NUTRITION AND FEEDING OF FISH

Second Edition

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edited by

Tom Lovell
Auburn University
Auburn, Alabama

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To My Graduate Students

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LIST OF CONTRIBUTORS

Ronald W. Hardy, Ph.D.
Director, Hagerman Fish Culture Experiment Station
University of Idaho
Hagerman, Idaho

Meng H. Li, Ph. D.
Thad Cochran National Warmwater Aquaculture Center
Mississippi State University
Stoneville, Mississippi

Chhorn E. Lim, Ph. D.
USDA, ARS, Fish Diseases and Parasites Laboratory
Auburn University
Auburn, Alabama

Edwin H. Robinson, Ph. D.
Thad Cochran National Warmwater Aquaculture Center
Mississippi State University
Stoneville, Mississippi

Carl D. Webster, Ph. D.
Aquaculture Research Center
Kentucky State University
Frankfort, Kentucky

PREFACE

Aquaculture is now recognized as a viable and profitable enterprise worldwide. As aquaculture technology has evolved, the push toward higher yields and faster growth has involved the enhancement or replacement of natural foods with prepared diets. In many aquaculture operations today, feed accounts for more than one-half the variable operating cost. Therefore, knowledge on nutrition and practical feeding of fish is essential to successful aquaculture.

This book was not written exclusively for scientists but for students, practicing nutritionists, and aquaculturists. It covers the known nutrient requirements and deficiency effects for different fishes, and digestion and metabolism of nutrients and energy. It discusses nutrient sources and preparation of practical and research feeds. It gives direction for conducting fish nutrition and feeding experiments. Feeding practices for salmonids, channel catfish, tilapias, shrimps and hybrid striped bass are presented.

Since the first edition of this book was printed, the National Research Council of the National Academy of Sciences has revised the nutrient requirements for fish. These revisions are in the present edition. Other additions to this revised edition are chapters on nutrition and fish health, and bioavailability of nutrients. Each original chapter has been meticulously revised and updated with new information. Aquaculture is a dynamic area and new technologies are being introduced continuously; therefore, some of the material discussed in this revised edition may become obsolete quickly. Nonetheless, the material presented has been thoughtfully selected and updated so that it will be of maximum use to persons whose interests range from general aquaculture to animal nutrition to feed manufacture.

The author deeply appreciates the assistance given by the five contributing authors, each being pre-eminent in the area he discussed.

1 THE CONCEPT OF FEEDING FISH

EVOLUTION OF AQUACULTURE

Shell (1993) defines aquaculture as "the planned and purposeful intervention in the production of aquatic animals," and explains that the underlying reason for the evolution of aquaculture was to reduce the uncertainty and unpredictability of production in natural systems. One of the major driving forces in human development has been the effort to seek ways to reduce uncertainties in food supply. This was an obvious reason for domesticating plants and animals some 10,000 years ago. In contrast to terrestrial animals, there has been virtually no domestication of aquatic animals; there are few recognized varieties, strains, or breeds of aquatic animals. Historically, as the demand for aquatic animals increased, people simply harvested more or harvested more efficiently from natural waters. This trend generally persisted until the latter half of the 20th century at which point the harvest of fish from wild populations could no longer keep pace with the world demand for fish. It became apparent that significant increases in the supply of aquatic animals could only be achieved by direct intervention in the production process, thus, through aquaculture.

Although aquaculture made its greatest advancements in the latter part of the 20th century, fish farming is believed to have been practiced in China as early as 2000 B.C., and a classical account of the culture of common carp was written by Fan Lei in 475 B.C. (Villaluz 1953). The Romans built fish ponds during the first century A.D. and during the Middle Ages fish ponds for carp farming were built throughout Eastern Europe by religious men (Lovell, Shell, and Smitherman 1978). Carp farming in Eastern European countries was popular in the 12th and 13th centuries. In Southeast Asia, fish ponds were believed to have evolved naturally along with salt-making in the coastal areas; the salt beds were utilized to grow milkfish during the rainy season. This practice was originated by the Malay natives before A.D. 1400 (Schuster 1952). Early interest in fish culture in the United States was carried over from England before 1800 and was concentrated on propagation and culture of trout and salmon.

By early in the 20th century, several forms of fish culture were fairly well established, such as milkfish farming in Southeast Asia, carp polyculture in China, carp monoculture in Europe, tilapia culture in tropical Africa, culture of indigenous finfish and crustaceans in estuarine impoundments in Asian and Southeast Asian coastal areas, and hatchery rearing of salmonids in North America and Western Europe. With the exception of salmonid culture, these forms of aquaculture were generally extensive, where the nutrient inputs into the system were restricted or limited to fertilizers and crude sources of foods, and yields were low.

Aquaculture has made its greatest advancements during the latter part of the 20th century. New species are being cultured, new technologies for more intensive culture have been introduced, a large research base has been established, and commercial investment is being attracted into aquaculture. Aquaculture is now recognized as a viable and profitable enterprise worldwide. For example, channel catfish farming in the United States has grown from almost obscurity in 1970 to an annual yield of over 223,000 tons in 1996 (USDA 1997). Farming of penaeid (marine) shrimp, primarily in South and Central America and Asia, is the fastest growing aquaculture enterprise worldwide, supplying approximately 43% of the world's consumption. Ocean pen culture of salmon is a thriving industry in Norway and other areas of Western Europe, where it provides 90% of the salmon consumed, and in regions of North and South America and Australia. High value marine species, such as sea breams, sea basses, turbot, and yellow tail tuna, are being cultured on a large commercial scale in Europe and Japan. Tilapia species are produced for export from tropical areas of America and Asia.

Aquaculture will continue to grow and supply an increasingly larger percentage of fishery products consumed. This is assured because supply, price and quality of marine fish fluctuate considerably because the ocean is inadequately managed and its yield is unpredictable. But when fish are cultured, like corn in a field, supply can be controlled more effectively. With the present technology and research base, yields and risks for a number of aquaculture enterprises are now predictable, which makes them attractive investment opportunities.

LEVELS OF AQUACULTURE

Several authors have recommended different systems of classification of the various stages of aquaculture. Some classify according to level of intervention, i.e., how much the culture environment has been modified. This ranges from impounding natural waters and harvesting any and all animals therein, without adding seed stock or nutrients, to a closed system where water is recirculated. Some classify on the basis of quantity and quality of nutrients utilized by aquaculturists, such as extensive (no nutrients added), fertilization (to enhance production of aquatic organisms), supplemental feeding (incomplete feeds), intensive feeding (nutritionally balanced feeds), and hyperintensive feeding (high inputs of concentrated, nutritionally complete feeds). Some classify on the basis of energy inputs (labor, fossil fuel, feed) or technology input (harvesting, stocking, feeding, pumping, aeration, biofiltration). Generally, the higher the level of intervention in production of aquatic animals, the more important is the feed to the success of the operation.

Figure 1.1 Low-level aquaculture, where no nutrients are added to the culture system. Compost of vegetation and manure is placed in the edge of the pond; as it decomposes, nutrients leach into the water and stimulate plankton growth which provides food for the fish.

Production of Fish Exclusively From Natural Aquatic Foods

Some fish obtain their food exclusively from plankton. These fish are usually continuous grazers and have mechanisms for filtering and concentrating the suspended animal and plant organisms from the water. An example is the silver carp. Others, such as some of the tilapias, have the ability to feed on plankton, but also feed on bottom materials. The common carp is an efficient bottom feeder. Some fishes, such as grass carp, have herbivorous appetites and consume large quantities of higher aquatic plants. Such fishes can be cultured without artificial feeds, as shown in Figure 1.1, but usually with pond fertilization. This level of production is most applicable in countries where supplemental feeds are expensive or unavailable.

Supplementing Natural Foods with Feed

This level of fish farming essentially involves taking full advantage of natural aquatic productivity and using various feedstuffs or prepared feeds as a supplement to increase yield further. An example is shrimp culture in Central and South America (Figure 1.2) where large ponds are fertilized to enhance natural productivity and also receive pelleted feeds. Usually with species that will accept supplemental feeds, the additional yield of fish resulting from the additional feeding is profitable. For example, the yield of common carp in fertilized ponds was 390 kg ha^{-1}; the addition of grain or grain byproducts increased yield to 1,530 kg ha^{-1}; and high quality formulated feed further improved yield to 3,000 kg ha^{-1} (Lovell, Shell, and Smitherman 1978).

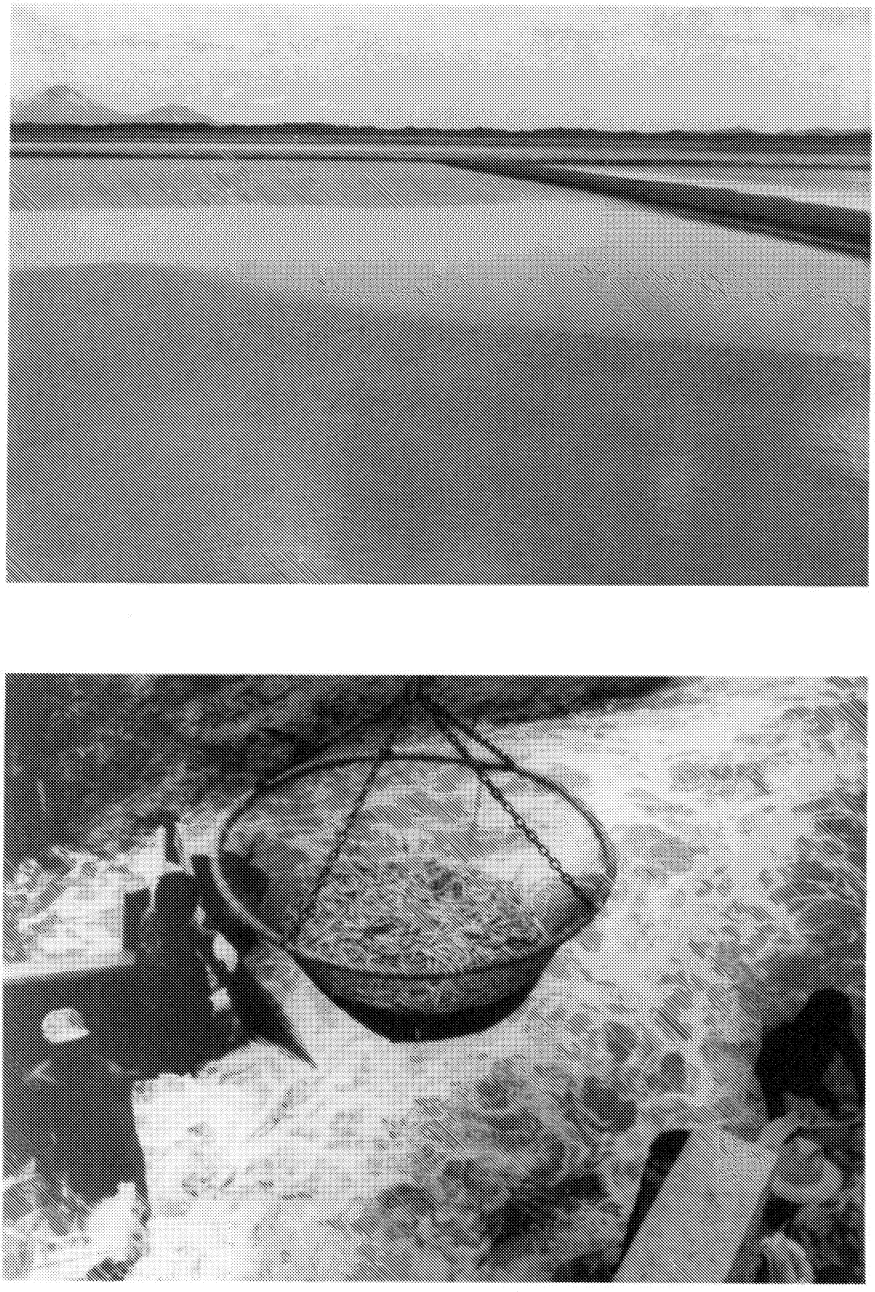


Figure 1.2 Semi-intensive pond aquaculture is represented by large (25-hectare) shrimp ponds in Honduras (top) which are stocked with 5 to 10 shrimp per square meter. The ponds are fertilized to produce a significant amount of food for the shrimp. Pelleted feed is added as a supplement. Yield at harvest (bottom) is 800 to 1200 kg per hectare per crop.

Where natural aquatic food may make a relatively small contribution to the total protein and energy requirements of the cultured fish, it can provide essential micronutrients that will allow nutritionally incomplete supplemental feeds to be used. As biomass of fish in the pond increases, however, the fish will become more dependent on the supplemental feed for all nutrients. Channel catfish grown in earthen ponds to maximum standing crops of 2,000 kg ha^{-1} grew normally and showed no deficiency signs when vitamin C was deleted from their feed. However, when fish density was increased to 4,000 kg ha^{-1} and above, growth was normal but resistance to bacterial infection was reduced and subclinical deficiency signs occurred.

Intensive Culture of Fish in Highly Modified Environments

With these systems, natural foods are an insignificant source of nutrients. Maximum yield per unit of space and effort, and minimum accumulation of unretained nutrients in the culture system are primary concerns. Thus, highly concentrated, nutritionally complete feeds are justified. Examples of this type of production are rainbow trout cultured in spring-fed raceways and Atlantic salmon grown in net pens in the coastal areas of the sea, as shown in Figure 1.3. Also, channel catfish or marine shrimp in intensively stocked ponds may obtain negligible amounts of nutrients from natural foods. Figure 1.4 shows intensively stocked catfish ponds in the southern United States.

COMPARISON OF FEEDING FISH AND LAND ANIMALS

Feeding fish in their aqueous environment takes on dimensions beyond those considered in feeding warm blooded food animals. These include the nutrient contribution of natural aquatic organisms in pond cultures, the effects of feeding on water quality, and the loss of nutrients if the feed is not consumed immediately. Because fish cannot be fed *ad libitum*, the feeder, not the fish, decides how much feed is fed and thus a higher level of management is required to feed fish. However, the concept of feeding is the same as that applied in feeding other food animals; to nourish the animal to the desired level or form of productivity as profitably as possible. Thus, application of knowledge on the nutritional requirements of fish and the husbandry of feeding various cultured species is essential to successful aquaculture.

Nutrient Requirements

The nutrients required by fish (finfish and crustaceans) for growth, reproduction, and other normal physiological functions are similar to those of land animals. They need protein, minerals, vitamins and growth factors, and energy sources. In many cases, the qualitative and quantitative requirements are similar between fish and land animals. For example, all fishes investigated have dietary requirements for the same 10 amino acids as mammals.

Notable nutritional differences between fish and land animals are the following: (a) energy requirements are lower for fish than for warm-blooded animals, thus giving fish a higher dietary protein to energy ratio; (b) fish require some lipids that warmblooded animals do not, such as omega-3 (n-3) series fatty acids for some species and sterols for crustaceans; (c) the ability of fish to absorb soluble minerals from the water negates the dietary need for some minerals; and (d)

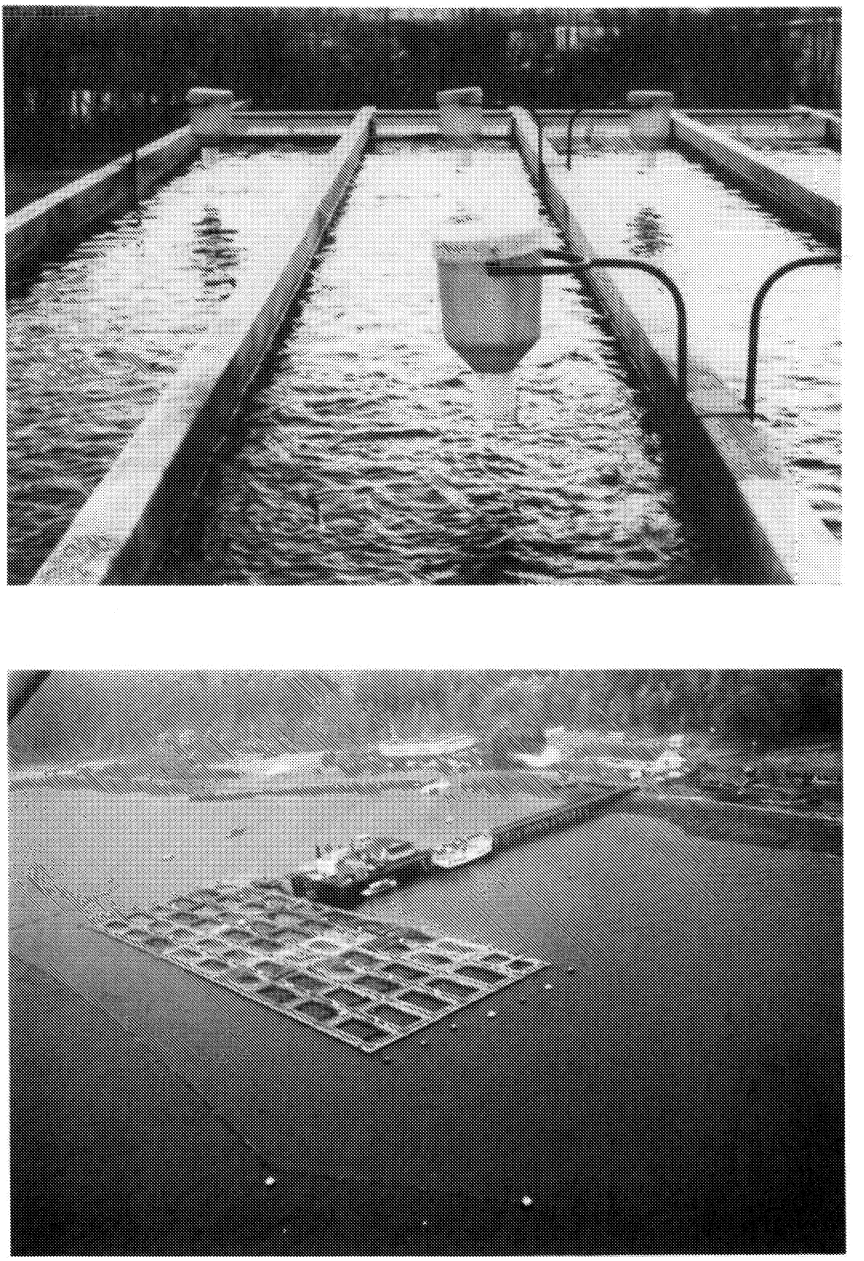


Figure 1.3 Intensive aquaculture where no natural food is available to the fish is represented by rainbow trout farming in Idaho (top) where fish are grown in raceways through which constant temperature ground water flows continuously. Nutritionally complete feed is placed in the demand feeders and the fish feed *ad libitum*. Net pen culture of salmon in Puget Sound on the coast of Washington (bottom) is also an example of intensive aquaculture.

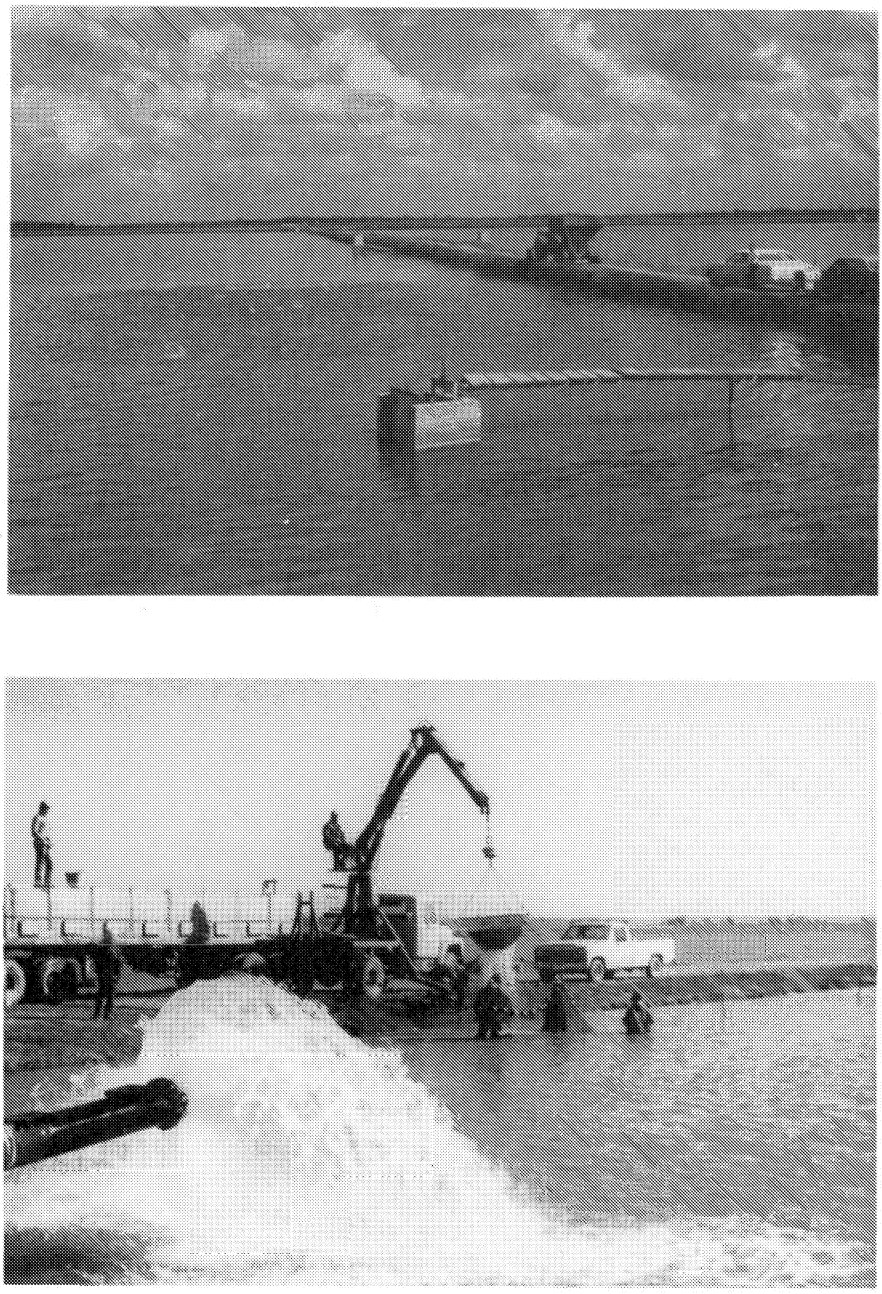


Figure 1.4 Intensive pond aquaculture is represented by commercial catfish farming in the Mississippi Delta. The top photograph shows a 10-hectare pond receiving feed. The pond will yield 4000 to 7000 kg per hectare at harvest (bottom photograph). Most of the nutrients consumed by the fish come from the processed feed.

most fish have limited ability to synthesize ascorbic acid and must depend upon dietary sources.

Also, fish are not fed *ad libitum*, as are livestock and poultry; their feed allowance is based upon the discretion of the feeder. Feed allowance influcenes the dietary nutrient requirements for maximum growth for fish. For example, Rumsey (1993) reported that the arginine requirement for maximum growth for young rainbow trout was significantly lower when the fish were fed to satiation than when the fish were given a restricted feed allowance. This interaction between feed allowance and optimum dietary nutrient concentration makes formulation of commercial feeds more difficult for fish than for farm animals. Unlike intensively reared livestock and poultry, which are fed ad libitum, fish are given a restricted feed allowance that will minimize waste. Because feedlot farm animals eat as much and as often as they want, their nutrient allowances are based upon satiation feeding. Fish are often (perhaps usually) not fed to satiation and daily feed allowance has been shown to affect fish response to various dietary nutrient concentrations. Li and Lovell (1992) found that the optimum dietary protein allowance for channel catfish fed to satiety was 26% while fish fed to less than satiation responded to higher protein concentrations.

Nutritional requirements of fish do not vary greatly among species. There are exceptions, such as differences in essential fatty acids, requirement for sterols, and ability to assimilate carbohydrates, but these often can be identified with warmwater or coldwater, finfish or crustacean, and marine or freshwater species. The quantitative nutrient requirements that have been derived for several species have served adequately as a basis for estimating the nutrient needs of others. As more information becomes available on nutrient requirements of various species, the recommended nutrient allowances of diets for specific needs of individual species will become more refined.

Feeding Practices

Because fish are fed in water, feed that is not consumed within a reasonable time represents not only an economic loss, but can reduce water quality. Therefore, feed allowance, feeding method, and water stability of the feed are factors that the fish culturist must consider, but that the livestock feeder does not. The culture environment may make valuable nutrient contributions to the fish. For example, most waters contain enough dissolved calcium to provide most of the fish's requirement. For fish that feed low on the food chain, such as shrimp and some tilapias, the pond environment can be a valuable source of protein, energy, and other nutrients.

Efficiency

Fish convert practical feeds into body tissue more efficiently than do farm animals. Cultured catfish can gain approximately 0.84 g of weight per gram of practical diet, whereas chickens, the most efficient warmblooded food animal, gain about 0.48 g of weight per gram of diet (Table 1.1). The reason for the superior food conversion efficiency of fish is that they are able to assimilate diets with higher percentages of protein, apparently because of their lower dietary energy requirement. Fish have a lower energy requirement than terrestrial animals because of a lower maintenance requirement and lower heat increment. Fish, however, do not hold an advantage over monogastric farm animals in protein conversion; as shown in Table 1.1,

Table 1.1. EFFICIENCY OF UTILIZATION OF FEED AND DIETARY PROTEIN AND METABOLIZABLE ENERGY (ME) BY FISH, CHICKEN AND CATTLE.

	Feed composition			Efficiency		
Animal	Protein (%)	ME (kcal/g)	ME-protein ratio (kcal/g)	Weight gain per g of food consumed (g)	Protein gain per g of protein consumed (g)	ME required per g of protein gain (kcal)
Channel catfish	32	2.7	8.5	0.75	0.36	21
Broiler chicken	18	2.8	16 0	0.48	0.33	43
Beef cattle	11	2.6	24 0	0.13	0.15	167

Source: Lovell (1991).

poultry convert dietary protein to body protein at nearly the same rate as fish. The primary advantage of fish over land animals is lower energy cost of protein gain rather than the superior food conversion efficiency. Protein gain per megacalorie of energy consumed is 47 for channel catfish versus 23 for the broiler chicken.

NUTRITIONAL VALUE OF FISH AS A HUMAN FOOD

The percentage of edible lean tissue in fish is appreciably greater than that in beef, pork, or poultry. For example, more than 80% of the dressed carcass of channel catfish is lean tissue; only 13.7% is bone, tendon, and waste fat (Lovell 1993). The caloric value of dressed fish is less than that of the edible portion of beef or pork. The net protein utilization (NPU) value of fish flesh, 83 (as compared to 100 for eggs), is about the same as that of red meat, 80, although the essential amino acid profiles of fish and red meat both reflect high protein quality.

Fish as well as other animal flesh, is a fair to good source of all of the nutrients except calcium and vitamins A and C. For example, 8-ounce servings of catfish and hamburger would each provide 100% of the recommended daily allowance (RDA) for an adult male of protein, niacin (vitamin), vitamin B_{12} and phosphorus; 25% to 50% of the iron, zinc, and copper; and about 25% of the vitamins thiamine, B_6, and riboflavin. However, this size serving of fish would contain only 280 calories as compared to 750 calories for a similar portion of hamburger. Nutritional values for channel catfish are presented in Tables 1.2 and 1.3. These values are fairly similar to those of other fish with the exception of lipids. Fish vary in quantity and quality of lipids; some have less than 1% total lipid and some have over 20% in their flesh. As shown in Table 1.4, wild salmon contain a high concentration of n-3 highly unsaturated fatty acids while freshwater channel catfish contain very little unless they are fed marine fish oil. By feeding fish oil, the concentration of n-3 highly unsaturated fatty acids in channel catfish can be increased significantly.

Table 1.2. PROXIMATE AND LIPID COMPOSITION OF CULTURED CHANNEL CATFISH FILLETS.

Item	Composition (g 100 g^{-1} of raw fillet)
Calories	128 0
Protein	15 6
Water	76.4
Ash	1.0
Total lipid	6.9
Saturated fatty acids	1.51
Highly unsaturated n-3 fatty acids	0.10
Cholesterol (mg)	33.40

Fish size was 0.45-0.68 kg. Values are means of four collections made at different times during the year.
Source: Nettleton et al. (1990).

Table 1.3. VITAMIN AND MINERAL CONTENTS OF RAW FILLETS OF CULTURED CHANNEL CATFISH

Vitamin	Content (mg 100 g^{-1})	Mineral	Content (mg 100 g^{-1})
Vitamin A	< 100 IU	Sodium	33
Vitamin E	< 1 IU	Potassium	315
Thiamin	0 34	Iron	0 34
Riboflavin	0 07	Copper	< 0.06
Niacin	2.30	Manganese	< 0.83
Vitamin B_6	0.19	Chromium	< 1.00
Pantothenic acid	0 57	Selenium	0.013
Ascorbic acid	< 1.00	Calcium	7 1
		Phosphorus	1.84
		Magnesium	23.00
		Zinc	0.57

Fish size was 0.45-0.68 kg Values are means of four collections made at different times during the year.
Source: Nettleton et al. (1990).

Table 1.4. TOTAL LIPID AND HIGHLY UNSATURATED FATTY ACID (HUFA) CONTENTS (AS PERCENTAGE OF RAW FILLET) OF CULTURED CHANNEL CATFISH FED A PRACTICAL DIET SUPPLEMENTED WITH THREE CONCENTRATIONS OF MENHADEN OIL, AND OF SEA-CAUGHT SALMON

	Content				
	Cultured channel catfish				
	Control	Fish oil supplement			Sea-caught
Lipid	feed	2%	4%	6%	salmon
Total fat	8 6	10 5	11 5	12 1	15
Total HUFAs	17 0	19 0	20 5	21 5	21
n-3 HUFAs	3 0	5 7	8 4	10 1	15
n-6 HUFAs	12 3	10 4	9 8	9 0	5
n-3/n-6 ratio	0 2	0 5	0 9	1 1	3

Source: Lovell (1991).

REFERENCES

LI, M. and R. T. LOVELL. 1992. Comparison of satiate feeding and restricted feeding of channel catfish with different percentages of dietary protein. *Aquac.* 103: 165-175.

LOVELL, R. T. 1991. Foods from Aquaculture. *Food Tech.* 45: 87-91.

LOVELL, R. T. 1993. Nutritional value of fish: A scientific status summary. *Food Tech.* 50: 20-30.

LOVELL, R. T., E. W. SHELL, and R. O. SMITHERMAN. 1978. Progress and prospects in fish farming. In New protein foods, eds. A.M. Altschul and H. Wilke, p. 262. Academic Press, Inc., New York.

NATIONAL RESEARCH COUNCIL. 1983. Nutrient requirements of warmwater fish and shellfish. National Academy of Sciences, Washington, D. C.

NETTLETON, J. A., W. H. ALLEN, L. V. KLATT. 1990. Nutrients and chemical residues in Mississippi farm-raised catfish. *J. Food Sc.* 55: 594-599.

RUMSEY, GARY. 1993. Protein quality in fish feeds. 20th Amm. Meeting Fish Feeding and Nutrition Workshop. Cornell University, Ithica, New York, Oct. 3, 1993.

SCHUSTER, W. H. 1952. Milkfish farming in southeast Asia. In Proc. of Indo-Pacific Fish Council, Southeast Asian Fish. Dev. Ctr., Spec. Pub.

SHELL, E. W. 1993. *The Development of Aquaculture: An Ecosystems Perspective.* Craftmaster, Inc., Opelika, AL.

USDA. 1997. National Aquaculture Statistical Service Sp. Cr., U. S. Department of Agriculture, Washington, D. C.

VILLALUZ, D. K. 1953. *Fish Farming in the Philippines.* Manila, Philippines: Bookman Co.

2 DIETARY REQUIREMENTS

Fish require dietary sources of energy and nutrients for growth, reproduction, and health. Dietary requirements for energy, protein and amino acids, vitamins, essential lipids and minerals have been established for several fish species of commercial importance. With a few exceptions, the nutrient requirements for fish are similar to those for terrestrial animals although energy requirements for fish are lower. Assimilation and metabolism of nutrients and energy are similar in these animal groups; however, there are some that are unique to fish, such as processes in osmoregulation, nitrogen excretion, and energy expenditures. To perform optimally, the fish must have all of its necessary nutrients and a supply of energy in optimum balance and quantity. These nutrients and energy may come from natural aquatic organisms or prepared feeds; however, in contemporary aquaculture, prepared feeds from commercial feedstuffs are the primary source. Thus a familiarization of the nutrients and their sources, requirements, and roles in metabolism are necessary for successful aquaculture.

ENERGY

Energy is not a nutrient--it is released during metabolic oxidation of carbohydrates, fats, and amino acids. Absolute energy requirements of the animal can be quantified by measuring either oxygen consumption or heat production. However, estimates of dietary allowances must be determined by equating animal performance with feed materials in which the amount of available energy is accountable.

Nutritional Energetics

Nutritional energetics, or bioenergetics, is the study of the balance between energy intake, in the form of food, and energy utilization by animals for life-sustaining processes such as maintenance, activity, and tissue synthesis. The original source of food energy is the sun: through photosynthesis, chloroplasts in green plants capture

radiant energy from the sun and convert it into chemical energy through the synthesis of glucose. This compound serves as the hydrocarbon source from which plants synthesize other organic compounds, primarily carbohydrates, proteins and lipids, which are the primary energy sources for fish and other animals.

The basic unit of heat energy is the calorie, defined as the amount of heat required to raise the temperature of 1 g of water 1°C, measured from 14.5°C to 15.5°C. This unit is too small for most convenient use in nutrition, therefore the kilocalorie (kcal), or 1,000 calories, is more commonly used. The international unit of work and energy, the joule, is also used: 1 joule = 0.239 calories or 1 calorie = 4.184 joules.

Gross Energy. Energy content of a substance is determined by completely oxidizing the compound to carbon dioxide, water, and other gases and measuring the heat released, which is called the gross energy of the product. This is done with an instrument called an adiabatic bomb calorimeter (Figure 2.1). Gross energy values for several pure compounds and feedstuffs are presented in Table 2.1. Note that fats (triglycerides) have approximately twice as much gross energy as carbohydrates. This is related to the relative contents of oxygen, hydrogen, and carbon in the compounds. Heat is released only when hydrogen or carbon can react with oxygen from outside of the molecule. Glucose, for example, has enough endogenous oxygen to react with all of the hydrogen in the molecule; therefore, only carbon is oxidized by exogenous oxygen. Because oxidation of a gram of carbon produces only approximately one-forth as much heat as oxidation of a gram of hydrogen, fats, which have much less endogenous oxygen than carbohydrates, yield more heat upon oxidation than carbohydrates.

Available Energy. Gross energy content of a food is not an accurate measure of its energy value to the consuming animal. Difference between gross energy and energy available to the animal for productive purposes varies widely among food materials. Digestibility accounts for most of the differences in available energy among feedstuffs for fishes.

Apparent digestible energy is the difference between the gross energy of the food consumed and the energy lost in the feces:

$$\%ADE = \frac{Food\ energy - Feces\ energy}{Food\ energy} X\ 100 \qquad (2.1)$$

Apparent digestible energy in fishes can be determined directly or indirectly. In the direct method, total food consumed and total feces excreted are measured. The indirect method involves collecting only a sample of the food and feces, and apparent digestion coefficients are calculated on the basis of ratios of energy to indicator in the food and feces. An indicator is an inert, indigestible compound in the food; it may be a natural component such as ash or fiber, or it may be an added component such as chromic oxide. Procedures for calculating apparent digestiblity of nutrients and energy in feedstuffs for fish are discussed in Chapter 3.

Metabolizable energy, which represents digestible energy less energy lost from the body through gill and urinary wastes, is more difficult to determine. The fish must be confined in a metabolism chamber to collect gill and urine wastes. The

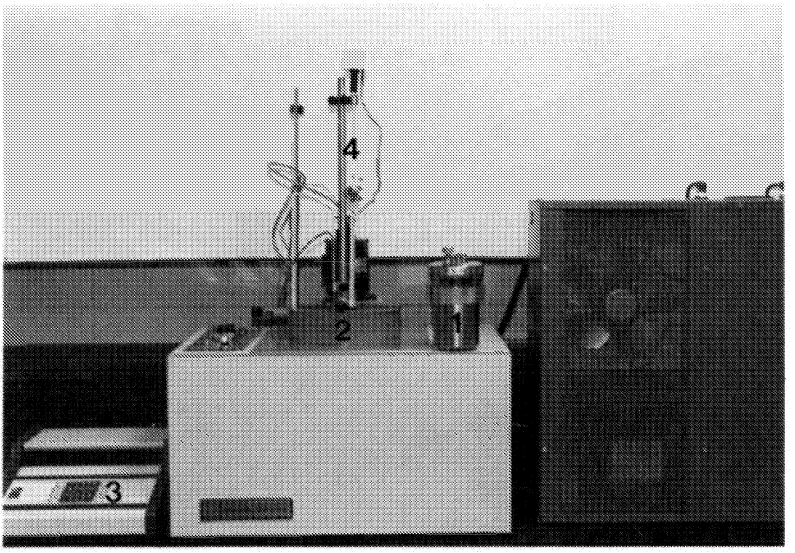


Figure 2.1 An oxygen bomb calorimeter is used to measure gross energy values of foods. It consists of a bomb (1), in which the food is burned in a concentrated oxygen atmosphere, enclosed in an insulated jacket (2) containing water which absorbs the heat of combustion, controller (3), and mercury (4) or electronic (3) thermometry to indicate temperature rise of water.

fish are force-fed and total fecal, gill, and urinary wastes are collected. Reliable metabolizable energy values have been determined for feedstuffs with rainbow trout; however, some species, including channel catfish, will not adapt to a metabolism chamber. Apparent metabolizable energy (AME) is defined according to equation (2.2):

$$\%AME = \frac{Food\ energy - (Energy\ lost\ in\ feces, urine, gills)}{Food\ energy} X\ 100 \quad (2.2)$$

Use of metabolizable energy instead of digestible energy to evaluate fish feeds would allow a more absolute estimate of the dietary energy metabolized by the tissue of the animal; also, the National Research Council Committee on Animal Nutrition has adopted this system. Practically, however, metabolizable energy offers little advantage over digestible energy in evaluating useful energy in feeds for fish because energy loss in digestion accounts for most of the variation in recoverable energy among foods. Energy losses through gill and urinary excretions by fish do not vary among foods nearly as much as fecal energy losses and are smaller than nonfecal energy losses by mammals and birds. Furthermore, confinement of the fish in metabolism chambers to determine metabolizable energy is difficult for some species and stresses the fish. Digestible energy is easier to determine and the fish are not stressed when allowed to feed voluntarily. Comparison of metabolizable energy

Table 2.1. GROSS ENERGY VALUES FOR SOURCES OF CARBOHYDRATES, FATS, AND PROTEIN DETERMINED BY BOMB CALORIMETER

Substrate	kcal g^{-1}
Glucose	3.77
Corn starch	4.21
Triglyceride:	
Beef fat	9.44
Soybean oil	9.28
Casein	5.84

and digestible energy values for various feedstuffs for rainbown trout (Smith, 1997) show that the ratio of digestible energy to gross energy varies greatly among feedstuffs, but the ratio of metabolizable energy to digestible energy varies only slightly. This indicates clearly that digestion accounts for most of the variation in available energy among foods for fish.

Energy Balance in Fish. The fate of ingested energy for fish is illustrated in Figure 2.2. There are several places where energy is lost between ingestion and recovery in animal products. Losses occur in feces, in gill and urine excretions, and as heat. Digestible energy represents ingested energy corrected for fecal energy loss; metabolizable energy represents digestible energy corrected for energy lost through gill and urine excretions; and recovered energy, such as weight gain, represents metabolizable energy less energy lost as heat. Heat losses occur primarily by two processes: the heat increment, which represents energy cost of digestion, nutrient metabolism and excretion; and maintenance, which includes basal metabolism and voluntary activity.

Heat increment is the increase in heat production subsequent to ingestion of feed. Factors contributing to heat increment include the digestion and absorption processes, the transformation and interconversion of the substrates and their retention in tissues, and the formation and excretion of metabolic wastes. The main biochemical basis for heat increment in mammals and birds is the energy required for the ingested amino nitrogen to be deaminated and excreted; however, this represents less of an energy loss in fish because they can eliminate nitrogenous end products of protein metabolism (ammonia) without the need to synthesize urea, uric acid, or other similar compounds. Energy expenditures associated with diet ingestion and digestion are small compared with that associated with metabolic work.

The energy cost of synthesis for urea and uric acid is 3.1 and 2.4 kcal g^{-1} N, respectively (Martin and Blaxter, 1965). In contrast, ammonia, the primary nitrogenous waste product of protein catabolism in fish (Goldstein and Forster, 1970), can be readily released into the water through the gills. Thus, energy expenditure on urea or uric acid synthesis is not needed (Cowey, 1975). Cho et al. (1982) found that heat increment for rainbow trout at 15°C was 5 to 15 percent of the gross energy consumed and fell as the ratio of protein to energy decreased. The heat increment for livestock can be as much as 20 to 30 percent of the ingested energy (Farrell, 1974; National Research Council, 1984).

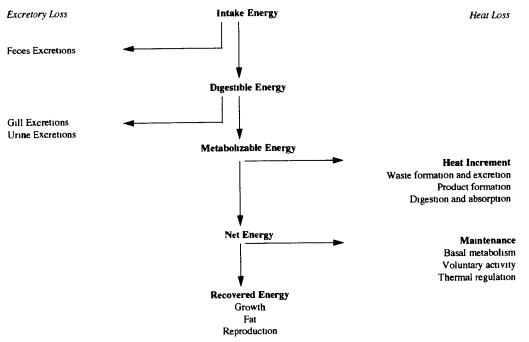


Figure 2.2 Partitioning gross energy in food consumed by fish.
Source: National Research Council, 1993.

Maintenance energy is that spent for basal metabolism, such as respiration, transport of ions and metabolites, body constituent turnover, circulation, voluntary or resting activity and, in the case of homeothermic animals, thermoregulation of body temperature. Since fish do not regulate body temperature and they expend less energy in maintaining position in the water than do terrestrial animals in maintaining their posture, the maintenance energy requirement of fish is lower than for land animals. Cho and Kaushik (1990) determined fasting metabolic heat production in rainbow trout (16 to 145 g in size), in kcal/fish/day, to be $8.85W^{0.82}$, where W is body weight. This was determined indirectly from oxygen consumption measured. Smith (1989) reported a fasting heat production value of $4.41W^{0.63}$ for rainbow trout weighing 4 to 50 g at 15°C where fasting heat production was measured directly by placing the fish in a calorimeter. When these fasting heat production values for fish are compared with $70W^{0.75}$ for mammals and $83W^{0.75}$ for birds (Brody, 1945), it is apparent that the fasting heat production of fish is much lower. The maintenance energy requirements of fish are one-tenth to one-twentieth of those of homeothermic animals of similar size in a thermoneutral environment (Brett, 1973). The lower maintenance requirement for fish means a greater percentage of the net energy that is not dissipated as heat but retained within the body as new tissue or recovered energy.

Energy Sources

Because fishes evolved in an aqueous environment where carbohydrates were scarce, their digestive and metabolic systems seem to be better adapted to utilization of protein and lipids for energy than carbohydrates. Some fishes, however, such as warm-water herbivores or omnivores, can digest and metabolize carbohydrates relatively well. Salmonids utilize carbohydrates poorly. Table 3.1 in chapter 3 compares digestibility of protein, fat and carbohydrate in various feedstuffs for channel catfish, Nile tilapia, and rainbow trout. The digestion coefficients for the high protein feedstuffs are similar, but the digestion coefficients for the carbohydrate sources are markedly lower for the rainbow trout. Although some fishes consume macrophytes (higher aquatic plants) readily, they digest native cellulose poorly.

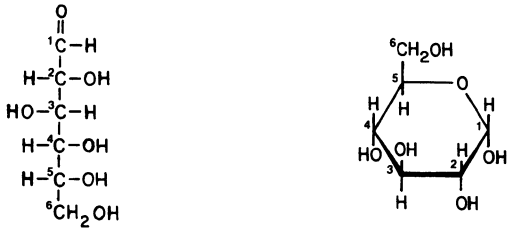


Figure 2.3 Glucose.

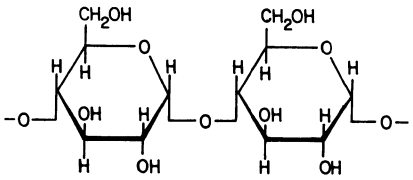


Figure 2.4 Starch (α 1-4 linkage).

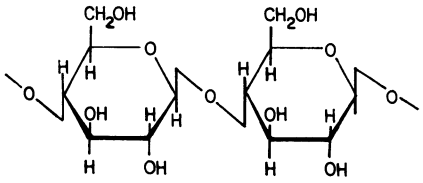


Figure 2.5 Cellulose (ß 1-4 linkage).

Carbohydrates. The basic chemical structure of carbohydrates consists of sugar units that are aldehyde or ketone derivatives of polyhydric alcohols containing carbon, hydrogen, and oxygen. Hydrogen and oxygen are usually in the same ratio as in water and as in glucose (Figure 2.3). Carbohydrates exist in nature as ringed structures and are more accurately depicted in Haworth perspective on the right.

Carbohydrates are classified by the number of "sugar" units in the molecule. *Monosaccharides* are one-sugar units, such as glucose (6-carbon) and ribose (5-carbon). *Disaccharides* are conjugates of two monosaccharides. Examples are maltose, composed of two glucose units, and sucrose, composed of glucose plus fructose. *Polysaccharides* are long-chain polymers of monosaccharides. The two most important carbohydrates in animal nutrition are starch and cellulose, each being polymers of glucose units; the difference between the two is the type of glucose molecules. Starch (Figure 2.4) contains α-D-glucose (glucose units are joined by a 1-4 linkage) and cellulose contains ß-D-glucose (glucose units are joined by 1-4 linkage).

Cellulose (Figure 2.5) is the major structural component of plant cell walls. It is highly insoluble at neutral pH and is indigestible to monogastric animals, including fish. Cellulose has a flat, band-like structure, instead of a helical form as starch, and the molecules are held more firmly to each other by hydrogen bonding.

The endosperm of grains is composed mostly of starch and this is the major source in animal feeding. Two forms of starch are found in the starch granules in grains: amylose and amylopectin. Amylose is a straight-chain, α-1,4-glucose polymer. It comprises 20% to 30% of the starch granule and is soluble in warm water. The less soluble amylopectin is a branched chain glucose polymer; the α-1,4 straight chain is branched by an α-1,6 linkage to a side chain. Starch granules are different in morphology and solubility among plant species. Some are quite resistant to rupture, which is necessary for digestion. Moist heating ruptures, or gelatinizes, the granule and increases solubility and digestibility of the starch. Glycogen is the carbohydrate energy reserve in animal tissue, mainly the liver. It is similar to amylopectin in molecular weight, but its 1,6 linked side chains are shorter and it is more soluble in water.

Although carbohydrates are a significant source of energy and are components of a number of body metabolites, such as blood glucose, nucleotides, and glycoproteins, they are not essential nutrients. Brambila and Hill (1966) showed that chicks can grow normally on carbohydrate-free diets if the calorie/protein ratio is optimum and if triglycerides are included in the diet to supply glycerol for carbohydrate synthesis. Several studies have shown that fish grow satisfactorily and show no pathologies when fed carbohydrate-free diets. However, if carbohydrates are not provided in the diet, other compounds, such as protein or lipids are metabolized for energy.

Enzymes for digestion of carbohydrates appear to be present in fish. Also, the enzymes for the major carbohydrate metabolic pathways, such as glycolysis, glycogen synthesis, gluconeogenesis and the tricarboxylic acid cycle, have been found in fish. However, the relative activity of these enzymes and hormonal regulation of carbohydrate metabolism in fish is not well known and may be somewhat different than in mammals.

Ability to utilize carbohydrates differs among fish species. Numerous studies have indicated that the freshwater species, channel catfish, common carp and

Nile tilapia, use higher dietary levels than salmonids or various marine species, such as yellowtail. A general recommendation has been to not use over 25% starch in salmonid and marine fish diets but the freshwater species can use more than this.

Glucose clearance from the blood subsequent to oral administration of glucose or starch is much slower in fish than in warm blooded animals. A glucose diet was more hyperglycemic than a starch diet in channel catfish, apparently because starch is absorbed more slowly than glucose. The reason for the prolonged hyperglycemia in fish following a carbohydrate meal is not well understood. It has been assumed to be due to low levels of endogenous insulin; however, determinations have shown insulin levels in fish comparable to those in mammals. Another explanation offered is fewer insulin receptors in muscles; however, this proposition has not been consistent when evaluated with high and low carbohydrate diets. Possibly other factors besides impaired insulin release and receptor binding are responsible for slow blood glucose clearance in fish.

Lipids as Energy Sources. Lipids are a large, varied group of organic compounds that are insoluble in water but soluble in organic (nonpolar) solvents. They represent concentrated energy sources, pigments, and essential growth factors for fish. The lipids that are important energy sources are fats, or triglycerides. Chemically, these are esters of fatty acids with glycerol. One mole of glycerol unites with three similar or different fatty acids, with the loss of three moles of water (Figure 2.6). R in the model represents the hydrocarbon chain in the fatty acid. Length of the carbon chain in most of the fatty acids in land plants and animals is 14 to 18 carbons and in fish, up to 22 carbons.

The chain length and number of double (unsaturated) bonds determines physical and nutritional properties of fats. Fatty acid composition of triglycerides from several sources is presented in Table A.3 in Appendix A on Composition of Feed Ingredients. Generally, the fat stores in warmblooded animals are highly saturated (few double bonds), while fats from plants are more unsaturated; however, chain length of fatty acids from land animals or plants is seldom longer than 18 carbons. Fats of farm-raised channel catfish resemble those of grain-fed livestock. Fats from marine fishes, however, have significant amounts of polyunsaturated fatty acids 20 carbons and longer in length. They obtain these through the food chain from marine algae. Cultured salmonids, which are fed marine fish oil in their diets, will have large amounts of polyunsaturated fatty acids in their body fat.

Proteins as Energy Sources. Fish use protein efficiently as a source of energy. A higher percentage of the digested energy in proteins is metabolizable in fish than in land animals which gives protein a higher productive energy value for fish. This is attributed to the efficient manner of nitrogen excretion in fish, where 80 to 90% of the nitrogen is excreted as ammonia through the gills and requires much less energy than excretion as urea or uric acid. Although fish can catabolize protein more efficiently than land animals, excessive amounts of protein in the diet, in relation to nonprotein energy, suppresses growth rate of fish. Studies with channel catfish showed that increases in dietary protein above 45%, without proportionate increases in nonprotein energy, reduced rate of weight gain.

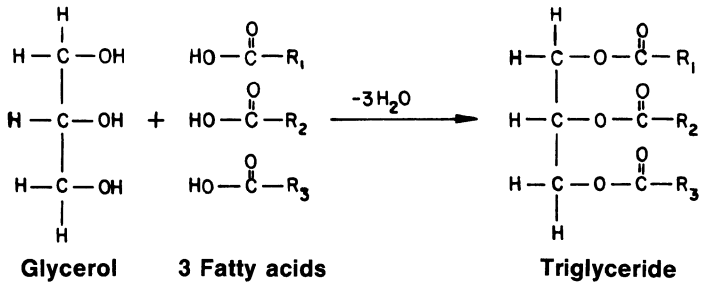


Figure 2.6 Lipids.

Energy Requirements of Fish

A dietary excess or deficiency of useful energy can reduce growth rate. Because energy needs for maintenance and voluntary activity must be satisfied before energy is available for growth, dietary protein will be used for energy when the diet is deficient in energy in relation to protein. On the other hand, a diet containing excess energy can restrict food consumption and thus prevent the intake of the necessary amounts of protein and other nutrients for maximum growth. Excessively high energy/nutrient ratios can also lead to deposition of large amounts of body fat. This can be undesirable in food fish if it reduces the dress-out yield and shortens shelf life of the frozen fish; however, large body fat stores may be desirable in hatchery fish raised for release.

Information on energy requirements of fish has been slow to accumulate. In practice, fish nutritionists have given priority to meeting the requirements for protein, major minerals, and the vitamins, and generally have allowed energy to take care of itself. A deficiency or excess of energy will not have great effect on the health of fish. Also, practical feeds for most species made with commonly available ingredients are not likely to be extremely high or low in energy when the protein requirement is met. For example, a 32% crude protein catfish feed containing soybean meal (50%), grain (40%), and animal byproduct (8%), plus vitamin and mineral supplements (2%), contains approximately 2.9 kcal of digestible energy per gram; this provides a digestible energy (kcal) to digestible protein (grams) ratio of approximately 10 to 1, which seems to be near optimum.

Livestock and poultry nutritionists have long recognized the importance of meeting energy requirements in formulating practical feeds. Some feeding tables present protein or amino acid allowances as a function of dietary energy plane; that is, as the energy concentration of the diet increases, the protein percentage increases proportionally. The rationale here is that in *ad libitum* feeding, energy intake regulates food consumption and thus the amount of nutrients the animal ingests daily. Fish, however, are not fed *ad libitum*, and often not to satiety, so nutrient consumption would be controlled by feed allowance and not energy concentration of the diet.

Ratios of digestible energy to digestible protein (kcal g^{-1}) for maximum weight gain for several fish species have been measured in growth studies and are presented in Table 2.2. Values range from 8.5 to 12.3 kcal/g and are substantially lower than energy-protein ratios for swine and poultry, which range from 16.6 to 25.0 kcal g^{-1} (National Research Council, 1988, 1994). The reason the energy-

Table 2.2. OPTIMUM PROTEIN : ENERGY RATIO FOR GROWTH FOR DIFFERENT FISH

Species	Digestible Protein (DP) (%)	Digestible Energy (DE) (kcal g^{-1})	DE/DP (kcal g^{-1})	Fish Size (g)
Channel catfish	22.2	2.33	10.5	526
	28.8	3.07	10.6	34
	27.0	2.78	10.3	10
	27.0	3.14	11.6	266
	24.4	3.05	12.3	600
Red Drum	31.5	3.20	10.2	43
Hybrid bass	31.5	2.80	8.9	35
Nile tilapia	30.0	2.90	9.7	50
Common carp	31.5	2.90	9.3	20
Rainbow trout	33.0	3.60	10.9	90
	42.0	4.10	9.5	94

Adapted from National Research Council, 1993.

protein ratio for fish is lower than that for farm animals is not because fish have a higher protein requirement (fish convert dietary protein into tissue protein about as efficiently as warm blooded animals [Smith, 1989]), but because fish require less energy for maintenance and nitrogen excretion.

Since lipid is the primary nonprotein energy source in salmonid diets, the energy-protein allowance for these diets is sometimes reported as the ratio of lipid to protein. The optimum combination for weight gain for rainbow trout was 35 to 36% protein and 15 to 20% lipid (Cho et al.1982).

Mangalik (1986) determined energy and protein requirements of channel catfish by growth trials. He fed channel catfish of three sizes (1 g, 20 g and 100 g) as much as they would consume with diets containing various energy and protein concentrations and used protein gain as a measure of growth rate. Daily digestible energy requirement for maximum growth was 16.8 kcal 100 g^{-1} weight for 1-g to 3-g fish, decreasing to 5 kcal 100 g^{-1} weight for 100-g to 250-g fish. As shown in Table 2.3, protein requirement changed at almost the same rate as the energy requirement with increase in fish size so that the optimum ratio of digestible energy to protein increased only slightly as fish size changed from 3 g to 266 g in size (Table 2.3).

Energy requirements of fish may be calculated empirically, based on energy losses and expected energy recovery, if reliable information on energy balances in the animal under a given set of conditions is available. Cho and Kaushik (1990) constructed a model for calculating the digestible energy required to grow 1 kg of rainbow trout, from 1 g to 100 g size at 15°C, based on derived heat and excretory losses and estimated recovery of energy in the fish. The model indicated that 3.56 Mcal of digestible energy would be required to produce 1.91 Mcal of recovered energy in 1 kg of fish biomass with a recovered energy/digestible energy efficiency ratio of 0.54, which is comparable to a value of 0.56 from growth studies reported for channel catfish (Gatlin et al., 1986a). However, several factors significantly affect energy balance in fish, such as diet composition, feeding rate, and composition of body gain. Therefore, this approach to calculating energy requirements for production diets must be used cautiously until sufficient information is available to establish reliable energy budgets for a variety of production conditions for a specific aquaculture species.

Table 2.3. PROTEIN AND DIGESTIBLE ENERGY (DE) REQUIREMENTS BY VARIOUS SIZES OF CHANNEL CATFISH FOR MAXIMUM PROTEIN SYNTHESIS

Fish size (g)	Protein (g/100g fish/day)	DE (kcal/100g fish/day)	DE:Protein ratio (kcal/g)
3	1.64	16.8	10.2
10	1.11	11.4	10.3
56	0.79	9.0	11.4
198	0.52	6.1	11.7
266	0.43	5.0	11.6

PROTEINS AND AMINO ACIDS

Amino acids are the structural components of proteins. The basic structure of an amino acid is illustrated in Figure 2.7. The essential components are a carboxyl group (-COOH) and an amino group ($-NH_2$) on the alpha carbon atom. All have the basic structure shown where R is the remainder of the molecule attached to the alpha carbon. Amino acids are linked together by a peptide bond to form proteins. Proteins contain carbon (50-55%), hydrogen (6.5-7.5%), nitrogen (15.5-18%; an average value of 16% is assumed), oxygen (21.5-23.5%), and usually sulfur (0.5-2.0%).

There are 18 amino acids that can be found in most any plant or animal protein, although proteins usually contain 22 to 26 amino acids. Amino acids can be conveniently classified into groups according to the series of organic compounds in which they belong. The formulas of 22 are presented in Figures 2.8 through 2.13.

Types of protein found in the fish body are generally based upon function or solubility. *Fibrous proteins* are highly insoluble (indigestible) proteins and include collagen, elastin, and keratin. Collagen is the component of connective tissues, bone matrix, skin, scar tissue, fins, gill operculum, and blood vessels. Elastins are found in arteries, tendons, and other stretch tissues. Keratins are found in hair and hooves of land animals, but in only small amounts in fish. *Contractile protein* is the muscle protein complex. Three proteins, actin, tropomyosin B, and myosin, take part in muscle contraction. Muscle protein is highly digestible and has high nutritional value. *Globular proteins* are proteins extractable from tissue with water or dilute salt solutions. They represent enzymes, protein hormones, and proteins of the serum (soluble) fraction of blood.

Essential Amino Acids

Amino acids can be divided into two nutritional groups, *essential* and *nonessential*. The essential amino acids are those that the animal cannot synthesize or cannot synthesize in sufficient quantity to support maximum growth. The nonessential amino acids are those that can be synthesized by the animal in quantity to support maximum growth, provided that amino nitrogen is available. Most monogastric animals, including fish, require the same 10 essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In the rat, several of the essential amino acids, arginine, histidine, isoleucine, leucine, methionine, phenylalanine, tryptophan, and valine, can be replaced by their corresponding α-hydroxy or α-keto analogs, indicating that

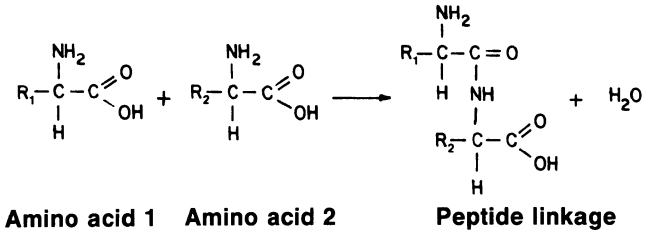


Figure 2.7 Amino acids are linked together by peptide bonds to form proteins.

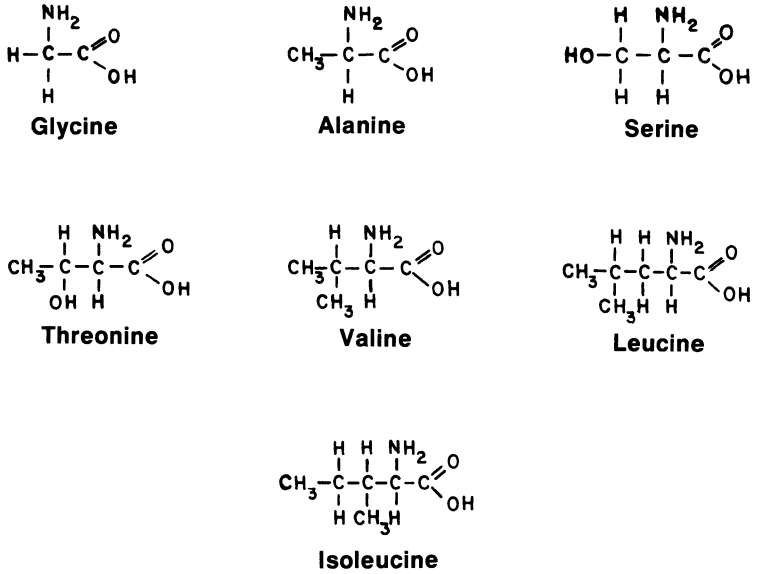


Figure 2.8 Aliphatic series of amino acids.

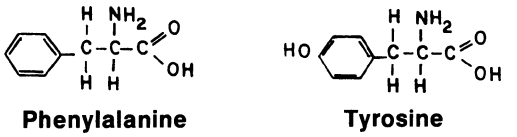


Figure 2.9 Aromatic series of amino acids.

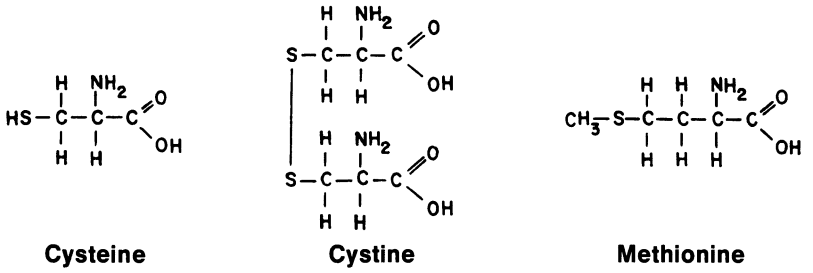


Figure 2.10 Sulfur-containing series of amino acids.

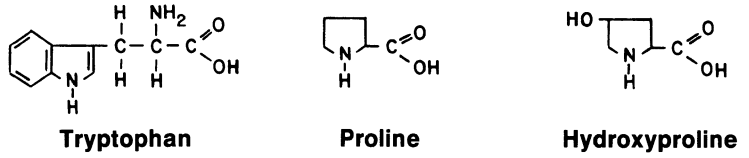


Figure 2.11 Heterocyclic series of amino acids.

Figure 2.12 Acidic series of amino acids.

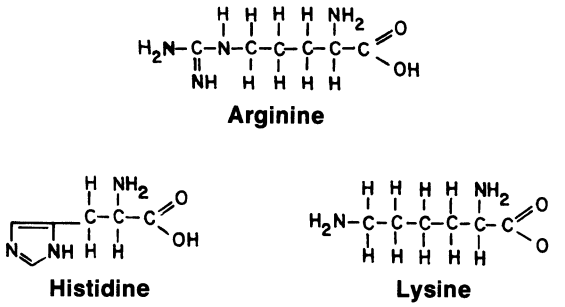


Figure 2.13 Basic series of amino acid.

the carbon skeleton is what the animal is unable to synthesize. However, these analogs for threonine and lysine are not utilized for growth by the rat.

Qualitative requirements for amino acids are determined in fish by feeding a purified diet containing isolated crystalline amino acids as a control diet, and feeding test diets similar to the control except that one amino acid at a time has been removed. Test diets that produce no growth or markedly less than the control represent amino acids that are essential to the fish. Quantitative requirements for essential amino acids are determined by feeding graded levels of one amino acid at a time in a test diet containing crystalline amino acids or a combination of a purified protein and crystalline amino acids. The amino acid profile of the test diet is usually designed to be similar to that in the fish muscle. (In fact, the essential amino acid profile of fish muscle has been found to agree closely with the dietary amino acid profile for optimum growth of the fish.) Growth data from the amino acid feeding trials are equated to concentrations of the amino acid in the diet, and the optimum dietary requirement is determined by estimating or calculating the break point in the response curve. In early studies involving salmon, the requirement was estimated by visual inspection of the growth response curve. Later, studies with channel catfish used the regression analysis method of Robbins et al.(1979) to determine the break point in the growth response curve.

Actually, the response of fish to dietary increments of a limiting amino acid, or other nutrient, is exponential and does not break at one particular point. Thus, the "diminishing returns" characteristic of the response curve should be taken into account when assessing the efficiency of incremental increases in amino acids in the diet as the response approaches maximum. Derivation of a growth response function (nonlinear regression equation) and supplementing with economic data to determine the most economical concentration of the limiting amino acid to use is recommended by Zeitoun et al. (1976). Santiago (1985) determined two requirements for essential amino acids with Nile tilapia, one for maximum growth (Y_{max}) and one for a level of growth less than maximum (Y_1) but within the 95% confidence limit of Y_{max}. This is illustrated, using arginine as an example, in Figure 7.3 in chapter 7.

In some cases, fish can partially substitute a nonessential for an essential amino acid. Channel catfish grow satisfactorily when methionine is the only sulfur-containing amino acid in the diet, but not when cystine is the only sulfur-containing amino acid; however, cystine can replace about 60% of the methionine. Tyrosine, a nonessential aromatic amino acid, can replace about one-half of the channel catfish's requirement for phenylalanine, an essential aromatic amino acid.

Dietary imbalances of amino acids can cause reduced performance by animals through amino antagonism or toxicity. When some amino acids are fed in excess of their required levels, they cause an increase in the requirement for other structurally similar amino acids, or *amino acid antagonism*. In some instances, however, dietary excesses of certain amino acids are directly toxic and their negative effects cannot be ameliorated by additions of other amino acids; this is *amino acid toxicity*. Fish diets containing practical feedstuffs, such as grain byproducts, oil meals, and animal byproducts, are not likely to be so seriously imbalanced, but special diets could be.

For most essential amino acids, deficiency is manifest as a reduction in weight gain. In some species of fish, however, a deficiency of methionine or tryptophan leads to pathologies, because these amino acids are not only

incorporated into proteins but also used for the synthesis of other compounds. Salmonids suffer from cataracts when given a diet deficient in methionine or sulfur amino acids. Cataracts also occur as a consequence of tryptophan deficiency in rainbow trout; the developmental pattern of the cataracts is similar to that occurring in methionine deficiency. Tryptophan deficiency can lead to scoliosis (lateral curvature of the vertebral column) and to a derangement of mineral metabolism in some salmonids. The condition may be related to a decline in levels of the brain neurotransmitter serotonin, which is formed from tryptophan, because inclusion of serotonin in tryptophan-deficient diets greatly reduces the incidence of scoliosis (Akiyama et al., 1986).

The quantitative amino acid requirements of five fish species are presented in Table 2.4. The data are presented as a percentage of dietary protein. This is because different concentrations of dietary protein are used in practice, based upon such factors as fish size, feeding strategy, and economics. It is assumed that the amino acid requirement will change in proportion to the change in dietary protein concentration, over a practical range in protein percentages. Except for arginine, the amino acid requirements of fish follow a relatively similar trend as those for mammals. The arginine requirement of fish and birds is higher than that of mammals because they do not synthesize appreciable quantities of urea. Arginine is a byproduct of the ornithine cycle in the urea synthesis process in mammals. The amino acid requirements among the species presented in Table 2.4 are relatively similar; however, there are some exceptions, such as methionine plus cystine. There is probably less variation among fishes than these initial data indicate. Factors such as fish size, temperature, genetics, feeding rate, energy concentration and other diet factors, and method of data analysis can influence the reported requirement for amino acids.

Meeting Amino Acid Requirements in Practical Feeds

Amino acid requirements for fish of the National Research Council, shown in Table 2.4, are presented on the basis of being 100% available, whereas amino acid composition of practical feedstuffs is usually presented on a total content basis. Thus, in formulating fish feeds to meet amino acid requirements, the total amino acid content of the feed ingredients must be corrected for availability (digestibility). Digestion coefficients for individual amino acids in several feedstuffs were determined for channel catfish (Wilson et al. 1981) and Atlantic salmon (Anderson et al., 1981). Digestible amino acid content of these feedstuffs is presented in Table A.2 in Appendix A. Digestibility of some amino acids varies markedly among feed ingredients; for example, apparent digestibility of lysine is approximately 25% lower in cottonseed meal than soybean meal. Generally, though, the digestibility of the protein may be used in estimating the availability of amino acids in the feedstuff when digestibility of individual amino acids is not known, but digestibility of the protein is.

Use of Isolated Amino Acids in Fish Feeds

The research literature is unclear on the efficacy of supplementing fish feeds with isolated amino acids. Individual supplementation of soybean meal with lysine, methionine, histidine, or leucine did not improve growth rate of rainbow trout, but collective supplementation did increase growth rate. Methionine supplementation of commercial soybean meal improved growth rate of rainbow trout, but methionine

Table 2.4. ESSENTIAL AMINO ACID REQUIREMENTS OF CHANNEL CATFISH, RAINBOW TROUT, PACIFIC SALMON, COMMON CARP AND NILE TILAPIA (PERCENTAGE OF DIGESTIBLE PROTEIN)

Amino acid	Channel catfish	Rainbow trout	Pacific salmon	Common carp	Nile tilapia
Arginine	4.3	4.4	6.0	4.3	4.2
Histidine	1.5	2.1	1.8	2.1	1.7
Isoleucine	2.6	2.6	2.2	2.5	3.1
Leucine	3.5	4.1	3.9	3.3	3.4
Lysine	5.1	5.3	5.0	5.7	5.1
Methionine + cystine	2.3	2.9	4.0	3.1	3.2
Phenylalanine + tyrosine	5.0	5.3	5.1	6.5	5.5
Threonine	2.0	2.4	2.2	3.9	3.8
Tryptophan	0.5	0.6	0.5	0.8	1.0
Valine	3.0	3.5	3.2	3.6	2.8

Source: National Research Council (1993).
Note: These values have been determined with highly purified ingredients and are assumed to be near 100% digestible.

supplementation of reheated soybean meal did not. There was no benefit in supplementing soybean meal with methionine or lysine in catfish diets; however, supplementing soybean meal with both methionine and lysine improved carp diets. Several studies have established that feeding channel catfish supplemental crystalline lysine with peanut meal or cottonseed meal, both being severely deficient in lysine, improved growth response in the fish.

Apparently fish respond to supplementation of isolated amino acids when the diet is markedly deficient in the amino acid, but little is known about the relative bioavailability of isolated amino acids compared to protein-bound amino acids. There is concern that fish do not utilize dietary crystalline amino acids as well as chickens or swine, or at least not with conventional once-per-day fish feeding practices. Growth assays with swine (Batterham et al., 1979) and chickens (Izquierdo et al., 1988) showed that free lysine was utilized as efficiently as protein-bound lysine with *ad libitum* feeding. When swine were fed once daily, however, isolated lysine was inferior to protein-bound lysine. Young carp fed once daily on a diet containing a high level of crystalline amino acids excreted up to 40% of the free amino acids intact through the gills and kidneys (Murai 1985). Increasing feeding frequency of the carp to four times daily improved utilization of the crystalline amino acids. This supports the concept that fish, like swine, do not utilize supplemental crystalline amino acids well when fed once per day because the crystalline amino acids are not absorbed from the gut at the same time as amino acids from the ingested protein.

Studies were conducted by Zarate and Lovell (1997) to determine if free lysine was used as efficiently as protein-bound lysine in practical diets by channel catfish. A lysine-deficient basal diet with sesame meal as the primary protein source was supplemented with various levels of lysine by adding free lysine or by substituting increments of sesame meal with soybean meal. Sesame protein is deficient in lysine, while soybean protein is not; other essential amino acids are present in near equal amounts in the two proteins. Growth trials were conducted

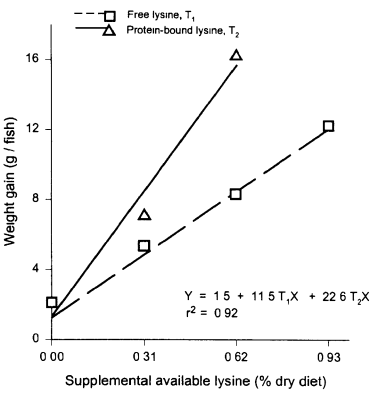


Figure 2.14 Growth response curves for channel catfish fed diets containing variable concentrations of free lysine or protein-bound lysine. Comparison of slopes of the response curves showed that protein-bound lysine (from soybean meal) was used 63% more efficiently than free lysine (lysine-HCl).
Source: Zarate and Lovell, 1997.

and regression of weight gain on dietary concentration of lysine was established. Comparison of the growth response curves, shown in Figure 2.14, indicated that protein-bound lysine from soybean meal was used 63% more efficiently than the free lysine. The research showed that free lysine disappeared from the stomach before the protein-bound lysine, which suggests that the free lysine was absorbed from the digestive tract faster than the protein-bound form.

Protein Requirements of Fish

When feeding guides recommend a minimum level of protein for a fish feed, it should be assumed that the protein is balanced in the essential amino acids. Reports in the technical literature have indicated that the optimum level of protein in feeds for growth of fish has ranged from 25% to 50%. In all of these studies the researchers were probably justified in making their conclusions that a specific percentage of protein was optimum under their experimental conditions because a number of factors influence the growth response of fish to feeds containing different levels of protein. Some of these are size of fish, water temperature, feed allowance, amount of nonprotein energy in the feed, quality of the protein, and natural food availability.

Fish have higher protein requirements during early growth than during later phases of growth. Mangalik (1986) showed that 3-g channel catfish required almost four times as much protein per 100 g body weight per day as 250-g fish for maximum growth, but ratio of protein to energy in the diet changed only slightly (as shown in Table 2.3). He also demonstrated that the smaller channel catfish could grow as well from a 27% protein diet as from a 38% protein diet when the energy level in the diet was low, but when the energy level increased, diet consumption decreased and the low protein diet would not support maximum growth.

Natural pond food consumed by fish can be an important protein source. This is influenced by feeding behavior of the fish, natural pond productivity, and density of fish in the pond. At low stocking densities, tilapia and shrimp obtain a

significant amount of protein from pond organisms. Pond sources of protein are primarily of animal origin and are high in quality and contain at least 50% protein on a moisture-free basis. Thus, a significant dietary contribution from this source would reduce the protein requirement of the supplemental diet. For example, tilapia and shrimp, which feed efficiently on pond organisms, grow as well on relatively low protein diets (25% or less) as on higher protein diets when natural pond food is a significant part of their diet, but they respond to higher protein diets in an environment with limited natural food.

When fish are underfed, they usually respond to higher amounts of dietary protein. This has been demonstrated in a series of pond feeding experiments with channel catfish (Li and Lovell, 1991). Reasons for interaction between feeding rate and dietary protein percentage for maximum growth are not completely clear. It is reasonable that a high protein diet will come closer than a low protein diet to supplying the fish's protein need for growth during restricted feeding. Also, when fish are underfed, a higher percentage of the dietary protein will be used to meet the metabolic energy needs of the fish unless the energy/protein ratio of the diet is increased.

VITAMINS

Vitamins are organic compounds required in the diet in relatively small quantities for growth, health, and function in animals. A vitamin that is a dietary essential for some animals may not be for other species. For example, humans and other primates, guinea pigs, and most fishes require vitamin C in the diet, but most land animals do not. Although the requirements for vitamins are small, deficiencies of these micronutrients can cause symptoms ranging from poor appetite to severe tissue deformities. Deficiency signs for various nutrients identified in several fish species are presented in Table 2.5.

Vitamins are classified as water soluble and fat soluble. Eight of the water soluble vitamins are required in relatively small quantities and have primarily coenzyme functions; they are known as the water-soluble B complex. Three water-soluble vitamins that have functions other than coenzymes and are sometimes required in larger quantities may be referred to as the macrovitamins. This group includes vitamin C, myo-inositol, and choline. For some warmwater fishes, intestinal synthesis by microorganisms supplies the requirement for certain vitamins. Thus, deficiency signs result only in those cases when antibiotics are fed along with a deficient diet. A constant supply of essential water-soluble vitamins is required to prevent deficiency signs in fish, since these vitamins are not stored very long in body tissues.

The fat-soluble vitamins, A, D, E, and K, are absorbed in the intestine along with dietary fats. These vitamins are stored by animals if dietary intake exceeds metabolic needs. Thus, animals can accumulate enough fat-soluble vitamins in their tissues to produce a toxic condition (hypervitaminosis). This has been demonstrated in the laboratory, but it is unlikely to occur under practical conditions. Since fat-soluble vitamins can be stored in the body, the nutritional history of experimental fish prior to their use in requirement studies becomes critical. The time required to deplete fish of their stored fat-soluble vitamins is highly variable. Differences in vitamin intake prior to an experiment may be responsible for some of the conflicting findings on the induction and severity of deficiency signs.

Table 2.5A. NUTRIENT DEFICIENCY SIGNS IN SALMONIDS[1]

Nutrient	Deficiency
Amino acids:	
Methionine	Cataracts
Tryptophan	Scoliosis, cataracts, fin erosion.
Lysine	Fin erosion, mortality.
Macrominerals:	
Calcium	Reduced growth (in water devoid of calcium).
Magnesium	Nephrocalcinosis, cataracts, degeneration of muscle fibers and epithelial cells of intestine and gill filaments. Skeletal deformity.
Phosphorus	Reduced growth, poor bone mineralization.
Potassium	Anorexia, convulsion, tetany.
Microminerals:	
Copper	Reduced liver superoxide dismutase and heart cytochrome c oxidase activity.
Iodine	Thyroid hyperplasia.
Iron	Hypochromic micro-cystic anemia.
Selenium	Muscular dystrophy, exudative diathesis, reduced glutathione peroxidase activity.
Zinc	Reduced growth, short-body dwarfism, fin erosion.

Table 2.5A. CONTINUED[1]

Nutrient	Deficiency
Vitamins:	
A	Skin depigmentation, exophthalmia, corneal thinning, retinal degeneration,edema and ascites.
D	Impaired calcium homeostasis, tetany of skeletal muscle and increase in liver lipid.
E	Skin depigmentation, ascites, muscular dystrophy.
K	Prolonged blood clotting, anemia, hemorrhagic gills and eyes.
Thiamin	Nervous disorder, loss of equilibrium, hyperirritability, convulsions, low transketolase activity in erythrocytes.
Riboflavin	Lethargy, dark pigmentation, spinal deformities, photophobia, corneal vascularization, reduced activity of erythrocytes glutathione reductase.
Pyridoxine	Nervous disorder, convulsions, hyperirritability, erratic spiral swimming.
Pantothenic acid	Clubbed gills, distended operculum, atrophied pancreatic acinar cells and mortality.
Niacin	Skin, fin and colon lesions, photosensitivity, sunburn and ascites.
Biotin	Degenerative gill lamellae, skin lesions, muscle atrophy, spastic convulsions, reduced hepatic acetyl COA carboxylase, and pyruvate carboxylase.
Folic acid	Lethargy, slow growth, dark skin, and anemia, and binucleated erythrocytes.
B_{12}	Microcytic hypochromic anemia and fragmented erythrocytes.
Choline	Fatty liver, exophthalmia, extended abdomen, hemorrhagic kidney and intestine.
Inositol	Dark skin coloration, distended abdomen, anemia, fin erosion, reduced activity of cholinesterase and transaminase.
Ascorbic acid	Intramuscular hemorrhage, distorted gill filaments, lordosis, scoliosis, ascites.

[1]*Does not include reduced growth and food consumption unless no other prominent signs were found.*

Table 2.5B. NUTRIENT DEFICIENCY SIGNS IN CHANNEL CATFISH[1]

Nutrient	Deficiency
Macrominerals:	
Magnesium	Reduced bone magnesium concentration, and lethargy.
Phosphorus	Poor bone mineralization, reduced bone strength, increased visceral fat, reduced resistance to bacterial infection.
Microminerals:	
Copper	Reduced cytochrome C oxidase activity.
Iron	Anemia and reduced nonspecific immune responses.
Selenium	Reduced glutathione peroxidase activity and resistance to bacterial infection.
Zinc	Reduced growth, reduced bone calcium and zinc concentration, reduced resistance to bacterial infection.
Vitamins:	
A	Exophthalmia, edema, hemorrhagic kidney and skin depigmentation.
D	Low bone ash.
E	Skin depigmentation, exudative diathesis, muscular dystrophy, pancreatic atrophy and ceroid deposition.
K	Hemorrhagic skin.
Thiamin	Dark skin coloration,loss of equilibrium and hypersensitivity.
Riboflavin	Short-body dwarfism, cloudy lens.
Pyridoxine	Nervous disorders, tetany, erratic swimming.
Pantothenic acid	Clubbed gills, eroded epidermis, anemia.
Niacin	Skin and fin lesions, exophthalmia, deformed jaws, anemia.
Biotin	Skin depigmentation, reduced hepatic pyruvate carboxylase activity.
Folic acid	Anemia, increased sensitivity to bacterial infection.
B_{12}	Reduced growth and anemia.
Choline	Enlarged liver, hemorrhagic kidney and intestine.
Inositol	No deficiency found.
Ascorbic acid	Internal and external hemorrhages, reduced bone collagen, lordosis, scoliosis, increased sensitivity to bacterial infection, slow wound repair and reduced hematocrit.

