Nutrient Requirements of Fish

Subcommittee on Fish Nutrition, National Research Council

ISBN: 0-309-59629-7, 124 pages, 8.5 x 11, (1993)

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Committee on Animal Nutrition
Board on Agriculture
National Research Council

NATIONAL ACADEMY PRESS
Washington, D.C. 1993

NATIONAL ACADEMY PRESS 2101 Constitution Avenue, NW Washington, D.C. 20418

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This study was supported by the Agricultural Research Service of the U.S. Department of Agriculture, under Agreement No. 59-32U4-5-6, and by the Center for Veterinary Medicine, Food and Drug Administration of the U.S. Department of Health and Human Services, under Cooperative Agreement No. FD-U-000006-10. Additional support was provided by the American Feed Industry Association. Dissemination of the report was supported in part by the W. K. Kellogg Foundation.

Library of Congress Cataloging-in-Publication Data

Nutrient requirements of fish / Committee on Animal Nutrition, Board on Agriculture, National Research Council.
p. cm.
Includes bibliographical references (p.) and index.

ISBN 0-309-04891-5

1. Fishes—Nutrition—Requirements. 2. Fishes—Feeding and feeds. I. National Research Council (U.S.). Committee on Animal Nutrition.
SH156.N86 1993
639.3—dc20 93-39031
CIP

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the organizations or agencies that provided support for this project.

Printed in the United States of America
First Printing, **December 1993**
Second Printing, **April 1999**

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Preface

The Subcommittee on Fish Nutrition was appointed in 1989 under the auspices of the Board on Agriculture's Committee on Animal Nutrition (CAN) to update and revise the 1981 edition of *Nutrient Requirements of Coldwater Fishes* and the 1983 edition of *Nutrient Requirements of Warmwater Fishes and Shellfishes*. New research indicates that similarities in the nutrition and feeding of cold-water and warm-water species do not warrant separate reports. Therefore, this edition combines all species of finish having commercial significance. (See Appendix Table A-1 for a list of fish discussed in this report.) It aims to expand knowledge on the nutrient requirements of finfish, diet formulations and preparation, and feeding practices.

The origins of CAN can be traced to the earliest days of the National Research Council, which was established in 1916 as the operating arm of the National Academy of Sciences. One of the major activities of the committee has been the development of nutrient requirement standards for domestic animals, including standards for laboratory animals. In 1943, the first appointments were made for subcommittees on the nutrition of poultry and swine. Since then, subcommittees have also been formed on the nutrient requirements of dairy cattle, beef cattle, sheep, horses, foxes and mink, dogs, cats, rabbits, laboratory animals, goats, nonhuman primates, warm-water fish and shellfish, and coldwater fish. These reports have been revised from time to time as new information on quantitative nutrient requirements has become available.

The Overview of this report is followed by eight chapters. Chapter 1 presents dietary requirements necessary for the normal health, growth, and reproduction of fish. Chapter 2 discusses materials in feedstuffs, other than nutrients, that may affect physical, palatability, or nutritional properties of the feed or metabolism of the fish. Chapter 3 covers antinutrients and adventitious toxins that may be present in feedstuffs and the varying susceptibilities of different fish to them.

Chapters 4, 5, and 6 focus on the nutrient availability of feedstuffs for various species as well as the formulation and feeding of commercial diets that are nutritionally balanced mixtures of feed ingredients with desired physical properties. Feeding practices for larval fish and various fish species are detailed.

Chapter 7 is a table of minimum nutrient requirements for maximum performance of five fish. These values represent minimum requirements for young, rapidly growing fish under optimal growing conditions. The values have not been increased to include a margin of safety commonly added in practice to compensate for ingredient variation, processing and storage losses, and variation in requirements caused by environmental effects. Therefore, those given here should be adjusted for practical allowances in feed formulation. Also, if the requirements are not given for a particular species, those established for a related species can often be discreetly substituted. As more information becomes available on nutrient requirements, the recommended allowances for specific needs will be refined.

Chapter 8 provides tables of feed ingredient composition for defining and formulating fish feeds for research and commercial practices. The nutrient composition data represent chemical analyses with no correction for availability to the animal. Therefore, the level of the feed ingredient, when used in diet formulation, must be adjusted to allow for availability to the fish.

Aquatic crustaceans (arthropods with mandibles, antennae, legs, or leg like appendages on the abdomen and thorax, and no wings) are not included in this edition because of the lack of published research data on their nutrient requirements. However, crustacean aquaculture is a viable commercial enterprise worldwide, and this subcommittee recognizes

the need for nutritional information for these species.

Most of the data presented in this report are for small fish, and it is recognized that nutrient requirements change as fish size increases. Also, environment influences nutrient requirements; therefore, future research should address effects of temperature, disease, and various other stresses on the nutritional needs of fish. More research is also needed on the nutrient requirements that have a large impact on feed costs, such as amino acids and energy-protein ratios, to refine or support existing values that in many cases are based on a single set of experimental conditions.

Richard T. Lovell, *Chair*

Subcommittee on Fish Nutrition

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Overview

Aquaculture is more than a science in its infancy; it 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.

To expand the knowledge on nutrition and feeding of fish, the Subcommittee on Fish Nutrition of the Committee on Animal Nutrition, under the auspices of the National Research Council, has examined the literature and current practices of aquaculture and prepared the latest recommendations on fish nutrition. It presents in this report a broad analysis of the nutrient requirements of various species, appropriate diet formulations and preparation, and feeding practices.

The subcommittee believes that the nutrient requirements derived for several fish species have served adequately as a basis for formulating productive and cost-effective diets for most commercial species. As more information becomes available, the requirements of various species and for specific productive functions will be refined.

Generally, nutrient requirements do not vary greatly among fish species. Any exceptions can often be identified with warm-water or cold-water, finfish or crustacean, carnivorous or omnivorous, and marine or freshwater fish. Therefore, the subcommittee recommends when nutrient requirements are not available that a prudent analogy be allowed to suffice.

Feed contaminants, from both human and natural sources, can dramatically affect the health, growth, and reproduction of fish. Because antinutrients and adventitious toxins are being given closer scrutiny in feedstuffs, the subcommittee believes that a greater awareness of their effects on fish is needed by nutritionists and the aquaculture industry. Solutions to problems, which vary depending on the contaminant, include development of alternative, contaminant-free feeds; alteration of feeding practices; and improvement of feed storage conditions.

Feeding fish in their aqueous environment involves considerations beyond those for feeding land animals. These aspects include the nutrient contribution of natural aquatic organisms in pond culture, the effects of feeding and diet composition on dissolved oxygen and other water quality factors, and the loss of nutrients if feed is not consumed immediately. Fish feeds require processing methods that provide special physical properties to facilitate feeding in water, and variation in feeding behavior requires special feeding regimens for various species. The effects of diet composition and feeding practice on the quality of the effluent from the culture system is also an important consideration.

The subcommittee has updated the nutrient composition of feed ingredients based on information provided by the feed composition data base of the National Agricultural Library of the U.S. Department of Agriculture. A comprehensive presentation of the fatty acid composition of various lipid sources has been included.

The nutrient requirements presented in this report were determined primarily with small fish and represent concentrations affecting maximum growth rate. Fish size, metabolic function, management, and environmental factors have slight to profound effects on nutrient requirements for optimum performance. Thus, these data represent approximations, and the subcommittee recommends that they be used with discretion. The requirement data do not include additional allowances for processing and storage losses, bioavailability of nutrients in feed ingredients, or economic considerations.

Aquaculture as a science and an industry will continue to grow. With increasing consumer demand and declining yields from natural waters, the aquaculture industry will

need to respond with an increasing supply of fish and fish products. These latest findings by the Subcommittee on Fish Nutrition, coupled with future research on nutrition and behavior, will provide the knowledge and techniques necessary to advance the science of aquaculture, ensuring its growth and success as an industry.

1

Dietary Requirements

Feeds and feedstuffs contain nutrients and energy sources essential for fish growth, reproduction, and health. Deficiencies of these substances can reduce growth rates or lead to diseases, and, in some cases, excesses can cause a reduction in growth rate. Dietary requirements can be established for energy, protein and amino acids, lipids, minerals, and vitamins.

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.

This section familiarizes the user with those aspects of nutritional energetics that deal with feed energy use by the animal, energy value of feedstuffs, and dietary energy requirements. Readers who require more detailed information on physiological energetics of fish may refer to the review of Brett and Groves (1979). Those who wish to read further on nutritional energetics, with an emphasis on determining dietary energy allowances for captive fish, should refer to the reviews of Smith (1989) and Cho and Kaushik (1990).

Partitioning of Dietary Energy

The energy of ingested feed is divided into many components in the animal's body. An illustration of energy flow in the animal with accepted abbreviations of energy metabolism terms (National Research Council, 1981) is shown in Figure 1-1. There are many places where energy is lost between intake and recovered products. Losses occur in feces, in urine and gill excretions, and as heat. Ideally, the fish feeder needs to minimize these losses and thereby obtain maximum return as useful products. The magnitude of these losses depends primarily on characteristics of the diet and the level of feeding. The difference between intake energy (IE) and digestible energy (DE) is energy lost in the feces (FE). The inclusion of fibrous materials that are poorly digested by fish will increase the FE loss. Metabolizable energy (ME) represents DE corrected for energy lost by

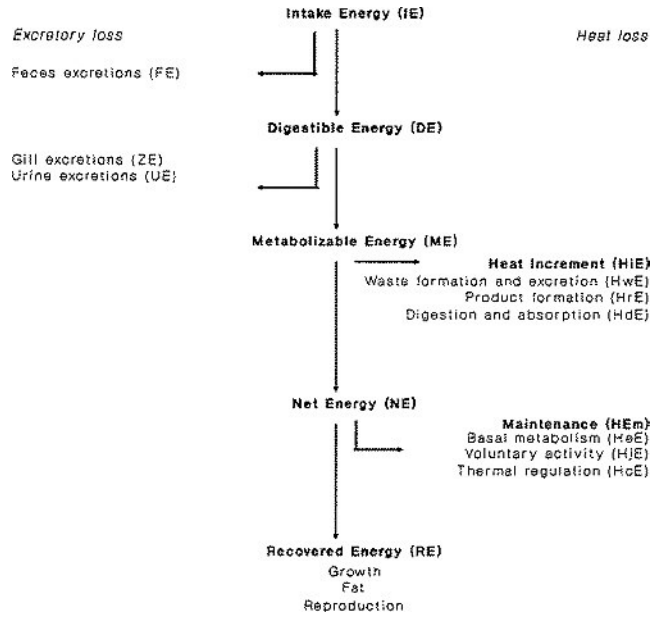

FIGURE 1-1 Schematic presentation of the fate of dietary energy for fish, categorizing the losses that occur as feed is digested and metabolized, leaving a fraction of the energy to be retained as new tissue. Source: Adapted from National Research Council. 1981. Nutritional Energetics of Domestic Animals and Glossary of Energy Terms. Washington, D.C.: National Academy Press.

excretion through the gills (ZE) and urine (UE). The difference between ME and energy recovered as growth and/or reproductive products (RE) is energy lost as heat (HE). Heat loss occurs primarily by two processes: the heat increment of feeding (HiE) and maintenance heat loss (HEm).

The HiE is the increase in heat production subsequent to ingestion of feed. The factors contributing to HiE are the digestion and absorption processes (HdE), the transformation and interconversion of the substrates and their retention in tissues (HrE), and the formation and excretion of metabolic wastes (HwE). The main biochemical basis for HiE in mammals and birds is the energy required for the ingested amino nitrogen (N) to be deaminated and excreted (Kleiber, 1975); however, this represents less of an energy loss in fish because they can eliminate and products of protein metabolism (ammonia, bicarbonate, and carbon dioxide) 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 (Brody, 1945). This conclusion has been reinforced by the observation that intravenous infusion of amino acids increases heat production to the same extent as does the oral administration of the amino acids (Benedict and Emmes, 1912; Borsook, 1936). HiE depends to a large extent on the balance of dietary nutrients and the plane of nutrition (Brody, 1945) and, in fish, the water temperature (Cho and Slinger, 1979). Thus, measurement of HiE for balanced feeds is more meaningful than measurement of the HiE of individual feed ingredients, because the metabolic fate of absorbed nutrients depends on the mixture absorbed and, hence, the variety of metabolic processes that are possible.

The HiE in fish is greater for diets with a high protein content than for diets with a low protein content (Cho, 1982). In mammals and birds, however, the effect of high dietary protein on heat increment is even more marked, partly because of the energy expenditure during synthesis of urea or uric acid from the deaminated nitrogen. The energy cost of synthesis for urea and uric acid is 3.1 and 2.4 kcal/g N, respectively (Martin and Blaxter, 1965). In contrast, ammonia is the primary nitrogenous waste product of protein catabolism in fish (Goldstein and Forster, 1970). Because this form of nitrogen can be readily released into the water, energy expenditure on urea or uric acid synthesis is not needed (Cowey, 1975). Cho et al. (1982) found that HiE for rainbow trout at 15°C was 5 to 15 percent of the gross energy consumed (IE) and fell as the ratio of protein to energy decreased. The HiE for livestock can be as much as 20 to 30 percent of the IE (Farrell, 1974; National Research Council, 1984). Thus, because of the lower heat increment of fish, the net energy (NE), which is the energy that is useful to the animal for maintenance and growth, in production diets is higher for fish than for warm-blooded animals.

Maintenance energy (HEm) is that required to maintain those functions of the body immediately essential to life. A major portion of this maintenance energy is spent for basal metabolism (HeE), such as respiration, transport of ions and metabolites, body constituent turnover, and circulation. A smaller portion is spent for voluntary or resting activity (HjE) 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 HEm requirement of fish is lower than for homeotherms. The fasting heat production (HEf) is an approximation of the HEm. Cho and Kaushik (1990) measured oxygen consumption of fasting rainbow trout weighing 96 to 145 g at 15°C and calculated their HEf, in kcal/fish/day to be 8.85 $W^{0.82}$ where W is body weight in kilograms. Smith (1989) reported an HEf value of 4.41 $W^{0.63}$ 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. Brett and Groves (1979) recommended the exponent 0.8 for metabolic body size for fish. When these HEf values for fish are compared with 70 $W^{0.75}$ for mammals and 83 $W^{0.75}$ 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 that the percentage of net energy that is not dissipated as heat but retained within the body as new tissue or recovered energy is greater.

Energy Value of Feedstuffs for Fish

The energy content of a diet depends on its chemical composition, with the mean values of heat of combustion of protein, lipid, and carbohydrate being 5.64, 9.44, and 4.11 kcal/g, respectively. However, the chemical makeup of the diet influences only its heat of combustion, or gross energy, and yields no information on whether the energy and nutrients are available to fish through the digestive process. Prior to formulating diets, therefore, it is necessary to know the bioavailability of the energy in the feedstuffs for the animal being fed.

Available energy values for feedstuffs for fish have been determined on a DE and ME basis. ME, where applicable, is a more exact measure of the energy value for a complete diet that becomes available for metabolism by the animal. Practically, ME offers little advantage over DE in evaluating useful energy in feedstuffs for fish because FE accounts for most of the excretory losses. Energy losses through ZE and UE by fish are smaller than nonfecal energy losses by mammals and birds, and they do not vary among feedstuffs as much as do FE losses. Furthermore, determining ME values with fish is difficult because of the need to force feed

and restrain the fish in metabolism chambers with the aid of a collar for simultaneous collection of fecal, gill, and urinary excretions (Smith, 1976). DE values are generally easier to determine and the fish feed voluntarily (Page and Andrews, 1973; Cruz, 1975; Cho and Slinger, 1979; Takeuchi et al., 1979). However, the use of proper techniques is necessary to give reliable DE values for fish. The collection of feces without the leaching of nutrients is important in determining DE with fish. Early studies (Smith and Lovell, 1973; Windell et al., 1978) showed that improper collection of feces, such as allowing feces to remain in the fish tank too long, caused serious overestimation of digestion coefficients. Methods for determining DE and ME in fish are discussed in Chapter 4.

Both proteins and lipids are highly available energy sources for fish (Cruz, 1975; Smith, 1976; Popma, 1982). The value of carbohydrate as an energy source is variable among species. Nile tilapia (Popma, 1982) and channel catfish (Wilson and Poe, 1985), which are warm-water omnivorous species, digest over 70 percent of the gross energy in noncooked starch while rainbow trout, a cold-water carnivore, may digest less than 50 percent (Cho and Slinger, 1979). Cooking, as in extrusion processing of feeds, increases digestibility of starch for fish. Extrusion processed corn had a 38 percent higher DE for channel catfish than compression pelleted corn (Wilson and Poe, 1985) and gelatinized starch had a 75 percent higher DE for rainbow trout than raw starch (Cho and Slinger, 1979).

Energy Requirements

Energy intake is a basic nutritional requirement because maintenance of life processes takes priority over growth and other functions. Thus, energy concentration should be the first nutritional consideration in diet formulation for fish. In practice, however, protein is usually given first priority because it is more expensive than other energy yielding components. Protein and energy should be kept in balance. A dietary deficiency or an excess of DE can reduce growth rates of fish. A diet deficient in energy in relation to protein will mean that protein is used for energy to satisfy maintenance before growth. In contrast, a diet containing excess energy can reduce feed consumption and thus lower the intake of the necessary amount of protein and other essential nutrients for maximum growth. Excessively high ratios of energy to nutrients can also lead to deposition of large amounts of body fat, which can be undesirable in food fish.

TABLE 1-1 Optimum Protein: Energy Ratio for Different Fish

Species	Digestible Protein (DP) (%)	Digestible Energy (DE) (kcal/g)	Final DP/DE (mg/kcal)	Weight (g)	Response Criteria	References
Channel catfish	22.2	2.33	95	526	Weight gain	Page and Andrews (1973)
	28.8[a]	3.07[a]	94	34	Weight gain	Garling and Wilson (1976)
	27.0	2.78	97	10	Protein gain	Mangalik (1986)
	27.0	3.14	86	266	Protein gain	Mangalik (1986)
	24.4[a]	3.05[a]	81	600	Weight gain	Li and Lovell (1992)
Red drum	31.5[a]	3.20[a]	98	43	Weight gain	Daniels and Robinson, (1986)
Hybrid bass	31.5[a]	2.80	112	35	Weight gain	Nematipour et al. (1992)
Nile tilapia	30	2.90	103	50	Weight gain	El-Sayed (1987)
Common carp	31.5[a]	2.90[a]	108	20	Weight gain	Takeuchi et al. (1979)
Rainbow trout	33	3.6	92	90	Weight gain	Cho and Kaushik (1985)
	42	4.10	105	94	Weight gain	Cho and Woodward (1989)

[a] Digestible protein and energy were estimated from ingredient composition of the diet.

Ratios of digestible protein to DE (mg/kcal) for maximum weight gain for several fish species have been measured in growth studies (Table 1-1). Values range from 81 mg/kcal to 117 mg/kcal and are substantially higher than protein-energy ratios for swine and poultry, which range from 40 to 60 mg/kcal (National Research Council, 1984, 1988). The reason the protein-energy ratio for fish is higher 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 the synthesis of uric acid.

Since lipid is the primary nonprotein energy source in salmonid diets, the protein-energy allowance for these diets is sometimes reported as the ratio of protein to lipid. The optimum combination for weight gain for rainbow trout was 35 to 36 percent protein and 15 to 16 percent lipid (Watanabe et al., 1979; Cho, 1982).

Empirical calculation of energy requirements of fish based on energy losses and expected energy recovery are possible with reliable information on energy balances in the

animal under a given set of conditions. Energy balances for rainbow trout have been established under laboratory conditions, as discussed previously. Cho and Kaushik (1990) constructed a model for calculating the DE 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 DE would be required to produce 1.91 Mcal of recovered energy in 1 kg of fish biomass with an RE:DE efficiency ratio of 0.54, which is comparable to a value of 0.56 reported for channel catfish (Gatlin et al., 1986). 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.

PROTIEN AND AMINO ACIDS

Proteins are composed of up to 20 α-amino acids linked into chains by peptide bonds. The chains are cross-linked by disulfide bridges, hydrogen bonds, and van der Waals forces. The amino acid content of proteins, particularly feed proteins, may differ markedly. Some, such as gelatin (a mixture of proteins derived from collagen) or zein (a protein from maize gluten), are largely, or even entirely, deficient in one or more amino acids. Others, such as fishmeal, have a balance of amino acids that more closely meets the requirements of fish. Consequently the capacity of different feed proteins to meet the amino acid needs of the fish will differ considerably. Ingested protein is hydrolyzed to free amino acids, dipeptides, and tripeptides by digestive enzymes secreted into the gastrointestinal tract. These products are absorbed by the mucosal cells where intracellular digestion of small peptides occurs; thus only amino acids appear to be released into the portal vein as products of protein digestion (Murai et al., 1987). Some evidence has shown that small amounts of certain whole proteins may be absorbed through the wall of the gastrointestinal tract, but the quantities involved have not been confirmed as being of any quantitative significance (Ash, 1985).

In the context of animal feeding, protein generally refers to crude protein (CP); that is, N × 6.25, a definition based on the assumption that proteins contain 16 percent N. The requirement for dietary protein has two components:

1. a need for indispensable amino acids that the fish cannot synthesize either at all or at a rate commensurate with its need for protein deposition or commensurate with the synthesis of a variety of other compounds with metabolic functions and
2. a supply of either dispensable amino acids or sufficient amino nitrogen to enable the fish to synthesize them.

Insofar as synthesis of dispensable amino acids requires expenditure of energy, feeding dietary proteins that most nearly meet the needs of fish for both indispensable and dispensable amino acids will result in the most efficient growth by the fish. Thus, the concept of balance or pattern of amino acids is basic to protein requirement.

Protein Requirements

The protein requirements, meaning the minimum amount needed to meet requirements for amino acids and to achieve maximum growth, have now been measured in juvenile fish of many species (see Tables 1-2 to 1-13). They have been obtained mainly from dose-response curves in which graded amounts of high-quality protein were fed in partially defined diets. The response measured was weight gain. The values are expressed as a percentage of dry diet. Although the expression of protein as a proportion of dietary energy would have focused attention on protein as a substantial source of dietary energy, this approach was not possible for many of the data because in formation on the DE content of the diets was unavailable and values used for the energy density of dietary components varied between authors.

The protein allowances in fish diets are appreciably higher than those in the diets of terrestrial warm-blooded animals. The methods used to determine protein requirements, however, may overestimate requirements, in that excess dietary protein or amino acids, which cannot be stored, are catabolized preferentially over carbohydrates and fats and used for energy by some fishes (Wilson, 1989). In addition, adequate consideration has not always been given to factors such as concentration of DE in the diet, amino acid composition of the dietary protein, and digestibility of the dietary protein (Wilson and Halver, 1986; Wilson, 1989). Understanding the nutritional constraints and limitations used in arriving at these reported protein requirements is important for their proper application.

Protein requirements, as a proportion of the diet, decrease as fish approach maturity. For example, 25 percent protein was adequate in the diet of channel catfish of 114 to 500 g, but 35 percent protein produced faster gains than did 25 percent protein in 14- to 100-g fish (Page and Andrews, 1973). Somewhat similar results have been obtained with salmonids, common carp, and tilapia (Wilson and Halver, 1986).

Little convincing evidence exists to show that protein requirement, expressed as a percentage of dry matter, is affected by water temperature. In general, all feeding and growth functions increase in parallel as water temperature rises, although growth rate may increase more rapidly because of an increased feed conversion efficiency coupled

with a higher intake per meal (Brett, 1979). Protein requirement for rainbow trout was unchanged from 35 percent (in diets containing 3,580 kcal DE/kg) at water temperatures ranging from 9° to 18°C (see Figure 5 in National Research Council, 1981).

TABLE 1-2 Estimated Dietary Protein Requirement for Maximal Growth of Some Species of Juvenile Fish (As Fed Basis)

Species	Protein Source	Estimated Protein Requirement (%)	Reference
Atlantic salmon	Casein and gelatin	45	Lall and Bishop (1977)
Channel catfish	Whole egg protein	32–36	Garling and Wilson (1976)
Chinook salmon	Casein, gelatin, and amino acids	40	DeLong et al. (1958)
Coho salmon	Casein	40	Zeitoun et al. (1974)
Common carp	Casein	31–38	Ogino and Saito (1970); Takeuchi et al. (1979)
Estuary grouper	Tuna muscle meal	40–50	Teng et al. (1978)
Gilthead sea bream	Casein, fish protein concentrate, and amino acids	40	Sabaut and Luquet (1973)
Grass carp	Casein	41–43	Dabrowski (1977)
Japanese eel	Casein and amino acids	44.5	Nose and Arai (1972)
Largemouth bass	Casein and fish protein concentrate	40	Anderson et al. (1981)
Milkfish	Casein	40	Lim et al. (1979)
Plaice	Cod muscle	50	Cowey et al. (1972)
Puffer fish	Casein	50	Kanazawa et al. (1980)
Rainbow trout	Fishmeal, casein, gelatin, and amino acids	40	Satia (1974)
Red sea bream	Casein	55	Yone (1976)
Smallmouth bass	Casein and fish protein concentrate	45	Anderson et al. (1981)
Snakehead	Fishmeal	52	Wee and Tacon (1982)
Sockeye salmon	Casein, gelatin, and amino acids	45	Halver et al. (1964)
Striped bass	Fishmeal and soy proteinate	47	Millikin (1983)
Blue tilapia	Casein and egg albumin	34	Winfree and Stickney (1981)
Mossambique tilapia	White fishmeal	40	Jauncey (1982)
Nile tilapia	Casein	30	Wang et al. (1985)
Zillii's tilapia	Casein	35	Mazid et al. (1979)
Yellowtail	Sand eel and fishmeal	55	Takeda et al. (1975)

The high concentrations of dietary protein necessary for maximal growth rates of fish do not mean that they use more protein as an energy source than is the case with homeothermic vertebrates. Values for net protein retention are in the range of 20 to 50 percent for both types of vertebrate; Bowen (1987) summarized a number of data that showed a median value for fish of 31 percent and for other

TABLE 1-3 Amino Acid Requirements of Juvenile Chinook Salmon

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	40	6.0	2.4	Chemically defined	Klein and Halver (1970)
Histidine	40	1.8	0.7	Chemically defined	Klein and Halver (1970)
Isoleucine	41	2.2	0.9	Chemically defined	Chance et al. (1964)
Leucine	41	3.9	1.6	Chemically defined	Chance et al. (1964)
Lysine	40	5.0	2.0	Purified	Halver et al. (1958)
Methionine[a]	40	4.0	1.6	Chemically defined	Halver et al. (1959)
Phenylalanine[b]	41	5.1	2.1	Chemically defined	Chance et al. (1964)
Threonine	40	2.2	0.9	Chemically defined	DeLong et al. (1962)
Tryptophan	40	0.5	0.2	Chemically defined	Halver (1965)
Valine	40	3.2	1.3	Chemically defined	Chance et al. (1964)

[a] Diet contained 1.0 percent cystine.
[b] Diet contained 0.4 percent tyrosine.

vertebrates of 29 percent. Broadly similar proportions of dietary protein are therefore used as an energy source in fish as in warm-blooded terrestrial vertebrates; this, notwithstanding the fact that fish have lower presumed energy requirements than do homeotherms. Attempts have been made to compare absolute protein intake rates (mg protein ingested/g body weight/day); this is a difficult undertaking both because accurate measurement of feed intake by fish is in itself difficult and because the use of data for fish of different physiological ages, held under different conditions of temperature and photoperiod, introduces considerable variation.

