

MILKFISH NUTRITION

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This paper reviews major contributions in the field of milkfish nutrition since the First International Milkfish Aquaculture Conference in 1976. Substantial progress has been made toward understanding the digestion, foods, and feeding behavior of milkfish, which in its natural habitat apparently feeds on planktonic microorganisms and is most frequently designated as a microphagous planktivore. It has fine, almost membranous gill rakers and a specialized epi-branchial organ that may help to concentrate microplankton. Vision seems to be the most important sensory mechanism for feeding in fry as well as in juveniles and larger milkfish. Active carbohydrases, proteases, and lipases have been detected in the pyloric caeca, pancreas, and intestines of milkfish. The most active carbohydrases were those involved in the hydrolysis of α -glucosidic bonds. Milkfish trypsin was inhibited by a tryptic inhibitor from *Chaetomorpha brachygonia*, one of the most commonly occurring filamentous algae in milkfish ponds. This may account for the slow growth rate of milkfish on this natural food base. There is very scant information on nutrient requirements and other important aspects of milkfish nutrition. A preliminary study on protein requirement showed that a dietary level of 40% protein was required by fry. Other studies showed that fry responded positively and were easily trained to accept artificial diets. The "deep water method" of growing milkfish practised in Taiwan

demonstrated that, with the use of formulated diets, productivity in milkfish aquaculture could be increased three-fold over traditional culture methods, which rely on natural food bases. Undoubtedly, productivity can still be enhanced through further improvement in the nutritional quality of diets.

INTRODUCTION

The intensification of milkfish aquaculture is a direction the milkfish industry may soon have to take. With the increase in the pressure from alternative uses of coastal land, suitable areas for aquaculture may steadily decrease. Productivity will have to be increased through a shift from extensive aquaculture, dependent on predominantly natural food bases, to intensive aquaculture, which utilizes formulated diets. Fundamental research on milkfish nutrition, including studies on digestibility, digestive enzymes, nutrient requirements, and metabolism, is necessary to be able to formulate economically feasible and nutritionally adequate milkfish diets.

Interest in research on milkfish nutrition was markedly enhanced after the First International Milkfish Aquaculture Conference in 1976, and this paper reviews the major contributions in the field since then. Substantial progress has been made toward understanding the digestion, food, and feeding behavior of milkfish.

FOOD AND FEEDING

Milkfish, in its natural habitat, apparently feeds on planktonic microorganisms and is most frequently designated as a microphagous planktivore (Smith 1980). All fish that share the unique characteristics of attaining very large body size on a diet of phytoplankton or zooplankton have very elaborate modified branchial structures — gill rakers or epibranchial organs. These special structures appear to function as food processing devices and serve to filter, collect, and concentrate the food (Nelson 1967, Hyatt 1979). Milkfish has fine, almost membranous gill rakers that suggest filter feeding. It also has a specialized epibranchial organ above and behind the gills that may help to concentrate microplankton (Kuwatani and Kafuku 1978, Smith 1980). A study of the food and feeding periodicity of milkfish fry collected from coastal waters suggested, however, that planktonic organisms constitute a minor component of the food intake of the fry (Banno 1980). Only about 3-11% of fry collected in marine waters and 5-9% of those collected from estuaries had planktonic organisms in their gut. The most common organisms were a centric diatom (*Coscinodiscus* sp.), a cyclopoid copepod (*Oithona* sp.), and calanoid copepods (*Paracalanus* sp. and *Calanus* sp.). Detrital materials suspended in the water column or settled at the bottom were suggested as a major source of food for the fry (Banno 1980).

Vision seems to be the most important sensory mechanism for feeding in milkfish fry and appears to develop before the other senses (Kawamura and Hara 1980). Gut content analysis of fry collected from both marine and estuarine waters suggested that milkfish fry are daytime visual feeders, with peak feeding at 0700 and 1300 h (Banno

1980). In the laboratory, the captive fry did not feed in the dark. However, the ability to feed in the dark increased as the fish grew to young juveniles, probably due to the development of chemosensory and auditory mechanisms (Kawamura and Hara 1980). An electrical device for recording feeding activity in larger milkfish that weigh about 2-3 kg showed that the fish also feed preferentially during the day. Feeding activity was significantly less at night (Kawamura and Castillo 1981).

