grown seaweed (*Gracilaria and Kappaphycus* sp.) have high water stability and have apparent dry matter digestibility comparable with those containing common binders like agar, bread flour, corn starch, and wheat gluten. Inclusion of the binder at 3-5% in the diet of *P. monodon* juveniles is recommended (Peñaflorida and Golez, in press).

Tolerance of *P. monodon* to various levels of aflatoxin present in feeds have been determined. Based on growth performance, pre-adult shrimp are able to tolerate aflatoxin ($AFLB_1$) levels of up to 50 ppb although histopathological changes are already evident in the tissues of shrimp given diets with 25 ppb $AFLB_1$ Survey of aflatoxin level in commercial shrimp feeds shows that 92% contains 40 ppb and below, indicating acceptable but narrow margin of safety for the shrimp (Bautista *et al.* 1994).

*Feed additives.* The addition of 1.5% chemoattractant, betaine/amino acid, to a diet with 38% plant protein (soybean meal) and 24% animal protein (fish meal and shrimp head meal) results in improved performance (growth, specific growth rate, and survival) ofjuvenile shrimps (Peñaflorida, unpubl.).

Newly processed and properly stored feeds do not need antioxidants if used within 1-2 months. The antioxidants tested include: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin (ETHQ), and propyl gallate (PG); added at 0.05%. BHA and BHT gave significantly higher weight gain and produced minimal lesion in the hepatopancreas of *P. monodon* compared with ETHQ and PG (Bautista *et al* 1992).

## Conclusion

The Feed Development Section of the SEAFDEC Aquaculture Department has endeavored to develop cost-effective practical diets for milkfish, sea bass, Nile tilapia, and tiger shrimp. Considerable research effort has been expended on understanding the nutrient requirements. The known nutrient requirements of these species are summarized in this paper. Little is known on their mineral and vitamin requirements, but studies are now underway. Emphasis has also been given to assessment of the nutritional value and apparent protein digestibility of feedstuffs used in diet formulation. Information on nutrient requirements are used in conjunction with feedstuff evaluation data in formulating cost-effective diets for the cultured species.

SEAFDEC/AQD has developed economical grow-out diets for milkfish, Nile tilapia, and tiger shrimp. These diets were formulated from inexpensive locally-available raw materials. Likewise, broodstock and larval diets have been formulated and tested. Diet formulation for sea bass is now being verified in cages and in pond. Future work on nutrient requirements and feed development shall focus on other economically important fish species such as grouper, red snapper, catfish, mullet, and the crustacean, mud crab, as alternative culture species to the tiger shrimp.

Although significant achievement has been made over the years, feed development and refinement continues to be a major research thrust at SEAFDEC/AQD. Diet refinement shall take into account the pond ecosystem and available natural food. Feed development is further geared towards reducing feed costs and safeguarding ecological balance. Hence, the development of environment-friendly feeds and application of nutritional strategies to decrease the impact of fish culture on the environment will be considered in future work.

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