TABLE 1-4 Amino Acid Requirements of Juvenile Common Carp

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	38.5	4.3	1.6	Chemically defined	Nose (1979)
Histidine	38.5	2.1	0.8	Chemically defined	Nose (1979)
Isoleucine	38.5	2.5	0.9	Chemically defined	Nose (1979)
Leucine	38.5	3.3	1.3	Chemically defined	Nose (1979)
Lysine	38.5	5.7	2.2	Chemically defined	Nose (1979)
Methionine[a]	38.5	3.1	1.2	Chemically defined	Nose (1979)
Phenylalanine[b]	38.5	6.5	2.5	Chemically defined	Nose (1979)
Threonine	38.5	3.9	1.5	Chemically defined	Nose (1979)
Tryptophan	38.5	0.8	0.3	Chemically defined	Nose (1979)
	42	0.3	0.1	Purified	Dabrowski (1981)
Valine	38.5	3.6	1.4	Chemically defined	Nose (1979)

[a]In the absence of dietary cystine.
[b]In the absence of tyrosine, with 1 percent tyrosine in the diet, phenylalanine requirement was 3.4 percent of protein or 1.3 percent of dry matter.

TABLE 1-5 Amino Acid Requirements of Juvenile Channel Catfish

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	24	4.3	1.0	Chemically defined	Robinson et al. (1981)
Histidine	24	1.5	0.4	Chemically defined	Wilson et al. (1980)
Isoleucine	24	2.6	0.6	Chemically defined	Wilson et al. (1980)
Leucine	24	3.5	0.8	Chemically defined	Wilson et al. (1980)
Lysine	24	5.1	1.2	Chemically defined	Wilson et al. (1977)
	30	5.0	1.5	Chemically defined	Robinson et al. (1980b)
Methionine[a]	24	2.3	0.6	Chemically defined	Harding et al. (1977)
Phenylalanine[b]	24	5.0	1.2	Chemically defined	Robinson et al. (1980a)
Threonine	24	2.0	0.5	Chemically defined	Wilson et al. (1978)
Tryptophan	24	0.5	0.12	Chemically defined	Wilson et al. (1978)
Valine	24	3.0	0.71	Chemically defined	Wilson et al. (1980)

[a]In the absence of dietary cystine.
[b]Diet contained 0.3 percent tyrosine. With 0.6 percent tyrosine in the diet, phenylalanine requirement was 2.0 percent of protein or 0.5 percent of dry matter.

Amino Acid Requirements

An absolute requirement for 10 amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) has been demonstrated in all fish species examined so far. Quantification of essential amino acid requirements has relied largely on dose-response curves in which the response measured has been weight gain. Various types of chemically defined, purified, and natural ingredient diets have been used to provide graded increments of the amino acid under test. Most studies have used test diets in which the nitrogen component consisted of either amino acids or a mixture of amino acids,

casein, and gelatin formulated to provide an indispensable amino acid composition identical with some reference protein (such as whole hen's egg protein or fish body protein) minus the amino acid under test. For many fish species, growth rates produced by diets with large amounts of free amino acids are inferior to diets of similar amino acid composition in which the nitrogen component is protein (Wilson et al., 1978; Robinson et al., 1981; Walton et al., 1982, 1986). Thus amino acid requirements obtained in this way are based on growth rates below the optimum.

TABLE 1-6 Amino Acid Requirements of Juvenile Japanese Eel

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	38	4.5	1.7	Chemically defined	Nose (1979)
Histidine	38	2.1	0.8	Chemically defined	Nose (1979)
Isoleucine	38	4.0	1.5	Chemically defined	Nose (1979)
Leucine	38	5.3	2.0	Chemically defined	Nose (1979)
Lysine	38	5.3	2.0	Chemically defined	Nose (1979)
Methionine[a]	38	3.2	1.2	Chemically defined	Nose (1979)
Phenylalanine[b]	38	5.8	2.2	Chemically defined	Nose (1979)
Threonine	38	4.0	1.5	Chemically defined	Nose (1979)
Tryptophan	38	1.1	0.4	Chemically defined	Nose (1979)
Valine	38	4.0	1.5	Chemically defined	Nose (1979)

[a] In the absence of dietary cystine.
[b] In the absence of tyrosine, with 2.0 percent tyrosine in the diet, phenylalanine requirement was 3.2 percent of protein or 1.2 percent of dry matter.

Other approaches to quantifying indispensable amino acid requirements have included using proteins with poor amino acid patterns that differ substantially from that required, such as zein (Dabrowski, 1981) or maize gluten (Halver et al., 1958; Ketola, 1983). Comparatively small amounts of crystalline amino acids are then added to balance the protein component, leaving it deficient only in one amino acid. Concerns about this approach center on protein digestibility, amino acid availability and rate of transit, and absorption of supplemented free amino acids compared with those from dietary protein. In addition, imbalanced proteins may have high percentages of certain amino acids, such as leucine, and these may depress the assimilation of other amino acids.

TABLE 1-7 Amino Acid Requirements of Juvenile Nile Tilapia

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	28	4.20	1.18	Chemically defined	Santiago and Lovell (1988)
Histidine	28	1.72	0.48	Chemically defined	Santiago and Lovell (1988)
Isoleucine	28	3.11	0.87	Chemically defined	Santiago and Lovell (1988)
Leucine	28	3.39	0.95	Chemically defined	Santiago and Lovell (1988)
Lysine	28	5.12	1.43	Chemically defined	Santiago and Lovell (1988)
Methionine[a]	28	2.68	0.75	Chemically defined	Santiago and Lovell (1988)
Phenylalanine[b]	28	3.75	1.05	Chemically defined	Santiago and Lovell (1988)
Threonine	28	3.75	1.05	Chemically defined	Santiago and Lovell (1988)
Tryptophan	28	1.00	0.28	Chemically defined	Santiago and Lovell (1988)
Valine	28	2.80	0.78	Chemically defined	Santiago and Lovell (1988)

[a] Cystine 0.54 percent of dietary protein, 0.15 percent of dry diet.
[b] Tyrosine 1.79 percent of dietary protein, 0.5 percent of dry diet.

Ogino (1980) measured the retention of indispensable amino acids in the whole body protein of carp and rainbow trout and used the increase in indispensable amino acid content measured over periods of 14 to 28 days to estimate requirements. This method assumes that the maintenance requirements of young growing fish are low (although it is not easy to reconcile this view with the fact that only 30 to 40 percent of dietary nitrogen is retained by growing fish), so

that the pattern of amino acids deposited in body weight gain is the main determinant of patterns of amino acids required.

TABLE 1-8 Amino Acid Requirements of Juvenile Rainbow Trout

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	36	3.3	1.2	Purified	Kaushik (1979)
	45	3.6	1.6	Purified	Walton et al. (1986)
	35	4.0	1.4	Chemically defined	Kim et al. (1983)
	33	4.7	1.6	Purified	Cho et al. (1989)
	47	5.9	2.8	Purified	Ketola (1983)
Lysine	35	3.7	1.3	Chemically defined	Kim and Kayes (1982)
	45	4.2	1.9	Purified	Walton et al. (1984a)
	47	6.1	2.9	Purified	Ketola (1983)
Methionine	46.4	2.2[a]	1.0[a]	Chemically defined	Walton et al. (1982)
	35	3.0[b]	1.1[b]	Chemically defined	Rumsey et al. (1983)
	35	2.9[c]	1.0[c]	Chemically defined	Kim et al. (1984)
	35	1.4	0.5	Chemically defined	Kim et al. (1992)
	41	1.5	0.6[d]	Purified	Cowey et al. (1992)
Tryptophan	55	0.5	0.3	Purified	Walton et al. (1984b)
	35	0.6	0.2	Chemically defined	Kim et al. (1987)
	42	1.4	0.6	Chemically defined	Poston and Rumsey (1983)

[a] Diet lacked cystine.
[b] Diet contained 0.3 percent cystine.
[c] Diet contained 0.5 percent cystine.
[d] Diet contained 0.16 percent cystine.

Relationship of Amino Acid Requirements to Protein Intake

In warm-blooded animals, a constant relationship was shown between indispensable amino acid requirements and protein intake up to the level of protein required for maximum growth (Almquist, 1972). For several indispensable amino acids, intake and weight gain were apparently linearly related and this relationship was presumed to hold for all indispensable amino acids. On this basis amino acid requirements of fish were expressed as a percentage of dietary protein as well as on a dry matter basis (National Research Council, 1981, 1983).

Later studies bear on the finding of Almquist (1972) in that they show the relationship to be not a linear but an exponential function (Finke et al., 1987). The response of an animal to dietary increments of a limiting nutrient does not break at one particular point. An accurate representation of the so-called "diminishing returns" area of the response curve is claimed (Finke et al., 1989) to be critical in assessing the efficiency of incremental increases of dietary amino acid concentration as the response approaches the maximum The use of a logistic model supports a more accurate assessment, than that provided by broken-line analysis, of the diminishing returns area of the response curve and of the maximum response (Finke et al., 1989).

TABLE 1-9 Amino Acid Requirements of Juvenile Coho Salmon

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	40	5.8	2.3	Chemically defined	Klein and Halver (1970)
Histidine	40	1.8	0.7	Chemically defined	Klein and Halver (1970)
Tryptophan	40	0.5	0.2	Chemically defined	Halver (1965)

The implication of these later studies is that indispensable amino acid requirements are not best expressed as a percentage of dietary protein. Nevertheless, because the dose-response relationship is, for all practical purposes, linear for much of its length (Gahl et al., 1991), amino acid

requirements in Tables 1-3 to 1-13 have again been expressed both as a percentage of dietary protein and on a dry matter basis.

TABLE 1-10 Amino Acid Requirements of Juvenile Chum Salmon

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	40	6.0	2.6	Chemically defined	Akiyama (1987)
Histidine	40	1.6	0.7	Chemically defined	Akiyama et al. (1985)
Isoleucine	40	2.4	1.0	Chemically defined	Akiyama (1987)
Leucine	40	3.8	1.5	Chemically defined	Akiyama (1987)
Lysine	40	4.8	1.9	Chemically defined	Akiyama et al. (1985)
Methionine + cystine	40	3.0	1.2	Chemically defined	Akiyama (1987)
Phenylalanine + tyrosine	40	6.3	2.5	Chemically defined	Akiyama (1987)
Threonine	40	3.0	1.2	Chemically defined	Akiyama et al. (1985)
Tryptophan	40	0.7	0.3	Chemically defined	Akiyama (1987)
Valine	40	3.0	1.2	Chemically defined	Akiyama (1987)

Diets in which the nitrogen component is made up of casein, gelatin, and crystalline amino acids have been referred to in the tables as chemically defined diets. Purified diets are those in which proteins, with an amino acid pattern (g amino acid/16 g nitrogen) that differs substantially from that required, supply the bulk of the nitrogen together with some supplementary amino acids. Natural ingredient diets use normal feed ingredients such as fishmeal, soya meal, blood meal, and wheat middlings.

The values in Tables 1-3 to 1-13 suggest that large differences exist among fish species in their requirements for certain amino acids. Where several estimates are available for one amino acid in a single species, as in the case of rainbow trout (Table 1-8), marked discrepancies occur. Some of these may be due to differences in growth rate, amino acid sources, feed intake, and other aspects of methodology.

Pathologies Resulting from Deficiencies

For most indispensable amino acids, deficiency is manifest as a reduction in weight gain. In certain 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 essential compounds.

TABLE 1-11 Amino Acid Requirements of Juvenile Mossambique Tilapia

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	40	4	1.6	Natural ingredient	Jackson and Capper (1982)
Lysine	40	4.1	1.6	Natural ingredient	Jackson and Capper (1982)
Methionine[a]	40	40	1.3	Natural ingredient	Jackson and Capper (1982)

[a] Diet contained 0.7 percent cystine.

Salmonids, including rainbow trout, Atlantic salmon (*Salmo salar*), and lake trout (*Salvelinus namaycush*), suffer from cataracts when given a diet deficient in methionine (Poston et al., 1977). The lens begins to become opaque after 2 to 3 months, depending on the extent to which the fish are deficient in sulfur amino acids. As the deficiency increases, lens opacity gradually progresses, causing a large reduction in light transmission. Cataracts also occur as a consequence of tryptophan deficiency in rainbow trout (Poston and Rumsey, 1983; Walton et al., 1984b); the developmental pattern of the cataracts is similar to that occurring in methionine deficiency (Poston and Rumsey, 1983).

Tryptophan deficiency leads to scoliosis (lateral curvature of the vertebral column) and to a derangement of mineral metabolism in certain salmonids, including rainbow trout (Walton et al., 1984b), sockeye salmon (*Oncorhynchus nerka*) (Halver and Shanks, 1960), and chum salmon (*Oncorhynchus keta*) (Akiyama et al., 1986). Scoliosis in chum salmon may be reversed by restoring tryptophan to normal concentrations in the diet. The condition may be related to a decline in levels of the brain neurotransmitter serotonin, which is formed from tryptophan. Thus, inclusion of serotonin

in tryptophan-deficient diets greatly reduces the incidence of scoliosis (Akiyama et al., 1986).

TABLE 1-12 Amino Acid Requirements of Juvenile Gilthead Sea Bream

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Arginine	34	5.0	1.7	Purified	Luquet and Sabaut (1974)
Lysine	34	5.0	1.7	Purified	Luquet and Sabaut (1974)
Methionine[a]	34	4.0	1.4	Purified	Luquet and Sabaut (1974)
Tryptophan	34	0.6	0.2	Purified	Luquet and Sabaut (1974)

[a] Cystine content of diet not stated.

Changes in mineral metabolism were observed in tryptophan-deficient rainbow trout (Walton et al., 1984b). Significantly greater concentrations of calcium (Ca) (a fourfold increase over control trout), sodium (Na), and potassium (K) were found in the kidneys of tryptophan-deficient trout. Concentrations of Ca, magnesium (Mg), Na, and K in the livers of tryptophan-deficient trout were also significantly greater than in normal trout. The metabolic lesion(s) responsible for these changes have not been resolved.

Relationships Among Amino Acids

Cystine can be formed metabolically from dietary methionine at a rate sufficient to meet the requirements of fish. The reverse sequence of reactions does not occur, however, and fish have an absolute requirement for methionine. Methionine can thus meet the total sulfur amino acid requirement of fish, although some of this requirement may be met by cystine.

Rainbow trout can use D-methionine to replace L-methionine on an equimolar basis (Kim et al., 1992). D-methionine is deaminated by D-amino acid oxidase and subsequently reaminated to L-methionine. This metabolic capacity is probably also characteristic of other fish.

A similar relationship exists between aromatic amino acids. Fish readily convert phenylalanine to tyrosine so that phenylalanine alone can meet requirements for aromatic amino acids. However, the presence of tyrosine in the diet will reduce some of the requirement for phenylalanine.

TABLE 1-13 Amino Acid Requirements of Juvenile Lake Trout

Amino Acid	Protein in Diet (%)	Requirement as Percentage of Dietary Protein	Requirement as Percentage of Dry Diet	Type of Diet	Reference
Isoleucine	27	2.0-2.6	0.5-0.7	Purified	Hughes et al. (1983)
Leucine	27	3.5-4.6	1.0-1.3	Purified	Hughes et al. (1983)
Valine	27	2.6-3.3	0.6-0.8	Purified	Hughes et al. (1983)

Some adverse interactions may occur between amino acids that are structurally related when their concentrations in the diet are imbalanced. Well-known examples in homeotherms are antagonisms arising from dietary imbalances of lysine-arginine and of leucine-valine. No convincing evidence exists, however, for lysine-arginine antagonism in fish. Robinson et al. (1981) could not demonstrate any effects when diets with excess lysine in the presence of adequate or marginal arginine were fed to channel catfish; diets containing excess arginine in the presence of adequate or marginal lysine similarly failed to show any antagonistic effect. Nor did excess lysine affect the growth rates of rainbow trout fed low concentrations of arginine (Kim et al., 1983).

Antagonism between branched-chain amino acids generally arises in mammals from an excess of leucine over isoleucine and valine; the first two steps of the catabolic breakdown of all three branched-chain amino acids are catalyzed by the same enzymes. Data on antagonisms among branched-chain amino acids in fish are not clear-cut and are inconsistent between species. Thus the isoleucine requirement of chinook salmon (*Oncorhynchus tshawytscha*) increased slightly with increasing concentrations of dietary leucine (Chance et al., 1964). Hughes et al. (1983) observed changes in concentrations of branched-chain amino acids in lake trout given diets containing increasing amounts of valine. Plasma isoleucine and leucine were both elevated in valine-deficient fish, and their concentrations decreased as

dietary valine was increased. No changes in plasma valine concentration occurred until valine in the diet reached the required concentration, after which the plasma valine increased about 2.5-fold. In contrast, rainbow trout showed a high tolerance for dietary leucine; no growth depression occurred with concentrations as high as 9.2 percent. Even with excessive dietary leucine concentrations (13.4 percent), which were overtly toxic, the concentrations of free valine and isoleucine in plasma, liver, and muscle were not depressed (Choo, 1990).

Another interaction characteristic of some homeotherms, and referred to as an imbalance, occurs when diets are supplemented with the second most limiting amino acid, or with all indispensable amino acids other than the first limiting amino acid. This leads to a fall in the concentration of the first limiting amino acid in the blood and eventually to reduced feed intake even though retention of the first limiting amino acid is not affected. No data are available on such interactions in fish. Nevertheless, oversupplementation with the second most limiting amino acid should be avoided as it may exacerbate a primary deficiency. There is considerable information available on amino acid interrelationships in mammals. Further information on these relationships can be found in Czarnecki et al. (1985), Baker (1987), and May et al. (1991).

LIPIDS

Dietary lipids are important sources of energy and of essential fatty acids (EFA) that are needed for normal growth and development. They also assist in the absorption of fat-soluble vitamins. Dietary lipids, mainly in the form of triacylglycerols, are hydrolyzed by digestive enzymes to a mixture of free fatty acids and 2-monoglycerides. These compounds are then absorbed and either used for the synthesis of various cellular components or catabolized for energy.

Dietary lipids contain both saturated and unsaturated fatty acids. Fatty acids may be designated by numbering either from the methyl or carboxyl terminal. The notation from the methyl terminal is most convenient for many nutritional purposes and is used here. It involves three numbers given in sequence, the first denoting the number of carbon atoms; the second, following a colon, the number of double bonds; and the third, designated as (n-) indicates the number of carbon atoms between the methyl terminal and the first double bond. The term polyunsaturated fatty acid (PUFA) normally refers to fatty acids with 18 or more carbon atoms and two or more double bonds.

Essential Fatty Acids

In common with other vertebrates, fish cannot synthesize either 18:2(n-6) or 18:3(n-3) de novo. Hence one or both of these fatty acids must be supplied preformed in the diet, depending on the EFA requirements. In addition, fish vary considerably in their ability to convert 18-carbon unsaturated fatty acids to longer-chain, more highly unsaturated fatty acids of the same series (Owen et al., 1975). The EFA requirement of the fish is thus related, to some extent, to their ability to modify these fatty acids metabolically.

The quantitative EFA requirements of several fish species are summarized in Table 1-14. A major difference appears to exist between freshwater and stenohaline marine fish (those unable to withstand a wide variation in water salinity). In general, freshwater fish require either dietary linoleic acid, 18:2(n-6), or linolenic acid, 18:3(n-3), or both, whereas stenohaline marine fish require dietary eicosapentaenoic acid (EPA), 20:5(n-3), and/or docosahexaenoic acid (DHA), 22:6 (n-3).

Among the freshwater species, the ayu, channel catfish, coho salmon, and rainbow trout require 18:3(n-3) or EPA and/or DHA. Chum salmon, common carp, and Japanese eel require an equal mixture of 18:2(n-6) and 18:3(n-3); whereas, Nile tilapia and Zillii's tilapia require only 18:2(n-6) for maximum growth and feed efficiency. Striped bass, however, require n-3 PUFA and cannot chain elongate 18:3(n-3) (Webster, 1989; Webster and Lovell, 1990).

The principal gross signs of EFA deficiency reported for various fishes are dermal signs (fin rot), a shock syndrome, myocarditis, reduced growth rate, reduced feed efficiency, and increased mortality (Castell et al., 1972; Takeuchi and Watanabe, 1977a,b; Takeuchi et al., 1980; Satoh et al., 1989). Essential fatty acid deficiency has also been shown to reduce the reproductive performance of common carp (Shimma et al., 1977), rainbow trout (Watanabe, 1982; Watanabe et al., 1984c; Leray et al., 1985) and red sea bream (Watanabe et al., 1984a,b).

In fish species that can further desaturate and chain elongate 18:2(n-6) or 18:3(n-3), an absence of either of these fatty acids in the diet leads to the desaturation and chain elongation of oleic acid, 18:1(n-9), to 20:3(n-9), which is characteristic of an EFA deficiency in many terrestrial animals. Thus when EFAs are deficient, increased concentrations of 20:3(n-9) are incorporated into tissue polar lipids in place of 20:4(n-6), 20:5(n-3), or 22:6(n-3). Castell et al. (1972) suggested that the ratio of 20:3(n-9)/20:5(n-3) in polar lipids from the liver of rainbow trout might be a useful index of EFA status. By analogy with mammals, the diet is considered satisfactory with respect to EFA if this ratio is not greater than 0.4.

Watanabe et al. (1983) have reported that n-3 PUFAs such as EPA and DHA, are required for normal growth and development of ayu and red sea bream larvae. High mortalities and abnormalities, such as underdeveloped swim bladder and scoliosis, have been observed in red sea bream larvae reared on rotifers and *Artemia* spp., either devoid of n-3 PUFAs or containing only low concentrations of n-3 PUFAs

(Watanabe et al., 1980; Kitajima et al., 1980a,b; Oka et al., 1980, 1982; Watanabe et al., 1982; Fukusho et al., 1984, 1985). Subsequently, EPA and/or DHA have been shown to be essential for various marine fish larvae, such as ayu (Kanazawa et al., 1981), red sea bream (Izquierdo et al., 1989; Watanabe et al., 1989b), striped jack (Watanabe et al., 1989c), and gilthead sea bream (Koven et al., 1989), as well as for the larvae of one freshwater fish, striped bass (Webster and Lovell, 1990).

TABLE 1-14 Essential Fatty Acid Requirement of Fish

Species	Fatty Acid Requirement	Reference
Freshwater fish		
Ayu	1 percent linolenic acid or 1 percent EPA	Kanazawa et al. (1982)
Channel catfish	1-2 percent linolenic acid or 0.5-0.75 percent EPA and DHA	Satoh et al. (1989)
Chum salmon	1 percent linoleic acid and 1 percent linolenic acid	Takeuchi and Watanabe (1982)
Coho salmon	1-2.5 percent linolenic acid	Yu and Sinnhuber(1979)
Common carp	1 percent linoleic acid and 1 percent linolenic acid	Watanabe et al. (1975); Takeuchi and Watanabe (1977a)
Japanese eel	0.5 percent linoleic acid and 0.5 percent linolenic acid	Takeuchi et al. (1980)
Rainbow trout	1 percent linolenic acid; 0.8 percent linolenic acid; 20 percent of lipid as linolenic acid or 10 percent of lipid as EPA and DHA	Castell et al. (1972); Watanabe et al. (1974); Takeuchi and Watanabe (1977b)
Nile tilapia	0.5 percent linoleic acid	Takeuchi et al. (1983)
Zillii's tilapia	1 percent linoleic acid or 1 percent arachidonic acid	Kanazawa et al. (1980)
Striped bass	0.5 percent of EPA and DHA	Webster and Lovell (1990)
Marine fish		
Red sea bream	0.5 percent EPA and DHA or 0.5 percent EPA	Yone et al. (1971)
Giant sea perch	1 percent EPA and DHA	Buranapanidgit et al. (1989)
Striped jack	1.7 percent EPA and DHA or 1.7 percent DHA	Watanabe et al. (1989a)
Turbot	0.8 percent EPA and DHA	Gatesoupe et al. (1977)
Marine fish	2 percent EPA and DHA	Deshimaru and Kuroki (1983)

NOTE: Linolenic acid, 18:3(n-3); EPA (eicosapentaenoic acid), 20:5(n-3); DHA (docosahexaenoic acid), 22:6(n-3); linoleic acid, 18:2 (n-6); and arachidonic acid, 20:4(n-6).

The essential fatty acids function as components of phospholipids in all biomembranes and as precursors for eicosanoids that fulfill a variety of metabolic functions. Biomembranes must be in a fluid state to function properly at various temperatures. Membrane fluidity depends on the proper balance of saturated and unsaturated fatty acids as components of membrane phospholipids (Bell et al., 1986). The role of dietary n-3 PUFA during homeoviscous regulation, whereby fish alter their biomembrane phospholipid composition in response to changes in environmental temperature, has been demonstrated by Hazel (1984). During acclimation to cold-water temperatures the total amount of phospholipid in the fish biomembrane does not change, however changes occur in the relative proportion of individual phospholipids, in the fatty acid composition of the phospholipids, and in the distribution of fatty acids within the phospholipid molecules. For example, in rainbow trout transferred from 20° to 5°C, the proportion of phosphatidylethanolamine increases with a corresponding decrease in phosphatidylcholine in both liver and gills (Hazel, 1979, 1985). Similar changes have been observed during cold adaptation in membranes of goldfish intestine (Miller et al., 1976) and carp muscle (Wodtke, 1981). Conversely, during adaptation to higher temperatures, the proportion of phosphatidylethanolamine decreases in trout gill membranes and phosphatidylcholine increases (Hazel and Carpenter, 1985). Thus changes in the ratio of the two major membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, can be used in fish as an index of proper adaptation to changes in environmental temperatures.

During cold adaptation, the relative amount of n-3 PUFA increases in trout liver membrane phospholipids, whereas the amount of saturated fatty acids decreases and the amount

of monounsaturated fatty acids remains relatively constant (Hazel, 1979; Sellner and Hazel, 1982). A large increase in 22:6 (n-3) occurs in phosphatidylcholine, whereas 20:5(n-3) instead of 22:6(n-3) increases in phosphatidylethanolamine in response to lower temperature in trout membranes (Hazel, 1979).

Practical Use of Lipids in Fish Diets

Lipids serve as an important source of dietary energy for all fish, but perhaps to a greater extent for cold-water and marine fish, which have a limited ability to use dietary carbohydrates for energy. Takeuchi et al. (1978) found that the protein content of rainbow trout diets could be reduced from 48 to 35 percent without any reduction in weight gain if the lipid concentration was increased from 15 to 20 percent. Takeda et al. (1975) were able to decrease the protein concentration in yellowtail diets from 70 to 55 percent without any reduction in growth rate by increasing the lipid content. These observations support the recommendation that all diets should be formulated not only to meet the optimum ratio of energy to protein for that species, but also to contain an adequate amount of lipid.