[1]*Does not include reduced growth and food consumption unless no other prominent signs were found.*

Table 2.5C. NUTRIENT DEFICIENCY SIGNS IN COMMON CARP[1]

Nutrient	Deficiency
Macrominerals:	
Magnesium	Convulsions, cataracts, and reduced bone magnesium concentration.
Phosphorus	Poor bone mineralization, skeletal and cranial deformity and increased visceral fat.
Microminerals:	
Iron	Hypochromic microcytic anemia.
Selenium	Anemia and cataracts.
Zinc	Cataracts, fin and skin erosion.
Vitamins:	
A	Skin depigmentation, exophthalmia, twisted operculum and hemorrhagic fins and skin.
D	Not tested.
E	Exophthalmia, lordosis, muscular dystrophy, kidney and pancreatic degeneration.
K	Not tested.
Thiamin	Hypersensitivity, skin depigmentation, subcutaneous hemorrhage.
Riboflavin	Photophobia, hypersensitivity hemorrhage of skin and fins, and anterior kidney necrosis.
Pyridoxine	Nervous disorder, anemia, and low hepatopancreatic transferase.
Pantothenic acid	Gill hyperplasia, lethargy, exophthalmia, and skin hemorrhage.
Niacin	Skin hemorrhage.
Biotin	Poor growth, lethargy and increased number of dermal mucous cells.
Folic acid	None detected.
B_{12}	None detected.
Choline	Fatty liver and vacuolation of hepatic cells.
Inositol	Loss of skin mucosa.
Ascorbic acid	Poor growth.

[1]*Does not include reduced growth and food consumption unless no other prominent signs were found.*

Table 2.6 VITAMIN REQUIREMENTS OF CHANNEL CATFISH, RAINBOW TROUT, PACIFIC SALMON, COMMON CARP AND NILE TILAPIA (AMOUNT PER KG OF DIET)

Vitamin	Channel catfish	Rainbow trout	Pacific salmon	Common carp	Nile tilapia
A, IU	1-2,000	2,500	2,500	4,000	NT
D, IU	500	2,400	NT	NT	NT
E, IU	50	50	50	100	50
K, mg	R	R	R	NT	NT
Riboflavin, mg	9	4	7	7	6
Pantothenic acid, mg	15	20	20	30	10
Niacin, mg	14	10	R	28	NT
Vitamin B_{12}, mg	R	0.01E	R	NR	NR
Choline, mg	400	1,000	800	500	NT
Biotin, mg	R	0.15	R	1	NT
Folate, mg	1.5	1.0	2	NR	NT
Thiamin, mg	1	1	R	0.5	NT
Vitamin B_6, mg	3	3	6	NT	
Myoinositol, mg	NR	300	300	440	NT
Vitamin C, mg	25-50	50	50	R	50

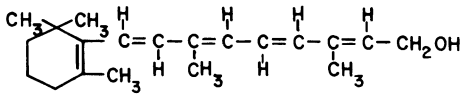
Source: National Research Council (1993).
Note: These values have been determined with highly purified ingredients and are assumed to be near 100% digestible.
R = required; NT = not tested; E = estimated; NR = not required.

Essentiality of all of the 15 vitamins has been established for fish, although all fish species do not seem to have a dietary requirement for all 15 of the vitamins. Qualitative and quantitative needs for vitamins have been studied with several species using the controlled environment, purified diet approach, similar to the previously described procedure for determining amino acid requirements. Vitamin requirements for fish vary with species, size, growth rate, nutrient interrelationships, environmental toxicants, and metabolic function (growth, disease resistance, etc.) Intestinal microorganism synthesis is a significant source of vitamins for some species. Culture system and feeding habits of the fish influence the need for vitamin supplementation of practical fish feeds; fish feeding actively on natural aquatic organisms may not need certain vitamins in the supplemental diet.

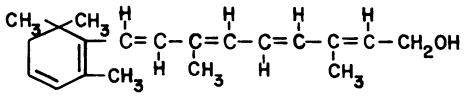
Vitamin requirements for channel catfish, common carp, and salmonids are presented in Table 2.6. These values represent minimum requirements for growth of young fish, determined under laboratory conditions. Little information is available on vitamin requirements of large fish approaching marketable size, but most vitamin requirements probably decrease as fish size increases. Vitamin C requirements for channel catfish less than 10 g in size was reported to be 30 to 50 mg kg^{-1} of diet, while the requirement for 25 to 50 g fish was 10 to 20 mg kg^{-1}. The values in Table 2.6 make no allowance for processing or storage losses. Thus, the vitamin levels chosen for the feed should be increased by 25% or more, depending upon the vitamin, to allow for losses in feed processing and storage, and possible increased needs by the fish due to stress, infection, or interaction with other substances in the feed.

Vitamin A

Vitamin A is found only in the animal kingdom. It exists in the free alcohol form as retinol and as esters of higher fatty acids. One International Unit (IU) of vitamin A



Vitamin A_1



Vitamin A_2

Figure 2.15 Vitamin A.

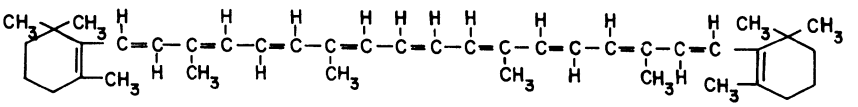


Figure 2.16 Beta-carotene.

is equal to 0.3 μg of all-trans retinol. Vitamin A_1, $C_{20}H_{30}O$, has been isolated from lipids of many fishes and land animals, whereas vitamin A_2, $C_{20}H_{28}O$, has been isolated only from freshwater fishes (Figure 2.15).

Plants produce red-to-yellow pigmented compounds called carotenoids, some of which have vitamin A activity. Beta-carotene (Figure 2.16) has by far the highest vitamin A activity. This compound appears to be capable of yielding two moles of retinol upon simple hydrolytic cleavage; however, the vitamin A activity of beta-carotene is less than this for most animals. Several fishes have been found capable of using beta-carotene for vitamin A activity; however, Poston et al.(1977) found that coldwater fish could utilize beta-carotene at 14°C, but not at 9°C. Lee (1987) found that channel catfish readily converted beta-carotene into vitamins A_1 (retinol) and A_2 (3-dehydroretinol) in almost a 1 to 1 ratio. Recent research with tilapia has shown that other carotenoids besides b-carotene, such as astaxanthin, zeaxanthin, and lutein, could be bioconverted into vitamin A.

Vitamin A, like other fat soluble vitamins, is stored in large amounts in the body (liver) if intake exceeds metabolic need. It is possible for fish to store enough vitamin A to produce a toxic condition (hypervitaminosis); however, prolonged consumption of a diet with an unusually high level of vitamin A would be required to produce this. Poston et al. (1977) found that 2.2 million IU of vitamin A per kg in experimental diets was toxic to rainbow trout; the dietary requirement is 1 to 2 thousand IU kg^{-1}.

An established physiological function of vitamin A is its role in vision. Retinol is combined with a protein, opsin, to form rhodopsin, which is the compound involved in the photochemical reaction in the retina of the eye in the

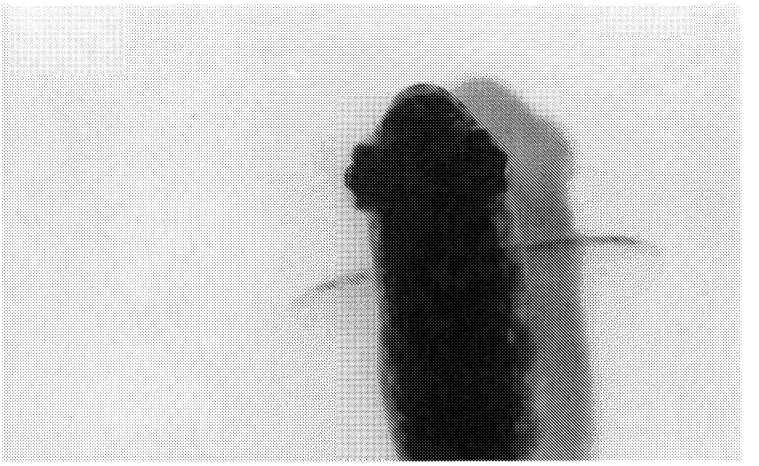


Figure 2.17 Dietary deficiency of vitamin A causes hypothalmia characterized by edema in the left eye.

process of vision. Other metabolic roles of vitamin A are less well understood. It is used for maintenance of mucosal membranes that line many body organs, the gastrointestinal tract, the respiratory tract, and the eye. In vitamin A deficiency, epithelial cells fail to differentiate beyond the squamous type to mucus-secreting type, and mesenchymal cells fail to differentiate beyond the blast stage. Epithelial cells from the eye and many other areas of the body keratinize and this lowers resistance to infection. Several deficiency symptoms appear to be related to impaired function of epithelial tissue. Reproduction is impaired in most animals.

Vitamin A deficiency causes poor growth rate in fish. In salmonids, deficiency signs are described as light skin color, ascites (fluid in abdominal cavity), anemia, and pathological condition of the eye characterized by exophthalmos, hemorrhagic eyes, eye lenses displacement (see Figure 2.17), thinning of cornea, degradation of the retina, and twisted gill opercula. Channel catfish fed vitamin A deficient diets over a long period (2 years) developed exophthalmos, edema (collection of fluid in tissues) and kidney hemorrhage. Common carp showed deficiency signs of light skin color, fin and skin hemorrhages, exophthalmos, and deformed gill opercula.

Plant carotenoids have been found to fill biological roles other than as a vitamin A precursor. A number of reports are in the literature on beneficial effects of carotenoids with no vitamin A activity on reproduction and resistance to infectious and noninfectious diseases. Several reports have indicated that various carotenoids with limited provitamin A potency are immunostimulatory when fed to fish. It is assumed that this is due to an antioxidative property which protects cell membranes against oxidation by free radicals.

Retinol and beta-carotene are sensitive to oxidation, so natural sources of vitamin A are relatively labile. Thus, supplemental vitamin A should be added to fish feeds. Synthetic vitamin A used in fish feeds is in stabilized forms, usually as palmitate, acetate, or propionate esters. Dry additives are usually in beadlet form, where the retinol is coated with gelatin or some other oxygen barrier.

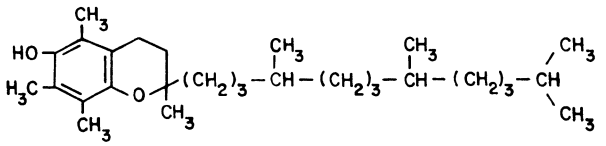


Figure 2.18 Alpha-tocopherol.

Vitamin E

Vitamin E is present in at least eight tocopherols which occur in plants. The name *tocopherol* is from the Greek word *tocos*, which means childbirth. Alpha-tocopherol (Figure 2.18) has the highest vitamin E activity. Vitamin E activity of compounds is measured in International Unit (IU), with 1 IU being equivalent to the biological activity of 1 mg of D-α-tocopherol. Traditionally, biological activity has been based on prevention of fetal resorption in rats.

Commercial antioxidants, like ethoxyquin (1,2-dihydro-2,2,4-trimethylquinoline), which is used in feeds as a preservative and has no chemical relationship to the tocopherols, also have vitamin E activity in fish and other animals. Lovell et al. (1984) found that 125 mg of ethoxyquin kg^{-1} of diet prevented deficiency signs in channel catfish, which included nutritional muscular dystrophy, but was not as effective as 25 mg of alpha-tocopherol kg^{-1} of diet for growth of the fish. Thus, under certain economic conditions, commercial antioxidants may spare alpha-tocopherol in fish feeds.

A major function of vitamin E is its role as a metabolic antioxidant with a specific role in preventing oxidation of unsaturated phospholipids in cellular membranes, such as erythrocytes, and subcellular membranes such as mitochondria. It is often referred to as a metabolic free radical scavenger or peroxide scavenger. In most animal species, a dietary increase in polyunsaturated fatty acids, especially when partially oxidized, produces an increase in dietary need for vitamin E. The function of vitamin E as an antioxidant is evidenced by an increased need in the absence of selenium. Selenium is a component of the enzyme, glutathione peroxidase, which catalyzes the removal of metabolic peroxides. A specific role for vitamin E in an enzyme system has not been identified, although impairment of several enzyme systems, such as those involved in porphyrin and heme synthesis, has been identified in vitamin E deficiencies in various animals.

Diverse physiologic abnormalities have been demonstrated with vitamin E deprivation in animals. Common are nutritional muscular dystrophy, characterized by atrophy and necrosis of muscle fibers and which has been produced in several fishes and terrestrial animals (see Figure 2.19); and pathological conditions of male and female reproductive organs, causing reduced fertility and reproduction. Increased permeability of capillaries, which result in hemorrhages and edema in various body areas, has been caused by vitamin E deficiency in various animals. The syndrome, exudative diathesis, manifested by accumulations of fluid under skin or in the abdominal cavity (sometimes of a greenish color caused by decomposed hemoglobin), has been produced in channel catfish and salmonids fed vitamin E-deficient diets. Vitamin E deficiency causes reduction in ability of erythrocytes to

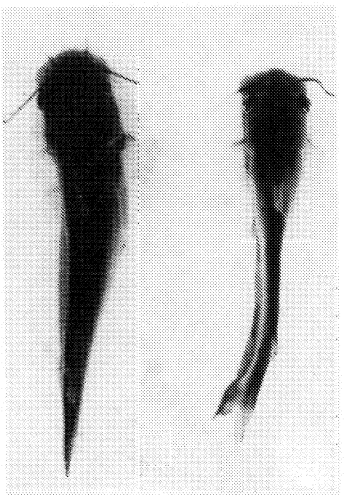


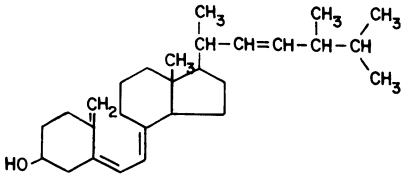
Figure 2.19 Channel catfish fed a vitamin E deficient diet (right) show nutritional muscular dystrophy, characterized by sever atrophy of skeletal muscles. Fish on left received a nutritionally complete diet. (*Courtesy of Delbert M. Gatlin III.*)

withstand peroxide deterioration of membranes. Severe anemia, characterized by immature, irregularly shaped and sized erythrocytes, is produced in vitamin E deficiency. Other vitamin E deficiency signs described for several fish species include fatty livers and ceroid (dark lipoid) bodies in liver. "Sekoke disease" in common carp, characterized by thinning of flesh on the back of the fish, was caused by feeding oxidized silkworm pupae, but corrected by supplementing the diet with vitamin E.

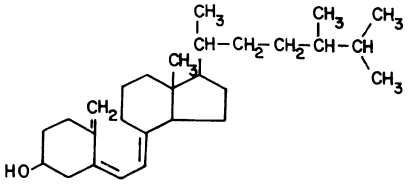
Subclinical measurements used to detect vitamin E deficiency include erythrocyte fragility and histological examination of tissues for necrosis of muscle fibers and ceroid concentration in liver and kidney.

In most experimental and practical situations where classical vitamin E-related myopathy has been produced in fish, inclusion of high levels of polyunsaturated fatty acids or omission of selenium from the diet has been necessary. However, Lovell et al. (1984) fed channel catfish diets low in polyunsaturated fatty acids, using stripped lard as the lipid source, and produced fish with severe nutritional muscular dystrophy and other signs of vitamin E deficiency. This implies that fish feeds not containing high levels of polyunsaturated lipids, such as many commercial warmwater fish feeds, can cause reduced growth rate and various pathologies when deficient in vitamin E. Rainbow trout were found to be relatively insensitive to vitamin E deficiency at optimum growth temperature (15°C) unless oxidized polyunsaturated fats were included in the diet. Cowey et al. (1984) reduced the water temperature to 6°C and produced severe myopathy in the fish. This suggested that maintenance of fluidity in biomembranes is more demanding in fish at lower temperatures.

Hypervitaminosis E can be produced in fish. Rainbow trout fed about 100 times the requirement, 5,000 mg of DL-α-tocopherol kg^{-1} of diet, showed reduced blood concentration of erythrocytes.



Vitamin D_2



Vitamin D_3

Figure 2.20 Two forms of vitamin D.

Alpha-tocopherol is found in most plant seeds. Significant sources are plant oils, germ or gluten meals, distillery or brewery dried products, and grain brans and by-products. Whole grains, fish meal, and solvent-extracted oilseed meals are poor sources and need supplementation for feeding fish. Free alpha-tocopherol is unstable to oxidation; therefore acetate or succinate esters, which are very stable during feed processing and storage, are the commercial forms of alpha-tocopherol.

Vitamin D

Vitamin D is found in nature in two forms: ergocalciferol (D_2) and cholecalciferol (D_3) (Figure 2.20). Ultraviolet irradiation of two provitamins, ergosterol (found in plants) and 7-dehydrocholesterol (found in animals), will produce vitamins D_2 and D_3, respectively. Animals not exposed to sunlight need a dietary source of vitamin D. Fish get relatively little ultraviolet energy from the sun because of the shallow depth of penetration of these rays in natural waters. Most land animals, except chickens, can use D_2 and D_3 interchangeably. Fish, however, utilize D_2 poorly or not at all. Rainbow trout used D_3 about three times more efficiently than D_2. One I.U. of vitamin D activity is equivalent to the antirachitic effect of 0.025 mg of cholecalciferol.

At least two fish species have shown a dietary need for vitamin D_3. Although little research has been done on vitamin D metabolism in fish, it can be assumed that a physiological role of vitamin D_3 in fish is similar to that in warmblooded animals, where it is the precursor to 1, 25-dihydroxycholecalciferol, important calcium-regulating and phosphate-regulating hormones. Vitamin D, after absorption from the intestine, is converted in the liver to 25-hydroxycholecalciferol and subsequently in the kidney to 1, 25-dihydroxycholecalciferol, the active hormone. This hormone is responsible for maintaining serum calcium and phosphorus levels by altering rate of intestinal absorption, renal resorption, and bone mobilization. It is also thought to play a role in the synthesis of calcium and phosphorus transport proteins.

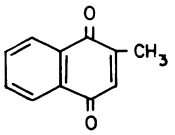


Figure 2.21 Vitamin K_3 (menadione).

Channel catfish fed a vitamin D-deficient diet for 16 weeks showed reduced weight gain and decreased body levels of ash, calcium, and phosphorus. Signs of vitamin D deficiency in rainbow trout have been described as slow growth rate, tetany of white muscle, ultrastructural changes in epaxial white muscle fibers, impaired calcium homeostasis, high liver lipid content, and elevated levels of triiodothyronine in blood. Feeding one million IU of D_3 per kg of diet, or more will cause hypervitaminosis D in rainbow trout. This compares with a normal dietary requirement of 2,400 IU kg^{-1}. Channel catfish raised in calcium-free water seemed to be less sensitive to excessive dietary levels of vitamin D_3 than when raised in water with calcium ions.

Fish oil is usually a good source of vitamin D_3 but other animal products are generally poor. Plant products are devoid of D_3. Because of the scarcity of vitamin D_3 in feedstuffs, this vitamin should be supplemented in commercial fish feeds.

Vitamin K

The name *vitamin K* was given to a fat-soluble factor necessary in the diet of chicks to prevent hemorrhage and for normal blood clotting. Several compounds with vitamin K activity have been isolated or synthesized. These include phylloquinone (vitamin K_1), found in green plant leaves; menaquinone (vitamin K_2), synthesized by bacteria and found in animal feces; and menadione (K_3), a synthetic compound with more vitamin K activity than K_1 or K_2 (Figure 2.21). Presently, the term vitamin K is used as a generic descriptor for 2-methyl-1,4-naphthoquinone and all 3-substituted derivatives of this compound, which exhibit antihemorrhagic activity in animals.

Vitamin K is necessary for normal blood clotting in all animals, including fish. Several proteins necessary for blood coagulation are dependent upon vitamin K for their synthesis. These include prothrombin, proconvertin, plasma thromboplastin antecedent, and Stewart-Prower factor. The metabolic role of vitamin K involves a vitamin K-dependent carboxylase enzyme which carries out the carboxylation of the glutamic acid residues in the active proteins to form γ-carboxyglutamic acid. The γ-carboxyglutamyl residues are functional in the interaction of the clotting factors with phospholipid cell surfaces. Other proteins, such as in bone and kidney, have been found that contain γ-carboxyglutamic acid residues, so it is presumed that vitamin K is involved in other carboxylations besides blood clotting.

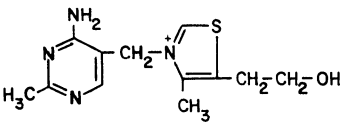


Figure 2.22 Thiamin.

Channel catfish and trout have been shown to require dietary vitamin K for normal blood coagulation; however, growth rate was not affected in either species when vitamin K was deleted from the diet. Quantitative requirement for vitamin K has not been determined for warmwater fishes. Levels of 0.5 mg to 1 mg of menadione per kg of diet is sufficient to maintain normal blood coagulation in fingerling trout.

Intestinal synthesis is an important source of vitamin K in some animals. This source has not been demonstrated in fish. However, dietary sulfaguanidine (an antibiotic) and low water temperature each prolonged blood coagulation time in trout, which suggests that bacterial synthesis of vitamin K may have been involved.

Fish meal and alfalfa meal are good sources of vitamin K. Vitamin K is added to fish feeds as a menadione salt: menadione sodium bisulfite or menadione dimethylpyrimidinal bisulfite; the latter is more heat stable during feed proceeding.

Thiamin

Thiamin is found in many grains and seeds, being most concentrated in the seed coat. Discovery of this growth factor began when persons consuming polished rice developed beriberi but recovered when a factor in the seed coat was added to the diet. Thiamin is composed of a pyrimidine ring and a thiazole ring, as shown in Figure 2.22. It is synthesized by higher plants, but not by animals. It is relatively sensitive to heat and moisture at pH above 5, so the commercial vitamin is sold in the hydrochloride form to ensure stability.

The active form of the vitamin is thiamin pyrophosphate. Phosphorylation occurs in the liver. Thiamin pyrophosphate acts as a coenzyme for several metabolic decarboxylation and transketolation reactions. It is involved in decarboxylation of pyruvic acid and a-ketoglutaric acid in aerobic glycolysis. It also acts as a coenzyme in transketolation in metabolism of glucose through the pentose phosphate shunt. Transketolase activity in erythrocytes and kidney is a sensitive indicator for thiamin status in rainbow trout.

Thiamin deficiency affects the central nervous system in fish, birds, and mammals. Polyneuritis in chicks (lack of control of position of the head) and Chastek paralysis in mink and foxes are caused by thiamin deficiency. Thiamin deficiency in fish causes hypersensitivity to disturbance, loss of equilibrium, and convulsions. Fish show thiamin deficiency quickly: channel catfish in 6 to 8 weeks, carp in 8 weeks, and Japanese eel in 10 weeks.

Tissues of most fishes contain thiaminase, an enzyme that can destroy thiamin in nonliving tissue by splitting it into its two component ring structures. Heating the fish destroys the enzyme. Feeding fish or fish visceral organs without

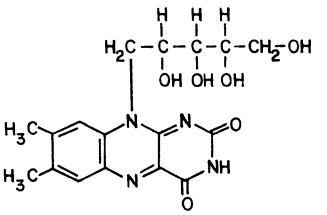


Figure 2.23 Riboflavin.

heat treatment has produced thiamin deficiency problems in mink and foxes and in channel catfish. The thiamin is destroyed prior to ingestion, when the enzyme (thiaminase) and substrate (thiamin) are in contact for a period of time. Channel catfish fed diets containing 40% nonheated fish viscera developed thiamin deficiency in 10 weeks, but when the fish were fed an additional diet containing thiamin in a separate meal daily, no deficiency occurred.

Riboflavin

Riboflavin is widely found in nature; green plants, seed coat of grains, and yeast are rich sources. It is synthesized by higher plants or microorganisms, but not by animals. Thus, nonruminant animals require it in their diet. Chemical structure of riboflavin (Figure 2.23) consists of a dimethyl-isoalloxazine moiety conjugated with ribose through which the vitamin is linked to phosphate in the intestinal wall to form the active coenzyme.

Riboflavin is a component of two flavoprotein coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are components of prosthetic groups of oxidases and reductases that act upon metabolic degradation products of proteins, carbohydrates, and lipids. The coenzymes function in the intermediary transfer of electrons in the mitochondrial electron transport system.

A number of riboflavin deficiency signs have been identified in animals but none have been related to a specific biochemical role of the vitamin. Crooked and stiff legs occur in swine and chickens (curled-toe paralysis). Eye problems occur in humans, farm animals, and fish. Photophobia and cataracts have been found in several fish deprived of riboflavin (Figure 2.24). Skin discoloration has been observed in salmonids; short, stubby bodies associated with vertebral compaction were described in riboflavin-deprived channel catfish and tilapia; dermatitis and hemorrhagic fins were found in eel; and necrosis of head kidney was observed in carp.

Sensitive subclinical tests for riboflavin deficiency in fish are *in vitro* measurement of erythrocyte glutathione reductase (EGR) or D-amino acid oxidase activity in presence of added FAD. Approximately 3 mg of riboflavin kg^{-1} of diet is sufficient to prevent change in EGR activity in rainbow trout. The minimum dietary requirement for normal growth and to prevent dwarfism in channel catfish is 9 mg kg^{-1} of body weight. Rainbow trout fed up to 600 mg of riboflavin kg^{-1} of diet showed no adverse effects.

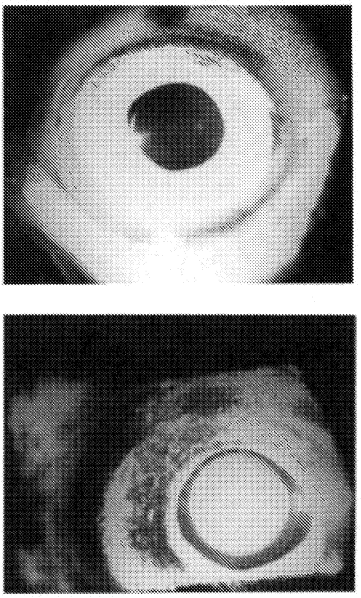


Figure 2.24 Normal lens (above). Severe eye cataract in rainbow trout fed riboflavin-deficient diet (below).

Many feedstuffs are reasonably good sources of riboflavin. Whole grains are poor sources, whereas bran or polishings from grains and distillery grain byproducts are fair to good sources. Synthetic riboflavin, which has equal bioactivity as natural sources, is usually supplemented in fish feeds.

Niacin

Niacin, nicotinic acid, and nicotinamide are often used interchangeably. Niacin is actually the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting biological activity of nicotinamide (Figure 2.25). Nicotinamide is found in plant and animal tissues as a component of two coenzymes of the hydrogen transport system, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These coenzymes are involved in a number of oxidation-reduction reactions. NAD is specific for hydrogenases involved in passing electrons on to oxygen in the mitochondrial electron transport systems. NADP is specific in other hydrogenases such as in fatty acid synthesis and the pentose phosphate shunt in glucose metabolism.

Niacin deficiency causes pellagra in humans, which is characterized primarily by dermatological problems, and is commonly found in situations where the diet is limited to corn or polished rice. Most fish show niacin deficiency signs rather quickly, which indicates the inability to synthesize niacin. Skin lesions are a common deficiency sign in fish. Channel catfish show skin and fin lesions along with deformed jaws, exophthalmic, anemia, and high mortality rate. Eels show skin lesions, dark pigmentation, and ataxia. Salmonids show sensitivity of skin to sunburn along with fin erosion, intestinal lesions, and muscle weakness. Exposure

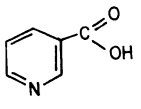


Figure 2.25 Niacin.

of rainbow trout to ultraviolet irradiation caused loss of mucus-producing cells of the epidermis, and enhanced the effects of niacin deficiency and increased niacin requirement (Figure 2.26). High dietary intake of niacin alters lipid metabolism. Megadose prescription of thiamin is frequently used to lower cholesterol in humans.

Although plant seeds, especially the outer coat, contain substantial amounts of niacin, the niacin occurs mostly in bound form and is highly unavailable to animals. Most land animals can convert the amino acid tryptophan to niacin and this source can contribute to their niacin requirement. However, fish appear to have limited ability to make this conversion. Brook trout were found incapable of converting tryptophan to niacin efficiently. Also, niacin deficiency can be demonstrated rapidly in fishes fed tryptophan in the diet. Because of the presumed limited availability of niacin in grains and oil seed meals to fish, vitamin supplementation of practical fish feeds is recommended.

Pantothenic Acid

Pantothenic acid is synthesized by plants and microorganisms. Chemically, it is composed of pantoic acid (2,4-dihydroxy-3,3-dimethyl butyric acid) linked through

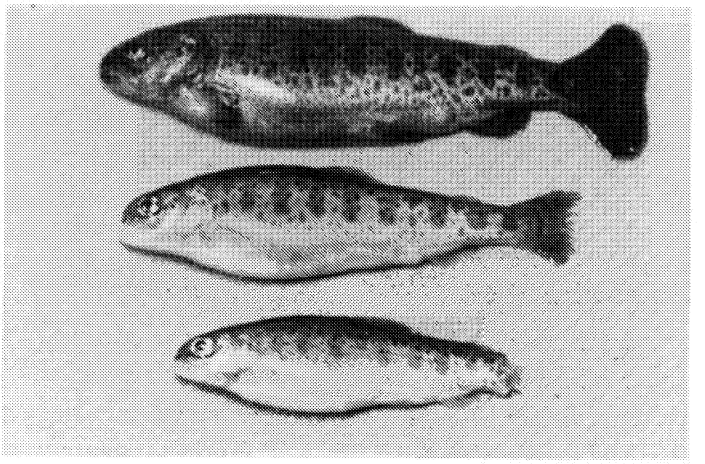


Figure 2.26 Niacin deficiency in rainbow trout. Top fish is control; two lower fish were fed a niacin-deficient diet for 16 weeks. Bottom fish was exposed to 20 hr/day of ultraviolet irradiation and had more serious fin erosion.

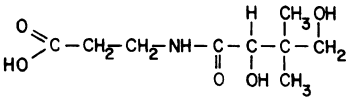


Figure 2.27 Pantothenic acid.

a peptide bond to b-alanine (Figure 2.27). In nature, it occurs largely in bound form as coenzyme A. Because of its sensitivity to destruction by heat and high or low pH, it is available commercially as calcium or sodium salt.

The only known function of pantothenic acid is as a component of coenzyme A (CoA), which is involved in transfer of acetyl (2-carbon) units in numerous reactions in the metabolism of proteins, carbohydrates, and lipids. Coenzyme A contains pantothenic acid linked through pyrophosphate to adenosine 3'-phosphate on one side and ß-mercaptoethylamine on the other. The acceptance of the acetyl units, which help form acetyl CoA, is through the sulfhydryl group of ß-mercaptoethylamine. Coenzyme A formation is an important route for carbohydrates and fatty acids to enter the tricarboxylic acid cycle. Coenzyme A has manifold functions, anabolic and catabolic, involving cellular energy release and synthesis of fatty acids, steroids, sphingosine, and many other compounds.

Pantothenic acid is a dietary essential for monogastric animals, including fish, and a dietary deficiency results in growth failure in all. Skin and hair problems are common deficiency characteristics. Pantothenic acid deficiency results in impaired functions of mitochondrial-rich, high energy expenditure cells. This may partially explain why gill lamellae, kidney tubules, and acinar cells in pancreas, all of which carry on a high level of metabolic activity, are all sensitive to pantothenic acid deficiency.

Fish show pantothenic acid deficiency signs quickly. Young channel catfish and yellowtail have shown deficiency signs within 2 weeks. Deficiency causes dietary gill disease in several fish species, which is characterized by clubbed, exudate-covered gill lamellae, fused gill filaments, and swollen operculums (Figure 2.28). Lesions on skin and fins, anemia, anorexia and poor growth are also found in fish deprived of pantothenic acid.

Pantothenic acid is widely found in commercial feedstuffs, but the level and availability in processed feed are likely to be lower than the requirement for most fishes. Supplemental pantothenic acid, as calcium d-pantothenate (92% vitamin activity) or calcium DL-panthenate (46% activity), is recommended for commercial fish feeds.

Vitamin B_6 (Pyridoxine)

The term B_6 is the generic descriptor for the 2-methylpyridine derivatives that have biological activity of pyridoxine. Three chemically related compounds that have similar metabolic functions have been identified: pyridoxine, pyridoxamine, and pyridoxal. Pyridoxine (Figure 2.29) was the first identified and was given this name. Later, the other two were identified and the name B_6 was given to this group of compounds. Vitamin B_6 is now the approved name for this vitamin. Vitamin B_6 is a dietary requirement of all nonruminant mammals, birds, and fish. Vitamin B_6

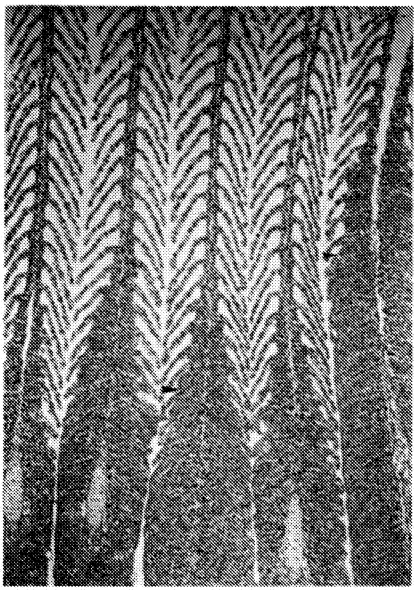


Figure 2.28 Gill filaments of channel catfish fed a pantothenic acid-deficient diet for two weeks showing hyperplasia of gill lamellae (arrows). Continuation on this diet resulted in fusion of all lamella on each filament and ultimate death.

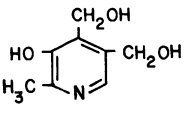


Figure 2.29 Vitamin B_6 (Pyridoxine).

compounds are synthesized by plants and some microorganisms. It is widely found in feedstuffs of plant and animal origin.

The metabolically active B_6 coenzyme is pyridoxal or pyridoxamine phosphate. It is functional in a number of enzymes in which amino acids are metabolized, including decarboxylases, transaminases, sulfahydrases, and hydroxylases. Increasing protein percentage in the diet causes an increase in the B_6 requirement of salmonids. Because fish are fed much higher protein diets than land animals, it should be expected that the B_6 requirement would be higher for fish. Vitamin B_6 is involved in metabolism of carbohydrates and lipids. It is essential for the synthesis of heme (in hemiglobin), and in the synthesis of serotonin from tryptophan, which may explain why B_6 deficiency causes nervous disorders.

Deficiency signs in fish develop quickly. Young channel catfish, salmonids, and carp show deficiency signs in 4 to 8 weeks. These include nervous

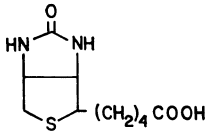


Figure 2.30 Biotin.

disorders, such as hypersensitivity to disturbances; poor swimming coordination; convulsion; and tetany when handled. Channel catfish develop a greenish-blue sheen to the skin. Common carp show edema, exophthalmos, and skin lesions.

The requirement for fish is 3 mg kg^{-1} to 10 mg kg^{-1} of diet, and most commercial feedstuffs contain this amount without B_6 supplementation. However, because of variation and uncertainty of content in feedstuffs and in processed, stored feeds, vitamin B_6 is often supplemented in commercial fish feeds as pyridoxine hydrochloride.

Biotin

Biotin (Figure 2.30) was first recognized in its association to "egg white injury" in rats because typical deficiency signs resulted when raw egg white was supplemented in the diet. When liver was fed, egg white injury was prevented. The preventive and causative factors were subsequently identified as biotin and avidin, the latter a heat labile protein in egg white that combines with biotin and renders it inactive.

Biotin functions as a coenzyme in carboxylation (transfer of carbon dioxide) reactions. Enzyme systems containing biotin are acetyl-CoA carboxylase, propionyl-CoA carboxylase, and pyruvate carboxylase. Metabolic functions requiring biotin are many and include synthesis of fatty acids, oxidation of energy yielding compounds, synthesis of purines, and deamination of certain amino acids. A reliable indicator of biotin status that has been used in fish and warm blooded animals is pyruvate carboxylase or acetyl-CoA carboxylase activity.

Biotin deficiency is difficult to produce in nonruminant mammals without feeding raw egg white. Deficiency signs in chicks and several fishes have been produced, however, without feeding a biotin antagonist. Fish vary in sensitivity to biotin deficiency. Channel catfish are relatively insensitive to biotin deficiency, but rainbow trout are highly sensitive (Figure 2.31). Young channel catfish required 24 weeks, while young rainbow trout required only 4 weeks to show deficiency signs, each under optimum environment for growth. Channel catfish showed reduced growth rate, light skin color, and hypersensitivity to abrupt noise or movement. Rainbow trout showed poor growth, degeneration of gill lamellae and epithelium, and enlarged, pale livers. Subclinical signs in fish are reduced carboxylase activities in liver and, in trout, abnormal synthesis of glycogen and fatty acids, degeneration of acinar cells of pancreas, and glycogen deposition in kidney tubules.

Bioavailability of biotin in many feedstuffs is limited. For example, one-half or less of the total biotin in wheat, barley, sorghum, meat and bone meal, and fish meal is available to chickens. Biotin availability in corn and soybean meal, however, is higher. Channel catfish do not require supplemental biotin in practical corn-soybean meal or corn-soybean meal-fishmeal diets for normal growth and pyruvate carboxylase activity. Rainbow trout also do not benefit from biotin

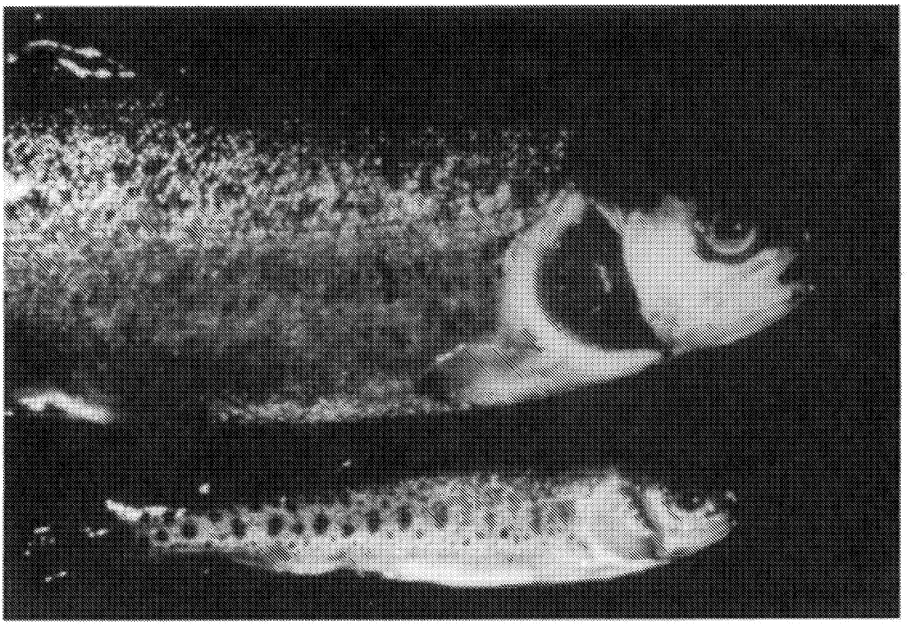


Figure 2.31 Rainbow trout are highly sensitive to biotin deficiency in the diet. The fish on top was fed a nutritionally complete diet while the one on bottom received the same ration except deprived of biotin. (*Courtesy of W.D. Woodward.*)

supplementation of practical diets. The dietary requirement for fish is low, about 0.25 mg kg^{-1} diet, so practical fish diets do not usually need supplemental biotin.

Folic Acid

When chemically identified, folic acid was named pteroyglutamic acid. As seen in Figure 2.32, it is composed of glutamic acid (right) plus pteroic acid, the latter being made up of paraminobenzoic acid and a pteridine nucleus. A number of biologically active forms or derivatives of folic acid exist in nature. These include tetrahydrofolic acid, which is the active coenzyme form; 5-methyl-tetrahydrofolic acid; folic acid glutamates; and others.

Folic acid is metabolically reduced to tetrahydrofolic acid which is functional in the transfer of single-carbon units, a role analogous to that of pantothenic acid in the transfer of two-carbon units. The one-carbon units may be

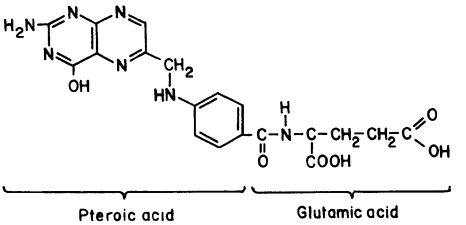


Figure 2.32 Folic acid.

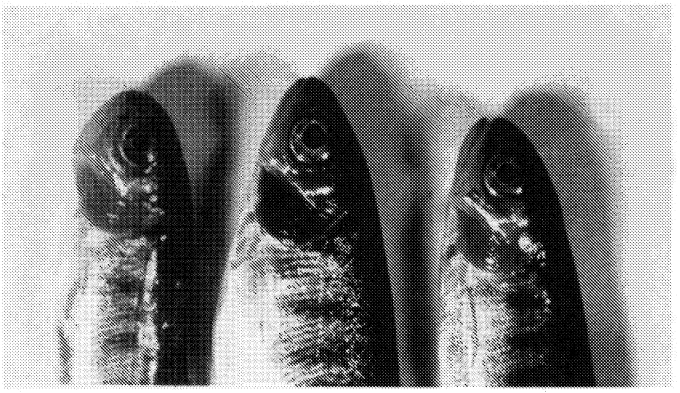


Figure 2.33 Coho salmon on left and right were deprived of dietary folic acid. Note pale gills, associated with severe anemia, in these fish compared to the control in the center. (*Courtesy of Charlie E. Smith.*)

formyl, methyl, hydroxymethyl, or others. Among the reactions involving tetrahydrofolic acid are the synthesis of purines and pyrimidines for formation of nucleic acids.

In animal species where folic acid deficiencies occur, megablastic anemia is usually found, characterized by large, immature erythrocytes. Megablastic anemia has been produced in salmonids, characterized by reduced production of erythrocytes causing pale gills (Figure 2.33), large and segmented erythrocytes with constricted nuclei, and abnormally large proerythrocytes in the erythropoietic tissues. Poor growth and reduced resistance to bacterial infection was seen in folic acid-deficient channel catfish; however, folic acid deficiency could not be demonstrated in common carp. Duncan and Lovell (1994) demonstrated interaction between allowances of folic acid and vitamin C in channel catfish diets; the folic acid requirement for maximum growth increased inversely with dietary level of vitamin C. They concluded that vitamin C was functional in reducing folic acid to the active coenzyme form.

In rats and pigs, folic acid deficiency could not be produced without inclusion of an antibiotic in the diet to inhibit intestinal synthesis of the vitamin. Kashiwada et al. (1970) showed that folic acid is synthesized by intestinal bacteria in common carp; this may explain why the researchers were unable to demonstrate dietary folic acid deficiency. Duncan and Lovell (1994) found that inclusion of the antibiotic succinylsulfathiazole, enhanced folic acid deficiency signs in channel catfish fed diets without folic acid supplementation.

Butterworth et al. (1986) associated an ideopathic anemia, called "no blood disease" in practice, in channel catfish with folic acid degradation in the feed. The disease is characterized by very low erythrocyte concentrations in blood, kidneys, liver, and gills, and abnormal erythrocytes characteristic of those in folic acid deficient rainbow trout. They isolated bacteria from feeds fed to affected fish (there are also molds) that are capable of degrading folic acid to glutamic acid and pteroic acid. Pteroic acid not only has no folate activity, but is antagonistic to absorption and metabolism of folic acid in warm blooded animals. Duncan and Lovell (1994) fed pteroic acid and methtrexate, which is also a folic acid antagonist,

and produced the "no blood" syndrome in channel catfish. They claimed that an environmental agent that interfered with folic acid absorption or metabolism could cause the practical anemic problem.

Folic acid is synthesized by plants and microorganisms. Grains, oilseed meals, and animal byproducts are all good sources. The needs of farm animals are readily met with practical feeds. Presently, supplemental folic acid is added to most fish feeds. One to five milligrams per kilogram of diet is sufficient for young salmonids and channel catfish.

Vitamin B_{12} (Cyanocobalamin)

This was the last of the 15 recognized vitamins to be identified; it is also the most chemically complex. For many years it was known that liver contained a factor that would correct pernicious anemia in laboratory animals. Supplementation of all-plant diets with animal by-products provided a missing growth factor for chickens and pigs. In 1948 the factor was isolated from liver and chemical identification was completed in 1955. The molecule, cyanocobalamin (Figure 2.34), which has a molecular weight of 1,354, has a cobalt nucleus in a tetra-ring porphyrin structure. Several similar compounds, where the cyanide or nucleotide is substituted, and have the biological activity of cyanocobalamin are found in nature and are collectively called cobalamins or B_{12}.

Vitamin B_{12} functions as a coenzyme in a number of metabolic reactions. A specific function is to act in concert with folic acid in the transfer of single-carbon units, such as methylation of uracil to form thymine in DNA synthesis and in methyl transfer in methionine synthesis from homocystine. Vitamin B_{12} is necessary for normal maturation of erythrocytes and healthy nervous tissue. A B_{12} deficiency can cause a folic acid deficiency because it is necessary for conversion of

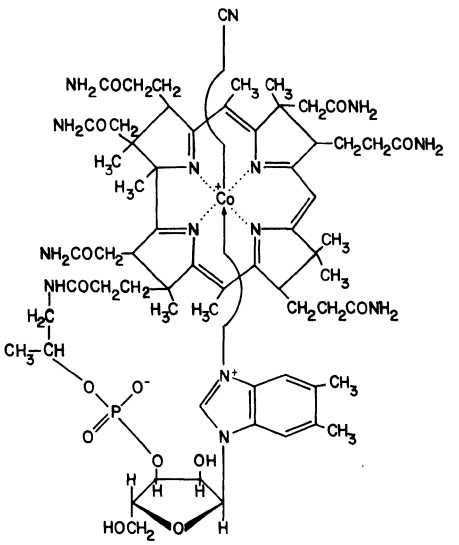


Figure 2.34 Vitamin B_{12} (Cyanocobalamin).

Figure 2.35 Inositol and its hexaphosphate ester, phytic acid.

tetrahydrofolic acid to its coenzyme form. Intrinsic factor, a mucoprotein in the digestive tract, is necessary for proper absorption of B_{12}. It has been found in the gut of most animals.

Vitamin B_{12} deficiency causes pernicious anemia in humans, characterized by macrocytic anemia and nervous disorders. In other animals there is usually microcytic anemia and suppressed growth. In fish, salmonids showed microcytic anemia and fragmented erythrocytes with low hemoglobin values, and reduced growth rate. Anemia was not found in B_{12}-deficient channel catfish, but slight reduction in growth occurred in the channel catfish after 36 weeks. No effects of B_{12} deficiency were found in common carp or Nile tilapia, apparently due to intestinal synthesis of a sufficient supply of the vitamin. Evidence has been presented that intestinal synthesis is a significant source of B_{12} in several species.

Vitamin B_{12} is assumed to be synthesized only by microorganisms. Neither higher plants nor animals can synthesize the vitamin; thus, it is a metabolic essential for all animals. A vitamin B_{12} supplement in the diet is unnecessary for some fishes, provided the diet contains cobalt, because of intestinal microfloral synthesis. However, this source of B_{12} varies among species and with environment; therefore, the vitamin is usually included in fish vitamin premixes.

Myoinositol

Inositol is widely distributed in plant and animal tissues. In animals it occurs freely as myoinositol, or as a structural component of biological membranes as phosphatidylinositol. In plants, it is most concentrated in seeds and about two thirds of it is in the form of a hexaphosphate ester, phytic acid, which is highly indigestible to fish and other monogastric animals.

Myoinositol (Figure 2.35) has no known coenzyme function. Besides being a component of cell membranes, it apparently has some lipotropic action. Rainbow trout fed inositol-deficient diets had large accumulations of triglycerides and cholesterol but low levels of phospholipids in the liver. Recently, phosphatidylinositol was shown to be involved in signal transduction of several metabolic processes (Matthews and van Holde, 1990). Inositol 1,4,5-triphosphate stimulates the release of calcium from its intracellular stores in the endoplasmic reticulum, and *sn*-1,2-diacylglycerol activates protein kinase C to phosphorylate specific target proteins. Examples of cellular processes controlled by the phosphoinositide second messenger system include amylase secretion, insulin

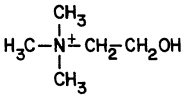


Figure 2.36 Choline.

release, smooth muscle contraction, liver glycogenolysis, platelet aggregation, histamine secretion, and DNA synthesis in fibroblasts and lymphoblasts.

In several fish species reduced growth, anemia, fin erosion, and slow rate of gut emptying have been reported with inositol deficiency. Inositol deficiency is difficult to produce in some animals when the diet is nutritionally complete otherwise. Burtle (1981) was unable to demonstrate inositol deficiency in channel catfish, and found a significant rate (equal to that in rat) of inositol synthetase activity in the liver. Although much of the inositol in plant feed ingredients is in the form of phytic acid, there is usually enough free inositol to supply the dietary needs of fish so that supplementation in commercial feeds is unnecessary.

Choline

Choline (Figure 2.36) has no known coenzyme function, but does have several metabolic roles. It reacts with acetyl coenzyme A to form acetylcholine, the neurotransmitter. It is a component of phosphatidylcholine (lecithin), which has structural functions in biological membranes and facilitates lipid transport in metabolism. Its three methyl groups ($-CH_3$) in the molecule make it an important methyl donor for numerous methylation reactions, such as formation of methionine from homocystine.

Choline can be synthesized in the body if "labile" methyl groups are available. Methionine or cystine may contribute methyl groups to ethanolamine to form choline. Although synthesis in the body is usually not fast enough to meet the choline requirement for normal growth, the dietary content of methionine or cystine as methyl donors or of folic acid and vitamin B_{12} for de novo synthesis of methyl groups can influence the dietary requirement of choline. Channel catfish fed choline-free purified diets containing excess methionine did not develop choline deficiency, but when fed diets not excessive in methionine, the fish developed deficiency signs (Wilson and Poe, 1988).

Choline deficiency has been produced in most animals with the exception of humans. A common deficiency sign is fatty livers. Choline deficiency signs have been demonstrated in all fish species evaluated. In addition to fatty livers, channel catfish had hemorrhagic kidneys and intestines, eels had white-gray colored intestines, and sturgeon showed a thinning of the intestinal wall and degeneration of exocrine pancreas.

Choline is widely found in plant seeds. This fact, plus the ability of animals to synthesize it should limit the need to supplement fish feeds with choline. However, feeds that contain fat-extracted oilseed as a major ingredient may require choline supplementation because choline is removed during fat extraction. In some milling processes the choline containing component (lecithin gum) is added back to the meal, and in others it is marketed separately. If the feed

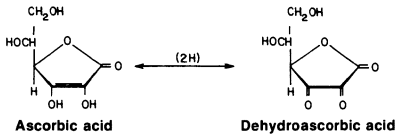


Figure 2.37 Ascorbic acid is the reduced, biologically active form of vitamin C. Dehydroascorbic acid can be reduced in body tissue and converted back to ascorbic acid.

ingredients are low in choline, a choline supplement such as choline chloride should be included.

Vitamin C (Ascorbic Acid)

Most animals can synthesize vitamin C in sufficient quantity for normal growth and function, but a few, such as primates, guinea pigs, some birds, and many fishes, cannot because they lack the enzyme L-gulonolactone oxidase for synthesis of vitamin C from glucose. The vitamin occurs in two forms, a reduced form (ascorbic acid) and an oxidized form (dehydroascorbic acid) (Fig. 2.37). The reduced form predominates, but the forms are biologically reversible, so both have vitamin C activity. If the dehydro- form is further oxidized to diketogulonic acid, it loses its activity and the reaction is irreversible.

Vitamin C is a strong metabolic reducing agent. Its role in hydroxylation of proline and lysine to the hydroxy-amino acids for the conversion of procollagen to collagen has long been recognized. Many of the deficiency signs in fish are related to malsynthesis of collagen, which is a component of bone, gill operculum, support cartilage, blood vessels, skin, fins, and wound scar tissue (see Figure 2.38).

Vitamin C has many other metabolic roles. Bone calcification is impaired when vitamin C is deficient. It is necessary in iron metabolism, probably to convert transferrin iron from oxidized to reduced form for metabolic transport. It is required in conversion of folic to folinic acid. It is required in tyrosine metabolism; a deficiency causes tyrosine excretion in the urine. Vitamin C deficiency increases blood clotting time. It can spare vitamin E in reducing peroxidation of lipid cellular and subcellular membranes. It is essential for maximum rate of immune responses, and has a role in detoxification of various xenobiotics.

Curvature of the spinal column is a prominent, early sign of vitamin C deficiency in finfishes. Scoliosis and lordosis (lateral and vertical curvature of spinal column, respectively) have been produced by feeding vitamin C-deficient diets to rainbow trout, brook trout, coho salmon, tilapia, channel catfish, and young carp (see Figure 2.39). Lim and Lovell (1978) described the pathology of vitamin C deficiency syndrome in channel catfish as deformed spinal columns, external and internal hemorrhages, erosion of fins, depigmented vertical bands around the midsection, distorted gill filament cartilage, and reduced rate of wound healing. Deformed head and gill operculums occur in rainbow trout deprived of dietary vitamin C. Lightner et al. (1979) demonstrated that without sufficient vitamin C in the diet, penaeid shrimp died of "black death," a condition characterized by melanized hemocytic lesions distributed throughout the collagenous tissues.

Dietary requirement of vitamin C varies with metabolic function. Hilton et al. (1978) found that 20 mg of vitamin C per kg of diet was sufficient for normal growth in young rainbow trout, but 40 mg kg^{-1} was necessary to prevent gross

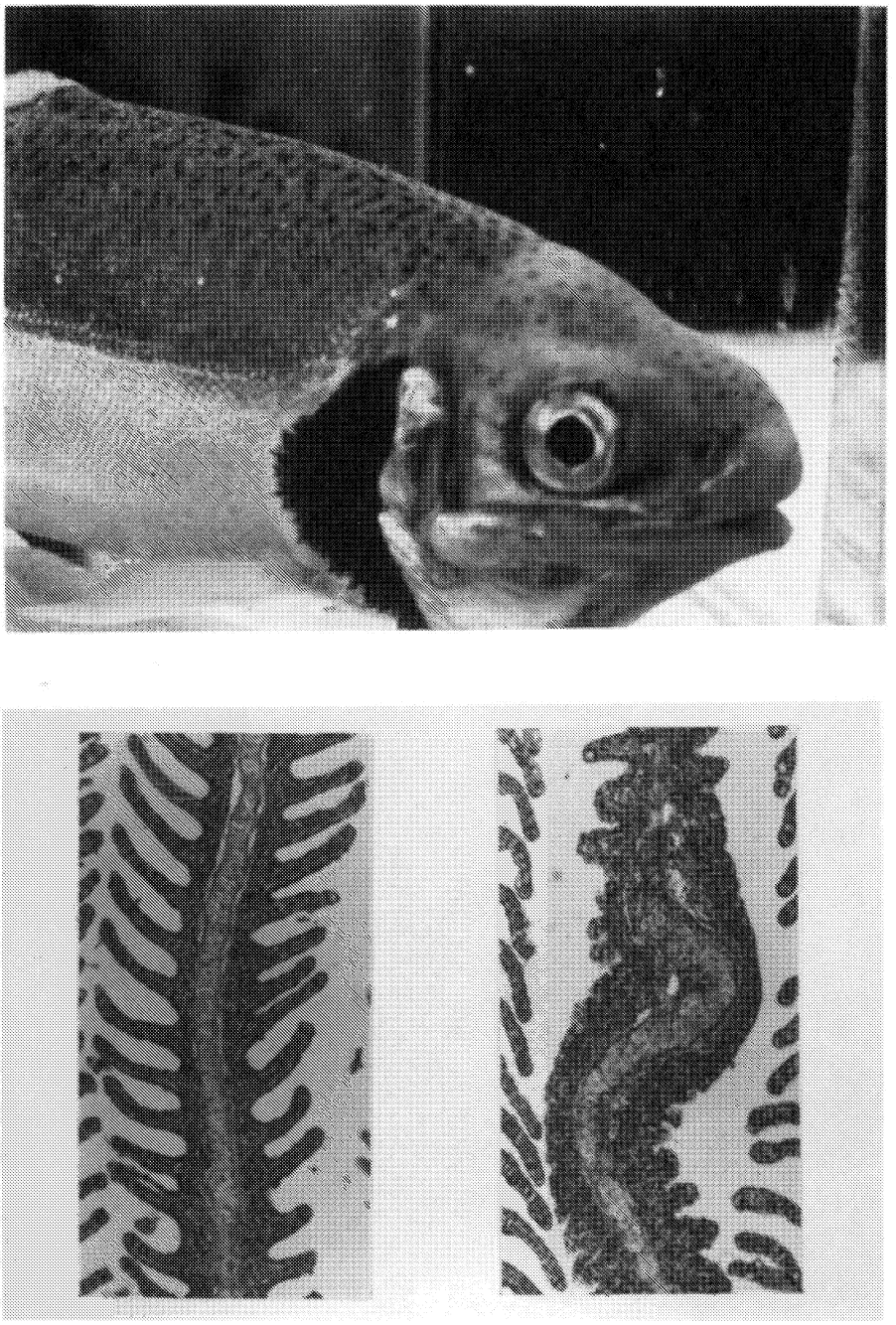


Figure 2.38 Vitamin C is necessary for proper development of connective tissue. Deformed gill operculum (top) can often be seen in fish deprived of a dietary source of the vitamin. Also, deformed gill support cartilage usually occurs, as shown bottom right; bottom left is normal gill filament.

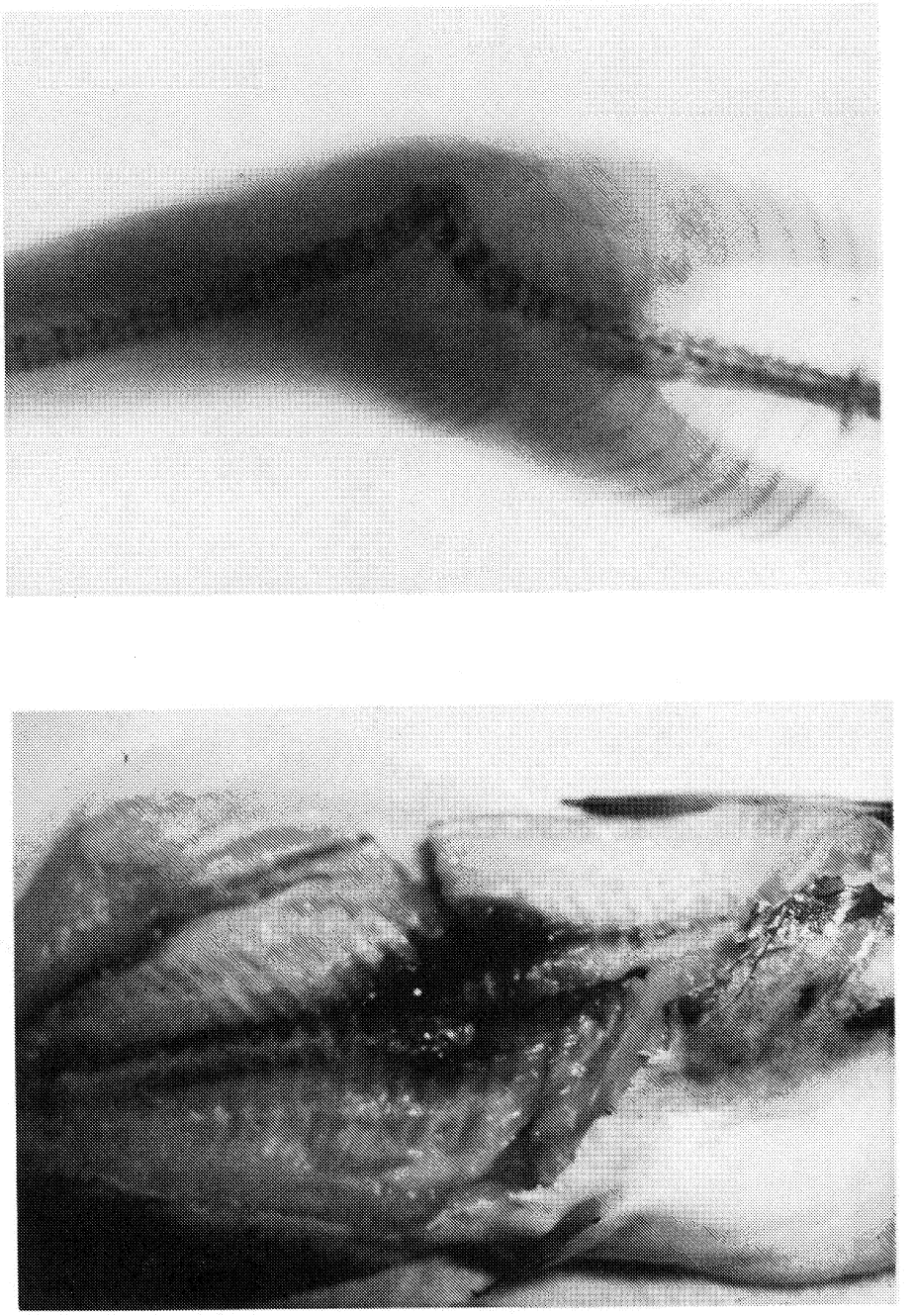


Figure 2.39 Deformed vertebral column is a common vitamin C deficiency sign in fish. Above is a radiograph of a channel catfish fed a diet devoid of the vitamin, showing lateral spinal curvature (scoliosis). Below is a channel catfish with severe vitamin C deficiency, showing complete separation of vertebral column.

deficiency signs. Li and Lovell (1984) found similar results with channel catfish. Halver et al. (1969) reported that 50 mg kg^{-1} (the lowest level fed) was sufficient for normal growth and bone development in coho salmon, but 400 mg kg^{-1} were required for maximum rate of wound healing. Sublethal levels of various pesticides in water increase the requirement of vitamin C by several fishes. Increasing dietary vitamin C reduced incidence of vertebral damage and concentration of toxaphene in the fish, indicating that vitamin C is a factor in detoxification of toxaphene.

Dietary requirement for vitamin C by fish seems to decrease with age. Sato et al. (1978) found that young trout (6 weeks) fed vitamin C-free diets grew slowly and developed scurvy, whereas older trout (19 months) developed none of these problems. Li and Lovell (1984) found that the dietary vitamin C content required for normal growth and bone development for small channel catfish, grown from 1 to 10 grams, was approximately twice that of larger catfish grown from 20 to 100 grams.

Two indicators have been suggested as reliable measures of vitamin C deficiency. Liver concentrations of ascorbic acid of 20 to 26 $\mu g\ g^{-1}$ of tissue have coincided with vitamin C deficiency signs in salmonids and channel catfish. Vertebral collagen level has been shown to be a sensitive index of vitamin C status in channel catfish and rainbow trout. Bone collagen does not increase as dietary vitamin C increases above the dietary requirement, but decreases when the diet becomes deficient in vitamin C. El Naggar and Lovell (1991) found that collagen concentrations below 25% of the dry vertebrae were associated with overt signs of scurvy in channel catfish.

Ascorbic acid is highly sensitive to oxidation; a significant amount is lost during feed processing and storage. Lim and Lovell (1978) found that approximately 50% is destroyed during extrusion and 30% during steam pelleting, and that the half-life of ascorbic acid in the finished feed is about 60 days. Because of the lability of crystalline ascorbic acid, more stable derivatives have been developed. Conjugation of L-ascorbic acid, at the number 2 carbon position, with phosphate or sulfate produces a compound that is highly resistant to oxidation. Rainbow trout can use L-ascorbate-2-sulfate as a dietary replacement for ascorbic acid (Tucker and Halver 1984); channel catfish can also use L-ascorbate-2-sulfate, but it has only about 7% of the potency of L-ascorbic acid (El Naggar and Lovell 1991). All species investigated seem to use L-ascorbate-2-phosphate as a vitamin C source.

Fish fed diets deficient in vitamin C have reduced resistance to bacterial diseases. One role of vitamin C in immune is to protect phagocytic and surrounding cells from oxidative damage. Phagocytic cells in the immune system of fish produce reactive oxygen radicals to kill pathogens but which can damage host cells. Elevated dietary doses of ascorbic acid, much beyond the requirement for normal growth, increased the resistance of young channel catfish and rainbow trout to bacterial infections under laboratory conditions. Fish exposed to natural epizootics of the pathogens under production conditions, however, were less responsive to high dose feeding of vitamin C.

Commercial feed ingredients are almost completely devoid of vitamin C, so the vitamin must be supplemented into practical feeds. L-ascorbic acid is highly sensitive to oxidative destruction during processing and subsequent storage of feeds; thus, overfortification is necessary unless the stabilized vitamin is used. The phosphate and sulfate conjugates of ascorbic acid, which are much more stable

against oxidation during processing and storage than L-ascorbic acid, are presently relatively expensive but will likely replace the less stable form at some time.

Natural Sources of Vitamins

Vitamins most likely to be deficient or of limited availability in commercial fish feeds that contain oilseed meals, animal byproducts, and grains or grain byproducts are vitamins C, A, D, niacin, pantothenic acid, riboflavin, and possibly vitamins E and K. Inositol, biotin, folic acid, pyridoxine, and thiamin are widely found in plant feedstuffs, and vitamin B_{12} is present in animal byproducts. Oil seeds are rich in choline, found in phosphatidylcholine (lecithin), but this component is extracted with the oil during solvent extraction, and may or may not be added back to the meal.

It is possible to formulate a fish feed from commercial ingredients that is adequate in all essential vitamins except vitamin C. Brewer's yeast or distillery dried grains are good sources, and grain byproducts that include the seed coat are fair sources, of the water-soluble vitamins (except C); alfalfa meal is a good source of vitamins E and K; fish meal is a good source of B_{12}; and fish liver oil can supply A and D.

Intestinal Synthesis of Vitamins

Microbial synthesis of vitamins in the gut has not been researched well in fish, although significant amounts of the B vitamins and vitamin K are attributed to this source in warmblooded animals. Studies conducted at Auburn University in which the ratio of vitamin to indigestible dry matter (IDM) were measured in the diet and in the feces showed significant increases in inositol (Burtle 1981) and vitamin B_{12} (Limsuwan and Lovell 1981) in the digestive tract of channel catfish and tilapia. There was no increase in biotin. Addition of antibiotics to the diet significantly reduced the ratio of vitamin to IDM in feces. Increase in vitamin B_{12} in the digestive tract of Nile tilapia, which has a very long digestive tract, was about five times higher than in the digestive tract of channel catfish which has a relatively short tract. Significant absorption of intestinally synthesized B_{12} by channel catfish was revealed by feeding the fish ^{60}Co in the diet and recovering radio-labeled vitamin B_{12} in the blood, liver, kidneys, and spleen (Limsuwan and Lovell 1981).

ESSENTIAL LIPIDS

In addition to being a concentrated energy source, lipids have other nutritional functions. They provide a vehicle for absorption of fat-soluble nutrients such as sterols and vitamins. They play a role in the structure of cell and subcellular membranes. They are components of hormones and precursors for synthesis of various functional metabolites such as prostaglandins and eicosonoids. Some lipids, such as sterols and certain fatty acids, must be provided preformed in the diet and, thus, are essential nutrients for fishes.

Essential Fatty Acids

Essentiality of a fatty acid depends upon its chemical structure, specifically the position of the first double bond proximal to the terminal methyl carbon in the fatty acid chain. Fatty acids are designated by the use of three numbers: the first indicates the number of carbon atoms, the second, the number of double bonds, and

the third, the position of the first double bond in relation to the terminal methyl carbon. For example, 18:2 (n-6) is linolenic acid which has 18 carbons, two double bonds and the first double bond is at the number 6 carbon.

Homeothermic animals have a dietary requirement for fatty acids with a double (unsaturated) bond in the n-6 position which is at the sixth carbon from the terminal (methyl) end of the fatty acid chain. Several fatty acids with similar n-6 end structures [18:2 (n-6), 20:2 (n-6), or 20:4 (n-6)] can satisfy this requirement. Salmonids, however, require n-3 fatty acids. A possible explanation for the difference in fatty acid requirement is that the omega-3 structure permits a greater degree of unsaturation, which is necessary in the membrane phospholipids to maintain flexibility and permeability characteristics in the fish at low temperatures. Several studies have shown that adipose as well as membrane lipids in fish are affected by temperature.

A function of essential fatty acids is to serve as a precursor to metabolic eicosonoids (20-carbon compounds). The eicosonoids are components of biomembrane phospholipids, and are mobilized from this site to be metabolized into other products. Eicosonoids derived from n-6 fatty acids are of the arachidonic acid [20:4 (n-6)] series, and those derived from n-3 fatty acids belong to the eicosopentaenoic acid [20:5 (n-3)] family. Some fish species can convert 18-carbon fatty acids to longer chain, more unsaturated fatty acids. Freshwater fishes can do this, including freshwater salmonids which can chain elongate and desaturate linolenic acid, 18:3 (n-3), to eicosopentaenoic acid. Marine fishes, however, seem to lack the ability to convert the 18-carbon n-3 fatty acids and require 20:5 (n-3) or 22:6 (n-3) in their diet. An absence of n-6 or n-3 fatty acid in the diet leads to the synthesis of n-9 fatty acids in the fish. Thus, 20:3 (n-9) fatty acids will be incorporated into the polar membrane lipids in place of 20:4 (n-6), 20:5 (n-3) or 22:6 (n-3). A ratio of 20:3 (n-9) to 20:3 (n-6) or 20:3 (n-3) higher than 0.4 in the polar lipids of rainbow trout has been suggested as an indication of essential fatty acid deficiency (Castell, et al. 1972).

Biomembranes in fish must be in a fluid state to function properly at various temperatures. Membrane fluidity is dependent on the fatty acid composition of the membrane phospholipids: the highly unsaturated fatty acids, such as 20:5 (n-3) and 22:6 (n-3), are more fluid than more saturated fatty acids at low temperatures. As water temperature changes, the amount of phospholipid in the biomembrane does not change but the fatty acid composition changes. As water temperature decreases, the ratio of n-3 polyunsaturated fatty acids to more saturated fatty acids has been found to increase in liver of rainbow trout (Sellner and Hazel 1982).

Lipids have a special role in immune responses in animals in that some fatty acids are precursors of leukotrienes, 20-carbon compounds produced by macrophages and neutrophiles and which have immunostimulatory functions. The usual fatty acid precursor for leukotrienes in warmblooded animals is linolenic acid [18:2 (n-6)] which through arachidonic acid [20:4 (n-6)] leads to the production of leukotriene B_4, a potent immune stimulator. When warmblooded animals are given n-3 fatty acids, such as 20:5 (n-3) or 22:6 (n-3), they will produce leukotriene B_5 which is antagonistic to the activity of leukotriene B_4. Studies by Fracalossi et al. (1994) showed that channel catfish fed diets containing fish oil or linseed oil, both high in n-3 unsaturated fatty acids, were less resistant to experimental infection with the bacterial pathogen *Edwardsiella ictaluri* than fish fed diets with corn oil, which is high in n-6 fatty acids. This indicates that channel catfish, a warmwater

species, require n-6 fatty acids as do warm blooded animals for optimum immune responses.

In general, freshwater fish from warm or temperate environments have a requirement n-6 fatty acids. Some warmwater fish, such as tilapias which are indigenous to tropical regions, require principally n-6 fatty acids. Some warmwater fish such as channel catfish and carp seem to favor a combination of n-6 and n-3 fatty acids. Some shrimp, such as *Palaemon serrratus* and *Penaeus indicus*, also seem to require both n-3 and n-6 fatty acids. Coldwater species and most marine fishes, even some which are from warm or temperate waters, have a major n-3 fatty acid requirement.

Determining requirements for fatty acids is difficult for fish because the metabolic requirement is very small and fatty acids stored in the body or even carried over from the egg yolk can influence performance of the experimental fish. Fish used in fatty acid experiments should be carefully depleted of the fatty acids to be tested prior to the experiment.

Fatty acid deficiency signs reported for various fishes are reduced growth, dermal anomalies (such as fin rot), elevated muscle water (edema), increased susceptibility to bacterial infection, increased permeability of cellular and subcellular (mitochondrial) membranes, and reduced reproductive performance. Deficient fish exhibited a fainting or shock syndrome. Larvae of red sea bream, ayu, and striped bass fed rotifers or artemia low in 20:5 (n-3) or 22:6 (n-3) fatty acids showed high mortality and underdeveloped swim bladders.

Most fish respond to lipids in the diet. How much of this response is caused by supplying essential fatty acids and how much is due to a readily available energy source is difficult to discern. The dietary requirement for n-3 fatty acids, for species that require this form, seems to be about 0.5% to 1% if the fatty acids are greater than 18-carbon (20:5 and 22:6). For fishes requiring n-6 fatty acids, or a combination of n-3 and n-6, the requirement appears to be in the range of 0.5% to 1%. Determined essential fatty acid requirements for various fishes are presented in Table 2.7.

Table 2.7. ESSENTIAL FATTY ACID REQUIREMENTS OF CHANNEL CATFISH, RAINBOW TROUT, PACIFIC SALMON, COMMON CARP AND NILE TILAPIA (AMOUNT PER KG OF DIET).

Fatty acid	Channel catfish	Rainbow trout	Red sea bream	Common carp	Nile tilapia
n-3 Fatty acids:					
C18:3, C20:5 or C22:6	-	1	1	-	-
C20:5 or C22:6	0.5	-	-	-	-
n-6 Fatty acid:					
C18:2	0.5	0.5	-	1	0.5

Adapted from National Research Council, 1993.

Fatty acid composition of adipose lipid in fish is influenced primarily by diet, whereas membrane lipids are more characteristic of environment and species. Higher plants synthesize primarily fatty acids that are 18-carbon or less in chain length, usually with the unsaturated bonds in n-6 and n-9 positions, so "grain-fed" fish will store primarily these types. Some plant oils, however, such as linseed oil, contain significant amounts of n-3 fatty acids. The source of long chain, polyunsaturated n-3 fatty acids (C20:5, C22:6) is freshwater or marine algae, and wild fish obtain these fatty acids through the food chain. Thus, oil of fish consuming lipids originating from marine or freshwater algae is the primary source of n-3 highly unsaturated fatty acids for fish or human diets.

Sterols and Phospholipids

Finfish readily synthesize sterols from acetate and mevalonic acid; however, crustaceans have limited ability to do this and therefore have a dietary requirement for preformed sterols. The absence of sterol from diets of crustaceans that do not have access to natural foods results in mortality in a short time. The dietary requirement for sterol (as cholesterol) is around 0.5% for penaeid shrimp and lobsters. Marine crustaceans seem to also require the phospholipid lecithin in their diet for maximum growth. Growth rate of penaeid shrimp in a laboratory environment was improved by adding 1% lecithin to the diet, and growth and survival of lobsters were improved with a 7% supplement of soybean lecithin.

MINERALS

Not all inorganic elements found in an animal's body are essential in its diet. However, dietary need for 22 minerals has been demonstrated in one or more animal species. Those required in large quantities are termed major and those required in trace quantities are called trace minerals. The major minerals are calcium, phosphorus, magnesium, sodium, potassium, chlorine, and sulfur. Trace minerals are iron, copper, zinc, manganese, selenium, iodine, cobalt, fluorine, molybdenum, aluminum, nickel, vanadium, silicon, tin, chromium, and possibly others. Determination of dietary mineral requirements is difficult, especially with fish. In addition to the usual problems encountered in mineral nutrition research, such as formulating absolute mineral-free diets and overcoming tissue stores of minerals, fish can absorb dissolved minerals from the water.

A major difference between mineral metabolism in fish and land animals is osmoregulation, or maintenance of osmotic balance between body fluids in the fish and the water around the fish. Other biochemical functions of minerals in fish are similar to those in warmblooded animals. Some minerals are constituents of hard tissues, such as bone, fins, and scales, and some are components of soft tissues, such as sulfur in protein and iron in hemoglobin. Some minerals function as components or activators of enzymes and hormones, such as zinc which activates alkaline phosphatase, and iodine which is a component of the hormone thyroxine. Some soluble elements, such as calcium, sodium, potassium, and chloride have functions in the blood or body fluids such as osmoregulation, acid-base balance, electron transfer, and sensitizing muscle fibers.

Fish can absorb dissolved minerals from the water across the gill membrane or, in the case of marine fishes that drink water, through the digestive tract. Most of the calcium requirement for fishes comes from the water. In seawater,

significant amounts of iron, magnesium, cobalt, potassium, sodium, copper, selenium, and zinc can be obtained from the water. Berg (1968) provided data that indicated that goldfish obtained 50% to 80% of their calcium from the water when fed a calcium-adequate diet. Fish require a dietary source of phosphorus to meet their relatively high metabolic requirement because levels of dissolved phosphorus in natural waters are relatively low.

Fish require the same minerals as warm blooded animals for tissue formation and various metabolic processes. Table 2.8 shows mineral requirements for several fish species.

Calcium and Phosphorus. Most of the calcium found in the fish body, perhaps 99%, is in skeletal tissue and scales. For scaled fish, from 20% to 40% of the total calcium is in scales. During fasting, calcium is resorbed from the hard tissues for physiological functions. The percentage of calcium in the whole, fresh (wet) body of finfish ranges from 0.5% to 1% with a ratio of calcium to phosphorus of 0.7 to 1.6. In addition to its structural functions in bones and scales, calcium is essential for blood clotting, muscle function, nerve impulse transmission, osmoregulation, and as a cofactor during various enzymatic processes.

There is little exchange of bone calcium with body fluids in marine fish; but in low-calcium fresh water, where fish must extract calcium against a steep gradient, mobilization of calcium stores from bones and scales may be necessary under certain conditions (Ichii and Mugiya, 1983), like during ovarian maturation. The calcium exchange rate of fish scales is three times that of bones (Berg, 1968).

The calcium requirement in fish is met in large part by absorption through gills and skin in fresh water and by drinking seawater. The concentration of dietary calcium rarely seems critical for salmonids, and a dietary requirement has not been demonstrated. Catfish and tilapia reared in calcium-free water require calcium in the diet for optimum growth. Calcium deficiency has not been detected in carp and catfish in fresh water. Generally, calcium from water and natural ingredients in the diet supply sufficient calcium to meet the requirements of fish.

Approximately 85% to 90% of the phosphorus in fish is in bone complexed with calcium to form apatite, or tricalcium phosphate. Phosphorus is also a component of adenosine triphosphate (ATP), which is involved in energy-releasing cellular reactions, nucleic acids such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and phospholipids in cell and subcellular membranes. Phosphorus functions in a number of phosphorylations in the metabolism of carbohydrates, lipids and amino acids. The phosphate buffer system maintains normal pH in body fluids.

Prominent signs of dietary phosphorus deficiency in fish are poor growth and bone mineralization. Further signs of deficiency observed in carp include increases in body fat, reduced blood phosphate levels, deformed head (frontal bone) and spinal column, and abnormal calcification of ribs and soft rays of the pectoral fins. Reduced breaking strength of rib bones and increased body fat were reported in channel catfish, and red sea bream showed deformed vertebrae with increased body fat content and decreased glycogen content in the liver. Eya and Lovell (1997) reported that resistance to bacterial infection was reduced in phosphorus deprived channel catfish. They also found that body fat percentage decreased linearly as dietary phosphorus increased above the requirement for normal growth.

Table 2.8. DIETARY REQUIREMENTS FOR MINERALS FOR CHANNEL CATFISH, RAINBOW TROUT, PACIFIC SALMON, COMMON CARP AND NILE TILAPIA (AMOUNT PER KG OF DIET)

Mineral	Channel catfish	Rainbow trout	Pacific salmon	Common carp	Nile tilapia
Calcium	-[a]	-[a]	-[a]	-[a]	-[a]
Chlorine, %	R[b]	0.9 E[b]	NT[b]	NT	NT
Magnesium, %	0.04	0.05	NT	0.05	0.06
Phosphorus, %	0.45	0.60	0.60	0.60	0.50
Potassium, %	R	0.70	0.80	NT	NT
Sodium, %	R	0.6 E	NT	NT	NT
Copper, mg	5.0	3.0	NT	3.0	R
Iodine, mg	1.1 E	1.1	0.6-1.1	NT	NT
Iron, mg	30.0	60	NT	150	NT
Manganese, mg	2.4	13	R	13	R
Zinc, mg	20.0	30	R	30	20
Selenium, mg	0.25	0.3	R	NT	NT

Adapted from National Research Council, 1993.

[a]*Dietary calcium not required unless water is unusually low in calcium.*

[b]*R = required; NT = not tested; E = estimated.*

Minimum requirement of available phosphorus in diets for young channel catfish was determined in several labatory experiments, with purified diets to be 0.4 to 0.45%. Requirements for young common carp, Nile tilapia, red sea bream, and Japanese eel have been determined under laboratory conditions to be 0.6%, 0.9%, 0.68% and 0.29% of the diet, respectively. Recently, two independent studies demonstrated that the dietary available phosphorus requirement of large channel catfish, grown to marketable size in commercial-type ponds, was 0.3%. This represents a 25 to 33% lower phosphorus requirement for large catfish fed in ponds than was determined for small fish in laboratory studies.