The eyes of milkfish larvae are pigmented on the second day after hatching, and feeding on exogenous food starts on the third day when the yolk is completely absorbed (Liao et al 1979). At about 3 weeks of age, wild fry were observed to have well-developed, regionally differentiated retinæ with all retinal elements already present (Kawamura and Hara 1980). A retinal tapetum was also observed in the pigment epithelium, and its presence suggested that the fry may be adapted for vision in optically turbid shore water or in dim or subdued light (Kawamura and Hara 1980). The sensory mechanisms associated with feeding activity are described by Kawamura (1984) in greater detail elsewhere in this volume.

Biochemical studies showed a high correlation between the intestinal feeding index and amylase activity in milkfish grown in fishponds (Chiu and Benitez 1981). The intestinal amylase activity consistently peaked daily at about noon, when the milkfish gut was full. In contrast, enzyme activity was significantly lower at 0030 h, when the gut was empty. These data confirm that milkfish is a daytime feeder and suggest an efficient digestive capacity in which the periodicity of secretion of digestive enzymes is in phase with the daytime feeding activity of the fish (Chiu and Benitez 1981).

Milkfish aquaculture in the Philippines depends on the utilization of either of two natural food bases, one consisting predominantly of unicellular algae, diatoms, and other organisms associated with the algal community and locally known as *lablab*, the other consisting mainly of fibrous filamentous green algae, mostly *Chaetomorpha brachygonia* (Rabanal 1966, Vicencio 1978, Guerrero 1979). Most fish farmers concede that, on the basis of faster growth rate and therefore better productivity, *lablab* appears to be a better food base for milkfish. Consequently, a number of scientific research studies were made on the nature of *lablab* as food for milkfish. Both floating *lablab* and the benthic algal mat designated here as adhering *lablab* were studied.

Sampling of *lablab* posed technical difficulties until a standard sampling procedure was designed. With this procedure, it was shown that floating and adhering *lablab* differ markedly in quality and quantity. The quantity of *lablab* could be assessed with reasonable precision by analysis of ash-free dry weight. Analysis of protein content appeared to be the most appropriate indicator of *lablab* quality. Floating *lablab* had higher mean protein content (14.98%) than adhering *lablab* (5.99%). There was no detectable difference in lipid content among samples, but at about 1.0% lipid was considered a minor component (Jumalon 1978). More detailed chemical analyses, however, showed differences in sterol and fatty acid composition of floating and adhering *lablab* (Teshima et al 1981). Adhering *lablab* had a relatively higher concentration of 24-E-ethylidenecholesterol (13.3%), while floating *lablab* contained only 1.0%. Cholesterol, at about 40% of total sterols, was the predominant sterol in both floating and adhering *lablab*. The high cholesterol content was

attributed to zooplankton since blue-green algae, the major algal constituent of *lablab*, generally contains cholesterol as a minor sterol. On the other hand, the 24-methyl, 24-ethyl-sterols and their Δ^2 derivatives detected in *lablab* were suspected to be derived mainly from blue-green algae and phytoplankton.

The major fatty acids in both floating and adhering *lablab* were palmitic acid (16:0) and palmitoleic acid (16:1); while *lablab* contained 3.6% 18:2 ω_6 and 4.5% 18:3 ω_3 long-chain polyunsaturated fatty acids, 20:5 ω_3 and 22:6 ω_3 were present at extremely low levels of about 0.5% and 0.6%, respectively (Teshima et al 1981). These quantities were essentially confirmed by subsequent studies which showed that *lablab* contains about 2.0% 18:2 ω_6 and 4.0% 18:3 ω_3 but no detectable amounts of 20:5 ω_3 (Gorriceta 1982, Benitez and Gorriceta 1983).