No definite percentage of dietary lipids can be given for fish diets without considering the type of lipid as well as the protein and energy content of the diet. Lipid concentrations of up to 20 percent give optimum results with some species (Yone et al., 1971; Stickney and Andrews, 1972; Lee and Putman, 1973; Adron et al., 1976; Takeuchi et al., 1978). However, too much dietary lipid may result in an imbalance of the DE/CP ratio and in excessive fat deposition in the visceral cavity and tissues, which would adversely affect yield, product quality, and storage. The fatty acid composition of the dietary lipid has a significant influence on the tissue fatty acid composition of the fish (Watanabe, 1982; Henderson and Tocher, 1987; Sargent et al., 1989).

The use of lipids in fish diets also requires the use of appropriate antioxidants. The proper use of antioxidants and the deleterious effects of lipid autoxidation in fish diets are discussed in Chapter 2.

CARBOHYDRATES

The nutritional value of carbohydrates varies among fish. Warm-water fish can use much greater amounts of dietary carbohydrate than cold-water and marine fish. No dietary requirement for carbohydrates has been demonstrated in fish; however, if carbohydrates are not provided in the diet, other compounds, such as protein and lipids, are catabolized for energy and for the synthesis of various biologically important compounds usually derived from carbohydrates. Thus, it is important to provide the appropriate concentration of carbohydrate in the diet of the fish species being cultured.

Enzymes for carbohydrate digestion are apparently present in fish. The enzymes for the major carbohydrate metabolic pathways, such as glycolysis, tricarboxylic acid cycle, pentose phosphate shunt, gluconeogenesis, and glycogen synthesis, have been demonstrated (Shimeno, 1974). Only a limited number of studies have reported on the kinetic properties of the various enzymes, and the results are consistent with evolutionary development. Even though the various enzymes and pathways for glucose metabolism have been detected, the role of dietary carbohydrates and the contribution of glucose to the total energy requirement of fish remain unclear. Studies have indicated that the hormonal and metabolic regulation of carbohydrate and energy metabolism varies among fish and may be somewhat different than in mammals (Shimeno, 1974; Cowey and Walton, 1989).

Utilization of Carbohydrates

The relative use of dietary carbohydrates by fish varies and appears to be associated with the complexity of the carbohydrate. Glucose, maltose, and sucrose resulted in the best growth rates, followed in descending order by dextrin and fructose, galactose and potato starch, and glucosamine when various carbohydrate sources were fed to young chinook salmon at a concentration of 10 percent of the diet (Buhler and Halver, 1961). Rainbow trout have been reported to use 30 percent glucose in a 45 percent protein diet, whereas a concentration of 30 percent glucose in a 30 percent protein diet had a negative effect on growth and feed efficiency (Bergot, 1979a,b). A similar trend has been reported for sucrose use by rainbow trout when 35 and 55 percent protein diets were compared (Luquet, 1971). Fifty-seven and 64 percent of the gross energy of glucose and sucrose, respectively, were used by rainbow trout when these carbohydrates were included at a concentration of 30 percent in a 48 percent protein diet (Pieper and Pfeffer, 1979). In another study with rainbow trout, the replacement of dietary lipid with glucose at concentrations from 2.5 to 18.3 percent in natural ingredient diets containing 40 percent CP and similar ME content resulted in a significant linear decrease in weight gain as glucose increased (Hilton and Atkinson, 1982).

Feeding high concentrations of digestible carbohydrates has been reported to result in an increase in liver size and glycogen content in salmonids (Phillips et al., 1948; Buhler and Halver, 1961; Lee and Putnam, 1973; Bergot, 1979a,b; Pieper and Pfeffer, 1979). Similar effects have been reported in red sea bream (Furuichi and Yone, 1971a), plaice (Cowey et al., 1975), and yellowtail (Shimeno et al., 1979).

The relative utilization of dietary glucose, dextrin, and gelatinized starch has been compared in carp and red sea bream. Growth and feed efficiency of carp were highest when fed the gelatinized starch diet, followed by the dextrin

and glucose diets in decreasing order, whereas red sea bream did not show any significant difference in growth rates for the various carbohydrate sources (Furuichi and Yone, 1982a). Channel catfish used dextrin or starch for growth but not mono- and disaccharides (Wilson and Poe, 1987). Chum salmon fry used glucose, maltose, sucrose, dextrin, and gelatinized starch but not fructose, galactose, or lactose for growth (Akiyama et al., 1982). White sturgeon, however, used glucose and maltose better than dextrin or starch (Hung et al., 1989).

The ability of fish to use dietary carbohydrates differs among species. Studies have indicated that common carp (Shimeno et al., 1977; Takeuchi et al., 1979; Furuichi and Yone, 1980; Shimeno et al., 1981), channel catfish (Garling and Wilson, 1976, 1977), red sea bream (Furuichi and Yone, 1971a, 1980), and tilapia (Anderson et al., 1984; El-Sayed and Garling, 1988) use higher levels than yellowtail (Furuichi and Yone, 1981) and salmonids (Buhler and Halver, 1961; Edwards et al., 1977; Atkinson and Hilton, 1981). In general, a concentration of less than 25 percent dextrin or gelatinized starch appears to be used as an energy source by rainbow trout (Lee and Putnam, 1973), plaice (Cowey et al., 1975), and yellowtail (Takeda et al., 1975), whereas channel catfish (Page and Andrews, 1973; Garling and Wilson, 1977) and common carp (Takeuchi et al., 1979) can use a higher percentage.

Oral glucose tolerance tests have been conducted with brook trout (Phillips et al., 1948), rainbow trout (Palmer and Ryman, 1972), common carp (Furuichi and Yone, 1981), channel catfish (Wilson and Poe, 1987), red sea bream (Furuichi and Yone, 1971b, 1981), and yellowtail (Furuichi and Yone, 1981). In each case, the oral administration of glucose resulted in a persistent hyperglycemia. A similar outcome was observed when rainbow trout were fed diets containing 15 and 30 percent glucose (Bergot, 1979c). Furuichi and Yone (1981) determined the change in plasma insulin levels during glucose tolerance tests in common carp, red sea bream, and yellowtail. Insulin was measured by a radioimmunoassay procedure using antiskipjack insulin serum (Furuichi et al., 1980). The plasma insulin level reached a maximum about 2 hours after oral glucose administration in each species, paralleling the level of plasma glucose. The researchers point out that the plasma insulin pattern, with respect to both the time to reach maximum level and the maximum activity, was very similar to that observed for a diabetic human.

The prolonged hyperglycemia observed in fish following glucose tolerance tests and the relative inability of fish to utilize high concentrations of dietary carbohydrates has been assumed to be the result of low levels of endogenous insulin (Palmer and Ryman, 1972: Furuichi and Yone, 1982b; Wilson and Poe, 1987). However, the development of radioimmunoassay methods for the determination of insulin levels in fish have shown that these levels are similar to or often higher than those observed in mammals (Plisetskaya, 1990; Mommsen and Plisetskaya, 1991). The relative intolerance of fish to large doses of exogenous glucose despite the high levels of circulating insulin has been suggested to resemble conditions known as non-insulin-dependent diabetes mellitus rather than insulin-dependent diabetes (Hilton et al., 1987; Hertz et al., 1989). Rainbow trout muscle tissue has been shown to have from 3 to 10 percent of the insulin receptors per microgram of protein compared with those in either the white or red skeletal muscle of rats, with the overall insulin-receptor binding capacity in trout being lower than that reported for mammals (Gutierrez et al., 1991). However, these workers could not demonstrate a difference in insulin-receptor binding in skeletal muscle of trout fed a high-carbohydrate diet as compared to those fed a low-carbohydrate diet. Thus, they concluded that the high glycemic levels observed in trout fed the high-carbohydrate diet were not due to impaired binding of insulin to its receptors in skeletal muscle. It is apparent from this latest information that the hyperglycemia in fish fed high concentrations of carbohydrates is not solely due to impaired insulin release or receptor binding as previously thought.

Value of Carbohydrates in Fish Diets

Although no specific carbohydrate requirement has been established for fish, some form of digestible carbohydrate should be included in the diet. For example, the growth rate of channel catfish fingerlings was greater when their diets contained some carbohydrates rather than only lipids for all the nonprotein energy (Garling and Wilson, 1977). Carbohydrates may serve as precursors for the dispensable amino acids and nucleic acids, which are metabolic intermediates necessary for growth. Because carbohydrate is the least expensive source of dietary energy, the maximum tolerable concentration should be used with regard to the fish species. Cereal grains serve as inexpensive sources of carbohydrates for warm-water fish, but their use in cold-water fish feeds is limited. Starch is also important for the binding properties of extruded and pelleted feeds.

MINERALS

Fish, unlike most terrestrial animals, can absorb some minerals (inorganic elements) not only from their diets but also from their external aquatic environment. Calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), zinc (Zn), copper (Cu), and selenium (Se) are generally derived from the water to satisfy part of the nutritional requirements of fish. Phosphates and sulfates, however, are more effectively obtained from feed sources (as reviewed by Lall, 1989). Inorganic elements are required for the normal life processes of fish. Their main functions include the

formation of skeletal structure, electron transfer, regulation of acid-base equilibrium, and osmoregulation. Minerals are also important components of hormones and enzymes, and they activate enzymes. Complex biochemical mechanisms control and regulate the uptake, storage, and excretion of various inorganic elements, allowing fish to live in a dynamic equilibrium with their aquatic medium. The electrolytes Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and HCO_3^- play a major role in the osmotic and ionic regulation of extra- and intracellular fluids in fish.

The exchange of ions from the surrounding water across the gills and skin of fish complicates the measurement of mineral requirements. Although most essential elements known for terrestrial animals are also considered important for fish, quantitative requirements have been reported for only nine minerals (calcium, phosphorus, magnesium, iron, copper, manganese, zinc, selenium, and iodine) for selected fish species. The role of macrominerals (calcium, phosphorus, magnesium, sodium, potassium, and chlorine) and trace elements (iron, copper, zinc, manganese, selenium, iodine, fluorine, and chromium) in fish nutrition are discussed in this section and sources of the minerals are given in Table 8-3.

Calcium and Phosphorus

Calcium and phosphorus are directly involved in the development and maintenance of the skeletal system and participate in several physiological processes. Fish scales are also an important site of calcium metabolism and deposition. In addition to its structural functions, calcium plays an important role in muscle contraction, blood clot formation, nerve impulse transmission, the maintenance of cell integrity and acid-base equilibrium, and activation of several important enzymes.

Fish absorb calcium directly from their environment (Phillips et al., 1959) and rely entirely on calcium present in water during dietary calcium deprivation (Ogino and Takeda, 1976,1978; Ichii and Mugiya, 1983). The uptake of calcium occurs through gills, fins and oral epithelia, however gills are considered the most important site for calcium regulation. In marine fish, which drink copiously, the gut is not a major site of calcium absorption (Simkiss, 1974). Limited calcium homeostasis occurs in bone, which is acellular (bone without enclosed osteocytes) in most fish (Moss, 1963). There is little exchange of bone calcium with body fluids in marine fish (Simmons et al., 1970); but in a low-calcium environment, like 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). Decalcification of scales and bones occurs during ovarian maturation, starvation, and spawning migration (Crichton, 1935; Ichikawa, 1953; Yamada, 1956; Garrod and Newell, 1958; Mugiya and Watabe, 1977). The calcium exchange rate of fish scales is three times that of bones (Berg, 1968). Although fish bone is responsive to calcium-regulating hormones, sufficient evidence does not exist to suggest that bone has a major role in blood calcium regulation.

The calcium requirement of fish is met in large part by absorption through gills and skin in fresh water and by drinking sea water. The calcium requirement is affected by the water chemistry and species differences. The concentration of dietary calcium rarely seems critical for salmonids, and a dietary requirement has not been demonstrated. A low concentration of calcium (0.34 percent or less) is required in the diet of carp and eel (Ogino and Takeda, 1976) for optimum growth. Catfish and tilapia reared in calcium-free water require 0.45 percent and 0.7 percent calcium in the diet, respectively, for optimum growth (Robinson et al., 1986, 1987). Atlantic salmon derive calcium from sea water, thus making dietary supplementation unnecessary (Lall and Bishop, 1977). The uptake of calcium from sea water is not sufficient to meet the calcium requirement of red sea bream (Sakamoto and Yone, 1973, 1976b), which requires 0.34 percent calcium in the diet.

Calcium deficiency has not been detected in carp and catfish in fresh water (Andrews et al., 1973; Ogino and Takeda, 1976; Lovell, 1978) or in Atlantic salmon in sea water (Lall and Bishop, 1977). Generally, calcium from the feed ingredients of natural ingredient diets supplies sufficient calcium to meet the requirements of most finfish.

Phosphorus is an important constituent of nucleic acids and cell membranes, and is directly involved in all energy-producing cellular reactions. The role of phosphorus in carbohydrate, lipid, and amino acid metabolism, as well as in various metabolic processes involving buffers in body fluids, is also well established. Feed is the main source of phosphate for fish because the concentration of phosphate is low in natural waters. The dietary supply of phosphate is more critical than that of calcium because fish must effectively absorb, store, mobilize, and conserve phosphate in both freshwater and sea water environments.

In most fish, the main phosphorus deficiency signs include poor growth, feed efficiency, and bone mineralization. Other signs of deficiency in carp include increase in the activity of certain gluconeogenic enzymes in liver, increase in carcass fat with decrease in carcass water content, reduced blood phosphate levels, deformed head, and deformed vertebrae (Ogino and Takeda, 1976; Onishi et al., 1981; Takeuchi and Nakazoe, 1981). A reduction in hematocrit level of catfish may also occur (Andrews et al., 1973). A low-phosphorus intake by red sea bream also causes curved, enlarged vertebrae; increased serum alkaline phosphatase activity; higher lipid deposition in muscle, liver, and vertebrae; and reduction in liver glycogen content (Sakamoto and Yone, 1980).

Dietary phosphorus requirements ranging from 0.5 to 0.8 percent have been reported for rainbow trout (Ogino and

Takeda, 1978), Atlantic salmon (Ketola, 1975; Lall and Bishop, 1977), chum salmon (Watanabe et al., 1980), carp (Ogino and Takeda, 1976), and red sea bream (Sakamoto and Yone, 1978a). The phosphorus requirement of Atlantic salmon in either fresh water (Ketola, 1975) or sea water (Lall and Bishop, 1977) is 0.6 percent of the diet. Andrews et al. (1973) reported the phosphorus requirement of catfish is 0.8 percent of available phosphorus in a natural ingredient diet. Lovell (1978) and Wilson et al. (1982) reevaluated the phosphorus requirement using chemically defined diets and estimated it to be approximately 0.42 percent available phosphorus. The phosphorus requirement of Japanese eel is relatively low (0.29 percent) compared with that of other finfish (Nose and Arai, 1979). Several workers have reported that the concentration of dietary calcium has no effect on phosphorus requirement of catfish, carp, and rainbow trout (Andrews et al., 1973; Nose and Arai, 1976; Lall and Bishop, 1977; Lovell, 1978; Ogino and Takeda, 1978). However, optimum Ca:P ratio is important in the diet of red sea bream, 1:2 (Sakamoto and Yone, 1973), and eel, 1:1 (Nose and Arai, 1979).

Wide differences in the availability of phosphorus from different sources have been reported. In general, the more soluble the salt, the higher the availability of phosphorus for fish. Thus, the phosphorus of monocalcium or dicalcium phosphates is more readily available than that from tricalcium phosphate (Ogino et al., 1979; Sakamoto and Yone, 1979a). The availability of phosphorus in fishmeal for tilapia is low compared to that for rainbow trout and chum salmon (Watanabe et al., 1980). Also, the availability of phosphorus in fishmeal is significantly lower for carp than for rainbow trout (Ogino et al., 1979). 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 warm-water species (Ogino et al., 1979; Yone and Toshima, 1979). About 60 percent of the phosphorus in anchovy and menhaden fishmeals was available to channel catfish (Lovell, 1978).

Feedstuffs that originate from seeds contain phosphorus primarily as the calcium-magnesium salt of phytic acid known as phytin. Phytin phosphorus is unavailable to animals with simple stomachs because they lack the enzyme phytase in the gastrointestinal tract. Phytic acid also forms insoluble salts with free calcium in the digestive tract. Hence, the availability of phosphorus in most plant products is low; for example, that of soybean meal is between 29 and 54 percent (Lovell, 1978; Wilson et al., 1982).

Magnesium

Magnesium is an essential cofactor in many enzymatic reactions in intermediary metabolism. These enzymes include phosphokinases, thiokinases, phosphatases, pyrophosphatases, and amino acyl synthetases. Magnesium plays an important role in the respiratory adaptation of freshwater fish (Houston, 1985). It is also required in skeletal tissue metabolism, osmoregulation, and neuromuscular transmission. The quantitative dietary magnesium requirements of rainbow trout (Ogino et al., 1978; Knox et al., 1981a; Shearer, 1989), carp (Ogino and Chiou, 1976), channel catfish (Gatlin et al., 1982), eel (Nose and Arai, 1979), and guppy (Shim and Ng, 1988) have been estimated to range from 0.04 to 0.06 percent of the diet. A dietary magnesium content of 0.06 to 0.08 percent was required for tilapia (Dabrowska et al., 1989).

The magnesium requirement of fish can be met either from the diet or water. Shearer and Asgard (1992) found that in fresh water, a waterborne concentration of 46 mg per liter was sufficient to meet the magnesium requirement of rainbow trout fed a magnesium-free diet. In the marine environment, magnesium supplementation of diet may not be necessary (Lall and Bishop, 1977; Sakamoto and Yone, 1979b). The magnesium requirement of rainbow trout was not influenced by an increase in dietary calcium or phosphorus (Knox et al., 1981a). Inorganic magnesium salts are efficiently used by rainbow trout with an apparent magnesium retention of 76 percent from these compounds, but magnesium retention from the bone fraction of fishmeal is only 54 percent (Shearer and Asgard, 1990).

Magnesium deficiency causes anorexia, reduced growth, lethargy, and reduced tissue magnesium content in fish. In rainbow trout, magnesium deficiency also caused calcinosis of the kidney, vertebrae deformity, and degeneration of muscle fibers and epithelial cells of the pyloric cecum and gill filaments (Cowey et al., 1977; Ogino et al., 1978). Catfish and rainbow trout fed magnesium-deficient diets show flaccid appearance of their muscle (Knox et al., 1981a; Gatlin et al., 1982). Carp maintained on a low-magnesium diet also develop convulsions and cataracts (Ogino and Chiou, 1976). Magnesium deficiency has not been demonstrated in fish in a sea water environment, where they obtain magnesium by drinking the water. An interaction between dietary protein and magnesium concentrations has been demonstrated in tilapia (Dabrowska et al., 1989), where excess magnesium (0.32 percent) in a low-protein (24 percent) diet produced toxicity signs and where magnesium deficiency in a high-protein (44 percent) diet caused whole-body hypercalcinosis.

Sodium, Potassium, and Chlorine

Sodium, potassium, and chlorine are the most abundant electrolytes in the body. Sodium and chlorine are the principal cation and anion, respectively, in the extracellular fluid of the body; whereas, potassium is the major monovalent intracellular cation. Chloride ion is the major anion of gastric juice and blood. The deficiency signs of these elements are difficult to produce because fish readily absorb these elements from the surrounding aquatic medium.

The abundance of these elements in common feedstuffs used in fish diets means they need not be supplemented in most natural ingredient diets. However, potassium supplementation was found necessary in chemically defined diets for chinook salmon. Juvenile chinook salmon reared in fresh water required 0.8 percent potassium in their diet for maximum growth, and the whole-body potassium saturation was reached at a potassium concentration between 0.6 and 1.2 percent of the diet (Shearer, 1988). Red sea bream reared in sea water, where potassium concentration is much higher, did not require a dietary potassium supplement (Sakamoto and Yone, 1978b). The signs of potassium deficiency in chinook salmon included anorexia, convulsions, tetany, and death (Shearer, 1988).

Supplements of 1 to 4 percent of sodium chloride in natural ingredient diets had no beneficial effect on growth of rainbow trout (Salman and Eddy, 1988), coho salmon (Zaugg and McLain, 1969), Atlantic salmon (Basulto, 1976), channel catfish (Murray and Andrews, 1979), or red sea bream (Sakamoto and Yone, 1978b). However, higher supplements of salt adversely affected growth and feed efficiency of coho salmon and rainbow trout (Zaugg and McLain, 1969; Salman and Eddy, 1988). Atlantic salmon and coho salmon fed salt-enriched diets adapted well to seawater, with few mortalities (Zaugg and McLain, 1969; Basulto, 1976). The Na^+- and K^+-stimulated ATPase activity of gill microsomes is elevated by dietary salt supplementation, thus making saltwater adaptation easier physiologically (Zaugg and McLain, 1969).

Iron

Iron is an essential element in the cellular respiratory process through its oxidation-reduction activity and electron transfer. It is found in the body mainly in the complex form bound to proteins, such as heme compounds (hemoglobin and myoglobin), heme enzymes (cytochromes, catalase, peroxidase, and so on), and nonheme compounds (transferrin, ferritin, and iron-containing flavoproteins [ferredoxins, dehydrogenases]). Feed is considered the major source of iron for fish because natural waters usually contain low amounts of soluble iron. Fish can absorb soluble iron from the water through the gills because the addition of ferrous sulfate to water enhanced growth and hemoglobin level in sword tail and platyfish (Roeder and Roeder, 1966). Iron was absorbed from the peritoneal cavity in rainbow trout and stored in the liver, spleen, and anterior kidney (Walker and Fromm, 1976).

The iron requirements have been reported for catfish (Gatlin and Wilson, 1986a), Atlantic salmon (Lall and Hines, 1987), and eel (Nose and Arai, 1979), and they are 30, 60, and 170 mg/kg of diet, respectively. Iron deficiency causes characteristic microcytic anemia in brook trout (Kawatsu, 1972), red sea bream (Sakamoto and Yone, 1976a, 1978c), yellowtail (Ikeda et al., 1973), eel (Nose and Arai, 1979), and carp (Sakamoto and Yone, 1978d). In most cases, growth was not influenced by iron deficiency. The normal liver color changed to yellowish-white during iron deficiency in carp (Sakamoto and Yone, 1978d). In catfish, iron deficiency suppressed hematocrit, hemoglobin, and plasma iron concentrations and transferrin saturation (Gatlin and Wilson, 1986a). Sakamoto and Yone (1979c) found that ferrous chloride and ferrous sulfate were equally effective in preventing anemia in red sea bream; however, a somewhat higher concentration of ferric citrate was required. Dietary iron toxicity signs develop in rainbow trout fed more than 1,380 mg Fe/kg (Desjardins et al., 1987). The major effects of iron toxicity include reduced growth, increased mortality, diarrhea, and histopathological damage to liver cells.

Copper

Copper is a constituent of many enzymes and is essential for their activities. It is associated with cytochrome *c* oxidase of the electron transport chain in the cell. Other cuproenzymes found in fish tissues include superoxide dismutase, tyrosinase, lysyl oxidase, ceruloplasmin, and dopamine β-hydroxylase. Although Syed and Coombs (1982) found that the distribution of copper and copper-dependent enzymes is similar in plaice and mammals, copper metabolism of most fish is poorly defined. High concentrations of copper are found in the heart, liver, brain, and eyes. Copper is present as the copper-protein complex, ceruloplasmin, in plasma.

The dietary copper requirements of selected fish species have been reported: rainbow trout and common carp require 3 mg/kg (Ogino and Yang, 1980); channel catfish require 5 mg/kg (Gatlin and Wilson, 1986b); and Atlantic salmon require 5 mg/kg (Lall and Hines, 1987). Gatlin and Wilson (1986b) observed reduced heart cytochrome *c* oxidase and liver copper-zinc superoxide dismutase activities in copper-deficient catfish. Carp fed diets containing high-ash fishmeal without copper supplement showed reduced growth and cataract formation (Satoh et al., 1983a). A low concentration of copper was found in Atlantic salmon suffering from Hitra disease (Poppe et al., 1986), which is a cold-water bacterial disease caused by *Vibro salmonicida*.

Fish appear to be more tolerant of copper in the diet than of dissolved copper in the water. Concentrations of 0.8 to 1.0 mg copper per liter as copper sulfate in water are toxic to many species of fish (Friedman and Shibko, 1972). Ashley (1972), however, found that coho salmon tolerated copper at 1,000 mg/kg in the diet with only retarded growth and impaired pigmentation. Knox et al. (1982) found no deleterious effects of feeding rainbow trout diets containing 150 mg copper/kg for 20 weeks.

Copper toxicity has been experimentally produced in rainbow trout fed 730 mg Cu/kg of diet for 24 weeks (Lanno et al., 1985). The toxicity signs include reduced growth and

feed efficiency and elevated liver copper levels. However, a diet with up to 665 mg Cu/kg did not produce toxicity signs (Knox et al., 1982; Lanno et al., 1985). Wild and cultured salmonids accumulate exceptionally high copper levels in the liver without being exposed to elevated waterborne or dietary copper (Poppe, 1986).

Zinc

The essential function of zine for living organisms is based on its role as an integral part of a number (more than 70) of metalloenzymes, including dehydrogenases, aldolases, peptidases, and phosphatases. Fish accumulate zinc from both water and dietary sources; however, dietary zinc is more efficiently absorbed than waterborne zinc. The gills in rainbow trout play a major role in excretion of zinc (Hardy et al., 1987).

The zinc requirement of young rainbow trout and carp is 15 to 30 mg/kg of diet (Ogino and Yang, 1978, 1979), whereas channel catfish and blue tilapia require 20 mg/kg of diet (Gatlin and Wilson, 1983; McClain and Gatlin, 1988). Dietary calcium and phosphorus concentrations, presence of phytic acid, protein source, and form of zinc and calcium affect zinc absorption and use in fish (Takeda and Shimma, 1977; Gatlin and Wilson, 1984a; Hardy and Shearer, 1985; Richardson et al., 1985; Wekell et al., 1986; Satoh et al., 1987; McClain and Gatlin, 1988; Satoh et al., 1989). Phytate forms a complex with transitional elements, such as zinc, iron, and manganese, in the gastrointestinal tract and prevents their absorption. Calcium promotes the complexing of zinc to phytates. The bioavailability of zinc in fishmeal is inversely related to the tricalcium phosphate content (Satoh et al., 1987). This is presumably caused by absorption of zinc into insoluble calcium phosphate complexes in the intestine that are passed through the gut unabsorbed and excreted. Higher supplements of zinc should be included in natural ingredient fish diets to compensate for reduced zinc bioavailability caused by dietary phytate and tricalcium phosphate.

Rainbow trout and common carp tolerated 1,700 to 1,900 mg Zn/kg of diet without adverse effect on growth or survival (Jeng and Sun, 1981; Wekell et al., 1983). However, Knox et al. (1982, 1984) fed rainbow trout elevated concentrations of zinc to 1,000 mg/kg diet and observed reduced hemoglobin, hematocrit, and hepatic copper concentrations. Copper status of channel catfish was not impaired by diets containing 200 mg Zn/kg (Gatlin et al., 1989).

In rainbow trout, zinc deficiency caused growth suppression, mortality, lens cataracts, erosion of fins and skin, and short-body dwarfism (Ogino and Yang, 1979; Satoh et al., 1983b). High-ash (white) fishmeal may affect zinc absorption and use, resulting in lens cataracts (Ketola, 1979). When zinc supplements (40 mg/kg) were added to rainbow trout diets containing white fishmeal, dwarfism and cataract problems were alleviated (Satoh et al., 1987).

