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RESEARCH ON NUTRITION AND FEED DEVELOPMENT AT SEAFDEC/AQD

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ABSTRACT

The Feed Development Section at SEAFDEC/AQD conducts research on the nutritional requirements and the development of cost-effective practical diets for regionally important fishes (milkfish, tilapia, carp, and sea bass) and shrimp (*Penaeus monodon*).

Macronutrient requirements for protein, lipid, carbohydrate, energy, and optimum dietary protein to energy ratio have been defined. Essential fatty acids required by each species have been identified. Requirement levels for the ten essential amino acids in milkfish and tilapia have been established. In shrimp, requirements for other essential nutrients like phospholipid and cholesterol are known. Dietary calcium and phosphorous requirements of shrimp have been determined. Requirement for water-soluble vitamins and bioavailability of stable forms of vitamin C are being evaluated. However, much work remains to be done on the vitamin and mineral requirements of cultured species.

The major digestive enzymes, proteases, carbohydrases, and lipases in milkfish have been studied. Further, the apparent digestibility of commonly used feedstuffs were determined *in-vivo* and *in-vitro* for milkfish, and presently, for shrimp and sea bass.

In diet development, the formulation of supplemental grow-out feeds from inexpensive indigenous materials has been emphasized. Likewise, artificial diets for larvae and broodstock are being developed. Effects of feed additives like chemo-attractants and antioxidants were studied. In addition, studies on feed and feedstuff quality control and application of proper processing techniques are being pursued. At present, there are supplemental grow-out diets for the fishes that are commercially viable. Diets for all life stages (grow-out, larval, and broodstock) of shrimp are available. Improvement of these diets will continue as more information on the nutrient requirements are known.

Feed is considered a major input in semi-intensive and intensive pond culture systems because it represents 50% or more of the total production cost. The availability of a cost-effective feed is essential to the success of fish farming. However, feed development in developing countries is hindered by the lack of necessary expertise to formulate and test appropriate feeds to demonstrate their nutritional and economic values. Commercial feeds are available but these require ingredients that are not found locally or are too expensive for the fish farmer. Thus, the need for formulations based on locally available inexpensive ingredients is widely recognized.

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 fishes (milkfish, tilapia, carp, sea bass) and shrimp (*Penaeus monodon*). In diet development and improvement of a traditional diet, the primary concern is nutritionally balanced and low-cost feed that takes advantage of inexpensive, indigenous feed ingredients. Meanwhile, studies to improve feeding techniques and practices to minimize feeding costs are conducted.

Diets developed are continuously refined and improved. Effective diets are tested on-farm for technical and economic feasibility prior to dissemination to end users. The goal is to transfer feeding technology to fish farmers who may want to formulate and prepare his own feeds.

Highlights of recent work on nutrition and feed development conducted at SEAFDEC/AQD are reviewed.

Milkfish

Nutrient requirements. Milkfish (*Chanos chanos* Forsskal) 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% was found to be optimal for milkfish (Table 1).

Studies on milkfish requirement for the ten essential amino acids have been completed. The requirement levels (Table 2) correlate well with the tissue pattern except for lysine, leucine, tryptophan, and valine wherein the levels required are lower than those found in fish tissues. In diet formulation, caution should be exercised when using the tissue amino acid pattern because this might result in disproportionate amounts of dietary lysine, leucine, tryptophan, and valine, causing sub-optimal growth rates.

Studies on essential fatty acid requirements have shown that fish grown in seawater require n-3 fatty acids. Both linolenic acid (18:3n3) and n-3 highly unsaturated fatty acids (HUFA) gave good growth and survival. Linoleic acid (18:2n6) is less important in milkfish adapted to seawater compared with n-3 fatty acids.

Digestive enzymes and digestibility. Milkfish have substantial activities of the major digestive enzymes: carbohydrases, proteases, and lipases; however, cellulase activity is absent. Since milkfish reared in ponds rely mostly on filamentous algae and cellulosic plant materials for food, its cellulase activity is probably derived from the gut microflora.