Phytate phosphorus (approximately 67% of the phosphorus in grains is in the phytate form) is unavailable to fish. However, addition of a microbial phytase to catfish feeds approximately doubles the availability of phytate phosphorus. Phosphorus in fish meal is 40% to 75% available to fish with gastric stomachs, but less than 25% available to the stomachless common carp. The differences in the availability of phosphorus to salmonids and to carp and tilapia is probably due to the limited secretion of gastric juices by these warmwater species. Inorganic phosphorus from sodium or monocalcium phosphate is highly available to all fish; however, dicalcium phosphate is less available and tricalcium phosphate is poorly available. Availability of phosphorus in several feed ingredients for catfish, carp, and trout is presented in Table 3.3 in chapter 3.

Magnesium. About 70% of the magnesium in a fish's body is in the hard tissue. Other functions of magnesium are as enzyme activators in many carbohydrate metabolism and in protein synthesis reactions. Magnesium is necessary in body fluids for osmoregulation and, also, to maintain integrity of smooth muscle. Deficiency causes tetany in warmblooded animals and flaccid muscle in fish.

Magnesium deficiency in the diet of channel catfish causes poor growth, anorexia, lethargy, flaccid muscles, high mortality, and depressed magnesium levels in the whole body, blood serum, and bones. Deficiency of magnesium in the diet of common carp caused similar signs, in addition to convulsions and cataracts. Signs of deficiency in rainbow trout include reduced growth rate, vertebral curvature, degeneration of muscle fibers, calcinosis of the kidney, and degeneration of the epithelial cells of the pyrolic caecae and gill filaments. Magnesium deficiency has not been demonstrated in fish reared in seawater, which typically contains high levels of magnesium. Fish in fresh water, which only contains 1 to 3 mg L^{-1} of magnesium, require 0.025% to 0.07% magnesium in the diet. However, most foods, especially plants, are high in magnesium, which usually makes it unnecessary to supplement feeds made from natural ingredients. Purified diets must have a magnesium supplement.

Iron. A principal role of iron in a fish's body is as a component of hemoglobin. Hemoglobin is the oxygen-carrying pigment in red blood cells, and iron, though its oxidation-reduction activity, is the oxygen binding element in the molecule. Red blood cells are formed primarily in the spleen and anterior kidney in fish instead of the bone marrow, as in land animals. Red blood cells are regenerated periodically and most of the iron is recycled. That which is not recycled is excreted through the bile into the intestine. Another role of iron is as a component of a number of heme enzymes, such as cytochromes, which are involved in the electron transport process in cellular oxidation.

Iron, like other elements of low solubility, such as zinc and copper, is absorbed and transported in the body in protein-bound form. In the intestinal cell mucosa, iron combines with a protein, apoferritin, to form ferritin. The amount of apoferritin in the mucosa is regulated by body need for iron. Iron is in the oxidized form (Fe^{+++}), when combined with the protein in the mucosa. When liberated into blood, it is reduced to Fe^{++} by reducing agents, such as vitamin C. It is transported in the blood bound to another protein as transferrin and stored in the liver and hemopoietic tissues as Fe^{+++} in combination with a protein until used. Iron and other minerals of low solubility are not excreted through the urine, but are returned to the digestive tract through the bile duct.

Dietary deficiency of iron, or factors facilitating its absorption, cause microcytic anemia in fish. In some experiments to evaluate effects of iron deficiency, growth was not affected, however, Lim et al. (1996) found channel catfish were highly sensitive to dietary iron deficiency. Dietary requirement for iron by channel catfish is 30 mg kg^{-1} of diet and by Atlantic salmon is 60 mg kg^{-1} of diet. Dissolved iron in the water can serve as a source of iron for fish metabolism, although iron in water often precipitates out as ferric hydroxide and levels in solution are low. Iron is widespread in feedstuffs; however, its availability from plant feeds is relatively low. Unless fish feeds contain significant amounts of animal byproducts, supplemental iron should be used. Ferrous sulfate (36% Fe) is highly available to animals and is the major iron supplement used in animal feeds. Ferrous carbonate is also an acceptable iron supplement, but iron oxide has limited availability. Iron chelates, where iron is chelated to an amino acid or protein, are commercially available but usually more expensive than inorganic sources. Trace mineral chelates are usually much more bioavailable than inorganic iron.

Copper. Copper is involved with iron absorption and metabolism. When the diet is deficient in copper, iron levels in body tissues decrease. Copper plays a role in hematopoiesis (hemoglobin synthesis). It is a component of a number of enzyme systems, such as cytochrome C oxidase of the electron transport system superoxide dismutase, and tyrosinase. It is essential in bone development, perhaps through its role in collagen synthesis. Some sea animals, mollusks, and crustaceans contain copper as the metal nucleus of the oxygen-carrying pigment in the blood, hemocyanin or cyanidin, which has an analogous role to hemoglobin in red-blooded animals. Like iron, copper is absorbed and transported as a copper-protein complex.

Common carp require approximately 3 mg of copper per kilogram of diet for normal growth. Channel catfish require 1.5 mg kg^{-1} to 5 mg kg^{-1} for normal growth and blood cell formation, but 32 mg kg^{-1} caused growth depression and anemia. This indicates a rather narrow range of dietary copper tolerance. Approximately 100 mg to 250 mg copper kg^{-1} of diet is toxic to mammals.

Iodine. Iodine is a component of thyroxine and the thyroxine derivative, triiodothyronine, thyroid hormones that regulate the rate of metabolism. If the amino acid tyrosine and iodine are supplied, the body can synthesize these compounds. Deficiency of iodine results in hyperplasia of the thyroid gland, or goiter, in fishes. Fish obtain iodine from water via branchial pumps and from feed sources. Sea water is a good source of iodine but freshwater is usually limited. Iodine is transported in the body bound to a protein and excreted in the urine rather than through the bile duct which is the usual route of excretion for metal elements.

The minimum requirement of fish for dietary iodine has not been defined; however, 1 to 5 mg kg^{-1} feed has been found to be an adequate level for several species. Increases in dietary iodine concentrations have been observed for salmon smoltification and for Atlantic salmon exposed to bacterial kidney disease infection. Fish meal is a rich source of iodine. Fish feeds not containing fish meal should probably be supplemented with iodine in the absence of natural aquatic foods.

Zinc. Zinc has several functions. It serves as a cofactor in a number of enzyme systems, including carbonic anhydrase found in red blood cells, and enzymes in protein and carbohydrate metabolism. Zinc plays a role in preventing keratinization of epithelial tissue. Insulin is stored in the body as a zinc complex.

Gatlin and Wilson (1983) demonstrated that zinc-deficient channel catfish had depressed growth, appetite, and serum alkaline phosphatase activity, and reduced levels of zinc and calcium in bones. They showed that channel catfish had a minimum dietary zinc requirement of 20 mg kg^{-1} to prevent deficiency signs. Zinc deficiency in common carp caused slow growth, loss of appetite, high mortality, and erosion of the skin and fins. Cataracts and short-body dwarfism were reported in rainbow trout fed zinc deficient diets. Reduced reproductive efficiency can be caused by zinc deficiency. The dietary zinc requirement for most fish species fed diets free of zinc-bonding components, such as phytic acid, is around 15 to 30 mg kg^{-1}.

Ketola (1979) produced bilateral lens cataracts in rainbow trout by feeding diets with normal requirements of zinc but which contained fish meal with high bone ash. He corrected the problem by supplementing 150 mg kg^{-1} zinc into the diet, and speculated that the high level of calcium or other minerals in the fish meal impaired zinc absorption.

Dietary calcium and phosphorus concentrations, presence of phytic acid, and source of zinc affect zinc absorption in fish. Phytate forms a complex with transitional elements, such as zinc and iron, and impedes their absorption. Calcium promotes the complexing of zinc to phytates. The bioavailability of zinc in fishmeal is inversely related to the tricalcium phosphate content. This is presumably caused by adsorption of zinc onto insoluble calcium phosphate complexes in the intestine that are unabsorbed. Thus, dietary supplementation of zinc will depend upon the amount of fish meal, meat and bone meal, and phytic acid in the diet. Gatlin and Wilson (1984) demonstrated that the zinc allowance in practical catfish feeds should be several times higher than in purified diets because of the phytic acid in soybean meal. They recommended that the zinc allowance be increaed from 20 mg kg^{-1} as determined in experimental diets to 80 to 100 mg kg^{-1} in practical feed to compensate for the zinc-binding property of soybean meal and other phytic acid sources.

Manganese. Manganese functions as a cofactor in a number of enzyme systems, including superoxide dismutase and those involved in amino acid, fatty acid, and glucose oxidation.

Manganese-deficient diets caused depressed growth in common carp and rainbow trout, and abnormal tail growth and shortening of the body in the latter. Supplementation of the diet to bring the level of manganese to 13 mg kg^{-1} improved growth in both species and prevented abnormalities in rainbow trout. However, Gatlin and Wilson (1984b) found that 2.4 mg manganese kg^{-1} diet was sufficient for normal growth and health of channel catfish. Sea water is a significant

source of manganese for fish, but fresh water is a poor source of soluble manganese. Animal protein feedstuffs are sources of manganese but may not be sufficient because of limited amounts of the feedstuff in the formula or limited availability of the element. Manganese sulfate is a highly available manganese supplement. It is usually more expensive than manganese oxide which has about 67% of the bioavailability of the sulfate form. Manganese dioxide is a poor source.

Selenium. The most notable function of selenium is as a component of the enzyme glutathione peroxidase, which reduces hydroperoxides, which are strong pro-oxidants, and thus protects polyunsaturated phospholipids in cellular and subcellular membranes from oxidation damage. Selenium has also been identified as a cofactor in glucose metabolism.

Selenium deficiency in Atlantic salmon caused reduced growth rate and suppressed plasma glutathione peroxidase activity. Selenium deficiency alone does not produce pathological signs in fish, but selenium and vitamin E deficiency combined caused nutritional muscular dystrophy. Maximum plasma glutathione peroxidase activity occurred on rainbow trout fed diets containing 0.38 mg of selenium kg^{-1}. A level of 13 mg of selenium kg^{-1}, as sodium selenite, in trout diets caused suppressed growth and increased mortality. Gatlin and Wilson (1984a) found that maximum growth and glutathione peroxidase activity occurred in channel catfish fed diets containing 0.25 mg of selenium kg^{-1} as selenite, and that 15 mg of selenium kg^{-1} was toxic to the catfish.

In the United States, selenite selenium is routinely supplemented in corn-soybean meal feeds for poultry and swine to compensate for deficiencies in feedstuffs produced on selenium-deficient soils. Fish feeds containing predominately plant ingredients should contain a selenium supplement.

Sodium, potassium, and chloride. Dietary deficiencies of sodium, potassium, and chloride are not readily produced in fishes, although these elements are necessary for osmoregulation, electrolyte balance and pH balance in the body fluids, nerve impulse transmissions, and other functions. Sodium and potassium are the major extracellular and intracellular cations, respectively, and chloride is the major extracellular anion. The chloride ion is also a component of hydrochloric acid, which is secreted in the stomach. Common feedstuffs are rich in these elements; this source, plus the fact that most freshwater and all seawater contain significant amounts, make supplementation of practical diets with these three minerals unnecessary. Some fish have been found to require potassium supplementation in purified diets fed in freshwater but not when fed in sea water. Red sea bream did not require potassium in the diet in sea water but did in fresh water. Fish absorb the ions through the gills in freshwater and through the gut in seawater. Because most fish can excrete large dietary intakes of salt effectively, dietary levels of up to 4 percent salt have had no adverse effect on subadult or adult fish fed in fresh or seawater.

Chromium. Chromium has been identified as a cofactor in enzymes in carbohydrate, lipid, protein, sterol and nucleic acid metabolism in laboratory animals. The role of chromium in potentiating the action of insulin for glucose metabolism is well known. In the absence of dietary chromium, glucose tolerance is reduced and lipid and glycogen synthesis from glucose is impaired. Field studies

have shown supplementation of practical, basal diet with 0.2 to 0.4 mg of chromium kg^{-1} as chromium picolinate increased gain and muscle mass, and reduced carcass fat in pigs, lambs and broiler chickens.

Less is known about chromium requirements of fish. They apparently have a dietary requirement and metabolic need. Tilapia fed high (40%) glucose diets showed delayed plasma glucose plateau, higher content of liver glycogen and immune weight gain when fed chromium in the diet. Chromium chloride is the usual inorganic source of chromium for animal diets. Chromium picolinate, chromium proteinate and chromium yeast are organic sources which have been reported to have higher bioavailability for animals than chromium chloride.

REFERENCES

AKIYAMA, T., T. MURAI, and T. NOSE. 1986. Oral administration of serotonin against spinal deformity of chum salmon fry induced by tryptophan deficiency. Bull. Jpn. Soc Sci Fish. 52: 1249-54.

ANDERSON, R. J., E. W. KIENHOLZ, and S. A. FLICKINGER. 1981. Protein requirements of smallmouth bass and largemouth bass. J. Nutr. 111: 1085-1097.

BATTERHAM, E. S., R. D. MURISON, and C. E. LEWIS. 1979. Availability of lysine in protein concentrates as determined by the slope-ratio assay with growing pigs and rats and by chemical techniques. Br. J. Nutr. 41: 383-391.

BERG, A. 1968. Studies on the metabolism of calcium and strontium in freshwater fish. I. Relative contribution of direct and intestinal absorption. Mem. Ist Ital. Idrobiol. Dott. Macro de Marchi 23: 161-196.

BRAMBILA, S. and F. W. HILL. 1966. Carbohydrate requirement of chickens. J. Nutr. 88: 84-89.

BRETT, J. R. 1973. Energy expenditure of sockeye salmon during sustained performance. J. Fish. Res. Board Can. 30: 1799-1809.

BURTLE, G. J. 1981. Essentiality of dietary inositol for channel catfish. Ph.D. diss., Auburn University, Auburn, AL.

BUTTERWORTH, C. E., JR., J. A. PLUMB, and J. M. GRIZZLE. 1986. Abnormal folate metabolism in feed-related anemia in cultured catfish. Proc. Soc. Exp. Biol. Med. 181: 210-216.

CASTELL, J. D., R. O. SINNHUBER, J. H. WALES, and D. J. LEE. 1972. Essential fatty acid requirements of juvenille seabass, Lates calcarifer. Paper presented at Third International Symposium on Feeding and Nutrition in Fish. Toba, Japan. August 28-September 1, 1989.

CHO, C. Y. and S. J. KAUSHIK. 1990. Nutritional energetics in fish: Energy and protein utilization in rainbow trout (salmo gairdneri). World Rev. Nutr. Diet. 61: 132-172.

CHO. C. Y., S. T. SLINGER, and H. S. BAYLEY. 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. Comp. Biochem. Phys. 73B: 25-41.

COWEY, C. B. 1975. Aspects of protein utilization by fish. Proc. Nutr. Sci. 34: 57-63.

COWEY, C. B., E. DEGENER, A. G. T. TACON, A. YOUNGSON, and J. G. BELL. 1984. Effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout grown at natural and varying water temperatures. Brit. J. Nutr. 51: 443-451.

DUNCAN, P. L. and R. T. LOVELL. 1994. Influence of vitamin C on the folate requirement of channel catfish, Ictalurus punctatus, for growth, hematopoiesis, and resistance to Edwardsiella ictaluri infection. Aquac. 127: 233-244.

EL NAGGAR, G. O. and R. T. LOVELL. 1991. L-Ascorbyl-2-monphosphate has equal antiscorbutic activity as L-ascorbic acid but L-ascorbyl-2-sulfate is inferior to L-ascorbic acid for channel catfish. J. Nutr. 116: 1061-1067.

EYA, J. and R. T. LOVELL. 1997. Available phosphorus requirements of food-size channel catfish fed practical diets in ponds. Aquac. (In Press).

FARRELL, D. J. 1974. General principles and assumptions of calorimetry. In Energy requirements of poultry, ed. T. R. Morris and B. M. Freeman, Edinburgh, UK: British Poultry Science.

FRACALOSSI, D. M., M. C. CRAIG-SCHMIDT, and, R. T. LOVELL. 1994. Effects of dietary lipid sources on production of leukotriene B by head kidney of channel catfish held at different water temperatures. J. Of Aquatic Animal Health. 6: 242-250.

GATLIN, D. M., III, and R. P. WILSON. 1983. Dietary zinc requirements of channel catfish. J. Nutr. 113: 630-635.

GATLIN, D. M., III, and R. P. WILSON. 1984a. Dietary selenium requirement of fingerling channel catfish. J. Nutr. 113: 627-633.

GATLIN, D. M., III, and R. P. WILSON. 1984b. Studies on manganese requirement of fingerling channel catfish. Aquac. 41: 85-92.

GATLIN, D. M., III, and R. P. WILSON. 1986a. Dietary copper requirements for channel catfish. Aquac. 54: 277-285.

GATLIN, D. M., III, and R. P. WILSON. 1986b. Characterization of iron deficiency and dietary requirements for channel catfish. Aquac. 52: 191-198.

GOLDSTEIN, L. and R. P. FORSTER. 1970. Nitrogen metabolism in fish. In Comparative biochemistry of nitrogen metabolism, ed. J. W. Campbell, New York: Academic Press.

HALVER, J. E., L. M. ASHLEY, and R. R. SMITH. 1969. Ascorbic acid requirements of coho salmon and rainbow trout. Trans. Am. Fish. Soc. 98: 762-771.

HILTON, J. W., C. Y. CHO, and S. J. SLINGER. 1978. Effect of graded levels of supplemental ascorbic acid in practical diets of rainbow trout. J. Fish. Res. Bd. Can. 35: 431-436.

ICHII, T. and Y. MUGIYA. 1983. Effects of dietary deficiency in calcium on growth and calcium

uptake from the aquatic environment in the goldfish, Carassius auratus. Comp. Biochem. Physiol. 74A: 259-262.
IZQUIERDO, O. A., C. M. PARSONS, and D. H. BAKER. 1988. Bioavailability of lysine in L-lysine-HCl. J. Animal Sci. 66: 2590-2597.
KASHIWADA, K., S. TESHIMA, and A. KANAZAWA. 1970. Studies on the production of B vitamins by intestinal bacteria of fish. V. Evidence of the production of vitamin B12 by microorganisms in the intestinal canal of carp, Cyprinus carpio. Bull. Jpn. Soc. Sci. Fish. 36: 421-424.
KETOLA, H. G. 1979. Influence of dietary zinc on cataracts in rainbow trout (Salmo gairdneri). J. Nutr. 109: 965-969.
LI, M. and R. T. LOVELL. 1991. Comparison of satiate and restricted feeding of channel catfish with various protein concentrations in ponds. Aquac. 103: 165-175.
LI, Y. and R. T. LOVELL. 1984. Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J. Nutr. 115: 123-131.
LIGHTNER, D. V., B. H. HUNER, P. C. MAGERELLI, and L. B. CALVIN. 1979. Ascorbic acid: Nutritional requirement and role in wound repair in shrimp. Proc. World Maric. Soc. 9: 447-458.
LIM, C., and R. T. LOVELL. 1978. Pathology of the vitamin C deficiency syndrome in channel catfish (Ictalurus punctatus). J. Nutr. 108: 1137-1146.
LIM, C., W. M. SEALEY, and P. H. KLESIUS. 1996. Iron methionine and iron sulfate as sources of dietary iron for channel catfish Ictalurus punctatus. J. World Aquac. Soc. 27: 290-296.
LIMSUWAN, T., and R. T. LOVELL. 1981. Intestinal synthesis and absorption of vitamin B12 in channel catfish. J. Nutr. 111: 2125-2132.
LOVELL, R. T., T. MIYAZAKI, and S. REBEGNATOR. 1984. Requirement of alpha-tocopherol by channel catfish fed diets low in polyunsaturated fatty acids. J. Nutr. 114: 894-901.
MANGALIK, A. 1986. Dietary energy requirements for channel catfish. Ph. D. diss., Auburn University, Auburn, AL.
MARTIN, A. K. and K. L. BLAXTER. 1965. The energy cost of urea synthesis in sheep. In Energy metabolism, ed. K. L. Blaxter, New York: Academic Press.
MATTHEWS, C. K. and E. J. van HOLDE. 1990. Biochemistry, Redwood City, Calif.: Benjamin/Cummings.
MURAI, T. 1985. Biological assessment of nutrient requirements and availability of fish. Special workshop at the International Congress on Nutrition, August 19-25, Brighton, England.
NATIONAL RESEARCH COUNCIL. 1988. Nutrient requirements of swine. Washington, D.C.: National Academy of Sciences.
NATIONAL RESEARCH COUNCIL. 1994. Nutrient requirements of poultry. Washington, D.C.: National Academy of Sciences.
NATIONAL RESEARCH COUNCIL. 1993. Nutrient requirements of fish. Washington, D.C.: National Academy of Sciences.
POSTON, H. A., R. C. RIIS, G. L. RUMSEY, and H. G. KETOLA. 1977. The effect of supplemental dietary amino acids, minerals and vitamins on salmonids fed cataractogenic diets. Cornell Vet. 67: 472-509.
ROBBINS, K. R., H. W. NORTON, and D. H. BAKER. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109: 1710-1714.
SANTIAGO, C. B. 1985. Amino acid requirements of Nile tilapia. Ph. D. diss., Auburn University, Auburn, AL.
SATO, M., R. YOSHINAKA, and S. YAMAMOTO. 1978. Nonessentiality of ascorbic acid in the diet of carp. Bull. Jpn. Soc. Sci. Fish. 44: 1151-1156.
SELLNER, P. A. and J. R. HAZEL. 1982. Time course of changes in fatty acid composition of gills and liver from rainbow trout (Salmo gairdneir) during thermal acclimation. J. Exp. Zool. 221: 159-168.
SMITH, R. R. 1976. Metabolizable energy of feedstuffs for trout. Feedstuffs. 48: 16-21.
SMITH, R. R. 1989. Nutritional energetics. In Fish nutrition, ed. J. E. Halver, San Diego, Calif: Academic Press.
TUCKER, B. W., and J. E. HALVER. 1984. Ascorbate-2-sulfate metabolism in fish. Nutr. Rev. 45: 173-179.
WILSON, R. P. and W. E. POE. 1988. Choline nutrition of fingerling channel catfish. Aquac. 68: 65-71.
WILSON, R. P., E. H. ROBINSON, and W. E. POE. 1981. Apparent and true availability of amino acids from common feed ingredients for channel catfish. J. Nutr. 111: 923-929.
ZARATE, D. D. and R. T. LOVELL. 1997. Free lysine (L-lysine-HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (Ictalurus punctatus). Aquaculture 159:87-100.
ZIETOUN, I. H., D. E. ULBREY, and W. T. MAGEE. 1976. Quantifying nutrient requirements of fish. J. Fish. Res. Bd. Can. 33: 167-172.

3 DIGESTION AND METABOLISM

DIGESTION

Digestion is classically defined as the preparation of food by the animal for absorption. As such, this may include mechanical reduction of particle size (grinding by pharyngeal teeth or gizzard), enzyme solubilization of organics, pH solubilization of inorganics, and emulsification of lipids. Thus, absorption includes the various processes that allow ions and molecules to pass through membranes of the intestinal tract into the blood to be metabolized by the animal. In a less formal context, digestion is often broadly used to describe both processes.

The Digestive Tract

Fish vary tremendously in morphology and physiology of digestive tracts, and in feeding behavior. Most fishes do not have teeth or gizzards for grinding food; examples of exceptions are the grass carp (which has pharyngeal teeth) and the gizzard shad. Crustaceans grind food prior to ingestion. The most pronounced distinction among fishes with different digestive process is that some, such as channel catfish, have digestive tracts less than the length of their body and others, such as tilapias, have tracts six to eight times their body length. Some fish, such as silver carp, feed exclusively on plankton concentrated from the water; others, like common carp, are strictly bottom feeders, and many species feed only on large prey animals. It is indeed hazardous to make a generalization about the digestive system and feeding behavior of fishes. Figure 3.1 shows the digestive tracts of tilapia, which has a modified stomach, carp, which has no stomach, and channel catfish, which has a well developed gastric section.

The major divisions of the digestive tract in vertebrate animals are mouth, esophagus, pharynx, stomach, intestine, rectum, and secretory glands, which include the liver and pancreas. Not all fish have functionally divided parts; in some

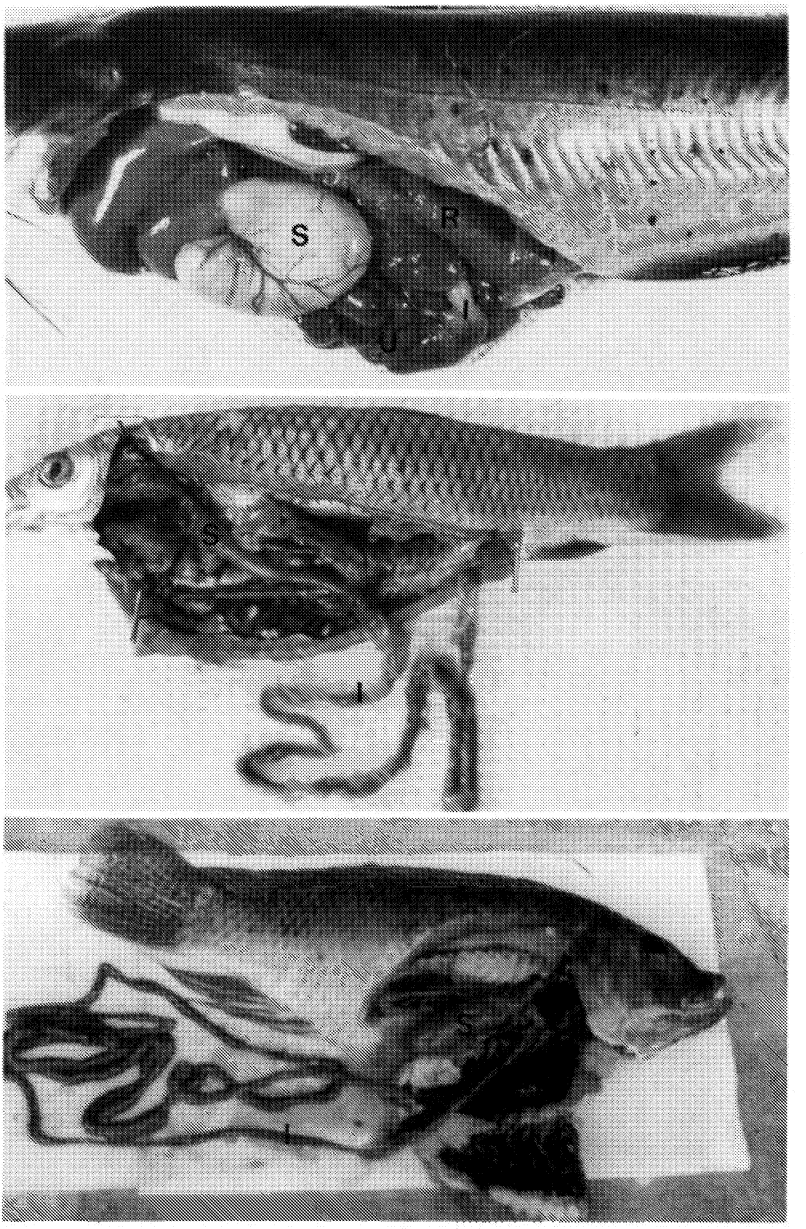


Figure 3.1 The channel catfish (top) has a large, highly functional stomach (S), a relatively short upper intestine (U), and a distinctive rectal intestine (R) separated by the intestinal sphincter (I). The grass carp (middle) has a poorly defined stomach area (S), a relatively long intestine (I), and no defined rectum. The Nile tilapia (bottom) has a modified stomach (S), which appears to be less functional than that in channel catfish, a very long intestine (I), and no distinctive rectal area.

fish, the digestive tract is a long tube from mouth to anus. Usually, however, the esophagus, stomach, and intestine can be distinguished. The principal layers of the wall of the digestive tract are the mucosa (or inner epithelium), submucosa (mainly connective tissue and blood vessels), the muscularis (two or three layers of smooth muscle), and the serosa or outside layer (fibrous connective tissue).

Mouth and Esophagus

Predaceous fish, such as solmonids and channel catfish (catfish are only slightly predaceous when supplemental feed is offered) have a large mouth and esophagus for capturing prey.

The mouth has no teeth but an abrasive plate and there are pads but no teeth in the pharynx; there is no gizzard; the esophagus is separated from the stomach by a cardiac sphincter which effectively separates water from ingested food and prevents food from backing out of the stomach; gill rakers are not arranged for filter feeding; and, catfish have chemosensory barbels that aid in finding food.

The common carp has a small mouth designed for bottom feeding, moderately developed pharyngeal teeth, two pairs of chemosensory barbels, and coarse gill rakers. The grass carp has large pharyngeal teeth attached to branchial bones which serve as grinders to reduce plant tissues to smaller particles sizes.

Tilapias have mouths intermediate between bottom feeders and predaceous fish. Most tilapias are efficient plankton feeders, although not all have closely spaced gill rakers. An important mechanism for concentrating plankton is secretion of pharyngeal mucus that coalesces the plankton so the fish can swallow it.

Stomach

Channel catfish, salmonids, and most predacious fish have true stomachs that secrete hydrochloric acid and pepsinogen, and provide an acidic environment. Bile and pancreatic (digestive) secretions empty into the catfish intestine just posterior to the pyloric sphincter valve, which separates the stomach from the intestine. Common carp has no stomach, but a slightly enlarged "bulb" exists at the anterior end of the digestive tract. There is no gastric (low pH) section in the carp's gut. Digestive secretions enter the digestive tract immediately posterior to the cardiac sphincter, which separates the esophagus from the intestine.

Tilapias have a modified stomach which secretes hydrochloric acid. There is a well defined compartment, however, which appears to be more of a pocket through which ingesta may divert as it moves through the gut rather than a specialized section, as in catfish. The pH of the ingesta in the stomach is dependent upon the amount of food passing through the gut. When the stomach is empty, the first food eaten may reach pH 1.4 but as more is eaten, gastric secretion is unable to maintain the low pH. In stomached fish and higher animals, the pyloric sphincter generally remains closed until the stomach contents (chyme) reach a sufficient solubility and low pH to be released into the lower intestine.

Intestine

The intestine of the channel catfish is not separated into a large and small intestine as in mammals. The catfish's intestine, except for a short rectum, is similar with regard to pH (slightly above 7), digestive secretions, and nutrient absorption to the small intestine in warm blooded animals. Although the intestine is relatively short, it has many folds, which provide a large surface area for absorption (Figure 3.2).

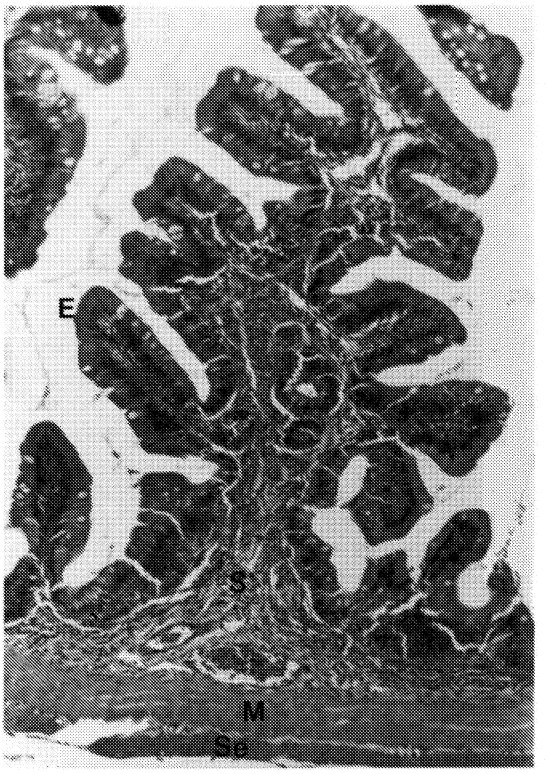


Figure 3.2 Histological section of wall of intestine of channel catfish. The sections, from inside outward, are epithelium (E), submucosa (S), mucosa (M), and serosa (Se). Note the large amounts of folds and epithelial surface in the lumen of the intestine to facilitate absorption of nutrients.

The entire digestive tract of common carp, which is about three times the length of its body, is like the intestine (posterior to the stomach) of catfish in alkalinity, secretions, and absorptive functions. Tilapias have a long digestive tract that is six to eight times the length of their bodies. The intestine posterior to the stomach is similar to that of the carp except for being longer.

Liver and Pancreas

These organs produce digestive secretions. The liver produces bile in addition to being the primary organ for synthesis, detoxification, and storage for many nutrients. The pancreas is the primary source of digestive enzymes (found as precursors or "zymogens") in most animals. In teleost fishes the pancreatic tissue is usually quite diffuse. It consists of acini (ramified tubules) scattered in the mesenteries, along the intestinal surface, and within the liver and spleen. Crustaceans have a hepatopancreas, a distinct organ that functions as liver and pancreas.

Digestive Processes

Digestive processes in the channel catfish, beyond the mouth, are relatively similar to those in warmblooded monogastric animals; these will be described and exceptions in other cultured fishes will be mentioned.

When food enters the stomach, neural and hormonal processes stimulate digestive secretions. Distension of the stomach by entering food starts hydrochloric acid secretion by parietal cells. Pepsinogen is secreted by chief cells and is quickly hydrolyzed to pepsin, an active proteolytic enzyme. Mucous cells begin to secrete. In mammals, the hormone gastrin is secreted into the stomach to stimulate release of gastric juices; however, in fishes, it is believed that other hormones or compounds (histamine, cerulein) may be more functional. The pyloric sphincter at the posterior end of the stomach holds food until it is sufficiently fluid to be passed into the small intestine. Water must be secreted into the stomach of freshwater fishes, but saltwater fishes drink water. Pepsin and hydrochloric acid partially hydrolyze proteins into shorter chain polypeptides. Pepsin, which is active at pH 1.5 to 3.0, works mainly at the site of aromatic amino acids (phenylalanine and tyrosine). Minerals and mineralized tissue are solubilized in the acid stomach, but no fat or carbohydrate breakdown occur. The mixture of food, mucus, and gastric juices becomes a slurry, called chyme. When food materials become sufficiently fluid, they are released from the stomach into the anterior intestine. Rate of release is apparently related to solubility. Zarate (1997) fed channel catfish intact protein, from soybean meal, and isolated lysine. He found that disappearance of lysine from the stomach was markedly faster in fish fed the highly soluble free lysine than in fish fed the protein bound lysine.

In mammals, chyme entering the intestine initiates release of secretions from the pancreas and gall bladder (bile). Pancreatic secretions include bicarbonate buffering compounds, which neutralize the acidic chyme, and the zymogens of enzymes, which digest proteins, carbohydrates, lipids, chitin, and nucleotides. Trypsinogen, the zymogen of trypsin, is activated by enterokinase, which is secreted by the intestinal mucosa. Chymotrypsinogen also comes from the pancreas and is activated to chymotrypsin when it comes in contact with trypsin. These two proteolytic enzymes cleave polypeptides into shorter chain peptides. Carboxypeptidases and aminopeptidases may also come from the pancreas. They split off individual amino acids, containing the free carboxyl or the free amino group, from a peptide chain.

Chitinase activity has been found in pancreas extracts from many fishes. This enzyme hydrolyzes the chitinous exoskeleton of insects and crustaceans. The pancreas also produces amylase, which hydrolyzes starch, and nucleases, which degrade nucleic acids. Pancreatic lipases split ester bonds and partially or completely hydrolyze fats, phospholipids, and other lipid esters. Cellulase activity has been reported in intestinal extracts from a few fishes, but this probably came from intestinal bacteria.

Cells of the intestinal mucosa of most vertebrates secrete a number of enzymes. These include carboxy- and aminopeptidases, amylases, lipases, lecithinase, nucleases, and others. However, the quantitative importance of intestinal mucosa enzymes to digestion in fish is not known. Grizzle and Rogers (1976) found no secretory glands in the intestine of channel catfish.

Bile is produced in the liver and is secreted into the intestine, usually via the gall bladder. It contains bile salts, cholesterol, phospholipids, pigments, and

other compounds and ions. Its primary function is to emulsify fats into small globules (chylomicrons) for absorption or to make hydrolysis by lipases easier.

Food moves through the digestive tract by peristaltic waves or constrictions that move along the intestine. The food is churned in the foregut buy various independent movements of the layers of smooth muscle in the intestinal wall. This aids in exposing the nutrients to the intestinal mucosa for absorption.

Most nutrient absorption occurs in the intestine. In the lumen of the intestine, there are many folds to provide for a large surface area for nutrient absorption. Some highly soluble nutrients, such as electrolytes, monosaccharides, and some of the vitamins and amino acids, diffuse across the mucosal cell membranes because of concentration gradient (passive diffusion). Other nutrients must be actively transported into the cell. Another mechanism of cell absorption is pinocytosis, where the cell engulfs large molecules in amoeba-like fashion.

Proteins are absorbed primarily as free amino acids, but also as low molecular weight peptides. Triglycerides are absorbed as small fat particles (micelles) and as free fatty acids and glycerol.

Water-soluble vitamins are absorbed in an aquous medium, but fat-soluble vitamins are solubilized in lipids when absorbed. Carbohydrates are absorbed as glucose or other monosaccharides. Electrolytes are absorbed in aquous medium whereas most of the trace minerals (iron, copper, zinc, etc.) have low solubility and are coupled with proteins to pass into the mucosal cells.

All nutrients, except possibly lipids, are absorbed from the mucosal cells into the blood that moves from the intestine through the portal vein to the liver. In birds and mammals, lipids are picked up by the lymph as triglycerides, which are formed after absorption into the mucosal cells, and later enter the blood for transport to the liver. Teleost fishes have a well defined lymph system, but the system's role in lipid absorption in fish is not clear.

Fishes do not have a well defined large intestine, as do higher animals, where bile salts, some minerals, most of the water, and nutrients synthesized in the intestine are absorbed. However, these processes apparently do occur in fishes in the posterior section of the intestine.

MEASURING NUTRIENT BIOAVAILABILITY BY DIGESTION TRIALS

The bioavailability of nutrients or energy in feedstuffs for fish may be defined in terms of digestibility or, in the case of energy, metabolizability. Digestibility describes the fraction of the nutrient or energy in the ingested feedstuff that is not excreted in the feces. Metabolizability describes the fraction of the digested energy that is not excreted in the urine and through the gills. Both digestible energy and metabolizable energy have been used to express feedstuff values for fish, but many researchers use and report only DE values because of difficulties in obtaining ME values for fish. This subject has been discussed in greater detail in Chapter 2.

Percentage apparent digestibility (AD) of a nutrient is expressed by:

$$\%AD = 100\ x\ \frac{Food\ nutrient - Feces\ nutrient}{Food\ nutrient} \qquad (3.1)$$

Apparent digestibility does not take into account nutrient losses losses of endogenous origin which are part of the feces. "Corrected" or "true" digestibility calculations exclude the endogenous materials from the feces. The endogenous materials are primarily nitrogenous compounds, such as enzymes, peptides, and epithelial cells, or nutrients that are excreted through the intestine instead of in the urine, such as non-ionizable inorganic materials. These excretions must be determined in a separate study in which a nitrogen-free diet is fed. Apparent digestibility is of more practical importance than corrected digestibility because the endogenous losses are minor if the animal is not fed, therefore these losses must be charged to ingestion of the food.

Quantitative recovery of the fed nutrient voided in the feces is difficult in fish because of the aqueous environment. Several methods of direct and indirect measurement of digestibility have been used with fish. The direct method involves measuring directly all of the food consumed and all of the feces excreted. Care must be taken when feces are collected from the water so that leaching of nutrients into the water is prevented. Methods for making total fecal collections are described by Cho et al. (1982).

The indirect method has the advantages that it eliminates the need to quantitatively collect all of the excreta, and the fish can eat voluntarily. It involves measurement of the ratios of nutrient to some indigestible component (indicator) in the feed and in the feces. The indicator must be indigestible, unaltered chemically, nontoxic to the fish, conveniently analyzed, and able to pass through the gut uniformly with other ingesta. As the dietary nutrient is absorbed in the gut, the ratio of nutrient to indicator will be less in the feces than in the feed. Digestibility has been determined indirectly in fishes and shrimps, using internal indicators such as ash, crude fiber or plant chromagens, or diet additives such as chromic oxide. The equation for calculating percentage apparent digestibility (AD) by the indirect method is as follows:

$$\%AD = 100\left[1 - \frac{\textit{Nutrient in feces}}{\textit{Nutrient in diet}} x \frac{\textit{Indicator in diet}}{\textit{Indicator in feces}}\right] \qquad (3.2)$$

Procedures for measuring apparent digestibility indirectly, using chromic oxide as an indicator, are described for channel catfish by Smith and Lovell (1973).

Popma (1982) showed that natural plant chromagens (chlorophyll derivatives) are suitable reference compounds and may be used to measure nutrient digestion in naturally feeding fish when the ingested food contains green plant tissue. He sampled ingested food from the esophagus of Nile tilapia before digestion had commenced and fecal material from the rectal area of the fish from fish free in a pond with access to a variety of foods. It is important that the species or particle composition of the sampled ingesta be identified in order to know what the determined digestion coefficients represent.

Because few feedstuffs are fed as the sole component of a fish diet, Cho et al. (1982) recommends to evaluate the apparent digestibility of a feedstuff in combination with other ingredients in the assay diet. This is done by comparing the apparent digestibility of a reference diet with that of an assay diet that contains a mixture of the reference diet (70%) and the test ingredient (30%). The reference diet is composed of ingredients similar to a commercial diet. Apparent digestion

coefficients are determined for the reference and assay diets by the indirect method described in equation 3.2, and these coefficients are used to calculate the apparent digestibility of the test ingredient according to equation 3.3:

$$\%AD = \frac{100}{30}[\%D_t - \frac{70}{100}\%D_r] \quad (3.3)$$

where D represents the digestion coefficient of the test ingredient, D_t the digestion coefficient of the test diet, and D_r the digestion coefficient of the reference diet. This method has advantages over testing ingredients singly in that any effect of feeding the ingredient in combination with other diet components may be realized. Also, the test ingredient may be more acceptable to the fish when fed in combination with other ingredients.

Percentage apparent digestibility of protein, lipid and carbolydrate in several feed ingredients for channel catfish, rainbow trout and Nile tilapia are presented in Table 3.1.

NET RETENTION OF MINERALS

The net retention, or absorption, of minerals in fish feeds is of increasing concern because of environmental pollution by discharge from aquaculture and hatchery operations. Inorganic nutrients stimulate growth of algae and higher aquatic plants. Phosphorus is generally regarded as the first limiting nutrient for aquatic plants, and in some areas the amount of phosphorus released from aquaculture operations is restricted.

The relative availability of dietary phosphorus is affected by both chemical and physical forms, and fish species. Generally, phosphorus absorption by channel catfish, rainbow trout and other fished with a well defined gastric (acidic) section in the digestive tract is higher than that of the stomachless carp. Monobasic phosphates of sodium, potassium, and calcium appear to be highly available sources to all of the species noted. Dibasic calcium phosphates, commonly used in commercial fish feeds, are generally less available than the monobasic form. Tribasic phosphate is poorly available to all species. The availability of phosphorus from fishmeal, which is primarily of bone (tribasic phosphate) origin, is generally lower than that of certain other high-protein feedstuffs, such as casein. The availability of phosphorus from bone is markedly lower for carps than for fish with acidic stomachs. Apparent net absorption of phosphorus from various feed ingredients by channel catfish is presented in Table 3.2.

Phytate is the primary form of phosphorus in grains, and its availability to fish is very low. For this reason, most of the phosphorus, approximately two-thirds to three-fourths, from grain sources is excreted in the feces and may contribute to effluent pollution problems. Eya and Lovell (1997) found that supplementation of an all-plant feed with a phytase enzyme of microbial origin increased phosphorus availability from 31% to 62% and negated the need to add an inorganic phosphorus supplement to the feed.

Table 3.1. PERCENTAGE APPARENT DIGESTIBILITY OF PROTEIN, FAT, AND CARBOHYDRATE FOR CHANNEL CATFISH (CC), RAINBOW TROUT (RT), AND NILE TILAPIA (NT).

	Protein			Lipid			Carbohydrate		
Ingredient	CC	RT	NT	CC	RT	NT	CC	RT	NT
Alfalfa meal	-	61	66	51	71	-	12	-	27
Casein	97	95	-	-	-	-	-	-	-
Corn, ground:									
raw	-	60	79	76	-	90	66	-	45
cooked	-	66	84	96	-	-	78	-	72
Cottonseed meal	83	76	-	88	-	-	17	-	-
Fish meal:									
herring	-	87	-	-	97	-	-	-	-
menhaden	88	-	85	-	97	98	-	-	-
Soybean meal (48%)	93	83	-	-	-	-	-	-	54
Starch, purified, corn:									
raw	-	-	-	-	-	-	55	29	-
cooked	-	-	-	-	-	-	66	52	-

Sources: Catfish, Cruz (1975); Wilson and Poe (1985); Saad (1989). Trout, Smith (1977); Cho et al. (1982). Tilapia, Popma (1982).

Table 3.2. PERCENTAGE APPARENT NET RETENTION OF PHOSPHORUS FROM VARIOUS SOURCES BY THREE FISH SPECIES.

Source	Channel Catfish	Rainbow Trout	Common Carp
Animal products			
Casein	90	90	97
Brown fishmeal	-	74	24
Menhaden fishmeal	60	66	-
Inorganic phosphates			
Calcium, monobasic	94	94	94
Calcium, dibasic	65	46	71
Calcium, tribasic	-	13	64
Potassium, monobasic	-	94	98
Sodium, monobasic	90	94	98

Sources: Channel catfish, Wilson et al. (1982); Eya and Lovell (1997). Rainbow trout, Ogino et al. (1979). Common carp, Ogino et al. (1979).

Differential absorption of different forms of trace minerals has been noted in several fish species. Examples of this include the higher availability in Atlantic salmon of selenomenthionine as compared with other sources of selenium (Bell and Cowey, 1989). Paripatananont and Lovell (1997) demonstrated that organic (Chelated) forms of trace minerals were more digestible than the inorganic sources commonly used in fish feeds (Table 3.3).

Some feed components are antagonistic to the absorption of minerals. The bioavailability of zinc in diets containing fishmeal is inversely correlated with the ash content. Rainbow trout and carp that had been fed diets containing high ash fishmeal exhibited signs of zinc deficiency even though the zinc content of the diets was adequate. The deficiency signs were alleviated by the addition of more zinc to the diet. Zinc bioavailability is also reduced by presence of phytate in the diet; therefore, the zinc allowance in commercial fish feeds should be increased to three or four times the requirement established with purified diets. Procedures for measuring net retention of minerals, by correcting for residual levels of minerals in the basal diet, are discussed in Chapter 5.

Table 3.3. PERCENTAGE APPARENT NET RETENTION OF MINERALS FROM ORGANIC (CHELATED) AND INORGANIC SOURCES ADDED TO A SOYBEAN MEAL BASED DIET FOR CHANNEL CATFISH.

Mineral Source	Organic[1]	Inorganic[2]
Copper	89	40
Iron	84	51
Manganese	87	40
Selenium	89	70
Zinc	90	52

[1]Protein chelates

[2]Sources of copper, iron, manganese and zinc were sulfate forms; selenium was from sodium selenite.

Source: Paripatananont and Lovell (1997).

BIOAVAILABILITY BY GROWTH TRIALS

Digestion trials reveal the fraction of the consumed nutrient that is absorbed by the animal. A more absolute evaluation of bioavailability of a nutrient in a feed ingredient is to measure the response of the animal. Growth trials can provide information that digestive trials do not. For example, two ingredients may have similar absorption values for a nutrient, but provide different responses in the animal, indicating that the nutrient is not metabolized the same in the two ingredients. Determination of nutrient bioavailability by growth trials will be discussed in Chapter 5.

METABOLISM

Metabolism may be defined as the biological processes of utilization of absorbed nutrients for growth and other synthesis and for energy expenditure.

Metabolism of Carbohydrates

The protein-sparing effect of carbohydrates varies from poor to relatively good among fish species. Fish have poorer control over blood glucose levels than do warm blooded animals; in fact, fish respond to glucose loading like a diabetic mammal. Following glucose ingestion, blood glucose level rises rapidly in fish, but takes many hours to decrease. Turnover rate of glucose in trout is about 10 times slower than in the rat. Fish oxidize deaminated amino acids for energy more efficiently than, and in preference to, glucose. Because fish evolved in a carbohydrate-poor environment, it can be appreciated that many fish do not utilize carbohydrates efficiently, although the reasons are not well understood.

Absorbed carbohydrates, primarily in the form of glucose, may have three major metabolic roles: (a) an immediate source of energy, (b) stored as glycogen as reserve energy, and (c) synthesized into compounds such as triglycerides, nonessential amino acids, and others. Pathways for metabolism of glucose are well defined for mammals and birds. Because the associated enzymes have been identified in fish (Shimeno et al. 1981) it is assumed that there is similarity in metabolism of glucose between fish and warmblooded animals although preference for various routes may differ between these groups of animals. For example, the pentose-phosphate shunt seems to be the preferred route for glycolysis in fish whereas the Embden-Meyerhof pathway is primary in mammals. Classical metabolism of glucose begins with glucose phosphorylation and proceeds with glucose being converted into glycogen or degraded by the Embden-Meyerhof (EM) pathway or the pentose-phosphate pathway (Figure 3.3). The EM pathway takes glucose-6-PO_4 to pyruvate (glycolysis), whereas the pentose-phosphate pathway takes it to ribose or other compounds which can go back into the EM pathway and proceed to pyruvate.

Pyruvate penetrates the mitochondrion and is decarboxylated to 2-carbon acetyl, which combines with coenzyme-A (CoA) to form acetyl-CoA in a reaction catalyzed by pyruvate dehydrogenase complex. Acetyl-CoA enters the tricarboxylic acid (TCA) cycle (Figure 3.4) by combining with oxaloacetate. In the TCA cycle, carbon is released as CO_2 and hydrogen is transferred to NAD^+ and FAD. These coenzymes transfer hydrogen atoms to the cytochrome enzyme system (electron transport chain) which in turn promotes the transfer of the hydrogen (electrons) to

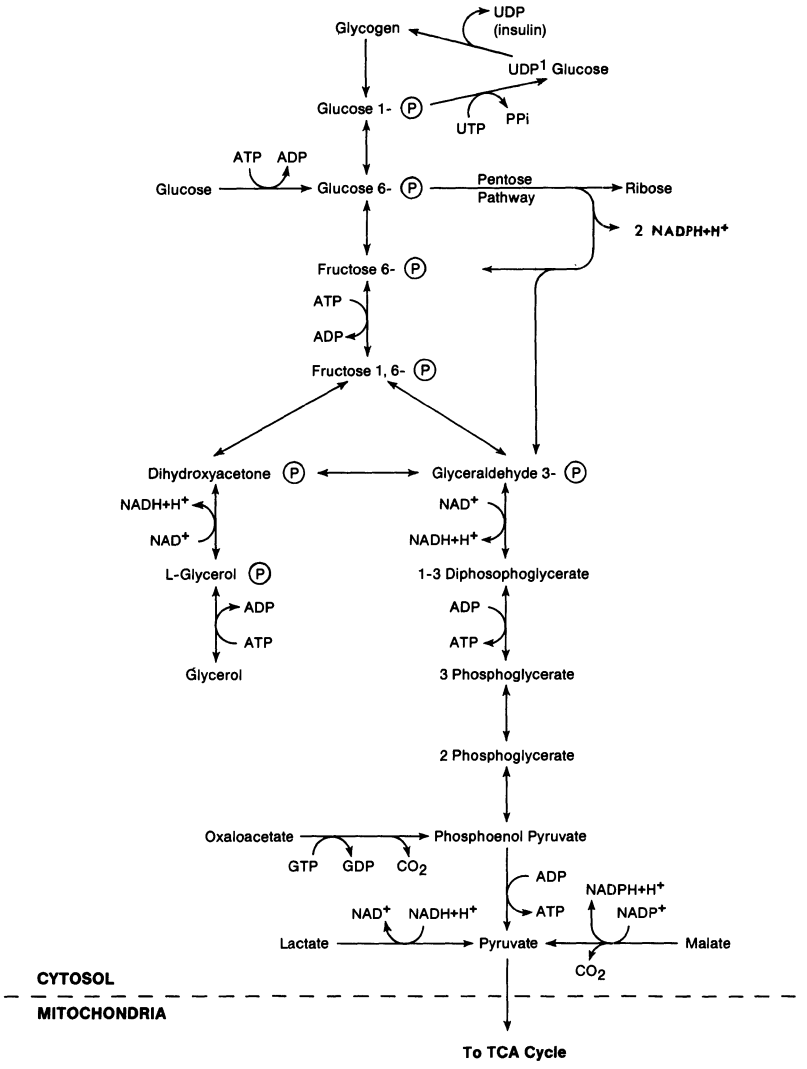


Figure 3.3 Glycolytic pathway.

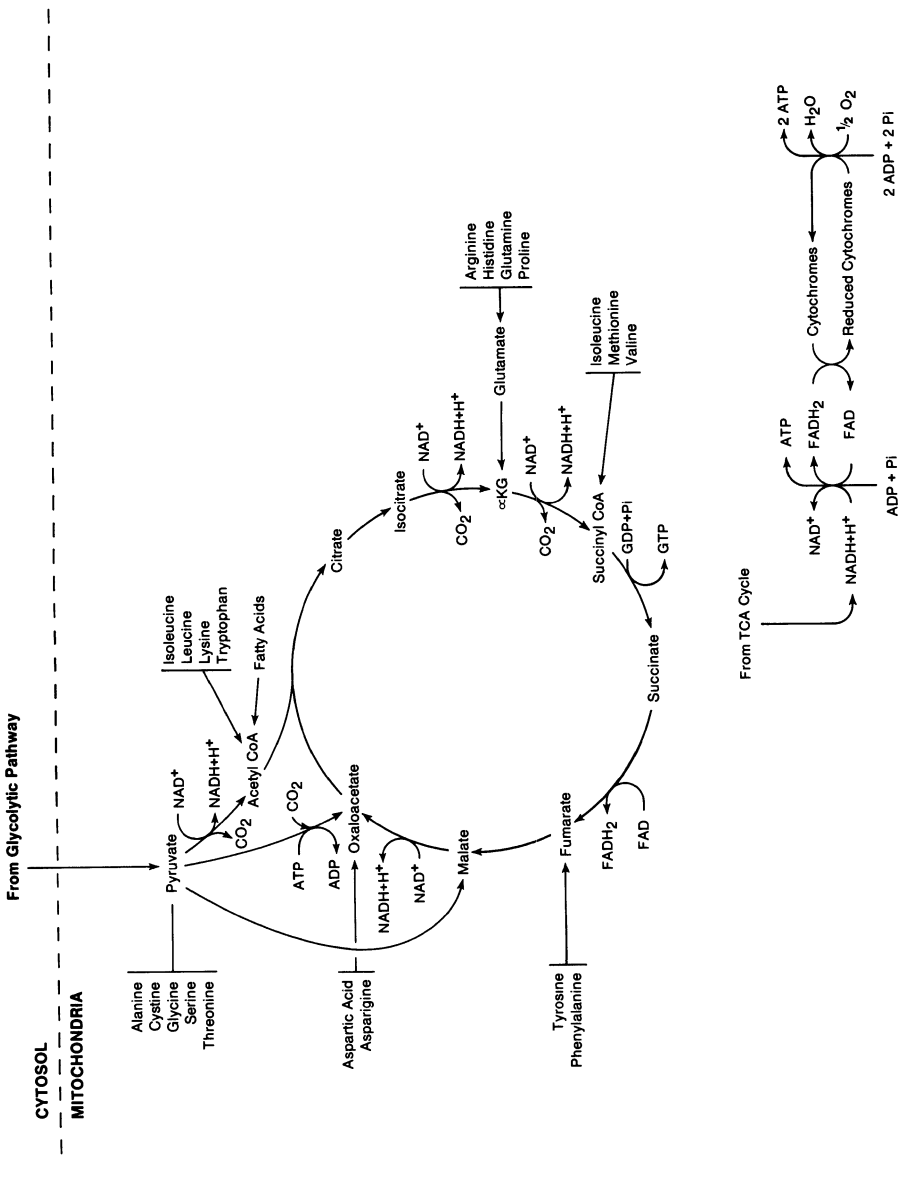


Figure 3.4 Tricarboxylic acid (TCA) or citric acid cycle.

molecular oxygen, ultimately forming H_2O. As electrons pass through the electron transport chain, energy is released; some is immediately lost as heat and some is captured by oxidative phosphorylation. In this process, energy is retained in the formation of phosphate bonds regenerating adenosine triphosphate (ATP) from ADP and inorganic phosphate. ATP, which provides the energy for endergonic metabolic processes, is composed of a nitrogenous base (adenine), a 5-carbon sugar (ribose), and three phosphate units (Figure 3.5). There are compounds analogous in function to ATP, but less prominent, which contain other nitrogenous bases, such as GTP (guanine), UTP (uracil), and CTP (cytosine).

Six to eight moles of ATP are produced in the glycolysis of 1 mole of glucose to 2 moles of pyruvate. For 2 moles of pyruvate oxidized, 30 moles of ATP are produced. Thus, the net gain from glycolysis and oxidation of 1 mole of glucose is 36 to 38 ATP's. If each mole of ATP represents 7.3 kcal of chemical energy captured through oxidative phosphorylation, the efficiency of energy conversion of glucose, which has a gross energy value of 673 kcal/mole, is about 40%.

$$100\,x\,\frac{38\ moles\ ATP\ x\ 7.3}{673} = 41.2\% \qquad (3.4)$$

Note in Figures 3.3 and 3.4 that keto acids, from deaminated amino acids, and triglycerides are also oxidized through the TCA cycle. Keto acids enter at pyruvate, acetyl-CoA, and several points in the TCA cycle. Fatty acids enter through acetyl-CoA. Glycerol (from hydrolyzed fats) enters through the glycolytic pathway at dihydroxyacetone phosphate.

All of the enzymes for glycolysis and biological oxidation have been found in fish tissues. The reader is referred to biochemistry textbooks for a discussion of these enzymes. Heart and white muscle usually have the highest rate of activity, followed by brain, kidneys, gills, and liver. The role of glycolysis in the liver is probably to supply precursors for various biosyntheses rather than to supply pyruvate for oxidation. Most of the TCA enzymes have been found in fish tissues

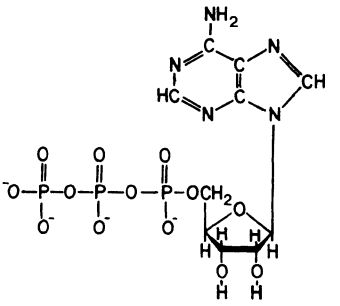


Figure 3.5 Adenosine triphosphate (ATP).

and intermediates in the TCA cycle have been recovered, which indicates that this cycle is functional in fish. Enzymes for gluconeogenesis, the synthesis of glucose from other nutrients, are found in fish. Several studies have found high liver glycogen stores in starved fish, which suggest that gluconeogenesis, probably from amino acids, preempts glycogen breakdown for glucose production.

Metabolism of Lipids

Lipogenesis in fish proceeds similar to that in mammals. Fatty acids are synthesized through acetyl-CoA from 2-carbon residues that come primarily from glucose and deaminated amino acids. The 2-carbon units (acetate) are synthesized into primarily palmitic acid (C 16:0), but to a lesser degree into stearic (C 18:0) and myristic (C 14:0) acids. Generally, the scheme is as follows: acetyl-CoA is converted to malonyl-CoA by addition of CO_2; malonyl-CoA combines with a carrier protein (ACP) to form malonyl-ACP; malonyl-ACP combines with acetyl-ACP , releasing CO_2 and ACP, to form acetoacetyl-ACP; acetoacetyl-ACP is reduced, dehydrated and again reduced to form butyl-ACP, which is sequentially elongated with malonyl CoA units until palmitate is formed. The enzyme systems responsible for fatty acid synthesis are acetyl-CoA carboxylase and the fatty acid synthetase complex. Palmitate can be elongated and the fatty acids can be desaturated (double bonds produced) by enzymes in the mitochondria. In mammals, chain elongation does not go beyond C 18 and desaturation is limited to the omega-9 (n-9) position. Some fish species, such as rainbow trout, can chain elongate beyond C 18 and some can desaturate at n-6 as well as at n-9. Most of the polyunsaturated, long-chain fatty acids found in fish tissues are of dietary origin and come originally from microscopic aquatic plants.

Three synthesized fatty acids, or fatty acyl-CoA's, are subsequently esterified with glycerol-1-phosphate (which can come from the glycolytic pathway through dihydroxyacetone-phosphate) to form triglycerides. Liver and adipose tissues are primary sites for fatty acid synthesis.

Catabolism of fats in fish seems to be similar to the classical scheme identified in mammals. After hydrolysis, glycerol is phosphorylated and converted to dihydroxyacetone-phosphate, which enters the Embden Meyerhof glycolytic pathway where it can go to glucose or to pyruvate and the TCA cycle. The fatty acid is first activated to fatty acid acyl-CoA, which combines with carnitine to diffuse into the mitochondrion for oxidation. The carnitine diffuses back to the cytosol and the fatty acid acyl-CoA is degraded, two carbons at a time, which go into the TCA cycle as acetyl-CoA. This process, known as beta-oxidation, proceeds until all carbons are removed.

Except for a short time following a meal, most of the fat oxidized for energy comes from body fat stores. Release of triglycerides from adipose tissue is under hormonal control. Hydrolysis of fat to glycerol and fatty acids occurs in adipose tissue. The fatty acids circulate in the blood complexed with a protein. During starvation, mammals often develop "ketosis", which results from more acetyl-CoA being presented to the TCA cycle than there is oxaloacetate to carry it into the cycle. The surplus acetyl-CoA is converted to ketones, which produce an acidic condition in the blood. Teleost fishes, however, have been found not to produce significant amounts of ketone bodies. This may indicated that they are more efficient than mammals in oxidizing fatty acids for energy.

Growth and Amino Acid Metabolism

Weight gain is not a measure of true growth. True growth is an increase in muscle (smooth and striated), skeletal and organ tissue whereas weight gain can represent an increase in adipose (fat) tissue with relatively less change in the other tissues. Because muscle tissue is the main marketable product in fish grown for food, the aquaculturist is less interested in fat gain (although body fat may be more important in hatchery fish produced for release.) Several factors, such as fish size, feeding rate and diet composition have marked effects on lipid content of the fish body. Fish tend to deposit more fat as they grow older. Heavily fed fish will be fatter than restricted fed fish. Often the nutritional quality of the diet will affect composition of gain. For example, feeding a diet deficient in an essential amino acid will result in a higher percentage of fat in the fish body than feeding an amino acid balanced diet. Also, dietary phosphorus deficiency has been found to cause increased fat deposition in several fish.

Feed conversion is more efficient in smaller, faster growing fish than in more mature fish. Lovell and Li (1992) showed that channel catfish grown from 20 to 462 g in ponds had a practical feed conversion of 1.35 whereas fish grown from 594 to 1,580 g had a conversion ratio of 1.78. A major reason for this difference is that small fish are gaining more protein and less fat than large fish. According to Jobling (1994) deposition of 1 g of fat represents a weight increase of 1 g, whereas deposition of 1 g of protein leads to the gain of about 4 g of tissue; this is because muscle contains approximately 75% water while fat tissue contains no water.

In most situations, protein gain (accretion) is an acceptable measure of growth in fish. In small fish, weight gain correlates well with protein gain but as fish increase in size there is less similarity. In basic nutrition studies, such as in the determination of nutrient requirements, protein gain is the preferred response measure. In applied feeding studies, however, weight gain is the appropriate measure because fish are marketed on the basis of weight.

Protein accretion (PA) is a balance between protein synthesis (PS) and protein degradation (PD); thus PA=PS-PD. This is because body protein in animals is in a state of flux, or turnover. Protein synthesis and degradation in fish have been measured by applying methods developed for mammals. These methods involve injecting or infusing a radio-labeled amino acid (isotope) into the animal and periodically measuring the amount of the isotope in protein (muscle) and the amount in the amino acid free pool. With fish, this usually entails the use of a number of experimental animals being slaughtered at appropriate times after administration of the isotope. This method has been applied with several fish species. Lovell and Mustin (1993) determined that protein accretion rate in fingerling (43 g) channel catfish was 555 mg day^{-1} while protein degradation rate was 452 mg day^{-1}. This is a net protein accretion of 103 mg day^{-1}, which indicates the high rate of protein turnover. Rate of protein synthesis varies among fishes but is generally lower than in land animals. For example, with intensive feeding, a broiler chicken will grow from 40 g to 1.8 kg, or increase its weight 45 times, in 7 to 8 weeks, where a young channel catfish (20 g) with intensive feeding will increase its weight only four to eight times during this time interval.

Amino Acid Degradation and Nitrogen Excretion

The liver is responsible for maintaining the body amino acid pool. Sources of these amino acids are the diet, which is usually intermittent, and catabolized body

proteins, which are in a continuous state of flux. In order to keep the pool relatively constant, amino acids are discretely released for synthetic purposes (proteins, nucleotides, etc.), oxidation for energy release, or conversion to fat. Amino acid not used for synthesis are first deaminated and the carbon residues are oxidized or converted to fats, carbohydrates, or other compounds. The amino group is removed from amino acids mainly by transamination or by oxidative deamination. Transamination, which seems to be the major initial deamination pathway in fish, involves the transfer of the amino group from an amino acid to an α-keto acid, usually α-ketoglutaric acid. Aminotransferase enzymes are specific for the amino acid being deaminated, and vitamin B_6 is a cofactor. The recipient α-keto acid then releases the amino group through oxidative deamination, for excretion or synthesis of other amino acids, and the α-keto acid is recycled through another transamination. The α-keto acid formed in the initial transamination of an amino acid can be oxidized, converted to fat, or used in synthesis of other compounds. Of the essential amino acids, only lysine and threonine do not participate in transamination. The carbon skeleton of the amino acid is the part that determines whether or not an animal can synthesize it. The a-keto acids of all essential amino acids except lysine and threonine can be aminated metabolically and serve as essential amino acid sources. Oxidative deamination is sometimes the initial deamination reaction. It is an energy yielding reaction catalyzed by dehydrogenase enzymes. The end products are an α-keto acid and free ammonia, because the amino group from the amino acid is not transferred to a keto acid.

Most terrestrial vertebrates excrete nitrogen in the urine as urea; however, birds and reptiles excrete uric acid, which requires less water. Teleost fishes excrete 60% to 90% of their waste nitrogen as ammonia, and most is eliminated through the gills. Other nitrogen excretory compounds for fishes are urea, uric acid, creatine, creatinine, trimethylamine oxide, and amino acids. Teleost fishes do not have the enzymes to synthesize urea by the ornithine-citrulline-arginine cycle,as do mammals. Because the essential amino acid, arginine, is a product of the ornithine cycle, the dietary arginine requirement for mammals is lower than for teleost fish. For example, the arginine requirement for pigs is approximately 1-2% of the dietary protein as compared to 4-6% of the protein for fish. Cartilaginous fish, however, can synthesize urea by the ornithine pathway, but urea serves as an osmolyte in the body fluids and is not a primary nitrogen excretory product. Some species, including the sharks, maintain high urea levels in the body fluids for osmoregulatory purposes.

Ammonia is an efficient route for nitrogen excretion. Less energy is expended and less water is lost from the body than in the synthesis and excretion of urea. Urea synthesis is an energy demanding (endergonic) process; ATP is required in the synthesis. Also, water is needed in urea excretion, which would be a serious liability for saltwater fishes which need to conserve body water. Ammonia (nonionized) is lipid soluble and moves across gill cell membranes easily without an exchange of water. An advantage of urea as a nitrogen excretory product is that urea is a weak base and is less toxic when it accumulates in the animal, whereas ammonia is a relatively strong base and will produce an alkaline condition in the body fluids unless eliminated quickly.

The physiological and bioenergetic mechanisms of ammonia excretion by fish are not well understood. Most deamination, or ammonia production, occurs in the liver but ammonia is also produced in muscle and other tissues. The ammonia

ionizes in body fluid to yield a combination of ammonia (NH_3) and ammonium ion (NH_4+). At body pH, near 7, the ratio of NH_3 to NH_4 is about 1/100. (The pka of ammonia, or pH at which NH_3/NH_4+ is 1/1, is 9.24.) Ammonia is transported to the gill in free, ionized form and also attached to amino acids which are deaminated at the gill surface. Goldstein, Forster, and Fanelli (1964) found that approximately 60% of the ammonia excreted from the gill of *Myoxocephalus scorpius* was from blood ammonia, while 40% was from plasma amino acids that were deaminated in the gill tissue.

Because nonionized ammonia is lipid-soluble, it diffuses across cell membranes easily, while the charged ammonium ion does not. The ammonium ion may, however, traverse cell membranes by ion exchange, probably with sodium ions. The relative contributions of the two methods of branchial excretion of nitrogen are not known. Because of the ease of diffusion of NH_3 across branchial membranes, this is probably the primary nitrogenous excretory route. Although only about 1% of the ammonia is in the NH_3 form in the blood, this is not a real problem because of the rapid conversion of NH_4^+ to NH_3 which diffuses passively from the extra-cellular body fluid into the gill epithelial cells and from the gill epithelium into the water.

The problem in ammonia excretion in fish is in the movement of ammonia from the gill to the surrounding water. If ammonia concentration and pH of the water are low in relation to the fish's body fluid, NH_3 diffuses rapidly from the gill into the water. Once NH_3 is across the gill membrane and into the water, it changes to NH_4^+, the rate being dependent upon water pH. As water pH increases, the concentration of NH_3 in relation to NH_4^+ increases and makes movement of NH_3 from the gill epithelium more difficult. In fact, as concentration of NH_3 in water increases, the outward flow of NH_3 from the gill epithelial cells can be reversed.

Rate of ammonia production in fish culture systems is dependent on amount and quality of protein fed. The amino acids of ingested protein that are not used in body synthesis are deaminated and the nitrogen is excreted. If a protein of poor amino acid balance is fed, less protein synthesis per unit of ingested protein occurs and the unused amino acids are deaminated and the nitrogen excreted. Typically, ammonia production by a 1-kg channel catfish fed to satiation on a 32% protein commercial feed is approximately 600 mg of ammonia per kilogram of fish weight per day. This is based on the following assumptions: The fish consumes 25 g of feed/day; 20% of the fed protein is undigested, 40% is retained by the fish, and the remaining 40% is excreted by the fish as ammonia or products that will rapidly be converted to ammonia by bacteria. Calculation is as follows:

$$\begin{aligned} &(25\ g\ food) \times (32\%\ protein) \times \\ &(16\%\ N\ in\ protein) \times (40\%\ N\ excreted) \times \\ &(1.2\ g\ NH_3\ per\ g\ N) = 610\ mg\ ammonia \end{aligned} \tag{3.5}$$

The undigested protein will also contribute to the ammonia load in the system unless removed before bacterial decomposition.

The lethal level of nonionized ammonia in water for channel catfish is around 2.5 mg L^{-1}. Sublethal levels causing gill tissue damage in channel catfish and rainbow trout are 0.12
mg L^{-1} to 0.4 mg L^{-1}.

RATE OF METABOLISM (OXYGEN CONSUMPTION) IN FISH

Rate of metabolism, or oxygen consumption, in fish is influenced primarily by water temperature, fish size, activity of the fish, feeding, and concentration of oxygen in the water. Concentration of ammonia, nitrite, or other adverse compounds in the water, nutritional deficiencies, hormone applications, and other factors can also affect rate of metabolism in fish.

Metabolic rate in mammals is usually reported as heat production (kcal kg body weight^{-1}hr^{-1}). This can be determined by placing the animal in a calorimeter and measuring heat production directly or by measuring oxygen consumption and calculating heat production from the calorie equivalent value of the oxygen consumed (4.6-5.0 kcal L^{-1} of oxygen). Oxygen consumption is the preferred method for measuring and reporting metabolic rate in fish because of accuracy and convenience of measurement. A number of respirometers have been designed and used successfully to measure oxygen consumption by fish under various conditions of activity, feeding, and temperature. If oxygen consumption is to be converted to heat production in fish, oxygen caloric equivalent values of 4.63 kcal L^{-1} of O_2 consumed should be used when the energy sources are protein and fat and 4.8 kcal L^{-1} of O_2 consumed when some carbohydrate is being oxidized.

Knowledge of metabolic rate of fish under various conditions has practical importance. It is valuable in determining dietary energy levels and daily feed allowances for fish under various conditions and for various production purposes. For example, maintenance energy requirements of fish and heat increment (loss) values for various feedstuffs can be derived from metabolic rate data. Oxygen consumption rate by fish is also useful in determining carrying capacity of fish culture systems and in predicting aeration needs or flow rates in various aquacultural environments.

Factors Influencing Oxygen Consumption by Fish

Temperature. Temperature has little effect on metabolic rate in warmblooded animals unless it is above or below the "comfort range" of the animal. In fishes, however, metabolic rate varies directly with temperature. Within the normal environmental temperature range of the fish, a Q_{10} value of 2.3 has been found to apply within a 20% span for nonfed brook trout, bullhead catfish, carp, and sockeye salmon (Brett 1979). Q_{10} represents the increase in metabolic rate for an increase of 10° C. Andrews and Matsuda (1975) found Q_{10} values of 2.3 and 1.9 for nonfed and fed channel catfish, respectively. Figure 3.6 shows increase in oxygen consumption with temperature increase.

Fish Size. Rate of metabolism per unit of body weight diminishes with increase in size. Andrews and Matsuda (1975) measured oxygen consumption by channel catfish, ranging in size from 2.5 g to 1,000 g, under feeding and nonfeeding conditions. As shown in Figure 3.7 oxygen consumption rate decreased for 1 kg

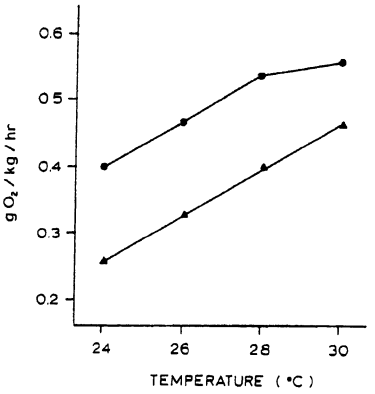


Figure 3.6 The relationship between temperature and oxygen consumption by 450-g channel catfish. The circles represent measurements taken 1 hour after feeding, and the triangles represent measurements from fish fasted overnight.
Source: Andrews and Matsuda (1975).

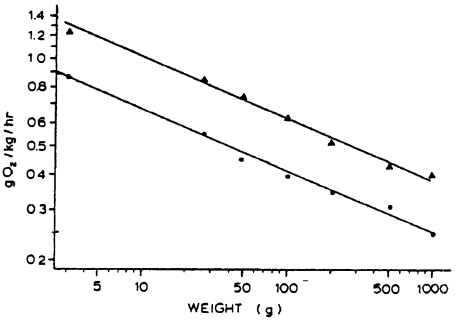


Figure 3.7 Relationship between body weight and oxygen consumption by fed (triangles) and fasted (circles) channel catfish. Temperature was 26°C.
Source: Andrews and Matsuda (1975).

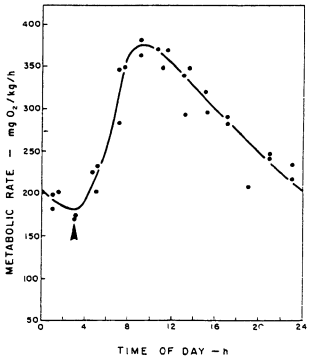


Figure 3.8 Effect of food intake (3% of body weight) on oxygen consumption by sockeye salmon. Fish were fed at 0300 hours (arrow).
Source: Brett and Zala, 1975.

fish to approximately 28% of that of 2.5 g fish. A similar rate of decrease in metabolic rate was found in Atlantic salmon as size increased from 1 g to 3 kg.

Feeding. Following ingestion of food, an increase in metabolic rate occurs in animals. This is known as the *heat increment.* In mammals, it can amount to up to 30% of the caloric content of the diet. In fish it is generally lower, usually 10% to 15%. This increase in metabolism is due primarily to metabolism of amino acids. A number of studies with fish and warmblooded animals have shown that heat increment is proportional to percentage of protein in the diet. Ingestion of carbohydrates and lipids will also elicit an increase in heat increment, but less than that from protein. The increase in oxygen consumption subsequent to feeding is large. As shown in Table 3.4, channel catfish increased oxygen consumption within 1 hr after feeding by 38% to 60%. Duration of heat increment varies with fish species, size and composition of meal consumed, and environmental conditions. Typically, when fish are fed once daily, heat increment begins immediately after feeding, peaks after 4 to 8 hours, and terminates within 18 to 24 hours. Figure 3.8 shows the changes in oxygen consumption by sockeye salmon following a meal (3% of their weight) at 0300 hours. By 0800 hours the oxygen consumption rate had essentially doubled, and by 2400 hours it was back to near the baseline value.

Table 3.4. OXYGEN CONSUMPTION RATE BY NONFED AND FED CHANNEL CATFISH OF FOUR SIZES MEASURED AT 26 °C.

Fish size (g)	Oxygen consumption rate (mg kg^{-1} hr^{-1})		Increase in oxygen consumption from feeding (%)
	Nonfed[1]	Fed	
2.5	880	1,230	40
100	400	620	55
500	320	440	38
1,000	250	400	60

[1] Nonfed fish were fasted 24 hr; fed fish were fed to satiation on a commercial feed 1 hr before measurement.
Source: Andrews and Matsuda (1975).

Dissolved Oxygen Concentration. As dissolved oxygen concentration decreases, oxygen consumption, as well as feeding activity, decreases. Andrews and Matsuda (1975) found that as dissolved oxygen concentration decreased below 7 mg L^{-1}, which was near saturation at 28°C, metabolic activity of channel catfish (200g) in raceways began to decrease. This is contrary to the claims of some fish culturists that dissolved oxygen is not an adverse factor until it decreases significantly below saturation.

Determining Oxygen Consumption by Fish

A number of studies to measure rate of oxygen consumption by fish have been made in closed containers and in raceways. These studies involved measuring oxygen concentration of incoming and outgoing water, and equating the amount of oxygen

removed to the mass of fish in the system. Metabolism chambers or other closed containers, allow control over variables such as temperature, atmospheric oxygen, and fish swimming activity. However, data collected from fish in raceways are probably more meaningful for practical aquaculture because the fish are not confined to a small space, and large numbers can be used. Oxygen consumption by channel catfish was determined by Andrews and Matsuda (1975) in 3-m^3 raceway tanks. They first determined oxygen absorption from the atmosphere by water in raceway tanks that contained no fish. This was small and considered negligible over an 8-hour period. Subsequently, fish of various sizes were placed in the tanks at practical stocking rates, and oxygen consumption by the fish was measured. The investigators examined effects of temperature, dissolved oxygen, fish size, and feeding on rate of oxygen consumption. By measuring decrease in oxygen concentration in the raceways containing known weights of fish at various time intervals, oxygen consumption rates, expressed as milligrams of dissolved oxygen consumed per kilogram of fish per hour (mg O_2/kg/hr), were determined. Table 3.4 shows oxygen consumption rates determined by Andrews and Matsuda (1975) for channel catfish of various sizes before and after feeding.

REFERENCES

ANDREWS, J. W., and Y. MATSUDA. 1975. The influence of various culture conditions on oxygen consumption of channel catfish. *Trans. Amer. Fish. Soc.* 104: 322-327.

BELL, J. G. and C. B. COWEY. 1989. Digestibility of dietary selenium from fish meal and selenomethionine in Atlantic salmon. *Aquac.* 81: 61-68.

BRETT, J. R. 1979. Physiological energetics. In Fish Physiology, vol. VII, ed. W. S. Hoard, D. J. Randall, and J. R. Brett. New York: Academic Press.

CHO. C. Y., S. T. SLINGER, and H. S. BAYLEY. 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *J. Biochem. Physiol.* 73B: 25-41.

CRUZ, E. M. 1975. Determination of nutrient digestibility in various feedstuffs for channel catfish. Ph.D. dissertation. Auburn University, AL.

EYA, J. and R. T. LOVELL. 1997. Available phosphorus requirements of food-size channel catfish fed practical diets in ponds. *Aquac.* (In Press).

GRIZZLE, J.M., and W. A. ROGERS. 1976. *Anatomy and histology of channel catfish.* Auburn, AL: Agricultural Experiment Station, Auburn University.

JOBLING, M. 1994. Fish bioenergetics. New York: Chapman & Hall.

LOVELL, R. T. and M. LI. 1992. Comparison of feed conversion, dressing yield, and muscle composition for second- and third-year channel catfish. *Prog. Fish Culturist.* 54: 171-173.

MUSTIN, W. T. and R. T. LOVELL. 1993. Feeding the repartitioning agent, ractopamine, to channel catfish increases weight gain and reduces fat deposition. *Aquac.* 109: 145-152.

NRC (National Research Council). 1993. Nutrient requirements of fish. National Academy Press, Washington, D.C.

OGINO, C., T. TAKEUCHI, H. TAKEDA, and T. WATANABE. 1979. Availability of dietary phosphorus in carp and rainbow trout. *Bull. Jpn. Soc. Sci. Fish.* 45: 1527-1532.

PARIPATANANONT, T. and R. T. LOVELL. 1997. Comparative net absorption of chelated and trace minerals in channel catfish diets. *J. World Aquac. Soc.* 28L62067.

POPMA, T. J. 1982. Digestibility of selected feedstuffs and naturally occurring algae by tilapia. Ph.D. dissertation, Auburn University, Auburn, AL.

SAAD, C. R. 1989. Carbohydrate metabolism in channel catfish. Ph.D. dissertation, Auburn University, AL.