Caudal fin zinc concentration is a good indicator of zinc status in rainbow trout (Wekell et al., 1986). In channel catfish, diets low in zinc reduced growth rate, appetite, and bone zinc, calcium, and serum zinc concentrations (Gatlin and Wilson, 1983). Broodstock diets low in zinc reduced egg production and hatchability (Takeuchi et al., 1981).

Manganese

Manganese functions either as a cofactor that activates metal-enzyme complexes or as an integral part of certain metalloenzymes in carbohydrate, lipid, and protein metabolism. Many kinases, transferases, hydrolases, and decarboxylases can be activated by either manganese or other divalent cations, such as magnesium, and their activity is not specific for manganese. However, enzymes such as glycosyl transferase are highly specific for manganese activation. Two important manganese metalloenzymes are pyruvate carboxylase and superoxide dismutase.

Although the uptake of manganese from water has been demonstrated (Miller et al., 1980; Srivastava and Agarwal, 1983), it is more efficiently absorbed from feed. The manganese requirements have been demonstrated for channel catfish, 2.4 mg/kg (Gatlin and Wilson, 1984b), and common carp and rainbow trout, 13 mg/kg (Ogino and Yang, 1980).

Manganese deficiency caused reduced growth and skeletal abnormalities in rainbow trout, carp, and tilapia (Ishak and Dollar, 1968; Ogino and Yang, 1980; Yamamoto et al., 1983). In rainbow trout, low manganese intake both decreased the activities of copper-zinc superoxide dismutase and manganese-superoxide dismutase in cardiac muscle and liver and suppressed manganese and calcium concentrations of the vertebrae (Knox et al., 1981b). Liver manganese-superoxide dismutase activity was not influenced in catfish fed a diet containing 2.4 mg Mn/kg of diet (Gatlin and Wilson, 1984b). In broodstock rainbow trout, a fishmeal-based diet without manganese supplement caused poor hatchability and low manganese concentration in the eggs (Takeuchi et al., 1981).

Selenium

Selenium is an integral part of the enzyme glutathione peroxidase (Rostruck et al., 1973). This enzyme can reduce hydrogen peroxide and fatty acyl hydroperoxides in water and fatty acyl alcohols, respectively, thereby protecting cells and membranes against peroxide damage.

Selenium deficiency causes growth depression in rainbow trout (Hilton et al., 1980) and catfish (Gatlin and Wilson, 1984c), but selenium deprivation alone does not produce pathological signs in these fish. Both selenium and vitamin E are required to prevent muscular dystrophy in Atlantic salmon (Poston et al., 1976) and exudative diathesis

in rainbow trout (Bell et al., 1985). Glutathione peroxidase activity in plasma and liver is a useful index of selenium status in fish (Poston et al., 1976; Hilton et al., 1980: Gatlin and Wilson, 1984c; Bell et al., 1985, 1986, 1987).

The selenium requirement of fish varies with the form of selenium ingested, polyunsaturated fatty acid and vitamin E content of the diet, and concentration of waterborne selenium. The selenium requirement determined on the basis of optimum growth and maximum plasma glutathione peroxidase activity was estimated to be 0.15 to 0.38 mg Se/kg diet for rainbow trout (Hilton et al., 1980) and 0.25 mg Se/kg for channel catfish (Gatlin and Wilson, 1984c). The biological availability of selenium differs in various selenium compounds and feed supplements. Bell and Cowey (1989) reported that selenium present in fishmeal has low digestibility, whereas selenomethionine is highly digestible. A low concentration of selenium is found extensively in aquatic ecosystems. The uptake of selenium across gills is very efficient at low-waterborne concentrations (Hodson and Hilton, 1983).

Selenium toxicity occurred in rainbow trout and catfish when dietary selenium exceeded 13 and 15 mg/kg dry feed, respectively (Hilton et al., 1980; Gatlin and Wilson, 1984c). Reduced growth, poor feed efficiency, and high mortality were the major effects. Trout reared on high-selenium diets (10 mg/kg) also showed renal calcinosis (Hilton and Hodson, 1983).

Iodine

Iodine is essential for the biosynthesis of the thyroid hormones, thyroxine and triiodothyronine. Fish obtain iodine from water via branchial pumps and from feed sources (Leloup, 1970). The total uptake of iodine depends on the iodine content of the feed and water (Gregory and Eales, 1975). Under laboratory conditions, rainbow trout derive 80 percent of their iodide from water, 19 percent from diet, and less than 1 percent by recycling iodide from thyroid hormone degradation (Hunt and Eales, 1979).

Iodine deficiency caused thyroid hyperplasia in brook trout (Marine, 1914). Thyroid hormone deficiency has been induced by glucosinolates in the diet (Higgs and Eales, 1978). A deficiency of ascorbic acid caused hypoactivity of the thyroid gland as demonstrated by a reduction in accumulation of ^{131}I by thyroid glands in scorbutic snakehead (Agrawal and Mahajan, 1981). The minimum iodine requirement of most fish species has not been established. Woodall and LaRoche (1964) reported higher iodine requirements for advanced chinook salmon parr compared with fingerlings due to increased thyroid activity during smoltification. Lall et al. (1985) observed that relatively high concentrations of iodine and fluorine (4.5 mg/kg of diet of each) were essential to protect Atlantic salmon from bacterial kidney disease infections.

Other Trace Elements

Information on the dietary requirements of other trace elements is limited. Increased dietary fluoride enhanced fluoride accumulation in the vertebrae of rainbow trout (Tiews et al., 1982; Bowser et al., 1988). Evidence also indicated that elevated concentrations of fluoride may reduce the prevalence of bacterial kidney disease (Lall et al., 1985; Bowser et al., 1988). The importance of chromium and other trace elements essential for other animals and humans is also recognized in fish nutrition, although the effects of their deficiencies have not been reported. Tacon and Beveridge (1982) found that rainbow trout fed a low-chromium chemically defined diet did not show deficiency signs or change in tissue chromium distribution.

VITAMINS

Vitamins are organic compounds, distinct from amino acids, carbohydrates, and lipids, that are required in trace amounts from an exogenous source (usually the diet) for normal growth, reproduction, and health. Vitamins are classified as water-soluble and fat soluble. Eight of the water-soluble vitamins are required in relatively small amounts, have primarily coenzyme functions, and are known as the vitamin B complex. Three of the water-soluble vitamins, choline, inositol, and vitamin C, are required in larger quantities and have functions other than coenzymes. Vitamins A, D, E, and K are the fat soluble vitamins that function independent of enzymes or, in some cases such as vitamin K, may have coenzyme roles. In mammals the absence of vitamins leads to characteristic deficiency diseases, but in fish such diseases are less specifically identified.

Some vitamins may be synthesized from other essential nutrients to spare a portion of the dietary requirement. For example, channel catfish appear to synthesize choline if adequate methyl donors such as methionine are present in the diet; however, if the concentration of dietary methionine is limiting, a choline requirement can be demonstrated (Wilson and Poe, 1988). An exogenous source of some water-soluble vitamins for certain warm-water fish has been shown to be derived from microorganisms in the gastrointestinal tract (Limsuwan and Lovell, 1981; Lovell and Limsuwan, 1982; Burtle and Lovell, 1989). In cold-water carnivorous fish microorganisms are not a significant source of vitamins (Hepher, 1988).

Both qualitative and quantitative vitamin requirements of fish have been determined by feeding chemically defined diets deficient in a specific vitamin. The quantitative requirements for most of the vitamins have been established for chinook salmon, rainbow trout, common carp, channel catfish, and yellowtail, while only some of the requirements are known for red sea bream and tilapia. Qualitative requirements

have been identified in several other species. The requirements are affected by size, age, and growth rates as well as by various environmental factors and nutrient interrelationships. Thus, different researchers have reported fairly wide ranges in requirement values for growth in the same species (see Table 1-15). Recent studies with spring chinook salmon indicate that the dietary requirements for certain vitamins may be lower than previously reported for this species (Leith et al., 1990). In addition, the requirement values listed in Table 1-15, as determined by maximum liver storage or based on certain enzyme data, are often much higher than the requirement values based on weight gain and absence of deficiency signs; therefore, professional judgment must be used in selecting which requirement value best fits the user's needs. Thus, more studies are needed to refine the requirements for various species for normal growth, health, and enhancement of defense mechanisms, as suggested by Ikeda (1985). A summary of vitamin deficiency signs reported in several cultured fishes are presented in Appendix Table A-3. Further information on vitamin nutrition research in fishes is discussed by Halver (1989).

Fat-Soluble Vitamins

The fat-soluble vitamins, A, D, E, and K, are absorbed in the intestine along with dietary fats; therefore, conditions favorable for fat absorption also enhance the absorption of fat-soluble vitamins. Fat-soluble 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 with trout for vitamins A, D, and E, but it is unlikely to occur under practical conditions (Poston et al., 1966; Poston, 1969a; Poston and Livingston, 1969).

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.

VITAMIN A

Vitamin A is required in vertebrates for the regeneration of the light-sensitive compound rhodopsin in the retina of the eye. Vitamin A has also been shown to be essential for proper growth, reproduction, resistance to infection, and the maintenance of differentiated epithelia and mucus secretions. Blomhoff et al. (1992) have presented a recent review of metabolic functions of vitamin A in vertebrates.

Vitamin A occurs in three forms: as an alcohol (retinol), an aldehyde (retinal), and an acid (retinoic acid). Vitamin A_1 (retinol) is found in mammals and marine fishes, whereas both vitamin A_1 and vitamin A_2 (3-dehydroretinol) are found in freshwater fishes (Braekkan et al., 1969; Lee, 1987). In freshwater fish, the oxidative conversion of retinol to 3-dehydroretinol occurs (Goswami, 1984) as well as the reversible oxidation and reduction reactions of retinol to retinal and of 3-dehydroretinol to 3-dehydroretinal (Wald, 1945-1946). For example, tilapia has been shown to convert dietary retinol into 3-dehydroretinol and retinal into 3-dehydroretinal (Katsuyama and Matsuno, 1988). Channel catfish were found to convert β-carotene to vitamin A_1 and A_2 in about a 1:1 ratio (Lee, 1987).

Cold-water fish can use β-carotene as a vitamin A precursor (Poston et al., 1977). Dupree (1970) found that channel catfish could use β-carotene as a vitamin A source only if the dietary concentration exceeded 2,000 international units per kilogram (IU/kg). It has recently been shown that β-carotene and canthaxanthin can be biotransformed in the liver of tilapia into vitamin A_1 and that dihydroxycarotenoids such as astaxanthin, zeaxanthin, lutein, and tunaxanthin were directly bioconverted into vitamin A_2 (Katsuyama and Matsuno, 1988). In mammals, carotenoids have been found to fulfill various biological functions independent of vitamin A (Olson, 1989). Thus, more studies are needed on the metabolic role of carotenoids in fish and on the possibility that carotenoids serve as a provitamin A.

Vitamin A deficiency in rainbow trout causes anemia, twisted gill opercula, and hemorrhages in the eyes and base of fins (Kitamura et al., 1967a). Brook trout exhibited poor growth, high mortality, and eye lesions, such as edematous eyes, displaced lens, and degeneration of the retina, when fed a vitamin A-deficient, purified diet from first feeding (Poston et al., 1977). Channel catfish fed 0.4 mg of β-carotene/kg of diet for 3 years developed exophthalmia, edema, and hemorrhagic kidney (Dupree, 1966). Anorexia, pale body color, hemorrhagic skin and fins, exophthalmia, and twisted gill opercula occurred in common carp fed a vitamin A-deficient diet after 8 to 11 weeks (Aoe et al., 1968). Rapidly growing yellowtail fingerlings fed a vitamin A-deficient diet developed deficiency signs in 20 days including arrested growth of gill opercula, dark pigmentation, anemia, and hemorrhage in the eyes and liver, accompanied by high mortality (Hosokawa, 1989).

High dietary intake (2.2 million IU/kg diet) of retinyl palmitate caused slow growth, anemia, and severe necrosis of the caudal fin of brook trout at 8.3°C (Poston et al., 1966). Feeding up to 2.5 million IU retinyl palmitate to trout at 12.4°C also reduced body fat and liver size (Poston, 1971a). A high intake of dietary protein (Poston and Livingston, 1971) or methionine (Eckhert and Kemmerer, 1974) by young trout reduced the toxicity of excess dietary vitamin A observed in fish fed a low-protein diet.

TABLE 1-15 Vitamin Requirements for Growing Fish Determined with Chemically Defined Diets in a Controlled Environment

Vitamin and Fish	Requirement (units/kg diet)	Response Criteria	Reference
Vitamin A			
Pacific salmon	R		Halver (1972)
Rainbow trout	2,500 IU	WG, ADS	Kitamura et al. (1967a)
Channel catfish	1,000-2,000 IU	WG	Dupree (1970)
Common carp	4,000-20,000 IU	WG, MLS	Aoe et al. (1968)
Yellowtail	5.68 mg	WG, MLS	Shimeno (1991)
Vitamin D			
Pacific salmon	NR		Halver (1972)
Rainbow trout	1,600-2,400 IU	WG, FE	Barnett et al. (1982a)
Channel catfish	500 IU	WG	Lovell and Li (1978)
	1,000 IU	WG	Andrews et al. (1980)
	250 IU	WG	Brown (1988)
Yellowtail	NR		Shimeno (1991)
Vitamin E			
Atlantic salmon	35 mg	WG, ADS	Lall et al. (1988)
Pacific salmon	30 IU	WG, ADS	Woodall et al. (1964)
	40-50 mg	WG, MLS	Halver (1972)
Rainbow trout	30 IU	WG, ADS	Woodall et al. (1964)
	25 mg	WG, ADS	Hung et al. (1980)
	100 mg	MLS	Watanabe et al. (1981b)
	50 mg	AASLP	Cowey et al. (1983)
Channel catfish	25 mg	WG, ADS	Murai and Andrews (1974)
	50 mg	AASLP	Wilson et al. (1984)
Common carp	100 mg	WG, ADS	Watanabe et al. (1970b)
Yellowtail	119 mg	MLS	Shimeno (1991)
Blue tilapia	25 mg	WG	Roem et al. (1990)
Nile tilapia	50-100 mg	WG, ADS	Satoh et al. (1987)
Vitamin K			
Pacific salmon	R		Halver (1972)
Lake trout	0.5-1 mg	NHV	Poston (1976a)
Channel catfish	R		Dupree (1966)
	NR		Murai and Andrews (1977)
Yellowtail	NR		Shimeno (1991)
Thiamin			
Pacific salmon	10-15 mg	MLS	Halver (1972)
Rainbow trout	1-10 mg	WG, ADS	McLaren et al. (1947)
	1 mg	WG, ED	Morito et al. (1986)
Channel catfish	1 mg	WG, ADS	Murai and Andrews (1978b)
Common carp	0.5 mg	WG, ADS	Aoe et al. (1969)
Yellowtail	11.2 mg	MLS	Shimeno (1991)
Riboflavin			
Pacific salmon	20-25 mg	MLS	Halver (1972)
	7 mg	WG, ADS	Leith et al. (1990)
Rainbow trout	5-15 mg	WG, ADS	McLaren et al. (1947)
	6 mg	MLS	Takeuchi et al. (1980)
	3 mg	ED	Hughes et al. (1981a)
	2.7 mg	MLS, ED	Amezaga and Knox (1990)
Channel catfish	9 mg	WG, ADS	Murai and Andrews (1978a)
Common carp	4 mg	WG, ADS	Aoe et al. (1967c)
	6.2 mg	MLS	Aoe et al. (1967c)
	7 mg	MLS	Takeuchi et al. (1980)
Yellowtail	11 mg	MLS	Shimeno (1991)
Blue tilapia	6 mg	WG, ADS	Soliman and Wilson (1992b)

Vitamin and Fish	Requirement (units/kg diet)	Response Criteria	Reference
Vitamin B_6			
Atlantic salmon	5 mg	WG, ADS	Lall and Weerakoon (1990)
Pacific salmon	10-20 mg	MLS	Halver (1972)
	6 mg	WG, ADS	Leith et al. (1990)
Rainbow trout	1-10 mg	WG, ADS	McLaren et al. (1947)
	2 mg	WG, ADS	Woodward (1990)
	3-6 mg	ED	Woodward (1990)
Channel catfish	3 mg	WG, ADS	Andrews and Murai (1979)
Common carp	5-6 mg	WG, ADS	Ogino (1965)
Yellowtail	11.7 mg	MLS	Shimeno (1991)
Pantothenic acid			
Pacific salmon	40-50 mg	MLS	Halver (1972)
	17 mg	WG, ADS	Leith et al. (1990)
Rainbow trout	10-20 mg	WG, ADS	McLaren et al. (1947)
	20 mg	WG, ADS	Cho and Woodward (1990)
Channel catfish	10 mg	WG, ADS	Murai and Andrews (1979)
	15 mg	WG, ADS	Wilson et al. (1983)
Common carp	30-50 mg	WG, ADS	Ogino (1967)
Yellowtail	35.9 mg	MLS	Shimeno (1991)
Blue tilapia	10 mg	WG, ADS	Soliman and Wilson (1992a)
Niacin			
Pacific salmon	150-200 mg	MLS	Halver (1972)
Rainbow trout	1-5 mg	WG, ADS	McLaren et al. (1947)
	10 mg	WG, ADS	Poston and Wolfe (1985)
Channel catfish	14 mg	WG, ADS	Andrews and Murai (1978)
Common carp	28 mg	WG, ADS	Aoe et al. (1967b)
Yellowtail	12 mg	MLS	Shimeno (1991)
Biotin			
Pacific salmon	1-1.5 mg	MLS	Halver (1972)
Rainbow trout	0.05-0.25 mg	WG, ADS	McLaren et al. (1947)
	0.08 mg	WG, ADS	Woodward and Frigg (1989)
	0.14 mg	ED	Woodward and Frigg (1989)
Lake trout	0.1 mg	WG, ADS	Poston (1976b)
	0.5-1 mg	OSS	Poston (1976b)
Channel catfish	R		Robinson and Lovell (1978)
Common carp	1 mg	WG, ADS	Ogino et al. (1970b)
Yellowtail	0.67 mg	MLS	Shimeno (1991)
Vitamin B_{12}			
Pacific salmon	0.015-0.02 mg	MLS	Halver (1972)
Rainbow trout	R		Phillips et al. (1964)
Channel catfish	R		Limsuwan and Lovell (1981)
Common carp	NR		Kashiwada et al. (1970)
Yellowtail	0.053 mg	MLS	Shimeno (1991)
Nile tilapia	NR		Lovell and Limsuwan (1982)
Folate			
Pacific salmon	6-10 mg	MLS	Halver (1972)
	2 mg	WG, ADS	Leith et al. (1990)
Rainbow trout	1.0 mg	WG, ADS	Cowey and Woodward (1993)
Channel catfish	1.5 mg	WG, NHV	Duncan and Lovell (1991)
Common carp	NR		Aoe et al. (1967a)
Yellowtail	1.2 mg	MLS	Shimeno (1991)
Choline			
Pacific salmon	600-800 mg	MLS	Halver (1972)
Rainbow trout	50-100 mg	WG, ADS	McLaren et al. (1947)
	714-813 mg	WG, LLC	Rumsey (1991)

Vitamin and Fish	Requirement (units/kg diet)	Response Criteria	Reference
Choline			
Lake trout	1,000 mg	WG	Ketola (1976)
Channel catfish	400 mg	WG, LLC	Wilson and Poe (1988)
Common carp	1,500 mg	WG, LLC	Ogino et al. (1970a)
Yellowtail	2,920 mg	MLS	Shimeno (1991)
Myoinositol			
Pacific salmon	300-400 mg	MLS	Halver (1972)
Rainbow trout	250-500 mg	WG, ADS	McLaren et al. (1947)
Channel catfish	NR		Burtle and Lovell (1989)
Common carp	440 mg	WG, ADS	Aoe and Masuda (1967)
Yellowtail	423 mg	MLS	Shimeno (1991)
Vitamin C			
Atlantic salmon	50 mg	WG, ADS	Lall et al. (1990)
Pacific salmon	50 mg	MKS	Halver et al. (1969)
Rainbow trout	250-500 mg	WG, ADS	McLaren et al. (1947)
	100 mg	MKS	Halver et al. (1969)
	40 mg	WG, ADS	Hilton et al. (1978)
Channel catfish	60 mg	WG, ADS, VC	Lim and Lovell (1978)
	45 mg	WG, ADS	Robinson (1990)
	11 mg	WG, ADS, VC	El Naggar and Lovell (1991)
Common carp	R		Dabrowski et al. (1988)
Yellowtail	122 mg	WG, ADS	Shimeno (1991)
Blue tilapia	50 mg	WG, ADS	Stickney et al. (1984)

NOTE: Abbreviations: AASLP, ascorbic acid stimulated lipid peroxidation; ADS, absence of deficiency signs; ED, enzyme data; FE, feed efficiency; LLC, liver lipid content; MLS, maximum liver storage; MKS, maximum kidney storage; NHV, normal hematocrit values; NR, no requirement determined; OSS, optimum swimming stamina; R, required but no value determined; VC, vertebral collagen content; and WG, weight gain.

Vitamin A is added to fish feeds as the acetate, palmitate, or propionate ester in the form of free-flowing beadlets in a multivitamin premix.

Vitamin D

The two major natural sources of vitamin D are ergocalciferol (vitamin D_2, which occurs predominantly in plants) and cholecalciferol (vitamin D_3, which occurs in animals). Both forms of vitamin D are hydroxylated in the liver to the 25-hydroxy forms. The 25-hydroxy-D_3 is further hydroxylated in the kidney to 1,25-dihydroxyvitamin D_3, which is the biologically active form of vitamin D responsible for facilitating mobilization, transport, absorption, and use of calcium and phosphorus in concert with the actions of parathyroid hormone and calcitonin.

Cholecalciferol has been shown to be at least three times more effective than ergocalciferol in meeting the vitamin D requirement of rainbow trout (Barnett et al., 1982a). Andrews et al. (1980) found that vitamin D_3 was used more effectively by catfish than vitamin D_2 at dietary concentrations of 2,000 IU/kg of diet and that high concentrations of vitamin D_3 (20,000 to 50,000 IU/kg of diet) reduced weight gain. Brown (1988), however, found that vitamin D_2 was utilized as well as vitamin D_3 up to 1,500 IU/kg of diet, but higher concentrations of vitamin D_2 depressed weight gain and feed efficiency in channel catfish reared in calcium-free water.

Rainbow trout fed a vitamin D-deficient diet exhibited poor growth, elevated liver lipid content, impaired calcium homeostasis manifested by tetany of white skeletal muscles, and ultrastructural changes in the white muscle fibers of the epaxial musculature (George et al., 1981). However, in a similar study also with rainbow trout, no hypocalcemia or changes in bone ash were observed (Barnett et al., 1982a). A lordosis-like droopy tail syndrome observed in vitamin D-deficient trout (Barnett et al., 1982b) was suggested to be related to an epaxial muscle weakness. Channel catfish fed a vitamin D-deficient diet for 16 weeks showed poor growth, lowered body calcium and phosphorus levels, and lowered total body ash (Lovell and Li, 1978). Andrews et al. (1980) reported that vertebral ash level in channel catfish was not significantly affected by vitamin D deficiency.

Fingerling brook trout fed 3.75×10^6 IU vitamin D3/kg diet for 40 weeks had hypercalcemia and increased hematocrit levels but no difference in rates of growth and survival (Poston, 1969a). However, Hilton and Ferguson (1982) did not detect any incidence of renal calcinosis in rainbow trout

fed a diet containing up to 1×10^6 IU vitamin D_3/kg diet. Supplementation of 50,000 IU vitamin D_3/kg diet significantly depressed the growth rate of channel catfish (Andrews et al., 1980). By contrast, a diet of 1×10^6 IU vitamin D_3/kg has been reported to show no toxic effects in channel catfish reared in calcium-free water for 14 weeks (Brown, 1988).

Vitamin D_3 is added to fish feeds either in a beadlet with vitamin A or as a spray or drum-dried powder in a multivitamin premix.

VITAMIN E

Vitamin E is a generic descriptor for all the molecules that possess the biological activity of α-tocopherol. Natural forms of vitamin E are all d-stereoisomers and consist of a substituted aromatic ring and a long isoprenoid side chain. There are eight naturally occurring compounds with vitamin E activity: d-α-; d-β-; d-γ-; d-δ-tocopherols, which differ in the number and position of the methyl groups in the aromatic ring; and their corresponding tocotrienols. The compound with the highest biopotency is d-α-tocopherol. The other tocopherol isomers have some, but very low, biological activity. No interconversion between a-tocopherol and the other tocopherol forms has been detected in liver or muscle tissue of rainbow trout (Watanabe et al., 1981c). The free tocopherol form of vitamin E is unstable to oxidizing conditions; whereas the acetate and succinate esters are quite stable. These ester forms possess no antioxidant activity, but they are readily hydrolyzed in the digestive tract to the biologically active free tocopherol. One IU of vitamin E is defined as the biological activity of 1 mg of DL-α-tocopheryl.

Vitamin E functions in vitro as a very good antioxidant in a manner similar to several synthetic antioxidants. In vivo, vitamin E and selenium (via glutathione peroxidase) function as parts of a multicomponent antioxidant defense system. This system protects the cell against the adverse effects of reactive oxygen and other free radical initiators of the oxidation of polyunsaturated membrane phospholipids, critical proteins, or both.

Vitamin E deficiency signs have been described for chinook salmon (Woodall et al., 1964), Atlantic salmon (Poston et al., 1976), channel catfish (Dupree, 1968; Murai and Andrews, 1974; Lovell et al., 1984; Wilson et al., 1984), common carp (Watanabe et al., 1970a,b, 1981a), rainbow trout (Cowey et al., 1981, 1983; Hung et al., 1981; Watanabe et al., 1981b; Moccia et al., 1984) and yellowtail (Toyoda, 1985). The deficiency signs of vitamin E in various fishes are similar and include muscular dystrophy involving atrophy and necrosis of white muscle fibers; edema of heart, muscle, and other tissues due to increased capillary permeability allowing exudates to escape and accumulate, which are often green in color as a result of hemoglobin breakdown; anemia and impaired erythropoiesis; depigmentation; and ceroid pigment in the liver. The incidence and severity of these deficiency signs have been shown to be enhanced when diets deficient in both vitamin E and selenium were fed to Atlantic salmon (Poston et al., 1976), rainbow trout (Bell et al., 1985), and channel catfish (Gatlin et al., 1986). These latter observations demonstrated a significant interaction between selenium and vitamin E in the nutrition of fish.

Erythrocyte fragility has been used as an indicator of vitamin E status in some animals (Draper and Csallany, 1969). Peroxide hemolysis of red blood cells has been used to determine vitamin E deficiency in rainbow trout (Hung et al., 1981); however, this procedure was not sensitive enough to aid in determining the vitamin E requirement in rainbow trout (Cowey et al., 1981) and channel catfish (Wilson et al., 1984). Cowey et al. (1981) found that in vitro ascorbic acidstimulated lipid peroxidation in liver microsomes of rainbow trout accurately reflected α-tocopherol status. This latter procedure has also been used to assess vitamin E status in channel catfish (Wilson et al., 1984; Gatlin et al., 1986).