Most fish show a greater requirement for ω_3 fatty acids such as 18:3 ω_3 , 20:5 ω_3 , and 22:6 ω_3 than for ω_6 fatty acids such as 18:2 ω_6 and 20:4 ω_6 (Cowey and Sargent 1977, Watanabe 1982) except *Tilapia zillii*, a tropical euryhaline herbivore that appears to require ω_6 rather than ω_3 fatty acids (Kanazawa et al 1980). Since *lablab*, the primary food of captive milkfish, contains very small amounts of 20:5 ω_3 and 22:6 ω_3 , the question was posed whether milkfish, like other marine fish, has a strict requirement for 20:5 ω_3 as an essential fatty acid or instead possesses the ability to convert the dietary fatty acid 18:3 ω_3 to 20:5 ω_3 and 22:6 ω_3 (Teshima et al 1981). Subsequent studies detected significant quantities of long-chain polyunsaturated ω_3 fatty acids such as 20:5 ω_3 and 22:5 ω_3 in the livers of milkfish grown in *lablab* ponds (Benitez and Gorriceta 1983). The presence of these long-chain fatty acids in the liver, despite their absence in *lablab*, suggests that milkfish have the capacity to convert dietary ω_3 and ω_6 fatty acids, through chain elongation and desaturation, into long-chain ω_3 and ω_6 polyunsaturated fatty acids and implies further that the major site of such metabolic transformation is the liver (Benitez and Gorriceta 1983).

Milkfish is cultured mainly in coastal ponds, where salinity can fluctuate from 10 ppt during the rainy season to 60 ppt during the dry months. As a euryhaline species, it is known to thrive successfully in natural waters of 0-100 ppt. The highly productive freshwater pen culture of milkfish in Laguna de Bay in the Philippines is widely recognized (Felix 1975, Dalagan 1980, Pullin 1981). On the other extreme, milkfish are known to thrive in landlocked hypersaline ponds on Christmas Island (Crear 1980). The highest salinity at which an individual milkfish was found was 158 ppt, a value which exceeds salinity tolerances previously reported for other vertebrate species. In the hypersaline ponds, milkfish fed extensively on benthic mats composed of halophilic bacteria, blue-green algae, diatoms, and fungi. *Artemia salina*, which was introduced in 1971, has subsequently colonized many hypersaline ponds on Christmas Island. Several inspections of the alimentary tract of milkfish from these ponds indicated that brine shrimp accounted for 25% (by volume) of their diet (Crear 1980).

An unusual feature of the milkfish population in some hypersaline ponds of Christmas Island is the stunted size of the adults, possibly the first report of salinity stunting in a natural vertebrate population (Crear 1980). High salinity appears to inhibit growth significantly but does not impede fat deposition and the maintenance of a generally sound physiologic balance within the organism. Aside from their reduced size, the milkfish in these hypersaline ponds exhibit no externally

evident detrimental effects from osmotic stress. A review of the salinity data for the isolated hypersaline ponds and the gonadosomatic indices of their milkfish populations suggested that reproductive readiness is controlled by the interaction of salinity and diet (Crear 1980). A diet of benthic mat and brine shrimp produced a positive response in reproductive readiness even in the hypersaline environment, while a diet without brine shrimp stimulated reproductive readiness only at lower salinities when the osmoregulatory stresses on the fish were reduced. Undoubtedly, the study of the nature and composition of food ingested by milkfish in its natural habitat can provide important clues about its nutrient requirements.

DIGESTION

Carbohydrases

Crude extracts from various regions of the digestive tract of pond-grown milkfish were tested for their ability to catalyze the hydrolysis of various carbohydrates (Chiu and Benitez 1981). Nine distinct regions of the digestive tract that were tested were the epibranchial organ, esophagus, cardiac stomach, pyloric stomach, pyloric caeca, anterior intestine, posterior intestine, pancreas, and liver with gall bladder. The most active carbohydrases were those involved in the hydrolysis of α -glucosidic bonds. Maltose, trehalose, dextrin, starch, and glycogen were rapidly hydrolyzed by crude extracts from the intestines and pyloric caeca. Amylase was found to be a major digestive enzyme in milkfish and was detected in almost all regions of the digestive tract. High activity was observed in extracts from the intestines, pancreas, pyloric caeca, and liver. The intestinal amylase of milkfish had an activity optimum of about pH 6.2 and a temperature optimum of about 50°C. This temperature is above the usual range observed in fishponds. The digestion of starch can apparently proceed even at high temperature. This is advantageous since fish in general have very limited ability to thermoregulate. The amylase activity of a number of warmwater fishes has a temperature optimum of 50°C or above (Morishita et al 1964).