SHIMENO, D. M., S. TAKEDA, S. TAKAYAMA, A. FUKUI, H. SASAKI, and H. KAJIYAMA. 1981. Adapatation of hepatopancreatic enzymes to dietary carbohydrates in carp. *Bull. Jpn. Soc. Sci. Fish.* 47: 71-77.

SMITH, B. W., and R. T. LOVELL. 1973. Determination of apparent protein digestibility in feeds for channel catfish. *Trans. Am. Fish. Soc.* 102: 831-835.

SMITH, R. R. 1977. Recent research showing full-fat soybean meal in salmonid diets. *Salmonid.* 1:8,18.

WILSON, R. P. and W. E. POE. 1985. Apparent digestibility of protein and energy in feed ingredients for channel catfish. *Prog. Fish. Cult.* 47: 154-158.

ZARATE, D. D. 1977. Utilization of free and protein bound lysine in diets by channel catfish. Ph.D. dissertation, Auburn University, AL.

4 NONNUTRIENT DIET COMPONENTS

In addition to the essential nutrients, feeds may contain organic and inorganic materials that have beneficial, negligible, or deleterious effects on the growth or health of the fish or the sensory quality of the processed fish. These may be naturally occurring, intentionally or unintentionally added, or products produced through chemical change or microbial growth. Some components affect the legal status of fish feeds.

TOXINS AND ANTIMETABOLITES

Microbial Toxins

The microbial toxins of greatest economic importance in animal feeding are mycotoxins--metabolites of toxigenic molds. Those of current concern in the United States are produced by certain species of three genera of molds: *Asperigillus, Penicillium,* and *Fusarium.* These molds are ubiquitous and grow and produce toxins under conditions that include adequate substrate (carbohydrate), minimum moisture in the substrate of 14%, relative humidity of 70% or above, adequate temperature (varies widely with different molds), and oxygen. Mycotoxins are usually produced in feedstuffs prior to harvest, but can be produced in the feedstuffs or finished feeds during improper storage.

Aflatoxin. Aflatoxins, produced by *Aspergillus* sp., are the mycotoxins of greatest concern in animal feeding in the United States because of their toxic and carcinogenic properties and frequent occurrence, especially in the Southeast. Common effects of aflatoxin consumption among farm animals are poor growth, liver damage, impaired blood clotting, decreased immune responsiveness, and increased mortality.

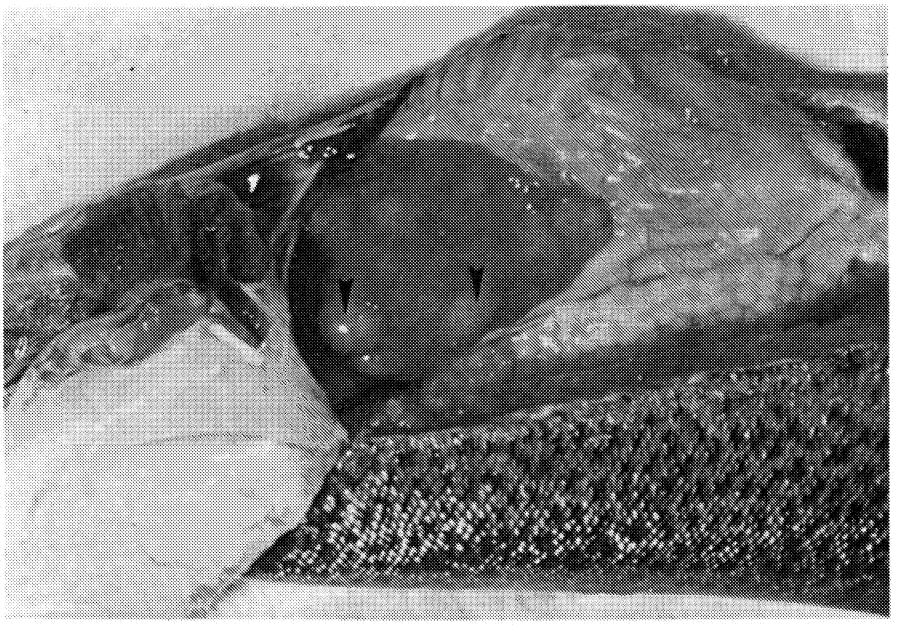


Figure 4.1 Aflatoxins are carcinogenic to animals. This rainbow trout developed hepatoma (arrows) in 20 weeks when fed a diet containing 1 mg*kg^{-1} of aflatoxin B_1. *(Courtesy of John E. Halver.)*

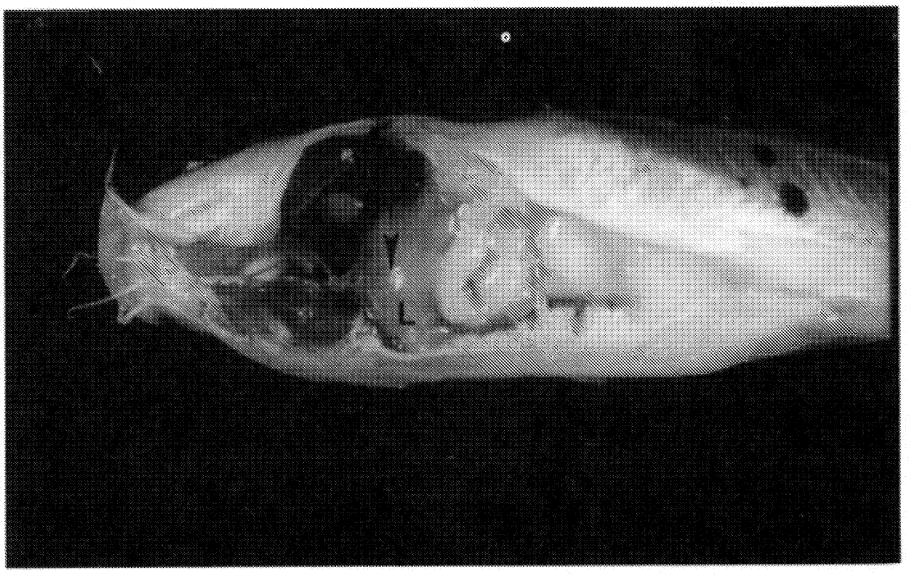


Figure 4.2 Feeding channel catfish fumonsin B_1 (80 mg*kg^{-1} diet) produced white nodules (arrow) on the liver (L) but no hepatoma. The catfish liver shown contains 2 to 4-mm diameter white foci of sucopular adipocytes. *(Source: Lumlertdacha et al., 1995.)*

Young rainbow trout has one of the highest sensitivities to aflatoxin of all animals (Halver and Mitchell 1967). Less than 1 μg kg^{-1} of diet will cause liver tumors with long term feeding (Figure 4.1). The LD_{50} (dose causing death in 50% of the subjects) for aflatoxin in 50-g rainbow trout is 0.5 mg kg^{-1} to 1.0 mg kg^{-1} of diet. Signs of severe aflatoxicosis in rainbow trout are liver damage, pale gills, and reduced red blood cell concentration. Warmwater fish are less sensitive to aflatoxin. Jantrarotai and Lovell (1990) found that channel catfish regurgitated feed containing above chromic doses of aflatoxin. They were unable to determine an oral LD_{50} and had to determine intraperitoneal LD_{50} by injecting aflatoxin B_1 into the fish. Pathological signs in channel catfish injected with an LD_{50} dose of aflatoxin are lesions in liver, stomach, intestines, spleen, heart, and kidney and severe erosion of intestinal mucosa.

Traditionally, feed sources in the United States most likely to be contaminated with aflatoxin are corn, cottonseed, and peanuts. Corn from the Southeast is more likely to be contaminated with aflatoxin than that from the Midwest due to climatic conditions. During an exceptionally bad year for aflatoxin contamination in corn in the Southeast, concentrations of the mycotoxin exceeding 400 μg kg^{-1} are not unusual; 20 μg kg^{-1}, is the action level prescribed by the United States Food and Drug Administration (FDA). Normally, only sporadic aflatoxin problems are found in corn from the Midwest. Cotton from the Southeast rarely contains aflatoxin; that which does is generally from the arid, irrigated regions of the West.

Cyclopiazonic Acid. Cyclopiazonic Acid (CPA) is produced by several species of *Aspergillus* and *Penicillium* fungi. It is indigenous to warmer latitudes and appears to be fairly widespread. It is often found in combination with aflatoxins, and has been reported to occur more frequently in field crops contaminated with *Aspergillus* flavus. The intraperitoneal LD_{50} for CPA was found to be 2.82 mg kg^{-1} with channel catfish. The effects of CPA are characteristic of neurotoxin. Affected fish show severe convulsions and die within 30 minutes following intraperitoneal injection. There are no changes in organs of intoxicated fish. Diet concentrations of CPA as low as 0.1 mg kg^{-1} reduce growth rate in channel catfish and subacute diet levels of 10 mg kg^{-1} cause necrosis of the epithelium of the digestive tract. Apparently, CPA is more toxic to channel catfish than aflatoxin; the intraperitoneal LD_{50} for aflatoxin is 11.5 mg kg^{-1} body weight and for CPA is 2.8 mg kg^{-1} body weight. The subacute toxicity dietary dose for the two toxins are 2,000 to 10,000 mg kg^{-1} for aflatoxin and approximately 100 mg kg^{-1} for CPA.

Fusarium. The *Fusarium* toxins that are most harmful to animal health are zearalenones, tricothecenes (T_2 toxin), vomitoxin, fumonsins, and moniliformin. The zearalenones are a group of estrogenic metabolites, some of which cause reproductive problems in farm animals consuming 0.6 mg kg^{-1} to 5 mg kg^{-1} in diets. The tricothecenes usually develop in corn in storage in the Midwest, with alternating cooling and warming trends in the fall. Toxicity signs in fish have not been described, but in livestock and poultry they are reduced growth, reduced red blood cell formation, widespread hemorrhage, slow blood clotting, and impaired immune responses.

The mold, *Fusarium moniliforme*, produces toxins called fumonsins; fumonsin B_1 is the most toxic and is usually in highest concentration. Since 1990, fumonsin B_1 has received a great amount of attention because field cases of equine leucoencephalomalacia (often fatal to horses) and porcine pulmonary edema (a respiratory disorder in pigs) have been linked to this toxin. The toxin is found primarily in corn and especially in corn screenings which contain broken grains. Because these products are used in catfish feeds, studies were initiated in Alabama and Mississippi to investigate toxicity of fumonsins to channel catfish. Feeding trials showed that dietary levels of 40 mg kg^{-1} of the crude toxin (from feeding corn culture material) would suppress growth rate, reduce resistance to bacterial infection, and cause liver damage in year-2 catfish fingerlings (Table 4.1). Lower dietary concentration (20 mg kg^{-1}) interfered with sphingolipid biosynthesis in the fish. Fumonsin B_1 is a specific inhibitor of sphingosine N-acyltransferase, the enzyme that converts sphinganine to sphingosine. Histopathological lesions were found in livers of fish fed 20 mg kg^{-1} of the toxin. Clinical (overt) signs of liver damage, observed in fish fed more than 20 mg of fumonsin B_1 kg^{-1}, were small (2-4 mm diameter), white, subcapsular foci of adipose tissue, as shown in Figure 4.2; the lesions were not carcinomatous.

Manning (1998) found that pure fumonsin B_1, extracted from corn culture material, was much less toxic (only about 20% as toxic) to channel catfish than crude fumonsin B_1 fed in corn culture material. This indicated that other materials in the *F. moniliforme* culture material have a synergistic effect on fumonsin B_1 or supplement its toxicity.

Ochratoxin. Ochratoxins are produced by *Aspergillus* and *Penicillium* mold species that are widely found in nature. Although not recognized as causing widespread problems in animal feeding in the United States, it is suspected that these toxins cause poor growth and feed conversion in livestock in undetected cases

Table 4.1. RESPONSES OF YEAR-2 CHANNEL CATFISH FED FUMONISIN-CONTAMINATED CORN.

Dietary fumonisin (mg kg^{-1})	Weight gain[1] (g)	Survival of infected fish[1, 2] (%)	Liver damage	
			Clinical signs	Histological signs
0	140[a]	80[a]	no	no
20	148[a]	70[a]	no	yes
40	120[b]	20[b]	yes	yes
80	100[b]	12[c]	yes	yes
320	10[d]	0[d]	yes	yes

Source: Lumlertdacha et al. (1995a, b).

[1] Values in columns with the same letters are not different (P > 5%).

[2] Fish were experimentally infected with Edwardsiella ictaluri.

because of widespread occurrence. Ochratoxins are recognized as kidney toxins, causing pale, swollen kidneys and renal tubular failure in swine, rats, and mice. The intraperitoneal LD_{50} for ochratoxin A in 6-month-old rainbow trout is 4.7 mg kg^{-1} of bodyweight. Pathological signs in trout fed ochratoxin A are severe necrosis of liver and kidney tissues, pale kidneys, pale, swollen livers, and death.

Other Microbial Toxins. There are other mold toxins, such as slaframine and citrinin, whose pathologies have been demonstrated in warm blooded animals but not in fish. Some molds and bacteria can destroy nutrients in feeds. An example is *Pseudomonas* species of bacteria and *Aspergillus* species of molds, which can separate glutamic acid from pteroic acid in the vitamin, folic acid, thus causing folic acid deficiency.

Microbial Toxins in Commercial Fish Feeds

Aflatoxin in cottonseed meal was responsible for serious economic losses in hatchery-raised trout in the 1960s until research revealed the intense sensitivity of rainbow trout to the toxin. Mycotoxins have not been conclusively identified as problem contaminants in other fish feeds, although situations have occurred where mycotoxicosis was suspected. Table 4.2 shows toxic dietary concentrations of several mycotoxins for fish. These concentrations, especially at subchronic doses, have been found in feedstuffs, so the potential for mycotoxicosis in fish from contaminated feed should encourage careful monitoring of feed ingredients.

Although FDA requires that grains shipped interstate contain less than 20 ppb of aflatoxin, grains produced and sold within the state are not subject to FDA regulation. Thus, feed manufacturers should require all feedstuffs that are traditionally associated with mycotoxins be tested before using. Reliable mycotoxin assay kits are available for screening feed ingredients for most of the commercially significant mold toxins. Feed manufacturers should avoid using feedstuffs that are

Table 4.2. SENSITIVITY OF FISH TO ACUTE AND SUBCHRONIC MYCOTOXINS: MG OF TOXIN PER KG OF DIET OR BODY WEIGHT. BODY WEIGHT VALUES ARE INDICATED (BW).

	Acute		Subchronic	
Mycotoxin	Channel catfish	Rainbow trout	Channel catfish	Rainbow trout
Aflatoxin B_1	11.5 (BW)	500	2-10	0.005-0.020
Cyclopiazonic acid (CPA)	2.8 (BW)	-	0.10	-
Fumonisin				
Crude	>720	-	20-40	-
Pure FB_1	-	-	>250	-
Vomitoxin	-	-	-	13
Tricothecene (T_2)	-	-	-	2.5
Ochratoxin A	-	4.7 (BW)	-	-

Sources: Channel catfish - aflatoxin and cyclopiazonic acid, Jantrarotai and Lovell (1990); fumonisin, Manning (1997); Rainbow trout - aflatoxin, Halver (1969); vomitoxin, Woodward et al. (1983); T_2 toxin, Poston (1983); ochratoxin, Doster et al. (1972).

suspected of having any trace of mycotoxins in feeds for newly hatched fry fish because of the increased sensitivity of young fish.

Steam pelleting or even extrusion processing of feeds does not destroy most of the mold toxins because they are relatively heat stable. Even though most mycotoxins are produced in the ingredient before the feed is processed, aflatoxins also can develop in fish feeds after processing if they are poorly dried or stored under highly humid conditions. The use of an anti-mold additive in the feed will minimize this possibility.

Histamine and Gizzerosine

These are moderately toxic products that have been found in fish meals. Histamine is a ptomaine, produced from bacterial and autolytic decarboxylation of the amino acid histidine. This compound is produced during decomposition of improperly stored fish prior to reduction to fish meal. It has been found in higher concentrations in meal from dark-fleshed than from light-fleshed fish. Reduction in growth rate has been reported in fish fed diets containing high concentrations of histamine.

Gizzerosine [2-amino-9-(4-imidazolyl)-y-azanonanoic acid] was isolated from fish meal and identified as a causative compound for gizzard erosion in broiler chickens fed diets containing heavily heated fish meal (Okayaki et al. 1983). Gizzerosine is produced by the reaction between free histidine and some side chain in the protein; the reaction is enhanced by heating. The toxic substance is in the protein and not as a free histidine derivative. Gizzerosine and histamine both derive from the amino acid histidine, but the former appears to result from a heat induced reaction, whereas the latter is produced from autolytic or microbial enzyme action.

Phytic Acid

Phytic acid, found in most plant feedstuffs, is a hexaphosphoric acid ester of inositol. It occurs as salts of calcium, magnesium, and other divalent cations. Approximately 60% to 70% of the phosphorous in plant feedstuffs is in phytic acid and is poorly available to fish. This is well known and is usually considered when determining phosphorus allowances for fish feeds. Phytic acid complexes with zinc and other divalent elements and reduces bioavailability. Gatlin and Wilson (1984) showed that channel catfish fed a soybean meal based diet, containing 1.1% phytic acid, required supplementation with approximately 100 mg kg^{-1} of zinc above the normal requirement of 20 mg kg^{-1} of diet. Phytic acid also depresses protein digestibility, as has been demonstrated with rainbow trout.

Gossypol and Cyclopropionic Acid in Cottonseed

Gossypol is found in the pigment glands in most commercial cotton varieties. Free gossypol is moderately toxic to nonruminant animals and can limit the quantity of cottonseed meal used in swine, poultry, and fish feeds. A dietary level of 0.03% free gossypol suppresses growth rate and a level as low as 0.01% will cause liver damage in rainbow trout (Herman 1970). A concentration of between 0.09 and 0.12% free gossypol is the minimum toxicity level for channel catfish (Robinson and Li 1994). However, Tilapia have been fed up to 0.18% free gossypol with no observed effect.

The amount of free gossypol in cottonseed meal depends upon the methods of processing the seed. Generally, free gossypol content of cottonseed meal is highest in direct solvent extracted meal (0.1 to 0.5%) and lowest in screwpress meal

(0.02 to 0.05)(Eng, 1990). Most cottonseed meal in the southern United States is made by expander-solvent or prepress-solvent extraction processes and contains 0.02 to 0.21% free gossypol. Available lysine content of cottonseed meal is inversely related to free-gossypol content; this is because lysine is a binding site for gossypol in protein in cottonseed meal. Thus, the amount of cottonseed meal that can be used in fish feeds depends upon the levels of free gossypol and lysine in the finished feed. Dorsa et al. (1982) fed 17% prepress-solvent cottonseed meal to channel catfish with no adverse effects. Levels of 27 to 34% cottonseed meal were fed to salmonids satisfactorily when lysine allowance in the diet was sufficient.

All varieties of cottonseeds contain cyclopropenoic fatty acids that produce several undesirable effects in birds, mammals, and fish. Increased saturated fatty acids in body lipids and delayed sexual maturity occurred in female rats; increased cholesterol levels, aortic atherosclerosis, and liver damage were found in rabbits. Liver damage, increased glycogen deposition, and elevated saturated fatty acid levels in lipids resulted when rainbow trout were experimentally fed cyclopropenoic fatty acids. No cyclopropenoic acid related problems have been specifically identified in fish fed commercial feeds containing cottonseed meal.

Oxidized Fish Oil

Marine fish oils contain 20% to 25% polyunsaturated fatty acids. Autoxidation of unsaturated fatty acids produces a large number of free radicals and peroxide compounds, which are active pro-oxidants. These components may react with other diet ingredients and reduce their nutritional value or, after ingestion, react with oxidation-sensitive phospholipid cellular and subcellular membranes and cause damage. Ingestion of oxidized fish oils has caused reduced growth rate, anemia, nutritional muscular dystrophy, and lesions and ceroidosis in liver of fishes. Increasing dietary levels of vitamin E reduces severity of these toxicity effects.

Antiproteases

Nonheated legume seeds, especially soybeans, contain globulin protein that combines with and inactivates the digestive enzymes trypsin and chymotrypsin. This significantly reduces growth rate in monogastric animals. In addition to reduction in protein digestion, hypertrophy and excess secretion from the pancreas have been observed in rats fed either nonheated soybeans or isolated protease inhibitor. Fish do not utilize nonheated soybean products well. In fact, additional heating of commercially processed soybean meal has reduced activity of the protease inhibitors and increased growth rate of rainbow trout and channel catfish over that obtained from feeding the commercial meal without further heating.

Thiaminases

Tissues of most freshwater fishes and some saltwater species contain an enzyme that can hydrolyze thiamine. The enzyme is heat sensitive and can be inactivated by moderate heating of the fish flesh before feeding. Thiamine in prepared fish diets is only destroyed after contact with the thiaminase for a period before being consumed. Raw fish products can be fed to fish if a source of thiamine is fed in a separate diet. Otherwise, the fish products should be heat treated before feeding.

FIBER

Fiber is not a specific chemical compound, but a mixture of plant components such as lignin, cellulose, hemicellulose, pentosans, and other components that are generally indigestible to monogastric animals, including fish. Fiber has no functional value in fish feeds except possibly to control rate of movement of ingesta through the digestive tract, and this is probably unnecessary in practical fish diets. Leary and Lovell (1975) found no benefit in increasing fiber in practical catfish diets above the basal content of 2.8% and that increasing fiber beyond 14% reduced growth rate, probably by diluting nutrients in the diet. In practical diets, which will contain 3 to 6% crude fiber, adding fiber is unlikely to have any measurable benefit. Because fiber is indigestible, it adds to the fecal waste and increases the biological oxygen demand (BOD) in the culture system.

PIGMENTS

Pigmentation is highly important in flesh of some fish, such as salmon, or in skin of others, such as ornamental fish, red sea bream and shrimps. Conversely, pigmentation is undesirable in flesh of some fish where consumers expect white flesh. Fish cannot synthesize these pigments; therefore they must be present in the diet. Many plants and animals contain natural pigments, called carotenoids, that impart yellow, orange, and red colors to the flesh, skin, and eggs of fish when consumed. In salmonids, two carotenoids, astaxanthin and canthaxanthin, are responsible for the red to orange coloring of the flesh. Astaxanthin is the main carotenoid pigment of wild salmonids which the fish obtains through the aquatic food chain. Salmonids cannot convert the yellow (lutein) and orange (zeaxanthin) carotenoids, of plant origin, to the red astaxanthin or canthaxanthin; however, microcrustaceans (zooplankton) can and through the food chain they serve as the source of red pigment for salmonids. The pigments of plant origin do not produce the desired color of salmon flesh.

Salmonids absorb astaxanthin and canthaxanthin 10 to 20 times more efficiently than lutein and zeaxanthin, while chickens absorb zeaxanthin and lutein at three times the rate of astaxanthin. However, many warmwater fish, such as channel catfish, absorb lutein and zeaxanthin easily. Yellow pigment deposition in the flesh of channel catfish, which is produced by zeaxanthin and lutein (Lee, 1987), is considered undesirable. Lee (1987) found that a concentration of 0.6 g carotenoid/g of flesh produced a distinguished yellow color in the fillet, which is commercially undesirable. A discernible concentration of carotenoid is deposited in catfish flesh when feeds contain 11 mg or more of carotenoid (xanthophyll) per kg.

The continuous feeding of 100 mg of the paprika *(Capsicum sp.)* extract, capxanthin, per kg of diet provided normal red color to skin of cherry barbs grown in tanks with no natural food organisms whereas fish in a similar environment without the pigment supplement were colorless. Feeding 400 mg of supplemental capxanthin for two weeks gave the same effect. Salmon diets are supplemented with approximately 50 mg of astaxanthin or canthaxanthin per kg of feed for continuous feeding. Shrimp raised in intensive culture systems benefit from astaxanthin supplementation in the diet.

DIET ADDITIVES

Pellet Binders

Steam-pelleted fish feeds, especially those fed to crustaceans, usually contain added binders to improve water stability, increase pellet firmness, and reduce the amount of fines produced during processing and handling. Among the most widely used binders are sodium and calcium bentonites, lignosulfonates, hemicellulose, carboxymethylcellulose, alginate, and guar gum. More recently, some inert polymeric binders have been introduced, but limited information is available on their composition or toxicity to commonly cultured fish. Most binder additives are considered to be inert and have limited or no nutritional or toxicological effect. Cereal grains provide starch that, when gelatinized, gives a durable, water-stable pellet. Certain feed ingredients, such as whey, wheat gluten, pregelatinized starches, and molasses, have good binding properties.

Hormones

Hormone implants have been advantageously used to enhance growth (synthetic androgens and estrogens) and milk production (somatotropin, or growth hormone) in livestock. Various natural and synthetic hormones have been evaluated in fish growth experiments, including growth hormone, thyroid hormones, insulin, and various sex steroids. Experimental feeding of synthetic androgens has enhanced growth and improved feed conversion in some species, especially in salmonids. Some warmwater species, however, such as channel catfish, have responded negatively to the feeding of androgens. Prolonged steroid treatment or feeding at too high diet concentration for growth acceleration may cause detrimental side-effects including hypotrophy of gonadal development, skeletal deformity, increased susceptibility to infections, and pathological changes in the liver, kidney, and digestive tract. None of these hormones has been approved by the United States Food and Drug Administration for growth enhancement in food fish.

Feeding sex hormones has been used to control sexual development in species where it is desirous to grow monosex (one sex) fish in the culture system to prevent reproduction or increase growth rate. Incorporating androgenic steroids (ethyltestosterone and methyltestosterone) in diets (30 to 60 mg kg^{-1}) fed as first food to tilapia fry and continued for 14 to 21 days results in 90% to 100% development of male fish. This method of sex reversal in tilapia is practiced commercially in several countries; however, feeding steroids for sex control is not presently approved for use in the United States. Experimental feeding of low doses of 17-ß-methyltestosterone to rainbow trout and Atlantic salmon fry for three months after the fish begin feeding resulted in all male fish. Feeding similar steroids to channel catfish fry, however, resulted in female development in all fed fish. Goudie et al. (1983) theorized that the fed androgen (17-ß-methyltestosterone) was enzymically changed to a compound with estrogenic properties in the channel catfish. Feeding estrogenic steroids to tilapia fry (ehtylnylestradiol, estrone, diethylstilbestrol) and salmonid fry (17-ß-estradiol) caused development of all females.

Hormones have been successfully used to induce or synchronize ovulation and the stimulation of spermiation in fish. The most commonly used preparations are pituitary extracts and human chorionic gonadotropin. Administration is usually by injection rather than feeding or implant. These hormones have been effective in

inducing ovulation and spawning in salmonids, cyprinids, catfishes, flounders, plaice, mullets, milkfish, sea basses, sea breams, and others.

Repartitioning Agents

A group of phenethanolamine compounds have demonstrated efficacy in increasing growth and protein accumulation while depressing lipid deposition when fed to livestock and laboratory animals. These compounds act as nutrient repartitioning agents in intermediary metabolism by redirecting nutrients from fat deposition to muscle protein synthesis. Ractopamine (Figure 4.3) is one of the phenethanolamines that has shown promising responses in livestock, and which the manufacturer has petitioned to the Food and Drug Administration for permission to use in commercial animal feeds. Generally, supplementing feed with 10 to 20 mg kg^{-1} of ractopamine increases growth, nitrogen retention and feed efficiency while reducing carcass fat in pigs, cattle and turkeys. Ractopamine has an advantage over other repartitioning agents, like cimaterol, in being biodegradable and, thus, will produce no residue in water or soil when used.

Mustin and Lovell (1993) supplemented diets for channel catfish with 20 mg kg^{-1} of ractopamine and found 17% increase in weight and 24% reduction in body fat content when compared to untreated controls. They found that ractopamine was more effective when fed in high protein (36%) than in lower protein (30%) feeds, and when the fish were fed to satiation rather than fed at a restricted rate. The mode of action of such a repartitioning agent is not generally agreed on. There is evidence that protein synthesis in the animal is enhanced and also evidence that protein degradation is reduced. Some research suggests that rate of lipogenesis is depressed. Energetically, the increase in weight from feeding a repartitioning compound provides no significant benefit to the animal when the gain is primarily muscle protein rather than fat because muscle is 80% water whereas fat contains no water. As a human food product, however, animal flesh with more muscle and less fat is desirable. None of the repartitioning compounds have been government approved for use in food animals in the United States.

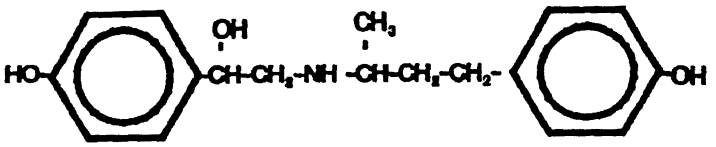


Figure 4.3 Ractopamine.

Antibiotics

A wide range of antibiotics is used for therapeutic purposes in livestock production; however, at present only two compounds, sulfadimethoxine/ormetoprim (Romet-30) and oxytetracycline (Terramycin), have been approved by the Food and Drug Administration for use in fish in the United States. Generally these compounds are stable during steam-pellet processing, however extrusion processing will inactivate a significant amount of the oxytetracycline but very little of the sulfadimethoxine/ormetoprim. Proper feeding rate and withdrawal time are required

to minimize residues from these compounds in tissues of food fish. Dose rate for Romet-30 is 50 mg kg^{-1} of fish weight daily for 5 days, and withdrawal time is 21 days for salmonids and 3 days for catfish. Dose rate for terramycin is 55 to 82.5 mg kg^{-1} of fish daily for 10 days, and withdrawal time is 21 days. Antibiotics may only be added to feeds in the United States by a licensed manufacturer.

Subtherapeutic concentrations of antibiotics in the diets of poultry, swine, and other farm animals influence bacterial microflora of the gut and stimulate growth and feed efficiency. However, oxytetracycline and chlortetracycline in the diets of salmonid fish showed no appreciable benefit (Herman, 1969). Chemotherapeutic compounds may be toxic when administered for an extended period or at high dose. Rainbow trout fed therapeutic concentrations of erythromycin for 10 weeks showed vascular degeneration of proximal renal tubules, and prolonged sulfonamide therapy in salmonids caused growth retardation and necrosis in liver and posterior kidneys.

Immunostimulants

Enhancement of nonspecific immune responses and protection against infection have been shown in fish administered glucans, chitin or other complex polysaccharides or conjugates, by injection, bath immersion and feeding. Mode of action of the immunostimulants is not well understood. Duncan and Klesius (1996) suggested that the ingested compounds are processed in the mucosal immune system of the digestive tract where the immune response is initiated. These researchers fed channel catfish glucopolysaccharides and found no that chemotactic and phagocytic activity of macrophages and neutrophylls were enhanced. Increased protection against bacterial infection has been reported in rainbow trout fed similar materials. Because glucans, yeast, chitin and other polysaccharides are not under FDA regulation and are considered nontoxic and noncarcinogenic, they may be added to fish feeds if they are found to be efficacious.

Attractants and Feeding Stimulants

Some materials, with or without nutritive value, are added to fish feeds to serve as attractants or to enhance palatability. Squid meal, shrimp head meal or extracts have been reported to improve acceptance of feed to some penaeid shrimps. Fish meal is considered an attractant in many fish feeds. Some chemical compounds, such as free amino acids, betaine and the nucleic acid, inosine, are highly effective olfactory and gustatory stimuli for fishes.

Carr (1982) identified four major characteristics of feeding stimulants for fish that were derived from animal tissues: (1) they have a low molecular weight (<1,000), (2) they contain nitrogen, (3) they are nonvolatile and water soluble, and (4) they have both acid and base properties simultaneously. Several substances or groups of substances for which these generalizations apply have stimulated feeding activity in carnivorous and omnivorous species. Few data exist on feeding stimulants for herbivorous species, but organic acids along with certain amino acids were found to be stimulatory. Feeding was stimulated by the organic acid, dimethyl-ß-propiothetin, in goldfish, common carp, and tilapia. A pattern seems to emerge relating to the feeding behavior of the fish and the type of compounds that are stimulatory. In general, more carnivorous species show the greatest positive response to alkaline and neutral substances, such as glycine, proline, taurine, valine, and betaine, while herbivorous species respond more to acidic compounds such as aspartic and glutamic acids.

Most of the research with chemical stimulants has been in the laboratory. Whether additives, like alanine and arginine, which are highly taste effective to channel catfish in aquaria, signal a food source in a pond environment is not determined. The efficacy of using such compounds in practical feeding should be evaluated in a commercial type setting.

Antioxidants

Oxidation of lipids in feeds or feedstuffs can cause reduction of the nutritional value of certain essential lipids and vitamins. This can also produce pro-oxidative compounds that are toxic to the fish, especially if vitamin E or selenium is marginal or deficient in the diet. To preserve oxidation-sensitive nutrients and prevent formation of toxic peroxide compounds, synthetic antioxidants should be included in fish feeds. Natural antioxidant compounds, like the vitamins ascorbic acid and alpha-tocopherol, are not stable in feeds for very long, and thus are not good preservatives. Commercial stabilized sources of ascorbic acid, such as l-ascorbyl-2-phosphate, and alpha-tocopherol, such as alpha-tocopherol acetate, have little antioxidative activity. Synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), which are approved for use in human foods, are sometimes used in animal feeds. Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyl quinoline), which is approved only for use in animal feeds, is commonly used in fish feeds. Maximum levels of synthetic antioxidants permitted by the Food and Drug Administration is 0.02% of the fat content for BHA and BHT and 150 mg kg^{-1} feed for ethoxyquin.

ACCIDENTAL CONTAMINANTS

These include elements or compounds such as heavy metals, pesticides, and industrial chemicals that enter the feed or its ingredients unintentionally or by accident during production, processing, or storage. Examples that have been identified are fish meal containing mercury accumulated from the sea, plant products containing pesticide residues from excessive farm application, overfortification of trace mineral or medicinal supplements in the feed, dioxin contamination of zeolite clays used as anti-caking additives to soybean meal, and contamination of the feed from pesticide applications during storage. These are highly uncommon, but have occurred and caused significant inconvenience and economic loss. Feed ingredients may occasionally exceed FDA tolerance levels for certain pesticides or other environmental contaminants. The feed manufacturer should be aware of these tolerances and have ingredients, especially those with high lipid concentrations such as fish oil, monitored periodically for pesticide residues.

The feed manufacturer must be vigilant for these various unexpected contaminants which could make the feed, and possibly the fish consuming the feed, adulterated and subject to condemnation by the FDA.

REFERENCES

CARR, W. E. S. 1982. Chemical stimulation of feeding behavior. In Chemoreception in fishes, ed. T. J. Hara, 259-273. Amsterdam: Elsevier.

DORSA, W. J., H. R. ROBINETTE, E. H. ROBINSON, and W. E. POE. 1982. Effects of dietary cottenseed meal and gossypol on growth of young channel catfish. *Trans. Am. Fish Soc.* 111: 6 5 1 655.

DOSTER, R.C., R. O. SINNHUBER, and J. H. WALES. 1972.
Acute intraperitoneal toxicity of ochratoxins A and B in rainbow trout (*Salmo gairdneri). Fd Cosmetl Toxicol.* 10: 85-92.

DUNCAN, P. L. and P. H. KLESIUS. 1996. Dietary immunostimulants enhance nonspecific immune responses in channel catfish but not resistance to *Edwardsiella ictaluri. J. Aquatic Animal Health.* 8: 241-248.

ENG, K. 1990. Gossypol content of cottonseed meal. *Feedstuffs.* 64: 62-63.

GATLIN, D. M. III, and R. P. WILSON. 1984. Zinc supplementation of practical catfish diets. *Aquac.* 41: 31-36.

GOUDIE, C, A., B. D. REDNER, B. A. SIMCA, and K. B. KAVIS. 1983. Feminization of channel catfish by oral administration of steroid sex hormones. *Trans. Am. Fish. Soc.* 112: 670-672.

HALVER, J. E. 1969. Crystalline aflatoxin and other vectors for trout hepatoma. In Troup hepatoma research conference papers. Washington, D.C.:Bureau of Sport Fisheries and Wildlife. 78-102.

HALVER, J. E., and I. A. MITCHELL. 1967. Trout hepatoma research conference papers. Bur. Sport Fish and Wildlife Rep. 70. Washington, DC: U.S. Department of the Interior.

HERMAN, R. L. 1969. Oxytetracycline in fish culture - a review. Pp. 1-9 in Tech. Paper 51. U.S. Fish and Wildlife Service, Washington, D. C.

HERMAN, R. L. 1970. Effects of gossypol on rainbow trout, *Salmo gairdneri. J. Fish Biol.* 2: 293-303.

JANTRAROTAI, W. and R. T. LOVELL. 1990. Subchronic toxicity of aflatoxin B_1 to channel catfish. *J. Aquatic Animal Health.* 2:248-254.

LAMBERTDACHA, S. and R. T. LOVELL. 1995. Fumonisin-contaminated dietary corn reduced survival and antibody production by channel catfish challenged with *Edwardsiella ictaluri. J. Aquatic Animal Health.* 7:1-8.

LAMBERTDACHA, S., R. T. LOVELL, R. A. SHELBY, S. D. LENZ, and B. W. KEMPPAINEN. 1995. Growth, hematology, and histopathology of channel catfish, *Ictalurus punctatus,* fed toxins from *Fusarium moniliforme. Aquac.* 130: 201-218.

LEARY, D. F. and R. T. LOVELL. 1975. Value of fiber in production diets for channel catfish. *Trans. Am. Fish. Soc.* 104: 328-332.

LEE, P. H. 1987. Carotenoids in cultured channel catfish. Ph.D. dissertation, Auburn University, Auburn, AL.

MANNING, B. 1998. Comparison of toxicity of pure and crude fumonsin B_1 in channel catfish. Ph.D. dissertation, Auburn University, AL.

MUSTIN, W.T. and R. T. LOVELL. 1993. Feeding the repartitioning agent, ractopamine, to channel catfish increases weight gain and reduces fat deposition. *Aquac.* 109:145-152.

OKAYAKI, T., T. NOGUCHI, K. IGAARASHI, Y. SAKAGAMI, and H. SETO. 1983. Gizzerosine, a new toxic substance in fish meal, causes severe gizzard erosion in chicks. *Agric. Biol. Chem.* 47: 2949-2952.

POSTON, H. A. 1983. Biological effect of dietary T_2 toxin on rainbow trout. *Aquat. Toxicol.* 2:79-88.

ROBINETTE, H. R. 1981. Use of cottonseed meal in catfish feeds. Proc. Catfish Farmers of Amer. Res. Workshop 3: 26.

ROBINSON, E. H. and M. LI. 1994. Use of plant proteins in catfish feeds: replacement of soybean meal with cottonseed meal and replacement of fish meal with soybean meal and cottonseed meal. *J. World Aquac.* 25: 271-276.

WOODWARD, B. L.,L. G. YOUNG, and A. K. LUN. 1983. Vomitoxin in diets for rainbow trout (*Salmo gairdneri). Aquac.* 35: 93-101.

5 BIOAVAILABILITY OF NUTRIENTS

Nutrients must be provided in appropriate amounts and in forms that are biologically usable for optimum performance by the animal. Therefore it is as important to know the bioavailability of the nutrient as the dietary requirement. A respectable amount of data is available on digestibility of gross energy and crude protein in commercial ingredients used in fish feeds. There is, however, much less information on bioavailability of vitamins, minerals and amino acids from various natural and synthetic sources. In many cases, assumed availability values for nutrients are used to formulate fish feeds which are probably far from accurate. Examination of data presently available supports this contention.

DEFINING BIOAVAILABILITY

Other terms often used synonymously with bioavailability are availability, bioactivity, biopotency, bioefficacy, net retention and digestibility (Ammerman et al., 1995). Bioavailability may be generally defined as the degree to which a nutrient in a foodstuff is absorbed in a form that can be metabolized by the animal. In some instances this definition is insufficient if utilization of absorbed nutrients is expected to vary among nutrient sources or conditions of the test. Thus, two general approaches are used to determine bioavailability. One is based on (true or apparent) digestibility; procedures for this determination are described in Chapter 3. The second approach is based on animal response, such as growth. Ideally, digestibility data should be validated with growth assays.

Bioavailability values are usually expressed as percentage units or in relative terms. Percentage means the proportion of the total nutrient in the source that is absorbed by the animal. This determination is appropriate and generally used for energy and protein. Bioavailability values are also expressed in relation to the response obtained with a standard nutrient source; thus, the term relative bioavailability is used. The standard reference material should be a material

commonly used in practical or experimental feeds. It is desirable that the reference material be highly available to the animal, but if a less available nutrient source is a more practical association, it can be used. Relative bioavailability is often used to compare various sources of micronutrients, which may vary in efficiency of metabolism subsequent to absorption.

DETERMINING BIOAVAILABILITY

Digestion or Net Absorption

Procedures for determining digestibility or net absorption of energy and nutrients in feedstuffs are discussed in Chapter 3. Factors affecting digestibility of energy or nutrients by fish, other than dietary sources, are size of meal, frequency of feeding, temperature, method of collecting feces, concentration of nutrient in the diet and other diet components. Early procedures for determining digestibility in fish involved feeding the test material alone; later, Cho et al. (1982) recommended feeding the test material in combination with other diet ingredients in the proportion that it would be included in a practical feed. (Described in Chapter 3). This provides a more accurate assessment of the digestibility of the nutrient in a practical feed.

When net absorption of a supplemental nutrient, such as a trace mineral supplement, is determined, the supplement must be fed in a basal diet. It is important that the net absorption coefficient derived for the test nutrient be corrected for the residual nutrient in the basal diet. This is addressed by determining digestion coefficients for the nutrient (mineral) in the basal diet and the test (supplemented) diet by equation 5.1:

$$\%NA = 100\left[1 - \frac{Mineral\ in\ feces}{Mineral\ in\ diet} x \frac{Indicator\ in\ diet}{Indicator\ in\ feces}\right] \quad (5.1)$$

where %NA = percentage net absorption of mineral in the diet. Subsequently, net absorption of the supplemented mineral is corrected for residual amounts of the element in the basal diet to provide corrected net absorption, CNA, calculated from equation 5.2:

$$CNA = 100\left[\frac{(NA_{test})(Min_{test}) - (NA_{basal})(Min_{basal})}{Min_{suppl}}\right] \quad (5.2)$$

Growth or Response Assays

This procedure for determining nutrient bioavailability involves feeding trials. Test animals are fed various levels of the test nutrient or a standard reference nutrient in a basal diet that is deficient in the test nutrient but otherwise nutritionally adequate. Dose-response data are obtained and subjected to regression analysis. The slope of the regression curve for the test material is compared with the slope of the regression curve for the standard. Response criteria include primarily growth (weight gain), but may be other nutrient-sensitive responses. Bioavailability of the test nutrient is presented in relation to that of the standard. More details of the "slope ratio" procedure for determining bioavailability is presented in Chapter 7.

Table 5.1. COMPARISON OF BIOAVAILABILITY OF ZINC FROM ZINC METHIONINE (ORGANIC) AND ZINC SULFATE (INORGANIC) BY NET ABSORPTION DETERMINATION AND BY GROWTH ASSAY FOR CHANNEL CATFISH

Zinc Source	Relative growth response (%)	Relative net absorption (%)
Zinc sulfate	100	100
Zinc methionine	352	173

Sources: Paripatananont and Lovell (1995; 1997).

Table 5.2. MINIMUM DIETARY REQUIREMENT FOR OPTIMUM GROWTH AND RELATIVE BIOAVAILABILITY VALUES FOR INORGANIC AND ORGANIC SOURCES OF ZINC AND SELENIUM

Mineral	Source	Minimum requirement[1] (mg kg^{-1})	Relative bioavailability[2] (%)
Zinc	Inorganic - $ZnSO_4$	18.90	100
	Organic - Zinc methionine	5.60	352
Selenium	Inorganic - Na_2SeO_3	0.28	100
	Organic - Selenomethionine	0.09	356

Sources: Zinc, Paripatananont and Lovell (1995); Selenium, Wang and Lovell (1997).

[1]Determined by breakpoint in growth response regression line.

[2]The ratio of the slope of organic element regression line to the slope of the inorganic element regression line.

BIOAVAILABILITY OF MINERAL SUPPLEMENTS

Bioavailability of minerals to fish varies among species and among mineral sources. Digestion of inorganic feed components is generally more complete for fish with well-developed gastric areas in the digestive tract. For example, carps (stomachless) digest phosphorus in bone (in fish meal) about one-third as efficiently as salmonids. It is well known that net absorption of phosphorus from various sources varies appreciably. This was shown in Table 3.2, in chapter 3, which presented data indicating that dicalcium phosphate is approximately 80% as digestible as monosodium phosphate. Li et al. (1996) compared phosphorus sources for channel catfish by growth assay and found that bioavailability of dicalcium phosphate was 80% of that of monosodium phosphate, indicating close agreement between net absorption and utilization for growth.

There is not always agreement between net absorption of a mineral and growth response. Paripatananont and Lovell (1997) reported net absorption coefficients for organic and inorganic sources of trace elements, as shown in Table 3.3 in chapter 3. Net absorption data indicated that the net absorption of organic zinc in relation to net absorption of inorganic is 173%. Subsequently, they derived bioavailability values for organic zinc (zinc methionine) relative to an inorganic source (zinc sulfate) by growth assay. The data, presented in Table 5.1 shows that the weight gain from organic zinc was 352% of that from inorganic zinc. Thus, both methods, net absorption and growth assay, showed that the chelated minerals were more available than the inorganic sources; however, the growth response data showed a larger difference in bioavailability between the two zinc sources than the net absorption data. This indicated that digestion and absorption only partially explained the superior bioavailability of chelated zinc. Evidently the chelated mineral was metabolized more efficiently after absorption than the inorganic source. In this instance, growth assay was a more accurate determination of bioavailability of a mineral than measuring net absorption.

Table 5.2 compares bioavailability of chelated and inorganic sources of selenium for channel catfish determined from growth assay. The data indicate that the organic sources of zinc and selenium are markedly more bioavailable to fish than the inorganic sources traditionally used in fish feeds. This means less of the mineral needs to be added to the feed when the chelated form is used. This may not be economical if the chelated mineral is much greater in cost, but may be important environmentally as less chelated mineral will go into the culture system as a feed supplement.

BIOAVAILABILITY OF VITAMIN SOURCES

Bioavailability of vitamins in natural feedstuffs varies among vitamins and among sources. Availability of niacin in grains is generally low for farm animals, less than 50%, and availability of biotin ranges from good in corn and soybean meal to poor in small grains. In most instances, the vitamin content of a feedstuff determined by chemical analysis is not 100% available to the animal. Because of the variations in bioavailability of vitamins in natural feedstuffs, vitamin supplements from synthetic sources are usually used in fish feeds, which often results in the total vitamin content being in excess of the fish's requirement. There is relatively little information on bioavailability of vitamins in natural feedstuffs for fish.

Considerable variation in bioavailability was found among different synthetic sources of vitamin C in fish. Because L-ascorbic acid, the traditional source of vitamin C, is relatively unstable during processing and storage of feeds, stable sources, such as L-ascorbyl-2-sulfate and L-ascorbyl-2-phosphate, were evaluated as sources of vitamin C for fish. The sulfate form appeared to be comparable in bioavailability to L-ascorbic acid for salmonids (Tucker and Halver, 1984). For channel catfish, however, results among studies varied. Some experiments indicated that the sulfate form was equal to L-ascorbic acid and results from others indicated that it was not. Careful review of the studies indicated that when an excessively high concentration of the different vitamin sources were fed, there was no difference in fish response among the sources. However, when dietary concentrations equal to or lower than the fish's requirement was fed, a difference among the sources was found. El Naggar and Lovell (1991) determined growth responses of channel catfish fed various concentrations, ranging from less than to more than the fish's requirement, of three sources of vitamin C. They derived dose-response curves for each and compared the slopes of the linear parts of the regression curves. Slope-ratio analysis revealed that ascorbyl-2-phosphate had essentially 100% of the bioavailability of ascorbic acid, but ascorbyl-2-sulfate had only 7% of the bioavailability of ascorbic acid.

Bioavailability of vitamins and trace minerals varies among natural and synthetic sources and, in instances, variation can be great. Because of the paucity of information on availability of these micronutrients from various sources to fish, oversupplementation is common practice. Supplementation of micronutrients, especially if this is done to a number of them, can increase feed costs appreciably. More attention should be given to micronutrient bioavailability to fish.

REFERENCES

AMMERMAN, C. W., D. H. BAKER, and A. J. LEWIS. 1995. Bioavailability of nutrients for animals - amino acids, minerals, and vitamins. San Diego: Academic Press.

CHO, C. Y., S. T. SLINGER, AND H. S. BAYLEY. 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. J. Biochem. Physiol. 73B: 25-41.

EL NAG GAR, G. O. and R. T. LOVELL. 1991. L-ascorby-2-monophosphate has equal antiscorbutic activity as L-ascorbic acid but L-ascorbyl- 2-sulfate is inferior to L-ascorbic acid for channel catfish. American Institute of Nutrition, 1991, pp. 1622-1626.

LI, M. H. and E. H. ROBINSON . 1996. Phosphorus availability of common feedstuffs to channel catfish Ictalurus punctatus as measured by weight gain and bone mineralization. J. World Aquac. Soc. 27: 297-302.

PARIPATANANONT, T. and R. T. LOVELL. 1995. Responses of channel catfish fed organic and inorganic sources of zinc to Edwardsiella ictaluri challenge. J. Aquatic Animal Health. 7: 147-154.

PARIPATANANONT, T. and R. T. LOVELL. 1997. Comparative net absorption of chelated and inorganic trace minerals in channel catfish Ictalurus punctatus diets. J. World Aquac. Soc. 28: 62-67.

TUCKER, B. W. and J. E. HALVER. 1984. Ascorbate-2-sulphate metabolism in fish. Nutr. Rev. 45: 173-179.

WANG, C. and R. T. LOVELL. 1997. Organic selenium sources, selenomethionine and selenoyeast, have higher bioavailability than inorganic selenium sources, sodium selenite, in diets for channel catfish (Ictalurus punctatus). Aquac.152: 223-234.

6 NUTRITION AND FISH HEALTH

Fish in the wild seldom show signs of nutritional disease. This is because natural aquatic foods are not deficient in essential nutrients; the diet may be deficient in quantity but not quality. It is when fish are confined to an artificial environment and intensively fed for rapid weight gain that nutrition-related diseases can occur. Nutrition-related diseases can be described as a) physiological or morphological anomalies resulting from dietary nutrient deficiency; b) materials in the diet which are toxic to the fish; and c) reduction in resistance to infection caused by diet quality or feeding practice. Anomalies resulting directly from nutrient deficiencies have been described in chapter 2 and diet related toxins are discussed in chapter 4. Effects of nutrition on resistance of fish to infectious diseases are discussed in this chapter.

There is a significant relationship between nutritional status and resistance to infectious diseases in animals. This is well established in warm blooded animals and some information has been obtained on this subject in fish. Several areas where effects of nutrition or feeding have been found to influence immune responses in fish are discussed in the following.

LIPIDS

Quantity and quality of dietary fatty acids influence immune responses in fish, and there appears to be variation among fishes, especially between warm and cold water species, and with temperature in response to dietary lipids.

Fracalossi et al. (1994) demonstrated that channel catfish fed menhaden fish oil under warmwater conditions (29-30°C) had lower survival following challenge with *Edwardsiella ictaluri,* than fish fed corn oil or a mixture of corn and fish oils. Menhaden oil contains approximately 23% of n-3 highly unsaturated fatty acids (HUFAs), namely 20:5 n-3 and 22:6 n-3. Whereas corn oil contains no n-3 HUFAs but a large amount of the n-6 fatty acid, 20:2 n-6. The study found

that fish oil in the diet caused a lower production than corn oil of leucotriene B, an immunostimulant derived from arachidonic acid (20:4 n-6) and which is important in immune function in warm blooded animals. This was interpreted to indicate that under warmwater conditions in channel catfish there may be competitive inhibition of arachidonic acid metabolism by n-3 fatty acids from fish oil, as occurs in warm blooded animals. Li et al. (1994) also found reduced resistance to *E. ictaluri* by channel catfish fed menhaden oil. Conversely, cold water fish appear to have greater resistance to bacterial infection with n-3 polyunsaturated fatty acids in the diet. The role of dietary fatty acids in immunity in fish appears to be related to production of eicosonoids, such as leucotrienes. Leucotrienes are potent immunostimulators, produced by macrophages and neutrophils, and, in humans, have been found to induce suppressor cell activity, increase production of interleukin, and act as chemotactic and chemokinetic agents for neutrophils.

At low water temperature (17-20°) Fracalossi et al. (1994) found no effect of the dietary n-6 or n-3 fatty acid sources on eicosonoid production in channel catfish. Sheldon and Blazer (1991) found that channel catfish macrophages showed higher bacteriocidal activity with menhaden fish oil in the diet than with beef tallow or soybean oil. They used a water temperature of 25°C, which may explain why they found different results from Fracalossi et al. (1994) at 29-30°C. Water temperature may play a role in the immune responses of fish fed various fatty acids in the diet.

VITAMINS

Deficiency of the antioxidant vitamins, vitamins A, C, E and b-carotene, generally reduces resistance of farm and laboratory animals to bacterial infections. Recently, reports have indicated enhanced immunocompetence in animals fed higher than normal doses of these vitamins. Li and Lovell (1984) showed that channel catfish fed diets deplete in vitamin C were more sensitive to *E. ictaluri* infection than fish fed diets replete in the vitamin (30 mg kg^{-1} diet). They also found that megadoses of the vitamin (300 mg kg^{-1} diet) increased survival, antibody production, complement activity and phagocytosis above rates measured in fish fed the level required for normal growth (30 mg kg^{-1} diet). Wise et al. (1993) found that dietary vitamin E increased the ability of macrophages to phagocytize *Edwardsiella ictaluri* cells, and that elevated levels of the vitamin (2,500 mg kg^{-1} diet) increased the rate of phagocytosis above that associated with the normal growth requirement of 60 mg kg^{-1} diet.

Duncan and Lovell (1994) demonstrated high rate of mortality when young channel catfish were fed a diet devoid of folic acid as compared to fish fed a diet replete with folic acid. They also found that there was interaction between dietary folic acid and vitamin C. Table 6.1 shows that as the dietary content of vitamin C increased above the normal requirement for growth (20 mg kg^{-1}), the folic acid requirement for maximum resistance against *E. ictaluri* decreased. The interaction between vitamin C and folic acid is apparently caused by the necessity of a reducing agent, such as vitamin C, to convert folic acid to the active coenzyme, 5- methyl-tetrahydrofolic acid. These results indicate that dietary folic acid influences immune responses in channel catfish, and that the dietary concentration of vitamin C influences the response of the fish to different levels of folic acid.

Table 6.1. EFFECT OF VITAMIN C AND FOLIC ACID ON RESISTANCE TO INFECTION AND ANTIBODY PRODUCTION BY CHANNEL CATFISH CHALLENGED WITH *EDWARDSIELLA ICTALURI*

Dietary vitamin C (mg kg^{-1})	Dietary folic acid (mg kg^{-1})	Mortality[1] (%)
20	0.0	88[a]
20	0.4	63[a]
20	4.0	31[b]
200	0.0	75[a]
200	0.4	34[b]
200	4.0	31[b]

Source: Duncan and Lovell (1994).
[1] Values with same leter are not different (P > 5%).

MINERALS

Dietary deficiency of zinc increased mortality of channel catfish challenged with *Aeromonas hydrophila* (Scarpa et al., 1992) and *E. ictaluri* (Paripatananont and Lovell, 1995). The latter study showed that chelated zinc (zinc-methionine) was three to six times more potent than inorganic zinc (zinc sulfate) in protecting channel catfish against experimental challenge with *E. ictaluri*. Selenium deficiency reduced resistance of channel catfish to *E. ictaluri* infection, and chelated selenium (selenium-proteinate) was found to be approximately two times as bioavailable for protection against infection as inorganic selenium (sodium selenite) (Wang et al. 1997).

Scarpa et al. (1992) found that dietary calcium deficiency reduced survival of channel catfish challenged with *A. hydrophila* when the fish were fed in low-calcium water (<1 mg L^{-1} as $CaCo_3$). Because natural waters contain enough dissolved calcium for normal growth of most fish without dietary supplementation, calcium deficiency may not be a practical problem in disease resistance of fish.

Eya and Lovell (1997) found that dietary phosphorus deficiency reduces resistance and decreases antibody production in channel catfish infected with *E. ictaluri*. They also found that the available phosphorus requirement for optimum growth and bone development was sufficient for maximum resistance against *E. ictaluri* infection. The amount of available phosphorus in an all-plant, commercial type feed without a phosphorus supplement is not sufficient to provide maximum immunity against *E. ictaluri* infection for young (year-1) channel catfish. It is not known if an all-plant feed without phosphorus supplementation is sufficient for food-size fish (year-2,3) which have a lower phosphorus requirement for growth than young fish.

MYCOTOXINS

Toxins produced by *Aspergillus, Fusarium* and *Penicillium* species of molds, which are commonly found in feedstuffs, have been shown to have immunosuppressing effects in chickens and livestock. Few reports are available of effects of feed molds on resistance of fish to bacterial infections. Lumlertdacha and Lovell (1995) fed channel catfish with diets containing various amounts of corn cultured with *Fusarium moniliforme*, a mold that produces the mycotoxin, fumonsin, then infected the fish with a virulent strain of *E. ictaluri*. The fish fed 80 mg of fumonsin per kg of diet had higher mortality and lower antibody production than fish fed 0 or 20 mg of fumonsin per kg of diet. The results indicate that feedstuffs containing fumonsins at concentrations that have been found to suppress growth rate will reduce resistance to *E. ictaluri* infection in channel catfish.

DIETARY IMMUNOSTIMULANTS

Various diet additions have shown efficacy in enhancing nonspecific immune responses in several fish species. These include glucans, chitin, dried *Spirulina* species of blue-green algae, and baker's yeast (*Sarcharomyces cerevisiae).* Increases in chemotactic and phagocytic activities of macrophages and neutrophils have been reported when these compounds have been included in feed for several fish species. In some cases, increased protection against infection was realized and in some there was only stimulation of nonspecific immune responses with no enhanced protection against infection. Mechanisms for the observed enhancement in immune responses when these materials are fed are not well understood. Cell wall polysaccharides from *Spirulina* caused an increase in rate of phagocytosis by chicken macrophages when the macrophages were exposed to the polysaccharides *in vitro.* The immunostimulants cited above are not restricted by FDA and can be used in fish feeds. Concentrations used experimentally range from 0.2% to 2.7% of the diet.

FEED DEPRIVATION

It is well documented that some degree of caloric restriction improves resistance against communicable and noncommunicable diseases in humans. Reducing the ration size has been found to enhance immune systems in laboratory animals. Reducing the ration of a nutritionally balanced diet by 40% decreased growth rate but also impeded the development of diseases associated with aging of laboratory mice (Good 1984). Wing et al. (1983) claim that fasting has differential effects rather than a uniform deleterious effect on the host animal, and that nutritional alteration can enhance or suppress certain functions, including various immune responses. There is evidence that availability of trace elements in various tissues in the host animal, as influenced by food intake or fasting, may be crucial for either a) immune functions in the host animal or b) the pathogenicity of the invading microorganism. For example, in warmblooded animals a low iron concentration in tissues of the host animal suppresses the multiplication and virulence of certain pathogenic microorganisms. Long or short term deprivation of food from the animal could

Table 6.2. WEIGHT GAIN AND MORTALITY RATE, FOLLOWING EXPERIMENTAL CHALLENGE, BY TWO SIZE GROUPS OF CHANNEL CATFISH MANAGED UNDER THREE FEEDING REGIMENS OVER WINTER (MID-NOVEMBER THROUGH MID-APRIL) IN TWO CONSECUTIVE EXPERIMENTS[1]

Fish size groups	Feeding regimens	Weight gain (%)		Mortality (%)	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
Year-1	Non-fed	-9[a]	-12[a]	90[a]	98[a]
	Partially fed	50[b]	93[b]	48[b]	55[b]
	Full fed	64[b]	107[b]	50[b]	52[b]
Year-2,3	Nonfed	-10[a]	-7[a]	33[a]	9[a]
	Partially fed	42[b]	38[b]	90[b]	69[b]
	Full fed	49[b]	39[b]	92[b]	77[b]

Sources: Experiment 1, Kim and Lovell (1995); Experiment 2, Okwoche and Lovell (1997).

[1]Values among treatments (feeding regimens) within a year group are not different if they are followed by same letter (P >5%).

reduce the concentration of critical materials in serum or other tissues and, thus, influence immune response or pathogenicity of the infectious organism.

Pond experiments in Alabama and in Mississippi have shown that restricting feed allowance for channel catfish increases their resistance against *E. ictaluri* infection. Two studies during separate years were conducted at Auburn University in Alabama on effects of over-winter feeding regimen (no feeding, restricted feeding, continuous feeding) on weight change and resistance to bacterial infection by channel catfish. As shown in Table 6.2, mortality from *E. ictaluri* infection among fingerlings (year-1) fish was significantly higher for fish not fed over winter, as expected. However, among larger fish (year 2 or 3), mortality was higher in the fish fed during winter. The fed fish gained 40 to 50% in body weight while nonfed fish lost 9 to 10% of their weight. The results were similar in both experiments and indicate that while feed deprivation was immunosuppressive in small channel catfish, it enhanced resistance to bacterial infection in larger fish.

The short-term study in Mississippi evaluated effects of restricted feeding on channel catfish fingerlings exposed to a natural epizootic of *E. ictaluri* in ponds. Lowest survival was in treatments where fish were continued on daily feeding with or without antibiotic. Highest survival was in treatments where fish were not fed or where fish were fed every second or third day with antibiotic. These data indicate that restricted feeding appears to be a sound strategy for natural *E. ictaluri* epizootics in commercial channel catfish ponds.

Each of the above experiments showed that feed deprivation affected resistance of channel catfish to *E. ictaluri* challenge. In the over-winter studies, fish were deprived for a long period and the fish showed some weight loss; while in the summer study, fish were deprived only during the challenge. The over-winter study

revealed that fish size (or age) was a factor. The studies suggest that feed restriction or deprivation when a bacterial epizootic occurs or is anticipated can be a prudent strategy with channel catfish and possibly other fish species. However, fish size or age, length and rate of feed deprivation, and pathogen species must be considered.

MEGADOSES OF NUTRIENTS AND DISEASE RESISTANCE

Studies have shown that higher than normal dietary requirements of vitamins, such as vitamins C and E, enhance protection of animals against infectious and noninfectious diseases. While the essentiality of these vitamins for maximum immune responses in fish has been amply demonstrated by research, the practical efficacy of feeding higher than the requirement for normal growth to increase resistance against infection in a commercial setting is questionable. Early studies with small channel catfish under laboratory conditions showed that increasing the dietary vitamin C allowance ten-fold above the requirement for growth reduced mortality when the fish were experimentally challenged with a virulent strain of *E. ictaluri*. Associated with this reduction in mortality were increases in antibody production, phagocytosis and serum complement activity. A similar response was obtained when young rainbow trout were fed ten times the normal requirement of vitamin C and experimentally challenged with *Vibrio anguilarum*. However, when channel catfish were fed normal and above normal levels of vitamin C in a pond environment and exposed to a natural epizootic of *E. ictaluri*, the additional vitamin C supplementation provided only a slight improvement in protection for the exposed fish. This suggested that increasing the vitamin C allowance in commercial catfish feeds for protection against natural infections from *E. ictaluri* is probably not economically feasible in most cases. However, small starved fish that are in poor nutritional status will likely benefit from megadoses of vitamin C if an *E. ictaluri* epizootic is anticipated.

There have been several studies to determine if the dietary requirements for certain vitamins or minerals for maximum immune responses against invading pathogens may be higher than the requirement for optimum growth. Duncan and Lovell (1994) showed that the dietary concentration of folic acid that provided for optimum weight gain was the same as that which allowed maximum survival of fish experimentally infected with *E. ictaluri*. Thus, the dietary requirements of folic acid for growth and disease resistance were the same. Studies were conducted at Auburn University in which the minimum dietary requirements for phosphorus, selenium and zinc by small channel catfish were determined for growth and resistance to *E. ictaluri* infection. For each mineral, the dietary requirements for optimum weight gain and for minimum mortality of experimentally infected fish were not significantly different, as shown in Table 6.3. These studies indicate that when the dietary requirements for growth for these three elements are met, the requirements for maximum resistance against *E. ictaluri* infection will be satisfied. It would be speculative to imply that this relationship between requirements for growth and immune responses exists for other nutrients or other fish species. However, it would be safe to imply that a diet that contains less than the requirement of a nutrient for optimum growth of the fish may not provide maximum protection against infection.

Table 6.3. COMPARISON OF DIETARY REQUIREMENTS OF MINERALS FOR OPTIMUM GROWTH AND MAXIMUM RESISTANCE AGAINST BACTERIAL (*EDWARDSIELLA ICTALURI*) INFECTION IN CHANNEL CATFISH, DETERMINED FROM BREAKPOINT IN DOSE RESPONSE REGRESSION LINE

Mineral	Response	Breakpoint
		(Percent of diet)
Phosphorus	Weight gain	0.42
	Survival of infected fish	0.40
Selenium ($NaSeO_3$)	Weight gain	0.28
	Survival of infected fish	0.34
Zinc ($ZnSO_4$)	Weight gain	18.90
	Survival of infected dish	22.00

Sources: Phosphorus, Eya and Lovell (1998); Selenium, Wang and Lovell (1997); Zinc, Paripatonanont and Lovell (1995).

REFERENCES

DUNCAN, P. L. and R. T. LOVELL. 1994. Influence of vitamin C on the folate requirement of channel catfish, *Ictalurus punctatus*, for growth, hematopoiesis, and resistance to *Edwardsiella ictaluri* infection. *Aquac.* 127: 233-244.

EYA, J. C. and R. T. LOVELL. 1997. Effects of dietary phosphorus on resistance of channel catfish to Edwardsiella ictaluri challenge. Ph.D. dissertation, Auburn University, Auburn, AL.

FRACALOSSI, D. M., M. C. CRAIG-SCHMIDT, and R. T. LOVELL. 1994. Effects of dietary lipid sources on production of leukotriene B by head kidney of channel catfish held at different water temperatures. *J. Aquatic Animal Health.* 6: 242-250.

KIM, M.K. and R. T. LOVELL. 1995. Effect of overwinter feeding regimen on body weight and resistance to *Edwardsiella ictaluri* in channel catfish. *Aquac.* 134: 237-246.

LI, Y. and R. T. LOVELL. 1984. Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. *J. Nutr.* 115: 123-131.

LI, M. H., D. J. WISE, M. R. JOHNSON, and E. H. ROBINSON. 1994. Dietary menhaden oil reduced resistance of channel catfish (*Ictalurus punctatus)* to *Edwardsiella ictaluri. Aquac.* 128: 335-344.

LUMLERTDACHA, S. and R. T. LOVELL. 1995. Funonisin-contaminated dietary corn reduced survival and antibody production by channel catfish challenged with *Edwardsiella ictaluri. J. Aquatic Animal Health.* 7: 1-8.

OKWOCHE, V.O. and R. T. LOVELL. 1997. Cool weather feeding influences responses of channel catfish to *Edwardsiella ictaluri* challenge. *J. Aquatic Animal Health.* 9: 163-171.

PARIPATANANONT, T. and R. T. LOVELL. 1995. Responses of channel catfish fed organic and inorganic sources of zinc to Edwardsiella ictaluri challenge. *J. Aquatic Animal Health.* 7: 147-154.

SCARPA, J., D. M. GATLIN III, and D. H. LEWIS. 1992. Effects of dietary zinc and calcium on select immune functions of channel catfish. *J. Aquatic Animal Health.* 4: 24-31.

SELDON, W. M., JR. and V. S. BLAZER. 1991. Influence of dietary lipid and temperature on bactericidal activity of channel catfish macrophages. *J. Aquatic Animal Health.* 3:87-93.

WANG, C., R. T. LOVELL, and P. H. KLESIUS. 1997. Response to *Edwardsiella ictaluri* challenge by channel catfish fed organic and inorganic sources of selenium. *J. Aquatic Animal Health.* 9: 172-179.

WING, E. J., R. T. STANKO, A. WINKELSTEIN, and S. A. ADIBI. 1983. Fasting enhanced immune effector mechanisms in obese subjects. *Am. J. Med.* 75: 91-96.

WISE, D. J., J. R. TOMASSO, D. M. GATLIN III, S. C. BAI, and V. S. BLAZER. 1993. Effects of dietary selenium and vitamin E on red blood cell peroxidation, glutathione peroxidase activity, and macrophage superoxide anion production in channel catfish. *J. Aquatic Animal Health.* 5: 177-182.

7 FISH NUTRITION AND FEEDING EXPERIMENTS

Nutrient requirements cannot be determined unless nutrient consumption by the fish is precisely known. Such studies are conducted in a controlled environment, such as aquaria or tanks, to prevent interaction of environmental effects, such as natural food organisms, temperature, and water quality, with the nutrient variable being studied in the experiment. Some studies, however, which involve evaluation of practical feed formulations or feeding regimes, should be conducted under conditions as similar as possible to the conditions where the results will be applied while at the same time allowing for accurate collection and analysis of data. These studies are conducted in experimental ponds, raceways, pens, and similar enclosures of water.

CONTROLLED ENVIRONMENT STUDIES

The requirement or effect of a specific nutrient or compound in the diet must be determined in a controlled environment. The overriding consideration in any feeding experiment is to keep all controllable variables equal among treatments except the one being tested. The physical, palatability, and nutritional properties of each experimental diet should be equal except for the nutrient or component that is being tested. Characteristics of controlled environment feeding studies that will yield reliable and useful information are presented in the following.

Rearing Facilities

Size of rearing containers should be large enough to accommodate the originally stocked population after a 500% to 1,000% weight increase. Containers should be supplied with a continuous flow of good quality water with temperature regulated as desired or to that accepted as standard for the fish species (see Figure 7.1). If surface

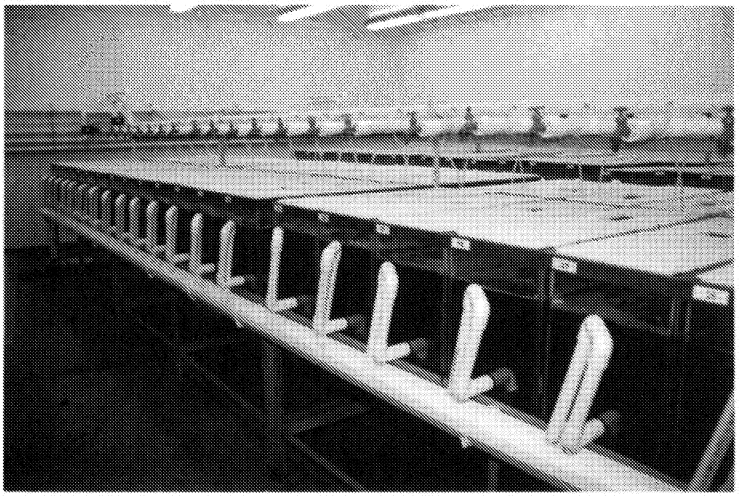


Figure 7.1 Experiments to evaluate the basic nutrition of fish must be conducted in a controlled environment like the laboratory above, where the aquariums have a continuous flow of temperature-controlled, nutrient-free water with sufficient dissolved oxygen. *(Courtesy of Meng H. Li.)*

water is used, it should be filtered to remove all natural sources of nutrients. A constant diurnal light/dark cycle of approximately 14:10 should be maintained for the experimental fish.

Test Fish

Full siblings of a fast-growing genetic strain are desirable. Small fish respond faster than large fish to nutritional variables. Also, small fish are more sensitive to diet differences; if small fish are unaffected by the treatments, it is a safe assumption that larger fish will not be. Higher numbers of small fish per rearing container can be used. However, where nutrient or energy requirements will change with size, different fish size groups should be evaluated. The minimum number of fish per rearing unit (tank or aquarium) should be high enough to negate effects of unequal sex ratio (males growing faster) or to prevent hierarchical feeding patterns. For statistical purposes, each treatment should be replicated in a minimum of three rearing containers.

Previous history of the experimental fish is important. Compensatory growth and tissue stores of nutrients can have significant effects on response to test diets. Shell (1963) showed that there is growth compensation in channel catfish. Two groups of fish were fed experimental diets, one that caused a significantly lower growth rate than the other. Subsequently, when both groups were placed on the same high quality diet, the group that grew slowly in the previous experiment grew faster than the other group in the second feeding trial.

In cases where the metabolic requirement for a nutrient is extremely low and the nutrient has a long residual time in fish tissues, such as with essential fatty acids, body stores of the nutrient may preclude use of the fish in the experiment without depleting the fish prior to the experiment.

Test Diets

Experimental diets for evaluating nutrient requirements should be prepared from highly purified ingredients to allow maximum control over the nutrient being tested. Preferably, all diets in an experiment should be alike in all respects, such as palatability, feedability (particle size, texture), water stability, and nutrient and energy contents (except for the nutrient being tested).

Casein and gelatin (4:1) are a good protein combination for purified diets; low-vitamin casein is available for use in vitamin experiments. Blood fibrin is a desirable protein for mineral studies. Egg protein is good for use in protein or amino acid experiments. All of these protein sources are available in highly purified forms. Dextrin is traditionally used as a carbohydrate source. Cooked starch is satisfactory for warmwater fishes, but starch is not utilized as well as dextrin by coldwater species. Some of the lipid should be from fish oil to provide n-3 fatty acids, and some from plant oils to provide n-6 fatty acids. Purified cellulose is used as a nonnutritive filler. Whether processed into moist or dry pellets, the feeds must contain a binding agent that will hold the particles together for a reasonable time in water. Gelatin, agar, alginic acid, or carboxymethylcellulose are used at levels of 2% to 5%.

Steps for preparing semipurified diets are as follows:

1. Mix the dry ingredients well before adding oil, then add oil and mix.
2. Add approximately 350 ml water per kg of diet mixture and stir. If gelatin is the binding agent, it should be added to the water and heated. The moist mixture should have a stiff, plastic consistency when compressed.
3. Extrude through a food grinder with proper diameter holes in the grinder plate.
4. Break extrusions into short lengths and dry or freeze moist. Moist diets should be stored frozen until 1 or 2 days prior to feeding and should be kept refrigerated until fed.

Model semipurified diets, made from highly purified ingredients for warmwater fish and salmonids are presented in Table 7.1. A standard mineral mixture for semipurified fish diets has not been developed. That presented in Table 7.2 has been used successfully in channel catfish feeds and should provide the minimum requirements for major and trace elements for finfish. Vitamin mixtures that meet the vitamin requirements for fish are available commercially or are referenced in research reports. The vitamins should be stabilized against deterioration during processing and storage. Most commercial vitamin sources available for research and practical diets are relatively stable. The stabilized, ascorbyl-2-phosphate should be used as the source of vitamin C instead of the highly unstable ascorbic acid.

To estimate the amount of each diet required for the experiment, estimate the amount of weight the fish will gain during the experiment and assume 1.5 g of dry diet is required for 1 g of weight gain. Thus, if the initial weight of the total number of fish is 1 kg and weight is expected to increase by 500%, the amount of diet needed would equal (1.5)(5)(1) = 7.5 kg.

Table 7.1. EXAMPLES OF PURIFIED REFERENCE DIETS FOR WARMWATER AND COLDWATER FINFISH

Ingredient	Warmwater finfish[1]	Coldwater finfish[2]
	(%)	(%)
Casein	32	40.8
Gelatin	8	8.0
Dextrin	33	16.0
Alpha-cellulose	14	11.6
Carboxymethyl-cellulose	2	-
Amino acid mixture[3]	-	1.5
Vitamin premix[5]	1	3.1
Mineral premix[5]	4	4.0
Marine fish oil	3	15.0
Vegetable oil	3	-

Source: National Research Council (1993).
[1] Contains 34% digestible protein and 3.1 kcal digestible energy per g.
[2] Contains 40% digestible protein and 3.6 kcal digestible energy per g.
[3] Amino acid mixture provides 0.5% methionine and 1.0% arginine.
[4] Use a vitamin premix that meets the National Research Council's recommended allowances for all of the vitamins for the test fish or similar species.
[5] Use the mineral premix presented in Table 7.2.

Table 7.2. MINERAL PREMIX FOR SEMIPURIFIED DIETS FOR FISHES. TO BE USED AT THE RATE OF 4% OF THE DRY DIET

Ingredients	Percentage of premix
Aluminum potassium sulfate	0.159
Calcium carbonate	18.101
Calcium diphosphate	44.601
Cupric sulfate .$5H_2O$	0.075
Cobaltous chloride	0.070
Ferric citrate .$5H_2O$	1.338
Magnesium sulfate	5.216
Manganese sulfate .H_2O	0.070
Potassium chloride	16.553
Potassium iodide	0.014
Zinc carbonate	0.192
Sodium diphosphate	13.605
Sodium selenite	0.006

Note: Mineral premix provides the following amounts in mg/kg of the dry diet: aluminum, 7; calcium, 8,140; chloride, 6,008; cobalt, 12; copper, 8; iron, 104; iodine, 4; magnesium, 421; manganese, 10; phosphorus, 5,250; potassium, 3,474; sodium, 1,932; selenium, 1; zinc, 40.

Management

The fish should be conditioned to the rearing environment for 1 to 2 weeks prior to beginning the experiment. During this time they can be given chemical baths for external pathogens and acclimated to experimental diets. Sometimes it is necessary to feed all of the experimental diets for a short preliminary period to be sure there is no difference in diet acceptability.

During the experiment, the fish should be fed for maximum growth rate. This enhances sensitivity to diet differences. Also, fish response may be different if the fish are underfed. Thus, satiation feeding is desirable. Accurate measurement of amount of diet eaten is essential for measuring feed efficiency. This means uneaten feed offered must be measured and with satiation feeding this is not an easy task. Feeding frequency depends on fish size and feeding behavior. More frequent feeding encourages faster growth. Length of the feeding trial will depend upon the time required to get statistical differences among the test diets. This will be influenced by the sensitivity of the fish to the limiting nutrient tested, fish species, size of fish, and other factors that affect growth rate. Sometimes the termination date of the experiment should not be established when the experiment is initiated.

If disease or other environmental problems not related to the diet occur in any of the rearing containers, feeding in all containers should be discontinued until the affected fish return to normal feeding; otherwise unequal feeding activity among sick and healthy fish may confound treatment effects.

Fish should be sampled at the beginning of the experiment for analysis for initial condition or body composition of the fish. Biweekly weighings will allow comparison of weight gain over the course of the experiment; this will also be useful in feeding the fish.

Response Data

Growth is usually the most important criterion for measuring fish response to experimental diets. In research studies such as determining nutrient requirements, growth is a sensitive and practical indicator of the adequacy of the diet for a particular nutrient or energy level. True growth in animals involves an increase in structural tissues, such as muscle and bone, and the circulating and organs tissues. This should be distinguished from an increase in fat deposition in adipose tissue. Thus, growth can be characterized reliably by an increase in protein because muscle, organs, and demineralized bone tissue contain primarily protein. It is often assumed that weight gain is synonymous with growth or that composition of gain (protein and fat gain) is the same among fish fed different diets. This, however, is not a safe assumption because diet composition can influence how much of the absorbed nutrients go to protein and how much go to fat. Weight gain is a reliable indicator for growth as long as the experimental variable is not expected to affect composition of gain in the fish. But if the variable being tested does affect composition of gain, erroneous conclusions can be drawn from evaluating weight gain alone.

Unless there is reason to justify not measuring composition of gain, fish from both aquarium and production type experiments should be chemically analyzed at the beginning and end of the feeding trials for fat, protein, and moisture. If there is a difference in final body composition of fish fed the different diets, protein gain should be reported as well as weight gain. Fortunately, fish are small animals, in contrast to warmblooded food animals, and whole body analysis is usually

possible. For food-size fish, dressing yield as well as body proximate composition should be reported.

Presence of clinical signs may be indicators of treatment effects in nutrition experiments. Lesions, hemorrhage, abnormalities in pigmentation, gill hyperplasia, cataracts, and skeletal and cartilage deformities are things to be observed. Subclinical signs or measurements, such as enzyme activity, tissue or cell histopathology, tissue levels of the test nutrient or its metabolites, and a number of others, are often more sensitive than growth response and should complement growth in evaluating the experimental effects. Specific subclinical indicators for various nutrient deficiencies are discussed in chapter 2.

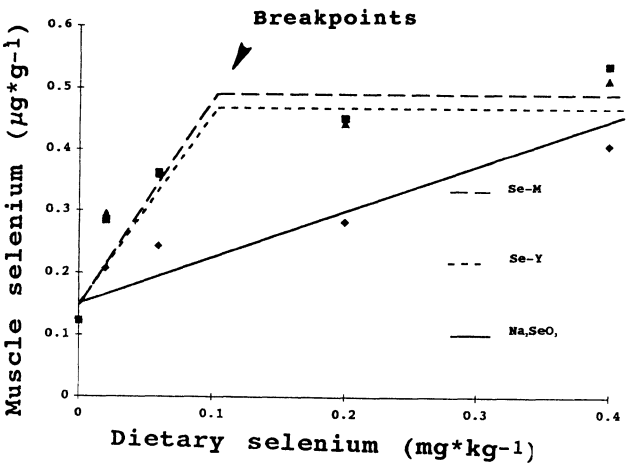


Figure 7.2 The optimum dietary concentration for a nutrient is determined by subjecting dose response data to broken-line regression. The graph above shows that the breakpoint concentrations for two organic sources of selenium, selenomethionine (Se-M) and selenoyeast (Se-Y), for maximum muscle selenium, were 0.09 and 0.11 mg*kg-1, respectively. There was no breakpoint in the response curve for inorganic selenium (Na_2SeO_3). *(From Wang et al., 1997.)*

Quantification of Nutrient Requirements

Determination of the quantity of a nutrient required in the diet by the fish generally involves feeding a series of diets containing various levels of the tested nutrient in a controlled environment and finding the lowest level that will elicit optimum rate of response from the fish. Statistical handling of the diet response data is as important as any other part of the research. Traditionally, the lowest level of the test nutrient fed that produces the maximum or acceptable response in a series of diets containing different levels of the nutrient is considered to be the required level. Regression analysis by the broken line method of Robbins et al.(1979) is often used. The point of intersection of the lines representing the linear and horizontal parts of the growth curve is derived mathematically and is considered to be the optimum dietary requirement for the nutrient as shown in Figure 7.2.