When high concentrations of dietary polyunsaturated fatty acids are involved in the diets of common carp (Watanabe et al., 1981a) and rainbow trout (Watanabe et al., 1981b; Cowey et al., 1983), the requirement for vitamin E is increased. Vitamin E-deficient rainbow trout have been reported to have significantly reduced immune and nonspecific responses to infection (Blazer and Wolke, 1984a); however, Salte et al. (1988) could show no beneficial effect of dietary vitamin E supplementation alone or in combination with selenium as a prophylaxis for Hitra disease in Atlantic salmon.

High dietary concentrations of vitamin E (5,000 mg of DL-α-tocopherol/kg of diet) have been shown to cause reduced concentrations of erythrocytes in trout blood (Poston and Livingston, 1969).

Vitamin E is added to fish feeds as a dry powder form of DL-α-tocopheryl acetate.

VITAMIN K

Vitamin K is required for stimulation of prothrombin activity in plasma and synthesis of blood clotting factors VII, IX, and X. The metabolic role of vitamin K involves the vitamin K-dependent carboxylase, which carries out the posttranslational conversion of specific glutamyl residues in the vitamin K-dependent plasma proteins to γ-carboxy-glutamyl residues. These residues are essential for the normal, Ca^{2+}-dependent, interaction of the vitamin K-dependent clotting factors with phospholipid surfaces (Suttie, 1985).

The term vitamin K is used as a generic descriptor for both 2-methyl-1,4-naphthoquinone and all 3-substituted derivatives of this compound, which exhibit an antihemorrhagic activity in animals fed a vitamin K-deficient diet. The three major forms of vitamin K include: vitamin K_1 or

phylloquinone, which can be isolated from plants; vitamin K_2 or the menaquinones, which are synthesized by bacteria; and vitamin K_3 or menadione which is a synthetic product.

Many animals do not require vitamin K in the diet because of bacterial synthesis in the intestinal tract, but intestinal vitamin K-synthesizing microflora have not been described in fish (Margolis, 1953). Supplementation of sulfaguanidine to a vitamin K-deficient diet and low water temperature caused prolonged blood coagulation time and low hematocrit values without affecting growth performance of trout (Poston, 1964). Dupree (1966) reported hemorrhages in channel catfish fed a vitamin K-deficient diet. However, Murai and Andrews (1977) failed to detect any deficiency signs in channel catfish fed a diet devoid of vitamin K and supplemented with sulfaguanidine. The addition of dicumarol, a vitamin K antagonist, did not increase prothrombin time in catfish. The addition of pivalyl, a stronger (20 times) vitamin K antagonist than dicumarol, completely blocked the blood coagulation of channel catfish (Murai and Andrews, 1977). High-dietary concentrations of menadione sodium bisulfite (2,400 mg/kg of diet) had no adverse affect on growth, survival, blood coagulation, or the number of erythrocytes of young trout (Poston, 1971b).

Vitamin K is added to fish feeds as a menadione salt—menadione sodium bisulfite (50 percent K_3), menadione sodium bisulfite complex (33 percent K_3), or menadione dimethylpyrimidinol bisulfite (45.5 percent K_3).

Water-Soluble Vitamins

The water-soluble vitamins, with the exception of two water-soluble growth factors (choline and myoinositol) and ascorbic acid, have unique coenzyme functions in cellular metabolism. Yet, it is not always possible to correlate a sign of deficiency with a diminished function of an enzyme system for which that vitamin is essential. For some warm-water 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 in body tissues.

THIMAIN

The coenzyme form of thiamin is thiamin pyrophosphate. Thiamin pyrophosphate functions in the oxidative decarboxylation of α-keto acids, such as pyruvate and α-ketoglutarate, and in the transketolase reaction in the pentose shunt.

Dietary thiamin deficiency has been shown to result in neurological disorders such as hyperirritability in salmonids (Halver, 1957; Coates and Halver, 1958; Kitamura et al., 1967b; Lehmitz and Spannhof, 1977), channel catfish (Dupree, 1966; Comacho, 1978), Japanese eel (Hashimoto et al., 1970), and Japanese parrotfish (Ikeda et al., 1988). However, Murai and Andrews (1978a) did not observe neurological disorders in thiamin-deficient channel catfish. Arai et al. (1972) found only subcutaneous hemorrhages and congested fins in subadult Japanese eels, and Hashimoto et al. (1970) observed neurological disorders in small Japanese eels. Similar deficiency signs with varying degrees of mortality have been reported in common carp (Aoe et al., 1969), red sea bream (Yone and Fujii, 1974), turbot (Cowey et al., 1975), and yellowtail (Hosokawa, 1989).

Erythrocyte transketolase activity has been used as a specific indicator of thiamin status in the turbot (Cowey et al., 1975). Kidney or liver transketolase activity in rainbow trout (Lehmitz and Spannhof, 1977; Masumoto et al., 1987) and thiamin content in the blood of yellowtail (Hosokawa, 1989) also have been shown to decrease much earlier than the appearance of external deficiency signs.

Thiamin is added to fish feeds as thiamin mononitrate, which is 91.9 percent thiamin. Thiamin mononitrate is stable in vitamin premixes that do not contain trace minerals and choline chloride.

RIBOFLAVIN

Riboflavin functions in the intermediary transfer of electrons in metabolic oxidation-reduction reactions as a component of two coenzymes, flavin monouncleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes serve as prosthetic groups of oxidation-reduction enzymes involved in the metabolism of keto-acids, fatty acids, and amino acids in the mitochondrial electron transport system.

Species-specific deficiency signs are found in fish. The only common signs are anorexia and poor growth. The first sign of riboflavin deficiency observed in salmonids (McLaren et al., 1947; Halver, 1957; Steffens, 1970; Takeuchi et al., 1980; Hughes et al., 1981a,b) appeared in the eyes and included photophobia, cataracts, corneal vascularization, and hemorrhages. Lack of coordinated swimming and dark skin coloration have also been reported for riboflavin-deficient chinook salmon (Halver, 1957) and rainbow trout (Kitamura et al., 1967b; Steffens, 1970). In contrast, Woodward (1984) did not observe cataracts or corneal occlusion in riboflavin-deficient rainbow trout fry and fingerlings; however, severe fin erosion and light skin coloration accompanied by high mortality were observed. The eye lesions and dark skin coloration followed by high mortality have also been observed in riboflavin-deficient yellowtail fingerlings (Hosokawa, 1989). Riboflavin-deficient common carp (Aoe et al., 1967c; Ogino, 1967; Takeuchi et al., 1980) and Japanese eel (Arai et al., 1972) exhibited hemorrhages in various parts of the body, nervousness, and photophobia but no evidence of cataract development. Monolateral or bilateral

cataracts have been reported in riboflavin-deficient channel catfish (Dupree, 1966), but Murai and Andrews (1978b) found only poor growth and short-body dwarfism in two independent feeding trials with channel catfish. Lethargy and high mortality have been reported in Japanese parrotfish fed riboflavin-deficient diets (Ikeda et al., 1988).

Hughes et al. (1981a) used the activation coefficient (ratio of activity following preincubation with FAD:basal activity) of erythrocyte glutathione reductase to measure the riboflavin status of rainbow trout. However, Woodward (1983) found the activity of D-amino acid oxidase to be a more sensitive indicator of the riboflavin status in rainbow trout, since the low activity of erythrocyte glutathione reductase made its quantification difficult. Amezaga and Knox (1990) also found that hepatic D-amino acid oxidase was a reliable indicator of riboflavin status in rainbow trout. They pointed out, however, that an assay for glutathione reductase activity in erythrocytes would be advantageous since it could be used on live fish. Woodward (1985) reported that the riboflavin requirement was not affected by temperature or by genetic differences in growth rate. This might be one reason why the riboflavin requirement values shown in Table 1-15 agree fairly well even among different species.

Hughes (1984) found that feeding high concentrations of riboflavin (up to 600 mg/kg diet) had no adverse effects on growth of rainbow trout. These results were expected since riboflavin has not been shown to cause hypervitaminosis in other animals. However, two previous studies (McLaren et al., 1947; Woodward, 1982) had reported depressed growth in rainbow trout fed moderate concentrations of riboflavin. It was concluded that the growth depression observed in the earlier studies must have resulted from some factor other than riboflavin.

Riboflavin is added to fish feeds as a dry powder in a multivitamin premix.

VITAMIN B_6 (PYRIDOXINE)

The term vitamin B_6 is the generic descriptor for the 2-methylpyridine derivatives that have the biological activity of pyridoxine. Pyridoxine is the main form found in plant products, whereas pyridoxal and pyridoxamine are the principal forms found in animal tissue. All three forms are readily converted in animal tissue to the coenzyme forms, pyridoxal phosphate and pyridoxamine phosphate. Pyridoxal phosphate is required for many enzymatic reactions involving amino acids such as transamination, decarboxylation, and dehydration. Pyridoxal phosphate also functions in the biosynthesis of porphyrins and in the catabolism of glycogen.

Pyridoxal phosphate is required for the synthesis of the neurotransmitters—5-hydroxytryptamine and serotonin—from tryptophan. Consequently, signs of pyridoxine deficiency include nervous disorders—erratic swimming, hyperirritability, and convulsions—that have been observed in salmonids (Halver, 1957; Coates and Halver, 1958), gilthead sea bream (Kissil et al., 1981), channel catfish (Andrews and Murai, 1979), common carp (Ogino, 1965), yellowtail (Sakaguchi et al., 1969), and Japanese eel (Arai et al., 1972).

Other deficiency signs such as anorexia and poor growth usually appear in the fish within 3 to 6 weeks after being fed a pyridoxine-deficient diet. Pyridoxine deficiency has been reported to cause various histopathological changes in rainbow trout liver (Jurss and Jonas, 1981) and kidney (Smith et al., 1974) and in the intestinal tissue of both rainbow trout (Smith et al., 1974) and gilthead sea bream (Kissil et al., 1981).

The activity of certain aminotransferase enzymes that require pyridoxal phosphate as a coenzyme has been used as an index of pyridoxine status in fish. Serum or tissue alanine and/or aspartate aminotransferase activities have been used to evaluate pyridoxine status in common carp (Ogino, 1965), rainbow trout (Smith et al., 1974; Jurss, 1978), chinook salmon (Hardy et al., 1979), turbot (Adron et al., 1978), and gilthead sea bream (Kissil et al., 1981).

Vitamin B_6 is added to fish feeds as pyridoxine hydrochloride in a dry form as part of a multivitamin premix.

PANTOTHENIC ACID

Pantothenic acid is a component of coenzyme A (CoA), acyl CoA synthetase, and acyl carrier protein. The coenzyme form of the vitamin is therefore responsible for acyl group transfer reactions. Coenzyme A is required in reactions in which the carbon skeletons of glucose, fatty acids, and amino acids enter into the energy-yielding tricarboxylic acid cycle. Acyl carrier protein is required for fatty acid synthesis.

A deficiency of this vitamin impairs the metabolism of mitochondria-rich cells that undergo rapid mitosis and high-energy expenditure. Thus, deficiency signs have been found to appear within 10 to 14 days in rapidly growing fish such as fingerling yellowtail (Hosokawa, 1989). Gill lamellar hyperplasia or clubbed gills is a characteristic sign of pantothenic acid deficiency in most fish. In addition to clubbed gills, anemia and high mortality have been observed in pantothenic acid-deficient salmonids (Phillips et al., 1945; McLaren et al., 1947; Coates and Halver, 1958; Kitamura et al., 1967b; Poston and Page, 1982; Karges and Woodward, 1984), channel catfish (Dupree, 1966; Murai and Andrews, 1979; Brunson et al., 1983; Wilson et al., 1983), and yellowtail (Hosokawa, 1989). Pantothenic acid-deficient Japanese parrotfish exhibited anorexia, convulsions, and cessation of growth followed by high mortality (Ikeda et al., 1988). Similar deficiency signs were observed in red sea bream (Yone and Fujii, 1974). Slow growth, anorexia, lethargy, and anemia were observed in common carp (Ogino, 1967). Poor growth, hemorrhage, skin lesions, and abnormal swimming were found in Japanese eel (Arai et al., 1972) fed pantothenic acid-deficient diets.

Pantothenic acid is added to fish feeds as either calcium *d*-pantothenate (92 percent activity) or calcium DL-pantothenate (46 percent activity) as a dry powder in a multivitamin premix.

Niacin

Niacin is used as the generic descriptor of pyridine 3-carboxylic acids and their derivatives that exhibit the biological activity of nicotinamide (the amide of nicotinic acid). Of the compounds with niacin activity, nicotinic acid and nicotinamide have the greatest biological activity. Niacin is widely distributed in both plant and animal tissue. Much of the niacin in plant material, however, is present in bound forms that have limited availability to fish.

Niacin is a component of the two coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These coenzymes are essential for several oxidation-reduction reactions involving the transfer of hydrogen and electrons in carbohydrate, lipid, and amino acid metabolism. They are also involved in various energy yielding and biosynthetic pathways including the mitochondrial electron transport system. Tryptophan can be metabolically converted to niacin in many animals, but not in certain salmonid fish (Poston and DiLorenzo, 1973; Poston and Combs, 1980). The fact that niacin deficiency can readily be induced in various fish indicates that most if not all fish lack the capacity for niacin synthesis.

Trout and salmon fed niacin-deficient diets exhibited anorexia, poor growth, poor feed conversion, photosensitivity or sunburn, intestinal lesions, abdominal edema, muscular weakness, spasms, and increased mortality (McLaren et al., 1947; Phillips and Brockway, 1947; Halver, 1957). Channel catfish (Andrews and Murai, 1978) and common carp (Aoe et al., 1967b) showed skin and fin lesions, high mortality, skin hemorrhages, anemia, and deformed jaws when fed niacin-deficient diets for 2 to 6 weeks. Skin hemorrhages, dermatitis, anemia, abnormal swimming, and ataxia were observed in Japanese eels fed a niacin-deficient diet for 14 weeks (Arai et al., 1972).

Poston and Wolfe (1985) have experimentally demonstrated the interaction between the occurrence of dermal lesions and niacin deficiency. Two weeks after exposure of niacin-deficient rainbow trout to ultraviolet radiation, a total loss of mucus-producing cells was observed in histopathological sections of the epidermis.

High dietary intake of niacin (10,000 mg/kg) increased liver fat, decreased body fat, and tended to reduce growth rate in fingerling brook trout (Poston, 1969b).

Niacin is added to fish feeds as either nicotinic acid or niacinamide; both have similar biological activity. Nicotinic acid or niacinamide is added to the multivitamin premix in a dry form.

BIOTIN

Biotin acts in certain metabolic reactions as an intermediate carrier of carbon dioxide during carboxylation and decarboxylation reactions. Specific enzymes that require biotin include acetyl-CoA carboxylase, pyruvate carboxylase, and propionyl-CoA carboxylase. Metabolic pathways requiring biotin include the biosynthesis of long-chain fatty acids and the synthesis of purines.

In many animals, a biotin deficiency can only be induced by feeding avidin, a glycoprotein found in raw chicken egg white that binds biotin and prevents absorption of the vitamin from the intestine. Robinson and Lovell (1978) fed avidin in a biotin-free chemically defined diet to channel catfish and noted a growth suppression that led them to suggest some biotin synthesis by intestinal microflora in this species. However, in a later study by Lovell and Buston (1984) no synthesis of biotin by the intestinal microflora in channel catfish could be detected.

Common carp required 8 to 12 weeks (Ogino et al., 1970a) and channel catfish took 11 weeks (Lovell and Buston, 1984) to show growth depression when fed biotindeficient diets. A similar effect in rainbow trout took only 4 to 8 weeks in water temperatures of 15°C (Woodward and Frigg, 1989). Anorexia, reduced weight gain, and higher feed conversion were more noticeable in smaller than in larger rainbow trout fed biotin-deficient diets (Walton et al., 1984). Biotin-deficient channel catfish exhibited skin depigmentation (Robinson and Lovell, 1978), whereas biotin-deficient Japanese eels had darker skin coloration (Arai et al., 1972). Histological signs of biotin deficiency were not detected after 12 weeks in rainbow trout having an initial weight of 25 g (Walton et al., 1984). However, severe deficiency signs were produced in rainbow trout and lake trout having initial weights of 1.3 and 6.7 g, respectively (Poston and Page, 1982; Woodward and Frigg, 1989). Rainbow trout and lake trout developed biotin-related histopathological signs in the gills (Castledine et al., 1978; Poston and Page, 1982), liver (Poston, 1976b; Poston and Page, 1982), and kidney (Poston and Page, 1982).

Hepatic pyruvate carboxylase activity in rainbow trout fed a lipid-free and biotin-deficient diet decreased to 3.3 percent of that in fish fed a diet sufficient in lipid and biotin, although the enzyme activity was restored to about 50 percent of normal following the addition of lipid to the diet (Walton et al., 1984). In contrast, lipid supplementation of biotin-deficient diets did not increase hepatic pyruvate carboxylase activity in channel catfish (Robinson and Lovell, 1978).

Signs of biotin deficiency were not detected in rainbow trout (Castledine et al., 1978) or channel catfish (Lovell and Buston, 1984) fed natural ingredient diets without supplemented biotin for 24 and 17 weeks, respectively. These studies concluded that adequate biotin was available in the

various feed ingredients in the natural ingredient diets used to meet the requirements of the fish.

Biotin is added to fish feeds when necessary as D-biotin in a dry form in the multivitamin premix.

FOLATE

The term folate is used as the generic descriptror for folic acid and related compounds exhibiting qualitatively the biological activity of folic acid. Folic acid is composed of a pteridine ring linked through a methylene bridge to *p*-aminobenzoic acid to form pteroic acid, which is in turn linked as an amide to glutamic acid. Folic acid undergoes enzymatic reduction in the tissues to its active coenzyme form, tetrahydrofolic acid. It functions as an intermediate carrier of one-carbon groups in a number of complex enzymatic reactions. In these reactions, methyl, methylene, and other one-carbon groups are transferred from one molecule to another. These reactions are found in the metabolism of certain amino acids and the biosynthesis of purines and pyrimidines along with the nucleotides found in DNA and RNA.

Trout and salmon fed folate-deficient diets exhibited anorexia; reduced growth; poor feed conversion; and macrocytic normochromic, megaloblastic anemia (Smith, 1968; Smith and Halver, 1969) characterized by pale gills, anisocytosis, and poikilocytosis. The erythrocytes were large with abnormally segmented and constricted nuclei, and a large number of megaloblastic proerythrocytes were present in the erythropoietic tissue of the anterior kidney. Production of erythrocytes decreased with time in fish fed the folate-deficient diet. Some of these signs have also been observed in the rohu (John and Mahajan, 1979).

Poor growth and dark skin coloration were noted in Japanese eels fed a folate-deficient diet for 10 weeks (Arai et al., 1972). Folate-deficient yellowtail fingerlings also showed congestion in fins and bronchial mantle, dark skin coloration, and anemia (Hosokawa, 1989). Folate deficiency signs in channel catfish included reduced growth, anemia, and increased sensitivity to bacterial infection (Duncan and Lovell, 1991). Deficiency signs were not observed in common carp (Aoe et al., 1967a) fed a folate-free diet, presumably due to bacterial synthesis of folate in the intestine (Kashiwada et al., 1971).

Folate is added to fish feeds as folic acid as a dry powder in a multivitamin premix.

VITAMIN B_{12}

The term vitamin B_{12} should be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. This vitamin was previously known as vitamin B_{12} or cyanocobalamin. Vitamin B_{12} is a large molecule (molecular weight 1355) that contains a cobalt atom. Neither higher plants nor animals can synthesize vitamin B_{12}, but both depend on certain microorganisms for the trace amounts required. Vitamin B_{12} is required for normal maturation and development of erythrocytes, for the metabolism of fatty acids, in the methylation of homocysteine to methionine, and for the normal recycling of tetrahydrofolic acid. Thus, a deficiency of vitamin B_{12} can result in signs similar to folate deficiency.

Salmon (Halver, 1957) and trout (Phillips et al., 1964) fed low amounts of vitamin B_{12} showed a high variability in numbers of fragmented erythrocytes and in hemoglobin values, with a tendency for a microcytic, hypochromic anemia. Channel catfish fed a vitamin B_{12}-deficient diet for 36 weeks exhibited reduced growth but no other clinical deficiency signs (Dupree, 1966). John and Mahajan (1979) observed reduced growth and lower hematocrit in rohu fed a vitamin B_{12}-deficient diet. Japanese eel were found to require vitamin B_{12} for normal appetite and growth (Arai et al., 1972).

Intestinal microfloral synthesis appeared to satisfy the B_{12} requirement of Nile tilapia (Lovell and Limsuwan, 1982), but channel catfish required dietary supplementation of B_{12} to prevent anemia (Limsuwan and Lovell, 1981). Intestinal microfloral synthesis of vitamin B_{12} has been demonstrated in common carp (Kashiwada et al., 1970; Sugita et al., 1991a), channel catfish (Limsuwan and Lovell, 1981; Sugita et al., 1990, 1991a), Nile tilapia (Lovell and Limsuwan, 1982; Sugita et al., 1990, 1991a), rainbow trout (Sugita et al., 1991b), and ayu and goldfish (Sugita et al., 1991a). Sugita et al. (1991a) found a close relationship between the amount of vitamin B_{12} and the viable counts of *Bacteroides* type A in the intestinal contents of the various fish studied. They found that this bacterium was present in the intestinal contents of fish that do not require vitamin B_{12} and absent in those fish that do require vitamin B_{12}.

Vitamin B_{12} is added to fish feeds when necessary in a dry form as part of a multivitamin premix.

CHOLINE

Unlike the other water-soluble vitamins, choline has no known coenzyme function. Choline has three major metabolic functions: as a component of phosphatidylcholine, which has structural functions in biological membranes and in tissue lipid utilization; as a precursor of the neurotransmitter acetylcholine; and as a precursor of betaine, which serves as a source of labile methyl groups for methylation reactions such as the formation of methionine from homocysteine and creatine from guanidoacetic acid.

Rainbow trout fed a choline-deficient diet developed light yellow-colored livers, protruded eyes, anemia, and extended abdomens (kitamura et al., 1967a). Lake trout fed a choline-deficient diet for 12 weeks had depressed growth rate and increased liver fat content (Ketola, 1976). Depressed

growth, loss of appetite, and white-gray colored intestines were observed in Japanese eels fed a choline-deficient diet (Arai et al., 1972). Increased liver lipid content has been observed in common carp and channel catfish fed choline-deficient diets (Ogino et al., 1970b; Wilson and Poe, 1988). In addition, common carp developed vacuolization of hepatic cells after being on such a diet for 10 weeks (Ogino et al., 1970b). A thinning of the intestinal wall muscle and focal degeneration of the exocrine pancreas were observed in choline-deficient sturgeon (Hung, 1989).

Channel catfish fed casein-gelatin diets containing excess methionine did not develop signs of choline deficiency; however, catfish fed diets adequate but not excessive in methionine did develop deficiency signs (Wilson and Poe, 1988). Rumsey (1991) has suggested that 50 percent of the choline requirement of rainbow trout can be met from betaine. These observations indicate that certain fish can meet a part of their choline needs through the synthesis of choline by the methylation of ethanolamine, which uses methyl groups from S-adenosyl methionine.

Choline is added to fish feeds as a 70 percent choline chloride solution or a 25 to 60 percent dry powder. Choline chloride can decrease the stability of other vitamins in a multivitamin premix during prolonged storage.

MYOINOSITOL

Inositol may exist in one of seven optically inactive forms and as one pair of optically active isomers. Only one of these forms, myoinositol, possesses biological activity. Inositol is a biologically active cyclohexitol and occurs as a structural component in biological membranes as phosphatidylinositol. Recently, phosphatidylinositol was shown to be involved in signal transduction of several metabolic processes (Mathews and van Holde, 1990). Although similar in many respects to the adenylate cyclase transduction system, the phosphoinositide system is distinctive in that the hormonal stimulus activates a reaction that generates two second messengers. Membrane bound phosphatidylinositol 4,5-bisphosphate is cleaved to release *sn*-1,2-diacylglycerol and inositol 1,4,5-triphosphate, following the interaction of a hormone or agonist with the receptor on the cell membrane. 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 release, smooth muscle contraction, liver glycogenolysis, platelet aggregation, histamine secretion, and DNA synthesis in fibroblasts and lymphoblasts.

Signs of inositol deficiency have been reported to include poor appetite, anemia, poor growth, fin erosion, dark skin coloration, slow gastric emptying, and decreased cholinesterase and certain aminotransferase activities in trout (McLaren et al., 1947; Kitamura et al., 1967b), red sea bream (Yone et al., 1971), Japanese eel (Arai et al., 1972), Japanese parrotfish (Ikeda et al., 1988), and yellowtail (Hosokawa, 1989). Rainbow trout fed a diet devoid of inositol had large accumulations of neutral lipids in the liver, increased levels of cholesterol and triglycerides, but decreased amounts of total phospholipid, phosphotidylcholine, phosphotidylethanolamine, and phosphotidylinositol (Holub et al., 1982).

Inositol appears to be synthesized in common carp intestine (Aoe and Masuda, 1967), but not in amounts sufficient to sustain normal growth of young fish without an exogenous source of this vitamin, because younger carp require a higher level of inositol than older fish. Burtle and Lovell (1989) demonstrated de novo synthesis of inositol in the liver of channel catfish, as well as intestinal synthesis. High concentrations of dietary glucose may increase the need for inositol in some fish (Yone et al., 1971).

Myoinositol is added to fish feeds when necessary as a dry powder in a multivitamin premix.

VITAMIN C

Most animals can synthesize vitamin C, or L-ascorbic acid, from *D*-glucose, but many fish cannot (Kitamura et al., 1965; Poston, 1967; Halver et al., 1969; Wilson, 1973; Dabrowski, 1990). Ascorbic acid is a strong reducing agent and is readily oxidized to dehydroascorbic acid. Dehydroascorbic acid can be enzymatically reduced back to ascorbic acid in animal tissue with glutathione or reduced NADP. Ascorbic acid is a cofactor in the hydroxylation of proline and lysine to hydroxyproline and hydroxylysine in procollagen, which is the precursor of collagen and thus is necessary for the formation of connective tissues, scar tissue in wound repair, and bone matrix (Sandel and Daniel, 1988). Ascorbic acid also facilitates the absorption of iron, thus preventing the anemia often observed in ascorbic acid-deficient fish. In addition, ascorbic acid functions with vitamin E to minimize peroxidation of lipids in fish tissues (Heikkila and Manzino, 1987).