Milkfish digestive enzymes have two well-defined pH optima, one at

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

Nutrient	Requirement	Reference
Milkfish, *Chanos chanos* Forsskal		
Protein	30-40%, juveniles	Pascual 1989
	44% protein: energy	Coloso et al. 1988
Essential amino acids	See Table 2	
Lipid	7-10%	Pascual 1989
Essential fatty acids	n-3, 1-1.5%	Borlongan 1992
Carbohydrate	25%	Pascual 1989
Digestible energy	2,500-3,500 Kcal/kg	
Sea bass. *Lates calcarifer* Bloch		
Protein	43%, juveniles	Alava, unpubl.
	50% protein: energy	Catacutan, unpubl.
Essential amino acids		
Methionine	2.4% of protein	Coloso et al. unpubl.
Lysine	4.7%	
Lipid	10%	Catacutan, unpubl.
Essential fatty acids	n-3, 0.5%; n-6, 0.5%	Borlongan and Parazo 1991
	n3/n6 ratio, 1.0	
Carbohydrate	20-25%	Catacutan, unpubl.
Tilapia. *Oreochromis niloticus*		
Protein	25%, fingerlings	Santiago et al. 1982
Essential amino acids	See Table 2	
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		
Arginine	5.8% of protein	Pascual, unpubl.
Threonine	3.1%	Millamena, unpubl.
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	Millamena 1990
	22:6n-3	
	n-3 HUFA, 2.6%	Catacutan 1991
	18:2 n-6, < 5%	
Cholesterol	1.0%	Nalzaro 1982
Lecithin	1-2%	Pascual 1989
Carbohydrate	20%	Bautista 1986
Digestible energy	2,850-3,700 Kcal/kg	
Vitamin C (phosphated)	100 mg/kg diet	Catacutan, unpubl.
Ca/P ratio	1.0	Bautista and Baticados 1990

Table 2. Amino acid requirements of milkfish and tilapia

Amino acid	Percentage of protein	
	Milkfish[a]	Tilapia[b]
Arg	5.2	4.2
His	2.0	1.7
Ile	4.0	3.1
Leu	5.1	3.4
Lys	4.0	5.1
Met[c] (cys, 0.5)	2.5	3.2 (0.8)
Phe[d] (tyr, 1.8)	4.2	5.5 (1.0)
Thr	4.5	3.8
Trp	0.6	1.0
Val	3.6	2.8

[a]Borlongan and Coloso, in press. [b]Santiago and Lovell 1988.
[c]Methionine + cystine. [d]Phenylalanine + tyrosine.

slightly acidic and the other at alkaline pH. The optimum pH values of the various digestive enzymes are pH 6.2 to 7.2 and pH 8 to 9, and optimum temperature is 45-60°C. Thus, increased water temperature (30-34°C) during the dry season is favorable for growth of milkfish because of more active digestive enzymes.

Studies on diurnal variations of amylase activity in milkfish and feeding habits reveal a high correlation between amylase activity and feeding index. Peak enzyme activity occurs at noon time (1230 H) when feeding activity is maximal. This confirms earlier observations that milkfish is a daytime feeder.

Apparent protein digestibility values of commonly used feed ingredients for milkfish have been determined (Table 3). The digestibility of gelatin in milkfish is 90-98% while casein, defatted soybean meal, and fish meal are moderately digestible (50-90%). Ipil-ipil leaf meal is the least digestible at 10-40%.

Table 3. Apparent protein digestibility coefficients (%) of some feedstuffs for milkfish

Feedstuff	Freshwater fish (g)			Seawater fish (g)		
	2	60	175	2	60	175
Casein	58	83	87	73	49	65
Fish meal	45	65	73	71	62	71
Gelatin	94	94	94	96.5	98.5	97.5
Ipil-ipil	47	41	41.6	60.5	30.5	-10
Soybean meal (defatted)	53	62	94.3	74	54	60

Source: Ferraris et al. 1986.

Practical diet. Supplementary feeding for milkfish (27% protein) has been tried in brackishwater ponds with encouraging results. Undoubtedly, productivity in milkfish culture can be increased further with the use of supplementary feeds. At present, practical diet development using alternative protein sources are being undertaken. Leaf meals (kangkong, camote, cassava, ipil-ipil, and papaya) and legumes (cowpea, mungbean, and soybean) have been used to replace part (15-20%) of fish meal in the diet.

An artificial diet for milkfish fry reared in sea water with crude protein of 40.8% has been developed. Results showed that soybean meal can replace corn gluten meal and meat and bone meal can substitute for shrimp head meal up to 8% of the crude protein in the diet.

Sea bass

Nutrient requirements. Sea bass (*Lates calcarifer* Bloch) juveniles require 43% protein, 10% lipid, and 20-25% carbohydrate (Table 1). Optimum protein to energy ratio is about 50%.