As the concentration of an essential nutrient increases from below to above the optimum, fish response, such as growth, will generally show sharp initial increase as nutrient level in the diet increases, then a slower rate of increase with

gradual leveling off, and subsequently (but not always) a decrease at the higher nutrient levels. Choosing the point on the growth response curve that represents the minimum level of nutrient for the desired rate of growth is critical because the nutrient level for maximum growth may be notably higher than that for economically optimum growth. For nutrients where the recommended dietary level has greater physiological than economical importance, as for most micronutrients, maximum growth is a suitable response point. But for nutrients that are of significant economic importance, such as proteins or amino acids, a response less than maximum may result in significant reduction in feed cost without significant loss in growth rate. Quadratic regression analysis of growth response data can yield nutrient requirements for maximum and less than maximum rates of growth. The growth curve in Figure 7.3 represents response of Nile tilapia to dietary levels of the amino acid arginine ranging from below to above the requirement for optimum

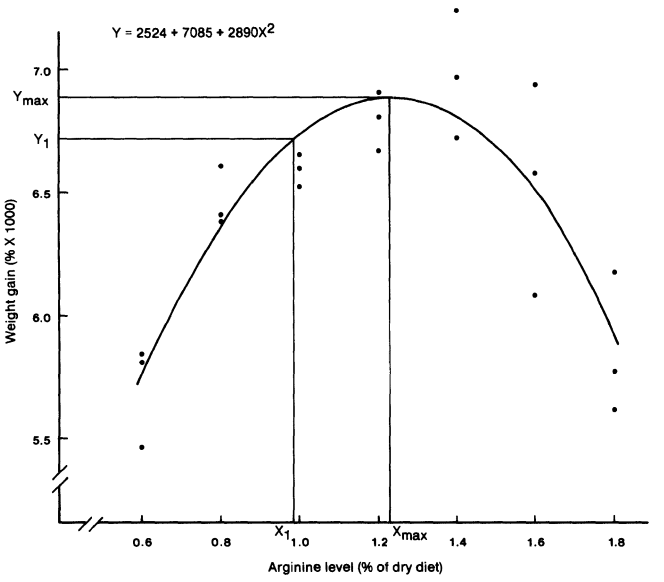


Figure 7.3 Relationship between weight gain by Nile tilapia and dietary arginine level as described by quadratic regression, which allows derivation of X_{max}, which is the requirement for maximum growth (Y_{max}), and X_1 which is the requirement associated with a rate of growth below maximum (Y_1) but within the 95% confidence interval. *(From Santiago, 1995.)*

growth (Santiago 1985). X_{max} on the quadratic regression curve represents the level of amino acid associated with maximum growth (Y_{max}); X_1 represents the quantity of amino acid associated with a rate of growth lower but within the 95% confidence limit of Y_{max}. The level of arginine at X_{max} is 4.4% of the protein, while the level at X_1 is 3.5%, 20% less. Thus, 20% less arginine can be fed with a significant reduction in feed cost with only a 5% likelihood of reducing growth rate. Nutrient cost and fish price data can be factored into the growth response equation to determine the most profitable nutrient concentration to use.

Determining Bioavailability Through Growth Assays

Determination of nutrient bioavailability through digestibility trials, which measure the percentage of the nutrient absorbed from the digestive tract, was discussed in Chapter 3. Bioavailability is also determined from dose-response data obtained through feeding trials where the animal is exposed to graded concentrations of the test nutrient in a basal diet and the bioavailability of the test nutrient is based upon rate of response in comparison with that of a standard material. Growth assays are expensive and time consuming; however, they have two attractive features. First, they measure a response (growth) that has practical and economic value. Second, they demonstrate the net effect of all the components that can affect bioavailability (digestion, absorption and utilization). The significance of the variation in bioavailability among sources of nutrients in fish feeding is discussed in chapter 5.

The basal diet used in a growth assay should be adequate in all nutrients except the one under investigation, which should be deficient or in low concentration. Graded concentrations of the test nutrient, ranging from below to above the anticipated optimum dietary level, from the source to be evaluated are added to the basal diet. Similar concentrations of the test nutrient from a standard source are added to the basal diet. The standard should be a source traditionally used and/or one that is high in bioavailability. The two diet series are fed at satiation rate to young fish in aquaria for a period of time and weight gain, or other responses, are measured for each replicate group of fish. The response data from each set of diets are subjected to regression analysis where dietary nutrient concentration is the independent variable. The linear segments of the regression lines are used to compare bioavailability of nutrient from the test material with that from the standard material by deriving the ratio of the slopes of the lines, as described by Littell et al. (1995).

Paripatananont and Lovell (1994) determined relative bioavailability of zinc from zinc methionine compared to zinc from the traditional source, zinc sulfate, for young channel catfish. They fed two basal diets, an egg white-based purified diet and a soybean meal-based practical diet, each containing serial concentrations of zinc from each source, for 10 weeks. Weight gain data was analyzed by slope-ratio regression analysis. The response curves for the two zinc sources fed in the purified diets are shown in Figure 7.4. Relative bioavailability of zinc methionine was 352% of that of the standard zinc sulfate in the purified diet, and 482% in the practical diet. This indicates that the chelated zinc had approximately 3.5 times the potency of zinc sulfate in a purified diet and 4.8 times the potency of zinc sulfate in the practical diet (which contained phytic acid) for channel catfish.

PRACTICAL ENVIRONMENT STUDIES

Research to evaluate practical diets or feeding regimes should involve fish fed in an environment similar to commercial practice, but which is amenable to sound statistical evaluation.

Pond Experiments

Pond feeds should be evaluated in experimental ponds. The results of pond experiments represent the combined and inseparable effects of nutrients from the pond and the test feed. Some fish species consume relatively insignificant amounts of pond food organisms, while others consume significant amounts. Channel catfish

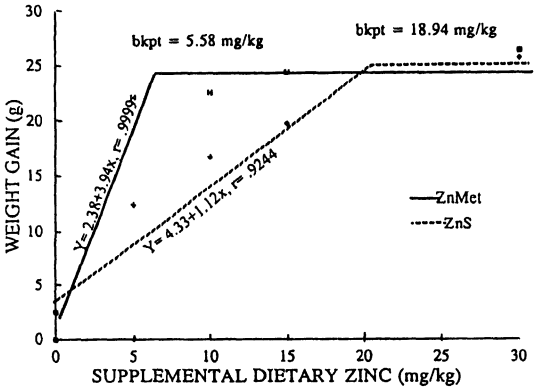


Figure 7.4 The relative bioavailability of a nutrient from a given source may be compared to that of a standard or conventional source from the ratio of the slopes of the linear part of the response curves. Here, the slope of the response curve for zinc methionine (ZnMet) is markedly greater than that for the conventional zinc sulfate (ZnS). The slope ratio, ZnMet/ZnS, is 3.52, indicating that ZnMet has over three times the bioavailability of ZnS for growth of channel catfish. *(From Paripatananont and Lovell, 1994.)*

will gain approximately 250 kg/hectare in ponds without feeding, while tilapia can gain 1,000 to 2,000 kg/hectare from natural pond food alone (Lovell et al. 1978). Other confounding effects of pond environments for feeding experiments are changes in temperature and water quality. Water quality usually deteriorates as the feeding period progresses and this may influence feeding activity of fish or may interact with experimental diets or feeding regimes. However, these environmental effects are characteristic of pond cultures and will be present in practice.

An experimental pond should represent a commercial pond. Concrete or plastic-lined ponds do not represent earthen culture ponds. Minimum size should be approximately 0.05 hectare. A source of water should be available to replace evaporation loss or to flush the pond in the event of oxygen depletion, and to rapidly refill ponds when restocking. Aeration should be available for each pond at all times. The ponds should be easily drained and harvested. See Figure 7.5, which shows a series of experimental earthen ponds.

Pond experiments are expensive. Because of great variation among individual ponds managed similarly, a number of replicates are needed per treatment to discern meaningful differences among treatments. Shell (1983) reported that the coefficient of variation (100 X standard deviation/mean) of experimental ponds treated alike was 10 to 15 for channel catfish ponds in Alabama and 8 to 16 for common carp ponds in Israel. Thus, small differences in treatment effects on fish production in ponds are not practical to measure without an excessively (and expensively) large number of ponds. With knowledge of the amount of experimental error associated with a set of experimental ponds, the number of replications required to demonstrate a desired percentage difference among treatments can be determined statistically. Ordinarily, a minimum of three replicate ponds per treatment are used, and because of costs, more than four or five ponds per treatment are seldom used. Thus, small differences should not be sought using earthen ponds.

Figure 7.5 Experimental earthen ponds are used to evaluate pond feeds and feeding strategies. These are 0.04-hectare research ponds at the Delta Western Research Facility, Indianola, Mississippi. (Courtesy of Delta Western Feed Company.)

Experimental ponds should be stocked and managed similarly to existing or potential commercial conditions while allowing for accurate collection of data. In most cases, the fish should be fed over a growing season. Because of changes in temperature and water quality, a short-term feeding trial over a limited segment of the normal growing period may not give results applicable to practical conditions.

Fish in pond experiments may be fed as much as they will consume or fed on a restricted basis. Feeding to satiation allows the full benefit of the superior diets to be realized. A problem with restricted feeding is that most fish species exhibit hierarchial feeding order and when a limited amount of feed is fed, the more aggressive feeders will consume as much feed as they want while some fish will get little. Most commercial fish farmers feed as much as the fish will consume to obtain maximum growth rate. Another advantage of feeding as much as the fish will consume is that if the numbers of fish in the experimental ponds become unequal, due to predation, mortality, stocking discrepancy, or other reasons, satiation feeding still insures all fish in the pond an opportunity to eat and respond maximally.

Feeding fish to satiation can be done conveniently if floating (extruded) feeds are used so that the feed allowance can be adjusted periodically, based on observed feeding activity of the fish. Floating feeds also allow the removal of unconsumed feed so that feed conversion can be measured more accurately. When sinking pellets are used, the fish should be sampled periodically to adjust the feed allowance according to feed allowance tables which have been developed for various species.

Sometimes, a restricted feeding strategy is desirable. In practice, fish farmers may set a limit on the maximum amount of feed they want to put in the pond daily, regardless of the amount of fish in the pond, in order to avoid water quality problems. However, feeding experiments where feed allowance is restricted are complicated. This method penalizes the faster gaining treatments; also, there may be interaction between diet and feeding rate on fish response. Li and Lovell (1992) found that daily feed allowance influenced the response of channel catfish to

various levels of protein in the feed; satiation-fed fish required less protein in the feed for maximum gain than did restricted-fed fish.

Weight gain is usually considered the most important measurement of the productivity of experimental feeds. It is assumed that the weight of the fish is a reliable indicator of "marketable product." Fish weight does represent marketable product to the producer, but not necessarily to the processor. If yield of marketable carcass and quality of the final food product are to be considered, dressing yield and proximate composition of the fish flesh should be measured.

Weight gain can be presented in several ways: harvest weight (standing crop), net gain, percentage gain, gain per day, specific growth rate, or by growth curves. Standing crop, which is the total weight of the fish in the pond, is meaningful to the commercial culturist in that this indicates the total weight of fish that can be harvested in terms of yield per hectare from the pond. If there are unequal numbers of fish in the ponds, fish response should be reported as average gain per fish rather than gain per pond. Percentage gain gives an indication of how much the fish has increased in size in relation to its initial weight and it is usually of more interest to a researcher than a farmer.

Average daily gain or specific growth is a commonly used measure of livestock response in feeding trials. It has limited application to fish feeding experiments because daily or specific gain is related to size of fish; large fish gain more per day than small ones. Thus, data must be collected under standardized conditions with regard to fish stocking size, length of feeding trial, harvest size, etc., and at present there are no standardized conditions for fish "feedlot" studies.

Other desirable measurements for pond feeding experiments are feed conversion ratio, fish size variation within a pond or treatment (the number or percentage of fish in various size groups), and possible clinical and subclinical nutrient deficiency signs. Feed conversion, or efficiency (reciprocal of conversion), cannot be accurately determined if the amount of feed consumed cannot be accurately ascertained. Thus, unless the researcher has a measure or reliable estimate of the net amount of feed consumed, he or she should not bother calculating feed conversion coefficients.

Raceways and Pens

Raceways, tanks, pens, and cages, which simulate commercial culture conditions, may be used as experimental growing units to evaluate practical feeds or feeding regimes. These units represent highly artificial culture conditions, and environmental interaction with the test feeds is usually minimal. Variation in fish response among rearing units within treatments will be less than in earthen ponds, thus smaller differences among test feeds should be detectable in these units than in ponds using the same number of replicates. The experimental diets must be nutritionally complete.

REFERENCES

LI, M. and R. T. LOVELL. 1992. Comparison of satiate feeding and restricted feeding of channel catfish with different percentages of dietary protein. Aquac. 103: 165-175.

LITTELL, R. C., A. J. LEWIS, and P. R. HENRY. 1995. Statistical evaluation of bioavailability assays. In Bioavailability of nutrients for animals, eds. C. B. Ammerman, D. H. Baker, and A. J. Lewis. New York: Academic Press.

LOVELL, R. T., E. W. SHELL, and R. O. SMITHERMAN. 1978. Progress and prospects in fish farming. In New protein foods, eds. A. M. Altschul and H. L. Wilke, pp. 261-292. New York: Academic Press.

NATIONAL RESEARCH COUNCIL. 1993. Nutrient requirements of fish. Washington, D.C.: National Academy of Sciences.

PARIPATANANONT, T. and R. T. LOVELL. 1995. Responses of channel catfish fed organic and inorganic sources of zinc to Edwardsiella ictaluri challenge. J. Aquatic Animal Health. 7: 147-154.

ROBBINS, K. R., H. W. NORTON, and D. H. BAKER. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109: 1710-1714.

SANTIAGO, C. R. 1985. Essential amino acid requirements of Nile tilapia. Ph.D. dissertation, Auburn University, Auburn, AL.

SHELL, E. W. 1963. Effects of changed diets on growth of channel catfish. Trans. Amer. Fish. Soc. 92: 432-434.

SHELL, E. W. 1983. Fish farming research. Auburn, AL: Alabama Agricultural Experiment Station.

8 FEED FORMULATION AND PROCESSING

Meng H. Li

Fish feed manufacturing involves the processing of mixtures of feedstuffs and feed additives into a usable form. There are several goals and considerations in feed manufacturing, some of which are nutritional and some of which are nonnutritional. The primary goal is to increase profits of fish production by maximizing the nutritional value of a feedstuff or a mixture of feedstuffs at minimum cost. Depending on the fish species and size, this process may range from a simple reduction of particle size to forming feed pellets through steam pelleting or extrusion. Fish feeds are unique compared to feeds used for terrestrial animals grown for food because fish feeds must be processed into water stable pellets, and for many species, must float on the water surface.

NUTRITIONAL CONSIDERATIONS

All animals including fish require protein, lipids, vitamins, minerals, and energy for normal growth and other physiological functions. Depending on the culture system, nutritionally complete feeds are necessary for fish raised in a system where natural foods are absent, such as trout raised in raceways, and where the contribution of natural food organisms is minimal, such as in intensive catfish farming. However, where natural foods are abundant, such as in extensive carp, tilapia, or shrimp culture, supplemental feeds that provide primarily protein and energy can be used.

Nutrient requirements for several fish species, particularly small fish, have been discussed in Chapter 2. In formulating and manufacturing fish feeds, it is essential that the finished feed meets these requirements and be manufactured in a form that is readily consumed, using feedstuffs that are highly digestible. In feed processing, the conditions of high temperature, pressure, and moisture encountered

during pelleting and extrusion destroy certain nutrients and improve the availability of others. Some vitamins are sensitive to destruction, thus, fish feeds are normally overfortified with most vitamins to account for losses during feed manufacture and storage. Energy digestibility of starch (carbohydrate) appears to be enhanced by the extrusion process.

NONNUTRITIONAL CONSIDERATIONS

Although nutritional considerations are important, nonnutritional factors often influence the composition of the final product. The logistics of procuring and storing feedstuffs and feed additives are primary nonnutritional considerations. In general, feed ingredients must be available on a consistent basis, be easily handled in the manufacturing process, be able to withstand the rigors of the manufacturing process, and be economical. These characteristics are the main reasons that soybean meal, corn grain, and grain milling byproducts have been the primary feedstuffs used for aquaculture feeds in the United States. Other ingredients such as peanut meal and cottonseed meal are often priced economically and could be used in fish feeds, but their use is limited because they are not available on a consistent basis. Even if a large number of feedstuffs were available for use in fish feeds, lack of ingredient storage bins would limit their use. Most fish feed mills, even high volume mills, have storage bins for only six to seven feedstuffs.

When formulating fish feeds, the feed manufacturing process must be considered because there is an interrelationship between feed formulation and feed manufacturing. For example, extrusion generally requires that at least 25% of the feed be composed of grains or grain milling by-products for proper starch gelatinization for the expansion necessary for the pellet to float. This is generally not a problem, but the type and amount of grain or grain milling by-products that are used may be affected by humidity in the air. Levels of wheat middlings up to 25-30% can generally be used in catfish feeds except at high air humidity. In humid conditions, wheat middlings are reduced to 10 to 15%, and the amount of corn grain increased to avoid making the feed too sticky and difficult to handle. High fat feedstuffs, such as rice bran, are generally limited to 5 to 10% of the feed because high levels of fat make the feed more difficult to pellet or extrude. Supplemental fat is usually sprayed on the finished feeds. Highly fibrous feedstuffs must be limited to rather low levels because high levels of fiber negatively impact pellet quality. Feed processing involves high temperatures and pressures, and a high level of moisture which may inactivate antinutritional substances in feedstuffs, such as trypinase, and reduces the occurrence of molds and bacteria in the feed.

PRACTICAL FEED INGREDIENTS

No single feedstuff can supply all of the nutrients and energy required for optimum growth of fish. Thus, commercial fish feeds are comprised of a mixture of feedstuffs and vitamin and mineral premixes that provide adequate amounts of essential nutrients as well as the energy necessary for their utilization. Ingredients used in practical fish feeds can be classified as protein sources, energy sources, vitamin supplements, mineral supplements, and specific feed additives. Nutrient composition of various feed ingredients is presented in Appendix A.

Protein Sources

Feed ingredients containing 20% crude protein or more are considered protein sources. Protein sources may be classified as animal and plant proteins. Animal proteins, from animal byproducts such as fish meal, are generally considered to be of higher quality than plant proteins, primarily because of their superior complement of essential amino acids. Animal protein, especially fish meal, has been considered to be essential in fish feeds. However, recent research has shown that animal protein is not essential for normal growth of channel catfish under typical commercial culture conditions provided the amino acid requirements are met (Robinson and Li 1994).

The primary plant protein sources used in fish feeds are oilseed meals, such as soybean meal, cottonseed meal, and peanut meal. Compared to animal proteins, many plant proteins are deficient in lysine and methionine, the two most limiting amino acids in fish feeds. Also, certain plant proteins contain toxins and antinutritional factors that may or may not be inactivated during processing of the meal. A brief description of various animal and plant protein sources that can be used in fish feeds is given below.

Fish meal. Fish meal is prepared from dried, ground tissues of undecomposed whole marine fish or fish cuttings. Whole fish meal contains 60 to 80% protein of excellent quality that is highly palatable to fish. Since fish meal is a good source of essential amino acids, it is often used to supplement feeds containing plant proteins. Fish meal is also rich in energy, minerals, and essential fatty acids. Fish meal made from waste from fish processing and canning plants has a lower quality and quantity of protein and higher ash content. Fish meal is used sparingly in many commercial fish feeds because of its high cost. However, feeds for eels, pen-raised salmon and trout contain high levels of fish meal. Research with catfish has shown that other animal proteins can replace fish meal without detrimental effect.

Fish solubles, condensed. Condensed fish solubles are a semisolid byproduct obtained by evaporating the liquid remaining from the steam rendering of fish, primarily sardines, menhaden, and redfish. It contains approximately 50% dry matter, 30% protein, and 4.0% fat.

Meat and bone meal. Meat and bone meal is the rendered product from beef or pork tissues and should not contain blood, hair, hoof, horn, hide trimmings, manure, stomach and rumen contents except in amounts as may be unavoidable during processing. Meat and bone meal contains approximately 50% crude protein. Its protein quality is inferior to whole fish and the consistency of the product may vary considerably. It is a good source of minerals. Its high ash content may limit its use because of possible mineral imbalance.

Blood meal. Blood meal is prepared from clean, fresh animal blood, a by-product from meat packing plants. It contains 80 to 86% crude protein and is an excellent source of lysine. It is deficient in methionine.

Meat and bone/blood meal blend. Special products are available for use in fish feeds that are mixtures of meat and bone meal and blood meal. Because blood meal is high in protein and lysine, the two feedstuffs are blended to mimic the nutritional profile of fish meal and to provide 60-65% protein.

Poultry byproduct meal. Poultry byproduct meal is made up of ground, rendered clean parts of the carcass of slaughtered poultry. It contains heads, feet, underdeveloped eggs, and visceral organs but does not contain feathers. The product contains approximately 59% good quality protein.

Poultry feathers, hydrolyzed. Hydrolyzed poultry feathers prepared by the high-pressure treatment of clean, undecomposed feathers from slaughtered poultry. At least 75% of the protein should be digestible as measured by pepsin digestion. It is high in protein (85%), but protein quality is poor because of deficiencies in several essential amino acids, especially lysine.

Shrimp waste meal. Shrimp waste meal including the head is a useful feedstuff. The exoskeleton is primary chitin and has limited nutritional value. Shrimp waste meal contains approximately 40% crude protein; however, chitin accounts for 10 to 15% of the total nitrogen in the meal which lowers the true protein content. Shrimp waste meal is a good source of n-3 fatty acids, cholesterol (essential for crustaceans), and astaxanthin (for red pigment in salmonids). It is highly palatable and may serve as an attractant in feeds for fishes and crustaceans.

Soybean meal. Soybean meal is prepared by grinding the flakes after removal of the oil from soybeans by solvent extraction or the expeller process. There are three types of soybean meal that can be used in fish feeds: dehulled and solvent extracted, solvent extracted, and expeller extracted. These contain 48, 44, and 42% protein, respectively. Soybean meal is the major protein source used in aquaculture feeds. It has the best amino acid profile of all common plant protein sources. Based on NRC (1993) requirements, soybean protein is sufficient in all essential amino acids for many fish species. It is highly palatable to most warmwater fish, but less palatable for salmonids. Antinutritional factors in soybeans, mainly trypsin inhibitor, are destroyed or reduced to insignificant levels by heating during oil extraction and during extrusion of fish feed.

Heated, full-fat soybean meal. Full-fat soybean meal is prepared by grinding heated full-fat soybeans. The meal contains approximately 39% protein and 18% fat. It is rarely used in warmwater fish feeds because of its high fat content, although a limited amount can be used as long as the total fat level in the finished feed does not exceed about 6%. More can be used in salmonid feeds. Research by U.S. Fish and Wildlife Service showed that heating full-fat soybean meal to > 177°C improved the nutritional value for trout as compared to that of solvent-extract soybean meal.

Cottonseed meal. Cottonseed meal is obtained by grinding the cake remaining after the oil has been extracted hydraulically, by screw press extraction, prepress solvent extraction, direct solvent extraction, or expander solvent extraction. The products generally contain 41% protein but are severely deficient in lysine. They are highly palatable to catfish, salmonids, and most other fish. Cottonseed meal contains free gossypol and cylcopropenoic acids which can be toxic to monogastric animals. The amount of free gossypol in cottonseed meal depends upon processing method (Robinson and Li 1995). Free gossypol contents of five types of cottonseed meal are as follows: screw press, 0.02% to 0.05%; hydraulic, 0.04% to 0.10%;

prepress solvent, 0.02% to 0.07%; expander solvent, 0.06% to 0.21%; direct solvent, 0.10% to 0.50%. Currently, the expander solvent method is the method of choice for processing cottonseed into meal. Levels of cottonseed meal in fish feeds should not exceed 30%. Cottonseed meal is generally used in catfish feeds at a level of 10 to 15%.

Peanut meal. Peanut meal is obtained by grinding shelled peanuts with the oil removed either mechanically or by solvent extraction. Solvent extracted peanut meal contains about 48% protein and the mechanically extracted product contains near 45% protein. Peanut meal is highly palatable to fish and contains no known antinutritional factors. It is seriously deficient in lysine. Levels used in fish feeds are usually restricted to 15 to 20% without lysine supplementation.

Distillers' dried grains with solubles. Distillers' dried grains with solubles are the primary fermentation residues, after removal of the alcohol by distillation, from the yeast fermentation of cereal grains. The product contains approximately 27% protein and is highly palatable to fish; however, it is relatively low in lysine.

Sunflower meal. Sunflower meal is prepared by grinding the residue remaining after mechanical or solvent extraction of the oil from sunflower seeds. Dehulled sunflower meal is prepared from sunflower seed after the hull is removed. Solvent extracted sunflower meal contains about 44% protein. The hulls are not easily removed so even the dehulled meal contains around 13% fiber. Higher levels of fiber are found in meals which are not dehulled. Sunflower meal can be used in fish feeds to replace part of the soybean meal. Its low lysine content and high level of fiber limit its usefulness in feeds. A level of up to about 20% without lysine supplementation is acceptable for catfish feeds.

Canola meal. Canola meal is prepared from a special rapeseed by solvent extraction to remove the oil. Compared to typical rapeseed meal, canola meal is low in glucosinolates and erucic acid which may be detrimental to fish growth. Canola meal contains about 38% protein and is relatively low in lysine compared to soybean meal, but is higher than other oilseed meals. It is palatable to fish and can be used at levels up to about 20 to 25% in fish feeds without detrimental effects.

Energy Supplements

Energy supplements are feedstuffs that contain less than 20% crude protein. These include grain and grain by-products, and fat or oil of animal or plant origin. Energy sources typically used in commercial fish feeds include corn, wheat and rice products, animal fat, and fish oil.

Corn products. Corn grain and corn screenings are used interchangeably in commercial catfish feeds as a relatively inexpensive source of energy. Whole corn is ground prior to use. Corn screenings are obtained in the cleaning of corn and include light and broken grains. Cooking improves energy digestibility of corn for fish. Corn contains a yellow pigment, xanthophyll, which at high levels has been shown to accumulate in catfish giving the flesh a yellowish coloration that is undesirable to consumers. Corn products have been used in warmwater fish feeds up to 45% of the feed without adverse effects.

Wheat products. Wheat grain is a good source of energy for warmwater fish and is a good pellet binder, but is generally more expensive than corn. As a result, wheat grain is usually used sparingly (2-5%) in fish feeds, and is used primarily for its pellet binding properties. Wheat middlings are fine particles of wheat bran, shorts, germ, and flour recovered from milling wheat grain. Depending on cost, wheat middlings can replace corn or corn screenings as an energy source in fish feed. If used primarily as a binder, a level of 2-5% is generally used.

Rice products. Rice bran is the bran layer and germ of rice grain with hulls or broken rice at levels only that are unavoidable in milling rice grain. It is high in fat and fiber which limits its use in fish feeds to less than 7% of catfish feeds. Defatted rice bran is used in higher quantities.

Sorghum. Nutrient composition of sorghum grain is similar to corn, but slightly higher in protein (11%). When milo is substituted for corn, the feeds are darker and more dense. Some varieties of milo have a high tannin concentration in the seed coat and cause reduction in palatability. Sorghums do not provide binding and expansion properties during extrusion comparable with corn or wheat products.

Animal and plant fats and oils. Animal and plant fats and oils are highly concentrated sources of energy as well as a source of essential fatty acids. Animal fats used in fish feeds include beef tallow, poultry fat, and fish oils. Plant oils can be used, but animal fats and oils are generally preferred because they are generally less expensive. Lipids are the primary nonprotein energy sources in salmonid feeds because of the limited ability of these species to utilize carbohydrate. Relatively large amounts of marine fish oils are used in feeds for trout, salmon, and cultured marine fishes, not only because they are a good source of energy, but they provide essential (n-3) highly unsaturated fatty acids. There is evidence that levels of menhaden oil, as the primary lipid source, of 2% or higher may reduce disease resistance in catfish (Fracalossi and Lovell 1994; Li et al. 1994). Therefore a blend of approximately equal parts of catfish oil, which is low in n-3 fatty acids, and menhaden oil are often used in fish feeds. Supplemental fat is generally sprayed on the surface of finished feed pellets.

Vitamins and Minerals

Most commercial feedstuffs used in fish feeds contain vitamins and minerals, but the amount and bioavailability, especially for vitamins, are usually unknown. Thus commercial fish feeds are supplemented with vitamin and trace mineral premixes as well as phosphorus that provide vitamins and minerals in quantities necessary to meet dietary requirements including losses of vitamins due to feed processing. Table 8.1 shows rates of retention of vitamins during extrusion processing of catfish feeds.

Binding Agents

Steam-pelleted fish feeds require the addition of binding agents to improve water stability of the finished pellet, whereas extruded fish feeds depend on starches inherent in feedstuffs for pellet binding. Binding agents are especially valuable in

Table 8.1. RETENTION OF VITAMINS IN EXTRUSION PROCESSED CATFISH FEED

Vitamin	Retention (%)
Vitamin C (fat-coated)	57[a]
Vitamin C (ethylcellulose-coated)	48[a], 43[b]
Vitamin C (L-ascorbyl-2-polyphosphate)	77[b], 96[c]
Vitamin A (vitamin A acetate)	65[b]
Vitamin E (DL-alpha-tocopherol acetate)	100[b]
Vitamin B1 (thiamin mononitrate)	64[b], 67[d]
Vitamin B2 (riboflavin)	100[d]
Vitamin B6 (pyrodoxine hydrochloride)	67[b], 70[d]
Folic acid	91[b]
Niacin	96[d]
Pantothenic acid	100[d]

[a]From Robinson (1992).
[b]From Producer's Feed Company, Belzoni, MS; assayed by Hoffman-LaRoche, Inc., Nutley, NJ.
[c]From Robinson et al. (1989).
[d]From Li et al. (1996).

Table 8.2. BINDING AGENTS USED IN STEAM PELLETED FEEDS FOR FINFISH AND CRUSTACEANS. REMARKS FOR EFFECTIVENESS ARE FOR SHRIMP FEEDS

Binder	Amount to use (%)	Nutritive Value	Relative Cost	Relative Effectiveness
Guar gum	1-3	No	Expensive	Good
Lignon sulfonate	2-4	No	Inexpensive	Poor
Carboxymethyl-cellulose	2-6	No	Expensive	Fair
Hemicellulose	2-3	No	Moderate	Poor
Bentonite	2-3	No	Inexpensive	Poor
Alginate	2-3	No	Expensive	Good
Agar	2-3	No	Expensive	Good
Carrageenan	0.5-1	No	Expensive	Good
Ethylene/vinyl acetate copolymer	2-4	No	Expensive	Good
Wheat gluten	3-5	Yes	Moderate	Good
High gluten wheat flour	20	Yes	Moderate	Good
Collagen	0.5-3	Yes	Moderate	Good
Processed milo	3-5	Yes	Moderate	Fair
Cooked starch	> 20	Yes	Inexpensive	Fair

steam-pelleted crustacean feeds, which must remain stable in water for several hours. Binding agents commonly used in commercial and laboratory pelleted feeds for finfish and crustaceans are presented in Table 8.2. The stability of steam-pelleted feeds in water is dependent on the type and amount of binder used.

Carboxymethylcellulose and guar gum have been used successfully in laboratory finfish diets but are too expensive for commercial feeds. Hemicellulose and bentonite are relatively inexpensive and are usually suitable for finfish feeds that are consumed within 20 to 30 minutes after feeding, but may not suitable for crustacean feeds. Cooked starches are good binding materials and are also nutritious. However, they are highly hygroscopic and will absorb water which causes the pellet to swell and disintegrate after a time in the water. Wheat gluten, agar, alginate, carrageenan, and ethylene/vinyl acetate copolymer (synthetic) are good binders for crustacean, but they are too expensive to use in commercial feeds. Lignin sulfonate, processed milo, and collagen are fair to good binding agents and are relatively inexpensive. High-gluten wheat flour is the most commonly used binder for shrimp feeds because it is cost effective.

FEED FORMULATION

Fish feeds have generally been based on a fixed formula with little use of a least-cost approach as is used in other animal industries. In the past, fixed formulas were used because of the lack of sufficient nutritional information. Presently nutritional data are available to allow nutritionists to formulate fish feeds on a least-cost basis. To use a least-cost computer program to formulate feeds, the following information is needed: 1) cost of feed ingredients; 2) nutrient concentrations in feedstuffs; 3) Table

Figure 8.1 Catfish feed mill located in Mississippi Delta. It can produce up to 1500 metric tons of extruded (floating) feed daily. The feed is hauled in bulk directly to catfish farms nearby. Most catfish feed mills are owned by catfish farmers.

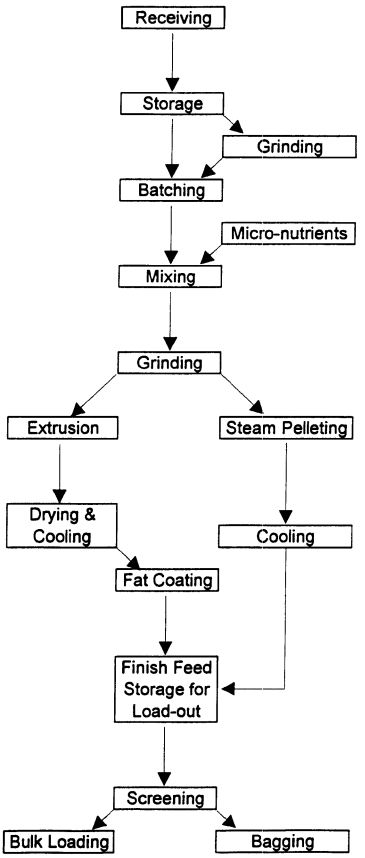


Figure 8.2 Typical flow scheme for manufacturing fish feeds.

nutrient requirements; 4) nutrient availability from feedstuffs; and 5) nutritional and nonnutritional restrictions.

There are several constraints that inhibit the widespread use of least-cost formulation of fish feeds, in addition to the limited number of suitable feedstuffs available. These include a lack of knowledge of the nutrient levels that result in maximum profit as opposed to levels that maximize weight gain, a lack of capacity to store large number of different ingredients at the feed mills, and the logistics of obtaining a wide assortment of feedstuffs on a timely basis. Examples of restrictions placed on nutrients and feed ingredients for least-cost formulation of catfish feeds are presented in Table 8.3. These restrictions are based either on nutritional requirements or on constraints of characteristics of individual feedstuffs, or on constraints due to the manufacturing process.

Table 8.3. RESTRICTIONS FOR LEAST-COST FORMULATION OF A 28% PROTEIN PRODUCTION FEED FOR CHANNEL CATFISH. UNITS ARE EXPRESSED AS A PERCENTAGE OF THE FEED[a]

Item	Restriction	Amount	Unit
Crude protein	Min.	28.0	%
Crude fiber	Max.	7.0	%
Lipid	Max.	6.0	%
Available phosphorus	Min.	0.3	%
Available phosphorus	Max.	0.5	%
Digestible energy	Min.	2.8	kcal/g
Digestible energy	Max.	3.0	kcal/g
Available lysine	Min.	1.43	%
Available methionine	Min.	0.26	%
Available methionine + cystine	Min.	0.65	%
Grain or grain by-products	Min.	25.0	%
Cottonseed meal[b]	Max.	15.0	%
Whole fish meal	Min.	2.0	%
Non-fish animal protein	Min.	2.0	%
Xanthophyll	Max.	11.0	mg/kg
Vitamin premix[c]	Include		
Trace mineral premix[c]	Include		

[a]Adapted from Robinson and Li (1996).
[b]Higher levels may be used if supplemental lysine is used.
[c]Meet dietary allowances for catfish given in Chapter 2.

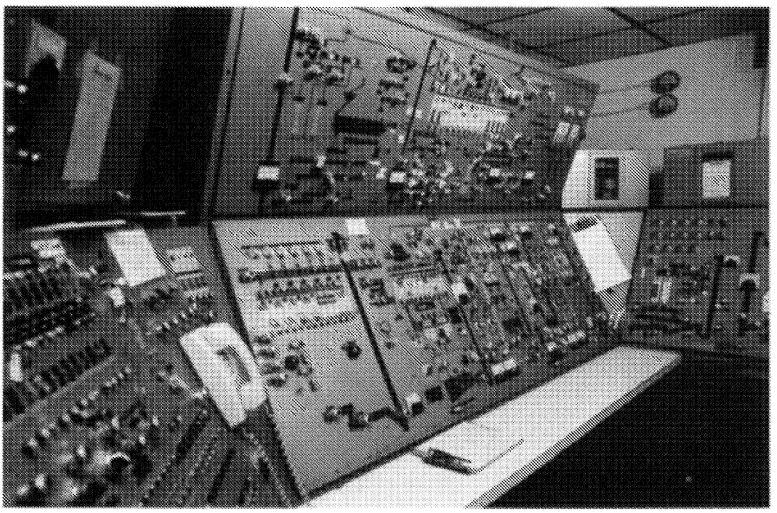


Figure 8.3 Control center for fish feed mill.

MANUFACTURING PROCESSES

Fish feeds are manufactured in modern feed mills (Figure 8.1). Regardless of whether a feed is floating or sinking, the general scheme of feed manufacture is similar (Figure 8.2). Whole grains are ground through a hammer mill prior to batching. The feed ingredients are batched, weighed, mixed, and then reground. After regrinding, mixed feeds are either extruded or steam pelleted and then cooled or dried, screened, and fat coated. Fat is generally sprayed on to the finished feed pellet just prior to being loaded into trucks for bulk delivery or prior to bagging. Operation of the various phases of feed manufacture are controlled by operators from a control center (Figure 8.3). Typical processes of catfish feed manufacture are presented in the following.

Receiving and Storage

Feedstuffs and other ingredients are either received at the mill by rail or by truck. Rail is generally more economical. Feedstuffs are unloaded from the railcars or trucks and transferred to storage houses or bins. As feedstuffs are needed they are moved by conveyers or screws to the appropriate section of the feed mill for processing.

Grinding, Batching, and Mixing

Whole grains are ground though a number 7 screen (2.8 mm) in a hammer mill prior to batching and mixing. During batching, feedstuffs are moved into a hopper above the mixer and weighed prior to mixing. After batching, the batch is dropped into a mixer and mixed for 2 to 3 minutes. After mixing, the feed mix is re-ground through a smaller screen, a number 4 or 6 (1.6-2.4 mm) depending on the type of feed being manufactured. After regrinding, the feed mixture is moved into hoppers above the extruders or the pellet mill.

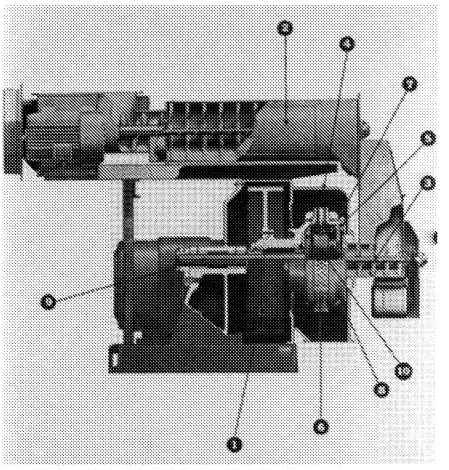


Figure 8.4 Pellet mill: (1) Transmission; (2) feed conditioner (mixes steam); (3) feed distributor (to the pellet chamber); (4) pelleting chamber; (5) adjustable feed plow; (6) pellet die ring; (7) die holding bolt; (8) die stiffener ring; (9) shaft; and (10) roll assembly (presses feed through holes in the die ring). (Courtesy of Sprout Waldron Co.)

Steam Pelleting

Steam-pelleted (sinking) feeds are manufactured by using moisture, heat, and pressure to form ground feed ingredients into larger homogenous feed particles. Steam is added to the ground feed ingredients to increase the moisture level to 15 to 18% and temperature to 75 to 80°C. Steam helps to gelatinize starches, which bind the feed particles together. The hot mash is then forced through a pellet die in a pellet mill (Figure 8.4). Die size is dependent on the size of pellet desired. The pellets exit the die at about 10% moisture; thus, require little drying but must be cooled. Steam-pelleted feeds are generally less expensive to manufacture than extruded feeds because less energy is expended in their manufacture. Also, less destruction of nutrients occurs during steam pelleting as compared to extrusion.

Extrusion

Extrusion cooking is a process which involves the plasticizing and cooking of feed ingredients in the extruder barrel by a combination of pressure, heat, and friction. Fish feed ingredients are a mixture of starchy and proteinaceous materials that are moistened to form a mash. The mash may be preconditioned in a conditioning chamber for 3 to 5 minutes during which moisture is added in the form of steam (water can also be injected) to increase the moisture level of the mash to about 25%. During this period, the mash is cooked as moisture penetrates the feed particles. Preconditioning may improve flavor development and feed digestibility, reduce extruder barrel wear, and allow for increased volume through the extruder. After preconditioning, the mash enters the extruder, which is a jacketed barrel that contains a rotating screw. Temperatures in the extruder generally range from 90 to 150°C and are generated from friction in the extruder (Figure 8.5). The superheated mixture is then forced through a die (about 3 to 6 mm in diameter) located at the end of the extruder barrel. The die restricts product flow thus causing development of the necessary pressure and shear. The die is also used to shape the product

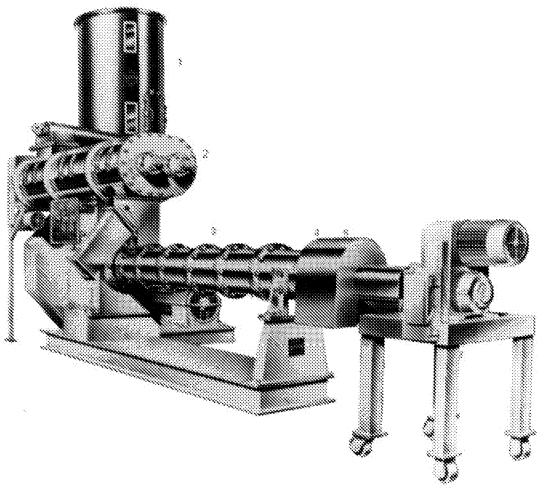


Figure 8.5 Cooker-extruder for processing expanded (floating) fish feeds: (1) Bin-discharger; (2) preconditioner where steam and/or water are added; (3) extruder barrel where temperature is quickly elevated through friction; (4) die for shaping feed particles; and (5) knife for cutting off extruded feed particles. (Courtesy of Wenger Manufacturing Inc.)

(extrudate) passing through it. As the product passes through the die, a sudden reduction in pressure results in the vaporization of part of the water in the mixture and the feed pellets expand. The moisture level of the pellets leaving the extruder is higher (18-21%) than that of steam-pelleted feed; thus, extruded pellets must be dried at high temperatures for longer periods of time.

Drying and Cooling

Some moisture is lost by flash evaporation as the feed pellet exits the die and by evaporative cooling after the feed pellets are exposed to air. Steam-pelleted feeds exit the die at a moisture level of about 10% and do not require heat for drying. Extruded feeds also lose moisture by flash evaporation and evaporative cooling (about 2%), but require additional drying since they contain 20% or more moisture. Extruded fish feeds should be dried to a moisture content of 8 to 10%. Drying is generally accomplished using a multi-stage dryer which has different temperature zones. For extruded catfish feeds, drying time is around 30 minutes. Dryer temperature ranges from 135 to 150°C.

Storage, Fat Coating, Screening, and Delivery

After drying, extruded catfish feeds are normally stored in bins awaiting loadout. Prior to bulk loadout or bagging, feed pellets are screened to remove fines and passed through a fat coater which applies a thin layer of fat to the pellet surface which helps reduce feed dust (fines). Fines are reclaimed and used as a feed ingredient. Almost all commercial catfish feeds are delivered to the farm in bulk by truck. A small quantity of catfish feed is bagged.

QUALITY ASSURANCE

Consistently manufacturing feeds that are cost effective, nutritionally balanced, free of contaminants and have proper physical properties requires stringent quality control methods. Feed mills should have a continuous and comprehensive quality-assurance program in place whereby various quality control measures are appropriately delegated and carried out. This must be the responsibility of all persons involved, from top management down, and encompass all aspects of feed production from ingredient purchase to delivery of the finished feed.

Ingredients

The purchasing agent ensures that high-quality ingredients are available on a timely basis at a reasonable cost by having an understanding of feed ingredients and by knowing which suppliers can consistently provide ingredients as needed. Working with the nutritionist and the production manager, the purchasing agent establishes and uses ingredient specifications to ensure that ingredients meet the standards desired. Ingredients are visually inspected for color, odor, and texture prior to acceptance. Each batch of feed should be screened for appropriate mycotoxins and moisture. Samples are taken for proximate chemical analysis and to determine presence of toxins, pesticides, or heavy metals. Periodically, especially if the type or source of ingredients changes, an amino acid analysis should be made. Since chemical tests lag behind ingredient use, a particular ingredient will be used prior to receiving the analytical results. However, if specifications are not met, a deficiency

claim is filed with the supplier. Ingredient inventories should be maintained which provide information on the amount of an ingredient used over a certain time period. This can be used to check and correct errors in the manufacturing process.

Feed Formulation

Fish feed formulations are based on nutrient requirements established by research. Nutrient requirement data are updated frequently to ensure current data are available for formulating least cost feeds. Nutrient profiles of feedstuffs are continually updated based on actual assays conducted over a number of years on feedstuffs used and on information supplied by various suppliers of feedstuffs. Feeds are generally formulated to meet nutrient requirements on a least cost basis. A safety margin is used to account for variations in the nutrient content of feed ingredients. Complete vitamin and mineral premixes are usually added to fish feeds. This is done primarily because of lack of knowledge on availability of nutrients in the major feed ingredients. As more information becomes available on nutrient availability in feed ingredients for fish, some of the vitamins and minerals in the premixes can probably be eliminated.

Manufacturing

Quality control measures continue during each phase of production to ensure that a feed containing the proper nutrient content with desirable physical characteristics is produced consistently. Ingredients should be properly ground, batched, mixed, re-ground, extruded or pelleted, dried, and fat coated prior to shipping. Equipment is continually checked and maintained at proper specifications. Since a uniform mix is essential, mixing is checked periodically by assaying for particular vitamins or other micronutrients.

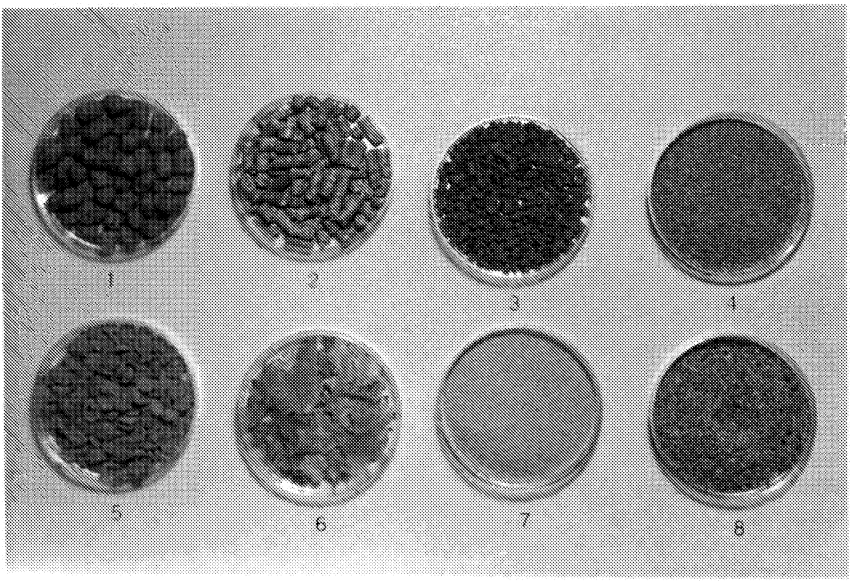


Figure 8.6 Various types of aquaculture feed: (1) extruded (floating); (2) steam pelleted (sinking); (3) large crumbles; (4) small crumbles; (5) extruded nonfloating (for shrimp); (6) flaked (drum dried); (7) fine meal (<0.5 mm); (8) coarse meal (0.5-1.0 mm).

Finished Feed

The finished product should be routinely assayed for moisture, protein, fat, and fiber, and periodically for selected micronutrients to ensure nutritional quality. Each batch of feed should be checked for physical characteristics, such as pellet stability and floatability. Most states require certain guaranteed analyses for commercial feeds. These include minimum crude protein, minimum crude fat, and maximum crude fiber.

FEED TYPES

Specialized feeds are discussed in the following section. These include larval feeds, meal and crumbles, microencapsulated feeds, flake feeds, moist feeds, crustacean feeds, and medicated feeds. Various forms of fish feeds are shown in Figure 8.6.

Larval Feeds

Many newly hatched larval fishes depend on live foods such as unicellular algae, rotifers, and brine shrimp nauplii for good survival. Complete replacement of live foods with prepared feeds has been successful for some species, such as channel catfish and rainbow trout, but not for others, such as various marine species. Because cultured live foods are expensive, prepared larval feeds have been developed to partially replace the live foods. Prepared feeds used to feed larval fish in the hatchery should be nutritional complete, palatable, water stable, and should be less than 0.5 mm in particle size. Methods to process larval feeds include finely grinding a steam-pelleted or extruded feed or grinding feed ingredients and mixing the ground ingredients into meal type feeds; flaking and regrinding to small particles; and microencapsulation.

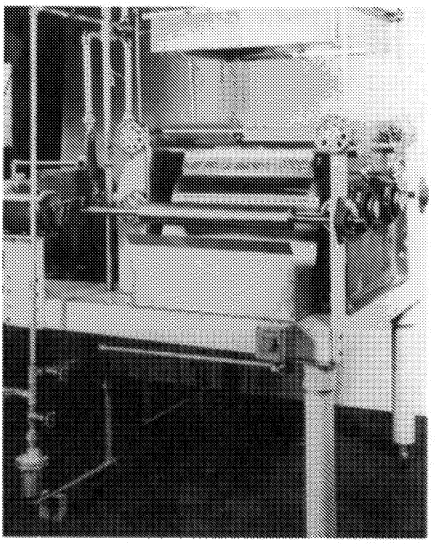


Figure 8.7 Drum dryer used to make flake feeds.

Meals and Crumbles

Feeds of a small particle size (meals or crumbles) are needed for feeding fish fry and small fingerlings. Meal type feeds are usually prepared by either reducing the particle size of a steam-pelleted or extruded feed by grinding and screening to the appropriate size or by finely grinding feed ingredients to a particle size of less than 0.5 mm and mixing the ground ingredients. Crumbles are prepared by crumbling pelleted feeds and screening for proper size. If meal-type feeds are made from pelleted or extruded feeds which are re-ground to the proper particle size instead of simply grinding and mixing, water soluble nutrients are less likely to be lost to the water. Since water soluble vitamins are easily to be lost to the water, fry feeds should be overfortified with those vitamins. Adding fat to meal or small particle feeds improves water stability and floatability, and reduces nutrient loss to the water.

Microencapsulated Feeds

Microencapsulation involves coating a small particle or beadlet of feed with a thin layer of a compound that will reduce dissolving and leaching of nutrients. There are several published and patented processes for microencapsulation of larval feeds, which vary with encapsulation materials, feed materials being coated, and manufacture process. Nylon (N-N bonds) cross-linked proteins, calcium alginate, and lipids have been used as encapsulation materials. The materials should be nontoxic, water insoluble, and digestible by the larval fish.

Flaked Feeds

Feeds for aquarium fishes should be nutritionally complete, palatable, should float or sink slowly, and should not disintegrate quickly in the water. The ingredients are ground to extremely fine particle size with an attrition mill and blended with water to form a slurry that is spread over the surface of a heated rotating cylinder (drum) to dry into a thin sheet. A drum dryer is shown in Figure 8.7. The dried sheet is continuously scraped off the rotating drum and crumbled into flakes. Some larval feeds are made from regrinding the flakes into small particles. Flaked feeds should contain ingredients with good hydrocolloidal properties as well as tensile strength. Chitin from shrimp shells is important for the desired physical properties of the flakes (Boonyaratpalin and Lovell 1977). Astaxanthin (in crustacean meal) and canthaxanthin (synthetic) are often added to aquarium feeds for enhancing pink-red color of fish. Xanthophylls from plant pigments are added for enhancing yellow-orange pigmentation of certain species of ornamental fish.

Moist Feeds

Some fish species, such as salmon smolts and eel, prefer a moist, soft diet to a dry diet. Moist feeds are prepared by adding moisture and a hydrocolloidal binding agent (e.g. carboxymethylcellulose, gelatinized starch, gelatin), or fresh tissues (e.g. liver, blood, ground fish, or fish processing waste) to dry ingredients and processing to form moist feed strands by using a food grinder. No pelleting machine or drying equipment is needed; however, moist diets are susceptible to microorganism or oxidation spoilage unless fed immediately or frozen. Fresh fish tissues should be heated to destroy possible pathogens and thiaminase (an enzyme that destroys thiamin).

Some moist diets contain humectants like propylene glycol and sodium chloride which lower water activity to prohibit bacterial growth. Fungistats like propionic acid or sorbic acid may be added to retard mold growth. These diets do not require frozen storage, but must be packaged in hermetically sealed containers and preferably stored at low temperatures to reduce the opportunity for mold growth. They also need to be overfortified with vitamins because moisture enhances oxidative loss of vitamins. Stabilized vitamin C should be used.

Eel feeds are processed and stored dry but moistened just prior to feeding. They generally contain 10 to 20% pregelatinized starch, which serves as a binding agent. About 5 to 6% fish oil and 50 to 100% water are added to the dry mix. The moist mixture can be fed in large balls or extruded through a food grinder into smaller particles.

Crustacean Feeds

Crustaceans are bottom feeders which feed slowly. They require diets that will remain stable in water for a longer time than those for most finfish. Feedstuffs with good binding properties along with special binding agents should be used in steam pelleted feeds. Extrusion processing improves water stability of crustacean feeds and may eliminate the need for binding agents. However, the expansion must be controlled, so the feed will not float. At present, the majority of crustacean feeds are produced by steam pelleting because extrusion increases feed cost. Thus, binding agents are important for water stability of the feeds. However, adequate binding of crustacean feeds depends not only on binding agents but also on the processing conditions and techniques. Steam pelleting machines that process crustacean feeds usually have a conditioning attachment that allows extra heating of the moistened feed to enhance gelatinization of starches. Feedstuff particle size, extrusion temperature, conditioning time, die size, and thickness of die can greatly affect water stability of the produced feed.

Medicated feeds

Presently, only two antibiotics, Terramycin™ (oxytetracycline, Pfizer, New York, New York) and Romet™ (sulfadimethoxine-ormetoprim, Hoffmann LaRoche Inc., Nutley, New Jersey) are approved by the FDA for use to treat fish bacterial infections. Medicated feeds containing these two antibiotics are available commercially. Because Terramycin is heat sensitive and is destroyed during extrusion, it is used in steam pelleted feeds. Romet is heat stable, so it can be used in extruded feeds. To improve the palatability of Romet feeds to channel catfish, the level of fish meal should be increased to about 16% (Robinson et al. 1990). Feed mills must be licensed to add these antibiotics to their feeds, and the quantities added are regulated.

REFERENCES

BOONYARATPALIN, M. and R. T. LOVELL. 1977. Diet preparation for aquarium fishes. Aquaculture 12:53-62.

FRACALOSSI, D. M. and R. T. LOVELL. 1994. Dietary lipid sources influence responses of channel catfish (Ictalurus punctatus) to challenge with pathogen Edwardsiella ictaluri. Aquaculture 119:287-292.

LI, M. H., D. J. WISE, M. R. JOHNSON, and E. H. ROBINSON. 1994. Dietary menhaden oil reduced resistance of channel catfish (Ictalurus punctatus) to Edwardsiella ictaluri. Aquaculture 128: 335-344.

LI, M. H., J. B. RUSHING, and E. H. ROBINSON. 1996. Stability of B-complex vitamins in extruded catfish feeds. J. Appl. Aquacult., 6(2):67-71.

NATIONAL RESEARCH COUNCIL. 1993. Nutrient requirements of fish. National Academy Press, Washington, D.C.

ROBINSON, E. H. 1992. Vitamin C studies with catfish. Tech. Bull. No. 182. Miss. Agri. Forest. Exp. Sta., Mississippi State University, Mississippi State, MS.

ROBINSON, E. H. and M. H. LI. 1994. Use of plant protein in catfish feeds: Replacement of soybean meal with cottonseed meal and replacement of fish meal with soybean meal and cottonseed meal. J. World Aquacult. Soc. 25(2):271-276.

ROBINSON, E. H. and M. H. LI. 1995. Use of cottonseed meal in aquaculture feeds. In Nutrition and Utilization Technology in Aquaculture, eds. C. E. Lim and D. J. Sessa. pp 157-165. Champaign: AOCS Press.

ROBINSON, E. H. and M. H. LI. 1996. A practical guide to nutrition, feeds, and feeding of catfish: Revised. Tech. Bull. No. 1041. Miss. Agri. Forest. Exp. Sta. Mississippi State University, Mississippi State, MS.

ROBINSON, E. H., J. R. BRENT, J. T. CRABTREE, and C. S. TUCKER. 1990. Improved palatability of channel catfish feeds containing Romet-30. J. Aquat. Anim. Health 2:43-48.

ROBINSON, E. H., J.R. BRENT, and J. T. CRABTREE. 1989. AsPP, an ascorbic acid, resists oxidation in fish feed. Feedstuffs 61(40): 64-66.

9 FEEDING CHANNEL CATFISH

Edwin H. Robinson

Culture of channel catfish (Figure 9.1) accounts for about two-thirds of the commercial aquacultural production in the United States. Sales of catfish (live weight) reached 225,000 tons in 1996 (USDA, 1997). Once considered to have primarily a regional appeal as a food, farm-raised catfish are now in national and international markets. Market expansion has been due largely to the efforts of The Catfish Institute, which was established in 1986 to educate consumers on the positive qualities of catfish. Catfish reach the processing plant alive and are kept alive until they are slaughtered, which takes less than 30 minutes. Farm-raised catfish are fed grain-based feeds which give the fish a mild flavor with the absence of a "fishy" odor. The flesh is mostly white muscle which is free of intramuscular bones. Nutritionally, an 84-gram serving of farm-raised catfish contains approximately 140 kcal, 17 grams of protein, 9 grams of fat, 50 milligrams of cholesterol, 40 milligrams of sodium, and a number of essential vitamins and minerals. The delicate flavor, light flesh, high nutritional value, and year-around availability make farm-raised catfish an appealing choice to the food service industry and to consumers.

Since catfish were popular sport fish in the southeastern and midwestern United States, state and federal hatcheries developed techniques to produce seed to stock reservoirs and sport fishing ponds. This work provided the basis on which catfish farming was built. Research conducted by Dr. Homer Swingle at Auburn University in Alabama in the 1950's on the potential for small-scale aquaculture of catfish in farm ponds provided more information. Subsequent research by various universities in the Southeast led to improvements necessary for large-scale culture of catfish.

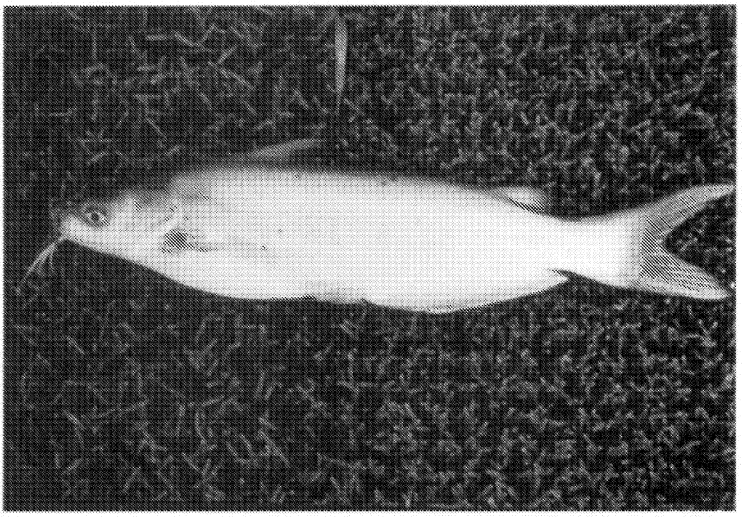


Figure 9.1 Channel catfish.

Channel catfish possess several qualities that make it amenable to culture. The fish normally does not reproduce in culture ponds, it is easy to spawn under hatchery conditions and produces a large number of fry, and it readily accepts a variety of prepared feeds. In addition, channel catfish tolerate water temperatures from near freezing to 34°C and wide fluctuations in water quality in production ponds. Channel catfish grow rapidly; a 10-gram fingerling reaches a harvestable size of 0.5 kg in about 6 months as long as water temperature remains above 23 C. Also, it converts feed efficiently; feed conversion ratios (feed/gain) of 1.4-1.5 can be achieved.

In the early 1970's when catfish farming was in its infancy, farmers stocked earthen ponds at rather low densities, ranging from 2,500 to 5,000 fingerlings per hectare, in the spring and harvested the fish in the fall. The fish were fed a pelleted, concentrated feed and yields of 1,000 to 2,000 kg per hectare were typical. Today, yields range from 4,000 to 7,000 kg per hectare. The increased yields can be attributed to higher stocking densities and to improvements in feeds, feeding practices, water quality management, and disease control. In addition, a multiple-batch cropping system is used in which fish of different sizes and ages are present in the pond simultaneously. Harvest-size fish are removed several times during the year, and ponds are restocked with fingerings without draining.

Catfish farming has become a major industry located primarily on the Mississippi River flood plain (the Delta) where a combination of physical and socioeconomic factors allowed the industry to develop. A typical farm is several hundred hectares in size (some may be 1500 hectares or more) with individual ponds of 5 to 10 hectares. Figure 9.2 presents an aerial view of a catfish farm in the Mississippi Delta. Large farms coupled with the development of local, specialized feed mills and catfish processing plants have made catfish farming profitable. Ninety-five percent of the catfish produced in the United States is produced in

Figure 9.2 Aerial view of a commercial catfish farm in the Mississippi Delta. Small ponds are nursery and fingerling ponds, and large ones (2 to 10 hectares) are for grow-out.

Mississippi, Arkansas, Alabama, and Louisiana in approximately 160,000 hectares of catfish ponds. Mississippi accounts for 75 percent of catfish production in the United States (USDA, 1997).

Even though catfish farming is successful, there are several problems that the industry faces. Management of water quality is of particular importance, and its management is becoming increasingly more troublesome as stocking densities and feeding rates continue to increase. Although nitrogenous and phosphorus compounds excreted from the fish or from uneaten feed are of concern, management of dissolved oxygen is the most critical pond environment problem. During periods of heavy feeding dissolved oxygen levels drop precipitously during the night. Permanent electrical aerators located in the ponds as well as temporary aeration devices powered by tractors are used to provide oxygen in emergencies. Infectious diseases are becoming more of a problem, and the increased occurrence of disease outbreaks appears to be related to high stocking rates and heavy feeding.

FEEDING PRACTICES

Even though catfish have been cultured for many years and considerable research has been conducted on nutrition and feeding of catfish, feeding is far from an exact science. There is still considerable variation in feeding practices on commercial catfish farms. Generally, catfish should be fed daily as much feed as they will consume without adversely affecting water quality. However, water quality or fish health problems may cause the farmer to restrict the daily feed allowance or to feed less frequently. Daily feed allowance and the frequency of feeding are decisions that must be made daily by catfish producers for each pond of fish. No two ponds are exactly alike, thus fish feeding behavior in individual ponds may differ greatly. Also, feeding activity in a particular pond may vary greatly from day to day (Figure 9.3).

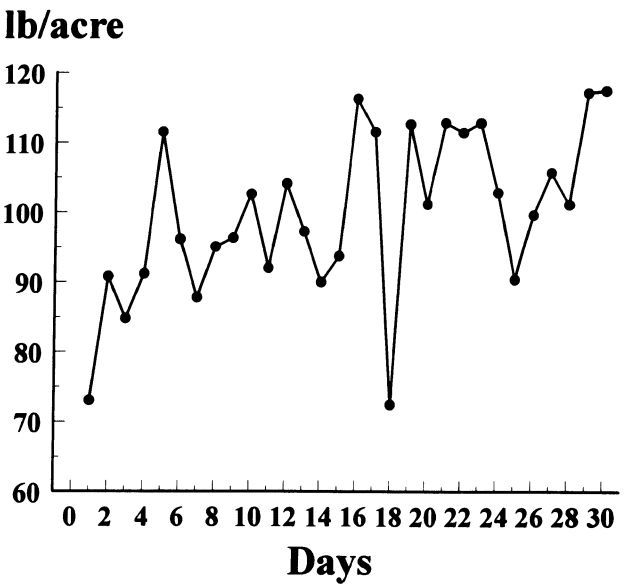


Figure 9.3 The graph shows daily variation in feed consumption by channel catfish fed to satiation in an intensively stocked, heavily fed pond.

Warm Weather Feeding

Fry. Newly hatched catfish fry, which are only about 25 mm in total length, are usually held in indoor troughs or tanks for about 10 days before releasing into outdoor nursery ponds. Initially, catfish fry use their yolk sac as an energy and nutrient source. Once the yolk sac is absorbed (approximately 3 to 5 days after hatching), fry begin to seek food. In the hatchery, fry are fed finely ground meal or flour type feeds (Table 9.1) containing 45 to 50% protein supplied primarily from fish meal at a daily rate equal to about 25% of body weight divided into 8 to 10 feedings.

It is difficult to effectively feed catfish fry that have recently been stocked into large nursery ponds. The tiny fish spread out over the pond and, because they are relatively weak swimmers, they are not able to move rapidly to areas where feeds are offered. The best way to ensure good growth and survival of recently stocked fry is to ensure that plenty of natural food is available in the nursery pond when the fish are stocked. Natural foods for channel catfish fry include microcrustaceans, insect larvae, and zooplankton. Even though fry presumably meet their nutrient needs from natural food organisms, they are fed once or twice daily using finely ground feed at a rate equal to 25 to 50% of fry biomass. Since the feed is a supplement to natural pond foods, it is not necessary to feed a high protein feed as is used in the hatchery. Fines (feed dust) from regular 28 or 32% growout feeds are suitable for catfish fry during this phase. After a few weeks, the fry reach 2.5 to 5 cm in length and will come to the pond surface seeking food. At this stage of development they are generally referred to as fingerlings.

TABLE 9.1. EXAMPLES OF TYPICAL CATFISH FRY AND FINGERLING FEEDS

Ingredients (%)	Fry feed (50% protein)	Fingerling feed[a] (35% protein)
Soybean meal (48%)[b]	–	38.8
Cottonseed meal (41%)	–	10.0
Menhaden meal (61%)	60.2	6.0
Meat/bone/blood (65%)	15.3	6.0
Corn grain	–	16.1
Wheat middlings	19.0	20.0
Dicalcium phosphate	–	1.0
Catfish vitamin mix[c]	include	include
Catfish mineral mix[c]	include	include
Catfish oil[d]	5.0	2.0

[a]For fingerlings less than 10 cm in length. After they reach 10-12 cm, a 32% protein grow-out feed (shown in Table 9.9) may be fed.
[b]Percentage protein.
[c]Commercial mix that meets or exceeds requirements for catfish.
[d]Sprayed on after extrusion to reduce feed dust.

Fingerlings. Initially, the small fingerlings are fed once or twice daily to satiation using a crumbled feed or small pellets (3 mm diameter) containing 35% protein (Table 9.1). The feed should contain some fish meal or other animal protein source. Some catfish producers feed fingerlings the same feed they feed to fish for grow out; however, the pellets may be so large that the fingerlings nibble on the feed after the pellets soften and begin to break up in the water. Fingerlings appear to grow satisfactorily using this feeding strategy, but nutrient losses are likely due to leaching because of the extended time the pellet is in the water. Fingerlings are generally fed a high-protein fingerling feed until they reach about 12 to 15 cm in length, at which time they are stocked in grow-out ponds to be grown to harvestable size.

Grow-out. Catfish grown for food are usually stocked as advanced fingerlings of about 12 to 15 cm in length (about 25 grams). They are typically fed a 28 to 32% floating feed with a pellet diameter of approximately 4 to 5 mm (Table 9.2). Low-protein feeds (24-28%) can be used if the fish are fed to satiation. Li and Lovell (1992) found that channel catfish grew maximally when fed 24 or 26% balanced protein feeds if fed as much as they would consume, but if fed to less than satiation, they required higher levels of protein for optimum growth. Because management practices vary greatly throughout the catfish industry, the choice of what protein percentage to use is up to the individual catfish producer.

On large commercial catfish farms, feed is typically blown onto the surface of the water using pneumatic dispensers mounted on or pulled by vehicles (Figure 9.4). Feed should be scattered over a large area to provide feeding opportunities for

as many fish as possible. It is desirable to feed on all sides of the pond, but this is generally not practical because prevailing winds dictate that feed must be distributed along the upwind side to prevent it from washing ashore.

Table 9.2. EXAMPLES OF TYPICAL CATFISH GROW-OUT FEEDS

	Percentage of feed					
Ingredient	(32%)[a]	(32%)	(32%)	(28%)	(28%)	(26%)
Soybean meal (48%)[a]	36.5	34.5	22.5	26.3	25.5	21.3
Cottonseed meal (41%)	10.0	12.0	27.5	10.0	10.0	12.0
Menhaden meal (61%)	4.0	–	4.0	4.0	–	4.0
Meat/bone/blood (65%)	4.0	8.0	4.0	4.0	4.0	4.0
Corn grain	22.9	22.4	21.1	30.6	31.4	51.4
Wheat middlings	20.0	20.0	18.0	22.5	22.5	4.0
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0
Lysine-HCl	–	–	0.275	–	–	–
Catfish vitamin mix[b]	include	include	include	include	include	include
Catfish mineral mix[b]	include	include	include	include	include	include
Catfish oil[c]	1.5	2.0	1.5	1.5	1.5	1.5

[a]Percentage protein.
[b]Commercial mix that meets or exceeds all requirements for channel catfish.
[c]Sprayed on finished feed pellet to reduce feed dust ("fines").

Figure 9.4 Catfish are being fed from a feeder drawn by a tractor along the levee separating large (10-hectare) ponds. The fish are fed an expanded floating pellet, enabling the farmer to gauge how much the fish consume.

Table 9.3. EXAMPLE OF FEEDING RATE FOR GROW-OUT CATFISH FED ONCE DAILY TO SATIATION FROM MAY TO OCTOBER IN PONDS STOCKED AT 25,000 FISH PER HECTARE IN A SINGLE-BATCH SYSTEM IN THE MISSISSIPPI DELTA

Date	Water temperature (°C)		Fish size (g)	Feeding rate (% body weight)
	7:00 am	4:00 pm		
May 1	20	23	50	2.1
May 15	23	26	62	3.4
June 1	21	25	82	2.9
June 15	27	30	111	3.2
July 1	27	31	143	2.7
July 15	28	31	176	2.4
August 1	28	32	233	1.8
August 15	27	30	285	2.0
September 1	25	30	336	1.5
September 15	25	30	382	1.3
October 1	20	22	463	1.1

Table 9.4. FEED CONSUMPTION AND FEED CONVERSION RATIOS FOR DIFFERENT SIZES OF CATFISH AT OPTIMUM TEMPERATURE

Fish body weight (g)	Feed consumption (% body weight)	Feed conversion ratio
27	4.0 - 4.5	1.1 - 1.2
45	3.5 - 4.0	1.3 - 1.4
136	2.5 - 3.0	1.4 - 1.6
272	2.0 - 2.5	1.6 - 1.8
340	1.5 - 2.0	1.8 - 1.9
454	1.3 - 1.5	1.9 - 2.0
908	1.1 - 1.2	2.0 - 2.2
1362	1.0 - 1.1	2.2 - 2.4

Typically, catfish producers feed once a day, seven days a week. Feeding twice a day when the water temperature is above 25 C will allow for a 20% higher rate of feed consumption and a corresponding faster growth rate (Lovell 1979). However, the logistics of multiple daily feedings on large catfish farms generally

make the practice impractical. During disease episodes or during extremely hot weather it may be beneficial to feed every other day or every third day.

Feed allowance is affected by several factors including fish standing crop, fish size, water temperature, and water quality. Water temperature and fish size have a profound affect on feeding rate (Tables 9.3, 9.4). Feed consumption increases as water temperature increases until a temperature of around 32 C is reached and consumption begins to decrease. As fish size increases feed consumption as percentage of body weight decreases and feed conversion increases (Table 9.4). In a study conducted at Auburn University during the summer months when water temperature is rather constant, small fish (45 g initial weight) consumed more feed, grew faster, and converted feed better than larger fish (150 g or 550 g initial weight). As fish size increased, feed consumption, feed efficiency, and weight gain decreased.

Because catfish are generally cultured using a multiple-batch production system in which several sizes of fish are present in the pond simultaneously, they should be fed to satiation. Offering as much feed as possible (without wasting feed) provides a better opportunity for the smaller, less aggressive fish to receive feed. Satiation feeding appears to be particularly important when catfish are fed less frequently than on a daily basis. Although it is recommended that catfish typically be fed as much feed as they will consume, at high standing crops of fish it may be impossible to satiate the fish and maintain water quality. Feeding rates should not exceed what can be assimilated by organisms in the pond and not require excessive use of aeration or cause toxic concentrations of waste metabolites, such as ammonia. This is difficult to judge, but generally long-term daily feed allowance should not exceed 100 to 120 kg per hectare. Overfeeding should definitely be avoided because wasted feed increases production cost by reducing feed efficiency and it also contributes to deterioration of water quality.

The best time of day to feed is still debated, but the point is more or less academic. On large catfish farms, the time fish are fed is largely dictated by the logistics required to feed many hectares of ponds in a limited time period. As a result, many catfish producers start feeding in early morning as soon as dissolved oxygen levels begin to increase. Figure 9.5 shows the diurnal variation in dissolved oxygen in typical catfish ponds. Research has shown that there are no significant differences in weight gain, feed consumption, feed conversion, and survival among catfish fed to satiation at 0800 hours, 1600 hours, or 2000 hours (Robinson et al. 1995). There were also no differences in emergency aeration time among treatments. However, feeding late evening or at night in commercial catfish ponds is not recommended because peak oxygen demand by the fish occurs 6-12 hours after feeding, when dissolved oxygen levels in the pond are low. Thus, if aeration is insufficient, fish may be stressed or die. Generally, it appears most practical, during the warm growing season, to begin feeding in the morning as the dissolved oxygen begins to increase. In cool weather, during fall, winter and spring, water temperature is usually higher in afternoon and fish will feed better.

Broodfish. Brood catfish are usually fed the same type feed as used for grow-out fish. Some catfish producers prefer feeding sinking or slow-sink feeds because broodfish are often hesitant to feed at the surface. However, because brooders generally feed slowly, sinking pellets may disintegrate before they can be consumed. Some catfish producers supplement commercial feeds for brood fish with live or frozen forage fish, such as golden shiners. However, based on research conducted at

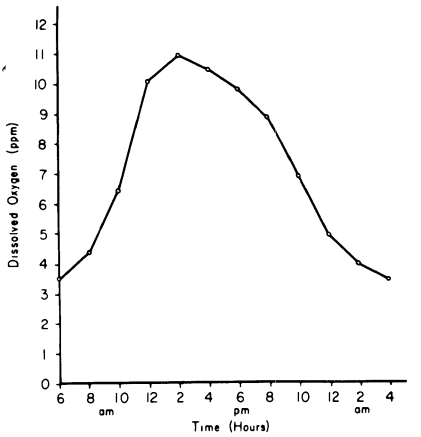


Figure 9.5 Typical diurnal variation in dissolved oxygen content in intensively fed catfish ponds. Oxygen concentrations of 6-7 ppm represent normal water saturation, and concentrations below 3mg/L are stressful to the fish.

Auburn University the practice of providing forage does not appear to be beneficial if broodfish are fed a nutritionally complete feed. It is recommended that catfish brooders be fed a typical 28 to 32% protein feed once daily at a rate of about 1% of fish body weight. The fish should be fed as much as they will consume during fall, winter and spring before spawning.

Winter Feeding

Although catfish feed inconsistently at water temperatures below 20 C, a winter feeding program appears to be beneficial to prevent weight loss and maintain fish health. A study conducted at Auburn University demonstrated that catfish (0.45 kg) held over winter in ponds without food lost 9% of their body weight; whereas, fish fed 1% body weight on days when the water temperature was above 12 C increased their body weight by 18%. The amount of gain or loss realized during winter depends on the severity of the winter. Fish will gain or lose more weight during a mild winter than during a cold winter.

The benefits of winter feeding on weight gain is fairly well documented; however, the health aspect of winter feeding is less well defined. Logically one would expect fish fed during the winter to be in better condition and perhaps more resistance to disease-causing organisms than fish that are not fed. Even so, many catfish producers do not feed during the winter months because inclement weather often prevents access to pond levees or because they do not see any benefit to winter feeding. Research at Auburn University has shown that food-size catfish that are not fed during the three coldest winter months (December, January, and February) can make up differences in weight gain as compared with fish that are fed during the winter months when satiate feeding is resumed in the spring and summer. If fish are to be marketed during winter, however, it would appear to be prudent to follow a winter feeding program, particularly during a mild winter. Also, small (fingerling) catfish are more disease resistant when fed during winter.

Table 9.5. WINTER FEEDING SCHEDULE FOR FINGERLING, GROW-OUT, AND BROOD CATFISH

Temperature	Fingerlings		Growout		Brood	
(°C)	%Body wt.	Frequency	%Body wt.	Frequency	%Body wt.	Frequency
< 13	0.5 - 1.0	1 - 2 days/wk	0.5 - 1.0	Weekly	0.5 - 1.0	Weekly
13 - 21	1.0 - 2.5	Daily or every other day	1.0 - 2.0	Every other day	1.0 - 2.0	2-3 times/wk

Table 9.6. TYPICAL SLOW-SINK WINTER FEED AND ANTIBIOTIC MEDICATED FEED FOR CHANNEL CATFISH

Ingredient	% of feed	
	Winter feed[a] (25% protein slow-sink)	Medicated feed (32% protein with Romet®)
Soybean meal (48%)[b]	18.3	26.8
Cottonseed meal (41%)	10.0	10.0
Menhaden meal (61%)	4.0	16.0
Meat/bone/blood (65%)	4.0	--
Corn grain	35.1	23.0
Wheat middlings	25.0	20.0
Dicalcium phosphate	1.0	1.0
Catfish vitamin mix[c]	0.1	0.1
Catfish mineral mix[c]	0.025	0.025
Catfish oil[d]	2.5	1.5
Romet® antibiotic	–	1.65

[a]Feed is extrusion processed but expanded less than grow-out floating feeds so that it will sink slowly.
[b]Percentage protein.
[c]Sprayed on after extrusion.
[d]Commercial mix that meets or exceeds the requirements of channel catfish.

Several schedules for winter feeding of fingerlings and food fish have been suggested. Generally water temperature dictates feeding allowance and frequency. A typical winter feeding schedule is shown in Table 9.5. The type of feed that should be fed during winter has not been precisely defined. A typical grow-out feed formula, containing 28 or 32% protein, or a 25% protein slow-sink feed (Table 9.6) will provide sufficient nutrition for overwintering catfish. Many farmers feed a slow-sink feed in cool weather because the fish seem reluctant to feed on the water surface, and wind may blow floating feed to shore before it is eaten. This feed is processed by extrusion, but density is greater than a floating feed and it sinks slowly.

Feeding rates and frequencies used in winter feeding of broodfish, as with fingerlings and growout fish, are based on water temperature. A suggested schedule is shown in Table 9.5. The most common broodfish feed used in the winter is the same feed used for grow out of fish--a 28 or 32% protein floating pellet. If broodfish appear to be reluctant to feed at the surface, the 25% slow-sink feed can be used. Research has shown that brood fish in ponds that are not fed or fed a nutrient deficient diet will not reproduce optimally. Some catfish producers also stock forage fish (e.g. fathead minnows) into ponds to ensure that adequate food is available during the winter.

Feeding Diseased Fish

Feeding diseased fish is difficult because fish that are sick feed poorly, if at all. However, offering medication through the feed is generally the only method available to treat bacterial infections. There is considerable debate over the efficacy of medicated feeds (feeds containing antibiotics) and the best method to treat diseased fish. There are catfish producers who do not feed during outbreaks of certain diseases and those who limit feed to every other day. Research has indicated that restricting feeding enhances resistance against certain bacterial infections. However, the efficacy of such practices is still under investigation.