Vitamin C-deficient salmon and trout exhibited structural deformities (scoliosis, lordosis, and abnormal support cartilage of the eye, gill, and fins) and internal hemorrhaging usually preceded by nonspecific signs such as anorexia and lethargy (Halver et al., 1969; Hilton et al., 1978; Tsujimura et al., 1978; Sato et al., 1983), ascites and hemorrhagic exophthalmia (Poston, 1967), and high level of plasma triglycerides and cholesterol (John et al., 1979). Similar structural deformities such as scoliosis and lordosis due to vitamin C deficiency have been observed in channel catfish (Wilson and Poe, 1973; Andrews and Murai, 1974; Lim and Lovell, 1978; Wilson et al., 1989), Indian major carp (Agrawal and Mahajan, 1980), common carp and roach (Dabrowski et al., 1988, 1989), blue tilapia (Stickney et al., 1984),

Nile tilapia (Soliman et al., 1986a,b), and yellowtail (Sakaguchi et al., 1969). Japanese eels fed a vitamin C-deficient diet showed reduced growth after 10 weeks and hemorrhage in the head and fins after 14 weeks (Arai et al., 1972). Opacity of the cornea and kidney granulomatosis associated with hypertyrosinemia have been described as signs of vitamin C deficiency in turbot (Messager, 1986; Messager et al., 1986).

Phagocytic activity of cells of the immune system in fish produce reactive oxygen radicals that are potent microbicidal factors, but also autotoxic to fish macrophages (Secombes et al., 1988). Vitamin C appears to protect phagocytic cells and surrounding tissues from oxidative damage. An increased immune response due to high concentrations of vitamin C supplementation has been demonstrated in channel catfish (Durve and Lovell, 1982; Li and Lovell, 1985) and rainbow trout (Blazer and Wolke 1984b; Wahli et al., 1986; Navarre and Halver, 1989). However, Lall et al. (1990) observed no differences in humoral response and the complement system in Atlantic salmon fed diets containing 0 to 2,000 mg of vitamin C/kg after vaccination and subsequent live challenge with *Aeromonas salmonicida* and *Vibrio anguillarum.* Dietary and environmental contaminants, such as heavy metals (Yamamoto and Inoue, 1985) and chlorinated hydrocarbon pesticides (Mayer et al., 1978), increase the vitamin C requirements of fish.

Reproduction appears to increase maternal demands for vitamin C. Female tilapia fed vitamin C-free diets for 21 weeks produced eggs and fry containing no detectable ascorbic acid (Soliman et al., 1986b). Reduced reproductive performance has also been reported in rainbow trout fed vitamin C-deficient diets (Sandnes et al., 1984). Ascorbic acid reserves are rapidly depleted during embryonic (Sato et al., 1987) and larval development of certain fish (Dabrowski et al., 1988, 1989; Dabrowski, 1990), suggesting that requirements during early life stages may be higher than for fingerlings or adults.

Liver (Hilton et al., 1977; Sato et al., 1983) and kidney (Halver et al., 1969) ascorbic acid concentrations of less than 20 µg/g have been suggested as an indicator of vitamin C deficiency in salmonid fish. A similar value of less than 26 µg/g of liver has been suggested to indicate vitamin C deficiency in channel catfish (Lim and Lovell, 1978). A much higher value of 100 µg/g of kidney coincided with signs of vitamin C deficiency in snakehead (Mahajan and Agrawal, 1979).

Vertebral collagen levels have been shown to be a sensitive index of vitamin C status in channel catfish (Wilson and Poe, 1973; Lim and Lovell, 1978; El Naggar and Lovell, 1991) and rainbow trout (Sato et al., 1978).

Various derivatives of ascorbic acid, which are more stable than the parent compound, have been shown to provide antiscorbutic activity in fish. These include L-ascorbate-2-sulfate in rainbow trout (Halver et al., 1975; Grant et al., 1989), channel catfish (Murai et al., 1978; Brandt et al., 1985; Wilson et al., 1989), and tilapia (Soliman et al., 1986a); L-ascorbyl-2-monophosphate in channel catfish (Brandt et al., 1985; Lovell and El Naggar, 1990); and L-ascorbyl-2-polyphosphate in rainbow trout (Grant et al., 1989) and channel catfish (Wilson et al., 1989). Ascorbate-2-sulfate does not appear to be used as well as other more stable forms of ascorbic acid by certain fish (Murai et al., 1978; Soliman et al., 1986a; Dabrowski and Kock, 1989; Dabrowski et al., 1990), and in channel catfish it accounted for only 7 percent as much vitamin C activity as L-ascorbic acid or L-ascorbyl-2-monophosphate (Lovell and El Naggar, 1990).

Ascorbic acid is very labile and thus readily destroyed in the manufacturing process, especially in extruded feeds. Therefore it is not usually added to multivitamin premixes for fish feeds. Various coated forms of ascorbic acid, such as ethylcellulose or fat-coated products, have been used to increase retention of the vitamin in fish feeds. Nevertheless, approximately 50 percent of the supplemental ascorbic acid is destroyed during the manufacture of extruded catfish feeds (Lovell and Lim, 1978), and excess ascorbic acid is added to commercial formulations to ensure that an adequate concentration of the vitamin is retained during processing. Phosphorylated ascorbic acid, which is stable during extrusion processing (El Naggar and Lovell, 1991), is available for use in fish feeds but is presently relatively expensive. The form of the vitamin selected depends on how the fish feed is to be manufactured and how long it is to be stored before being fed to the fish. At present, it is still more economical to overfortify channel catfish feeds with the ethylcellulose coated product than to use the phosphate derivatives of ascorbic acid.

2

Other Dietary Components

Diets and diet ingredients contain materials other than nutrients that may affect metabolism in a positive or negative way. These components may occur naturally in feedstuffs or may be added to meet physiological needs, to improve or preserve the quality of the diet, or to fulfill economic purposes. Included are such substances as water, hormones, antibiotics, fiber, pellet binding agents, synthetic antioxidants, and feeding stimulants.

WATER

Diets contain water as an added ingredient, a natural constituent of the feedstuffs, or an element adsorbed from the atmosphere. Moisture derived from the atmosphere is generally low, 6 to 10 percent by weight, and little significance is attributed to these low "air-dry" concentrations in diets or ingredients. Low-moisture concentrations permit relative ease of storage and handling. In contrast, feeds with 12 percent or more moisture are generally more susceptible to spoilage by microorganisms.

Moist (10 to 40 percent) diets have been used at first feeding for Pacific salmon (Hublou et al., 1959; Hublou, 1963; Fowler and Burrows, 1971; Crawford et al., 1973) and Atlantic salmon (Lemm and Hendrix, 1981; Lemm, 1983) because these fish prefer moist feeds under hatchery conditions, particularly at cold-water temperatures. Thus, in recent years, commercial semimoist (15 to 20 percent) diets have been introduced that do not require refrigeration. To reduce moisture loss during processing and storage and to improve feed texture, polyhydric alcohols (propylene glycol, glycerol, and sorbitol, for example) are incorporated into these diets. The addition of compounds that inhibit molds is also required. Although many reports attribute the higher palatability of semimoist and moist diets to the higher moisture content (Ghittino, 1979), these claims have not been supported by experiments where proper comparisons were made. Studies on Atlantic salmon, chinook salmon, coho salmon, pink salmon, brown trout, and turbot fingerlings show no apparent benefit from the addition of water to dry diets (Poston, 1974; Bromley, 1980; Higgs et al., 1985; Hughes, 1989). Other factors such as pellet hardness or feeding strategy may be more important in improving feed intake than moisture.

FIBER

Fiber refers to indigestible plant matter such as cellulose, hemicellulose, lignin, pentosans, and other complex carbohydrates found in feedstuffs. These components are indigestible unless bacterial action occurs within the digestive tract. Fish do not secrete cellulase (Lindsay and Harris, 1980; Bergot, 1981), therefore cellulose digestion does not play an important role in their nutrition.

Fiber provides physical bulk to the feed. Cellulose and hemicellulose have been used as diluents and binders in experimental fish diets. Dietary fiber improved gastric evacuation time of rainbow trout fed purified diets (Hilton et al., 1983). Buhler and Halver (1961) reported that small amounts of supplemental cellulose increased growth and the efficiency of protein utilization in laboratory diets. Most fish can tolerate up to 8 percent fiber in their diets, but higher concentrations (8 to 30 percent) depress growth (Buhler and Halver, 1961; Leary and Lovell, 1975; Edwards et al., 1977; Hilton et al., 1983; Poston, 1986). The poor performance of salmonids fed certain types of fiber may result from a combination of factors including poor digestion and faster gastric emptying rates, which in turn affect feed intake and utilization of nutrients (Davies, 1988). In natural ingredient diets that contain 3 to 5 percent fiber (derived mainly from plant ingredients), adding fiber is unlikely to have any measurable benefit. In most cases the concern is to formulate diets without excessive fiber content, which may reduce the

nutrient intake and increase fecal waste production. To limit environmental pollution from aquaculture waste, an important strategy is to use highly digestible feed ingredients and limit the fiber content of the diet.

HORMONES

Various natural and synthetic hormones have been evaluated in fish growth experiments, including growth hormone, thyroid hormones, gonadotropin (GnH), prolactin, insulin, and various steroids. Experimental feeding of synthetic androgens has enhanced growth and improved feed conversion in some species, especially in salmonids (Donaldson et al., 1979; Higgs et al., 1982; Matty, 1986). Approximately 20 fish species have shown anabolic responses to steroids (Donaldson et al., 1979; Matty, 1986). Some warm-water species, however, such as channel catfish (Gannam and Lovell, 1991a,b), have responded negatively to the feeding of androgens. Prolonged steroid treatment for growth acceleration may cause detrimental side-effects including early gonadal development, skeletal deformity, increased susceptibility to infections, and pathological changes in the liver, kidney, and digestive tract (Zohar, 1989; Gannam and Lovell, 1991a,b). None of these hormones has been approved by the U.S. Food and Drug Administration (FDA) for growth enhancement in food fish.

Hormones have been successfully used to induce or synchronize ovulation and the stimulation of spermiation. To increase gamete availability and fry production throughout the year, hormones have some application for the initiation and stimulation of oogenesis and spermatogenesis. The most commonly used preparations are pituitary extracts and human chorionic gonadotropin (Lam, 1982; Donaldson and Hunter, 1983). Failure of fish to release GnHs may be responsible for the lack of final oocyte maturation, ovulation, and spawning (Zohar, 1988). GnH-releasing hormones (GnRH) have been effective in inducing ovulation and spawning in salmonids, cyprinids, Indian catfish, winter flounder, plaice, grey mullet, milkfish, sea bass, red sea bream, sablefish, and herring (Donaldson and Hunter, 1983; Zohar, 1988).

Sex steroids have also been used to reverse the sex of some species of salmonids, carps, and tilapias. The objectives are to produce monosex and sterile fish of the faster growing sex, achieve better somatic growth, and prevent sexual maturation and the accompanying deterioration of flesh quality. This subject has been extensively reviewed by Hunter and Donaldson (1983). Feminization can be achieved by feeding estrogenic steroids (ethynylestradiol, esterone, and diethylstilbestrol) to tilapia fry and 17-β-estradiol to salmonid fry. Production of all-male populations of tilapia by feeding androgenic steroids to the fry is practiced in many countries. Generally, ethynyltestosterone or methyltestosterone (30 to 60 mg/kg of diet) is incorporated in the first feed of tilapia fry and fed for 14 to 21 days. This sex reversal method produces 90 to 100 percent male tilapia.

ANTIBIOTICS

A wide range of antibiotics are used for therapeutic purposes in livestock production; however, only two compounds, sulfadimethoxine/ormetoprim and oxytetracycline, have been approved by the FDA for use in fish. Generally these compounds are stable during compression pellet processing and storage. Extrusion processing, however, will inactivate some of the oxytetracycline but very little of the sulfadimethoxine/ormetoprim. The quantity of antibiotic fed must be controlled, and proper feeding rate and withdrawal time must be practiced to reduce the entry of such compounds into the tissues of food fish or into the surrounding water. 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 (National Research Council, 1980). However, oxytetracycline and chlortetracycline in the diets of salmonid fish showed no appreciable benefit (Wolf, 1952; Herman, 1969). In red sea bream, however, administration of a subtherapeutic concentration (0.01 percent) of furazolidone, a nitrofuran derivative used against salmonellosis and protozoan diseases, improved growth and feed efficiency (Yone, 1968). Chemotherapeutic compounds may also be toxic when administered for an extended period or at high doses. Hicks and Geraci (1984) found that rainbow trout fed therapeutic concentrations of erythromycin (110 mg/kg daily) for 10 weeks showed vascular degeneration of proximal renal tubules. Prolonged sulfonamide therapy in salmonids caused growth retardation (Gutsell and Snieszko, 1949), renal tubular casts, focal hepatic necrosis, and visceral arterial sclerosis (Wood et al., 1957).

ANTIOXIDANTS

Antioxidants are commonly used in fish feeds that contain a high concentration of polyenic fatty acids to prevent the oxidation of lipids. Oxidative rancidity, or lipid peroxidation, affects the nutritional value of lipids, oxidation sensitive vitamins, and other feed components. The breakdown products of lipid peroxidation can react with the epsilon amino group of lysine and reduce its nutritional value. Natural tocopherols (vitamin E) have antioxidant activity; however, synthetic vitamin E is usually supplied in the diet in ester form, which has little antioxidant activity until it is hydrolyzed in the gut to the alcohol form. Thus, synthetic

vitamin E has little antioxidant activity in the diet. More vitamin E is required alone than when used in combination with antioxidants. Murai and Andrews (1974) showed that the commercial antioxidant ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyl-quinoline) could physiological replace vitamin E in channel catfish. Lipoid degeneration of liver and other pathologies have been associated with the feeding of rancid fat and/or the absence of vitamin E from the diet (Smith, 1979; Moccia et al., 1984; Tacon, 1985).

Synthetic antioxidants used in fish feeds are ethoxyquin, BHT (3,5-di-tert-butyl-4-hydroxytoluene), BHA (2(3)-tertbutyl-4-hydroxyanisole), and propyl gallate. Several excellent reviews of antioxidant types and mechanisms have been published (Uri, 1961; Stuckey, 1968; Porter, 1980). The maximum concentration of BHA and BHT permitted by the FDA is 0.02 percent of the fat content; for ethoxyquin, it is 150 mg/kg diet (21 C.F.R. § 573.380, 582.3169, 582.3173 [1987]).

PIGMENTS

Many plants and animals contain a variety of natural pigments that impart yellow, orange, and red colors to the flesh, skin, and eggs of fish. One of the most important groups of natural pigments in the plant and animal kingdom is the carotenoids. Fish and birds use oxygenated carotenoids (xanthophylls) for pigmentation of skin, flesh, and plumage. Fish cannot synthesize these pigments; therefore, they must be present in the diet. In salmonids, two oxycarotenoids, astaxanthin (3,3'-dihydroxy-4,4'-diketo-β-carotene) and canthaxanthin (4-4'-diketo-β-carotene) are responsible for the red to orange coloring of the flesh, skin, and fins. Astaxanthin is the main carotenoid pigment of wild salmonids, and is derived mainly from zooplankton. Feedstuffs of plant origin contain pigments that do not produce the desired color of salmon flesh. The major plant carotenoids are lutein (3,3'-dihydroxy-a-carotene) and zeaxanthin (3R,R'-β,β-carotene-3,3'-diol), as found in alfalfa, yellow corn, and algae. Lutein produces a yellow color whereas zeaxanthin imparts a yellow-orange color. Carotenoid concentration of some animal and plant sources are presented in Tables 2-1 and 2-2.

The retention of carotenoids in tissues depends on absorption, transport, metabolism, and excretion of these compounds (as reviewed by Torrissen et al., 1989). The digestibility of astaxanthin found in yeast and shrimp waste meal is low. However, ensiling of shrimp by-products improves the digestibility of astaxanthin by degrading the chitincalcium-protein-carotenoid complex in shrimp shells (Torrissen et al., 1981). Free astaxanthin is absorbed more efficiently than the astaxanthin ester (Torrissen and Braekkan, 1979; Schiedt et al., 1985). It appears that the rate of hydrolysis of the astaxanthin ester to free astaxanthin in the digestive tract of salmonids is limited. Approximately 90 percent of astaxanthin in fish flesh is located in free form, while the ester form predominates in skin. Salmonids are not able to oxygenate carotenoids, but deposit ingested oxygenated carotenoids without modification. Wide differences in the accumulation of carotenoids may be due to the differences in absorption of these compounds. Apparently, absorption is enhanced by the incorporation of hydroxyl groups into the carotene skeleton because astaxanthin is deposited at significantly higher concentrations than canthaxanthin in both Atlantic salmon and rainbow trout (Torrissen, 1986, 1989; Choubert and Storebakken, 1989). In salmonids, the absorption of astaxanthin and canthaxanthin is 10 to 20 times more efficient than lutein and zeaxanthin, while chickens absorb zeaxanthin at three times the rate of astaxanthin (Schiedt et al., 1985).

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 of the fillet. A discernible concentration of carotenoid was deposited in catfish flesh from feeds containing 11 mg xanthophyll/kg.

TABLE 2-1 Astaxanthin Content of Selected Natural Materials Used for Pigmentation of Salmonids

Materials	Astaxanthin (mg/kg)
Capelin (*Mallotus villosus*), oil	6–94
Copepod (*Calanus finmarchicus*)	39–84
Copepod (*C. finmarchicus*), oil	520
Crab, red (*Pleuroncodes planipes*)	100–160
Crab, red (*P. planipes*), oil extract	1,550
Crab (*Chinochetes opilio*), vacuum dried	5
Crab (*Greyon quinquedens*), freeze dried	76
Crawfish (*Procambarus clarkii*), oil extract	750
Crawfish (*P. clarkii*) meal	137
Krill (*Euphausia pacifica*)	100–130
Krill (*E. pacifica*), co-dried with oil	200
Krill (*E. pacifica*), oil	727
Krill (*Megannyctiphanes norvegica*)	46–93
Mackerel (*Scomber scombrus*), oil	6–11
Shrimp (*Pandalus borealis*), shelled	20–128
Shrimp (*P. borealis*), silaged	74
Shrimp (*P. borealis*), vacuum dried[a]	100
Shrimp (*P. borealis*), steam dried[b]	192
Shrimp (*P. borealis*), carotenoprotein[b]	1,160
Shrimp (*P. borealis*), oil	1,095
Yeast (*Phaffia rhodozyma*)	30–800

[a] Stabilized with antioxidant.
[b] Freeze-dried carotenoprotein.
SOURCE: Torrissen, O. J., R. W. Hardy, and K. D. Shearer. 1989. Pigmentation of salmonids-carotenoid deposition and metabolism. Rev. Aquat. Sci. 1:209–225.

TABLE 2-2 Xanthophyll Content of Selected Plant Materials

Material	Xanthophyll (mg/kg)
Alfalfa meal, 17 percent protein	260
Alfalfa meal, 20 percent protein	280
Alfalfa meal, 22 percent protein	330
Alfalfa juice protein, 17 percent protein	800
Algae, common, dried	2,000
Algae (*Chlorella pyrenoidosa*)	4,000
Corn, yellow	17
Corn gluten meal, 41 percent protein	175
Corn gluten meal, 60 percent protein	290
Marigold petal meal	7,000
Paprika, spanish	275
Seaweed (*Ascphyllum nodosum*)	340
Seaweed (*Fucus vesiculosus*)	350
Seaweed (*Fucus serratus*)	920

SOURCES: National Research Council. 1984. Nutrient Requirements of Domestic Animals. Nutrient Requirements of Poultry, 8th ed. Washington, D.C.: National Academy Press. Data for algae (*Chlorella pyronoidosa*), paprika, and seaweed were taken from Scott, M. L., M. C. Nesheim, and R. J. Young. 1982. Nutrition of the Chicken, 3d ed. Ithaca, N.Y.: M. L. Scott.

Skin pigmentation is important in cultured yellowtail and red sea bream. These fish convert dietary astaxanthin largely into tunaxanthin and deposit it in their skin. Goldfish and fancy red carp are similar to the chicken in their absorption preference: zeaxanthin-astaxanthin-lutein. Hata and Hata (1972, 1973, 1976) showed that the yellow pigment, zeaxanthin, is readily metabolized to astaxanthin in goldfish and fancy red carp, which imparts red coloration. Goldfish metabolize little β-carotene and no lutein to astaxanthin (Hata and Hata, 1972).

The function of carotenoids other than as precursors of vitamin A in fish is not well defined and mostly speculative (Tacon, 1981). Although the mobilization of carotenoids from the flesh to skin and ovaries of salmonids during maturation is well documented, their role in reproduction is not clear. Schiedt et al. (1985) reported a biological function of astaxanthin, canthaxanthin, and zeaxanthin as vitamin A (retinol and 3,4-dihydroretinol) precursors for vitamin A-depleted rainbow trout.

PELLET BINDERS

Binders are incorporated into fish feeds to improve stability in water, 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. 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 will permit the production of good-quality pellets. Most binders are considered to be inert and have limited or no nutritional value. However, incorporation of alginate and guar gum in rainbow trout diets reduced feed intake, increased moisture content of feces, and lowered the digestibility of protein and lipids (Storebakken, 1985). Wood et al. (1954) showed that carboxymethylcellulose at 2 percent in the diet of trout caused no growth depression.

FEEDING STIMULANTS

The primary modes of feed detection by fish are through olfaction or sight, but the taste of the item is the key factor in determining whether the item is swallowed or rejected (Adron and Mackie, 1978). There appears to be a well-defined and species-specific tuning of the taste receptors of fish for the particular cues present in their feed items (Goh and Tamura, 1980). Many researchers and feed manufacturers have attempted to add substances to fish feeds to enhance palatability and feed acceptance. This focus has taken particular importance in the production of larval and starter feeds, where feed acceptability is a major concern.

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 are amphoteric (have both acid and base properties simultaneously). Several substances or groups of substances for which these generalizations apply, such as amino acids, betaine, and inosine, have improved feeding behavior in carnivorous and omnivorous species (as reviewed by Atema [1980], Carr [1982], Mackie [1982], Adams and Johnsen [1986a], Rumsey [1986]). Harada (1989) has shown that some dipeptides elicit a greater feeding response than either of the constituent amino acids presented alone for abalone. Few data exist on feeding stimulants for herbivorous species, but in four studies using Zillii's tilapia (Adams and Johnsen, 1986a,b; Johnsen and Adams, 1986; Adams et al., 1988), organic acids along with certain amino acids were found to be stimulatory. Feeding was stimulated by the organic acid, dimethyl-β-propiothetin, in goldfish, common carp, and tilapia (Nakajima et al., 1989).

When data on the effectiveness of the various feeding stimulants containing amino nitrogen are considered, a pattern seems to emerge relating to the feeding behavior of the fish and the type of compounds that are stimulatory. In general, carnivores show the greatest positive response to alkaline and neutral substances, such as glycine, proline, taurine, valine, and betaine, while herbivores respond more

to acidic substances, such as aspartic acid and glutamic acid. The pattern is reasonable considering the chemical characteristics of the feed items the fish would seek in the natural environment (Mackie, 1982; Adams and Johnsen, 1986a).

The presence of certain compounds can also act as feeding deterrents. This phenomenon has been shown to occur with certain combinations of amino acids (Adron and Mackie, 1978; Mackie and Adron, 1978; Mackie, 1982). Trimethylamine or its oxidation products, which are produced in decaying fish flesh, were shown to cause a decrease in feed consumption in turbot (Mackie and Adron, 1978), plaice (Mackie, 1982), and chinook salmon (Hughes, 1991) when these compounds were added to the diet. Salmonids (Hung and Slinger, 1980; Ketola et al., 1989) and yellowtail (Murai et al., 1988) show aversion to highly oxidized oils and fishmeals.

3

Antinutrients and Adventitious Toxins

A number of antinutrients and adventitious toxins may gain entry into fish diets. These components can be integral substances of a feedstuff, they may result from natural contamination, or they may be accidental contaminants derived from human sources. When these materials are present in sufficient concentrations, the effectiveness of the diet may be compromised or the diet may exceed legal restrictions for some substances. This translates into a need for continuous awareness of quality during the selection of ingredients and the processing and storage of the diets.

ANTINUTRIENTS PRESENT IN FEEDSTUFFS

Numerous substances that occur naturally in plant materials or raw fish preparations from which feedstuffs are derived can affect the performance of fish. These naturally occurring antinutrients include trypsin inhibitors, hemagglutinating agents, phytic acid, gossypol, cyclopropenoic fatty acids, glucosinolates, erucic acid, alkaloids, and thiaminase.

Trypsin Inhibitors

Raw soybeans contain crystalline globular proteins that act as trypsin inhibitors (Mickelsen and Yang, 1966; Liener and Kakade, 1980). These proteins, which form irreversible complexes with trypsin, can be inactivated through heat processing (Ham and Sandstedt, 1944); however, excessive heating reduces the availability of certain amino acids, particularly lysine. The growth inhibition of underheated soybean products for fingerling channel catfish (Robinson et al., 1981) and rainbow trout (Sandholm et al., 1976; Smith, 1977) has been presumed to be due to interference with protein utilization. Several studies, however, with common carp (Viola et al., 1982), channel catfish (Robinson et al., 1985; Wilson and Poe, 1985), and rainbow trout (Ketola, 1975; Rumsey and Ketola, 1975; Dabrowski and Wojno, 1977) have indicated that other antinutritional factors in the soybeans may also be responsible. Robinson et al. (1981) found that dietary soybean products decreased intestinal lipase activity in channel catfish. Research has shown that the sensitivity of various fish species to trypsin inhibitors varies, with salmonids being more sensitive (Sandholm et al., 1976; Smith, 1977) than either channel catfish (Robinson et al., 1985; Wilson and Poe, 1985) or carp (Dabrowski and Kozak, 1979). Channel catfish fed a 35 percent crude protein diet appeared to tolerate soybean meal with much higher trypsin inhibitor activity than fish fed a 25 percent crude protein diet (Wilson and Poe, 1985).

Hemagglutinating Agents

In addition to trypsin inhibitors, soybeans have been found to contain proteins called hemagglutinins, or lectins, that can cause in vitro agglutination of the red blood cells from various species of animals (Jaffe, 1980). Soybean hemagglutinin is readily inactivated by pepsin in the stomach (Mickelsen and Yang, 1966), and therefore would not appear to cause any significant problems for fish with true stomachs.

Phytic Acid

Approximately 70 percent of the phosphorous in soybean meal and many other feedstuffs of plant origin is in the form of phytate, and its availability to fish is negligible (Ketola, 1985; Ketola, in press). Phytates act as strong chelators and form protein-phytic acid complexes that may reduce the bioavailability of protein (Spinelli et al., 1983) and minerals, such as zinc, manganese, copper, molybdenum, calcium, magnesium, and iron (O'Dell and Savage, 1960; Rackis, 1974; Smith, 1977). Ketola (1975) postulated that this reduction in mineral bioavailability explains in part the need for additional mineral supplementation in soybean-based

diets as compared with those based on fishmeal. Phytates, in conjunction with high concentrations of dietary calcium, caused a zinc deficiency in chinook salmon fed a diet presumed to be adequate in zinc content (Richardson et al., 1985). The addition of 0.5 percent phytic acid to chemically defined diets fed to rainbow trout resulted in a 10 percent reduction in growth and feed conversion, but had no apparent effect on zinc absorption (Spinelli et al., 1983). The conclusion was that the growth retarding effect of phytic acid was related to reduced protein availability. Gatlin and Wilson (1984b) found that the zinc allowance in natural ingredient catfish diets containing about 50 percent of soybean meal should be increased to about five times the normal requirement for growth.