Although the milkfish studied by Chiu and Benitez (1981) fed mainly on naturally occurring algae and other plant materials in the ponds, no cellulase activity was detected in any region of the digestive tract. Complex polymeric substrates with β -glucosidic linkages such as microcrystalline cellulose and sodium carboxymethyl cellulose were not hydrolyzed. Similarly, no hydrolysis was detected with cellobiose as the substrate. Significant amounts of salicin and p-nitrophenyl- β -D-glucose were, however, hydrolyzed by crude extracts from the pyloric caeca and the intestines, confirming the presence of a specific β -glucosidase of limited substrate specificity. In general, milkfish can digest with ease most naturally occurring carbohydrates such as dextrin, starch, and glycogen. Evidently, substrates with β -glucosidic linkages are not as easily digested (Chiu and Benitez 1981).

Proteases

The protease activity of crude extracts from various organs of the digestive tract of two groups of milkfish was determined (Benitez and Tiro 1982). One group derived its food from ponds that had predominantly unicellular algae (*lablab*), while the other group was reared on ponds dominated by filamentous green algae, *C. brachygonia*.

Milkfish have proteases of varying specificities and activities. Proteases were most active in the pyloric caeca, intestines, and pancreas. At pH 7.6, no protease activity was observed in extracts from the cardiac and pyloric stomachs for both groups of fish. At about pH 2.0, however, slight peptic activity could be observed in the stomach extracts.

The intestinal proteases showed activity peaks at pH 7.2 and 9.3 for fish reared on *lablab*, indicating a predominance of alkaline proteases. The fish reared on filamentous green algae showed an activity peak at pH 10. The optimum temperature for milkfish intestinal protease activity was between 50 and 60°C for fish reared on *lablab*. A broader peak of 45-60°C was observed for fish reared on filamentous green algae. The two most common alkaline proteases are trypsin and chymotrypsin. Using specific chromogenic substrates, it was possible to detect the presence of these two proteases in crude extracts of pancreas, pyloric caeca, and intestines of milkfish. Milkfish chymotrypsin was detected in fish reared on *lablab* as well as in those grown on filamentous green algae (Benitez and Tiro 1982). Chymotryptic activity from the digestive tract of milkfish was inhibited by L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK). TPCK specifically and completely inhibits mammalian chymotrypsin by alkylation of a histidine residue at the active site of the enzyme (Schoellman and Shaw 1963, Neurath 1964). The inhibition of milkfish chymotrypsin by TPCK indicates that its active site may be structurally similar to the mammalian enzyme and that a histidine residue is likely involved in the proteolytic process (Benitez and Tiro 1982).

High tryptic activity was observed in extracts from the pancreas, pyloric caeca, and intestines of milkfish reared on *lablab*, but no tryptic activity could be detected in any of the extracts from the digestive tract of milkfish reared on *C. brachygona*. It is quite evident, however, that milkfish can synthesize trypsin. In an in vitro test, extracts from *C. brachygona* completely inhibited milkfish trypsin when pre-incubated with an equal volume of crude extract from milkfish pyloric caeca and pancreas, strongly indicating that the absence of tryptic activity is caused by the presence of a powerful tryptic inhibitor in the algal diet. A number of trypsin inhibitors have been isolated and purified from tissues of various plants (Kanamori et al 1976, Schwartz et al 1977, Tashino and Maki 1979). Seeds of leguminous plants are particularly rich in trypsin inhibitors (Wagner and Riehm 1967, Willson and Laskowski 1973, Odani and Ikanaka 1977). The *Choetomorpha* inhibitor may, however, be the first report of a trypsin inhibitor in an aquatic alga (Benitez and Tiro 1982, Tiro and Benitez 1982). Most fish farmers have observed that milkfish grown on filamentous algae have slow growth, which may be accounted for by the presence of a trypsin inhibitor in these algae.