Medicated feeds. Antibiotics can be administered to a large population of fish through the feed. Medicated feeds have been used to treat diseased fish for a number of years in other aquaculture industries (i.e. salmon and trout) and are currently accepted as the only viable alternative to treat systemic bacterial infections of catfish. Two antibiotics, Romet® (sulfadimethoxine-ormetroprim, Hoffmann-La Roche, Nutley, NJ) and Terramycin® (oxytetracycline, Pfizer, Inc., New York) are registered by the Food and Drug Administration (FDA) to treat bacterial infections of catfish through their incorporation into feeds.

Romet® is registered for control of enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri*, and has also been shown to be effective in treating motile aeromonad septicemia (MAS), caused by *Aeromonas hydrophila*, and systemic columnaris infections. Romet®-medicated feed (Table 9.6) is fed at a feeding rate (dependent on the formulation of Romet® used) sufficient to deliver 2.3 grams of antibiotic/45 kg of fish/day. Romet® is heat stable, so it can be used in extruded feeds. Research indicates that the level of fish meal should be increased to 16% to improve the palatability of feeds containing Romet® to catfish (Robinson et al. 1990). Romet® is registered by the FDA to be fed at the prescribed rate for 5 consecutive days. If the majority of fish affected by the disease in the pond are fingerlings, feeding crumbles or 3 mm diameter pellets may be beneficial. If fish mortality does not decrease after treatment, additional sick fish should be diagnosed to confirm the source of infection. An additional 5-day period of medicated feed may be prescribed. A 3-day mandatory withdrawal period is required before fish can be slaughtered.

Terramycin® is a broad-spectrum antibiotic which is registered by the FDA to treat MAS infections. Terramycin® has also been shown to be effective in treating other aeromonad infections, ESC, and systemic columnaris infections. The most common feed formulation currently used contains 22.7 kg of TM-100® premix/ton of finished feed, and delivers 2.5 grams antibiotic/45 kg of fish/day when fed at 1% of body weight/day. Terramycin® medicated feed is primarily

recommended to treat systemic columnaris infections or ESC infections caused by strains of E. ictaluri that are resistant to Romet®). Terramycin® is registered to be fed for 7 to 10 consecutive days. A 21-day withdrawal period is required before fish are slaughtered.

NUTRITIONAL REQUIREMENTS

Nutritional requirements for catfish have generally been based on studies with small fish conducted under conditions presumed to be near optimum; the requirement being based primarily on weight gain and feed efficiency. Presently, there is considerable interest in practical nutrient requirements for catfish. Recently, studies have been conducted to more precisely define nutrient requirements of catfish under conditions that reflect commercial culture practices. Nutrients recommended for catfish grow-out feeds are given in Table 9.7.

Energy requirements of catfish were largely neglected in the early stages of catfish feed development, primarily because an imbalance in dietary energy does not appreciably affect the health of the fish. Also, feeds prepared from feedstuffs typically used in catfish feeds, such as soybean meal, corn and fish meal, are unlikely to be extreme in respect to energy balance. However, correct balance of dietary energy is an important consideration when formulating catfish feeds, because too much energy can result in a reduction in food intake and thus reduce nutrient intake. Also, excess dietary energy may result in an increased deposition of body fat. If the dietary energy level is too low, protein will be used for energy instead of tissue synthesis. Based on current information, it appears that a digestible energy (DE) level of 8 to 9 kcal per g of protein is adequate for use in catfish feeds (Lovell 1989; Robinson and Li 1996). Thus a 32% protein feed should contain a digestible energy level of about 2,600 to 2,800 kcal per kg diet.

Essential fatty acid requirements (EFA) for catfish and most other warmwater fish have not been precisely defined, but catfish apparently require small amounts of n-3 and n-6 fatty acids. Because catfish apparently elongate and desaturate linolenic acid to synthesize highly unsaturated fatty acids, it appears that 1 to 2% dietary linolenic acid (18:3 n-3) or 0.5 to 0.75% highly unsaturated fatty acids will satisfy the n-3 EFA requirement (Satoh et al. 1989). The n-3 EFA requirement can be supplied by marine fish oil such as menhaden oil. The n-6 EFA requirement is usually met through the lipids in the plant ingredients in the feed. Natural pond food organisms may also be a source of EFA. When fish oil is the only source of fatty acids, immune response in channel catfish is compromised; however, when plant oil containing n-6 fatty acid is present, immune response is optimal.

Catfish appear to have the ability to synthesize most of their fatty acids; thus, nutritionally there may be no "best" level of dietary lipid except that needed to provide EFAs. Catfish have been fed diets containing up to 16% lipid without conclusive evidence as to which level is best for optimum growth. Even so, there is likely an optimum level of lipid to be used in catfish feeds with respect to protein sparing, product quality, and constraints of feed manufacture. Lipid levels in commercial catfish grow-out feeds rarely exceed 5 to 6%. About 3 to 4% of the lipid is inherent in the feed ingredients with the remaining 1 to 2% being sprayed onto the finished pellets to control feed dust.

Table 9.7. NUTRIENTS RECOMMENDED FOR CATFISH GROW-OUT FEEDS[a]

Nutrient	Recommended level	Comments
Protein (%)	26-32	Will vary depending on fish size, water temperature, dietary energy level, and daily feed allowance. As low as 16% protein feeds have provided for good performance.
Essential amino acids (% of protein):		Generally, if lysine and sulfur amino acid requirements are met other amino acids will be adequate with feedstuffs commonly used in catfish feeds. Cystine can replace about 60% of methionine requirement. Tyrosine can replace about 50% of phenylalanine requirement. Synthetic amino acids can be used to supplement deficient proteins.
Arginine	4.3	
Histidine	1.5	
Isoleucine	2.6	
Leucine	3.5	
Lysine	5.1	
Methionine	2.3	
Phenylalanine	5.0	
Threonine	2.0	
Tryptophan	0.5	
Valine	3.0	
Digestible energy (kcal*g^{-1} protein)	8-10	Use carbohydrate and lipid (fats or oils) as energy to spare protein for growth.

Table 9.7. Continued.

Nutrient	Recommended level	Comments
Lipid (%)	<6.0	Mixture of animal, vegetable, and fish oils may be used. High levels of marine fish oil impart a "fishy" flavor to the fish. Supplemental fat or oil should be sprayed on pellet surface.
Carbohydrate (%)	25-35	Floating feeds require at least 25% grain. Use grain byproducts for good expansion and bonding. Crude fiber should be maintained below 7%.
Vitamins ($mg*kg^{-1}$):		
Thiamin	5.5	Thiamin mononitrate is generally used.
Riboflavin	13.2	
Pyridoxine	11	Pyridoxine HCl is generally used.
Pantothenic acid	35	Calcium d-pantothenate generally used.
Nicotinic acid	22	Either nicotinic acid or nicotinamide may be used.
Biotin	None	Required, but feed contains adequate biotin without adding a supplement.
Folic acid	2.2	
B_{12}	0.01	Required, but amount not known. It is synthesized in intestine of catfish.
Choline	0-275	Required in low-methionine diets. It is abundant in most feedstuffs, therfore choline supplements do not appear to be necessary.

Table 9.7. Continued.

Nutrient	Recommended level	Comments
Inositol	None	No requirement demonstrated.
Ascorbic acid	50-100	Phosphorlayed form is stable during feed processing and storage. Metabolized forms will lose 40 to 60% of activity during processing.
A ($IU*kg^{-1}$)	2,200	Acetate ester is used to improve stability during feed processing.
D_3 ($IU*kg^{-1}$)	1,100	D-activated animal sterol used as source of D3.
E ($IU*kg^{-1}$)	66	DL-alpha-tocopheryl acetate is used for improved stability.
K	4.4	Required, but level for catfish not known. Menadione sodium bisulfite is added to ensure adequacy.
Minerals ($mg*kg^{-1}$):		
Calcium	None	Catfish usually absorb sufficient calcium from the water to meet their needs. Requirement of 0.45% for fish reared in calcium-free water.
Phosphorus, available	3,000-4,000	About 33% of plant phosphorus and about 50-70% of animal phosphorus is available to catfish. Dicalcium or defluorinated phosphates are generally used as a phosphate source in catfish feeds.
Magnesium	None	No supplement needed; abundant in feedstuffs.

Table 9.7. Continued.

Nutrient	Recommended level	Comments
Sodium, potassium, and chloride	None	No supplement necessary; abundant in feedstuffs.
Sulfur	None	No supplement needed.
Cobalt[b]	0.05	Cobalt carbonate used to insure adequacy.
Iodine[b]	2.4	Calcium iodate used to insure adequacy.
Zinc[b]	200	Phytic acid in feed reduces availability. Zinc sulfate is preferred source.
Selenium	0.1	Maximum allowable by FDA is 0.1 mg/kg. Sodium selenite used.
Manganese[b]	25	Phytic acid in feed reduces availability. Manganese oxide used.
Iron[b]	30	Ferrous sulfate and ferrous carbonate used.
Copper[b]	5	Copper sulfate used.

[a]*Recommendations are for advanced fingerlings (45 g) to market size (0.5 kg or larger). Adapted from Robinson, E. H. 1989.*
[b]*A supplement may not be needed when the diet contains 4% or above animal protein.*

Fish, animal, or vegetable oils can be used for catfish feeds. Marine fish oils may impart "fishy" flavor to the fish flesh if fed in high levels. There is evidence that when menhaden fish oil is the only lipid source in the diet, levels as low as 2% reduce survival of catfish exposed to the bacterial pathogen *Edwardsiella ictaluri* (Fracalossi et al. 1994, Li et al. 1994). This is likely caused by the immunosuppressive effect of highly unsaturated n-3 fatty acids. Catfish feeds manufactured in the Mississippi Delta are generally sprayed with catfish oil, a local product extracted from catfish offal, which is not highly unsaturated as are marine fish oils and does not have a fishy odor. In some cases, menhaden oil or a mixture of catfish oil and menhaden oil is used.

Research data from various studies indicate that the dietary protein requirement for catfish ranges from about 24 to 50%. Protein requirements vary because of numerous reasons including differences in water temperature, feed allowance, fish size, amount of nonprotein energy in the diet, protein quality, natural food available, and management practices. However, 28 or 32% protein feeds are typically used for fish during grow out (Robinson and Li 1996). Levels as low as 24% have been shown to be adequate for food-size catfish if the fish are fed to satiation (Li and Lovell 1992). Studies conducted at the Delta Research and Extension Center (DREC), Mississippi State University, indicate that weight gain of catfish is only slightly reduced using feeds containing as low as 16% protein. To maximize profits, the optimum dietary protein level should not necessarily be based on maximum weight gain but rather on the most economical gain.

Amino acid levels used in formulating practical catfish feeds are presented in Table 9.7. Lysine is generally the limiting amino acid for catfish, and if feeds are formulated to meet a minimum lysine requirement all other amino acid requirements are met or exceeded if traditional feed ingredients are used. Cystine can replace about 60% of the methionine and tyrosine can replace about 50% of the phenylalanine. In a practical feed, amino acid requirements are best met by feeding a mixture of feedstuffs or by using a mixture of feedstuffs supplemented with crystalline amino acids. Research has shown that synthetic amino acids, such as lysine HCl, are effectively used by catfish when supplemented in a practical feed (Robinson and Li 1993).

Catfish feeds are generally supplemented with a vitamin premix that contains all essential vitamins in sufficient quantities to meet requirements and to compensate for losses due to feed processing and storage. (Vitamin losses during storage are not a major factor in the catfish farming industry where feed is usually not stored for more than 2 to 3 days.)

Although the vitamins inherent in feedstuffs contribute to the nutrition of the catfish, they are not usually considered during feed formulation because their bioavailability is not known. Natural food organisms may also be a source of vitamins for catfish, because vitamins are relatively abundant in zooplankton (Table 9.8). Although many nutritionists discount the contribution of natural foods to the nutrition of catfish, there are indications that natural foods may contribute to their micronutrient requirements. Several studies have been conducted in Mississippi on the production of catfish in earthen ponds where the fish were fed diets without supplemental vitamins. The results have indicated that the level of supplemental vitamins used in catfish feeds may be reduced or certain supplemental vitamins may be omitted. In some cases the requirements could not be met with the vitamins inherent in the feed ingredients.

Table 9.8. NUTRIENT COMPOSITION (DRY MATTER BASIS) OF ZOOPLANKTON FROM CATFISH PONDS IN MISSISSIPPI. ZOOPLANKTON CONTAINED 7.7% DRY MATTER

Proximate composition (%):		**Vitamins (per kg):**		
Crude protein	72.5	D	245	IU
Crude fat	6.2	E	115	mg
Crude fiber	10.7	B_1	3.4	mg
Nitrogen-free extract	8.1	B_2	100	mg
Ash	2.6	B_6	2.5	mg
		B_{12}	2.2	mg
		Folic acid	1.2	mg
Amino acids (% protein):		Niacin	141	mg
Arginine	7.1	Pantothenic acid	20	mg
Histidine	3.0	Biotin	1.5	mg
Isoleucine	4.1	Inositol	1,565	mg
Leucine	7.3	C	164	mg
Lysine	6.8			
Methionine	2.3	**Minerals:**		
Cystine	1.1	Phosphorus	0.93	%
Phenylalanine	3.9	Calcium	0.39	%
Tyrosine	6.1	Sodium	0.15	%
Threonine	4.5	Potassium	0.38	%
Tryptophan	0.9	Sulfur	0.72	%
Valine	4.6	Magnesium	0.12	%
Alanine	8.0	Iron	622	$mg*kg^{-1}$
Aspartic acid	7.9	Manganese	113	$mg*kg^{-1}$
Glutamic acid	12.3	Zinc	76	$mg*kg^{-1}$
Glycine	4.8	Copper	16	$mg*kg^{-1}$
Proline	4.3			
Serine	4.1			
Fatty acids (% fat):				
14:0	1.3			
16:0	16.4			
16:1	2.9			
18:0	7.1			
18:1	6.2			
20:5 n-3	12.0			
22:5 n-6	4.3			
22:5 n-3	1.5			
22:6 n-3	13.9			
Total n-3 HUFA[a]	28.4			
Total n-6 HUFA	11.1			
n-3/n-6 HUFA ratio	2.6			

[a]HUFA - highly unsaturated fatty acids with 20 carbons or longer and 4 or more double bounds.

Studies have been conducted to evaluate the efficacy of feeding megadose levels of certain vitamins, particularly vitamin C, to enhance disease resistance in catfish. Early laboratory investigations indicated that high levels of vitamin C, 10 to 100 times the level needed for normal growth, reduced mortality in young channel catfish challenged with certain pathogenic bacteria (Durve and Lovell 1982; Li and Lovell 1984). Results from studies conducted at Mississippi State University have not shown an advantage to using high levels of dietary vitamin C for disease resistance in catfish. Data from these studies indicated that response to dietary vitamin C during disease challenge is an "all or none" type. That is, if vitamin C is not present, mortalities are increased during disease challenge but if vitamin C is present in the diet mortalities are significantly reduced. Concentrations as low as 25 $mg*kg^{-1}$ vitamin C has been shown to enhance survival of catfish during challenge with the bacterium Edwardsiella ictaluri. Commercial catfish feeds generally contain 80 to 100 $mg*kg^{-1}$ of vitamin C after processing, which should be sufficient for protection against bacterial infections without increased supplementation.

Phosphorus supplements are used in catfish feeds to provide the 0.3-0.4% biologically available phosphorus that is required. Data from both laboratory and pond studies have shown that dicalcium and defluorinated phosphates have equal biological value to catfish (Li et al. 1996; Robinson et al. 1996). However, because defluorinated phosphates are much less soluble in water than dicalcium phosphate, their use in catfish feeds may reduce phosphorus levels in pond water.

Plant feedstuffs are poor sources of phosphorus for animals. Approximately 2/3 of phosphorus in feedstuffs of plant origin is in the form of phytate, a bound form of phosphorus that is poorly available to fish. Laboratory studies have demonstrated that phytase enzymes can be used in catfish feeds to release phytate phosphorus making it available for use (Jackson et al. 1996).

Catfish feeds are typically supplemented with a trace mineral premix that contains essential trace minerals in sufficient amounts to meet or exceed dietary requirements of catfish (Table 9.7). There is evidence that supplemental trace minerals are not necessary in some catfish feeds, particularly those containing animal protein sources.

NATURAL FOOD

Because of the high level of nutrients introduced by feeding, commercial catfish ponds are fertile and normally contain large numbers of organisms including phytoplankton, zooplankton, and invertebrates such as insects and crustaceans. Many of these organisms are high in protein and other essential nutrients and may contribute to the diet of pond-raised catfish (Table 9.7).

While some commercially cultured fish which feed low on the food chain (such as tilapia and silver carp) make excellent gains on natural foods, channel catfish require prepared feeds for maximum yields, except for newly stocked catfish fry which appear to meet their nutrient requirements from natural food organisms. Although natural food organisms are abundant in most catfish ponds, their contribution to growth of fish during the growout phase has been generally thought to be minimal. For example, it is estimated that only 2.5% of the protein

requirement and 0.8% of the energy needed for catfish grown in intensively-fed ponds was obtained from natural food (Wiang 1977). Another study showed that only about 8 to 9% of the growth of channel catfish could be attributed to natural food (Lovell 1989).

The major contribution of natural food organisms to the nutrition of commercially cultured catfish may be from nutrients which are required in trace amounts such as vitamins, minerals, and essential fatty acids. Studies with catfish have shown that while vitamin deficiencies could be produced by feeding catfish purified diets devoid of various vitamins in aquaria under controlled laboratory conditions, the same deficiencies could not be produced in catfish raised in ponds fed practical feeds lacking a supplement of a specific vitamin. Thus, the vitamin requirement was met either from vitamins naturally occurring in feedstuffs, natural food organisms, or from a combination of the two. Studies have also been conducted with minerals and essential fatty acids with similar results.

EFFECT OF FEEDS ON SENSORY QUALITY OF PROCESSED CATFISH

Flavor

Commercial feeds composed of oilseed meals, grains, and animal products generally have little influence on flavor quality of farm-raised catfish. A study was conducted at the USDA Southern Regional Research Center, New Orleans, LA, and the USDI Fish Farming Experiment Laboratory, Stuttgart, AR, to evaluate the effects of feed ingredients on flavor quality of farm-raised catfish. Commonly used feed ingredients were substituted individually into semi-purified experimental diets at levels commonly used in commercial feeds. The diets were fed to catfish under laboratory conditions for two months and fish were evaluated for flavor quality by a trained panel using quantitative sensory techniques. Results showed no significant differences in flavor among fish fed the different experimental diets.

High levels of dietary marine fish oil may give catfish a "fishy" flavor that is undesirable, but catfish fed feeds containing 2% menhaden oil (this level is rarely exceeded in growout feeds for catfish) have no distinct "fishy" flavor. Off-flavor problems of farm-raised catfish are predominantly influenced by phytoplankton, some of which produce odorous metabolites that are absorbed by the fish.

Appearance

Consumer acceptance of farm-raised fish is significantly influenced by the color of the flesh. The preferred color of catfish flesh is white. At high dietary levels the yellow-orange carotenoids, xanthophylls, have been shown to concentrate in catfish giving the flesh a yellowish coloration that is undesirable (Lovell 1989). Corn gluten meal is limited as a feed ingredient because of its high concentration of xanthophyll. Corn and corn screenings contain the pigment, but it is present at concentrations that are not problematic. Xanthophyll concentration in catfish feed should not exceed 11 $mg*kg^{-1}$.

Fattiness

The amount of body fat found in catfish is influenced by several factors including the dietary energy/protein ratio (E/P), feeding rate, and fish size. Several studies have shown that as the dietary E/P ratio increases body fat increases, and, usually,

dressing yield decreases. However, fish size may impact fattiness more than diet (Robinson and Robinette 1994). There is evidence from studies conducted at Auburn University that feeding Ractopamine® may reduce body fat in catfish. Ractopamine® is a repartitioning agent that can repartition fat to synthesize protein. Carnitine is a natural compound that acts as a catalyst for fat metabolism and appears to reduce fat in catfish. These compounds are not approved for use by FDA.

A major concern about fattiness of catfish is that increasing fat in edible tissue may reduce the shelf-life of frozen fish. However, a cooperative study involved several universities has shown that variation in body fat content among catfish of the same age groups has little effect on frozen keeping quality of catfish fillets as long as the product was properly frozen. Large catfish, 1 to 1.5 kg in size, have a large amount of fat under the skin which, if not removed during skinning, can cause rancid flavor after prolonged frozen storage. Oxidized flavors are generally not a problem in medium and small catfish.

COMPENSATORY GROWTH

After a period of feed deprivation or restriction, animals have the potential to compensate or "catch up" resulting in increased growth rate after full feeding is resumed. This phenomenon is called compensatory growth. Research conducted at Auburn University demonstrated that catfish are able to make up weight gain following a 3-week restricted feeding regimen when the fish are returned to full feed. Also, catfish which are not fed during the winter months of December, January, and February can make up for the weight loss when full feeding resumes the following spring and summer. These studies clearly indicate that catfish exhibit compensatory growth. This is of practical importance because catfish are often not fed or fed infrequently during the winter. Also, during summer, there are occasions when the fish must be held off feed because of disease or poor water quality. If this period of feed deprivation is not too long, the fish can probably compensate for the missed feedings by eating more when feeding is resumed. However, it should be noted that these studies were conducted at relatively low standing crops with fish of a single size class. In the typical multiple-batch system used to raise catfish and at high standing crops, it may be difficult to feed enough feed to fully realize potential compensatory growth and avoid the negative effects of increased feed input on water quality. That is, feeding to satiation is essential for compensatory growth, but at high standing crops the amount of feed necessary may exceed the capacity of the pond to "assimilate" the input and avoid poor water quality conditions. It should not be assumed that severely restricting feed or not feeding at all will always be compensated once feeding resumes.

REFERENCES

DURVE, V. S., AND R. T. LOVELL. 1982. Vitamin C and disease resistance in channel catfish. Can. J. Fish. Aquat. Sci. 39:948-951.

FRACALOSSI, D. M., AND R. T. LOVELL. 1994. Dietary lipid sources influence responses of channel catfish (Ictalurus punctatus) to challenge with pathogen Edwardsiella ictaluri. Aquaculture 119:287-298.

JACKSON, L. S., M. H. LI, AND E. H. ROBINSON. 1996. Use of microbial phytase in channel catfish Ictalurus punctatus diets to improve utilization of phytate phosphorus. J. World Aquacult. Soc. 27(3):309-313.

LI, M. AND R. T. LOVELL. 1992. Comparison of satiate feeding and restricted feeding of channel catfish with various concentrations of dietary protein in production ponds. Aquaculture 103:165-176.

LI, Y., and R. T. LOVELL. 1984. Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J. Nutr. 115:123-131.

LI, M. H., M. R. JOHNSON, and E. H. ROBINSON. 1993. Elevated dietary vitamin C concentrations did not improve resistance of channel catfish, Ictalurus punctatus, against Edwardsiella ictaluri infection. Aquaculture 117:303-312.

LI, M. H., H. R. ROBINETTE, and E. H. ROBINSON. 1996. Efficacy of dicalcium and defluorinated rock phosphates for channel catfish (Ictalurus punctatus) and the relationship of phosphorus utilization to their solubility in neutral ammonium citrate. Aquaculture (in press).

LI, M. H., D. J. WISE, M. R. JOHNSON, and E. H. ROBINSON. 1994. Dietary menhaden oil reduced resistance of channel catfish (Ictalurus punctatus) to Edwardsiella ictaluri. Aquaculture 128:335-344.

LOVELL, R. T. 1979. Factors affecting voluntary food consumption by channel catfish. Proceedings of the World Symposium on Finfish Nutrition and Fish Feed Technology, pp. 556-561. Hamburg, 20-23 June, 1978.

LOVELL, R. T. 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York, NY.

ROBINSON, E. H., and H. R. ROBINETTE. 1994. Effects of dietary protein level and feeding regimen on growth and on fattiness of channel catfish, Ictalurus punctatus. J. Applied Aquacult. 3(5):67-89.

ROBINSON, E. H., and M. H. LI. 1993. Protein quantity and quality of catfish feeds. Technical Bulletin 189. Miss. Agri. & Forest. Exp. Sta., Mississippi State University, Mississippi State, MS.

ROBINSON, E. H., J. R. BRENT, J. T. CRABTREE, and C. S. TUCKER. 1990. Improved palatability of channel catfish feeds containing Romet-30®. J. Aquat. Anim. Health 2:43-48.

ROBINSON, E. H., L. S. JACKSON, and M. H. LI. 1996. Supplemental phosphorus in practical channel catfish diets. J. World Aquacult. Soc. 27(3):303-308.

ROBINSON, E. H., L. S. JACKSON, M. H. LI, S. K. KINGSBURY, and C. S. TUCKER. 1995. Effect of time of feeding on growth of channel catfish. J. World Aquacult. Soc. 26:320-322.

ROBINSON, E. H., and M. H. LI. 1996. A practical guide to nutrition, feeds, and feeding of catfish. Bull. No. 1041. Miss. Agri. Forest. Exp. Sta., Mississippi State University, Mississippi State, MS.

SATOH, S., W. E. POE, and R. P. WILSON. 1989. Effect of dietary n3 fatty acids on weight gain and liver polar lipid fatty acid composition of fingerling channel catfish. J. Nutr. 119:23-28.

TUCKER, C. S., and E. H. ROBINSON. 1990. Channel Catfish Farming Handbook. Van Nostrand Reinhold, New York, NY.

WIANG, C. 1977. Nutritional contribution of natural pond organisms to channel catfish growth in intensively fed ponds. Ph.D. Dissertation, Auburn University, Auburn, AL.

USDA. 1997. National Aquaculture Statistics Service Special Circular, U.S. Dept. of Agriculture, Washington, DC.

10 FEEDING SALMON AND TROUT

Ronald W. Hardy

During the past decade, salmon farming has grown from a relatively modest level of production, centered mainly in Norway and Scotland, to a global business producing nearly 500,000 metric tons per year (Tilseth et al., 1991; Torrissen et al., 1995). This growth has affected nearly all aspects of salmon and trout culture, including feeds and nutrition. Industrial growth of this magnitude has drawn large multi-national corporations into the salmon business, thereby bringing a business approach that has transformed the industry and placed unparalleled demands on scientists to provide information on feed ingredients, feed formulations, feed manufacturing processes, and feed quality or efficiency. This, in turn, has resulted in a significant increase in the amount of applied salmonid nutrition research. Consequently, the scientific literature has greatly expanded, with over 2000 papers published between 1987 and 1997, just in the area of salmon and trout nutrition. The number would be larger if studies done by research divisions of corporations or by institutions under contract where confidentiality is involved were also published. Privatization of research has resulted in some significant results not appearing in the scientific literature but rather being used by the corporations that supported the research. Overall, salmon and trout nutrition has advanced significantly in the last decade, and is striving to reach the level of sophistication and efficiency of poultry nutrition.

Salmonid aquaculture is the oldest form of fish rearing in Europe and North America with records of culture efforts dating back hundreds of years in Europe and nearly a century and a half in North America. For decades, salmon and trout were raised primarily for fishery enhancement (Stickney, 1996). State and federal government agencies raise salmon in freshwater hatcheries to the stage at which they

normally migrate to the sea. The juvenile fish are then released to complete their life cycle in the ocean and return to enhance coastal fishing. Trout, including anadromous rainbow trout (steelhead trout), are also raised to restock depleted lakes and streams, and to enhance public fishing. Until 15 years ago, most salmon and trout nutrition research was conducted on fry and fingerling fish, and intended to improve juvenile production. However, the rapid growth of the salmon farming industry from the early 1980s, producing 4-6 kg fish, has changed the character and focus of salmonid nutrition research. This industry has grown from virtually nothing in the 1970s to an industry now producing over 60% of the salmon consumed in restaurants and in the home. The main species being farmed are Atlantic salmon and rainbow trout, with Pacific salmon, coho and chinook, constituting a second, much lower tier of production. Arctic char and other species of trout are also raised for food production, but in relatively low numbers.

In North America, the rainbow trout is by far the most extensively cultured salmonid, both for stocking of public waters for recreational fishing and for food. Over 300 state and federal hatcheries rear trout for fisheries enhancement. The total public production of trout is about 200 million fish (70,000 metric tons) annually. Private trout farms raise an additional 25,000 metric tons each year for food. While this production is spread throughout many states in the Northern and Central United States, 70% of commercial trout culture is located along the Snake River aquifer in southern Idaho where the trout industry utilizes nearly 90,000 ft^3/sec of constant-temperature, fully oxygenated, spring water, which is returned to the Snake River.

In the United States, Pacific salmon are primarily cultured for fisheries enhancement. Pacific states rear and release about 600 million salmon fingerlings, or smolts, each year. British Columbia contributes an additional 400 million. Brood fish, such as in Figure 10.1, are taken from wild sources for spawning in hatcheries. The different species of Pacific salmon are reared in freshwater hatcheries for different lengths of time, depending upon their natural periods of freshwater residence (Table 10.1). During late spring and summer, as water temperature and photoperiod increase, salmon fry and fingerlings undergo a metamorphosis that results in a transformation from freshwater to saltwater-tolerant forms (smolts). The fish are released into streams and rivers and voluntarily migrate to the ocean. After a period of near-shore feeding, they move offshore into the North Pacific, where they remain for several years until they return to coastal areas during the summer and fall as mature adults preparing to migrate back to freshwater to spawn and die. It is at this point that adult salmon enter the commercial and sport fisheries. Each of the five species differs from the others in some aspect of its life history, most likely to avoid direct competition in the wild between species for resources such as food supply, freshwater rearing area, or spawning area. Culture practices used to rear Pacific salmon reflect these differences.

Hatchery-raised fish make significant contributions to many fisheries, especially in areas where freshwater habitat loss has reduced wild populations. Many state and federal hatcheries, such as that shown in Figure 10.2, have been established to produce fish for release. Without the contribution of hatchery-produced salmon, the annual catch of some species of Pacific salmon would be cut significantly. In Alaska, the numbers of hatchery-bred pink salmon returning to the fishery have been so high that catches have exceeded the demand for this species by

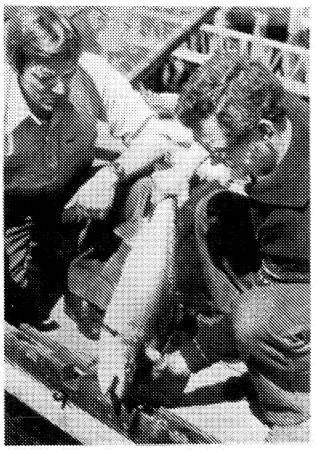


Figure 10.1 Atlantic salmon is the major salmonid species grown in ocean net pen culture. This female, taken from a net pen, is near marketable size.
Source: National Marine Fisheries Service.

Figure 10.2 Mature male chinook salmon taken from wild source for spawning.

fish processors, thus depressing the price received by fishermen. Nevertheless, serious questions have been raised in the Pacific areas of North America concerning the potential effects that hatchery-produced salmon have on wild salmon populations. These effects mainly revolve around adult straying and interbreeding between adult salmon of wild and hatchery origin, and management of mixed-stock fisheries, where wild and hatchery salmon are harvested. These issues are complicated, controversial, and beyond the scope of this chapter.

Table 10.1. PACIFIC SALMON (GENUS *ONCORHYNCHUS*) INDIGENOUS TO NORTH AMERICA[1]

Common name	Scientific name	Size at first feeding, g	Smolt wt, g (Age at smolting, wk)	Adult Size, kg	Age at maturity, yr
Chinook	*O. tshawytscha*	0.4	5-25[2]	6-15	3-5
Coho	*O. kisutch*	0.3	20-35 (1+)	3-6	3-4
Chum	*O. keta*	0.3	0.5-2(4-6)	2-10	3-4
Pink	*O. gorbuscha*	0.3	1-4 (2-6)	1-2	2
Sockeye	*O. nerka*	0.15	3-80[3] (2+)	2-4	3-5

[1]Values for size at first feeding, smolt weight, and adult weight are average values.
[2]Chinook salmon populations can be ocean or stream types, with ocean types migrating to the sea as sub-yearlings (90-120 days after swim-up) and stream types migrating to the ocean as yearlings (1+).
[3]Sockeye fry from southern part of range (Washington State) are generally larger than fry from Alaska, but considerable variation among populations exists, with Kodiak, and Kamchatka stocks being quite large.
Source: Groot and Margolis, 1995.

In Europe, commercial marine net-pen culture of Atlantic salmon and rainbow trout is a successful, mature industry. Figure 10.3 shows an Atlantic salmon near marketable size being removed from a net pen. Norway is the leader in salmon farming, producing over 250,000 in 1996. Salmon are now the second most important food production crop in Norway. Scotland is the other major producer of farmed salmon in Europe, with Ireland, Iceland, and the Faroe Islands producing lower quantities. Chile has the most rapidly growing salmon and trout farming industries, increasing from virtually nothing in 1986 to over 125,000 metric tons in 1996. Excellent environmental conditions, low labor costs, and favorable investment and regulatory climates have stimulated the rapid growth of salmon and trout farming in Chile (Hardy and Castro, 1994).

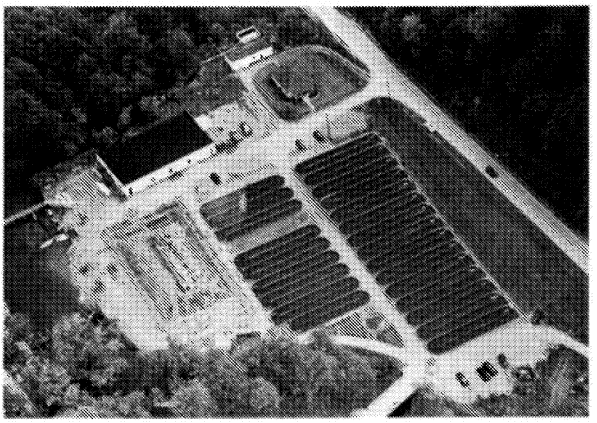


Figure 10.3 Many salmon and trout are produced in state and federal hatcheries for release to enhance commercial and recreational fisheries. Shown here is the Green River salmon hatchery, Auburn, Washington. *Source: Washington Department of Fisheries.*

GENERAL CULTURE METHODS

Salmon and trout have similar life histories and are relatively easy to culture compared to many fish. Relatively large eggs are spawned by salmon in the fall and early winter months. Hatching occurs about 30 days later in 10°C water, and yolk absorption occurs 20-30 days later, at which time the fish are ready for exogenous feed. At first feeding, salmon and trout fry range in weight from 100 mg to over 500 mg, depending on the species. Size at first feeding dictates the size of feed used at this stage, but fish are usually started on mash or small flakes. As the fish grow, the feed particle size is increased and feeding frequency is reduced. Feeding frequency is of critical importance to salmon and trout fry, and the small fry are fed almost continuously. As the fry grow, they are transferred from starter tanks to larger tanks and raceways supplied with constantly running fresh water. Salmon destined for release are fed so that they reach a target size by their hatchery release date. This may involve reducing the feeding rate so that the fish do not get too large, a factor that has been shown to increase the percentage of coho and chinook salmon that return as undersized, precocious males. Feeding rate is reduced during winter months and increased in early spring to ensure that the fish have adequate stores of energy for their downstream migration.

Salmon and trout reared for market are raised in ponds, tanks, raceways and net-pens (cages). Transfer to sea cages must be done when the fish are undergoing smoltification and are able to osmoregulate in the marine environment. Sea water tolerance occurs at the fry stage in pink and chum salmon, but chinook, coho and Atlantic salmon must live in freshwater for months to over a year, depending on the species, stock and details of rearing, before they can tolerate sea water. Rainbow trout can adapt to sea water after they reach approximately 100 g. After transfer to marine pens, salmon and trout are fed mainly extruded, slowly-sinking pelleted feeds until harvest, or, in the case of broodstock fish, until voluntary feeding ceases several months before final maturation and spawning.

NUTRIENT REQUIREMENTS

Salmon and trout have been shown to require 34 specific nutrients in their diets and probably require some trace elements not yet investigated. In addition, salmon and trout, like other animals, require dietary sources of energy and nitrogen to synthesize nonessential amino acids. Most of the research on the nutrient requirements of salmon and trout has been conducted using small fish raised in freshwater under laboratory conditions. Usually, nutrient requirements are determined by feeding small fish semi-purified diets which contain all essential nutrients at levels above the known dietary requirement, except the one being tested. The dietary level of the nutrient being tested is varied, and fish growth or some other physiological response variable, e.g., enzyme activity, tissue nutrient concentration, bone ash, is measured. The dietary level above at which additional supplementation does not result in increased growth or physiological response is considered to be the minimum dietary requirement.

Establishing an exact dietary nutrient requirement for salmonids is rarely simple. Among the numerous factors that complicate this task is the choice of physiological response variable. For example, the first studies of the quantitative

dietary requirements for water-soluble vitamins used fish weight gain, feed conversion ratio, and liver vitamin concentration as response variables (Halver, 1957). Subsequent research showed that liver vitamin concentration was not an accurate indicator of dietary sufficiency and that using liver vitamin concentration as a response variable resulted in the establishment of a dietary vitamin requirement above the levels that were required for optimum growth and health. Levels of activity of metabolic enzymes for which a specific vitamin is a co-factor have been demonstrated to be sensitive response variables for several dietary vitamins. However, enzyme activity levels must always be validated with other measures of vitamin status before being recommended for diagnostic use. Similarly, using whole body mineral concentration as a response variable is often a more sensitive measure of adequate dietary intake than is growth rate, because in many cases, fish can utilize tissue reserves to maintain growth rates even if they are in negative mineral balance, thereby lowering whole body levels. However, whole body mineral levels often simply reflect the withdrawal of minerals from storage tissues. Measuring mineral levels in the storage tissues is usually more sensitive than measuring whole body levels. In fact, using whole body levels to establish the dietary requirement for certain essential minerals sometimes leads to overestimation of the actual dietary requirement. Undoubtedly, our estimation of the nutritional requirements of salmon and trout will continue to improve as researchers refine their assessment methods.

Protein and Amino Acid Requirements

The dietary protein requirement of salmon and trout varies with age, dietary energy level, and dietary concentration and balance of amino acids. Theoretically, salmon and trout do not require dietary protein *per se*, but rather require only ten indispensable, or essential, amino acids contained in protein. Requirements for these amino acids are presented in Table 10.2. The amino acid requirements of trout and salmon are similar, with the possible exception of the requirement for arginine, which is reported to be 4% of dietary protein for rainbow trout (NRC, 1993), and 5% of dietary protein for juvenile Pacific salmon (Luzanna et al., 1998). When dietary amino acid requirements are expressed as a percentage of dietary protein, the values for salmon and trout are similar to those of swine and poultry.

When the amino acid requirements of salmon and trout are added up, they only total about 15% of the diet. Thus, theoretically, it should be possible to grow salmonids at an acceptable rate on a diet containing 15% essential amino acids, and an additional 10% or so of non-essential amino acids. In practical feeds, the dietary protein level is usually formulated to be around 45-50% for swim-up fry, and 42-45% for post-juveniles and maturing fish. The difference between these dietary protein levels and the level one would deem necessary based upon the sum of essential amino acids plus a generous addition of non-essential amino acids can be explained by differences in amino acid availability between purified and practical diets, and by the fact that salmonids are metabolically adept at using amino acids as metabolic energy sources. Protein retention rarely exceeds 50% in fish fed either practical or purified diets. An additional factor explaining the differences noted above is that in practical diets, it is nearly impossible to match the ratio of amino acids with the dietary requirement, and dietary amino acids present in excess of the amount needed for tissue protein synthesis are not stored for later use. The amount of each amino acid required for tissue protein synthesis is not precisely known, but

presumably the amount needed at any given moment is limited by the level of whatever amino acid is present in the lowest amount relative to its dietary requirement.

Table 10.2. QUANTITATIVE AMINO ACID REQUIREMENTS OF JUVENILE SALMON AND TROUT AS A PERCENTAGE OF DIETARY PROTEIN (PERCENTAGE OF DIET IN PARENTHESES)

Amino acid	Chinook Salmon	Coho Salmon	Rainbow Trout
Arginine	6.0 (2.4)	5.0 (2.3)	3.5 (1.5)
Histidine	1.8 (0.7)	1.8 (0.7)	1.6 (0.7)
Isoleucine	2.2 (0.9)		2.4 (0.9)
Leucine	3.9 (1.6)		4.4 (1.4)
Lysine	5.0 (2.0)		5.3 (1.8)
Methionine + cystine	4.0 (1.6)		1.8 (1.0)
Phenylalanine + tyrosine	5.1 (2.1)		3.1 (1.8)
Threonine	2.2 (0.9)		3.4 (0.8)
Tryptophan	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)
Valine	3.2 (1.3)		3.1 (1.2)

Source: NRC, 1993.

Energy

Wild salmon and trout derive nearly all of their dietary energy from protein and lipids; their prey are mainly insects, marine invertebrates, and small fish. Despite the fact that the average lipid content in the food consumed by Pacific salmon in the sea is over 25% lipid, expressed on a dry weight basis (Higgs et al., 1995), wild Pacific salmon do not accumulate substantial lipid stores in the tissues around their viscera. Muscle tissue is the main lipid storage site for wild Pacific salmon and trout, with the highest level present just before the fish undertake their upstream spawning migration. During the spawning migration, salmon and trout do not eat. The percentage of lipid in muscle stores varies with species and population of salmon (Table 10.3). Fish that migrate the longest distances generally have the highest lipid stores, and by the time the migrating fish spawn, they have exhausted over 90% of their lipid stores, with only 7-8% being transferred to developing ovaries (Idler and Bitners, 1959).

In contrast to wild fish, farmed salmon and trout accumulate lipid in both muscle and visceral stores, especially when fed a high energy diet. This difference is readily apparent to those in the fish processing and fish smoking industries, and is also noticeable to knowledgeable consumers. Farmed Pacific chinook and coho salmon (post-juveniles) appear to accumulate excessive amounts of visceral lipid when fed diets containing over 20-24% lipid (Silver et al., 1993). Juvenile salmon and trout raised in hatcheries also accumulate lipid when fed high energy diets;

hatchery fish are consistently found to have higher whole body lipid contents than their wild counterparts. Despite the efforts of many researchers, this difference in whole body lipid content has not been shown to reduce fish survival after hatchery release; in fact it seems to be associated with higher survival during downstream migration and higher adult returns.

Lipids are a much less expensive source of energy for salmonids than are protein and carbohydrates, when compared by cost of metabolizable energy (ME) from these various sources. For example, if fish oil and fish meal cost the same ($0.68/kg) and the ME content ($Kcal*kg^{-1}$) is 8,000 for fish oil and 4,500 for fish meal, then fish oil is clearly a less expensive source of energy and the cost of having salmon and trout utilize dietary protein for energy in a dietary formulation containing an insufficient level of energy is readily apparent. In a diet containing a well-balanced amino acid profile, the optimum ratio of protein to energy for trout fingerlings is provided by 35-40% protein to 15-20% lipid (Watanabe et al., 1979).

Table 10.3. LIPID CONTENT OF VARIOUS SPECIES OF PACIFIC SALMON WHEN TYPICALLY CAPTURED IN OCEAN FISHERIES

Species	Lipid content (% wet wt + SEM)	Range (%)
Chinook	11.5 + 2.4	2.2-19.0
Sockeye	7.5 + 1.2	1.6-19.2
Coho	5.7 + 0.5	3.1-9.0
Pink	5.3 + 0.4	2.0-9.4
Chum	4.3 + 0.6	1.3-4.3
Atlantic	11.0 + 1.1	8.7-14.0

Source: Sidwell et al., 1974.

Essential Fatty Acids

Salmon and trout require about 1-2% omega-3 fatty acids in the diet to prevent essential fatty acid deficiency signs (Castell et al., 1972; Watanabe et al., 1974). These signs include poor growth, pale liver, depigmentation, caudal fin necrosis, "shock syndrome" in which fish become unconscious during stress, and elevated tissue levels of n-9 fatty acids. In salmon and trout, these signs can be prevented by any of the n-3 fatty acids, although growth rates are reportedly higher when long-chain highly unsaturated fatty acids (HUFAs) of the n-3 group (C22:5, C22:6) are fed. Essential fatty acid deficiency signs can be exacerbated by high dietary levels of n-6 fatty acids. Usually, a level of 4-5% marine fish oil in the diet provides a sufficient dietary level of n-3 fatty acids for growing salmonids. Supplying the remaining dietary lipids from animal or plant oil sources greatly influences tissue fatty acid composition, but does not affect growth or gamete quality (Mugrditchian et al., 1981; Hardy et al., 1987; Hardy et al., 1989).

Table 10.4. VITAMIN REQUIREMENTS OF SALMONIDS

Vitamin	Mg or I.U. per kg diet[1]	Form commonly added to feeds (% active vitamin in parentheses)
Thiamin (B_1)	1	Thiamin mononitrate (92%)
Riboflavin (B_2)	4-7	Riboflavin (80%)
Pyridoxine (B_6)	3-6	Pyridoxine HCl (82%)
Pantothenic acid	20	D-calcium pantothenate (90%)
Niacin	10	Nicotinic acid (100%)
Biotin	0.15	D-biotin (2%)
Folic acid	1-2	Folic acid (80%)
Vitamin B_{12}	0.01	Cyanobalamine (1%)
Choline	1000	Choline Cl (70% liquid, 60% dry)
Myoinositol	300	M-inositol (100%)
Ascorbic acid	50	Crystalline (92%), or phosphate (35%)
Vitamin A	2500 I.U.	Acetate or palmitate (500 I.U. per g)
Vitamin D	2400 I.U.	Cholecalciferol (500 I.U. per g)
Vitamin E	50 I.U.	α-tocopheryl acetate
Vitamin K	10	Menadione sodium bisulfite (50%)

Source: NRC, 1993. Note: These values were determined using semi-purified diets fed to juvenile fish under laboratory conditions. Higher levels should be used in practical feeds to provide a margin of safety to account for losses during pelleting and storage of feeds.

Vitamin Requirements

Salmon and trout require 15 vitamins in their diet to ensure rapid growth and optimal health (NRC, 1993). The precise quantitative dietary vitamin requirements for salmon and trout of various sizes, fed various diet formulations, and reared in various environments have not yet been established, but the vitamin requirements of fry and fingerlings fed semi-purified diets in laboratory settings have been determined (Table 10.4), and these values are used in grower feeds for salmon and trout. Supplementing practical diets to ensure these levels after pelleting and storage has proven effective in preventing overt vitamin deficiency signs in practical fish culture. The actual vitamin requirements may be less than the recommended levels for some of the vitamins in certain situations; however, actual confirmation of this awaits verification of vitamin status by micro-chemical analysis of tissue levels or by measuring the activity of vitamin-dependent enzymes. In any event, the difference in cost between the recommended vitamin levels and precise dietary requirements is relatively small in practical feeds. The feeding period required to produce overt signs of vitamin deficiencies and the presence of unique or characteristic deficiency signs in fry fed vitamin-deficient semi-purified diets, can be useful in identifying certain vitamin deficiencies in salmonids. These are shown in Table 10.5. It is useful to note that weeks to months of feeding a completely deficient diet are needed to deplete tissue levels of vitamins to levels at which clinical signs appear. The time required to produce deficiency signs is influenced by fish size, environment, and other factors. For pantothenic acid, fry require 6-8

Table 10.5. CHARACTERISTIC VITAMIN DEFICIENCY SIGNS DETERMINED WITH YOUNG SALMONIDS

Vitamin	Period required to produce deficiency signs[1]	Characteristic deficiency signs
Thiamin	8-12 weeks	Hyperexcitability to sudden blow to fish tank
Riboflavin	8-12 weeks	Cataracts; photophobia
Pyridoxine	3-4 weeks	Convulsions; rapid and gasping breathing
Pantothenic acid	8-12 weeks	Clubbed gills
Niacin	14-16 weeks	Muscle spasm; poor growth and appetite
Biotin	8-12 weeks	Poor growth; skin lesions in trout
Folic acid	10-12 weeks	Anemia; absence of immature erythrocytes
Vitamin B_{12}	12-16 weeks	Fragmented erythrocytes; many immature erythrocytes
Choline	3-4 weeks	Poor growth and food conversion ratio
Inositol	8-12 weeks	Poor growth; distended abdomens; hemorrhages at base of fins
Ascorbic acid	12-20 weeks	Impaired collagen formation; spinal deformities
Vitamin A	>15 weeks	Exophthalmia; edema; acites
Vitamin D	12 weeks	Lethargy; tetany
Vitamin E1	12-20 weeks	Anemia; acites; dermal depigmentation
Vitamin K	10-14 weeks	Prolonged blood clotting time

[1]*Determined with highly purified diet, fed to juvenile fish in laboratory setting.*

weeks of feeding a completely deficient diet, while post-juvenile trout require over 28 weeks of feeding (Masumoto et al., 1994). Presumably, even longer periods of feeding would be required for clinical signs of deficiency to appear if practical diets were fed. Thus, if mortality increases in groups of salmon or trout fingerlings soon after a change in feed suppliers is made, it is extremely unlikely that a vitamin deficiency is the cause.

Mineral Requirements

Salmonids, like all animals, require dietary sources of minerals which they utilize for structural purposes, osmoregulation, and as co-factors in metabolic reactions. For many years, it was thought that salmonids obtained most of their mineral needs directly from the water in which they lived. Early studies showing significant uptake of radioactive minerals by trout directly from water supported this view. Subsequent studies have supported this view for many elements, but it is also now known that some essential mineral elements must be supplied by the diet (Lall, 1989), even when fish are reared in seawater or in high mineral content freshwater,

Table 10.6. DIETARY MINERAL REQUIREMENTS OF SALMONIDS

Mineral	Dietary requirement	Deficiency signs
Calcium	0.3%	None described
Phosphorus	0.5-0.7%	Cessation of feeding and growth; low phosphorus levels in skin and bone
Magnesium	0.05-0.07%	Poor growth; low tissue magnesium, especially bones, spinal deformity
Iron	25-50 mg/kg	Hypochromic microcytic anemia
Manganese	12-13 mg/kg	Short body dwarfism; depressed growth
Copper	1-4 mg/kg	Poor growth, low tissue copper levels
Selenium	0.03-0.1 mg/kg	High fry mortality; reduced serum glutathionine peroxidase activity
Iodine	0.1-0.3 mg/kg	Thyroid hyperplasia
Zinc	20-30 mg/kg	Lens cataracts; poor growth; low tissue zinc concentration

as shown in Table 10.6. As is the case with other animals, dietary mineral requirements will vary with fish size and reproductive status.

Determining the quantitative dietary requirements of salmon and trout for essential minerals is difficult because of the contribution of dissolved minerals in rearing water, which salmon and trout ingest with feeding. Generally, dietary sufficiency has been assessed by measuring the concentration of minerals in blood, muscle, liver, bone, and whole body of fish fed diets with increasing dietary levels (Lall, 1989). The most sensitive tissue response to measure depends on the specific mineral. In the case of phosphorus, for example, the hard tissues of the rainbow trout, e.g. bone, skin, and scales, are the main reservoirs of these mineral, containing nearly 70% of the total body phosphorus while constituting less than 20% of total body weight (Skonberg, 1997). Thus, one would expect skin/scales or bone to be more sensitive indicator tissues of phosphorus status than blood, muscle, liver, or whole body, and this is indeed the case (Skonberg, 1997).

Unlike catfish feeds, practical salmon and trout feeds contain sufficient levels of essential macro-elements, e.g., Ca, K, Mg, Na, P, to satisfy the requirements of growing salmon and trout. Fish meal is the main source of these minerals in practical feeds. Restrictions on phosphorus levels in salmon and trout hatchery effluents and increasing demand on finite world supplies of fish meal are stimulating efforts to replace a portion of the fish meal in salmonid feeds, and this trend, coupled with conversion to high-energy feeds, may require salmon and trout feeds to be supplemented with essential minerals. At present, salmon and trout feeds are routinely supplemented with several essential micro-elements (Cu, I, Mn, Se, Zn), mainly to guarantee that the fish receive sufficient amounts should the micro-elements in the diet formulation have reduced bioavailability. Bioavailability of minerals to monogastric animals varies with chemical form, with bioavailability

generally correlated with water solubility. Thus, chlorides, sulfates, and carbonates generally have a higher bioavailability than do oxides. Complicating this in salmonids is the fact that calcium phosphate, e.g. bone, is more soluble in the acidic environment of the stomach than in the neutral to basic environment of the intestine. Thus, calcium phosphate precipitates in the distal intestine, taking divalent cations, mainly Zn, out of solution at the same time. The result is that salmon and trout feeds containing fish meals having high levels of bone, such as fish scrap meal, require added micro-element supplementation to avoid mineral deficiencies, especially Zn (Ketola, 1979; Hardy and Shearer, 1985). Feeds containing high-phytate ingredients, such as plant protein supplements, make this problem worse (Richardson et al., 1985). Recently, studies have shown that supplementing trout feeds with citric acid increases the availability of Ca, P, and Zn, presumably by acidifying the feed and reducing the formation of precipitates (Suguira et al., 1998). Supplementing the trout feed with sodium bicarbonate has the opposite effect. High dietary levels of Fe reduce bioavailability of Zn and, to a lesser extent, Cu (Wekell et al., 1983; Lellis et al., 1998). Supplementing trout feeds with chelates and other compounds thought to improve the bioavailability of minerals has not had any detectable effect of mineral bioavailability (Suguira et al., 1998). In summary, overfortification of salmon and trout feeds with key essential trace minerals is recommended because it is a cost-effective way to avoid deficiencies caused by antagonistic interactions among feed ingredients.

FEED FORMULATION

Several important developments in salmonid nutrition, diet formulation and ingredient stability have occurred in the past 30 years which have enabled salmonid aquaculture to advance to today's levels of production and sophistication. They were: (1) the semi-purified test diet for salmonids that allowed the quantitative nutrient requirements to be determined; (2) the Oregon Moist Pellet, a highly functional prepared diet for salmonids; (3) steam-pelleted dry feeds for salmonids; (4) extruded feeds for salmonids, and (5) stable forms of ascorbic acid. These developments have resulted in continuous improvement in salmon and trout feed quality, with lower feed conversion ratios, higher protein retention values, and nutritional deficiencies.

Salmonid Feed Development

Prior to pelleted feeds, salmonids were fed wet feeds made primarily from beef liver, other slaughterhouse by-products, and various fish or plant products that were available to the hatchery (Hardy, 1989). Part of the work of any hatchery staff was to make fish food. Formulas such as 48% ground scrap fish, 28% liver, and 24% salmon eggs, or 47.5% fresh liver, 47.5% canned carp, and 5% dried brewer's yeast were used. The feed was formed by hand into chunks and fed fresh. As the demand for fresh ingredients outstripped supply, fresh ingredients were extended by combining them with dry feed mixtures. Fish diseases associated with poor water quality caused by soluble material leaching from feed and with the use of raw fish as a dietary component were common.

The development of the Oregon Moist Pellet (OMP) was a natural evolutionary step in production of salmonid diets made from wet-mix/dry-mix combinations, but it was a tremendous advancement. The major improvements

Table 10.7. OREGON MOIST PELLET FORMULATIONS FOR JUVENILE PACIFIC SALMON

Ingredient	Oregon mash OM-3 (%)	Oregon pellet OP-4 (%)	Oregon pellet OP-2 (%)
Herring meal	49.9	> 47.5	14.0
Other fish meal	–	–	14.0
Wheat germ meal	10.0	remainder	remainder
Dried whey	8.0	4.0	5.0
Cottonseed meal and poultry by-product meal	–	–	15.0[1]
Corn distillers dried solubles	–	–	4.0
Sodium bentonite	–	3.0	–
Vitamin premix	1.5	1.5	1.5
Mineral premix	0.1	0.1	0.1
Wet fish hydrolysate[2]	20.0	20.0	30.0
Fish oil	10.0	6.5-7.0	6.0-6.7
Choline chloride (70%)	0.5	0.5	0.5
Proximate composition:			
Crude protein		>35	>35
Crude fat		>10	>10
Moisture		<35	<35
Crude fiber		<4	<4

[1]*Use maximum of 10% cottonseed meal and 8% poultry byproduct meal.*
[2]*Ground, pasteurized fish or fish processing waste may also be used.*
Note: Diet must be stored frozen.

over earlier feeds were the hydrolysis and pasteurization of the wet mix, made from fish processing waste or underutilized fish, and the combination of ingredients used in the formulation that resulted in a wet mash that could be pelleted, frozen, sacked, and shipped to hatcheries from a feed plant. This eliminated the transmission of disease from the feed and the need for each hatchery to prepare its own feed. The OMP was modified from its original formulation to reflect changes in the availability and price of ingredients, as shown in Table 10.7. Eventually, other feed types evolved to the point where performance of juvenile salmon fed such feeds was equal to those fed the OMP, and the OMP was replaced by dry or semi-moist feeds. In commercial trout farming, dry pelleted feeds have been used since the industry first was developed. In Pacific salmon hatchery production, pelleted feeds based upon open formulas, such as the Abernathy diet shown in Table 10.8, and the West Vancouver salmon diet shown in Table 10.9, have replaced the OMP (Hardy, 1991). Federal trout hatcheries in the United States raising fish for stocking rivers and lakes used open-formula feeds for many years, but now purchase closed-formula feeds from commercial producers. In Canada, the Ministry of Natural Resources in

Table 10.8. ABERNATHY DIET FORMULATIONS FOR JUVENILE PACIFIC SALMON

Ingredient	S9 (92), Mash (%)	A2-2 (92), Starter (%)	A3-2 (92), Grower (%)
Fish meal (herring or anchovy)	58.0	55.0	46.5
Dried whey or wheat flour	5.0	5.0	5.0
Wheat middlings	6.17	4.5	13.0
Wheat germ meal	–	5.0	5.0
Spray-dried blood meal	2.5	2.5	2.5
Feather meal	7.5	10.0	10.0
Poultry by-product meal	3.0	3.0	3.0
Condensed fish solubles or poultry by-product meal or fish meal	1.5	1.5	1.5
Vitamin premix[1]	1.5	1.5	1.5
Mineral premix[2]	0.05	0.1	0.1
Choline chloride	0.58	0.58	0.58
Ascorbic acid	0.2	0.2	0.2
Calcium propionate (fungistat)	0.125	0.125	0.125
Lignon sulfonate pellet binder	2.0	2.0	2.0
Fish oil or fish oil plus soybean lecithin (max. 2%)	12.0	9.0	9.0
Proximate composition:			
Crude protein	>48	>45	>41
Crude fat	17-19	13-17	13-17
Moisture	<10	<10	<10

[1]Vitamin premix should meet or exceed NRC (1993) requirements, plus an allowance for processing and storage losses.

[2]Trace mineral premix supplies the following per kg diet: Zn (as $ZnSO_4 \cdot 7\ H_2O$), 75 mg; Mn (as $MnSO_4 \cdot H_2O$), 20 mg; Cu (as $CuSO_4 \cdot 5\ H_2O$), 1.54 mg; I (as KI), 10 mg.

Ontario publishes open-formula diets for trout and salmon, such as those is Table 10.9. Several feed manufacturers continue to produce semi-moist feeds (12-25% moisture) which contain mold inhibitors and preservatives, thus permitting extended storage without freezing. These feeds are commonly used as starter feeds for Pacific salmon.

When commercial Atlantic salmon farming began in Europe, high-protein pelleted dry feeds, and moist feed were used. The ratio of wet material to dry mix in the moist feeds was about 60:40, and these feeds were usually fed within two or three days of production. Today, nearly all feeds used in commercial salmon farming are made by cooking-extrusion. Cooking-extrusion was adopted over compression steam-pelleting for four reasons: (1) lower pellet density, reducing the sinking rate; (2) higher levels of fish oil could be top-dressed onto extruded pellets;

(3) cooking-extrusion gelatinized the starch in salmon feeds, thus increasing water stability and digestibility; and (4) extruded pellets are less likely to break during shipping and handling. Today, relatively low levels of starch are added to salmon and trout feeds because salmonids cannot tolerate high levels of available carbohydrates. A primary function of carbohydrates is as a binding agent in extruded feeds.

Table 10.9. WEST VANCOUVER DIET FORMULATION FOR JUVENILE PACIFIC SALMON AND ONTARIO MINISTRY OF NATURAL RESOURCES (MNR) DIET FORMULATIONS FOR RAINBOW TROUT

Ingredient	W. Vancouver (mg/kg)	MNR-89S (%)	MNR-89G (%)
Herring meal (steam-dried)	504.2	–	–
Fish meal	–	40.0	20.0
Soybean meal	–	8.0	12.0
Corn gluten meal	–	12.0	17.0
Brewer's yeast	–	5.0	–
Dried whey	76.9	9.5	7.0
Wheat middlings	69.7	–	20.0
Freeze-dried euphausids	20.6	–	–
Shrimp meal	31.5	–	–
Blood flour	50.2	10.0	9.0
Poultry by-product meal	73.5	–	–
Vitamin premix[1]	43.0	1.5	1.0
Mineral premix[2]	20.4	1.0	1.0
Choline chloride (60%)	4.7	–[3]	–[3]
Ascorbic acid	1.9	–[3]	–[3]
Lignon sulfonate pellet binder	18.9	–	–
Herring or salmon oil, antioxidant	84.5	13.0	13.0
Proximate composition:			
Crude protein, %	>48	>45	>41
Crude fat, %	17-19	13-17	13-17
Moisture, %	<10	<10	<10

[1]Vitamin premix should meet or exceed NRC (1993) requriements, plus an allowance for processing and storage losses.

[2]Trace mineral premix supplies the following per kg premix: Zn (as ZnSO4.7 H2O), 28.4 mg; Mn (as MnSO4.H2O), 69.2 mg; Cu (as CuSO4.5 H2O), 3.29 mg; I (as KI), 5.1 mg; Co (as CoCl.6 H2O), 0.94 mg; Fe (as FeSO4.7 H2O), 47.3 mg; and Na (as NaCl), 1951 mg.

[3]Included in vitamin premix to supply 600 and 400 mg of ascorbic acid, and 1500 and 1000 mg of choline chloride in diet formulations 89S and 89G, respectively.

Feed Ingredients in Salmonid Diets

The typical salmon or trout diet consists of fish meal, other high-protein plant or animal sources, fish oil, grain-derived products for binding, and micro-nutrient

premixes. Diet formulations for salmonids are very high in protein, fat, and digestible energy compared to other animal feeds, and this limits the use of many common feed ingredients, namely grains, used in conventional animal feeds. Another factor affecting feed ingredient choice is that undigested feed is excreted into the aqueous environment and becomes a pollutant. Restrictions on the concentration of suspended solids, and in many regions, phosphorus, in aquaculture effluents dictate that feeds be produced from highly digestible ingredients. Ingredients which contain relatively high amounts of crude fiber or starch, or which are low in protein and metabolizable energy, cannot be included in salmonid feeds. Thus, corn and other whole grains, the primary ingredients in feeds for domestic animals, are not used. Ground wheat is used sparingly at levels between 7-12% as a binder. The trend in commercial salmon feeds has been toward increasing the level of fish oil so that over the last 20 years, the lipid content of salmon feeds has increased from approximately 15% to over 30% (Torrisson et al., 1995). Over the same period, the quality of fish meal used in salmon feeds has increased and the use of other protein sources in salmon feeds decreased, so that by the mid-1990s, salmon feeds were comprised of fish meal, fish oil, ground wheat or some gelatinized grain by-product, and micro-nutrient premixes. There was no room in these feed formulations for any other ingredient, particularly if it contained less protein than premium grade fish meal. Feed conversion ratios decreased during this period from 1.8-2.0, to about 1.0 or less. Protein retention values increased from about 20% to nearly 50%.

An important reason for these changes to more concentrated feeds has been to comply with regulations devised to limit the levels of phosphorus in freshwater hatchery effluents, especially in Norway (Kossman, 1989). The approach by regulators has been to restrict the amount of feed an individual farmer can purchase to raise a fixed amount of fish. In order to remain profitable, farmers have had to use more efficient feeds, which in turn resulted in the changes in feed formulation and ingredient selection described above. In North America, regulators have taken a different approach, simply limiting effluent levels of various materials and letting fish culturists and the salmonid feed industry use their ingenuity to meet target effluent levels.

In addition to ingredient limitations caused by the high dietary protein and lipid levels in salmon feeds and by pollution concerns, the presence of antinutritional factors and compounds that reduce palatability have restricted the use of feed ingredients, mainly those derived from oilseeds. Fry and juvenile Pacific salmon find diets containing even low levels of soybean meal unpalatable, while trout appear to tolerate much higher dietary levels (Fowler, 1980; Bureau et al., 1996). Additional heating and adding krill to enhance palatability improves performance of juvenile Pacific salmon fed feeds containing soybean meal, but not to levels of fish fed fish meal-based feeds (Arndt et al., 1998). Older salmon and rainbow trout will consume diets in which fish meal in completely replaced by soybean meal, but performance of fish is improved if some fish meal remains in the feed (Watanabe and Pongmaneerat, 1993). Cottonseed meal can be fed at levels up to 15% of salmon and trout grower feeds but not broodstock feeds. Higher levels are not recommended due to the gossypol content of cottonseed meal. Canola meal contains compounds which impair thyroid function, so salmon diets should contain no more than 15% and trout diets should be restricted to 25%. However, protein concentrates made from soybeans and canola can completely replace fish meal in

grower feeds for salmon and trout (Higgs et al., 1995; Kaushik et al., 1995; Stickney et al., 1996; Weede, 1997). High-ash (>15%) fish meal, meat and bone meal, and poultry by-product meal are generally restricted in salmon and trout feed formulations because of concerns over high phosphorus levels in the hatchery or farm effluents.

Specialized Diets

Salmon and trout diets can be modified to enhance reproductive success, to alter the lipid level and fatty acid composition of the fish, and to modify sensory attributes , such as color, aroma and flavor. Broodstock diet formulations are similar to grower diet formulations, with additional supplements of carotenoid pigments, vitamins, and trace minerals. Pacific salmon and steelhead trout are raised in hatcheries for release as juveniles, and it is well known that hatchery fish contain higher amounts of lipid than their wild cohorts. Opinions differ concerning the effects of body lipid level on survival of the fish after hatchery release, but no data exists to support the hypothesis that high levels of body lipid reduce fish survival; in fact, as mentioned earlier, extant information argues the opposite. Nevertheless, adjusting feeding levels and dietary lipid levels to match the seasonal flux of protein and lipid accretion by juvenile Pacific salmon will likely produce healthier fish and reduce feed waste.

Modifications to salmon and trout feeds to influence sensory attributes of farmed products have mainly been limited to carotenoid supplementation. Salmonids cannot synthesize carotenoid pigments; they obtain these pigments from their prey (Torrisson et al., 1989). In nature, these pigments are synthesized by algae and obtained through the food chain. Farmed salmon must be fed diets containing carotenoid pigments to color the skin and flesh of the fish. Early salmon feeds contained carotenoid pigments derived from natural sources, mainly crustacea such as shrimp, crab, and krill. Today, nearly all carotenoid pigment supplementation to salmon and trout feeds is from Carophyll pink™, which is gelatin-encapsulated astaxanthin, the pigment found in crustacea and wild salmonids. Because the astaxanthin in Carophyll pink™ is produced by chemical synthesis, it contains a mixture of isomers that differs from the mixture of isomers in astaxanthin from natural sources. The ratio of astaxanthin isomers can be used to identify wild or farmed salmonids. For decades there has been speculation about whether or not astaxanthin was an essential dietary nutrient for salmonids, and speculation about the metabolic role of carotenoid pigments (Tacon, 1981). Recently, astaxanthin was shown to be essential in the diet of Atlantic salmon fry which were offspring of females reared throughout their life cycle on feeds devoid of carotenoid pigments (Christiansen et al., 1995).

Salmon and trout feeds can be modified to alter the lipid level and fatty acid composition, aroma and taste, fresh product shelf-life, and its frozen storage stability of products of salmon farming. Increasing the lipid level of the feed increases lipid storage levels in both viscera and muscle depots of salmon and trout. For example, wild trout typically contain about 4-5% lipid in muscle tissue compared to 5-8% in farmed fish fed dietary lipid levels similar to the lipid level in the natural diet of wild trout. However, feeding high-lipid diets to rainbow trout can result in muscle lipid levels in excess of 20%, on a wet-weight basis. Similarly, wild chinook salmon contain an average of 11.5% lipid in muscle tissue (range 2.2-19.0%, Sidwell et al., 1974) in the months prior to their spawning

migration. The average percentage lipid in muscle tissue of farmed chinook salmon increased from below 5% to 8% when dietary lipid level increased from 15% to 25% (Silver et al., 1993).

Tissue fatty acid composition of salmon and trout generally reflects the fatty acid profile of lipid sources used in the feed. Fillets of Pacific and Atlantic salmon fed feeds containing soybean oil in place of fish oil have higher levels of linoleic acid and lower levels of omega-3 fatty acids than do salmon fed diets containing fish oil (Hardy et al., 1987; 1991). Fillets of Atlantic salmon fed feeds containing menhaden oil have higher levels of omega-3 fatty acids than do fillets of fish fed feeds containing herring oil. Rainbow trout fed feeds containing sunflower oil in place of fish oil have much higher levels of oleic acid than control fish, and score higher in taste tests (Skonberg et al., 1995). In general, consumers in North America prefer fish that do not smell or taste fishy, and rainbow trout fed feeds in which fish oil is replaced with plant oils are less fishy than those fed feeds containing fish oil.

Supplementing rainbow trout feeds with a-tocopherol during the last few months of rearing greatly increases the concentration of a-tocopherol in the tissues (Boggio et al., 1985), which, in theory, should provide delay the onset of lipid oxidation in fresh or frozen fish products. However, Boggio et al. (1985) were unable to detect any difference in degree of lipid oxidation among high or low α-tocopherol fillets after 10 months of frozen storage. Skonberg et al. (1993) reported that fillets of fish fed diets containing sunflower oil had lower concentrations of polyunsaturated fatty acids and were resistant to lipid oxidation after thawing compared to fillets from fish fed feeds containing fish oil.

Low-polluting diets are formulated to reduce organic matter and phosphorus levels in hatchery and farm effluent by reducing the levels of indigestible material and unavailable phosphorus in the diet. Reducing phosphorus levels in hatchery and farm effluents involves careful formulation to match the available phosphorus concentration in the feed to the requirements of the fish and reducing the amount of phosphorus in the feed that cannot be digested by the fish. An additional reduction in the amount of phosphorus generated by salmon and trout farming can be made by feeding a phosphorus deficient diet during the final grow-out period of production (Lellis et al., 1998). Salmonids maintain a body reserve of phosphorus in their hard tissues (bone, skin, fins, scales) from which they can withdraw phosphorus to maintain plasma levels and maintain other critical functions when dietary intake is below their requirement (Hardy et al, 1991; Skonberg, 1997). Depending upon their growth rate, salmon and trout can continue growing normally when fed phosphorus-deficient feeds for some time. Eventually, the fish use up their body reserves, become anorexic, and stop growing. If the fish are harvested before they use up their body reserves, they suffer no ill effects, and if they are fed a phosphorus-sufficient feed, their body reserves are restored.

FEEDING PRACTICES

Feed is the largest single operating expense in salmonid aquaculture, averaging about 55% of the cost of rearing fish (Higgs et al., 1995). Unlike domestic animals, farmed fish do not feed themselves; rather they must be fed. How this is done affects the profitability of salmon and trout farming and affects the extent to which salmon and trout farming affect the aquatic environment. Feeding practices, e.g., feeding

level, feeding frequency, feed particle size, and feed delivery system, affect fish growth rates, feed conversion rates, uniformity of fish size within a pond, and, as mentioned, the cost of fish production and the amount of waste that must be captured before it leaves the farm and pollutes the environment.

Of the feeding practices, feeding level is the most important variable influencing fish growth and feed conversion values. Feeding level ranges from starvation to maximum feeding, as illustrated schematically in Figure 10.4. At some point, generally around 1% of fish biomass, the amount of feed fed to salmon and trout meets their metabolic and activity needs. This point is called the maintenance feeding level; the feeding level at which fish neither gain nor lose

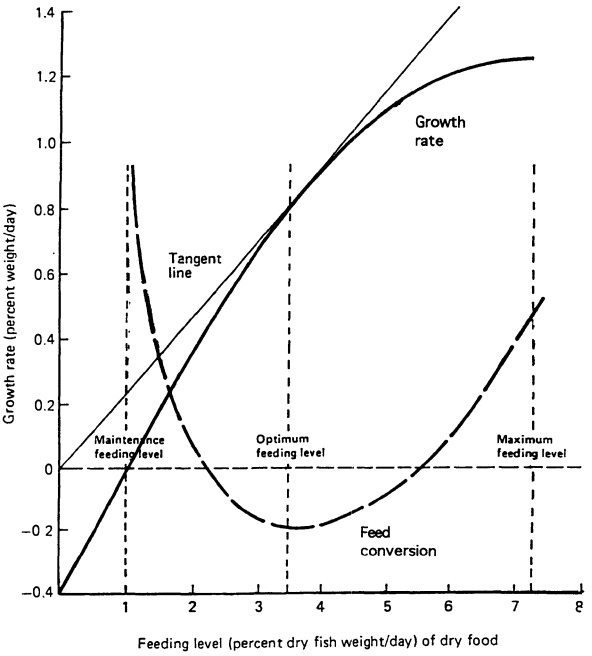


Figure 10.4 Theoretical relationship between feeding level, growth rate, and feed conversion.

weight. Obviously, the maintenance feeding level varies with water temperature, fish weight and life cycle stage, photoperiod, moisture and energy content of feed, rearing density, and water quality. As feeding level increases above the maintenance level, salmon and trout gain weight and length in a more-or-less linear fashion, until the feeding level approaches the maximum amount the fish can consume. Within this range of feeding levels, the efficiency of conversion of feed into fish weight gain diminishes and weight gain no longer increases in direct proportion to feeding level. Feeding level eventually reaches a maximum point beyond which fish simply cannot consume more feed.

Salmon and trout farmers generally control the amount that they feed their fish. Feeding charts are available from feed manufacturers and other sources that provide guidelines for determining appropriate feeding levels. In Norway, juvenile

Atlantic salmon are usually fed using automatic feeders, making it necessary to have an accurate estimate of expected feed intake at a given water temperature and fish size. Such estimates are available (Austreng, et al., 1987), and often fish are fed a ration that slightly exceeds the expected feed intake. Many Pacific salmon conservation hatcheries in North America calculate feeding levels using the method of Buterbaugh and Willoughby (1967). At a constant water temperature, the percent body weight to feed daily is calculated by dividing a hatchery constant by the fish length in inches. The hatchery constant is determined by multiplying 300 times the desired feed conversion value times the daily increase in fish length. This method assumes that the available protein and energy in feed is relatively constant

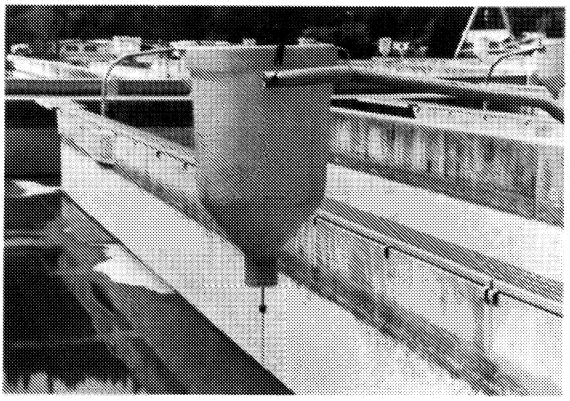


Figure 10.5 Demand feeder in trout raceway. Fish bumps the rod at bottom of the feeder and pellets fall into the water.

from year to year. In commercial salmon and trout farming, the daily feed ration is usually weighed and delivered to the fish using methods described below.

The relationship between feeding level and feed conversion ratio is well illustrated in Figure 10.4. Under any conditions and with any feed, there is a feeding level at which feed conversion ratio will be the lowest (most efficient) and a higher feeding level at which growth will be maximum.

Feed particle size should be increased as fish grow, and feeding charts such as Table 10.10, provide guidelines for selecting appropriate feed particle and pellet sizes during salmon and trout production cycles. Feeding a correct particle or pellet size is important because if a pellet is too small, feed will be wasted. If a particle or pellet is too large, the fish will have to break up the feed before swallowing, also increasing feed waste. A group of salmon or trout are never all the same size and, for this reason, farmers generally combine pellet sizes during the period that they are switching to a larger pellet. This allows the smaller fish within the group to keep up.

Feeding frequency is another operational variable in salmon and trout rearing. First-feeding fry require almost constant feeding, while grow-out fish are usually fed heavily once or twice a day. The point of optimizing feeding frequency is to ensure that aggressive fish within a tank, raceway, or pen are not the only fish consuming feed at each feeding. In other words, feeding frequency must be linked to

Table 10.10. GUIDELINES FOR APPROPRIATE FEED PARTICLE/PELLET SIZE FOR SALMON AND TROUT OF VARIOUS SIZES

Fish weight (g)	Particle/pellet size (mm)
<0.3	<0.5
0.3-0.5	0.5-0.8
0.5-0.9	0.8
0.9-2.0	1.0
2.0-4.0	1.5
4.0-8.0	2.5
8.0-20	3.0
20-50	3.5
50-125	4.0
125-500	4.5
500-1000	6.0
1000-2000	9.0

Note: Feed sizes may vary with species and with type of pellet, e.g. semi-moist, extruded, pelleted.

the amount fed at each feeding to make sure that all fish have an opportunity to feed. This prevents excessive size disparity within the group from too-frequent feeding or wasted feed and water pollution associated with a single or several large feedings where the size of the meal may exceed the amount the fish can consume.

Salmon and trout can be fed by hand, automatic feeders, or demand feeders. Hand feeding a series of discrete meals is common with fry and fingerling rearing, but once fish are transferred to larger tanks, the labor costs are too high. In some situations, fish in raceways may be fed using mechanical blowers that distribute a large amount of feed from a truck or tractor, but most salmon and trout farms now use automatic or demand feeders to reduce labor costs. Modern salmon farms often use automatic feeders which deliver feed to pens by blowing pellets through tubes. The daily ration for each pen is controlled by a computer. Before the use of feedback devices, such as underwater cameras and ultrasonic detectors, that measure uneaten feed falling to the bottom of net cages, automatic feeders wasted feed because they delivered feed to pens whether or not the salmon ate it. Now, the amount fed to each pen is based upon the computer program and nearly instantaneously adjusted to the appetite of the fish. In the United States, many trout farms use demand feeders, simple mechanical devices that deliver a small amount of feed in response to an action by the fish. Demand feeders consist of a feed hopper that tapers to a conical bottom with a narrow opening that allows feed to flow onto a movable platform. A rod extends from the platform into the water and when fish bump the rod, feed falls into the water. This device is illustrated in Figure 10.5. The daily feed ration is added to the hopper each morning, and the fish feed throughout the day, not just between 8:00 AM and 5:00 PM. Compared to hand feeding, using demand feeders reduces fluctuations in dissolved oxygen concentrations in the water associated with feeding activity by the fish.

REFERENCES

ARNDT, R., HARDY, R.W. and DONG, F.M., 1998. Soybean products as components of juvenile Pacific salmon feeds. Aquaculture, in press.

AUSTRENG, E., STOREBAKKEN, T. and ASGARD, T. 1987. Growth rate estimates for cultured Atlantic salmon and rainbow trout. Aquaculture, 60, 157-160.

BOGGIO, S.M., HARDY, R.W., BABBITT, J.K. and BRANNON, E.L., 1985. The influence of dietary lipid source and alpha-tocopheryl acetate level on product quality of rainbow trout (Salmo gairdneri). Aquaculture, 51: 13-24.

BUREAU, D.P., HARRIS, A.M. and CHO, C.Y., 1996. The effects of a saponin extract from soybean meal on feed intake and growth of chinook salmon and rainbow trout. Proc. VI. Int. Symp. on Feeding and Nutrition in Fish, College Station, Texas. (Abst.).

BUTERBAUGH, G.L. and WILLOUGHBY, H. 1967. A feeding guide for brook, brown, and rainbow trout. Prog. Fish-Cult. 29: 210-215.

CASTELL, J.D., SINNHUBER, R.O., WALES, J.H. and LEE, D.J., 1972. Essential fatty acids in the diet of rainbow trout (Salmo gairdneri): Growth, feed conversion and some gross deficiency signs. J. Nutr., 102: 77-86.

CHO, C.Y. and COWEY, C.B., 1991. Rainbow trout, Oncorhynchus mykiss. In: Handbook of Nutrient Requirements of Finfish, R.P.Wilson (ed.). CRC Press, Inc., Boca Raton., FL, pp.131-143.

CHRISTIANSEN et al., 1995.

FOWLER, L.G. and BURROWS, R.E., 1971. The Abernathy salmon diet. Prog. Fish-Cult., 33: 67-75.

FOWLER, L.G., 1980. Substitution of soybean meal and cottonseed products for fish meal in diets fed to chinook and coho salmon. Prog. Fish-Cult. 42: 87-91.

GROOT, C. and MARGOLIS, L., eds., 1995. Pacific Salmon Life Histories, UBC Press, Vancouver, BC.

HALVER, J.E., 1957. Nutrition of salmonid fishes. 4. Water-soluble vitamin requirements of chinook salmon. J. Nutr., 62: 225-243.

HARDY, R.W, 1989. Diet preparation. In Fish Nutrition, Second Edition, J. E. Halver (ed). Academic Press, NY, pp. 473-546.