Gossypol

The use of glanded cottonseed meal is limited in fish diets due to its gossypol content. Gossypol is found in the pigment glands of cotton and may account for as much as 2.4 percent of seed weight in certain varieties (Berardi and Goldblatt, 1980). Free gossypol is tolerated at varying amounts by different fish species, but excessive concentrations can depress growth and cause damage to various organ tissues. Gossypol has also been identified as a carcinogen with aflatoxin B in rainbow trout (Sinnhuber et al., 1968a).

Roehm et al. (1967) reported adverse effects of free gossypol on the growth of rainbow trout fed concentrations of 1,000 mg/kg of diet or higher, but not at 250 mg/kg of diet. Herman (1970) found that although growth depression did not occur at concentrations lower than 290 mg/kg of diet, histopathological changes were noted at 95 mg/kg of diet and included thickening of the glomerular basement membrane of the kidney and necrosis and ceroid deposition in the liver. Wood and Yasutake (1956) noted similar histopathology in rainbow trout. These results indicate that the maximum concentration of free gossypol in the diets of salmonids should be restricted to 100 mg/kg of diet or less.

Growth inhibition was observed in fingerling channel catfish fed more than 900 mg of free gossypol/kg of diet (Dorsa et al., 1982), but a portion of the depression was possibly due to a dietary lysine deficiency resulting from the irreversible binding of lysine and gossypol (Wilson et al., 1981; Dorsa et al., 1982). In commercial catfish diets in the United States, 10 to 20 percent cottonseed meal is commonly used. Robinson (1991) reported that the solvent extracted cottonseed meal used in catfish diets, which typically contains 400 to 800 mg of free gossypol/kg, would not provide toxic concentrations of free gossypol when mixed into catfish diets; however, the concentration of available lysine in the diet should be scrutinized when cottonseed meal replaces soybean meal. Blue tilapia have been shown to tolerate free gossypol concentrations as high as 1,800 mg/kg of diet with no apparent growth depression (Robinson et al., 1984).

Cyclopropenoic Fatty Acids

Cottonseed meal is the primary source of the cyclopropenoic fatty acids (CFAs) (sterculic acid and malvalic acid) in fish diets. CFAs are present in all varieties of cottonseed meal and are not completely removed by the oil extraction process (Mickelsen and Yang, 1966). Dietary CFAs caused lesions, increased glycogen deposition, and elevated saturated fatty acid concentration in the liver in rainbow trout (Roehm et al., 1970; Malevski et al., 1974; Scarpelli et al., 1974; Struthers et al., 1975a,b). The CFAs are powerful carcinogens when fed in combination with aflatoxins for rainbow trout (Lee et al., 1968, 1971; Hendricks et al., 1980) and sockeye salmon (Wales and Sinnhuber, 1972). These compounds also induced hepatomas in the absence of aflatoxins in rainbow trout (Sinnhuber et al., 1976; Hendricks et al., 1980). Dietary CFAs alter the activity of a number of liver enzymes (Taylor et al., 1973; Eisele et al., 1978, 1983), including the inhibition of fatty acid desaturases (Roehm et al., 1970) which may explain the accumulation of saturated fatty acids found in the liver of fish fed CFAs. No conclusive data exist on the effects of dietary CFAs on other fish species.

Glucosinolates

Glucosinolates are found naturally in oilseed crops such as rapeseed. The compounds are not themselves harmful, but upon enzymatic hydrolysis the products release thiocyanate ion, isothiocyanates, goitrin, and nitrites, all functioning as potent antithyroid agents. Thiocyanate ion inhibits the uptake of iodine by the thyroid while isothiocyanates and nitrites presumably are precursors to thiocyanate ion. The effects of these compounds can be reversed with additional iodine supplementation of the diet. Goitrin is the most potent glucosinolate; it inhibits the ability of the thyroid to bind iodine. The effects of goitrin cannot be reversed with dietary iodine (Tookey et al., 1980).

The glucosinolate content of meal from traditional rapeseed ranges from 3 to 8 percent (Fenwick and Hoggan, 1976; Langer, 1983; van Etten and Tookey, 1983), but selective breeding has led to the development of low glucosinolate varieties of rapeseed, referred to as canola, which provide meal with less than 0.2 mg/g glucosinolates (Higgs et al., 1982, 1983; Hardy and Sullivan, 1983). Yurkowski et al. (1978) showed that feeding rainbow trout traditional rapeseed meal caused thyroid hyperplasia and reduced plasma thyroxine concentration. Heat treatment inactivated the enzyme myrosinase, which hydrolizes the glucosinolates to their toxic by-products, but heating did not eliminate the glucosinolates or allow for growth equal to that of control fish. Extraction of rapeseed meal with water reduced the glucosinolate content and led to improved growth of rainbow trout (Yurkowski et al., 1978; Jones, 1979). Dabrowski and Kozlowska (1981) found that heat treatment of rapeseed

meal did not eliminate all the growth-suppressing factors for common carp.

Higgs et al. (1979) fed diets containing up to 22 percent (low glucosinolate) canola meal to coho salmon and obtained satisfactory growth, but elevated thyroid activity was observed in the fish. They claimed that higher amounts of canola meal could be used in salmon diets if 3,5,3-triiodo-L-thyronine was included in the diet to compensate for the loss of thyroid function (Higgs et al., 1982, 1983). Rainbow trout fed up to 20 percent canola meal showed normal growth rate, but thyroid hyperplasia and increased production of thyroid hormones was induced (Hardy and Sullivan, 1983).

Erucic Acid

Erucic acid, a constituent of rapeseed oil, is a 22-carbon monounsaturated fatty acid and may constitute from 20 to 55 percent of the oil (Kramer et al., 1975; Slinger, 1977). Diets with erucic acid-containing rapeseed oils were cardiotoxic to rats and caused lipid accumulation followed by necrosis of heart muscle fibers (Slinger, 1977). Unpublished work by Parker and Hendricks (as cited by Hendricks and Bailey [1988]) has shown that inclusion of erucic acid in the diet of coho salmon at 3 to 6 percent led to mortalities and pathologies of the skin, gill, kidneys, and heart. The selective breeding program that developed canola emphasized low erucic acid as well as low glucosinolates. No erucic acid-related pathologies have been associated with the inclusion of rapeseed meal in natural ingredient fish diets (Yurkowski et al., 1978; Higgs et al., 1979; Dabrowski and Kozlowska, 1981; Dabrowski et al., 1981, 1982; Higgs et al., 1982; Hardy and Sullivan, 1983; Higgs et al., 1983).

Alkaloids

The pyrrolizidine alkaloids are toxins found in several plant families, but not in any that are commonly used as feedstuffs in fish feeds. Many of these plants are grown in conjunction with soybeans and cotton, and therefore may find their way into fish diets as contaminants of soybean or cottonseed meal. The compounds are metabolized to toxic pyrroles (McLean, 1970) by mixed function oxidases in the liver. Pyrrolizidine alkaloids at 100 mg/kg of diet caused severe growth depression and mortality in rainbow trout (Hendricks et al., 1981); and at concentrations of 2 mg/kg of diet these toxins caused hepatic lesions including necrosis, megalocystis, fiber tissue scarring, and occlusion of the hepatic veins. Liver lesions and mortalities were also noted 6 months after the alkaloids were removed from the diet.

Thiaminase in Raw Fish

The thiamin-destroying enzyme thiaminase has long been recognized in some raw fish preparations (Green et al., 1941; Wooley, 1941; Wolf, 1942; Deutsch and Hasler, 1943; Lieck and Agren, 1944; Yudkin, 1945; Neilands, 1947). Species that contain thiaminase, as well as those that do not, were reviewed by the National Research Council (1983). The enzyme is found more commonly in freshwater fish than in marine fish. Thiamin is only destroyed after contact with the thiaminase for a period of time; therefore, feeding fresh fish or feeding thiamin in a separate diet from the raw fish will not cause a thiamin deficiency (Camacho, 1974). Heating or ensiling thiaminase-containing raw fish reduces thiaminase activity (Greig and Gnaedinger, 1971; Anglesea and Jackson, 1985).

NATURAL CONTAMINANTS OF FEEDSTUFFS

Some substances may be produced by natural processes in feedstuffs or in the aquatic environment that may affect fish performance. Feedstuffs can become contaminated with mycotoxins, dietary lipids can oxidize, and algal and other marine toxins can be produced in the water and impair proper nutrient uptake or metabolism.

Mycotoxins

Many fungi grow well on a number of diet ingredients or processed diets under proper temperature and moisture conditions. They produce mycotoxins that are carcinogenic, cytotoxic, or neurotoxic (Eiroa and Nelly, 1979; Lovell, 1991). Feeds contaminated with aflatoxins produced by the mold *Aspergillus flavus* were a major cause of liver hepatomes in rainbow trout (Wolf and Jackson, 1963; Halver, 1967). Of the different aflatoxins produced by the various strains of *Aspergillus*, the B_1 form was responsible for trout hepatoma (Halver, 1967) and can produce hepatoma at dietary concentrations as low as 0.5 µg/kg (Ashley et al., 1965; Sinnhuber et al., 1965).

The carcinogenic or toxic effects of aflatoxins in fish seem to be species specific. Coho salmon from the Great Lakes are similar to rainbow trout in sensitivity to aflatoxins (Black et al., 1988), but those from sea-run populations (Halver et al., 1967) and brook trout (Wolf and Jackson, 1967) are less sensitive than rainbow trout. Halver (1967) reported the 10-day oral LD_{50} of aflatoxin B_1 for rainbow trout to be 0.5 mg/kg of diet and cited an unpublished study by W. H. Hastings that showed the LD_{50} for channel catfish was 15 mg/kg of diet. Carp fed diets containing 2 mg/kg of aflatoxin B_1 showed no adverse effects. These observations may indicate that warm-water species are less sensitive to aflatoxins than cold-water fish. Jantrarotai and Lovell (1991a) found liver and kidney lesions and a reduction in growth rate and hematocrit but no mortalities in channel catfish fed aflatoxin B_1 at 10 mg/kg of diet for 10 weeks. The carcinogenicity

of aflatoxins is affected by other dietary factors such as the presence of cyclopropenoic fatty acids (Lee et al., 1968, 1971; Sinnhuber et al., 1968b) and gossypol (Sinnhuber et al., 1968a) from cottonseed meal and the pesticide dieldrin (Hendricks et al., 1979). Increasing the dietary concentration of protein also increased the carcinogenicity of aflatoxins (Lee et al., 1978; Stott and Sinnhuber, 1978).

Other mycotoxins are produced by molds and fungi that grow on feedstuffs. Friedman and Shibko (1972) identified 27 mycotoxin-producing fungi among 114 species isolated from 20 samples of dried shrimp. The tricothecene toxin, T_2, produced by the fungus *Fusarium tricintum* proved lethal to rainbow trout at a dietary concentration near 6 mg/kg body weight (Marasas et al., 1967). Poston et al. (1983), however, fed rainbow trout T_2 at 15 mg/kg of diet and found that the main effects were reduced feed consumption, reduced growth, lower hematocrit, and lower blood hemoglobin. Woodward et al. (1983) showed that rainbow trout had a sensitive taste acuity for vomitoxin produced by *Fusarium* and reduced their feed intake as the concentration of vomitoxin increased from 1 to 13 µg/g of diet; the fish refused to consume the diet with a vomitoxin concentration of 20 µg/g. Jantrarotai and Lovell (1991b) found that cyclopiazonic acid (CPA), a neurotoxin frequently found in association with aflatoxin, was more toxic to channel catfish than aflatoxins and is more frequently found than aflatoxins in feedstuffs in the southern United States. The minimum dietary concentration that caused a reduction in growth rate was 0.1 mg/kg for CPA as compared with 10 mg/kg for aflatoxin B_1.

Algal and Marine Toxins

The existence of toxic marine, estuarine, and freshwater algae has been well documented (Shilo, 1964; Fogg et al., 1973; Taylor and Seliger, 1979; Kungsuwan et al., 1987), and blooms of toxic algae may cause extensive mortality in fish culture facilities (Sparks, 1972; White, 1982; Meriwether et al., 1984; Saunders, 1988). Particular care must be taken to ensure that toxic algal species are not included in diets or nursery ponds of larval fish because the survival of many species depends on direct consumption of phytoplankton. Some mollusks can consume toxic algae and concentrate the toxins in their tissues (Sparks, 1972; White, 1982); therefore, contaminated mollusks must not be included in fish diets. Toxins of certain algae, such as *Microcystis aeruginosa*, do not seem to affect finfish (Phillips et al., 1985), but the toxins of other algal species, such as *Ganyaulax*and *Gyrodinium* spp., are highly toxic (Roberts et al., 1983).

Oxidative Rancidity

Autoxidation of unsaturated lipids produces a large number of chemical products, including free radicals, peroxides, hydroperoxides, aldehydes, and ketones. These compounds may be toxic to fish or react with other dietary components and reduce their nutritional value (Andrews et al., 1965; Crawford et al., 1966; Roubal and Tappel, 1966; Yamagita et al., 1973; Ko et al., 1975; Forster et al., 1988). The primary effect of feeding diets containing oxidized oils appears to be the interaction of the peroxidative decomposition compounds with vitamin E. Studies with rainbow trout (Sinnhuber et al., 1968b), channel catfish (Murai and Andrews, 1974), common carp (Hashimoto et al., 1966; Watanabe and Hashimoto, 1968; Iijima and Zama, 1979; Hata and Kaneda, 1980), and yellowtail (Ueda and Nagaoka, 1969; Park, 1978) have shown that the pathologies resulting from feeding oxidized oils were similar to those of vitamin E deficiency. Sinnhuber et al. (1968b) and Watanabe and Hashimoto (1968) demonstrated with rainbow trout and common carp that the toxic effects of feeding oxidized lipids could be prevented by increasing the supplement of vitamin E in the diet. Other studies with various fish species have shown that the toxic effects of oxidized oils can be ameliorated by additional vitamin E or a-tocopherol (Hashimoto et al., 1966; Watanabe and Hashimoto, 1968; Murai and Andrews, 1974). Hung and Slinger (1980), however, could show no toxic effects of adding oxidized fish oils to nutritionally complete natural ingredient diets for rainbow trout; the only adverse effect was a slight decrease in liver a-tocopherol level. Adding synthetic or natural antioxidants to feed lipids can prevent or minimize the adverse effects of autooxidized lipids. Antioxidants are discussed in Chapter 2.

HEAVY METALS

Metals may act as both nutrients and toxicants. A detailed discussion of maximum tolerable concentrations of the dietary essential metals is found in a report by the National Research Council (1980). Potential toxicity depends not only on their concentration in the diet but also on the concentration of other minerals, such as calcium and magnesium, in the rearing water (Spear and Pierce, 1978; Waiwood and Beamish, 1978; Carroll et al., 1979). The toxicity of metals can be reduced by other dietary components, such as phytin, which forms a nondigestible organic complex with certain metals. Metal chelators, such as ethylenediaminetetraacetic (EDTA), can reduce the toxicity of cadmium, copper, zinc, lead, and aluminum when added to the diet (Muramoto, 1980; 1981).

Mercury

The toxicity of mercury to fish depends on its chemical form. Rainbow trout are apparently unable to convert inorganic mercury to the more toxic methyl mercury (Pennacchioni et al., 1976) even though oral doses of inorganic mercury (mercuric chloride) increased tissue total mercury

concentration. Wobeser (1975) fed graded concentrations of methyl mercury chloride to rainbow trout and found that dietary concentrations of mercury up to 24 mg/kg did not cause mortalities, but that fish fed 16 mg/kg or more showed hyperplasia of the gill epithelium and reduced hematocrits. Coho salmon fed dogfishmeal with 2.3 mg total mercury/kg to replace 50 percent of the herring meal in an Oregon moist diet grew as large as control fish and did not accumulate total body mercury concentrations above the U.S. Food and Drug Administration (FDA) tolerance level of 0.5 mg/kg (Spinelli and Mahnken, 1976).

Fish accumulate mercury in muscle tissue and the rate is influenced by dietary form and concentration (Pennacchioni et al., 1976) and fish size (Scott and Armstrong, 1972). The bioaccumulation of mercury is directly correlated with fish size (Friedman and Shibko, 1972). Feedstuffs fed to fish grown for human consumption should be scrutinized for mercury concentration because mercury could possibly accumulate in the fish and exceed the FDA tolerance level. Selenium was found to reduce the toxicity of methyl mercury (Ganther et al., 1972; Friedman et al., 1978) and decrease the rate of mercury bioaccumulation in fish, crayfish, and lake sediment biota (Rudd et al., 1980).

Cadmium

Water-borne cadmium has been shown to be toxic to many fish species (Sangalang and O'Halloran, 1972; Kumada et al., 1973; Clearley and Coleman, 1974; Benoit et al., 1976; Smith et al., 1976). Cadmium absorbed through the gastrointestinal tract (by gastric intubation) was shown to cause liver necrosis and mortality at doses as low as 5 µg/g body weight.

Arsenic

The main source of arsenic in ingredients used in fish feeds comes from marine fishmeal. Reinke et al. (1975) reported arsenic concentrations in tissues of a number of commercial fish species from the North Atlantic ranging from 1.8 to 40 mg/kg; however, most of the arsenic was in the form of an organic complex rather than the highly toxic arsenite. The potential toxicity to fish of feeding diets containing organic arsenic compounds is not known.

Polychlorinated Biphenyls

The polychlorinated biphenyls (PCBs) are widely used industrially as plasticizers and as heat-transfer, dielectric, and hydraulic fluids. They are poorly biodegraded and accumulate in lipids, and have been found in marine and freshwater organisms from almost all areas of the United States (Addison, 1976; Ito and Konishi, 1980; Peneva, 1980; Brunn et al., 1981; Falandysz and Ganowiak, 1981; Veith et al., 1981). Fish oil and meal represent the primary sources for PCB contamination of fish diets (Hansen et al., 1976). A PCB dosage of 14.5 mg/kg body weight resulted in 100 percent mortality of coho salmon after 260 days (Mayer et al., 1977). Sublethal effects of PCB exposure in fish include liver enlargement, lesions in the liver ultrastructure, inhibition of hepatic aryl hydrocarbon hydroxylase and other hepatic microsomal enzymes (Lidman et al., 1976; Addison et al., 1977, 1978, 1979; Gruger et al., 1977; Shelton et al., 1984), and decreased thyroid activity (Leatherland and Sonstegard, 1978, 1980). These compounds accumulate in fish tissues (Guiney and Peterson, 1980); therefore, prolonged feeding of dietary concentrations below the toxicity level may result in tissue accumulation that would be toxic to the fish or that are above FDA-approved levels (0.2 mg/kg) for human food.

PESTICIDES

The FDA restrictions on the use and concentration of pesticides in agricultural products used for human or animal consumption make it unlikely that fish feeds will be sufficiently contaminated to cause acute toxicities. Fish are most likely to be exposed to pesticides through accidental contamination of feedstuffs with hazardous quantities of insecticides or rodenticides, or use of water that has been contaminated by these chemicals, such as through aerial spraying. Most pesticides bioaccumulate in fish, therefore, prolonged exposure to small amounts, from the water or the diet, may result in accumulations in the tissue that will affect the health of the fish or the marketability of the product for human food. Ashley (1972) evaluated the toxic effects of chlorinated hydrocarbons in several fishes. Toxicity was greatest in young fish, characterized by dysplasia and sterility of gonads, lethargy, nervous disorders, anorexia, and death. The toxicity to DDT decreased among fish species in the following order: rainbow trout, brown trout, guppy, bluegill, and channel catfish. The insecticide DDT caused inhibition of gill and kidney Na^+- and K^+-ATPase (Campbell et al., 1974), liver tumors (Halver, 1967), nervous disorders (Bahr and Ball, 1971), and acute toxicity (Buhler et al.,1 969) in various fishes.

4

Digestibility and Absorption

The nutritional value of a feedstuff is based not solely on its chemical composition but also on the amount of the nutrients or energy the fish can absorb and use. The bioavailability of nutrients or energy in feedstuffs for fish may be defined mainly 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 (DE) and metabolizable energy (ME) have been used to express feedstuff values for fish (National Research Council, 1981, 1983), 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 1.

METHODS OF DIGESTIBILITY DETERMINATION

Methods for determining digestibility coefficients involve either a direct or an indirect measurement of the amount of nutrient ingested and subsequently excreted. The test feedstuffs have been fed singly or in combination with other ingredients.

Indirect Method

This method involves the use of a nondigestible marker, such as chromic oxide (Cr_2O_3), which is included in the diet at a concentration of 0.5 to 1.0 percent. It is assumed that the amount of the marker in the feed and feces remains constant throughout the experimental period and that all of the ingested marker will appear in the feces. The digestibility of the nutrient in question can be determined by assessing the difference between the feed and fecal concentrations of the marker and the nutrient or energy. The percent nutrient digestibility can be estimated by use of the following formula:

$$\text{digestion coefficient of nutrient} = 100 - 100 \frac{\text{percent marker in feed} \times \text{percent nutrient in feces}}{\text{percent marker in feces} \times \text{percent nutrient in feed}}$$

This method has been used to determine the digestion coefficients for energy, crude protein, carbohydrates, lipids, and dry matter for several fish species (Nose, 1960a,b; Hastings 1966, 1969; Smith and Lovell, 1971, 1973; Windell et al., 1978a,b; Wilson et al., 1981; Cho et al., 1982). The indirect method has the advantages that it eliminates the need to quantitatively collect all of the excreta, and the test fish can eat voluntarily.

Proper fecal collection to prevent loss of soluble nutrients into the water is critical in digestion trials, whether the direct or indirect method is used. Data from Smith et al. (1980) indicate that a significant amount of the fecal nitrogen from rainbow trout is in liquid form and can leach out of the feces into the water prior to collection. The calculation of digestibility when a significant amount of soluble nutrients have leached from the feces gives digestion coefficients that are erroneously high (Smith and Lovell, 1971; Windell et al., 1978b). Some investigators have therefore chosen to collect the feces directly from the rectum using anal aspiration (Windell et al., 1978b), surgical excision (Smith and Lovell, 1971), or stripping (Nose, 1960a,b) to minimize the problem of nutrient leaching. Austreng (1978) cautioned that if stripping was used to remove the feces from the rectum, care should be taken not to obtain partially digested feed or biological fluids from the gut. Choubert et al. (1979) and Cho and Slinger (1979) have shown, however, that if excretions are removed from the fish tank soon after expulsion, the collection of passively excreted feces can give good digestibility data. Digestion coefficients determined

by the indirect method have been useful and feeding regimens based on these data have been successful (Takeuchi et al., 1979; Cho and Kaushik, 1985; Wilson and Poe, 1985; Mangalik, 1986; Satoh et al., 1992).

Direct Method

This method involves measuring all the feed consumed by the fish and all of the resulting excreta. Smith (1971, 1976) and Smith et al. (1980) used an aquatic modification of the metabolism chamber designed for terrestrial animal studies; it allowed for the separate quantitative collection of gill, urine, and fecal excretions of rainbow trout. The fish were force-fed a measured amount of feed, and the various excrements were subsequently collected and analyzed for their nutrient content. The amounts of the nutrients in the excrements were then subtracted directly from those in the feed to determine the amounts retained. This method allows for determining carbon and nitrogen balances as well as DE and ME values. Also, the problem of fecal leaching is eliminated because all of the water in the chamber is included in the analyses. The method is open to criticism, however, because the fish are immobilized and force-fed and so stressed that the utilization of the feed may be compromised. Use of this method has been restricted to rainbow trout and attempts to adapt it to other species have so far been unsuccessful.

Neither the direct nor indirect method accounts for the inclusion of materials of endogenous or metabolic origin in the excreta. Therefore, the data obtained are actually apparent rather than true digestibility values. Nose (1967) found that 3.1 percent of the fecal nitrogen collected from rainbow trout came from endogenous sources, and Foltz (1978) found that the endogenous protein content of the feces rose from 3.1 to 8.4 percent as water temperature increased from 7° to 19°C. This distinction between apparent and true digestibility probably has little practical impact on feeding practices, however, and thus, the reported apparent digestibility values are quite adequate.

Assay Diets

Very few feedstuffs are fed as the sole component of a fish diet; therefore, some researchers evaluate the digestibility of a feedstuff in combination with other ingredients in the assay diet. Cho et al. (1982) determined digestible energy and digestible protein coefficients for feed ingredients by comparing the digestibility of a reference diet with that of an assay diet that contained a mixture of the reference diet (70 percent) and the test ingredient (30 percent). The reference diet was composed of natural ingredients similar to a commercial diet. Digestion coefficients were determined for the reference and assay diets by the indirect method described above, and these coefficients were used to calculate the digestibility of the test ingredient according to the following expression:

$$\begin{aligned}\text{digestibility of test ingredient} &= \text{digestion coefficient of test diet} \\ &\quad - \frac{70}{100} \times \text{digestion coefficient of reference diet}\end{aligned}$$

Wilson and Poe (1985) also used this procedure to determine digestion coefficients for energy and protein in diets and diet ingredients for channel catfish. This method has advantages over testing ingredients singly in that any synergistic 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, which leads to a normal level of intake so that the difference between apparent and true digestibility is minimized and negative nitrogen balance is avoided.

FACTORS AFFECTING DIGESTIBILITY

No differences were found in protein and energy digestibility coefficients for rainbow trout as water temperature ranged from 7° to 18°C (Windell et al., 1978a; Cho and Slinger, 1979; Cho and Kaushik, 1990). The apparent lack of response to temperature change has also been found in common carp (National Research Council, 1977). Cho and Kaushik (1990) reported a small increase in crude fat digestibility at 18° versus 9°C when highly saturated animal fats were fed. The rate of movement of ingesta through the digestive tract of channel catfish was found to increase at temperatures above 26°C, but no increase in digestibility coefficients was noted (Shrable et al., 1969). It may be concluded, therefore, that nutrient digestibility is little affected by water temperature within the species' range of normal growth.

It has been found in a number of species that as meal size increases, digestive and absorptive efficiencies decrease (Kinne, 1960; Pandian, 1967; Solomon and Brafield, 1972; Elliot, 1976; Windell et al., 1978a; Andres, 1979). Wilson and Poe (1985) found that extrusion processing increased the digestibility of energy, but had no effect on the digestibility of protein as compared with pellet processing for catfish diets. Cruz (1975) found similar results with diet ingredients for catfish.

NUTRIENT DIGESTIBILITY

Table 4-1 presents digestibility coefficients for protein, lipids, and carbohydrates in various feedstuffs for chinook

salmon, rainbow trout, channel catfish, and blue tilapia. The DE and ME values for feed ingredients are given in Table 8-1. Amino acid availability and protein digestibility values for certain feed ingredients are given in Table 4-2 for Atlantic salmon and channel catfish.