Lipases

Lipase is widely distributed in the digestive tract of milkfish. For fish reared on *lablab*, as well as for those reared on *C. brachygona*, the highest enzyme activity was observed in intestinal extracts and, in both cases, the anterior portion had a stronger activity than the posterior (Gorriceta 1982). It has been suggested that lipase activity in the intestines of fish is due to the presence of pancreatic lipase. However, more recent histochemical studies showed that the intestinal mucosa of several teleost

fishes are truly capable of lipase secretion (Sastry 1974 a, b). Lipase activity was also observed in milkfish pancreatic extracts (Gorriceta 1982). As in most teleost fish, the pancreas of milkfish is diffused. The absence of a well-developed compact pancreas in teleost fish is partly compensated by the secretion of lipase by the intestinal mucosa, in addition to the secretory activity of the diffused pancreas (Sastry 1974b).

The lipase activity of extracts from the anterior intestines of milkfish shows two pH optima, one at about pH 6.8 and another at pH 8.0. This indicates the presence of both acidic and alkaline lipases and suggests a physiological versatility for digestion of lipids. Similarly, the pancreatic extract has two pH optima at pH 6.4 and 8.6 (Gorriceta 1982). The pH optima of intestinal lipase differ from those of pancreatic lipase, perhaps indicating that the pancreatic lipase is a different molecular entity from the intestinal lipase. Further characterization of milkfish lipases should be done to confirm this.

The optimum temperatures for milkfish intestinal and pancreatic lipase activity were found to be 45°C and 50°C, respectively. As was also true of milkfish amylase and proteases, milkfish lipases are quite active even at relatively high temperatures. From the temperature activity profile, it is clear that the digestive enzyme activity of milkfish is minimal at 0-25°C. This may in turn affect digestion and metabolism and may be manifested in terms of poor or reduced feeding activity of the fish at lower pond temperatures. These findings may account for the common observation of many fish farmers that growth rate and feeding activity during the cold months are lower than in warm months.

NUTRIENT REQUIREMENTS

There is very scant information on nutrient requirements and other important aspects of milkfish nutrition. A preliminary study on the protein requirement of fry under controlled laboratory conditions showed that a dietary level of 40% protein was required for maximum growth, efficient feed conversion, and high survival rate (Lim et al 1979). Forty fish with an average weight of 40 mg were stocked in a 60-liter aquarium filled with 30 liters of filtered seawater with a salinity of 32-34 ppt and a temperature of 25-28°C. They were fed diets containing 20, 30, 40, 50, and 60% protein and 2740 kcal of digestible energy per kg at a daily rate of 10% of biomass for a period of 30 days. The five semipurified diets contained casein as the protein source, dextrin as the carbohydrate source, equal parts of cod liver oil and corn oil as the lipid source, and a vitamin and mineral supplement. The fry were fed twice daily (half of the ration at 0900 h and half at 1700 h), 7 days per week, at the rate of 10% (dry matter) of their body weight per day. Fish that were fed the diet containing 40% protein had the highest weight gain (134.7 mg), which was significantly higher than those receiving the lower dietary levels of protein. Slightly lower weight gains were obtained when fish were fed diets containing 50 and 60% protein. Although feed conversion values were not statistically different among all treatments, the value for the 40% protein diet (1.96) was the best. There was no significant difference in the survival rates of fish receiving different diets; however, mean survival rates were low for all treatments, with the highest at 30% for the fish fed the 40% protein diet. The

relatively high mortality in the study may have been due to environmental stresses such as high salinity (32-34 ppt) and low water temperature (25-28°C), which might have caused the gradual death of the fry. However, the fact that 40% protein supported the highest survival rate suggests that this level of protein is adequate to maintain satisfactory resistance of milkfish fry against environmental stresses (Lim et al 1979).

Using a recirculating system, experiments were performed to determine the requirements of milkfish fry for protein, carbohydrates, fats, and vitamins (Camacho and Bien 1983). The test diets consisted of vitamin-free casein, gelatin, white dextrin, corn and cod liver oil, and vitamin and mineral mixtures. These diets were fed to milkfish fry (10-35 mg body weight) for 28-30 days at stocking densities ranging from 2000-4000/m³ of water, the salinity of which was maintained at 16-18 ppt. The results showed that a purified diet with the following formulation was required by milkfish fry for normal growth: 40-45% vitamin-free casein, 12-15% gelatin, 8-10% fat, and 3-4% vitamin mixture. Unexplained but consistent mortalities occurred during the third week after stocking; syndromes associated with vitamin and amino acid deficiencies were also observed (Camacho and Bien 1983).