HARDY, R.W, 1991. Nutrient Requirements of Pacific Salmon. In Nutrient Requirements of Fish, R. P. Wilson (ed). CRC Press, Inc., Boca Raton, FL, pp. 105-121.

HARDY, R.W. and SHEARER, K.D., 1985. Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (Salmo gairdneri). Can. J. Fish Aquat. Sci. 42: 181-184.

HARDY, R.W. and CASTRO, E., 1994. Characteristics of the Chilean salmon feed industry. Aquaculture, 124: 307-320.

HARDY, R.W., SCOTT, T.M. and HARRELL, L.W., 1987. Replacement of herring oil with menhaden oil, soybean oil, or tallow in the diets of Atlantic salmon raised in marine net-pens. Aquaculture, 62: 267-277.

HARDY, R.W., FAIRGRIEVE, W.T. and SCOTT, T.M., 1991. Periodic feeding of low-phosphorus diet and phosphorus retention in rainbow trout (Oncorhynchus mykiss). In Fish Nutrition in Practice, S. J. Kaushik and P. Luquet (eds). INRA, Paris, 1993 (Les Colloques, #61), pp. 403-412.

HARDY, R.W., MASUMOTO, T, FAIRGRIEVE, W.T. and STICKNEY, R.R., 1989. The effects of dietary lipid source on muscle and egg fatty acid composition and reproductive performance of coho salmon (Oncorhynchus kisutch). In Proc. Third Int. Symp. on Feeding and Nutri. in Fish, Aug. 28-Sept 1, Toba, Japan, pp. 347-355.

HIGGS, D.A., DOSANJH, B.S., PRENDERGAST, A.F., BEAMES, R.M., HARDY, R.W., RILEY, W and DEACON, G., 1995. Use of rapeseed/canola protein products in finfish diets. In: C.E. Lim and D.J. Sessa, (editors), Nutrition and Utilization Technology in Aquaculture. AOCS Press, Champaign, pp. 130-156.

HIGGS, D.A., MACDONALD, J.S., LEVINGS, C.D. and DOSANJH, B.S., 1995. Nutrition and feeding habits in relation to life history. Physiological Ecology of Pacific Salmon, C. Groot, L. Margolis, and W.C. Clark, eds. UBC Press, Vancouver, pp.159-315.

IDLER, D.R. and BITNERS, I., 1959. Biochemical studies on sockeye salmon during spawning migration. V. Cholesterol, fat, protein, and water in the body of the standard fish . J. Fish. Res. Bd. Can., 16: 235-241.

KAUSHIK, S.J., CRAVEDI, J.P., LALLES, J.P., SUMPTER, J., FAUCONNEAU, B., and LAROCH, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout,

Oncorhynchus mykiss. Aquaculture, 133: 257-274.
KETOLA, H.G., 1979. Influence of dietary zinc on cataracts in rainbow trout (Salmo gairdneri). J. Nutr., 109: 965-969.
KOSSMAN, H., 1989. Present status and problems of aquaculture in the Nordic countries with special reference to fish feed. Pp. 27-39 In: Proc. Third Int. Symp. on Feeding and Nutr. In Fish, Toba, Japan, Aug. 28-Sept. 1, 1989. M. Takeda and T. Watanabe, eds.
LALL, S.P., 1989. The Minerals. In Fish Nutrition, Second Edition, J. E. Halver (ed). Academic Press, NY, pp. 219-257.
LELLIS, W.A., BARROWS, F.T., DONG, F.M. and HARDY, R.W., 1998. Development of low-phosphorus finishing diets for rainbow trout (Oncorhynchus mykiss). Aquaculture, in press.
Luzzana, U., Hardy, R.W. and Halver, J.E., 1998. Dietary arginine requirement of fingerling coho salmon (Oncorhynchus kisutch). Aquaculture, in press.
MASUMOTO, T., HARDY, R.W. and STICKNEY, R.R., 1994. Pantothenic acid deficiency detection in rainbow trout (Oncorhynchus mykiss). J. Nutr. 124: 430-435.
MUGRDITCHIAN, D.S., HARDY, R.W. and IWAOKA, W.T., 1981. Linseed oil and animal fat as alternative lipid sources in dry diets in chinook salmon (O. tshawytscha). Aquaculture, 25: 161-172.
NATIONAL RESEARCH COUNCIL (NRC), 1993. Nutrient Requirements of Fish. National Academy Press, Washington, D.C., 114 pp.
RICHARDSON, N.L., HIGGS, D.A., BEAMES, R.M. and McBRIDE, J.R., 1985. Influence of dietary calcium, phosphorus, zinc, and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (Oncorhynchus tshawytscha). J. Nutr., 115: 553-567.
SIDWELL, V.D., FONCANNON, P.R., MOORE, N.S. and BONNET, J.C., 1974. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish, and mollusks. I. Protein, fat, moisture, ash, carbohydrate, energy value, and cholesterol. Mar. Fish. Rev., 36: 21-35.
SILVER, G.R., HIGGS, D.A., DOSANJH, B.S., McKEOWN, B.A., DEACON, G. and FRENCH, D., 1993. Effect of dietary protein to lipid ratio on growth and chemical composition of chinook salmon (Oncorhynchus tshawytschas) in sea water. In Fish Nutrition in Practice, S. J. Kaushik and P. Luquet (eds). INRA, Paris, 1993 (Les Colloques, #61), pp. 459-468.
SKONBERG, D.I., 1997. A nutritional approach to reduce phosphorus pollution in hatchery effluent. Ph.D. dissertation, University of Washington, Seattle, WA., 181 pp.
SKONBERG, D.I., RASCO, B.A. and DONG, F.M., 1993. Effects of feeding high monounsaturated sunflower oil diets on sensory attributes of salmonid fillets. J. Aquat. Food Product Tech., 2: 117-133.
SKONBERG, D.I., HARDY, R.W., BARROWS, F.T. and DONG, F.M., 1998. Color and flavor analyses of fillet from farm-raised rainbow trout fed low-polluting feeds. Aquaculture., in press.
Stickney, R.R., Hardy, R.W., Koch, K., Harrold, R., SEAWRIGHT, D. and MASSEE K.C., 1996. The effects of substituting selected oilseed protein concentrates for fish meal in rainbow trout diets. J. World Aqua. Soc., 27: 57-63.
STICKNEY, R.R., 1996. Aquaculture in the United States, a Historical Survey. John Wiley & Sons, Inc., New York. 371 pp.
SUGIURA, S.H., DONG, F.M., RATHBONE, C.K. and HARDY, R.W., 1997. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture, in press.
TACON, A.G.J., 1981. Speculative review of possible carotenoid function in fish. Prog. Fish-Cult., 43: 205-208.
TILSETH, S., HANSEN, T. and MOLLER, D., 1991. Historical development of salmon culture. Aquaculture, 98: 1-9.
TORRISSEN, O.J., HARDY, R.W. and SHEARER, K.D., 1989. Pigmentation of salmonids - Carotenoid deposition and metabolism. Rev. in Aquat. Sci., 1(2): 209-225.
TORRISSEN, O.J., HOLM, J.C., NAEVDAL, G. and HANSEN, T., 1995. Aquaculture in Norway. World Aquaculture, 26: 11-20.
WATANABE, T., OGINO, C., KOSHIISHI, Y. MATSUNAGA, T., 1974. Requirement of rainbow trout for essential fatty acids. Bull. Jpn. Soc. Sci. Fish., 40: 493-499.
WATANABE, T., TAKEUCHI, T. and OGINO, C., 1979. Studies on the Sparing Effect of Lipids on Dietary Protein in Rainbow Trout (Salmo gairdneri). Proc. World Symp. on Finfish Nutrition and Fishfeed Tech. Hamburg 20-23 June 1978. Vol. 1. Berlin 1979.
WATANABE, T. and PONGMANEERAT, J., 1993. Potential of soybean meal as a protein source in extruded pellets for rainbow trout. Nippon Suisan Gakkaishi, 59: 1415-1423.
WEEDE, N, 1997. Low phosphorus plant protein ingredients in finishing diets for rainbow trout (Oncorhynchus mykiss). M.S. thesis, University of Washington, Seattle, 147 pp.
WEKELL, J.C., SHEARER, K.D. and GAUGLITZ, E.J.JR., 1983. Zinc supplementation of trout diets: tissue indicators of body zinc status. Prog. Fish-Cult., 48: 205-212.

11 FEEDING HYBRID STRIPED BASS

Carl D. Webster

Hybrid striped bass is a rapidly emerging aquaculture species in the United States. In 1989, production of food-fish was 400,000 pounds with a majority of the fish being grown by one producer in California. However, by 1993, production was estimated at about 5 million pounds with a projected farm value of $11 million, while estimated production values for 1996 are 7.8 million pounds. The majority of the production is from tank-culture systems, however, production from ponds has been increased recently in several states, including Maryland, Mississippi, North Carolina, and Tennessee. Producers of hybrid striped bass are located throughout the United States with 40% of production coming from the Southwestern United States (with California as the leading producer), 30% produced in the Southeastern United States (with Mississippi as the leading producer), 15% from the Mid-Atlantic region of the United States (with North Carolina as the leading producer), and 15% from the Northeastern United states (with Massachusetts as the leading producer). The average FOB farm price for fresh or live product ranges between $2.50-3.50/lb.

Hybrid striped bass are produced by crossing the striped bass (*Morone saxatilis)* with white bass *(M. Chrysops)*. Two crosses are most commonly utilized by aquaculturists. One is called the "original cross" (or Palmetto bass) which is produced by crossing a female striped bass with a male white bass. The other cross is called the "reciprocal cross" (or Sunshine bass) and is produced by crossing the female white bass with the male striped bass. This cross is used by many producers due to the ease of obtaining suitable white bass females for spawning compared to the legal difficulties in obtaining broodstock female striped bass.

GENERAL CULTURE METHODS

Hybrid striped bass require warm water temperatures to achieve optimal growth (28-30°C). Most hybrid striped bass fingerlings sold for grow-out are produced in ponds, while most fish sold as foodfish are produced in tanks. However, hybrid striped bass can be cultured using numerous means including ponds, raceways, and cages. Hybrid striped bass culture is divided into four phases of production: the hatchery phase; phase I culture in which the fish are grown from larvae (fry) to fingerling (8 cm); phase II culture where the fish are reared for 5 to 9 months and grow from 8 to 22 cm in length; and phase III culture in which the fish are grown to market size (700-1200 g).

Spawning and Fry Production (Phase I)

Broodstock are placed in tanks, preferably circular tanks, and water temperature in the holding tanks should remain constant (17-20°C) and be similar to the water temperature that the eggs will be incubated. Various types and combinations of hormones have been used to induce ovulation of suitable striped bass females, but primarily human chorionic gonadotropin (HCG) is used. Recommended dose levels of HCG are between 50 and 100 IU per kilogram of body weight (Stevens, 1966; Bonn et al. 1976), although it appears that higher doses (up to 800 IU kg^{-1}) have no negative effect on ovulation. White bass females would be injected at a dose between 200 and 400 IU kg^{-1} (Bonn et al. 1976). Milt production in male striped bass can be enhanced by injection of 20 to 30 IU kg^{-1}.

Eggs are staged for incubation at various time intervals. When ready to ovulate, the eggs are easy to expel from the female with a slight pressure applied to the posterior of the abdomen. When ready to obtain eggs, the female is anesthetized and eggs are manually stripped into a container of water, milt from several males is then added to the egg/water mixture and gently stirred with a feather to ensure optimal fertilization. Another method can be used whereby eggs are stripped into a dry container, milt added, and then water is added to the egg/milt mixture. After stirring for 2-3 minutes, the eggs can be placed into incubation jars (Figure 11.1) and allowed to hatch. Time required for striped bass eggs to hatch will vary depending upon water temperature, but at water temperatures between 16 and 18°C, it should take between 48-56 hours, while white bass eggs take approximately 40 hours to hatch at 18-20°C. Upon hatching, the larvae can be held in any non-toxic container as long as water flow is sufficient to keep them suspended in the water column.

Newly-hatched larvae have incomplete mouth parts and depend upon their yolk sac and lipid globule for nutrients. At 5 days of age, palmetto bass larvae have functional mouth parts, a simple digestive tract, and began to seek food. Sunshine bass larvae have functional mouth parts and a digestive tract when 4 days old and are 60% smaller than palmetto bass larvae. The larval fish (or fry) are usually moved from the hatchery when they begin feeding to appropriately prepared ponds. The ponds should have an abundance of zooplankton, be free of predators and have suitable water quality (dissolved oxygen, temperature, pH).

Optimal water temperatures for two-day-old hybrid striped bass can range from 16-21°F. Dissolved oxygen levels should be maintained above 6.0 mg L^{-1} (ppm) while pH should range between 7.0 and 8.5. Alkalinity of the water should be between 40 and 330 mg L^{-1} $CaCO_3$ with alkalinity and total hardness of 150 mg

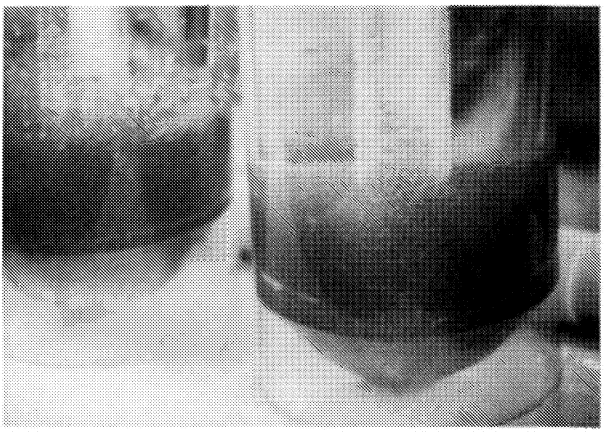


Figure 11.1 Incubation jar with striped bass eggs fertilized by white bass sperm. A continuous flow of water is supplied to the eggs. Upon hatching, the larvae swim out of the jar into a culture container.

L^{-1} as optimal. Pond stocking rates vary from 125,000 to 1,500,000 fry ha^{-1}, with an average of 250,000-625,000 ha^{-1} stocked for stock enhancement purposed and 125,000-300,000 ha^{-1} stocked for commercial fingerling production. Commercial producers often stock fewer fish to grow a larger fish at harvest. It is important when stocking fry into a pond to temper fish to the pond water. Fry can be either placed in plastic bags or plastic containers while slowly adding pond water to the container. After a period of time (up to 1 hour or more), the fry can be released directly into the pond. If possible, stocking of fry into a pond should be conducted at night. Direct sunlight should be avoided since it adversely affects fry health and survival.

Table 11.1 RECOMMENDED FEED SIZES AND DAILY FEEDING RATES (PERCENTAGE OF BODY WEIGHT) FOR VARIOUS SIZES OF HYBRID STRIPED BASS

Fish weight (g)	No of fish lb^{-1}	Diet size	Feeding rate (%)
0.4	1000	#2 meal/crumble	25.0
1.0	500	#3 meal/crumble	15.0
10.0	50	3/32" pellet	7.5
25.0	20	1/8" pellet	5.0
75.0	6	5/32" pellet	3.0
150.0	3	3/16" pellet	3.0

Source: Smith et al. (1992).

Supplemental feeding of a prepared diet to phase I hybrid striped bass raised in ponds can be used when fish are 21 to 26 days old, or when fish are greater than 2.5 cm in length. Most often, a high-protein salmon diet is fed, either small

crumbles or a fine meal (Table 11.1). Initial feeding rates are 1 to 5 kg ha^{-1} day^{-1}, and can be increased after a few days to range from 5 to 15 kg ha^{-1} day^{-1}. Feeding can be done by hand or blower one-to-three times per day. With supplemental feeding, the fish will be easier to train for more intensive grow-out situations and may reach a larger size than if the fish consume only zooplankton.

Production (Phase II)

Once phase I fish have been harvested from the pond, they can be restocked for grow-out to advanced (up to 2.5 cm in length) juveniles (phase II). Phase I juveniles to be grown to phase II juveniles should be fed at frequent intervals for at least two weeks before harvest, with at least two daily feedings. Stocking densities used to culture phase II juveniles varies. If a producer desires to produce a large number of fish which average 25-40 g, a high density (162,000 to 250,000 ha^{-1}) should be stocked. If larger fish (>80 g) are desired, fewer fish should be stocked (62,5000 to 112,5000 ha^{-1}). However, it is advisable not to stock phase II hybrid striped bass at very low densities (12,500 $hectare^{-1}$) due to the high probability of large size differences of the fish at harvest, with a large percentage of small fish (20 g) and a small percentage of large fish (>100 g). This could adversely affect survival as well because of cannibalism.

Production (Phase III)

Pond production of phase III hybrid striped bass involves growing fish to market-size as food fish, or to adults suitable for use as broodstock. Phase II juveniles (100-250 g) can be stocked at densities ranging from 12,500 to 37,500 ha^{-1}. Proper aquaculture methods need to be followed, including monitoring dissolved oxygen levels, feeding a nutritious diet and measuring water quality parameters (such as total ammonia nitrogen).

Figure 11.2 One person can harvest cages used to grow hybrid striped bass. Fish can then be sold at the pond bank or transported to markets or processing plants.

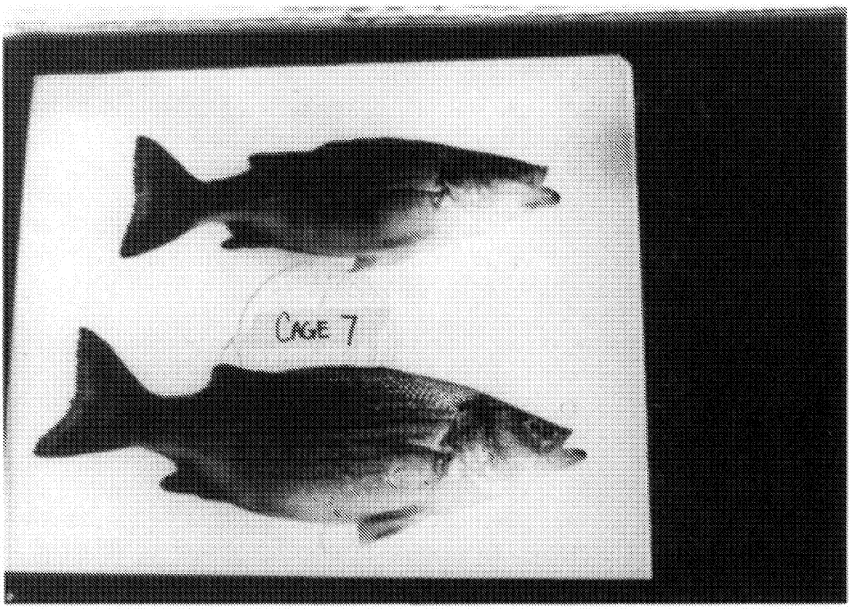


Figure 11.3 Sunshine bass grown in a cage. The fish at the bottom weighed 0.68 kg, and the fish at the top weighed 0.45 kg.

Intensive Culture Methods

Hybrid striped bass can be grown using intensive systems such as raceways, cages, or indoor recirculating systems; however, extra care must be used when raising fish by these methods. Aquaria can be used for holding larval fish, but long-term use of aquaria is not recommended because their rectangular shape results in inefficient water circulation. This causes uneaten food to collect in the corners. Circular tanks allow for water circulation to all areas within the tank and water currents help to remove wastes which reduces maintenance. Rectangular tanks are less desirable because corners tend to accumulate waste, and rectangular design does not allow for a continuous swimming path for the fish.

Cages have been used to grow hybrid striped bass for many years. Cages provide producers with a method to grow fish in ponds not suitable for conventional pond culture methods; the pond bottom may be irregularly shaped or the pond too deep to be seined (Figure 11.2). However, there are disadvantages with cage culture. One is that if the localized water quality is reduced, fish are not able to move to areas where water quality may be better. Secondly, fish may be more susceptible to disease infections, thereby having reduced growth or survival. Thirdly, careful feeding of the fish must be done so that all fish will have access to food. If all fish are not fed all they will eat, the fish may grow to different harvest sizes (Figure 11.3). Lastly, the cage mesh may become encrusted with algae or sessile organisms, such as freshwater sponges, that limit water exchange through the cage and may cause a deterioration in water quality in the cage.

Recirculating systems require biological and mechanical filtration so that waste solids and metabolites are removed from the culture water. Mechanical filtration can be accomplished through the use of sand filters, diatomaceous earth, coral, carbon, or various man-made "fabrics" or plastic beads. Biological filtration is the process where excreted nitrogenous compounds (ammonia and nitrate) are used by bacteria and converted to non-toxic forms (nitrate). Water quality parameters (temperature, dissolved oxygen, pH, ammonia, nitrite, chlorides, alkalinity, and hardness) need to be carefully monitored at all times to ensure optimal growing conditions for the fish. Adequate water flow also aids in providing optimal culture conditions.

NUTRIENT REQUIREMENTS

Nutrient requirements and practical diet formulation for hybrid striped bass have only recently been investigated, and much additional research needs to be conducted. Because of the scarcity of information, many of the nutrient requirements and diet formulations for salmonids have been used for hybrid striped bass feeds. However, recently information has begun to occur on the nutritional needs of hybrid striped bass. As more data is collected, it may be revealed that striped bass, palmetto bass, and sunshine bass have some unique nutritional needs.

Protein and Amino Acids

Dietary protein requirements can vary with age of the fish, energy level of the diet, amino acid balance, and culture conditions. Optimum percentage dietary protein reported for striped bass and hybrid striped bass has ranged from 36 to 55%. Millikin (1983) reported that a diet with 47% protein plus 12% lipid produced optimal growth, while feeding a diet with 37% protein and 7% lipid resulted in reduced growth. Optimal protein level has been reported to be 41% when sunshine bass fingerlings were fed diets containing menhaden fishmeal as the sole protein source (Brown et al. 1992).

Hybrid striped bass are presumed to require the same 10 essential amino acids as most fish and terrestrial animals; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The requirements for several essential amino acids are not known for hybrid striped bass. The lysine requirement has been estimated to be 1.4% of the diet (4% of dietary protein) for sunshine bass. Total sulfur amino acid requirement (methione and cystine) for sunshine bass has been reported as 0.87% of the diet as methionine when 0.13% of the diet is cystine; this results in a total sulfur amino acid requirement of 1.0% of the diet (2.9% of dietary protein). Research has found that cystine spared less than 40% of the methionine. A total sulfur animo acid requirement of 0.73% of the diet (2.1% of dietary protein) has been reported for palmetto bass fed purified diets. Arginine requirement for sunshine bass is 1.55% of the diet (4.4% of dietary protein). Amino acid requirements for hybrid striped bass are compared with those for other fish species in Table 11.2.

Apparent protein digestibility for practical feed ingredients for palmetto bass ranges from 71 to 93% (Table 11.3). Wheat flour, wheat middlings, menhaden fish meal, corn grain, and blood meal all have protein digestibilities greater than 85%. Rice bran and meat-and-bone meal have relatively low protein digestibilities of 71% and 73%, respectively.

Table 11.2. ARGININE, LYSINE, AND METHIONINE/CYSTINE REQUIREMENTS OF HYBRID STRIPED BASS AND OTHER AQUACULTURE SPECIES, PRESENTED AS PERCENTAGE OF DIET (PERCENTAGE OF DIGESTIBLE PROTEIN IN PARENTHESIS)

Amino acid	Hybrid striped bass	Channel catfish	Rainbow trout	Common carp	Nile tilapia
Arginine	1.5(4.3)[a]	1.2(4.2)[f]	1.5(4.4)[f]	1.3(4.2)[f]	1.2(4.2)[f]
Lysine	1.4(4.0)[b,c]	1.4(5.1)[f]	1.8(5.2)[f]	1.7(5.6)[f]	1.4(5.1)[f]
Methionine/ cystine	1.0(2.9)[e]	0.6(2.3)[f]	1.0(2.9)[f]	0.9(3.0)[f]	0.9(3.2)[f]

[a] *Griffin et al. (1994b).*
[b] *Griffin et al. (1992).*
[c] *Keembiyehetty and Gatlin (1992).*
[d] *Griffin et al. (1994b).*
[e] *Keembiyehetty and Gatlin (1993).*
[f] *From National Research Council (1993).*

Table 11.3. AVERAGE PERCENTAGE APPARENT DIGESTIBILITY OF PROTEIN FOR VARIOUS FEED INGREDIENTS FOR HYBRID STRIPED BASS AND OTHER FISH SPECIES

Ingredient	Hybrid striped bass[a]	Channel catfish[b,c]	Rainbow trout[d]
Fish meal, menhaden	88	86,76	-
Blood meal	86	74	69
Meat and bone meal	73	82,64	-
Cottonseed meal	84	80	76
Wheat middlings	92	88	76
Wheat flour (grain)	93	-	-
Soybean meal	80	85,93	83
Rice bran	71	78	-
Corn grain	87	92,96	95

[a] *Sullivan and Reigh (1995).*
[b] *Brown et al. (1985).*
[c] *Wilson and Poe (1985).*
[d] *Smith et al. (1980).*

Energy

Since protein is the most expensive component in a diet, nutritionists need to supply levels of protein sufficient to meet essential amino acid requirements, but not in excess because protein may be used for energy. Dietary lipids and carbohydrates can spare protein, however, excessive amounts of energy can lead to increased fat deposition and possibly reduced growth. The protein to energy (P:E) ratio of the diet influences on the efficiency of protein and energy utilization. The level of

digestible energy in the diet affects the amount of food consumed by fish, thus a low P:E ratio may cause the fish to consume less than the optimum amount of protein for fast growth, or may increase fat deposition. An excessively high P:E ratio may cause fish to utilize protein as an energy source, which is expensive. Optimal dietary energy-to-protein ratio for sunshine bass has been reported as 6-9 kcal g^{-1} protein for diets containing 35 to 45% crude protein. High percentages of abdominal fat are found in fish fed diets containing higher energy levels (lower P:E ratios). Woods et al. (1995) reported that a diet with 7 kcal g^{-1} protein was adequate for growth in juvenile striped bass. A diet with 40% protein and 8.6 kcal g^{-1} protein was found to be adequate for sunshine bass (Webster et al. 1995). If the diet contained less than this level of protein, an increase in lipid deposition was reported when fish were raised in cages.

It appears that carbohydrate and lipid in practical diets are utilized equally well for energy by sunshine bass (Webster et al. 1995). Digestion coefficients of several feedstuffs for hybrid striped bass are presented in Table 11.4.

Table 11.4. AVERAGE PERCENTAGE APPARENT DIGESTIBILITY OF GROSS ENERGY FOR VARIOUS FEED INGREDIENTS FOR HYBRID STRIPED BASS AND OTHER FISH SPECIES

Ingredient	Hybrid Striped bass[a]	Channel catfish[b]	Chinook salmon[c]
Fish meal, menhaden	96	95	84
Blood meal	-	-	32
Meat and bone meal	80	70	-
Cottonseed meal	73	65	-
Wheat middlings	61	49	45
Wheat flour (grain)	54	-	-
Soybean meal	55	73	66
Rice bran	47	53	-
Corn grain	41	79	-

[a] *Sullivan and Reigh (1995).*
[b] *Wilson and Poe (1985).*
[c] *Hagen et al. (1993).*

Essential Lipids

Highly unsaturated fatty acids (HUFA) of the omega-3 (n-3) group are essential for the proper growth and development of marine fish larvae. This is because most marine fish larvae have a limited ability, if any, to elongate (add carbon units) and desaturate (create molecular carbon-to-carbon bonds) short-chain (18 carbon) fatty acids. Thus, marine fish larvae must rely on obtaining these essential n-3 HUFAs from their food. Like marine fish larvae, striped bass and hybrid striped bass appear to have a limited ability to biosynthesize n-3 HUFAs and require a dietary source. Two of these HUFAs are eicosapentaenoic acid (EPA), 20:5(n-3), and docosahexaenoic acid (DHA), 22:6(n-3). It appears that essential fatty acid levels

higher than 5% of the total fatty acids are required for growth and survival of striped bass larvae (Webster and Lovell, 1990; Figure 11.4). Essential fatty acids are important for membrane fluidity, proper neurological and visual development, transportation of lipids, and as precursors for eicosinoids and prostaglandins.

This requirement for n-3 HUFAs in striped bass larvae may also be appropriate for hybrid striped bass. Palmetto bass larvae appear to be more susceptible to HUFA deficiency than striped bass during larval metamorphosis (Tuncer and Harrell, 1992). Since HUFAs are important components of membrane phospholipids, they are especially important to the fish during metamorphosis because there is extensive tissue development and change in the fish larvae. Phospholipids containing essential HUFAs appear to be vital for these tissue changes. High mortality rates occur if adequate levels of EPA or DHA are not present in the food of the larvae at metamorphosis. Failure of swim bladder to inflate is a common deficiency sign in the larvae.

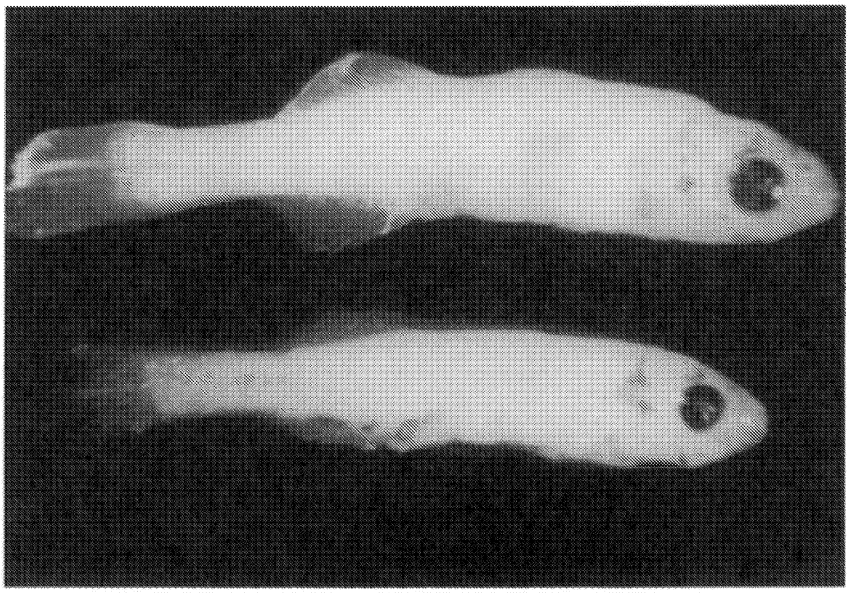


Figure 11.4 The larvae at the top was fed a strain of brine shrimp nauplii containing a high percentage of eicosapentanoic acid (20:5 n-3), an essential fatty acid for hybrid bass, while the one at the bottom was fed a strain with a low percentage of 20:5 n-3.

As fish grow, their EFA requirement may change. Sunshine bass (13 g) fed diets with 1.1% n-3 HUFAs had higher weight gains and protein efficiency ratios than fish fed diets with less than 1.1% or greater than 3.2% of n-3 HUFAs (Nematipour and Gatlin, 1993). However, palmetto bass (72 g) fed diets containing 40% crude protein and with either 0% or 4% menhaden oil did not show differences in growth when grown in ponds (Zhang et al. 1994).

Vitamins

Very little information is available on vitamin requirements of hybrid striped bass. One reason is that until recently, hybrid striped bass were fed commercial salmonid diets which are well fortified with all vitamins, and the fish appeared to grow satisfactorily. It is assumed that vitamin C is required, as it is for most teleost fish species. The dietary choline requirement for juvenile sunshine bass appears to be 500 mg choline kg^{-1} of diet (Griffin et al. 1994c). In view of the scarcity of information on vitamin requirements of hybrid striped bass, the vitamin allowances presented in Table 11.5 are proposed. The amounts of each vitamin listed, which allow for processing and storage losses, are based mostly on the requirements of salmonids and some warmwater species. These levels have been used successfully in commercial and experimental diets for hybrid striped bass.

Table 11.5. RECOMMENDED VITAMIN ALLOWANCES FOR A PRACTICAL HYBRID STRIPED BASS DIET

Vitamin	IU or mg kg^{-1} of diet
A	6,000 IU
D	2,200 IU
E	120 IU
K	10 mg
Niacin	200 mg
Pantothenic acid	60 mg
Thiamin	30 mg
Riboflavin	20 mg
B_6	20 mg
Folic acid	5 mg
B_{12}	0.1 mg
Biotin	2 mg
Vitamin C	1,000 mg
Choline	500-1,000 mg

Minerals

Hybrid striped bass probably require the same minerals as other fish for proper tissue and bone formation, metabolic activities, and to maintain osmotic balance between their bodily fluids and the surrounding water. Fish can derive a large proportion of certain minerals, like calcium, from the water; however, most minerals must be supplied in the diet since water is not a major source. Because of the lack of information on the mineral requirements for hybrid striped bass, the levels presented in Table 11.6 are recommended. These allowances have provided satisfactory growth in experimental and practical diets. Sunshine bass (3 g) require 0.54% available phosphorous in the diet for proper growth and bone and scale mineralization (Brown et al. 1993).

Table 11.6. RECOMMENDED MINERAL ALLOWANCES FOR A PRACTICAL HYBRID STRIPED BASS DIET

Mineral	mg kg^{-1} of diet
Phosphorus (available)	5,000
Manganese	180
Copper	8
Cobalt	1.5
Iron	66
Zinc	150
Iodine	6
Selenium	0.3

PRACTICAL DIET FORMULATION

When formulating diets for any fish species, careful consideration must be given to ingredient selection. Not only must the diets meet the protein (amino acid), essential fatty acid, vitamin, and mineral requirements of the fish, but the diets must be palatable. Further, the ingredients must be relatively digestible so that the nutrients can be utilized. It appears that hybrid striped bass have an ability to digest carbohydrates satisfactorily (Zhang et al. 1994; Sullivan and Reigh, 1995; Webster et al. 1995). Feeding diets having carbohydrate-to-lipid ratios of between 25:10 to 42:2.5 (grams:grams) resulted in similar growth, however, whole-body lipid increased as lipid increased (Nematipour et al. 1992). It appears that hybrid striped bass can digest protein from plant and animal sources equally well, as do channel catfish and rainbow trout; however, hybrid striped bass apparently can digest dietary starch more effectively than rainbow trout. Thus, digestibility coefficients for hybrid striped bass appear to be somewhere between an omnivorous species (channel catfish) and a carnivorous species (rainbow trout).

Since fish meal is the most expensive ingredient in most fish diets, it is important to formulate diets that utilize a minimum amount of fish meal. However, growth of some fish have been reduced as fish meal was replaced by plant-protein sources, primarily soybean meal. Recently, in an effort to reduce diet costs for hybrid striped bass, nutritionists have begun to determine the minimum level of fish meal in diets. Small palmetto bass (5 g) appear to require between 17 and 36% fish meal in the diet, while with larger fish (>150 g) soybean meal can replace 75% of the fish meal when the diet contains 17% fish meal and 35% protein (Gallagher, 1994). When small (20 g) palmetto bass were fed diets containing 40% protein, it was found that 15% fish meal was sufficient, but when fish meal was omitted from the diet there was reduced growth (Webster et al. 1997).

When a diet is unpalatable, hybrid striped bass take the pellet into the mouth and then quickly eject it back into the water. This action might be repeated several times before the fish either rejects or accepts the diet. Menhaden fish oil was

found not to be a good attractant for sunshine bass (5-10 g), but fish meal was a good attractant when used at a level of 10% of the diet (Brown et al. 1993). The use of marine fish oils at high concentrations in the diet may affect the flavor of hybrid striped bass fillets by imparting a fishy flavor (Best et al. In Press).

Hybrid striped bass will consume either floating or sinking pellets; however use of floating pellets allows the producer to observe feeding activity of the fish. This is advantageous to determine how much diet to feed to the fish as well as observe the condition of the fish. Physical properties of the diet are important, especially water stability and pellet size. Since hybrid striped bass are generally aggressive in their eating habits, water stability of the diet is not as important as it would be for crustacean diets; however, the diet must remain intact for a period of at least several minutes.

Diets formulated for intensive systems and for small hybrid striped bass in aquaria must be nutritionally complete, but diet formulations for pond-cultured fish may not. Certainly, small fish grown in properly prepared nursery ponds obtain significant nutrients from natural foods. However, intensive stocking in grow-out ponds may reduce the availability of natural foods so that nutritionally complete feeds are necessary.

FEEDING PRACTICES

Larvae

Brine shrimp nauplii are a significant part of the diet for first feeding hybrid striped bass larvae. Selection of a brine shrimp strain with a high (7%) content of n-3 HUFA was once considered essential to proper growth, development, and survival of larval palmetto bass. However, it has been found that enrichment of low n-3 HUFA brine shrimp nauplii with marine fish oils will allow the hybrid striped bass larvae to obtain sufficient essential fatty acids for normal development (Clawson and Lovell, 1992; Tuncer and Harrell, 1992). To accomplish this, newly-hatched brine shrimp nauplii are grown to the third nauplii stage (24 hours after hatch) and then placed into a container with fish oil emulsified in an aqueous medium. This medium is prepared by mixing the marine fish oil, salt (sea) water, and an emulsifier, such as gum xanthum. Baker's yeast can also be added to the mixture. Oxygen is provided to keep the nauplii suspended in the emulsion for up to 24 hours, allowing the nauplii to consume the yeast or the lipid droplets in the water.

Larval hybrid striped bass do not utilize prepared diets as well as live foods. There have been numerous attempts to feed hybrid striped bass larvae prepared diets but all have resulted in poor growth and survival of the larvae. It has been thought that the ability of larval fish to utilize prepared diets was due to a lack of digestive enzymes. However, larval striped bass appear to have the necessary enzymes (trypsin, chymotrypsin, pepsin, carboxypeptidase A, and a-amylase) to digest food (Baraji and Lovell, 1986). If a prepared diet is fed, brine shrimp nauplii should also be fed initially and the larvae are gradually weaned onto the prepared diet. Tuncer et al. (1990) recommended that brine shrimp nauplii be fed for 21 days and then gradually change to a prepared diet after that time period. When feeding sunshine bass larvae, rotifers are required for initial feeding because of the smaller mouth size; brine shrimp nauplii may be fed when the larvae become larger.

Juveniles

Feeding palmetto bass juveniles all they will eat produces larger fish than feeding a restricted ration of 2.5 or 5% of body weight (Tuncer et al. 1990). Diets used to feed phase II fish are generally high in protein (38-50% protein). A sinking, crumbled diet or small floating pellets can be used. As the fish grow, pellet size of the diet needs to be increased (Table 11.1). Hybrid striped bass in production or grow-out units should be fed at least twice daily. Small fish can be fed up to six times daily. However, frequent feedings are labor intensive, and on large farms it may be practical to feed only once daily. There is no information on which time(s) of day would be most advantageous to feed. Optimal temperature for growth of palmetto bass has been reported to be 27 to 28°C when the fish are fed to satiation (Woiwode and Adelman, 1991).

Fish can be fed by various methods, including hand feeding, use of blowers, automatic feeders, and demand feeders. The advantage of feeding by hand or by blower is that the person feeding can observe feeding activity, assess the amount of feed to give, and observe fish health. Use of a blower when feeding fish stocked in large ponds allows the diet to be spread over a larger area of the pond than feeding by hand. Automatic feeders are connected to timers which allow frequent feeding with less labor. Demand feeders drop feed into the water when the fish activates the dispensing mechanism.

The disadvantages of each method include the time expense of feeding fish by hand, especially when fish are large and consume large quantities of diet. Automatic and demand feeders may not function properly unless maintained regularly. Demand feeders with a "tickle bar" can be induced to drop feed into the pond on windy days so that no feed is wasted.

While a feeding chart such as in Table 11.1 can be used, feeding all the fish will consume (to satiation) can be done with a floating diet which allows the feeder to observe fish feeding activity. Even when using a feeding chart, fish feeding activity should still be observed carefully to avoid wasting feed or underfeeding, which could result in less than optimal growth.

Broodfish

Most broodstock used to produce hybrid striped bass are captured from the wild. If broodstock are kept at a hatchery, diets that supply all essential fatty acids, amino acids, and vitamins and minerals must be provided. Some nutrient requirements of striped bass broodstock are being investigated (at the University of Maryland). Generally, salmonid diets have been fed to striped or white bass broodstock. Special attention should be given to females to ensure adequate amounts of essential fatty acids to produce high quality eggs and larvae. Research on broodstock nutrient requirements needs to be conducted if domestic stocks of fish are used to produce larvae. Often brooders from domestic stocks do not produce the quantity and quality of larvae as wild broodstock.

EFFECTS OF DIET ON SENSORY QUALITIES OF PROCESSED FISH

Commercial diets may have an effect on the flavor of fish. While grains, oilseed meals and animal by-product meals usually do not add to the flavor of cultured fish, marine fish meal and oil can affect the flavor and storage quality of fish. Bett et al. (In press) stated that marine fish oil added to diets for sunshine bass caused the fish

to taste "fishy." Fish fed a diet with 10% menhaden oil had a stronger "fishy" taste than fish fed a diet with 2% menhaden oil. A strong "fishy" taste may not be a problem if the target consumer prefers a stronger flavor fish, but may adversely affect preference if a mild-flavor fish is desired.

Replacement of fish meal with oilseed meals can affect flavor of fish flesh. When soybean meal replaced fish meal in diets for palmetto bass, flavor and texture of fillets were improved compared to feeding a diet with high (47%) fish meal (Postel et al. 1996). While the evaluators could taste a difference between fish fed the high fish meal diet and those fed diets that contained soybean meal, all fillets were judged to be acceptable.

Fattiness in cultured fish is generally undesirable because consumers consider eating fish as part of a low-fat diet. Fat in fish may not only reduce consumer acceptance, but also may reduce storage time and decrease processed yield of the fish. There are several factors that affect the amount of fat in fish such as feeding rate, size of fish, and the ratio of protein to energy in the diet. When sunshine bass were grown in cages, fillet fat was less for fish fed a diet having a protein-to-energy ratio of 116 mg $kcal^{-1}$ than for fish fed diets with lower protein-to-energy ratios (Webster et al. 1995). Xiong et al. (1996) measured thiobarbituric acid reactive substances (TBARS), a measure of oxidative rancidity, in frozen sunshine bass that had been fed diets containing various levels of protein and subsequently frozen with or without skin. Figure 11.5 shows that the fish fed the lower protein diets (lower protein-to-energy ratios) had higher TBARS values. Removing the skin increased the rate of TBARS development during frozen storage.

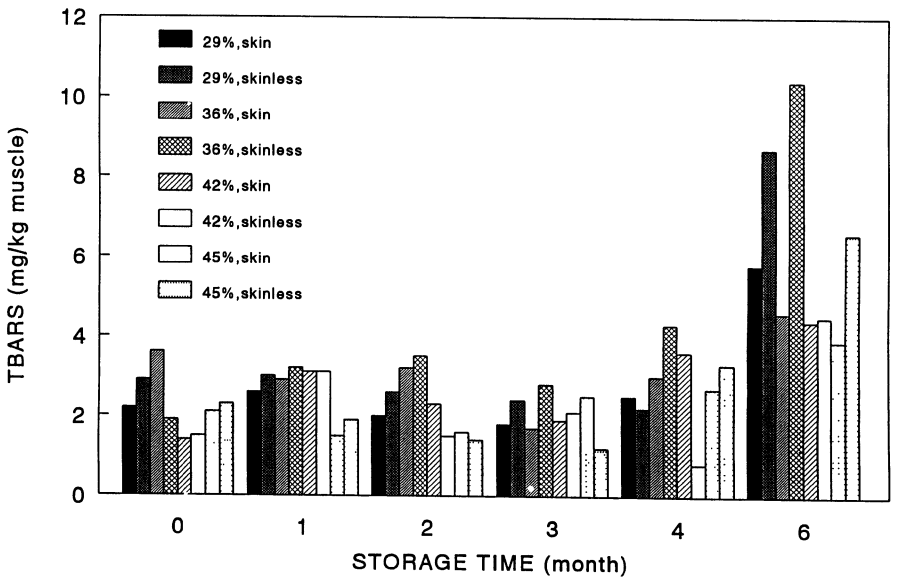


Figure 11.5 Changes in thiobarbaturic acid reactive substances (TBARS) in sunshine bass fillets stored for six months in a freezer with either the skin left on the fillet, or removed prior to freezing. The fish had been fed 29, 36, 42, or 45% protein feeds.

REFERENCES

BARAJI, V. and R. T. LOVELL. 1986. Digestive enzyme activities in striped bass from first feeding through larvae development. *Trans. Am. Fish. Soc.* 115: 478-484.

BETT, K. L., P. B. JOHNSEN, C. D. WEBSTER, L. G. TIU, Y. L. XIONG, and E. A. DECKER. Sensory and chemical evaluation of sunshine bass *(Morone chrysops x M. saxatilis)* fillets during frozen storage. In Press.

BONN, E., W. M. BAILEY, J. D. BAYLESS, K. E. ERICKSON, and R. E. STEVENS. 1976. Guidelines for striped bass culture. Striped Bass Committee, Southern Division, American Fisheries Society, Bethesda, MD.

BROWN, P. B., R. J. STRANGE, and K. R. ROBBINS. 1985. Protein digestibility coefficients for yearling channel catfish fed high protein feedstuffs. *Prog. Fish. Cult.* 47: 94-97.

BROWN, M. L., F. JARAMILLO, and D. M. GATLIN III. 1993. Dietary phosphorus requirement of juvenile sunshine bass, *Morone chrysops x M. saxatilis. Aquac.* 111: 355-363.

BROWN, P. B., M. E. GRIFFIN, and M. R. WHITE. 1993. Experimental and practical diet evaluations with juvenile hybrid striped bass. *J. World Aquat. Soc.* 24:80-89.

CLAWSON, J. A. and R. T. LOVELL. 1992. Improvement of nutritional value of artemic for hybrid striped bass/white bass *(Morone saxatilis x M. chrysops)* larvae by n-3 HUFA enrichment of nauplii with menhaden oil. *Aquac.* 108: 125-134.

GALLAGHER, M. L. 1994. The use of soybean meal as a replacement for fish meal in diets for hybrid striped bass *(Morone saxatilis x M. chrysops). Aquac.* 126: 119-127.

GRIFFIN, M. E., P. B. BROWN, and A. C. GRANT. 1992. The dietary requirement of juvenile hybrid striped bass. *J. Nutr.* 122: 1332-1337.

GRIFFIN, M. E., K. A. WILSON, and P. B. BROWN. 1994b. Dietary arginine requirement of juvenile hybrid striped bass. *J. Nutr.* 124: 888-893.

HAJEN, W. E., D. A. HIGGS, R. M. BEAMES, and B. S. DOSANGH. 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon *(Oncorhynchus tshawytscha)* in sea water measurement of digestibility. *Aquac.* 112:333-348.

KEEMBIYEHETTY, C. N. and D. M. GATLIN III. 1992. Dietary lysine requirement of juvenile hybrid striped bass *(Morone chrysops x M. saxatilis). Aquac.* 104: 271-277.

KEEMBIYEHETTY, C. N. and D. M. GATLIN III. 1993. Total sulfur amino acid requirement of juvenile hybrid striped bass *(Morone chrysops x M. Saxatilis). Aquac.* 110: 331-339.

MILLIKIN, M. R. 1983. Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. *Trans. Am. Fish. Soc.* 112: 185-193.

NATIONAL RESEARCH COUNCIL. 1993. *Nutrient requirements of fish.* Washington, D.C.: National Academy of Sciences.

NEMATIPOUR, G. R., M. L. BROWN, and D. M. GATLIN III. 1992. Effects of dietary energy:protein ratio on growth characters and body composition of hybrid striped bass *(Morone chrysops x M. saxatilis. Aquac.* 107: 359-368.

NEMATIPOUR, G. R. and D. M. GATLIN III. 1993. Requirement of hybrid striped bass for dietary (n-3) highly unsaturated fatty acids. *J. Nutr.* 123: 744-753.

POSTEL, E. T., M. LADOUCEUR, D. HOLBERT, and M. L. GALLAGHER. 1996. Texture and flavor of hybrid striped bass fed soybean meal diets. *J. Aquat. Food Product Technol.* 5(2): 83-91.

SMITH, R. R., M. S. PETERSON, and A. C. ALLRED. 1980. The effect of leaching on apparent digestion coefficients in determining digestibility and metabolizable energy of feedstuffs for salmonid. *Prog. Fish Cult.* 42: 195-199.

SMITH. T. I. J., W. E. JENKINS, and R. V. MINTAR. 1992. Production of advanced fingerling and subadult striped bass and striped bass hybrids in earthen ponds. In Culture and propagation of striped bass and its hybrids, eds. R. M. Harrell, J. H. Kerby, and R. V. Mintar. Bethesda, MD: American Fisheries Soc.

STEVENS, R. E. 1966. Hormone-induced spawning of striped bass for reservoir stocking. *Prog. Fish Cult.* 28:19-28.

SULLIVAN, J. A. and R. C. REIGH. 1995. Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone saxatilis x M. chrysops). Aquac.* 138: 313-322.

TUNCER, H., R. M. HARRELL, and E. D. HOUDE. 1990. Comparative energetics of striped bass (*Morone saxatilis)* and hybrid *(M. Saxatilis x M. chrysops). Aquac. 101: 105-121.*

WEBSTER, C. D. and R. T. LOVELL. 1990. Response of striped bass larvae fed brine shrimp from different sources containing different fatty acid composition. *Aquac.* 90: 49-61.

WEBSTER, C. D., L. G. TIU, J. H. TIDWELL, P. VAN WYK, and R. D. HOWERTON. 1995. Effects of dietary protein and lipid levels on growth and body composition of sunshine bass *(Morone chrysops x M. Saxatilis)* reared in cages. *Aquac.* 131: 291-301.

WEBSTER, C. D., TIU, L. G., and J. H. TIDWELL. 1997. Effects of replacing fish meal in diets on growth and body composition of palmetto bass *(Morone saxatilis x M. chrysops)* raised in cages. *J. Appl. Aquac.* 7(1): 53-67.

WILSON, R. P. and W. E. POE. 1985.Apparent digestible protein and energy coefficients of common feed ingredients for channel catfish. *Prog. Fish Cult. 47: 154-158.*

WOIWODE, J. G. and I. R. ADELMAN. 1991. Effects of temperatures, photoperiod, and ratio size on growth of hybrid striped bass x white bass. *Trans. Am. Fish. Soc.* 120: 217:229.

WOODS, L. C., D. YUST, C. MCLEOD, and M. SUBRAMANYAM. 1995. Effects of dietary protein:energy ratio on weight gain, body composition, serum glucose and triglyceride levels, and liver functions of striped bass. *Water Sic. Technol.* 31: 195-203.

XIONG, Y. L. E. A. DECKER, S. P. BLANCHARD, A. D. CRUM, N. C. SHANTHA, C. D. WEBSTER, L. G. TIU, and J. H. TIDWELL. 1996. Dietary protein level has minimal effects on flesh quality of frozen stored sunshine bass (*Morone chrysops x M. saxatilis). J. Appl. Aquac.* 6(1): 47-63.

ZHANG, Q., R. C. REIGH, and W. R. WOLTERS. 1994. Growth and body composition of pond-raised hybrid striped bass, *Morone saxatilis x M. chrysops and M. saxatilis x M. Mississippiensis,* fed low and moderate levels of dietary lipid. *Aquac.* 125: 119-129.

12 FEEDING TILAPIAS

Tilapias are tropical fish and will not survive temperature much below 12°C, therefore, their culture is limited to tropical and subtropical regions or temperature-controlled environments in temperate regions. They are endemic to Africa, but are presently found in most warm regions of the world. They are a popular fish for culture in the tropics and areas where supplemental feeds are cost restrictive because of their efficient use of natural aquatic foods, fast growth, propensity to consume a variety of supplemental feeds, herbivorous nature, resistance to diseases and handling, ease of reproduction in capacity, and tolerance to wide ranges of environmental conditions. Some of the cultured species have been shown to survive dissolved oxygen concentrations of 0.1 mg/L and tolerate unionized ammonia concentrations of 2.4 mg/L. Although indigenous to fresh water, tilapias are euryhaline and able to grow well in saline water if properly acclimated. However, their activity and feeding become reduced below 20°C and feeding stops around 16°C.

Most cultured tilapias are grouped into two genera (Trewavas 1982): *Tilapia*, which are macrophagous and substrate-spawners; and *Oreochromis*, which are microphagous and mouth-brooders. About 70 species have been identified under these two genera; however, only two *Tilapia* species, *rendalli* and *zillii*, and three *Oreochromis* species, *mossambicus*, *niloticus*, and *aureus*, have been used widely in practical culture. For practical purposes, and because most of the publications on tilapias still carry the old generic name, the common name of both genera is referred to here as tilapia. The most popular culture species is *Oreochromis niloticus*, the Nile tilapia, with natural dark color or red or gold pigmented skin. Figure 12.1 is a Nile tilapia with light red skin.

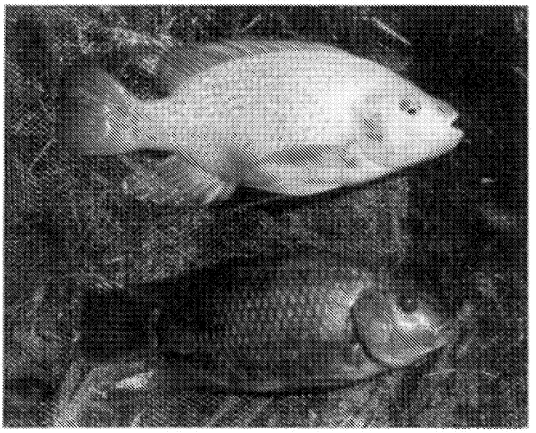


Figure 12.1 At bottom is Nile tilapia in natural, dark color. At top is genetically improved light (red) skin tilapia which has greater consumer appeal in some markets.

CULTURE PRACTICES

Seed Production

Most tilapias are able to reproduce at 5 to 6 months of age and can spawn every 6 to 8 weeks at water temperature between 25°C and 32°C. The total number of eggs produced per spawning is small and differs among species and size of fish. The mouth-brooding (*Oreochromis*) species lay fewer eggs than the substrate spawners (*Tilapia*). A large-size *Tilapia* species can lay as many as 7,000 eggs per spawning, whereas large Oreochromis brooders seldom produce more than 2,000 eggs.

Breeding of tilapias can be done in earthen ponds, nets, or tanks. Mature fish are stocked at a ratio of 2 to 5 females to 1 male. If females and males are of different species, the ratio may be as low as 1 to 1. The stocking density varies from 1 to 4 fish per square meter. Broodfish should be fed, and those spawned in an artificial environment should receive a nutritionally complete feed. At water temperatures of 25°C to 30°C, fry can be found in about 10 days to 14 days after the breeders are stocked. The fry should be removed from spawning facilities at regular intervals, usually weekly or biweekly, by use of a small (approximately 5/mm) mesh seine. The fry collected from the spawning areas are usually transferred to nursery ponds or tanks for rearing to a size suitable for stocking into production units.

Although the fecundity of tilapias is low, the early reproductive capability, high frequency of breeding and high rate of larval survival often create problems of overpopulation in ponds and stunting which can result in most of the fish not reaching marketable size. To overcome this problem, it is necessary to suppress reproduction or practice monosex culture.

In monosex culture, males are preferred because they have a faster growth rate than females. This can be managed by manual sexing, hybridization, or sex-reversal of genotypic females with the use of hormones. Manual sexing can be done by selecting the males after the fingerlings have reached 20 to 50 grams and

have well developed sexual parts. This is very labor intensive. Hybridization between certain species of tilapia can produce a high percentage of males (85-100%). However, a disadvantage of this technique is the difficulty in maintaining pure stocks that produce a high percentage of males. Sex reversal to produce a monosex male population can be accomplished by administering androgenic hormones during early larval stage by means of injection, submerging the fry in an aqueous hormone solution, or incorporating hormones into the diets. The latter is the most convenient and popular method. Methyltestosterone or ethynyltestosterone is incorporated at a concentration of 30 to 60 mg kg-1 of diet and fry are fed at 10% to 12% of their body weight per day, divided into three or four feedings, for approximately 4 weeks. This method of monosexing of tilapias for culture is widely used commercially and is practiced in most countries of the world. It has not been approved for use in the United States, but application for its use has been filed with the Food and Drug Administration.

In the United States, mixed sexes are often stocked in highly intensive culture systems, such as raceways or cages, without serious problems. Some reproduction may occur and females will grow slower than males, but marketable fish can be produced.

Culture Methods

Pond culture is the most commonly used system (Figure 12.2) in tropical and subtropical regions. Stocking rates vary depending on the size of fish stocked, size of fish to be marketed, type and level of nutrient inputs, desired standing crop, and various management practices. In many parts of the world, where commercial diets are unavailable or expensive, only manures (animal excreta or compost) or inorganic fertilizers are added to the pond. At low stocking densities, where natural food constitutes an important source of nutrients, supplementary feeding with locally available, inexpensive feed materials, such as rice bran, copra meal, brewery waste, coffee pulp, and similar materials, can increase production appreciably. As stocking rate increases, the natural food becomes less significant and better quality supplemental feeds are needed. The growing period may last from 3 months to 6 months, depending on preferred market size and management practice. Yields of 300 to 1,200 kg ha-1 have been obtained with only fertilization in ponds.

Integrated farming of tilapia with poultry or pigs is practiced in many developing countries. The manures of fed chicken, ducks, or pigs are used to fertilize the ponds or to serve directly as food for the fish. Fish yields of 250 to 1,500 kg ha-1 are obtained.

Intensive culture of tilapia has gained popularity in recent years because of the good market demand for tilapia fillets in the United States and other industrialized countries. Fish are stocked at very high densities in earthen ponds usually with flowing water and aeration, and fed with high quality pelleted feeds. All-male seedstocks are produced in hatcheries, grown to fingerling or sub-market size (10 to 50 grams) in nursery ponds, and finished to harvest size of 0.5 to 1.0 kg in production ponds. In tropical countries where favorable temperature allows year-round production, two or more crops of fish can be harvested in a year. Standing crops of 10,000 to 15,000 kg ha-1 are found on commercial farms; however, water exchange and aeration are required. As the rate of water exchange or aeration decreases fish stocking densities and yields decrease.

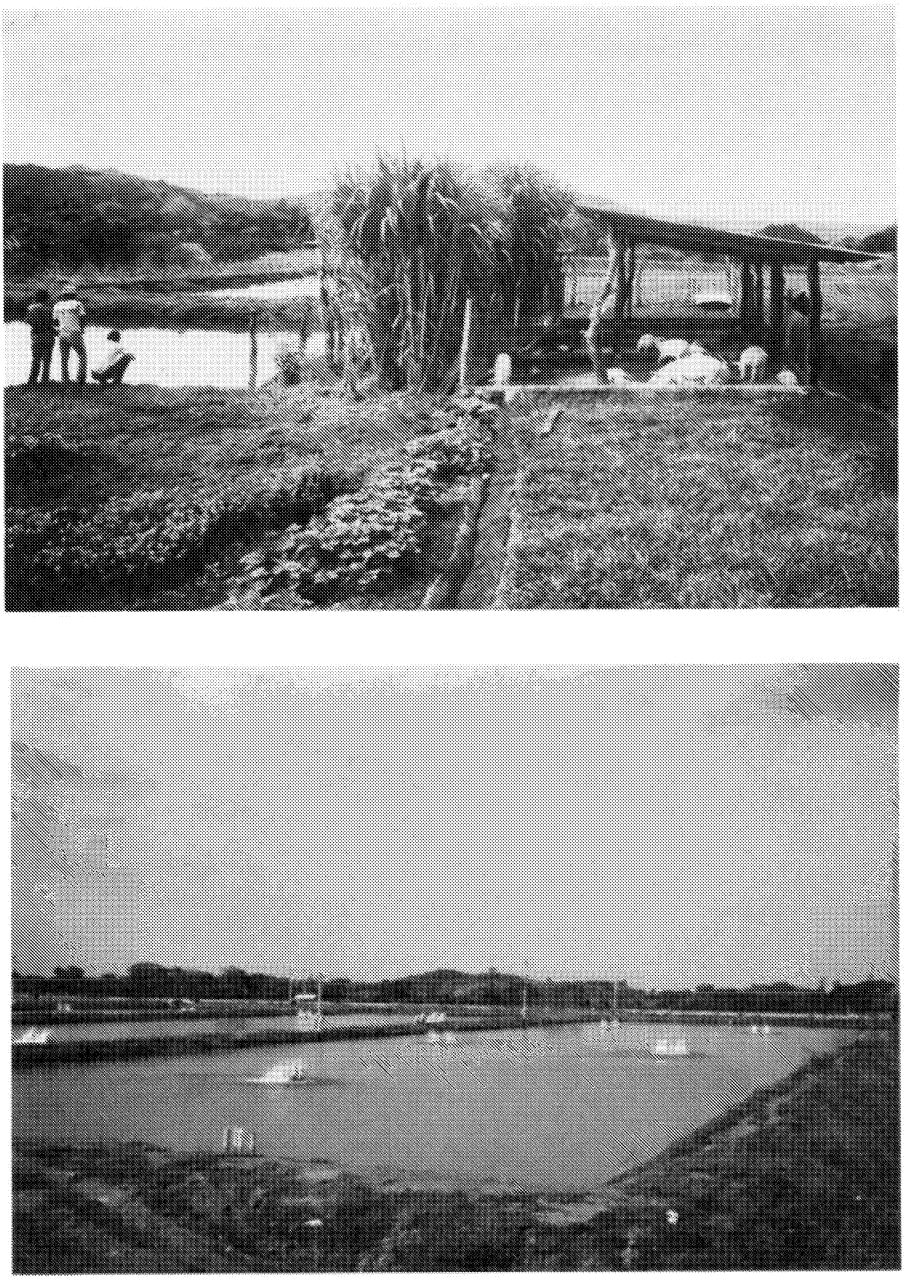


Figure 12.2 Pond culture of tilapia is productive at various levels of nutrient input. At top, the only nutrient input is waste from swine production. At bottom, the ponds are densely stocked and with aeration and nutritionally balanced feeds, can yield 15,000 kg/ha at each harvest.

Under intensive production, nutritionally complete feeds are generally used. Because tilapias, especially those of small size, can feed effectively on plankton and other natural pond foods, nutritionally complete feeds are probably not necessary in many instances. However, because of the uncertainty of the condition under which a nutrient is not needed in the diet, nutritionally balanced diets are usually fed in intensively stocked ponds.

In temperate regions, like in the United States, temperature controlled recirculating raceway systems are used to grow tilapia. The production units (raceways) are in an enclosed area, such as a greenhouse, to minimize heat loss. The water flows through biofilters to remove organic and inorganic wastes and to replenish oxygen (liquid oxygen is often diffused into the water). The fish are spawned in the hatchery, started in nursery tanks and finished in grow-out tanks. Most tilapia produced in the United States are marketed alive which allows a higher income than when the fish are processed for marketing. Marketable size ranges from 0.3 to 1 kg. Production rate from recirculating systems is variable, depending upon the desired market size, stocking density and the amount of feed the system will allow. Nutritionally complete feeds are necessary for recirculating systems.

NUTRIENT REQUIREMENTS

Protein and Amino Acids

Tilapias require the same 10 essential amino acids as other fishes and land animals. These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The quantitative requirements for these essential amino acids for growth by young Nile tilapia are presented in Table 2.1 in chapter 2. The requirement for the sulfur amino acids, methionine and cystine, can presumably be met by either methionine alone or a combination of methionine and cystine. Dietary cystine can substitute up to 50% of the total sulfur amino acid requirement for *O. Mossambicus* and presumably other tilapias. As with other warmwater fish, lysine is generally the first limiting amino acid in most practical feeds, followed by methionine plus cystine.

Fish meal is a better protein source than plant protein for tilapia, and soybean meal is a high quality protein (Jackson et al. 1982). When the 10 essential amino acids were individually added to an all-soybean protein diet, only histidine, isoleucine, phenylalanine, and valine increased growth of *O. aureus*. However, supplementation of the limiting essential amino acids is not required when soybean protein is supplemented with fish meal (Viola and Arieli 1983).

Tilapias digest the protein of fish meal and meat and bone meal well, equal to channel catfish. However, the digestibility of the protein in cereal grains and oilseed meals is higher for Nile tilapia than for channel catfish (Popma 1982). The digestibility of protein of some common feed ingredients by *O. niloticus* is given in Table 12.1.

Many studies have been reported on protein requirement of tilapias. Most have involved small fish. This is unfortunate because most of the commercial feed is consumed by large fish. Data on protein requirements for fish from 1-50 grams in size have ranged from 30 to 50%, varying with protein quality, dietary energy level, feeding rate, natural food, and fish size. Optimum protein levels reported for larger fish have ranged from 25 to 45%. A consensus of experimental data indicate that

Table 12.1. PERCENTAGE DIGESTIBILITY OF PROTEIN, FAT, CARBOHYDRATE, AND GROSS ENERGY IN FEED INGREDIENTS AND PHYTOPLANKTON BY *OREOCHROMIS NILOTICUS* AND *OREOCHROMIS AUREUS*

	O. niloticus				*O. aureus*
Item	Protein	Fat	Carbohydrate	Gross Energy	Protein
Feed ingredients:					
Fish meal	84.8	97.8	-	87.4	-
Fish meal plus corn	84.9	-	-	-	-
Meat and bone meal	77.7	-	-	68.7	-
Soybean meal	94.4	-	53.5	72.5	-
Corn (uncooked)	83.8	89.9	45.4	55.5	-
Corn (cooked)	78.6	-	72.2	67.8	-
Wheat	89.6	84.9	60.8	65.3	-
Wheat bran	70.7	-	-	-	-
Alfalfa meal	65.7	-	27.1	22.9	-
Coffee pulp	29.2	-	-	11.4	-
Phytoplankton:					
Filamentous green algae:					
Hydrodictyon sp.	-	-	-	-	70
Oedogonium sp.	-	-	-	-	63
Planktonic green algae:					
Scenedesmus sp.	-	-	-	-	42-64
Volvox sp.	-	-	-	-	68
Planktonic blue-green algae:					
Microcystis sp.	-	-	-	-	57-68

Sources: Manandhar (1977) and Popma (1982).

small fish should be fed approximately 35% balanced crude protein (without natural foods), and grow-out fish receive 30 to 32% protein.

Energy

The dietary protein to energy ratio required for maximum growth decreases with increasing size of tilapia. Winfree and Stickney (1981) found that small *O. aureus* grew best when the diet contained a digestible energy/protein (DE/P) ratio of 8.2 to 9.4 kcal/g of protein. Kubaryk (1980) reported that small *O. niloticus* grew maximally when the DE/P ratio was 8.3 kcal/g for a 36% protein diet. He also found that as DE content of the diet increased, food consumption decreased, but that the amount of protein in the diet did not affect consumption rate.

Tilapia digest the gross energy in most commercial feedstuffs relatively well (Table 12.1). They do not digest highly fibrous feedstuffs, such as alfalfa meal and coffee pulp, well for energy needs. They digest carbohydrates in feedstuffs relatively well, much better than salmonids. Fats or proteins are more digestible to tilapias than are carbohydrates.

Essential Fatty Acids

Tilapias appear to have a dietary requirement for fatty acids of the linoleic (n-6) family. Supplementation of tilapia diets with vegetable oils (soybean or corn oils) rich in 18:2 n-6 has given better performance than oils, such as marine fish oils, high in 20:5 and 22:6 n-3 fatty acids (Takeuchi, et al. 1983a). The optimum dietary level of n-6 fatty acid has been estimated to be about 0.5 to 1%. Deficiency signs observed in fish fed diets deficient in essential fatty acids were poor appetite, retarded growth, and fatty livers. Tilapias do not tolerate as high a level of dietary fat as do salmonids. A dietary lipid level in excess of 12% depressed growth of juvenile *O. aureus* x *O. niloticus* hybrids (Jauncey and Ross 1982).

Vitamins

Because tilapias are efficient feeders on natural aquatic organisms, they can obtain a significant amount of their vitamin needs from the environment when cultured in ponds, even at high densities. Vitamin supplements are often deleted from practical feeds for tilapias cultured under low fish density conditions in ponds. In intensive raceway systems, where no natural food organisms are present, supplemental vitamins must be added to commercial feeds. In intensive pond culture, a vitamin mixture is added to the feed to avoid a deficiency of some vitamin.

Metabolically, tilapias appear to have similar vitamin requirements as other warmwater species. They show the classical vitamin C deficiency signs when deprived of the vitamin in the absence of natural foods. Vitamin E deficiency causes reduced growth rate, ceroids in liver and kidney, failure of mature males to develop sexual coloration, and degenerative changes in skeletal muscle. Lovell and Limsuwan (1982) showed that *O. niloticus* produced vitamin B_{12} in their intestinal tract through bacterial synthesis and did not require the vitamin in their diet. Other B complex vitamins apparently are synthesized by the intestinal microorganisms. Until definitive information is available on the vitamins that are produced in sufficient quantity in the intestine, a complete vitamin supplement should be included in tilapia feeds that are fed in experimental or commercial cultures where natural food is absent or limited. Because of the lack of information of vitamin requirements for tilapias, the requirements for channel catfish have been used to successfully formulate tilapia feeds.

Minerals

Tilapias probably require the same minerals as other fish species for tissue formation, metabolism, and to maintain osmotic balance between the body fluid and the water. Like other finfishes, they probably get a significant amount of certain minerals, such as calcium, from the water. Although there is a lack of information on the mineral requirements for tilapias, it is likely that the quantitative requirements are similar to those of other finfish species. In the absence of information on specific mineral requirements for tilapia, the mineral requirements for channel catfish (Table 2.4 in chapter 2) can be used with reasonable assurance that

mineral needs are met. The dietary level of available phosphorus required for maximum growth and normal bone mineralization of *O. niloticus* was estimated to be 0.4%, similar to that of channel catfish.

FEEDS AND FEEDING

Natural Foods

Fish of the genus *Tilapia* are macrophyte-feeders, in which the adults feed mainly on filamentous algae and higher aquatic plants. Tilapias of the genus *Oreochromis* are microphagus; their feeding regime consists notably of phytoplankton, zooplankton, detritus, and benthic organisms. Species of this genus, such as *O. aureus*, *O. niloticus*, and *O. mossambicus*, are primarily omnivores. However, there is a great deal of overlapping among the diet compositions of various species of tilapias. For example, tilapias that feed on macrophytes also ingest the attached algae, bacteria, and detritus. Epiphyte consumers also frequently ingest the supporting macrophytes. Bacteria and protozoans, attached to detrital particles, are important sources of nutrients for benthic feeders. Animal components such as zooplankton and benthic organisms may also be eaten. The digestibility values of crude protein of various natural foods by tilapias are presented in Table 12.1. Some algae are relatively high in protein and energy with good digestibility. The animal organisms are very good sources of protein and lipid with high digestibility.

Most tilapias have short and widely spaced gill rakers, but are efficient in ingesting phytoplankton, even *Nannochloris*, a solitary coccoid green alga measuring less than 5 microns in diameter. The collecting processes of the minute food particles involve entrapment of algae in mucus secreted by mucous glands in the mouth and/or by filtration by microbranchiospines present on the outermost gill arches (Fryer and Illes 1972).

Most tilapia culture in the world is in ponds, fertilized with feed, manure or inorganic fertilizers. Under these conditions, natural food organisms supply substantial amounts of nutrients required by the fish. Schroeder (1983) used stable carbon isotope analyses of the fish and the food sources and found that natural foods contributed 50% to 70% of the growth of tilapia polycultured with carps in ponds receiving supplemental feeds. Stomach analysis showed that up to 50% of the stomach contents of the tilapia are natural food in the intensively fed pond cultures, indicating that the natural pond productivity contributed a substantial amount of nutrients. The size of this contribution to the fish's nutrient requirements for maximum growth will depend upon pond productivity and fish density in the pond.

Practical Feeds

Commercial pond feeds for tilapias usually contain 24% to 28% protein. Natural pond foods contribute a significant amount of protein, so this level is assumed to be high enough. However, few pond studies have been conducted to compare various diet formulations for extensive or semi-intensive culture of tilapias. A 25% protein pellet composed of 15% fish meal, 20% soybean meal, 20% ground wheat, and 45% ground sorghum has been used successfully in Israel in this type of production. The importance of micronutrient supplementation in pond feeds for tilapia is not well known. Due to the extreme variation in the culture practices used, formulation of practical feeds to efficiently supplement the nutrient contribution of the natural food is practically impossible.

In intensive cultures, such as raceways or cages, tilapias rely solely on the prepared feeds as a source of nutrients; thus, a nutritionally complete feed containing all essential nutrients is required. The protein content of cage or raceway feeds is usually 32%; however, there have been relatively few experiments on protein allowance for production feeds for tilapia. In recirculating systems, the overhead costs of operating the systems are such that maximum growth rate is of considerable economic importance. Thus, highly concentrated feeds may be desirable. Some recirculating culture operators use 36% protein feeds, with energy balanced with protein, and claim that the additional growth rate, as compared to a 32% protein feed, is economically beneficial. Model formulae for pond and raceway feeds for tilapia are given in Table 12.2. Commercial diets formulated for common carp and channel catfish have been fed successfully to tilapia.

Table 12.2. MODEL TILAPIA FEEDS FOR PONDS AND RACEWAYS

Ingredient	Ponds	Raceways	
	26% protein	32% protein	36% protein
	(%)	(%)	(%)
Soybean meal	38.3	48.5	50.8
Wheat middlings	4.0	20.0	18.0
Fish meal	4.0	6.0	12.0
Corn	50.8	22.6	16.5
Dicalcium phosphate	1.0	1.0	0.8
Plant oil (sprayed) on pellet surface	1.5	1.5	1.5
Vitamin mix[1]	0.2	0.2	0.2
Trace mineral mix[1]	0.2	0.2	0.2

[1] Vitamin and mineral allowances for channel catfish given in chapter 9 will be sufficient. Vitamin and trace mineral supplements not necessary in pond feeds if fish are small and density is low.

Tilapia accept a variety of feeds, in meal form and in sinking and floating pellets. Crude feedstuffs used in extensive culture, such as rice bran, are usually offered in meal form. Tilapia can utilize meal type feeds effectively, although they obviously do not eat all of the meal that is put into the water. Crude feed sources may be uneconomical when pelleted for pond feeding of tilapias. High quality feeds for intensive culture, however, should be processed into pellets to minimize waste.

Tilapia seem to prefer smaller pellets than channel catfish and salmonids of comparable size. They tend to chew the pellets rather than swallow them whole as do most finfish species. Unacceptable pellets are usually taken into the mouth and then rejected several times before they are finally consumed or discarded. For feeding tilapias to marketable size of 500 g, the most common pellet size is approximately 3mm to 4mm in diameter and 6mm to 10mm in length. Feeds in meal or crumble forms are used for fry and fingerlings. These are made by first pelleting or extruding the feed mixture and then reducing the particles to a desirable size by crumbling.

Table 12.3. FEEDING RATES AND FREQUENCIES FOR VARIOUS SIZES OF TILAPIAS AT 28°C

Size	Daily feeding (% of fish weight)	Times fed daily
2 days old to 1 g	30-10	8
1-5 g	10-6	6
5-20 g	6-4	4
20-100 g	4-3	3-4
>100 g	3	3

Source: Adapted from Jauncey and Ross (1982); Coche (1982); Kubaryk (1980).

Feeding rates for tilapia are affected by species, size, temperature, feeding frequency, and the availability of natural foods. *T. rendali* consume more feed than *O. niloticus* of comparable age (Balarin and Haller 1982). As with other fishes, feed consumption rate of tilapias is inversely related to fish size. Tilapia benefit from multiple daily feedings. Because of their continuous feeding behavior and smaller stomach capacity, tilapia respond to more frequent feeding than channel catfish and salmonids. Kubaryk (1980) found that *O. niloticus* grew faster when fed four times daily than when fed two times, but did not grow faster when fed eight times. Tilapia will consume more feed and grow faster than channel catfish or salmonids if given sufficient feeding opportunities. Recommended feeding rates and frequencies for various sizes of tilapia are given in Table 12.3.

Feeds are offered to fish by hand, blower, demand feeders, or automatic feeders. Hand feeding is labor intensive, but has the advantage over other methods in that the feeder can observe the fish feeding better. Demand feeders are feeding devices that deliver feed when fish activate the feed release device. Automatic feeders deliver measured quantities of feed at various time intervals. Meriwether (1986) showed that *O. niloticus* gained 72% more weight when fed by demand feeder than when fed by hand one time daily, but feed conversion was 45% poorer for the fish fed by demand feeder. Demand feeders have disadvantages in that fish may causes release of the feed without consuming it.

REFERENCES

BALARIN, J. D., and R. D. HALLER. 1982. The intensive culture of tilapia in tanks, raceways and cages. In Recent advances in aquaculture, eds. J. E. Muir and R. J. Roberts, 265-356. Boulder, CO: Westview Press.

COCHE, A. G. 1982. Cage culture of tilapias. In The biology and culture of Tilapia, eds. R. S. V. Pullin and R. H. Lowe-McConnel. Manila, Philippines: ICLARM.

FRYER, G., and T. D. ILLES. 1972. The cichlid fishes of the Great Lakes of Africa. Hong Kong: T.F.H. Publications, Inc. Ltd.

JACKSON, A. J., B. S. CAPPER, and A. J. MATTY. 1982. Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. Aquac. 27(2): 97-109.

JAUNCEY, K., and B. ROSS. 1982. A guide to tilapia feed and feeding. Institute of Aquaculture, University of Sterling, Scotland.

KUBARYK, J. M. 1980. Effect of diet, feeding schedule and sex on food consumption, growth and retention of protein and energy by tilapia. Ph.D. Diss., Auburn University, AL.

LOVELL, R. T., and T. LIMSUWAN. 1982. Intestinal synthesis and dietary nonessentiality of vitamin B_{12} for *Tilapia nilotica*. Trans. Am. Fish. Soc. 11: 485-490.

MANANDHAR, H. N. 1977. Digestibility of phytoplankton by silver carp and three tilapias in polyculture with channel catfish. Master's Thesis, Auburn University, AL.

MERIWETHER, F. H. 1986. An inexpensive demand feeder for cage-reared tilapia. Prog. Fish. Cult. 48: 226.

POPMA, T. J. 1982. Digestibility of selected feedstuffs and naturally occurring algae by tilapia. Ph.D. dissertation, Auburn University, AL. 93pp.

SCHROEDER, G. L. 1983. The role of natural foods in tilapia growth: A study based on stable isotope analyses. Proceeding of the International Symposium on Tilapia in Aquaculture, Nazareth, Israel, 8-13 May 1983: 313-322.

TAKEUCHI, T., S. SATOH, and W. WATANABE. 1983a. Dietary lipids suitable for practical feed of *Tilapia nilotica*. Bull. Jpn. Soc. Sci. Fish. 49 (9): 1361-1365.

TREWAVAS, E. 1982. Tilapias: Taxonomy and speciation. In The biology and culture of tilapias, eds. R.S.V. Pullin and R.H. Lowe-McConnel, 3-14. Manila, Philippines: ICLARM.

VIOLA, S. and Y. ARIELI. 1983. Nutrition studies with tilapia (*Sarotherodon*). 1-Replacement of fish meal by soybean meal in feeds for intensive tilapia culture. Bamidgeh 35 (1): 9-17.

WINFREE, R. A. and R. R. STICKNEY. 1981. Effect of dietary protein and energy on growth, feed conversion efficiency and body composition of *Tilapia aurea*. J. Nutr. III (6): 1001-1012.

13 FEEDING PENAEID SHRIMP

Chhorn E. Lim

Until about two decades ago, all shrimp supplied to the world markets was harvested from the oceans. Presently, the ocean fisheries for shrimp are at near maximum sustainable yield. Shrimp catches from the sea are unpredictable due to uncontrollable natural phenomena. Pollution and other human activities have disrupted the ecology of shrimp nursery grounds in many areas. Energy requirements for harvesting ocean shrimp are high; diesel fuel represents more than 50% of the total operating costs. Therefore, the increase in world demand for shrimp is dependent upon an increase in production through aquaculture. In 1986, pond-raised shrimp represented an estimated 6% to 8% of the world shrimp supply. By 1996, according to the Food and Agriculture Organization (FAO) of the United Nations, farmed shrimp accounted for 43% of the estimated world shrimp consumption.

Marine shrimp farming has been practiced for centuries in many Asian countries using the traditional method of trapping and holding wild shrimp which enter coastal ponds through the incoming tidal water exchange , as shown in Figure 13.1. However, the development of modern shrimp farming technology began in the 1930's when Hudinaga first successfully spawned and reared larvae of *Penaeus japonicus* in captivity. Since then technology has been developed for mass production of marine shrimp larvae. Within the past 20 years, several shrimp species of the penaeid family, such as *Metapenaeus ensis, Penaeus japonicus, P. monodon, P. indicus, P. mergiensis, P. aztecus, P. setiferus, P. schmitti, P. chinensis* (also known as *P. orientalis*), *P. penicillatus, P. stylirostris* and *P. vannamei,* have been matured, spawned and their larvae reared in captivity.

As shrimp farming has expanded, the production methods have shifted from traditional extensive to semi-intensive and intensive systems, utilizing modern facilities, equipment and management techniques aimed at high yield production. Natural food constitutes an important source of nutrients for extensive culture, whereas artificial feeds are the primary source of nutrients for semi-intensive and intensive practices. In semi-intensive and intensive culture systems, feed represents 30% to more than 50% of the total variable costs. Thus, the use of least-cost, nutritionally balanced feeds and good feeding practices are fundamental to successful shrimp farming. This chapter provides an overview of culture practices, nutrient requirements, feeds and feeding practices for penaeid shrimp.