Studies on the quantitative essential amino acid and fatty acid requirements of sea bass are ongoing. Initial results show the following requirements for essential amino acids (expressed as percentage of protein): methionine, 2.4% and lysine, 4.7%. Sea bass require both n-3 and n-6 highly unsaturated fatty acids (HUFA) at 0.5% in the diet or an n3/n6 ratio of 1.0.

Apparent digestibility. Studies on the apparent digestibility of commonly used feedstuffs for sea bass are currently being undertaken.

Practical diet. A formulated diet for sea bass grow-out has been tested under laboratory conditions with excellent results. The formulation will be verified in ponds.

Soybean meal (SBM), shrimp head meal (SHM), and meat and bone meal (MBM) have been shown to partially substitute fish meal protein in sea bass juveniles diet. SBM, SHM, and MBM can replace up to 10, 20, and 20%, respectively, of the 40% protein diet.

Dietary lipid sources - 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. Fry fatty acid profile reflects that of the dietary lipid. 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. Thus, savings in feed cost can be achieved if soybean oil is partly substituted for fish oil as dietary lipid source.

Tilapia

Nutrient requirements. Nile tilapia (*Oreochromis niloticus*) fingerlings require 25% dietary protein. For the red tilapia (a hybrid of Nile tilapia and one or more related species), diets with 35 to 40% protein with a protein to energy ratio of 111 mg/kcal gave the highest growth. The quantitative requirements of the fry for ten essential amino acids have been established (Table 2).

Practical diet. Practical diets for Nile tilapia fingerlings have been formulated and evaluated in feeding trials. Diets contain 20, 25, or 30% protein. Significant differences in weight gain are observed at each protein level and diets with higher protein content do not necessarily produce better growth. As to protein source, diets with 18% or more fish meal gave higher weight gain than diets with 5% or 0% fish meal. Those with copra meal or ipil-ipil leaf meal as major protein source gave the lowest growth. The results indicate that protein quality influence the growth of Nile tilapia fingerlings.

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 at two levels is found to be effective when used as supplemental feed of tilapia fingerlings in cages. However, ipil-ipil leaf meal exceeding 40% of the complete diet causes weight loss and cessation of reproduction among female broodstock, and drastic reduction in fry production.

The effect of dietary protein levels on the reproductive performance of Nile tilapia broodstock has been determined. Under laboratory conditions, the spawning frequency and number of eggs per spawning of broodstock fed diets containing 20-50% protein do not differ significantly. However, tilapia broodstock in cages and tanks have the best growth and fry production when fed a 40% crude protein diet.

Experiments on the influence of feeding rate and diet form on Nile tilapia fry have been conducted. Fry fed at 30% of the fish biomass daily grow fast and have efficient feed conversion rates. Between pellet crumbles and non-pelleted form of the same diet, the pellet crumbles slightly enhances growth and feed conversion ratio and significantly increases survival rates.

Fresh *Azolla pinnata* as supplemental feed can positively enhance the growth of Nile tilapia fingerlings in cages while dried *Azolla* is a suitable component of complete diets for the fry. Growth and feed conversion ratio improve as the level of *Azolla* meal increased from 8.5 to 42.5% of the diet. Survival rates are not affected by the levels of *Azolla* in the diets.

Bighead carp

Nutrient requirements. Bighead carp (*Aristichthys nobilis*) fry require 30% protein, 7-10% lipid, and 3,130 kcal metabolizable energy per kg diet. A dietary protein to energy ratio of 92 mg per kcal is required.

Practical diet. Bighead carp fry have been shown to attain high growth and survival when fed once daily at 30% of the fish biomass. However, higher feeding rates have to be tested. Fry grow best when fed a combination of natural food and artificial diet, particularly *Moina* or *Brachionus* and a 40% protein diet.

The benefit of giving supplemental diet to bighead carp broodstock in cages has been demonstrated. Although growth, fertilization, and hatching rates are not significantly increased by feeding, fecundity and fry quality (based on the ability to withstand starvation) are enhanced significantly. Fish fed the 40% protein diet have the highest fecundity, total weight of eggs per female, number of eggs per kg body weight, and number of 3-day old fry. Fish fed the 20% protein diet have the intermediate values while the control fish the lowest values for the same parameters.

Shrimp

Nutrient requirements. Shrimp, *Penaeus monodon* Fabricius, juveniles require 40% protein, 8-12% lipid, 20% carbohydrate, and 2,850-3,700 kcal digestible energy per kg diet. A protein to energy ratio of around 56% is required (Table 1). An amino acid test diet containing casein, gelatin, and amino acid mixture as protein sources promotes good growth and survival in shrimp juveniles. The formulation has been used in recent experiments to determine arginine, lysine, and threonine requirements of *P. monodon. A* study on the amino acid composition of *P. monodon* females during the reproductive cycle shows similar amino acid levels in tissues at different maturation stages.