RESPONSE TO ARTIFICIAL DIETS

The response of milkfish fry to artificial diets has been investigated both in diluted seawater and fresh water. In a feeding trial, milkfish fry with a mean weight of 7.00 mg were stocked at 4 fry/liter in 300-liter fiberglass tanks with filtered diluted seawater (14-18 ppt) in a flow-through system (Jamandre 1980). The fry were fed three artificial diets containing a minimum protein level of 42%. Training milkfish fry to receive artificial feed was accomplished with no difficulty; they were found to respond to conditioning and were observed to feed at a designated place.

Growth and survival were satisfactory up to the third week of feeding. At the end of the 28th day, the highest survival rate was about 63%. In a second experiment, using an airlift system for aeration, the highest survival rate after 42 days was about 18%. In both trials, after the first 3 weeks the fry lost weight and developed crooked backs and enlarged reddish heads—characteristic signs of vitamin C deficiency. The diets appeared satisfactory for the first 3 weeks but were not fit for the older and larger postlarvae (Jamandre 1980).

In another study, milkfish fry with a mean weight of about 15 mg were reared in fresh water for 5 weeks using four artificial diets, *Moina*, or blended water hyacinth leaves as feed (Santiago et al 1983). The artificial diets had an estimated crude protein content of 40%. The fry were stocked at 4 fry/liter in glass aquaria filled with 30 liters of filtered fresh water provided with aeration. After 5 weeks of feeding, the fry fed with the four artificial diets had significantly higher mean weight gains (0.16-0.18 g) than those fed with *Moina* (0.09 g) or blended water hyacinth leaves (0.06 g). Survival rates on the artificial diets were high (83-95%), while only about 16% survived with *Moina* and 22% with water hyacinth (Santiago et al 1983). The formulated diet containing 40% crude protein with fish meal as the major protein source appeared adequate for the fry. Substitution of up to 5% crude protein from plant sources did not appreciably affect growth (Santiago et al 1983).

The response of the hepatocytes of milkfish fry and fingerlings to starvation and subsequent feeding was evaluated by electron microscopy (Storch and Juario 1983). Large variations in the ultrastructure of the hepatocytes were observed and were attributed to differences in nutritional condition. Starvation promoted ultrastructural changes hitherto unknown for other teleost species; nevertheless, the hepatocytes could rapidly attain their prestarvation condition when fed live *Anemia nauplii* and *Brachionus plicatilis*.

In a subsequent study, it was observed that the hepatocytes of milkfish fry can alter their ultrastructure in response to artificial diets (Storch et al 1983). The hepatocytes showed patterns of alteration in cell size, nucleus, endoplasmic reticulum, mitochondria, glycogen, and lipids. A lipid-oriented diet resulted in an increased deposition of small lipid droplets in the cytoplasm and even in some nuclei of the hepatocytes. A carbohydrate-oriented diet did not, however, result in deposition of glycogen. A protein-rich diet resulted in the best recovery of hepatocytes. The hepatocyte ultrastructure may serve to determine acceptability and quality of food or may be used as an indicator of the general nutritional situation of the fish (Storch et al 1983).

The positive response of milkfish to artificial diets has been responsible for high productivity following a revolutionary change in the way milkfish is farmed in Taiwan (Chen 1981). In the so-called "deep water method," the depth of the grow-out pond is increased from the conventional 10-30 cm to as much as 2-3 m depending on the nature of the soil. The stocking rate is increased from the conventional annual total of 1 200 to a total of 20 000-40 000. The pond water is mechanically aerated. The fish are fed commercial pellet feed containing about 23.5-29.0% crude protein in daily amounts equal to 3-4% of their total weight. The feed conversion ratio is claimed to be 1:2-1:6 without taking into consideration the natural food produced in the ponds. The productivity of the deep water farming technique is three times that of the traditional culture technique, which relies mainly on natural food bases (Chen 1981). Undoubtedly, the productivity of milkfish aquaculture can still be enhanced through improvements in the nutritional quality of diets.

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