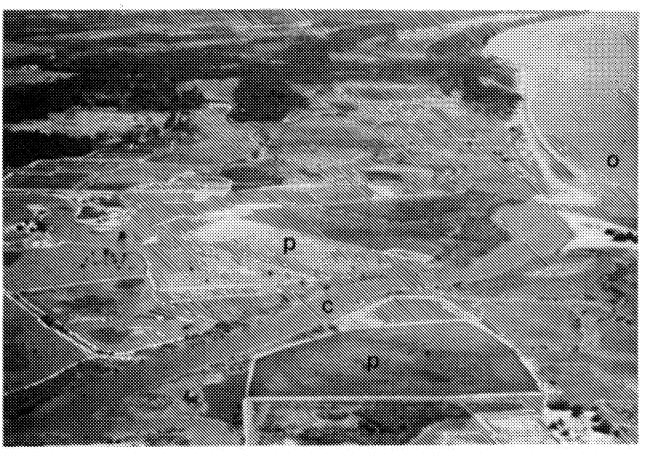


Figure 13.1 Coastal ponds in Indonesia where the incoming tide from the ocean fills the ponds (P) with water and wild shrimp. The water is impounded until the shrimp reach harvest size on natural foods, then the ponds are drained into the canal (C) with the outgoing tide and the shrimp are gathered.

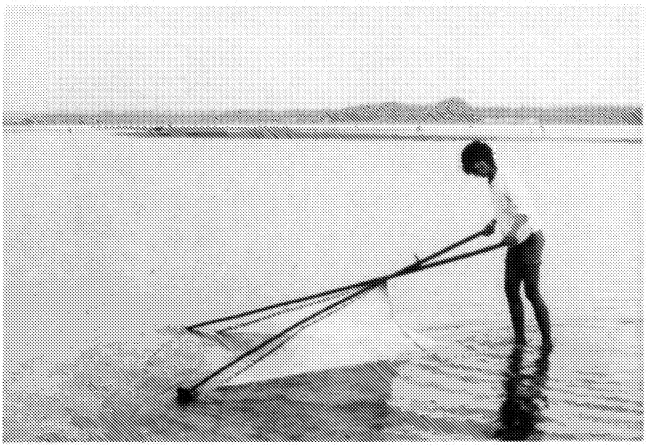


Figure 13.2 Post-larval *Penaeus vannamei* and *P. stylirostris* are taken from esturine waters in Honduras by artisan fishermen and sold to shrimp farmers to stock in production ponds. Wild caught post larvae are a significant source of seed shrimp for commercial pond stocking in may parts of the world.

CULTURE PRACTICES

Successful shrimp culture is dependent on the availability of good quality seed for stocking production ponds. Traditionally, wild caught postlarvae constitute the primary source of seed stock in many countries. Figure 13.2 shows an artisan fisherman collecting post larval shrimp from an estuary in Honduras. However, the quantity of wild seed is unpredictable and fluctuates considerably depending on the

ecology and productivity of the nursery grounds, weather conditions, and other factors. Therefore, shrimp farmers are currently relying more on hatchery-produced postlarvae for seed stock.

Broodstock Maturation and Spawning

One of the limiting factors in hatchery production of postlarval shrimp is the availability of spawners. Until the mid-1970's, the spawners used in hatcheries were gravid females taken from the wild. They were in advanced stages of reproductivity and most spawned the day they were captured. Since then, several species of penaeid shrimps have been induced to mature and spawn in captivity. Thus, gravid females may be produced in culture ponds or tanks, or caught from the oceans. Introduced species of broodstock must be spawned in captivity.

Ovarian maturation in captivity can be achieved by environmental manipulation, use of good quality feeds and eyestalk ablation (removal of eye). Some species of shrimp such as *P. indicus, P. merguiensis, P. californiensis, P. japonicus* and *P. chinensis* are easy to breed and can mature in captivity without requiring ablation. However, eyestalk ablation is usually performed even for the easy-to-breed species, to accelerate vitellogenesis and increase maturation and spawning rates. Unlike females, male shrimp do not require eyestalk ablation because they mature readily in captivity.

Brood shrimp are commonly stocked in indoor circular tanks (5-10 m^3 with 0.8-1.0 m water depth) at a rate of 5 to 10/m^2 and a sex ratio of 1-2 females to 1 male, depending on species and size. Maturation tanks are equipped with flow-through system (100-300% exchange rate/day) and continuous aeration, and provided with dark covers to reduce light intensity and control the photoperiod of about 13 hours light for tropical species, such as *P. monodon* and *P. vannamei,* and 8 hours light for subtropical species such as *P. chinensis* and *P. japonicus*. Water temperatures are maintained at 26-32°C and 20-28°C for tropical and subtropical species, respectively. Commonly used food sources for broodstock are fresh or frozen squid, mussel, clam, bloodworm, fish and pelleted feeds. Pelleted feeds are usually used in combination with fresh or frozen foods. Shrimp are fed to satiation 2 to 4 times daily. Feed consumption is regularly monitored and the amount of feed fed is adjusted so that the majority of the feed is consumed before the next feeding.

The first onset of maturation occurs 1 to 3 weeks following eyestalk ablation depending on the age and source of broodstock, stage of molting, and other factors. Females with advanced stage of ovary development are removed and transferred to spawning tanks. Spawning always occurs at night.

Larval Rearing

Fertilized eggs hatch into nauplii within 16 hours at a water temperature of 28°C to 30°C. Newly hatched nauplii do not feed but are nourished by the yolk nutrients. The nauplii metamorphose into protozoea (zoea) after five to six moltings within 48 hours. At this stage the larvae are fed primarily with planktonic diatoms such as *Skeletonema* sp., *Tetraselmis* sp., and *Chaetoceros* sp. Artificial feeds, such as egg yolk powder and yeast, are sometimes given as a supplement to the natural foods. The zoeal larvae have no reflex to search for food but must wait until, by chance, suitable food particles come in contact with the mouth. Thus, a sufficient quantity of food must be kept in suspension in the water in the culture tank at all times.

The zoea molt three more times within 4 to 5 days before transforming into myses. Mysis larvae resemble young shrimp, but they swim in a vertical position with head and tail downward. They are fed mainly with *Artemia* nauplii or zooplankton, especially *Brachionus* sp., in addition to the phytoplankton.

The myses metamorphose to postlarvae after three moltings within 3 to 4 days. During the first 5 days of the postlarval stage, they are usually fed with *Artemia*. Artificial feeds, such as small dry diet particles, microencapsulated diets, and minced fish flesh, are offered as partial substitutes for live foods as the larvae gradually acquire the benthic habit of living and feeding on the bottom of the tanks. The larvae are stocked in nursery or production ponds, depending upon the management practice, after 5 to 20 days at the postlarval stage.

Nursery Practices

Growing shrimp postlarvae to juveniles in nursery ponds prior to stocking in production ponds offers the advantage of having large size shrimp to stock. By stocking larger size shrimp, the culture period can be shortened or larger shrimp can be harvested. Survival rate is higher when postlarvae are grown in nursery ponds than when postlarvae are stocked directly into production ponds. Estimation of feed allowances and harvest yields is easier due to more accurate assessment of survival rate when juveniles (from nursery ponds) are stocked. Disadvantages of using nursery systems are that juveniles are more difficult to handle than postlarvae and higher mortality may result during transport. Additional land and capital are required for the construction and operation of nursery units.

Most modern, large-scale shrimp farms have incorporated nursery systems into the production scheme. The stocking rates used vary considerably depending on the systems, the management practices and the species cultured. Stocking rates in earthen nursery ponds are from 100 to 300 postlarvae/m^2. Higher stocking rates are used for intensive tank systems, ranging from 500 to 1,000 postlarvae/m^2. The postlarvae are fed two to four times daily with crumbled dry diets containing 35 to 45% crude protein or fresh, frozen foods such as chopped fish and mussel. Daily feeding rates vary considerably. When first placed in the nursery ponds, the small (0.001-0.01 g) postlarvae are fed 25% to 50% of their weight, divided into four or more daily feedings. As they grow, feed allowance and frequency decrease. Toward the end of the 30 to 45-day nursery period, the feed allowance may decrease to 10% or less of shrimp weight, depending upon the weight of shrimp in the pond. Final average shrimp weight in nursery ponds ranges from 0.5 to 1.5 g.

Growing Shrimp to Marketable Size

There are great differences among culture systems and management procedures being used in shrimp farming. These differences are mainly attributed to the availability and cost of land, seedstock, feed, electricity, fuel, and culture technology and value of the shrimp produced. The methods of shrimp culture may generally be classified under three categories: extensive, semi-intensive, and intensive techniques.

Extensive culture is characterized by low stocking density, usually less than 2.5 postlarvae or juveniles/m^2. Natural stocking with postlarvae coming in during high tides is still being practiced in some Asian countries. Supplementary feeding is seldom practiced and the shrimp depend mainly on natural foods available in the ponds. Organic and inorganic fertilizers are used to increase the productivity

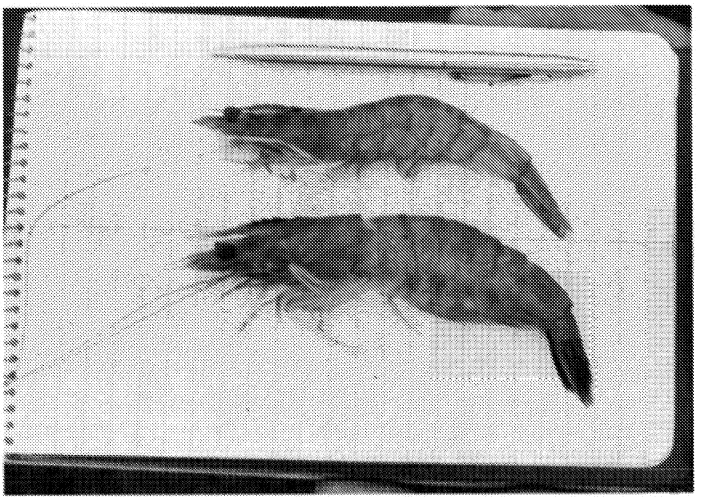


Figure 13.3 Harvestable size *Penaeus vannamei* (top) and *P. stylirostris* (bottom) from semi-intensive culture ponds in Honduras. Sizes are approximately 18 grams for *vannamei* and 23 grams for *stylirostris*.

of natural foods. Water management is done through tidal fluctuation. The yields obtained generally range from 150 to 500 kg/hectare/crop.

Most of the world's supply of farmed shrimp is produced in earthen ponds by semi-intensive management techniques. In semi-intensive culture operations the stocking rates per square meter vary from 3 to 15 juveniles, or up to twice this number if early postlarvae are stocked. Commercial feeds are given as supplements to the natural foods. Fertilizers are often applied initially to enhance the growth of natural food organisms. Water is pumped through the ponds at a rate 2% to 10% of the pond volume daily when the pond receives feed. Generally, the yields range from 600 to 2,000 kg/hectare/crop, with 2 to 2.5 crops per year. Average shrimp weight at harvest varies from 16 to 36 grams, depending upon stocking density, pond environment, and management. Price of shrimp is directly related to size. Figure 13.3 shows harvestable size *P. vannamei* and *P. stylirostris* from semi-intensive culture ponds.

In areas where land cost is very high, intensive culture practices in ponds or tanks may be economically feasible. Intensive culture operations require sophisticated management techniques and nutritionally complete, concentrated diets. In intensive pond culture, the shrimp are stocked at a rate of 20/m^2 to 150/m^2. Water is exchanged daily at a rate of 30% or higher and aeration is usually provided. The shrimp depend primarily on the commercial feed as their source of nutrients; thus, nutritionally complete, concentrated feeds are used. The production ranges from 2,500 to 6,000 kg/hectare/crop. In intensive tank cultures, stocking density goes up to 160 juveniles/m^2. High water exchange rate (100-300% daily), continuous aeration, and nutritionally complete, concentrated feeds are used. Production can reach as high as 24,000 kg/hectare/crop. Intensive tank culture techniques are practiced in Taiwan and Japan where shrimp market prices are high.

NUTRIENT REQUIREMENTS

Proteins and Amino Acids

Shrimps, like fishes and other animals, do not have an absolute requirement for protein per se, but require a balanced mixture of indispensable and dispensable amino acids. The optimum dietary protein level for growth of penaeid shrimps has been reported to range from 28% to 60%, as shown in Table 13.1. Reasons that these values differ are due to species, size, protein quality, level of nonprotein energy, physical quality of pellet, palatability of diet, feeding rate, water quality, and availability of natural food organisms. Most of the values in Table 13.1 were determined with small shrimp in tanks or aquaria in absence of natural aquatic foods. Shrimp grown to harvestable size, especially in ponds, require less protein than the higher values in Table 13.1.

The protein percentage in commercial feeds fed in intensive culture systems is usually 35% or above. That in semi-intensive culture feeds varies, generally from 25% to 35%. Research at the Enrique Ensenot Marine Laboratory in Panama showed that 25% protein feeds were as productive as higher protein feeds in semi-intensive culture ponds, which were also fertilized, containing 5 shrimp per m^2.

Several experiments have indicated that shrimp require the same 10 essential amino acids as do finfishes and terrestrial animals. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine have been found to be essential for *P. japonicus*, *P. azteus*, *Palaemon serratus*, *P. monodon*, and *P. chinensis*. However, except for the requirements of arginine, methionine, threonine and valine for *P. monodon* and lysine for *P. vannamei*, the quantitative requirements of essential amino acids for various shrimp species have not yet been determined. In absence of information on quantitative amino acid requirements, the essential amino acid profile of the protein in an animal's body, which closely approximates the dietary amino acid requirements of the animal, can be used. Table 13.2 shows the essential amino acid content of *P. japonicus* and *P. vannamei* muscle, and also of clam, squid, and whole-egg protein, which are high quality proteins for shrimps. A shrimp diet with an essential amino acid profile similar to that of shrimp muscle, clam or squid would likely provide good growth in the fed shrimp.

Numerous studies have been conducted to evaluate the nutritional values of various protein sources. Casein, which is generally used as a standard protein source for nutrient requirement studies of finfishes and other vertebrates, has been found to be poorly utilized by several shrimp species. This is possibly due to the low arginine content of casein. Fish meal, a high quality protein source for finfishes, seems to have lower nutritional value for shrimp, especially when fed as the sole protein source. This has been reported for several species. Shigueno (1975) has suggested that this may be due to a shortage of phenylalanine and the basic amino acids (arginine, histidine, and lysine) in some fish meals. Wheat gluten, one of the most commonly used natural binders in shrimp pellets, is deficient in arginine, lysine and methionine for *P. vannamei* with the order of limitation being lysine>methionine>arginine.

Soybean meal, the most commonly used plant protein in feeds for warmwater fish, has been found to be a relatively good protein source for shrimp. It provided better growth of *P. duorarum* than fish meal, shrimp meal, casein, or corn gluten meal (Sick and Andrews 1973). Soybean meal at levels of 20% to 50% of

Table 13.1. OPTIMUM DIETARY PROTEIN REQUIREMENTS FOR GROWTH OF YOUNG PENAEID SHRIMPS

Species	Protein source	Protein requirement (%)	Reference
Penaeus japonicus	Squid meal	60	Deshimaru & Shigueno 1972
	Shrimp meal	40	Balazs et al. 1973
	Casein and egg albumin	52-57	Deshimaru & Yone 1978a
	Casein	45-55	Teshima & Kanazawa 1984
Penaeus monodon	Casein and fish meal	45-50	Lee 1971
	Squid meal, shrimp meal, fish meal and casein	40-45	Alava & Lim 1983
Penaeus indicus	Prawn meal	43	Colvin 1976
Penaeus aztecus	Soy flour, fish meal and shrimp meal	51	Zein-Eldin and Corliss 1976
Penaeus merguiensis	Mussel meal	34-42	Sedgwick, 1979
Penaeus setiferus	Fish meal	28-32	Andrews et al., 1972
Palaemon serratus	Shrimp meal or fish meal	40	Forster and Beard, 1973
Penaeus stylirostris	Fish meal, shrimp meal and soybean meal	35	Colvin and Brand, 1977
Penaeus vannamei	Fish meal, shrimp meal and soybean meal	30	Colvin and Brand, 1977
	Fish meal, soybean meal, shrimp meal, wheat gluten and squid meal	30	Duerr and Lim, 1990

Table 13.2. ESSENTIAL AMINO ACID CONTENT OF PROTEINS FROM SHRIMP MUSCLE (*PENAEUS JAPONICUS* AND *P. VANNAMEI*), CLAM, SQUID AND WHOLE-EGG

Amino acid	Protein source					
	P. japonicus[1]	*P. vannamei*	Clam[1]	Squid	Casein	Whole-egg[1]
	(%)	(%)	(%)	(%)	(%)	(%)
Arginine	7.46	8.54	4.50	5.40	3.30	5.45
Histidine	1.66	1.86	1.27	1.50	2.65	1.71
Isoleucine	2.89	3.40	2.00	2.88	4.50	3.46
Leucine	7.04	6.28	4.01	5.79	8.76	6.47
Lysine	7.24	6.97	4.68	5.52	7.34	5.45
Methionine	2.92	2.48	1.70	2.30	2.51	3.01
Phenylalanine	3.90	3.39	2.13	2.86	4.75	4.15
Threonine	3.62	2.69	2.81	3.28	3.77	3.73
Tryptophan	0.52	1.27	0.51	0.72	1.21	0.79
Valine	2.87	3.38	2.18	2.66	5.83	3.76

[1]Deshimaru 1982.

the diet has been suggested as a replacement for much of the fish meal, shrimp meal, squid meal, or their combination in practical diets for shrimp. Lim and Dominy (1990), however, suggested that, if pellet palatability and water stability can be improved, the level of soybean meal in the diets of juvenile *P. vannamei* could be increased up to 56%. The nutritional value of extruded full-fat soybean meal for *P. vannamei* is essentially the same as that of commercial soybean meal reconstituted with soybean oil. The level of full-fat soybean meal that can be incorporated in marine shrimp diets, however, may be limited because of its high oil content which may result in imbalance of energy and essential fatty acids. Cottonseed meal has been shown to be relatively palatable for shrimp. However, glanded cottonseed meal (0.41% free gossypol) should not be included at more than 26%, or 1,100 ppm of free gossypol, in diets of juvenile *P. vannamei*. Also, cottonseed meal is very low in lysine and, for this reason, is not an equal substitute for soybean meal.

Attempts to supplement amino acid-deficient diets with crystalline amino acids have not been successful with shrimp. Basal diets consisting entirely of crystalline amino acids or a mixture of intact protein and amino acids have been evaluated in several shrimp species and have failed to provide good growth equal to that of intact protein of similar amino acid composition. However, when various protein sources are combined to provide an essential amino acid profile similar to that of a high quality protein, such as clam protein, growth rate of the shrimp is essentially the same as that obtained by using a diet with clam protein (Deshimaru 1982). Deshimaru (1982) showed that the rate of incorporation of free radioactive arginine into muscle protein was less than 1% as compared to incorporation of 90% of protein-bound arginine. Mai et al. (1988) showed that juvenile *P. orientalis* did not absorb free methionine and lysine simultaneously with the protein-bound amino acids. The inability of shrimps to utilize free amino acids as substitutes for protein-bound amino acids is probably due to differences in the rate of absorption of free and protein-bound amino acids. Another possible factor is the leaching of free amino acids from the feed into the water prior to ingestion since shrimp are slow feeders.

Various substances and techniques have been employed to encapsulate or bind synthetic amino acids to proteins to reduce their solubility and increase their retention time in the digestive tract, thus improving their bioavailability to shrimp. Teshima et al. (1992) found that soybean protein enriched with methionine by plastein reaction provided significantly better growth of *P. japonicus* than soybean protein supplemented with crystalline methionine. Supplementation of wheat gluten with covalently bonded lysine provided significantly better growth of *P. vannamei* than wheat gluten diets supplemented with crystalline free lysine (Fox et al. 1995).

Microencapsulating or coating amino acids to prevent leaching losses and to delay their release in the digestive tract, and using binders to improve the pellet water stability, have led to improved utilization of crystalline amino acids by shrimp. Using arginine encapsulated with cellulose acetate phthalate, Chen et al. (1992b) successfully determined the arginine requirement of juvenile *P. monodon* to be 5.47% of the dietary protein. Coating the amino acids with cooked carboxymethylcellulose allowed Millamena et al. (1996 and 1997) to quantify valine, methionine and threonine requirements of *P. monodon*. The requirements, expressed as percentage of dietary protein, are 3.40% for valine, 2.40% for methionine (in a diet containing 1.10% cystine) and 3.50% for threonine.

Energy

Generally, protein is given the first priority in feed formulation because shrimp utilize a high dietary level of protein and protein is the most expensive component of the prepared diets. However, providing the optimum amount of energy in the diet is important because a deficiency in non-protein energy means that part of the protein will be used for energy. Shrimp appear to utilize carbohydrates and lipids as energy sources to spare protein. Inclusion of an appropriate amount of carbohydrates and lipids in the diets of several species lowered the protein requirement without reducing growth performance. Excess energy in the diet, however, can limit feed consumption, thereby reducing the intake of protein and other nutrients. Sedgwick (1979) found that the amount of feed consumed by *P. merguiensis* is regulated by the dietary energy level irrespective of the protein content.

Relatively little is known about the energy requirements of shrimp. The optimum energy-to-protein ratios for various species of shrimp at different sizes have not been defined. The dietary protein to energy ratio required for optimum growth probably decreases with increasing size of shrimp, as has been reported for young fish. *P. indicus* grew best on a 42% crude protein diet containing 4.72 kcal gross energy per g (Colvin 1976) and small *P. merguiensis* had maximum growth with a 39.5% protein diet having a gross energy value of 4.42 kcal per g (Sedgwick 1979). The crude protein-to-gross energy ratio in these studies is approximately 90 mg per kcal, which is similar to the optimum protein-to-energy ratio for finfish.

Lipids

Lipids are required in the diets of shrimps for their energy value, and as sources of essential fatty acids, fat-soluble vitamins, sterols, and phospholipids. Shrimps appear to have a dietary requirement for fatty acids of the linoleic and linolenic series. Studies on the biosynthesis of fatty acids by *P. japonicus*, and *P. monodon* using labeled acetate or palmitate indicate that most of the radioactive compounds were incorporated into saturated and monosaturated fatty acids such as palmitic (16:0), palmitoleic (16:1 n-7), stearic (18:00), oleic (18:1 n-9), and eicosamonoenoic (20:1 n-9) acids; very little was converted into linoleic (18:2 n-6), linolenic (18:3 n-3), eicosapentaenoic (20:5 n-3) and docosahexaenoic (22:6 n-3) acids. This suggests that fatty acids of the linoleic (18:2 n-6, 20:4 n-6 or 22:5 n-6) and linolenic (18:3 n-3, 20:5 n-3 and 22:6 n-3) series are probably dietary essentials for penaeid shrimp. Feeding experiments have also shown that linoleic (n-6) and linolenic (n-3) series fatty acids are dietary essentials for *P. japonicus*, *P. indicus*, *Palaemon serratus*, *P. stylirostris* and *P. vannamei*. Fed alone, linolenic acid was found to be nutritionally superior to linoleic acid, while eicosapentaenoic or docosahexaenoic acids promoted better growth than linolenic acid. Shrimp appear to have limited ability to desaturate and elongate 18:3 n-3 to 20:5 n-3 and 22:6 n-3 fatty acids, which are the biologically active fatty acids. The optimum dietary level of 20:5 n-3 or 22:6 n-3 fatty acids for shrimps has been determined to range from 0.5% to 1.0%, while the optimum level of the n-6 series of fatty acids is estimated to be approximately 0.5%. Diets containing 0.5% n-6 and 0.5% n-3 fatty acids have provided for maximum growth of several shrimp species.

Dietary lipids have a sparing effect on the utilization of dietary protein. However, shrimp do not tolerate as high a dietary lipid level as salmonid fishes. Several studies using different lipid sources or combinations have suggested that a

lipid level of 6%-10% is optimum for various species of shrimp and a dietary lipid level in excess of 10% tends to depress growth.

Crustaceans do not synthesize sterols from acetate or mevalonate as do finfish, and therefore they require a dietary source. Cholesterol is the major sterol found in crustaceans and is a precursor of sex hormones, molting hormones, and a constituent of the hypodermis in crustaceans. Feeding experiments have indicated that sterols are dietary essential for growth and development of different crustacean species. Optimum dietary levels of cholesterol required by marine shrimps have been reported to be 0.5% to 1.0%. Crustaceans have the ability to metabolically convert some sterols to cholesterol. Phytosterols and fungal-sterols, such as ergosterol, stigmasterol, and beta-sistosterol can be partially substituted for cholesterol in the diets of juvenile *P. japonicus*. However, when used as the sole source, these sterols were not as effective as cholesterol in sustaining growth and survival of shrimp (Teshima et al. 1983 and 1989).

In addition to the essential fatty acids and sterols, marine shrimps seem to also have a dietary requirement for phospholipids, commonly known as lecithin. The optimum dietary levels of phospholipids for shrimp range from 1% to 3% depending on the species, life stages, source and nature of phospholipids, and possibly other dietary nutrients. Lecithin from clam, soybean, bonito egg, and phosphatidylinositol from soybean were suitable sources of phospholipids for shrimp. Effective phospholipids for shrimps appear to be those containing choline or inositol and polyunsaturated n-3 or n-6 series fatty acids. Finfishes do not have a dietary requirement for preformed phospholipids. The reason that marine shrimps have this requirement is apparently due to the slow rate of biosynthesis and the high metabolic requirement for phospholipids.

Carbohydrates

Shrimp utilize carbohydrates as energy sources but the efficiency of utilization varies depending on the source and level of carbohydrates. Polysaccharides such as dextrin and starch are better utilized by shrimp than monosaccharides such as glucose. Gelatinized bread flour at 25% (Shiau and Peng 1992) and corn starch or dextrin at 30% (Catacutan 1991) promoted good growth and survival of *P. monodon*. Carbohydrates have been shown to have a sparing effect on the utilization of dietary protein. The level of protein in the diet of *P. monodon* can be decreased from 40% to 30% by increasing the starch content from 20% to 30% (Shiau and Peng 1992).

A reason for the poor utilization of glucose by shrimp may be due to the rapid rate of absorption in the digestive tract. The serum glucose level of juvenile *P. japonicus* fed a diet containing glucose increased to a maximum level one hour after feeding and remained at that level for 24 hours (Abdel-Rahman et al. 1979). However, when shrimp were fed diets containing soluble polysaccharides, the maximum serum glucose level, which was reached after three hours, was much lower than in the glucose fed shrimp, and decreased to the original level within 12 hours. The plasma glucose concentrations of *P. monodon* fed glucose containing diets peaked earlier than those of shrimp fed dextrin or starch containing diets (Shiau and Peng 1992).

Chitin, a major component of exoskeleton of shrimp which is shed and replaced repeatedly, contains glucosamine. Shrimp can synthesize chitin from glucose via glucosamine. There is conflicting evidence on the essentiality of glucosamine in shrimp diets. Kitabayashi et al. (Kitabayashi et al. 1971) observed

a beneficial effect of glucosamine supplementation and reported that a dietary level of 0.53% is optimum for *P. japonicus*. However, Deshimaru and Kuroki (1974) failed to show a growth improvement of juvenile *P. japonucus* fed glucosamine supplemented diets. Inclusion of chitin in the diet depressed the growth of *P. japonicus* (Kitabayashi et al. 1971) but did not have any effect on the performance of *P. monodon,* even at a dietary level of up to 16% (Fox 1993).

Vitamins

Among the 15 vitamins which have been identified as essential for finfish, 14 have been demonstrated through research to be dietary essentials for shrimp. The essentiality and requirement data for these vitamins have generally been determined from weight gains.

Penaeid shrimp have a dietary requirement for the fat soluble vitamins, A, D, E and K. The optimum levels reported are 100 $mg*kg^{-1}$ of diet for vitamin E (Kanazawa 1985), 4,000 $IU*kg^{-1}$ for vitamin D (Shiau and Hwang 1994) and 30 $mg*kg^{-1}$ for vitamin K (Shiau and Liu 1994a). Quantitative requirement for vitamin A has not been determined.

Dietary requirements reported for water-soluble vitamins are 15-100 $mg*kg^{-1}$ for thiamin (Deshimaru and Kuroki 1979, and Chen et al. 1991), 20 $mg*kg^{-1}$ for riboflavin (Chen and Hwang 1992), 80 $mg*kg^{-1}$ for pyridoxine (Deshimaru and Kuroki 1979), 400 $mg*kg^{-1}$ for niacin (Kanazawa 1985), >4 $mg*kg^{-1}$ for biotin (Kanazawa 1985), 0.2 $mg*kg^{-1}$ for vitamin B_{12} (Shiau and Lung 1993), 2,000-4,000 $mg*kg^{-1}$ for inositol (Kanazawa et al. 1976) and 600 $mg*kg^{-1}$ for choline chloride (Kanazawa et al. 1976). Folic acid is dietary essential but the requirement value has not been determined. Pantothenic acid has not been tested in shrimp but is probably essential based on responses of several finfish species. When stable forms of vitamin C such as L-ascorbyl-2-phosphate are used, 20 to 215 mg of vitamin C per kg of diet appears to be adequate.

Shrimp fed diets deficient in vitamin C developed "black death" syndrome which is characterized by blackened lesions in the subcuticular tissues of the body surface; in the walls of the esophagus, stomach, and hindgut; and in the gills and gill cavity (Lightner et al. 1977). Vitamin C deficiency signs in *P. japonicus* are discoloration and development of a grayish-white on the margin of the carapace, the lower part of the abdomen, and on the tips of the walking legs (Deshimaru and Kuroki 1976).

The dietary levels of various vitamins reported for shrimps are considerably higher than those found for finfishes. Whether or not shrimp have a metabolic requirement for such high levels of these nutrients or whether a substantial quantity of nutrients is lost into the water during ingestion by the shrimp is unknown. Moreover, the information available are confined to postlarval or early juvenile stage cultured in the laboratory under well-controlled environmental conditions. Thus, in the absence of clear-cut information on the vitamin requirements of shrimps, vitamin allowances given in Table 13.3 may be used as guides.

Minerals

Shrimp, like other marine species, absorb several minerals from the surrounding sea water. *P. japonicus* grown in sea water do not have a dietary requirement for calcium, magnesium, iron or manganese, but require phosphorus, potassium and several trace minerals in the diet (Deshimaru and Yone 1978b, and Kanazawa et al.

Table 13.3. RECOMMENDED VITAMIN ALLOWANCES FOR SUPPLEMENTAL AND COMPLETE DIETS FOR SHRIMP

Vitamin	Amount per kilogram diet	
	Supplemental[1]	Complete[2]
Vitamin A	2,000 IU	4,000 IU
Vitamin D	1,000 IU	2,000 IU
Vitamin E	50 mg	100 mg
Vitamin K	10 mg	20 mg
Thiamin	20 mg	50 mg
Riboflavin	10 mg	30 mg
Pyridoxine	30 mg	60 mg
Pantothenic acid	30 mg	80 mg
Niacin	30 mg	80 mg
Biotin	0	2 mg
Folic acid	2 mg	5 mg
Vitamin B_{12}	0.01 mg	0.05 mg
Inositol	50 mg	200 mg
Vitamin C (stable form)	100 mg	200 mg
Choline chloride	500 mg	1,500 mg

[1] *Allowances in practical diets fed in semi-intensive ponds.*
[2] *Allowances in purified diets or feeds for highly intensive culture.*

1984). It has also been shown that *P. vannamei* do not have a dietary requirement for calcium and iron in a marine environment.

Although calcium is not a dietary essential for marine shrimp, this element is usually included in the diet to maintain a desired ratio of calcium to phosphorus of approximately 1:1 to 1:2. The phosphorus requirements of *P.vannamei* varied depending on the calcium content in the diet. Maximum growth was obtained with a dietary level of 0.34% phosphorus in the absence of calcium, but in the presence of 0.5% calcium maximum growth occurred with 1.0% dietary phosphorus (Davis et al. 1993b). Other minerals known to be essential for *P. vannamei* are copper at 32 mg, zinc at 33 mg and selenium at 0.2 to 0.4 $mg*kg^{-1}$ of diet. The dietary zinc

Table 13.4. RECOMMENDED MINERAL ALLOWANCES FOR NUTRITIONALLY COMPLETE SHRIMP DIETS

Mineral	Amount per kilogram dry diet
Macromineral (g)	
Calcium	10.0
Phosphorus[1]	10.0
Potassium	6.0
Magnesium	0.4
Micromineral (mg)	
Manganese	40.0
Zinc[2]	33.0 or 200.0
Iron	60.0
Copper	32.0
Iodine	5.0
Selenium	0.4
Cobalt	0.4

[1] Total P is used due to lack of information on phosphorus availability from various sources for shrimp.
[1] The higher level is recommended if the diet contains phytate.

requirement, however, increased to 218 mg/kg when the diet contained 1.5% phytate (Davis et al. 1993a). Table 13.4 presents recommended mineral allowances for nutritionally complete shrimp diets.

FEEDS AND FEEDING

Natural Foods

Penaeid shrimps are regarded as omnivorous scavengers that feed on a variety of benthic organisms and detritus, but they cannot be placed in any one trophic level because they are generally opportunistic feeders. The food habits of shrimps vary during the life stages. At zoea and mysis, the larvae feed on free swimming plankton. The postlarvae, being strictly demersal, are detritivores. The feeding habit of juveniles is at first omnivorous and then changes to carnivorous, and they prey mainly on slowly moving microinvertebrates. Adult penaeid shrimps are

opportunistic feeders, but seem to prefer foods of animal rather than plant origin. Small crustaceans, mollusks, fish, polychaetes, and annelids constitute the principal natural diet components of shrimps. Under pond conditions, the primary source of natural food for shrimps is the thin aerobic layer of the pond bottom. This layer consists of both living and dead algae and plankton, bacteria, detritus, and other benthos such as polychaetes and annelids. Bacteria have been found to comprise 10% to 20% of the total organic carbon in the stomach contents of several species of shrimps (Moriarty 1977).

Feeding Behavior

Shrimps find their foods mainly by chemosensory mechanisms rather than vision. The chemoreceptors are concentrated on the anterior appendages, antennae, and antennules. Once the scent is detected, the shrimps become alerted, move over the substrate toward the direction of the food, and rapidly seize the food with either of the first three pairs of the chelate pereiopods. Each pereiopod can work separately in either locating, gathering, holding, or conveying the food to the mouth parts. The mouth parts, which are comprised of three pairs of maxillipeds, two pairs of maxillae, and a pair of heavily chitinized mandibles, act together to reduce large food particles to a size suitable for ingestion. This apparently is an opportunity for loss of nutrients from processed feeds to the water.

The ingested food is further chewed to fine particulate size by the mandibles before being swallowed. The food passes through the esophagus and enters the anterior chamber of the proventriculus (foregut), where it is further reduced to a semifluid state mechanically and by digestive enzymes, and separated into fluid and coarse fractions by dense setae. The fluid passes into the posterior chamber and finally into the tubules of the hepatopancreas for further digestion and absorption. The coarser particulates pass directly to the midgut, where there is some digestion, and which is an important site of nutrient absorption. The undigested and unabsorbed portions of food enter into the hindgut, which serves mainly as a region for the compaction and transportation of fecal materials.

Practical Feeds

Traditionally, cultured shrimps were fed fresh or frozen trash fish, mussel, clam, or squid. Presently, these food items are used mainly for broodstock and occasionally at postlarval stages. Live foods, such as algae, rotifers, and *Artemia*, are still major sources of food for shrimp larvae although various types of artificial diets substitute for some of the live food.

Commercially processed feeds are successfully used in nurseries and semi-intensive and intensive grow-out operations. Many commercial shrimp feeds are available worldwide. Although research information is available on basic nutrient requirements of several shrimp species, there is a scarcity of research data on recommendations for pond feeds. Because the culture environment makes a valuable contribution to the nutrient requirements of shrimp, cost-effective feeds for the various culture systems and management practices are difficult to design. The necessity of supplementing various pond feeds with all nutrients, such as vitamins and essential lipids, has not been established. Most commercial feeds contain a vitamin premix. Generally, higher protein diets are fed during early postlarval stages and juvenile stages, and the protein percentage decreases during the grow-out period. Examples of formulas for practical shrimp diets are given in Table 13.5.

Table 13.5. MODEL FORMULAS OF PRACTICAL SHRIMP FEEDS FOR INTENSIVE CULTURE (38% PROTEIN) AND SEMI-INTENSIVE CULTURE (30 AND 25% PROTEIN)

Ingredient	Protein percentage		
	38	30	25
	(%)	(%)	(%)
Fish meal	19.0	12.0	10.0
Shrimp head meal	13.5	10.0	8.5
Squid meal	5.0	—	—
Soybean meal	24.5	28.6	22.5
Cereal products or by-products[1]	25-28	37-40	46-49
Fish oil	3.5	3.5	3.5
Soybean lecithin	1.0	—	—
Cholesterol	0.2	—	—
Binder[2]	1-4	1-4	1-4
Calcium phosphate, dibasic	2.0	2.4	2.9
Potassium phosphate, dibasic	1.3	1.5	1.6
Vitamin mix[3]	0.5	0.5	0.5
Trace mineral mix[4]	0.5	0.5	0.5

[1] Cereal products or by-products rich in starch, such as ground whole wheat or high gluten wheat flour, is recommended.
[2] Synthetic binders at 1% level or less, or wheat gluten at 4%. Other binders such as alginate, lignin sulfonate, carboxymethyl cellulose and benthonites can be used at 2%-3% level.
[3] Vitamin allowances recommended in Table 3 for complete diet should be used for the 38% protein diet whereas those for supplemental diet should be used for the 30 and 25% protein diets
[4] Trace mineral mix should contain the allowances recommended in Table 4.

Due to the benthic feeding behavior of shrimps, practical commercial feeds should be processed into sinking pellets. Sizes of the pellets vary depending on size of the shrimps. Crumbles are used during postlarval stages and pellets are fed from juvenile through marketable size. Recommended pellet diameter for various sizes of shrimps is given in Table 13.6.

Good water stability is very important for shrimp feeds. While finfishes usually swallow the whole feed pellet once they have learned to accept the feed, shrimps are selective and slow eaters. Thus, shrimp pellets should remain stable in the water for several hours until consumed by the shrimp. Shrimp take the feed

Table 13.6. RECOMMENDED PELLET DIAMETER FOR VARIOUS SIZES OF SHRIMP

Stage/size	Particle diameter
	(mm)
PL_{10} to PL_{30}[1]	<0.5
PL_{30} to 0.5 grams	0.0-0.8
0.5 to 2.0 grams	1-2
2.0 to 5.0 grams	2
5.0 to 10 grams	2-3
>10 grams	3-4

[1] PL means postlarvae and the subscript number represents the number of days the larvae have reached the postlarval stage.

with the chelate pereiopods, then convey it to the mouth parts, which act together to reduce the size of feed to small particles prior to ingestion.

Two manufacturing processes, cooked extrusion and steam pelleting, are commonly used to produce shrimp feeds. Extrusion processing is widely used to produce floating pellets for finfishes; however, sinking feeds can also be produced using a cooker extruder by reducing the expansion rate and pressure on the extrudate in the barrel. The resulting pellets usually have good water stability due to the high level of starch gelatinization. However, most shrimp feeds are processed with a compression pellet mill equipped with steam injectors and a steam jacketed pre-conditioner.

Various substances, either natural, modified or synthetic, have been used as binding agents for shrimp diets with varying degree of success. Wheat gluten, high gluten wheat flour, tuber and cereal starches, and/or their combination are the most commonly used natural binders in shrimp pellets. Commercial binders, such as lignin sulfonate and bentonite, which are commonly used for fish feeds, have been found to be less effective for shrimp feeds that require longer duration of water stability than finfish feeds. Organic hydrocolloids, such as carboxymethyl cellulose, alginate and gums, have been used successfully in laboratory prepared diets but their use in commercial feeds may be limited due to high costs and limited adaptability to large-scale production. Synthetic binders such as Aqua-firm 1A and Aqua-firm 2A, produced by Agresearch, Inc., Joliet, Illinois, are marketed for use in shrimp feeds.

Water stability values, determined by calculating the percentage of dry weight retained after submerging pellets in water for a predetermined time period, do not by themselves determine the nutritional quality of shrimp diets. The ability of diets placed in water to retain nutrients does not always correlate with the pellet dry weight retention. The percentages of methionine loss from a diet

Table 13.7. RECOMMENDED FEEDING RATES AND FREQUENCIES FOR VARIOUS SIZES OF SHRIMP

Stage/size	Daily feeding rate (% of body weight)	Feeding frequency (times per day)
PL_{10} to PL_{30}	30-20	6
PL_{30} to 0.5 grams	20-12	4
0.5 to 2 grams	12-8	3-4
2 to 5 grams	8-6	3
5 to 10 grams	6-4	3
10 to 20 grams	4-3	2-3
>20 grams	3-2	2-3

supplemented with free methionine and a shrimp meal control diet were 53% and 7%, respectively (Lim 1993). Thus, the nature of nutrient losses and the rate at which such losses occur should be evaluated in addition to dry matter losses in evaluating water stability of pellets.

Because the feeding response of shrimps is mainly chemosensory, attractants in the diets may increase their feeding activity and feed consumption. Various substances, such as amino acids; fatty acids; and extracts of fish, shrimp, squid, mussel, and clam, have been shown to stimulate feeding response in shrimps. These studies have been primarily under laboratory conditions and the value of attractants under pond feeding conditions is not clearly established.

Feeding

The daily amount of feed offered is affected by the size of shrimps, feeding schedule, stocking density, availability of the natural foods, dietary energy level, water quality, and shrimp health. Daily feed allowances for shrimps range from around 25% of body weight for larvae to less than 3% of body weight per day for market size. Since there are many factors which influence feed consumption of shrimp, satiation feeding may be a better alternative to feeding a prescribe amount based on percentage of biomass. Providing as much feed as the shrimp will eat provide a better opportunity for the smaller, less aggressive shrimp to receive their share. Because shrimp are slow feeders, satiation feeding is difficult and time consuming. Some farmers, however, feed their shrimp according to the demand. At each feeding, some feed is placed in feeding trays or on platforms located at different locations in the pond and these are checked one or two hours after feeding. The feed allowance will be increased or decreased on the basis of the amount of feed remaining on the feeding trays or platforms. Some farmers determine the daily feed

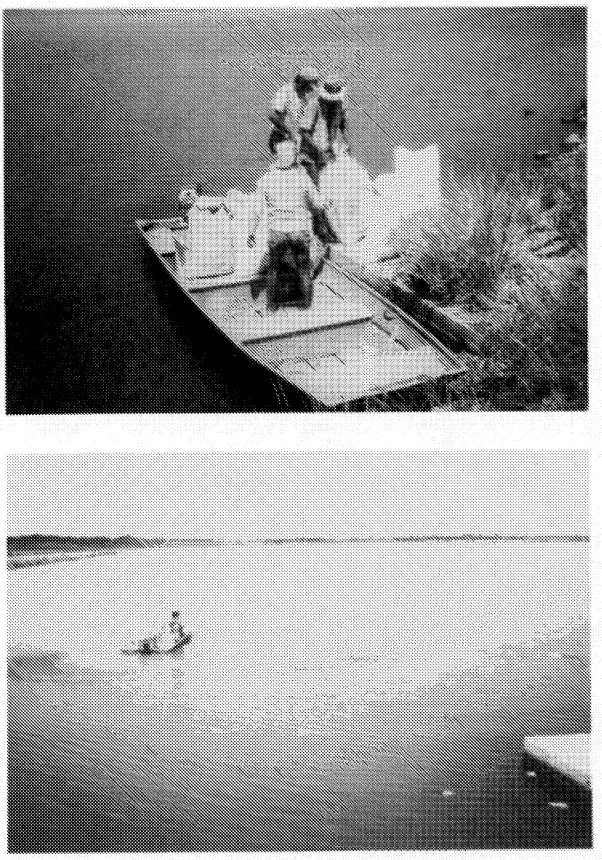


Figure 13.4 Shrimp in ponds do not move far for feed; therefore the feed must be distributed uniformly over the pond or the areas where the shrimp are located. Top photo shows feed loaded into a boat and lower photo shows feed being distributed over the pond.

allowance from the activity and behavior of the shrimp. One or two hours after feeding, if a significant number of shrimp are swimming actively along the pond banks, this is an indication of under-feeding. Other farmers collect a handful of bottom sediment from the feeding areas to detect leftover feed or odor of decomposing feed.

Although satiation feeding may be desirable, at high standing crop of shrimp it may be impossible to feed according to the demand and maintain water quality at an acceptable level. The amount of feed offered should not exceed the capacity of the system to assimilate the waste products and maintain a sufficient level of dissolved oxygen.

Because shrimps eat slowly and more or less continuously, multiple daily feeding is desirable. Under laboratory conditions, the optimum feeding frequency of *P. monodon* juveniles (2 g average weight) was three times per day (Lim and Pascual 1979). Disintegration of the feed and loss of the water soluble nutrients can

be minimized through multiple daily feeding. More frequent feeding also increases the rate of feed consumption. It was observed that shrimp prefer fresh pellets over those that have been exposed to the water for an extended period. Recommended feeding rates and frequencies for various sizes of shrimps are given in Table 13.7. Less frequent feeding may be desirable under certain circumstances such as during disease episodes, or unfavorable water quality conditions.

Unlike fish, shrimps are territorial and do not swim great distances to feed. Thus, it is important to distribute the feed uniformly over the pond or in areas where the shrimp are located. For small ponds feeding may be done by hand broadcasting from the pond bank. In large ponds, feeds are distributed from boats (Figure 13.4), tractor-mounted feed blowers, or airplanes. Feeding shrimp at the same time, in the same manner, and in the same area each day is important.

REFERENCES

Abdel-Rahman, S.H., A. Kanazawa, and S.I. Teshima. 1979. Effects of dietary carbohydrate on the growth and the levels of the hepatopancreatic glycogen and serum glucose of prawn. *Bul. Jap. Soc. Sci. Fish.* 45: 1491-1494.

Alava, V. R., and C. Lim. 1983. The quantitative dietary protein requirements of *Penaeus monodon* juveniles in a controlled environment. *Aquaculture* 30: 53-61.

Balazs, G. H., E. Ross, and C. C. Brooks. 1973. Preliminary studies on the preparation and feeding of crustacean diets. *Aquaculture* 2: 369-377.

Catacutan, M.R. 1991. Apparent digestibility of diets with various carbohydrate levels and the growth response of *Penaeus monodon. Aquaculture* 95: 89-96.

Chen, H., and G. Hwang. 1992. Estimation of the dietary riboflavin required to maximize tissue riboflavin concentration in juvenile shrimp (*Penaeus monodon*). *J. Nutr.* 122:2474-2478.

Chen, H.Y., Y.T. Leu, and I. Roelants. 1992. Quantification of arginine requirements of juvenile marine shrimp, *Penaeus monodon*, using microencapsulated arginine. *Mar. Biol.* 114: 229-233.

Chen, H., F. Wu and S. Tang.. 1991. Thiamin requirement of juvenile shrimp (*Penaeus monodon*). *J. Nutr.* 121:1984-1989.

Colvin, P. M. 1976. Nutritional studies on penaeid prawns: Protein requirements in compounded diets for juvenile *Penaeus indicus* (Milne Edwards). *Aquaculture* 7: 315-326.

Colvin, L. B., and C. W. Brand. 1977. The protein requirements of penaeid shrimp at various life-cycle stages in controlled environment systems. *Proc. Ann. Workshop World Maricull. Soc.* 8: 821-840.

Davis, D.A., A.L. Lawrence, and D.M. Gatlin, III. 1993a. Dietary zinc requirement of *P e n a e u s vannamei* and the effects of phytic acid on zinc and phosphorus bioavailability. *J. world Aquacult. Soc.* 24: 40-47.

Davis, D.A., A.L. Lawrence, and D.M. Gatlin, III. 1993b. Pesponse of *Penaeus vannamei* to diietary calcium, phosphorus, and calcium:phosporus ratio. *J. World Aquacult. Soc.* 24: 504-515.

Deshimaru, O. 1982. Protein and amino acid nutrition of the prawn, *Penaeus japonicus. Proc. Second Intl. Conf. in Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition*, 106-122 October 17-19, 1981. Rehoboth Beach, DE.: 106-122.

Deshimaru, O., and K. Kuroki. 1976. Studies on a purified diet for prawn-VIII. Adequate dietary levels of ascorbic acid and inositol. *Bul. Jap. Soc. Sci. Fish.* 42: 571-576.

Deshimaru, O., and K. Kuroki. 1979. Requirement of prawn for dietary thiamin, pyridoxine, and choline chloride. *Bul. Jap. Soc. Sci. Fish.* 45:363-367.

Deshimaru, O., and Y. Yone. 1978a. Optimum level of dietary protein for prawn. *Bul. Jap. Soc. Sci. Fish.* 44: 1395-1397.

Duerr, E.O., and C.Lim. 1990. Protein requirements of shrimp (*Penaeus vannamei*) grown in an outdoor microcosm. Abstracts, *World Aquacult. Conf.*, 10-14 June 1990, Halifax, Canada: 118.

Fox, C.J. 1993. The effect of chitin on growth, survival and chitinase levels in the digestive gland of juvenile *Penaeus monodon* (Fabricus). *Aquaculture* 109: 39-49.

Fox, J.M., A.L. Lawrence, and E. Li-chan. 1995. Dietary requirement for lysine by juvenile *Penaeus vannamei* using intact and free amino acid sources. *Aquaculture* 131: 279-290.

He, H. 1988. A study on the essential amino acids of the prawn, *Penaeus orientalis. Oceanol. Limnol. Sin.* 19:307-313.

He, H., and A.L. Lawrence. 1993a. Vitamin C requirement of shrimp *Penaeus vannamei. Aquaculture* 114: 305-316

He, H., A.L. Lawrence, and R. Liu. 1992. Evaluation of dietary essentiality of fat-soluble vitamins A, D, E and K for penaeid shrimp (*Penaeus vannamei*). *Aquaculture* 103: 177-185.

Kanazawa, A. 1985. Nutrition of penaeid prawns and shrimps. *Proc. First Intl. Conf. on the Culture of Penaeid Prawns/Shrimps*. Iloilo City, Philippines, 1984. SEAFDEC Aquaculture Department, Manila, Philippines: 123-130.

Kanazawa, A., S. Teshima, and N. Tanaka. 1976. Nutritional requirements of prawn-V. Requirements for choline and inositol. *Mem. Fac. Fish. Kagoshima Univ.* 25:47-51.

Kanazawa, A., S. Teshima, and M. Sakaki. 1984. Requirements of the juvenile prawn for calcium, phosphorus, magnesium, potassium, copper, manganese and iron. *Mem. Fac. Fish. Kagoshima Univ.* 33: 63-71.

Kanazawa, A., S. Tokiwa, M. Kayama, and M. Hirata. 1977. Essential fatty acids in the diet of prawn. I. Effects of linoleic and linolenic acids on growth. *Bul. Jap. Soc. Sci. Fish.* 43: 1111-1114.

Kitabayashi, K, H Kurata, K. Shudo, K. Nakamura, and S. Ishikawa. 1971. Studies on the formula feed for kuroma prawn. I. On the relationship among glucosamine, phosphorus, and calcium. *Bull. Tokai Regl. Fish. Res. Lab.* 65: 91-108.

Lee, D. L. 1971. Studies on the protein utilization related to growth in *Penaeus monodon* Fabricius. *Aquaculture* 1: 1-13.

Lightner, D. V., L. B. Colvin, C. Bran, and D. A. Donald. 1977. Black death, a disease syndrome of penaeid shrimp related to a dietary deficiency of ascorbic acid. *Proc. World Maricult. Soc.* 8: 611-624.

Lim, C., and D.M. Akiyama. 1995. Nutrient requirement of penaeid shrimp. *In*: Nutrition and Utilization Technology in Aquaculture, C. Lim and D.J. Sessa, eds. AOAC Press, Champaign, Il.: 60-73.

Lim, C., and W. Dominy. 1990. Evaluation of soybean meal as a replacement for marine animal protein for shrimp (*Penaeus vannamei*). *Aquaculture* 87: 53-63.

Mai, Y., A. Li and Lin Z. 1988. Studies on the absorption and utilization of amino acids in the test diets by prawn *Penaeus orientalis*. *Acta Oceanol. Sin.* 7:621-629.

Millamena, O.M., M.N. Bautista-Teruel, and A. Kanazawa. 1996. Methionine requirement of juvenile tiger shrimp *Penaeus monodon* Fabricius. *Aquaculture* 143: 403-410.

Millamena, O.M., M.N. Bautista, O.S. Reyes, and A. Kanazawa. 1997. Threonine requirement of juvenile marine shrimp *Penaeus monodon*. Aquaculture 151: 9-14.

Moriarty, D.J.W. 1977. Quantification of carbon, nitrogen and bacterial biomass in the food of some penaeid prawns. *Aust. J. Mar. Freshwater Res.* 28: 113-118.

Pascual, F.P., R.M. Coloso, and C.T. Tamse.1983. Survival and some histological changes in *Penaeus monodon* Fabricius juveniles fed various carbohydrates. *Aquaculture* 31: 169-180.

Read, G.H.I. 1981. The response of *Penaeus indicus* (Crustacean: Peneidea) to purified and compounded feed of varying fatty acid composition. *Aquaculture* 24: 245-256.

Sedgwick, R. W. 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merquiensis* de Man. *Aquaculture* 16: 7-30.

Shiau, S.Y., and J.Y. Hwang. 1994. The dietary requirement of juvenile grass shrimp (*Penaeus monodon*) for vitamin D. *J. Nutr.* 124:2445-2450.

Shiau, S.Y., and J.S. Liu. 1994. Estimation of the dietary vitamin K requirement of juvenile *Penaeus chinensis* using menadione. *Aquaculture* 126:129-135.

Shiau, S.Y., and J.F. Lin, and L.J. Lu. 1992. Effects of different types of wheat flour in feed for grass prawn *Penaeus monodon*. *Nippon Suisan Gakkaishi* 57: 705-710.

Shiau, S.Y., and C.Q. Lung. 1993. Estimation of the vitamin B_{12} requirement of the grass shrimp, *Penaeus monodon*. *Aquaculture* 117:157-163.

Shiau, S.Y., and C.Y. Peng. 1992. Utilization of different carbohydrates at different dietary protein levels in grass prawn, *Penaeus monodon*, reared in seawater. *Aquaculture* 101: 241-250.

Shigueno, K. 1975. Shrimp culture in Japan. Association for International Technical Promotion, Tokyo, Japan.

Teshima, S., A. Kanazawa, and K. Koshio. 1992. Supplemental effect of methionine-enriched plastein in *Penaeus japonicus* diets. *Aquaculture* 101: 85-93.

Teshima, S., A. Kanazawa, S. Koshio, and N. Kondo. 1989. Nutritive value of sitosterol for prawn, *Penaeus japonicus*. *Nippon Suisan Gakkaishi* 55: 153-157.

Teshima, S., A. Kanazawa, and H. Sasada. 1983. Nutritive value of dietary cholesterols and other sterols to larval prawn, *Penaeus japonicus* Bate. *Aquaculture* 31: 159-167.

Zein-Eldin, Z. P., and J. Corlis. 1976. The effect of protein levels and sources on growth of *Penaeus aztecus*. FAO Technical Conference on Aquaculture, Kyoto, Japan, 26 May-2 June 1976. FIR: AQ/Conf/76/E.33: 1-8.

APPENDIX A: COMPOSITION OF FEED INGREDIENTS

The purpose of the feed ingredient composition tables (Tables A.1 through A.4) is to provide information for formulating fish feeds for research and commercial practice that can be defined nutritionally as well as prepared economically. Data in the tables come from National Research Council/National Academy of Sciences tables and other published and unpublished sources considered to be reliable by the author. Where available, each feed ingredient is assigned an International Feed Number to facilitate identification.

With a few exceptions, proximate, amino acid, and mineral composition data represent chemical analyses with no correction for availability to the animal. Therefore, these values must be adjusted to allow for availability to the fish when they are used in diet formulation. This is because the nutrient requirements for fish have been determined with highly purified ingredients in which the nutrients are highly digestible and the nutrient requirement data are presented on the basis of being nearly 100% available. Available essential amino acid content is presented for several feeds based on digestibility to channel catfish. Similar amino acid availability in these feeds for other fish species is probably a safe assumption. Availability of minerals varies among sources and fish species. Availability of phosphorus in several feed ingredients is presented for three fish species in Table 3.2 of Chapter 3. Phosphorus availability in some feeds to the stomachless carp is much lower than to channel catfish or rainbow trout. Availability of minerals from technical or reagent grade chemical compounds will be higher and more consistent than from the feedstuffs.

Energy values for feedstuffs are presented on a metabolizable energy (ME) or digestible energy (DE) basis. ME values are presented for rainbow trout and DE values are presented for rainbow trout and channel catfish. Comparison of published research data indicates that cold- and warm-water species digest energy in proteins and fats relatively well and similarly, but energy in carbohydrates is more digestible to warm- than cold-water species. Thus, the DE values presented for grains, starch, and dextrin for channel catfish would probably be applicable for warm-water species, but should not be used for cold-water species.

Table A.1. PROXIMATE COMPOSITION AND ENERGY VALUES FOR PRACTICAL AND PURIFIED INGREDIENTS COMMONLY USED IN FISH FEEDS (AS FED BASIS)

Ingredient name	International feed number	Typical dry matter (%)	Energy (kcal kg^{-1}) ME (Trout)	DE (Trout)	DE (Channel)	Proximate composition (%) Crude protein	Crude fat	Crude fiber	Ash
PRACTICAL									
Alfalfa meal	1-00-023	92.0	510	560	730	17.4	2.8	24.1	9.8
Blood meal, spray, dehy.	5-00-381	93.0	3,440	3,410		86.5	1.3	1.0	6.6
Brewers grain, dehy.	5-02-141	92.0				27.0	6.6	13.2	3.6
Copra meal, solv. extr.	5-01-573	91.0				21.3	3.5	14.0	6.0
Corn distillers grain with solubles, dehy.	5-28-236	92.0				27.1	9.5	9.1	4.4
Corn distillers soluble, dehy.	5-28-237	93.0	2,280	2,440		26.6	8.6	4.7	7.3
Corn gluten meal	5-28-241	91.0	3,550	4,040		42.6	2.2	4.4	3.1
Corn, uncooked	4-02-935	89.0			1,240	9.7	3.8	2.6	1.3
Corn, extrusion cooked	4-02-935	89.0			2,840	9.7	3.8	2.6	1.3

Table A.1. Continued.

Ingredient name	International feed number	Typical dry matter (%)	Energy (kcal kg^{-1}) ME (Trout)	DE (Trout)	DE (Channel)	Proximate composition (%) Crude protein	Crude fat	Crude fiber	Ash
Cotton seed meal, solv. extr.	5-01-621	91.0	2,460	2,610	2,800	41.1	1.5	12.1	6.5
Crab meal, process residue	5-01663	92.0				32.0	1.9	10.7	41.0
Crab protein concentrate dehy.	5-16-422	90.0				60.34	0.5		6.1
Fish solubles, condensed	5-01-969	50.0				32.7	5.6	0.5	9.6
Fish solubles, dehy.	5-01-971	93.0	3,350	3,680		64.4	8.3	1.4	12.6
Fish meal, anchovy, mech. extr.	5-01-985	92.0	4,020	4,570		65.5	4.1	1.0	14.8
Fish meal, channel catfish, mech. extr.	5-09-835	92.0				50.9			

Table A.1. Continued.

Ingredient name	International feed number	Typical dry matter (%)	Energy (kcal kg^{-1}) ME (Trout)	DE (Trout)	DE (Channel)	Proximate composition (%) Crude protein	Crude fat	Crude fiber	Ash
Fish meal, herring, mech. extr.	5-02-000	92.0	4,130	4,720		72.0	8.5	0.6	10.5
Fish meal, menhaden, mech. extr.	5-02-009	92.0			4,240	61.4	9.7	0.9	19.1
Fish meal, tuna, mech.extr.	5-02-023	93.0				59.1	6.9	0.8	21.9
Fish meal, white, mech. extr.	5-02-025	91.0	2,970	3,490		62.1	4.6	0.7	23.1
Ipil-ipil (leucaena glauca) leaf meal	1-16-447	92.0				26.8	5.7	11.6	8.4
Meat meal	5-00-385	94.0				51.5	9.1	2.6	27.1
Meat and bone meal	5-00-388	93.0	3,240	3,390	3,730	50.3	9.7	2.2	29.3
Molasses, sugar cane, dehy.	4-04-695	94.0				9.7	0.8	6.3	12.5

Table A.1. Continued.

Ingredient name	International feed number	Typical dry matter (%)	Energy (kcal kg^{-1}) ME (Trout)	DE (Trout)	DE (Channel)	Proximate composition (%) Crude protein	Crude fat	Crude fiber	Ash
Peanut meal, mech. extr.	5-03-649	93.0				48.4	5.9	7.0	5.1
Peanut meal, solv. extr.	5-03-650	92.0				48.1	1.3	9.9	5.8
Poultry byproduct meal	5-03-798	93.0	2,980	3,720		58.4	13.1	2.2	15.6
Poultry feather meal, hydrolized	5-03-795	93.0			3,670	84.9	3.0	1.4	3.5
Rape seed meal, solv. extr.	5-03-871	91.0	2,710	2,990		37.2	1.6	12.0	6.8
Rice bran	4-03-928	91.0				12.8	13.7	11.6	11.6
Rice middlings	1-03-941	92.0				6.3	5.2	29.0	15.7
Rice polishings	4-03-943	90.0				12.1	12.5	3.2	7.5
Shrimp meal, process residue	4-04-226	90.0				39.8	3.9	14.0	26.7
Sorghum	4-04-383	90.0				11.2	2.8	2.3	1.8

Table A.1. Continued.

Ingredient name	International feed number	Typical dry matter (%)	Energy (kcal kg^{-1}) ME (Trout)	DE (Trout)	DE (Channel)	Proximate composition (%) Crude protein	Crude fat	Crude fiber	Ash
Soybean seed, dry roasted, 204°C, 12 min	5-04-597	90.0	3,840	4,190		38.0	18.0	5.0	4.6
Soybean meal, mech. extr.	5-04-600	90.0				42.9	4.8	5.9	6.0
Soybean meal, solv. extr.	5-04-604	90.0	2,570	2,980	2,870	44.9	1.3	5.9	6.3
Soybean protein concentrate	5-08-038	92.0				84.5	0.6	0.1	3.5
Sunflower meal, solv. extr.	5-04-739	93.0				46.3	2.9	11.3	7.5
Wheat bran	4-05-190	89.0			2,790	15.2	3.9	10.6	6.1
Wheat	4-05-268	88.0			2,900	12.7	1.6	2.5	1.7
Wheat flour	4-05-199	88.0				11.8	1.2	1.3	0.4
Wheat middlings	4-05-205	89.0	1,650	1,800		16.4	4.4	7.3	4.6
Yeast, brewers, dehy.	7-05-527	93.0	2,560	2,710		43.6	0.8	2.9	6.6
Yeast, torula, dehy.	7-05-534	93.0				49.0	1.6	2.2	7.7

Table A.1. Continued.

Ingredient name	International feed number	Typical dry matter (%)	Energy (kcal kg^{-1}) ME (Trout)	Energy (kcal kg^{-1}) DE (Trout)	Energy (kcal kg^{-1}) DE (Channel)	Proximate composition (%) Crude protein	Crude fat	Crude fiber	Ash
PURIFIED									
Casein	5-01-162	91.0			4,400	84.4	0.6	0.2	2.2
Cellulose powder		96.5						92.6	
Corn starch, raw		88.0			2,700	0.3	Trace	0.1	0.1
Corn starch, cooked		88.0				0.3	Trace	0.1	0.1
20% of diet					3.400				
30% of diet					3,000				
40% of diet					2,800				
Dextrin		90.2							
30% of diet					2,920				
60% of diet					1,920				
Gelatin	5-14-503	90.0			4,700	85.2	0.1		0.0
Glucose	4-02-891	90.0			3,380				

Source: National Research Council (1993).

Table A.2. AMINO ACID COMPOSITION OF INGREDIENTS COMMONLY USED IN FISH FEEDS, BY PERCENTAGE, DRY BASIS

Ingredient name	International feed no.	Typical dry matter	Crude protein	Argi-nine	Gly-cine	Histi-dine	Isoleu-cine	Leu-cine	Ly-sine	Methi-onine	Cys-tine	Phenyl-alanine	Tyro-sine	Ser-rine	Thre-onine	Trypto phan	Valine
Alfalfameal	1-00-023	92.0	18.9	0.84	0.91	0.36	0.88	1.39	0.93	0.29	0.31	0.87	0.59	0.77	0.77	0.37	0.96
Blood meal, spray dehydrated	5-00-381	93.0	93.0	3.88	4.14	5.59	0.98	11.86	8.04	0.95	0.78	6.36	2.44	3.82	3.93	1.13	8.13
Brewers grains, dehydrated	5-02-141	92.0	29.4	1.38	1.18	0.56	1.68	2.70	0.95	0.50	0.38	1.56	1.30	1.42	1.01	0.40	1.75
Casein	5-01-162	91.0	92.7	3.85	2.77	2.86	6.32	9.71	7.88	3.10	0.34	5.31	5.41	6.03	4.32	1.19	7.40
Copra meal, solvent extracted	5-01-573	91.0	23.4	2.65	1.14	0.41	0.91	1.59	0.66	0.35	0.27	0.95	0.63	-	0.73	0.22	1.14
Corn distillers grains with solubles	5-28-236	92.0	29.5	1.05	0.55	0.70	1.52	2.43	0.77	0.54	0.32	1.64	0.76	1.42	1.01	0.19	1.63
Corn gluten meal	5-28-241	91.0	46.8	1.53	1.65	1.06	2.46	7.92	0.87	1.14	0.73	3.05	1.11	1.97	1.56	0.23	2.40
Corn grain	4-02-935	89.0	10.9	0.48	0.42	0.29	0.39	1.37	0.28	0.19	0.25	0.54	0.43	0.57	0.40	0.90	0.50
				(0.39)		(0.26)	(0.27)	(1.19)	(0.27)	(0.13)		(0.44)	(0.33)		(0.27)		(0.37)
Cottonseed meal, solvent extracted	5-01-621	91.0	45.2	4.62	2.17	1.21	1.67	2.56	1.86	0.64	0.85	2.46	1.13	1.92	1.52	0.61	2.06
				(4.19)		(1.00)	(1.20)	(1.96)	(1.32)	(0.49)		(2.05)	(0.84)		(1.16)		(1.57)
Fish meal, anchovy	5-01-985	92.0	71.2	4.11	4.01	1.76	3.38	5.43	5.49	2.16	0.66	3.03	2.44	2.63	3.00	0.82	3.81
Fish meal, herring	5-02-000	92.0	78.3	5.02	4.80	1.80	3.41	5.64	5.83	2.27	0.81	2.94	2.39	2.88	3.16	0.83	4.68
Fish meal, menhaden	5-02-009	92.0	66.7	4.09	4.57	1.58	3.15	4.89	5.15	1.91	0.61	2.69	2.12	2.43	2.73	0.71	3.52
				(3.71)		(1.34)	(2.73)	(4.34)	(4.43)	(1.58)		(2.34)	(1.90)		(2.38)		(3.04)
Gelatin	5-14-503	90.0	97.4	7.75	21.48	0.85	1.54	3.24	3.95	0.81	0.15	1.99	0.58	3.45	1.96	0.05	2.33
Ipil-ipil (Leucaena glauca) leaf meal	1-16-447	92.0	29.1														
Meat and bone meal	5-00-388	93.0	54.1	3.75	6.93	1.04	1.76	3.29	3.11	0.70	0.53	1.83	0.85	1.94	1.77	0.32	2.63
				(3.30)		(0.85)	(1.43)	(2.71)	(2.70)	(0.56)		(1.56)	(0.71)		(1.35)		(2.13)

Table A.2. Continued.

Ingredient name	International feed no.	Typical dry matter	Crude protein	Arginine	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Cystine	Phenylalanine	Tyrosine	Serine	Threonine	Tryptophan	Valine
Peanut meal, solvent extracted	5-03-650	92.0	52.3	4.95 (4.84)	2.56	1.03 (0.92)	1.91 (1.78)	2.94 (2.79)	1.93 (1.82)	0.46 (0.41)	0.79	2.22 (2.13)	1.65 (1.59)	3.37	1.26 (1.17)	0.52	2.04 (1.90)
Poultry by-product meal	5-03-798	93.0	62.8	4.03	5.80	1.08	2.54	4.28	3.10	1.13	0.98	1.97	1.01	2.81	2.08	0.50	3.06
Rapeseed meal, solvent extracted	5-03-871	91.0	40.6	2.26	1.97	1.09	1.48	2.74	2.18	0.78	0-33	1.55	0-87	1.72	1.72	0.47	1.96
Rice bran	4-03-928	91.0	14.1	0.79 (0.75)	0.88	0.25 (0.21)	0.51 (0.44)	0.77 (0.69)	0.54 (0.51)	0.26 (0.22)	0.11	0.49 (0.43)	0.76 (0.71)	0.85	0.47 (0.42)	0.11	0.76 (0.68)
Shrimp meal, process residue	5-04-226	90.0	44.2	2.79	-	1.07	1.86	2.98	2.41	0.91	0.66	1.76	1.47	-	1.58	0.41	2.03
Soybean meal, solvent extracted	5-04-604	90.0	49.9	3.38 (3.26)	2.03	1.19 (1.04)	2.27 (1.80)	3.65 (3.03)	2.99 (2.80)	0.58 (0.49)	0.83	2.36 (1.98)	1.48 (1.23)	2.36	1.85 (1.51)	0.71	2.25 (1.77)
Sunflower meal, solvent extracted	5-04-739	93.0	49.8	4.75	3.03	1.32	2.42	4.12	2.06	1.25	0.79	2.54	1.49	2.37	2.07	0.65	2.80
Wheat	4-05-268	88.0	14.4	0.73	0.65	0.34	0.58	1.00	0.41	0.24	0.36	0.71	0.49	0.67	0.42	0.19	0.67
Wheat middlings	4-05-205	89.0	18.4	1.03 (0.98)	0.57	0.43 (0.40)	0.75 (0.66)	1.21 (1.09)	0.76 (0.73)	0.20 (0.17)	0.24	0.72 (0.67)	0.45 (0.40)	0.82	0.61 (0.54)	0.22	0.85 (0.76)
Yeast, brewers, dehydrated	7-05-527	93.0	46.9	2:35	1.87	1.17	2.37	3.45	3.33	0.79	0.53	1.96	1.60	-	2.27	0.55	2.52

Note: Data represent total and (available) contents. Availability is based on digestibility for channel catfish.

Table A.3. AMINO ACID AVAILABILITY AND PROTEIN DIGESTIBILITY VALUES FOR CERTAIN FEED INGREDIENTS FOR ATLANTIC SALMON AND CHANNEL CATFISH

Feed Ingredient Fish Species	International Feed No.	Protein (%)	ARG (%)	CYS (%)	HIS (%)	ILE (%)	LEU (%)	LYS (%)	MET (%)	PHE (%)	THR (%)	TRYP (%)	TRY (%)	VAL (%)
Corn, grain	4-02-935													
Channel catfish		-	-	82.0	90.3	67.9	87.5	96.5	70.5	81.8	69.8	-	77.5	74.4
Corn, gluten meal	5-28-241													
Atlantic salmon		95.0	99.9	90.8	94.5	90.4	88.4	99.9	93.8	91.2	92.0	-	92.0	91.3
Cottonseed, meal	5-01-621													
Channel catfish		-	90.6	-	81.6	71.7	76.4	71.2	75.8	83.5	76.7	-	73.4	76.1
Fish, herring meal	5-02-000													
Atlantic salmon		93.8	95.3	86.2	93.8	91.9	94.1	92.3	87.6	92.4	93.2	92.9	95.4	91.4
Fish, menhaden meal	5-02-009													
Atlantic salmon		88.5	86.8	92.0	91.1	88.5	90.1	87.6	83.6	87.4	88.4	89.0	92.1	86.3
Channel catfish		-	91.0	-	84.5	87.1	89.0	86.4	83.1	87.3	87.4	-	88.8	87.1
Meat and bone meal	5-00-388													
Channel catfish		-	87.9	-	88.2	80.8	82.4	86.7	80.4	85.4	76.3	-	83.1	80.8
Peanut meal	5-03-650													
Channel catfish		-	97.7	-	89.4	93.3	95.1	94.1	91.2	96.0	93.4	-	94.5	93.3
Soybean meal	5-04-604													
Atlantic salmon		88.3	86.7	-	86.4	79.2	75.9	83.6	94.0	78.7	84.5	50.3	83.0	77.3
Channel catfish		-	96.8	-	87.9	79.7	83.5	94.1	84.6	84.2	82.2	-	83.3	78.5
Wheat middlings	4-05-205													
Channel catfish		-	95.1	-	94.5	87.8	89.9	96.3	82.8	93.0	89.1	-	89.1	90.1

Source: Atlantic salmon, Anderson et al. (1992), Channel catfish, Wilson et al. (1981).

Notes: Amino acid abbreviation: ARG, arginine; CYS, cystine; HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE, phenylalanine, THR, threonine; TRYP, tryptophan; TYR, tyrosine; VAL, valine.

Table A.4. MINERAL COMPOSITION FOR MINERAL SUPPLEMENTS USED IN PRACTICAL AND EXPERIMENTAL FISH FEEDS

Mineral	International feed number	Typical dry matter	Macro minerals, dry basis (%)							Micro minerals, dry basis (mg/kg)						
			Cal-cium	Phos-phorus	Potas-slum	Chlo-rine	Magne-sium	So-dium	Sul-fur	Co-balt	Cop-per	Io-dine	Iron	Manga-nese	Sele-nium	Zinc
Bone meal, steamed	6-00-400	96.0	28.39	13.58	0.19	0.01	0.58	0.43	0.23	-	10	-	880	30	-	440
Calcium phosphate, monobasic (mono-calcium phosphate)	6-01-082	97.0	16.40	21.60 (20.30)2 (20.30)2 (20.30)3	0.08	-	0.61	0.06	1.22	10	10	-	15,800	360	-	90
Calcium phosphate, dibasic dicalcium phosphate)	6-01-080	97.0	22.0	19.30 (12.50)2 (8.88)2 (13.70)3	0.07	-	0.59	0.05	1.14	10	10	-	14,400	300	-	100
Calcium phosphate, tribasic	6-01-084	97.0	39.20	20.1 (2.61)2 (12.86)3	-	-	-	-	-	-	-	-	-	-	-	-
Copper oxide, CuO	6-01-712	99.0	-	-	-	-	-	-	-	-	798,800	-	-	-	-	-
Copper sulfate, pentahydrate, CUS04-5H20	6-01-720	100.0	-	-	-	-	-	-	12.84	-	254,500	-	-	-	-	-
Iron (ferrous) sulfate	6-20-734	98.0	-	-	-	-	-	-	12.35	-	-	-	218,400	-	-	-
Magnesium sulfate, heptahydrate, MgSO4-7H20	6-02-758	98.0	0.02	-	-	-	9.80	-	13.00	-	-	-	-	-	-	-

Table A.4. CONTINUED

Mineral	International feed number	Typical dry matter	Macro minerals, dry basis (%)							Micro minerals, dry basis (mg/kg)						
			Calcium	Phosphorus	Potasslum	Chlorine	Magnesium	Sodium	Sulfur	Cobalt	Copper	Iodine	Iron	Manganese	Selenium	Zinc
Manganese (manganous) oxide, MnO	6-03-056	99.0	-	-	-	-	-	-	-	-	-	-	-	774,500	-	-
Manganese sulfate, monohydrate, MnSO4-H2O	6-28-103	100.0	-	-	-	-	-	-	18.97	-	-	-	-	325,000	-	-
Phosphate, defluorinated	6-01-780	100.0	32.0	18.00	0.80	-	0.42	4.90	-	10	20	-	6,700	200	-	60
Potassium iodide, KI	6-03-759	100.0	-	-	21.00	-	-	-	-	-	-	681,700	-	-	-	-
Sodium chloride	6-04-152	100.0	-	-	-	60.66	-	39.34	-	-	-	-	-	-	-	-
Sodium phosphate, monobasic	6-04-288	97.0	-	22.50 (20.25)[1] (21.15)[2] (22.05)[3]	-	-	-	16.68	-	-	-	-	-	-	-	-
Sodium selenite	6-26-013	98.0	-	-	-	-	-	26.60	-	-	-	-	-	-	456,000	-
Zinc oxide, ZnO	6-05-553	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	780,000
Zinc sulfate	05-555	99.0	0.02	-	0.015	-	-	-	17.38	-	-	-	10	10	-	363,600

Note: Data represent total and (available) contents. Numbers in parentheses represent availability or absorption by various fish species.

[1]*Channel catfish.*

[2]*Common carp.*

[3]*Rainbow trout.*

Table A.5. FATTY ACID COMPOSITION OF THE TRIGLYCERIDE FRACTION OF INGREDIENTS USED IN FISH FEEDS

Ingredient name	International Feed No.	Fatty acid composition of triglyceride (%) C12 & C14.0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:2	C20:3	C20:4	C20:5	C22:4	C22:5	C26:6	Sat (%)	Mono-unsat. (%)	Poly-unsat. (%)	n-6 (%)	n-3 (%)	Ratio n-3:n-6 (%)
Fish meal or oil																					
Menhaden	7-08-049	8.0	28.9	7.9	4.0	13.4	1.1	0.9	0.5	-	1.2	10.2	0.7	1.6	12.8	40.9	21.3	29.0	3.9	23.9	8.44
Herring	5-02-00	7.6	18.3	8.3	2.2	16.9	1.6	0.6	-	-	0.4	8.6	-	1.3	7.6	28.1	25.2	20.1	3.3	16.8	5.09
Salmon-Sea caught	-	3.7	10.2	6.7	4.7	18.6	1.2	0.6	0.4	0.1	0.9	12.0	0.6	2.9	13.8	18.6	25.3	32.5	5.0	26.4	5.28
Channel catfish - cultured	5-09-835	1.0	14.1	2.2	4.6	41.1	25.5	2.5	-	1.1	0.8	0.7	-	0.5	1.7	19.7	43.3	33.8	26.3	6.4	0.24
Penaeied shrimp	5-04-226	1.1	15.5	7.5	8.2	12.8	4.3	1.0	-	-	8.7	11.2	-	1.9	11.0	24.8	20.3	28.1	14.9	23.2	1.56
Animal by-product meat or fat																					
Beef	4-08-127	3.0	27.0	-	21.0	40.0	2.0	0.5	-	-	-	-	-	-	-	51.0	40.0	2.5	2.0	0.5	0.25
Pork	4-04-790	1.5	32.2	3.0	7.8	48.0	11.0	0.6	-	-	-	-	-	-	-	41.5	51.0	11.6	11.0	0.6	0.05
Grain and seed meal or oil																					
Soybean	4-07-983	-	8.5	-	3.5	17.0	54.4	7.0	-	-	-	-	-	-	-	12.0	17.0	61.5	54.4	7.1	0.13
Corn	4-07-882	-	7.0	-	2.4	45.6	45.0	0.5	-	-	-	-	-	-	-	9.4	45.6	45.5	45.0	0.5	0.01
Coconut	4-09-320	65.5	8.0	-	2.8	5.6	1.6	-	-	-	-	-	-	-	-	28.8	5.6	1.6	1.6	0.0	0.00
Cottonseed	5-01-621	1.0	26.0	1.0	3.0	17.5	51.5	-	-	-	-	-	-	-	-	30.0	18.5	51.5	51.5	0.0	0.00
Linseed	4-14-502	-	6.0	-	3.5	20.0	14.5	56.0	-	-	-	-	-	-	-	9.5	20.0	70.5	15.5	56.0	3.86
Canola	4-06-144	-	3.0	-	1.5	32.0	19.0	10.0	-	-	10.5	-	23.5	-	-	4.5	55.5	39.5	29.5	10.0	0.34
Peanut	5-03-649	-	11.5	-	3.0	53.0	26.0	-	-	-	1.5	-	-	-	-	14.5	53.0	27.5	27.5	0.0	0.00

APPENDIX B: COMMON AND SCIENTIFIC NAMES OF SPECIES

Common name	Scientific name
VERTEBRATES	
Japanese eel	*Anguilla japonica*
Common carp	*Cyprinus carpio*
Silver carp	*Hypophthalmichthys molitrix*
Grass carp	*Ctenopharyngodon idella*
Sea bass	*Dicentrarchus labrax*
Channel catfish	*Ictalurus punctatus*
Coho salmon	*Onchorhynchus kisutch*
Chinook salmon	*Onchorhynchus tshawytscha*
Rainbow trout	*Salmo gairdneri*
Atlantic salmon	*Salmo salar*
Brook trout	*Salvelinus frontinalus*
Blue tilapia	*Oreochromis aurea*
Mossambique tilapia	*Oreochromis mossambica*
Nile tilapia	*Oreochromis niloticus*
Other tilapias	*Tilapia zilli*
	Tilapia rendalli
INVERTEBRATES	
Brine shrimp	*Artemia salina*
American lobster	*Homarus americanus*
Freshwater shrimp	*Macrobrachium rosembergi*
Marine shrimps	*Penaeus indicus*
	Penaeus vannamei
	Penaeus japonicus
	Penaeus stylirostris
	Penaeus monodon
	Penaeus serratus
	Palaemon serratus
Red crawfish	*Procambarus clarkii*

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