Studies on essential fatty acid requirement show that about 2.6% dietary HUFA enhances growth while levels of 18:2n6 greater than 5% have negative effect on growth of shrimp juveniles. Phospholipid requirement using soy lecithin as dietary source is 1-2% while cholesterol is required at 0.5-1.0%.

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 following highly unsaturated fatty acids (HUFA): arachidonic (20:4n6), eicosapentanoic (20:5n3), and docosahexanoic (22:6n3) acids. High levels of HUFA in the phospholipid fraction of maturing ovaries suggest that HUFA is significant to ovarian maturation. Likewise, fatty acid composition during larval development of *P. monodon* shows that levels of monoenoic fatty acids decreased with corresponding increase in HUFA, 20:5n3, and 22:6n3; this suggests the importance of the latter as dietary components.

Water-soluble vitamin-deficient diets have 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. Bioavailability of phosphated form of Vitamin C has also been tested. Results show that *P. monodon* can utilize the phosphated form as Vitamin C source. Shrimp without Vitamin C supplement develop blackened exoskeleton. Survival rate is significantly lower compared with those given the Vitamin-C supplemented diets.

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.*

Apparent digestibility. The protein digestibility coefficients of commonly used feedstuffs for *P. monodon* juveniles have been determined (Table 4). Purified forms of protein (casein and gelatin) are 98-99% digestible, while soybean meal is more digestible (88%) compared to Peruvian fish meal (61%). Dietary carbohydrate levels of 5-35% do not affect protein digestibility. Further, the apparent protein digestibility of whole and dehulled rice and cowpea has been compared. The dehulling process significantly increases the apparent protein digestibility of rice but not of cowpea. This is attributed to the removal of anti-nutritional factors like tannin and other poly-phenols present in rice hull.

Table 4. Apparent protein digestibility coefficients (APDC) of some feedstuffs for tiger shrimp

Feedstuff	APDC (%)
Pure protein source	
Casein	98.9
Gelatin	99.0
Animal protein source	
Fish meal (Peruvian)	60.8
Fish meal (white)	72.0
Meat and bone meal	73.8
Shrimp head meal	89.1
Shrimp meal (*Acetes* sp.)	95.4
Squid meal	96.0
Testis meal	93.4
Plant protein source	
Bean starch	84.7
Copra meal	75.2
Corn starch	72.0
Flour	69.0
Peanut cake	84.4
Rice bran	48.3
Soybean meal	88.1
Tapioca starch	32.1
Wheat germ meal	91.9
Yeast	86.8
Yeast (*Candida*)	93.0

Source: Catacutan, unpubl.

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) of juvenile shrimps. This information could open new avenues on the use of plant protein sources in feed formulation.

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); these have been added at 0.05%. BHA and BHT give significantly higher weight gain and show minimal lesion in the hepatopancreas of *P. monodon* compared with ETHQ and PG.

Practical diet. A refinement of supplemental grow-out diet for *P. monodon* significantly improves total yield in brackishwater ponds. The formulation is recommended at stocking densities of 5-10 shrimp per sq m.

Practical diets (53% protein) supplemented with various sources of lipids (cod liver oil, soybean lecithin, or their 1:1 combination) have been compared for 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 control (all-natural food diet).

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

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

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

Feed/feedstuff evaluation. The presence of anti-nutritional factors such as aflatoxin may lessen the nutritional quality of feeds and may result in huge losses to the feed industry.

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% contained 40 ppb and below, indicating acceptable but narrow margin of safety for the shrimp. Methods for removal or reduction of tannin and anti-tryptic factor in leaf meals by soaking and blanching are currently being evaluated.

Some local fish meals derived from herring, slipmouth, and tuna are found to be comparable with imported ones based on their essential amino acid index (EAAI). EAAI of local fish meals are 0.92-0.95 while those of white and Peruvian fish meals are 0.96 and 0.92, respectively.

Locally grown seaweeds (*Gracilaria* and *Eucheuma*) are found to have comparable water stability and higher apparent dry matter digestibility compared with common binders like agar, bread flour, corn starch, and wheat gluten. Inclusion at 3-5% in the diet of *P. monodon* juveniles is recommended.

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