TABLE 4-1 Apparent Digestibility of Protein, Fat, and Carbohydrate in Selected Diet Ingredients for Chinook Salmon, Rainbow Trout, Channel Catfish, and Blue Tilapia

Ingredient	International Feed Number	Protein (%)				Lipid (%)			Carbohydrate (%)		
		Chinook Salmon	Rainbow Trout	Channel Catfish	Blue Tilapia	Rainbow Trout	Channel Catfish	Blue Tilapia	Rainbow Trout	Channel Catfish	Blue Tilapia
Alfalfa meal	1-00-023	—	61[a]	—	66[b]	71[c]	51[d]	—	—	12[d]	27[b]
Blood meal	5-00-381	30[e]	69[a]	74	—	—	—	—	—	—	—
Casein	5-01-162	—	95[a]	97[f]	—	—	—	—	—	—	—
Canola meal	5-06-145	79[e]	—	—	—	—	—	—	—	—	—
Corn, grain	4-02-935	—	95[c]	60[d]	84[b]	—	76[d]	90[b]	—	66[d]	45[b]
cooked		—	—	66[d]	79[b]	—	96[d]	—	—	78[d]	72[b]
Corn gluten meal	5-28-242	—	87[a]	—	—	—	—	—	—	—	—
Cottonseed meal	5-01-621	—	76[a]	83[g]	—	—	88[d]		84[a]	17[d]	—
Fish, anchovy meal	5-0-985	92[e]	—	—	—	—	—	—	—	—	—
Fish, herring meal	5-02-000	91[e]	87[a]	—	—	97[c]	—	—	—	—	—
Fish, manhaden meal	5-02-009	83[e]	—	88[g]	85[b]	—	97[d]	98[b]	—	—	—
Meat and bone meal	5-00-388	85[c]	82[g]	78[b]	—	77[c]					
Poultry by-product meal	5-03-798	74[e]	68[c]	—	—	—	—	—	—	—	—
Poultry, feathers, hydrolyzed	5-03-795	71[e]	58[c]	74[d]	—	68[c]	83[d]	—	—	—	—
Soybean meal, 44 percent	5-04-604	—	—	—	94[b]	—	—	—	—	—	54[b]
Soybean meal, 48 percent	5-04-612	77[e]	83[a]	93[g]	—	—	81[d]	—	—	—	—
Starch, corn (uncooked)											
50 percent		—	—	—	—	—	—	—	24[a]	—	55[f]
25 percent		—	—	—	—	—	—	—	—	—	61[f]
Starch, corn (cooked)											
50 percent		—	—	—	—	—	—	—	52[a]	—	66[f]
25 percent		—	—	—	—	—	—	—	—	—	78[f]
Wheat middlings	4-05-205	86[e]	76[a]	72[d]	—	—	—	—			
Wheat grain	4-05-268	—	—	92[g]	90[b]	—	96[d]	85[b]	—	59[d]	61[b]
extruded		84[e]	—	—	—	—	—	—	—	—	—

NOTE: Dashes indicate data were not available.

[a]Smith (1977) and Smith et al. (1980) determined by metabolism chamber, single ingredient fed.
[b]Popma (1982) determined by indicator method, feces collected by frequent removal from water, ingredient fed in mixed diet.
[c]Cho et al. (1982) determined by indicator method, feces collected from a settling column outside the fish tank, ingredient fed in mixed diet.
[d]Cruz (1975) determined by indicator method, feces collected by surgical excision, single ingredient fed.
[e]Hajen et al. (1992) determined by indicator method, feces collected from water, ingredient fed in mixed diet.
[f]Saad (1989) determined by indicator method, feces collected by surgical excision, ingredient fed in mixed diet.
[g]Wilson and Poe (1985) determined by indicator method, feces collected by surgical excision, ingredient fed in mixed diet.

Protein

Proteins in most feedstuffs that have been properly processed are highly digestible to fish. The digestion coefficients for protein in protein-rich feedstuffs are usually in the range of 75 to 95 percent. Protein digestibility tends to be depressed as the concentration of dietary carbohydrate increases (Inaba et al., 1963; Kitamikado et al., 1964a,b; Nose, 1967; Page and Andrews, 1973; Smith and Lovell, 1973; Austreng et al., 1977; Rychly and Spannhof, 1979). Dabrowski et al. (1986) found no difference in protein utilization by rainbow trout held in either freshwater or sea water, and they concluded that salinity does not have an impact on protein digestibility. Overheating fishmeal during the drying process can greatly reduce its nutritive value (Finley, 1989). By contrast, insufficient heating of soybean meal decreases the availability of protein. Smith (1976) showed that increasing the heating temperature from 127° to 204°C increased protein digestibility in soybean meal from 45 to 75 percent.

TABLE 4-2 True Amino Acid Availability and Protein Digestibility Values for Certain Feed Ingredients for Atlantic Salmon[a] and Channel Catfish[b]

Feed Ingredient Fish Species	International Feed No.	Protein (%)	ARG (%)	CYS (%)	HIS (%)	ILE (%)	LEU (%)	LYS (%)	MET (%)	PHE (%)	THR (%)	TRYP (%)	TYR (%)	VAL (%)
Canola meal	5-06-145													
Atlantic salmon		91.4	96.7	97.1	95.0	87.3	85.0	92.0	99.9	89.2	93.2	—	92.9	83.8
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 (flame-dried)	5-02-000													
Atlantic salmon (steam-dried)		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
Atlantic salmon (low temperature)		82.6	94.1	94.1	88.2	89.0	89.0	90.1	88.6	88.9	94.9	56.7	90.2	88.3
Atlantic salmon		88.8	95.8	95.6	92.6	94.7	94.1	95.8	92.0	93.4	99.8	86.2	96.5	93.5
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	—	82.2	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
Rice bran	4-03-928													
Channel catfish		—	94.2	—	83.4	87.5	90.5	94.7	88.2	89.5	88.2	—	93.7	89.2
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

NOTES: Dash indicates data were not available. ARG, arginine; CYS, cystine; HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE, phenylalanine; THR, threonine; TRYP, tryptophan; TYR, tyrosine; VAL, valine.

[a]Data for Atlantic salmon from Anderson, J. S., S. P. Lall, D. M. Anderson, and J. Chandrasoma. 1992. Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo salar*) reared in seawater. Aquaculture 108:111–124.

[b]Data for channel catfish from 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.

Lipids

Fat when administered either alone or in a mixed diet routinely gives digestibility values of 85 to 95 percent for fish (Cho et al., 1974; Windell et al., 1974; Cruz, 1975; Austreng, 1978, 1979; Cho and Slinger, 1979; Takeuchi et al., 1979; Cho and Kaushik, 1990). The digestibility estimates for fats, however, often vary markedly when dietary concentrations are low (Smith et al., 1980). Takeuchi et al. (1979) reported that for common carp the digestibility coefficients for soybean, coconut, and pollack oils were about 90 percent, but that of beef tallow was only 76 percent.

The ability of channel catfish to digest beef tallow increased from 70 to 94 percent when the water temperature was raised from 23° to 28°C (Andrews et al., 1978). Similar results for rainbow trout were found when the temperature was raised from 9° to 18°C (Cho and Kaushik, 1990). Austreng (1979) showed that the ability of rainbow trout to digest fatty acids of the same chain length increased as the degree of unsaturation increased and decreased with increasing chain length up to a chain length of 22 carbons.

Carbohydrates

Salmonids absorb glucose well, but they are less efficient at utilizing dextrin or starch (Phillips et al., 1948; Buhler and Halver, 1961; Singh and Nose, 1967; McCartney, 1971; Cho and Slinger, 1979; Kaushik and Olivia-Teles, 1985). Cho and

Slinger (1979) showed that noncooked starch was only 49 percent digestible to rainbow trout. However, channel catfish (Wilson and Poe, 1985) and Nile tilapia (Popma, 1982) digested over 70 percent of the energy in noncooked starch. Cooked starch was more readily digested by trout (Smith, 1976; Smith et al., 1980) and channel catfish (Wilson and Poe, 1985). Falge et al. (1978) and McCartney (1971) showed that trout amylase activity was affected by the type and the amount of carbohydrate in the diet and that increasing carbohydrate load in the diet generally resulted in a decrease in enzyme activity. Saad (1989) showed that the digestibility of cooked corn starch decreased from 83 to 78 and 66 percent as the concentration of starch in the diet increased from 12.5 to 25 and 50 percent. Austreng et al. (1977) also showed a reduction in the ME value of the diet as carbohydrate increased.

TABLE 4-3 Net Absorption of Phosphorus from Various Sources by Channel Catfish, Common Carp, and Rainbow Trout

Source	International Feed Number	Channel Catfish (%)	Common Carp (%)	Rainbow Trout (%)
Animal products				
Casein	5-01-162	90[a]	97[b]	90[b]
Egg albumin	—	—	71[b]	—
Anchovy fishmeals	5-01-985	—	—	—
Brown fishmeals	—	—	24[b]	74[b]
Menhaden fishmeals	5-02-009	60[c]	—	—
White fishmeals	—	—	0[d]–18[b]	66[b]
Inorganic phosphates				
Calcium, monobasic	6-01-082	94[c]	94[b]	94[b]
Calcium, dibasic	6-01-080	65[c]	46[b]	71[b]
Calcium, tribasic	6-01-084	—	13[b]	64[b]
Potassium, monobasic	—	—	-94[b]	-98[b]
Sodium, monobasic	6-04-288	90[c]	-94[b]	-98[b]
Plant products				
Corn, ground	4-26-023	25[c]	—	—
Phytate	—	1[c]	8–38[b]	—
Rice bran	4-03-928	—	25[b]	19[b]
Soybean meal, dehulled	5-04-612	29[a]	—	—
Wheat germ	5-05-218	—	57[b]	58[b]
Wheat middlings	4-05-205	28[c]	—	—
Yeast, brewers	7-05-527	—	93[b]	—

[a] Data from Wilson, R. P., E. H. Robinson, D. M. Gatlin, III, and W. E. Poe. 1982. Dietary phosphorus requirement of channel catfish. J. Nutr. 112:1197–1202. Values are expressed as percent apparent absorption.
[b] Data from 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.
[c] Data from Lovell, R. T. 1978. Dietary phosphorus requirement of channel catfish (*Ictalurus punctatus*). Trans. Am. Fish. Soc. 107:617–621. Values are expressed as percent apparent absorption.
[d] Data from Yone, Y., and N. Toshima. 1979. The utilization of phosphorus in fishmeal by carp and black sea bream. Bull. Jpn. Soc. Sci. Fish. 45:753–756.

MINERALS

The relative availability of dietary phosphorus is affected by both chemical form and fish species. Table 4-3 presents the net absorption of various sources of phosphorus for four species of fish. Generally, phosphorus absorption by channel catfish and rainbow trout 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 and tribasic calcium phosphates vary in their availabilities but are generally less available than the monobasic form. The availability of phosphorus from fishmeal, which is primarily of bone origin, is generally lower than that of certain other high-protein feedstuffs, such as casein and yeast. The availability of phosphorus from fishmeals with high ash (bone) content (above 16

percent) varies among species, but it is generally lower than that of low-ash fishmeals (Yone and Toshima, 1979).

The bioavailability of phosphorus in fish diets is an important consideration because of the effect of phosphorus from the discharge of fish culture operations on stream eutrophication. Thus, highly available forms of phosphorus should be used and at minimum concentrations to meet dietary requirements. Phytate is the primary form of phosphorus in grains, and its availability to fish is very low. For this reason, most of the phosphorus from grain sources is excreted in the feces and may contribute to effluent pollution problems caused by hatcheries (Ketola, 1985).

Differential absorption of different forms of other minerals has also been noted in several fish species. Examples of this include the superior absorption by common carp of calcium in the lactate form as compared with the carbonate form (Nakamura and Yamada, 1980) and the higher availability in Atlantic salmon of selenomethionine as compared with other sources of selenium (Bell and Cowey, 1989).

The bioavailability of zinc from fishmeals appears to be inversely correlated with the ash content. White fishmeal is a by-product meal made from fish processing waste and, because of its high bone content, usually has ash values in excess of 16 percent. Rainbow trout that had been fed diets containing white fishmeal exhibited signs of zinc deficiency (Ketola, 1978, 1979; Ogino and Yang, 1978) even though it was calculated that the zinc content of the diets was adequate. The deficiency signs were alleviated by the addition of supplemental zinc to the diet. Ogino and Yang (1979) also reported that a similar condition developed in carp fed diets containing high-ash fishmeal. Satoh et al. (1987) reported a negative correlation between tricalcium phosphate content of the diet and zinc availability to rainbow trout. Gatlin and Wilson (1984) found that zinc bioavailability in channel catfish was reduced by dietary phytate, and recommended that the zinc allowance in commercial feeds be increased to five times the requirement established with purified diets. Satoh et al. (1983a,b) have shown that a similar pattern of nonavailability exists for magnesium in carp and trout fed diets containing high-ash fishmeals.

5

Diet Formulation and Processing

A primary objective in diet formulation for fish is to provide a nutritionally balanced mixture of ingredients to support the maintenance, growth, reproduction, and health of the animal at an acceptable cost. The mixture should also facilitate the manufacturing process to produce a diet with the desired physical properties. The diet should be palatable to the fish and not contain antinutritional components at concentrations that would impede the performance of the fish. The diet should be compatible with desirable flesh qualities of the fed fish and have minimum effect on water quality in the culture system. Readers needing to have more details on formulation and processing of fish diets may refer to recent publications of Cho et al. (1985), Halver (1989), Lovell (1989), Robinson (1991), and Wilson (1991) as well as the bulletin of the Food and Agriculture Organization (FAO) and the United Nations Development Program (UNDP) (1978).

USE OF NUTRIENT REQUIREMENT DATA

The energy and nutrient requirements presented in this report were determined primarily with small fish under optimum growth conditions and represent levels affecting maximum growth rate. Fish size, metabolic function, management, and environmental factors have slight to profound effects on dietary nutrient levels for optimum performance. Thus, these data represent approximations and should be used with discretion. Also, these requirement data were determined with diets containing chemically defined and purified, highly digestible ingredients; therefore, the data represent near 100 percent digestibility to the fish. Allowances should be made when formulating natural ingredient diets for bioavailability of nutrients, processing and storage losses, and cost.

If the dietary energy and nutrient requirements are not known for a species, the requirements established for a related species can be discretely substituted. Generally, variation among fishes should be expected between warm-water and cold-water species and between freshwater and saltwater ones. As more information becomes available on nutrient requirements of various species, the recommended nutrient allowances for specific needs will become refined and commercial feeds will be more cost-effective.

FORMULATING FISH DIETS

Protein is usually the first nutrient considered, with the level of energy in the diet being adjusted to provide the optimum ratio. The protein has to be balanced for essential amino acids. The amount of carbohydrate in the diet varies with fish species, depending on their ability to use it as an energy source, and processing requirements. The type and concentration of lipids used in the diet are selected to satisfy essential fatty acid (EFA) and energy requirements. The vitamin requirements are mostly supplied from a supplemental premix because of uncertainty over content and bioavailability of vitamins in the feedstuffs. Mineral contents of feedstuffs are more consistent, so mineral supplementation usually is made on the basis of the composition of the major ingredients. Overfortification of labile nutrients in processed fish feeds is necessary as a safety factor. Amino acids, several vitamins, and inorganic nutrients are relatively stable to heat, moisture, and oxidation that occur under normal processing and storage conditions. Some of the vitamins are subject to some loss, however, and should be used in excess of the requirement.

Least-cost formulation using linear programming methods is commonly used to derive minimum-cost diets for fish and other food animals. The following information must be available: nutrient requirements of the animal; bioavailable nutrient and energy content of the ingredients; minimum and maximum restrictions on concentrations of various ingredients;

and cost of ingredients. The bioavailability of nutrients to fish from various feedstuffs must be known in order to make computerized substitutions among ingredients. These values are often quite variable among fish and among feedstuffs. For example, it is well known that cold-water fishes to not utilize carbohydrates as energy sources as well as do warm-water species; digestibility of phosphorus is less for fish than for livestock, especially for fish without gastric secretions in the digestive tract (Nose and Arai, 1976); and the lysine in cottonseed meal is less digestible than the lysine in soybean meal (Wilson et al., 1981).

Restrictions can be placed on minimum or maximum concentration levels of certain ingredients because of their effects on manufacturing process and palatability or their potential adverse effects on fish performance, flesh quality, or water quality. For example, fishmeal and other animal protein sources have been found to be beneficial in catfish diets for reasons not explained on the basis of meeting amino acid requirements (Mohsen and Lovell, 1990), therefore, minimum levels are usually assigned. The content of cottonseed meal in fish diets is sometimes restricted because of free gossypol toxicity (Herman, 1970). Carotenoid concentrations should be controlled because xanthophylls impart undesirable yellow pigmentation to light-fleshed fish (Lee, 1987), whereas red pigmentation sources are necessary in the diets of salmonids.

COMMERCIAL-DIET INGREDIENTS

Ingredients used in commercial fish diets can be classed as sources of protein (amino acids), energy, EFA, vitamins, and minerals. Special ingredients may be used to enhance growth, pigmentation, or sexual development and to prepare diets having the required physical, palatability, and preservation properties. The nutrient composition of some ingredients commonly used in fish diets is presented in Chapter 8.

Fishmeal prepared from good-quality, whole fish is one of the highest-quality protein sources commonly available. It is also a rich source of energy, EFA, and minerals and is highly digestible and palatable to most fishes. fishmeal made from fish parts, such as waste from fish processing and canning plants, has a lower percentage of high-quality protein than that of meal from whole fish. It is also high in ash and should be used prudently in fish diets as it can produce mineral imbalances.

Other animal protein sources are by-products, such as meat and bone meal and poultry by-product meal, that contain about 45 to 55 percent crude protein. The quality of the protein in these by-products is less than that of whole fishmeal, and the ash content is usually high because a significant amount of the material comes from bone and other nonmuscle tissue. Flash or spray-dried blood meal is rich in protein (80 to 86 percent) but low in methionine and unbalanced in branched-chain amino acids. Feather meal is high in crude protein (80 percent) but, unless the feathers are thoroughly hydrolyzed during processing, digestibility is low (Cho and Slinger, 1979).

Soybean meal is universally available and has one of the best amino acid profiles of all protein-rich plant feedstuffs for meeting most of the essential amino acid requirements of fish (Mohsen, 1989). Some fish, such as young salmon, find soybean meal unpalatable (Hardy, 1989) while others, such as channel catfish, readily consume diets containing up to 50 percent soybean meal (Robinson, 1991). Soybeans contain several antinutritional factors (discussed in Chapter 3), but heating during commercial oil extraction destroys much of the activity. Meals from cottonseed and peanut (groundnut) are concentrated sources of protein and have been used in fish feeds in the United States. Compared with soybean meal, however, these meals are seriously limiting in lysine and methionine. Also, most cottonseed meals contain free gossypol, which is moderately toxic to monogastric animals and limits its use in fish feeds. Lupin flour effectively replaces full-fat soybean flour as a protein source in feeds for rainbow trout (Hughes, 1988). Meal from canola seed (low-glucosinolate rapeseed) has been used in experimental feeds for salmonids with success (Higgs et al., 1983). It has an amino acid profile comparable to soybean meal, but it is lower in protein and higher in fiber and tanins. When oilseed meals replace fishmeal or other animal by-product proteins in the diet, the losses in energy, minerals, and lipids should be considered. Dehulled soybean meal, for example, contains 25 percent less metabolizable energy (for rainbow trout), 86 percent less available phosphorus (for channel catfish), and 90 percent less (n-3) fatty acids than anchovy fishmeal on an equal dry matter basis (Lovell, 1984).

Carbohydrates are the primary nutritional contribution of grains. Whole grains contain 62 to 72 percent starch, which is 60 to 70 percent digestible by warm-water fish (Popma, 1982; Wilson and Poe, 1985) but markedly less digestible by salmonids (Smith, 1976; Cho and Slinger, 1979). Starch in grains is an important binding agent in steam-pelleted and extruded fish feeds. Fats and oils are used as energy sources, to provide EFA, and to coat the outside of pellets to reduce abrasiveness and dustiness. Marine fish oils are rich sources of essential (n-3) fatty acids, containing 10 to 25 percent of the highly unsaturated (n-3) fatty acids. Fatty acid composition of fats and oils from various sources is presented in Table 8-5.

QUALITY OF INGREDIENTS

Major ingredients used in fish diets should be analyzed regularly for proximate composition and for selected nutrients, such as limiting amino acids (usually lysine and sulfur amino acids) or EFA. Animal by-products that may contain

protein from bone, feathers, or connective tissues should be subjected to in vitro enzyme assays for an estimate of protein digestibility. All feed ingredients should be tested for mycotoxins before purchase. Periodic screening for pesticides and other contaminants (discussed in Chapter 3) are recommended. Standards may be established for some ingredients that vary considerably in quality and composition. Table 5-1 presents an example of quality standards for fishmeal and oil for use in salmonid diets.

TABLE 5-1 Suggested Quality Standards of fishmeal and Fish Oil for Salmonid Diets

Compound	Level
Fishmeal	
Crude protein[a]	>68 percent
Lipid	<10 percent
Ash	<13 percent
Sodium chloride	<3 percent
Moisture	<10 percent
Ammonia nitrogen	<0.2 percent
Antioxidant	200 mg/kg
Fish oil	
Peroxide value	<5 meq/kg
Anisidine value	<10 meq/kg
Nitrogen	<1 percent
Moisture	<1 percent
Antioxidant	500 mg/kg
Iodine value	>135
n-3 polyunsaturated fatty acids	>15 percent

[a] Percent nitrogen × 6.25.

SOURCE: Adapted from Cho, C. Y., C. B. Cowey, and T. Watanabe, eds. 1985. Finfish Nutrition in Asia. Methodological Approaches to Research and Development. Ottawa, Canada: International Development Research Center.

FEED PROCESSING

Fish feeds should be processed into water-stable, particulate forms (granules, pellets) for efficient consumption by the fish and to minimize fouling of the water. Most manufactured fish feed is processed by compression pelleting or extrusion; other manufactured forms include moist (or semimoist), microencapsulated, and micropulverized feeds. These processes will be introduced here; for more details on fish feed processing, refer to FAO/UNDP (1978), Hardy (1989), and Lovell (1989).

Steam pelleting, through compression, produces a dense pellet that sinks rapidly in water. This process involves the use of moisture, heat, and pressure to agglomerate ingredients into compact and larger particles. Steam added to the ground feed mixture (mash) during pelleting assists in partially gelatinizing starch, which aids in the binding of the ingredients. Generally, an amount of steam is added to the feed mixture to increase moisture content by approximately 5 to 6 percent and to assist in the elevation of temperature between 70° and 90°C. The pellets are cooled and dried by forcing air over the surface of the hot pellets immediately after they leave the pelleter. Steam-pelleted feeds must be firmly bonded to prevent rapid disintegration in water, which will reduce feed efficiency and water quality. Processing conditions and ingredient composition are both important. All ingredients should be finely ground to a particle size of 0.5 mm, or smaller, prior to pelleting. Starch and gluten are important for good pellet binding while fiber and fat are antagonistic to firm bonding. Thus, supplemental fats should not be added to the feed until after pelleting, and highly fibrous feedstuffs should not be used in large quantity. Special binding agents (discussed in Chapter 2) are sometimes used in quantities of 0.5 percent to 3 percent of the ingredient formula.

Extrusion is a process by which the feed mixture, in the form of a dough, is forced through a small orifice at high pressure and temperature. This process allows entrapment of water vapor by the feed particles, which on drying will float on water. Extrusion requires more elaborate equipment and higher inputs of moisture, heat, and pressure than pelleting. Usually the mixture of finely ground ingredients is conditioned with steam into a "mash" that may or may not be precooked before entering the extruder. The mash, which contains around 25 percent moisture, is compacted and heated to 104° to 148°C under pressure in the barrel of the extruder. As the material is squeezed through die holes at the end of the barrel, and external pressure decreases, part of the water in the superheated dough immediately vaporizes and causes expansion of the feed particles. The extruded particles contain more water than steam-pelleted particles and require external heat for drying. Thus, after extrusion, the particles must pass through a drying tunnel to reduce moisture to a safe storage level. Heat-sensitive vitamins, especially L-ascorbic acid, are added in excess prior to processing or applied to the surface after processing. Extruded feeds are firmly bound due to gelatinization of the starch and denaturation of the protein; this results in few fines and long water stability. Extruded feeds are preferred by many farmers, especially those feeding in large ponds, because they allow observation of the feeding process.

Granule diets for small fish are usually prepared by pelleting the ingredient mixture and subsequently reducing the size of the pellets by crumbling. The particles from the crumbled pellets are separated into various sizes by screening. Fat is usually sprayed onto the surface of the particles after processing. Considerable loss of water-soluble nutrients due to leaching may occur with small-particle diets because of the large amount of surface area, therefore,

overfortification of water-soluble vitamins is recommended to compensate this loss.

Microencapsulation involves coating a small particle of diet with a thin layer of a compound that will reduce disintegration, leaching, or, in some cases, bacterial degradation until the material is consumed by the fish or removed from the rearing container. The materials should be water insoluble but digestible by enzymes in the digestive tract of the fish. Several published and patented processes for microen-capsulation and microbinding vary with the encapsulation material and the substrate being coated. Nylon (N—N bonds) cross-linked proteins, calcium alginate, and oils have been used as encapsulation materials.

Moist or semimoist feeds are prepared by adding moisture and a hydrocolloidal binding agent, such as carboxymethylcellulose, gelatinized starch, or ground, wet animal tissue with the dry ingredients and forming the mixture into soft, moist pellets. The advantages of moist feeds are that some fish species find moist diets more palatable than dry diets, a steampelleting machine is not needed (a feed grinder will suffice), and heating and drying are avoided. Disadvantages of moist feeds are their susceptibility to microorganism or oxidation spoilage unless fed immediately or frozen. Fish parts going into moist feeds should be pasteurized to destroy possible pathogens and thiaminase. Some moist diets do not require frozen storage. They contain humectants, like propylene glycol, which lower water activity below that which will allow bacterial growth; they also contain fungistats, like propionic or sorbic acid, which retard mold growth (Lovell, 1989). These diets must be packaged in hermetically sealed containers, preferably under nitrogen, and stored at low temperatures for best keeping quality.

NATURAL INGREDIENT REFERENCE DIETS

Research laboratories and fish farming operations often have a requirement for a nutritionally complete, production-type reference diet to serve as a basis for developing and testing new feeds. Examples of production diets for salmonids and channel catfish are presented in Table 5-2. These diets contain commonly available ingredients, and they have been shown to produce good results under experimental and production conditions. Diets similar to the channel catfish diet have been used satisfactorily with other warm-water species such as tilapia and carp. It is important that these diets be produced with proper processing procedures and with high-quality ingredients, particularly the fishmeal and fish oil.

PURIFIED RESEARCH DIETS

All diets in an experiment should be alike in all respects except the variable being tested; this includes nutrient composition, energy level, palatability, water stability, and particle size. Experimental diets for evaluating nutrient requirements should be prepared from highly chemically defined ingredients. Casein and gelatin are a good protein combination for purified diets. Low-vitamin casein is available for use in vitamin experiments, however, regular casein is satisfactory for other experiments. Blood fibrin is a desirable protein for mineral studies, and chicken egg protein can be used in protein or amino acid experiments. These protein sources and others are available in highly purified forms. Dextrin is traditionally used as a carbohydrate source. Cooked starch is satisfactory for warm-water fish. Some of the lipid should be from fish oil to provide essential highly unsaturated (n-3) fatty acids. Purified cellulose can be used as a nonnutritive filler. The diets should contain a binding agent that will hold the particles together for a reasonable time in water. Gelatin, agar, alginic acid, wheat gluten, or carboxymethylcellulose can be used.

Examples of purified reference diets for salmonids and channel catfish are presented in Table 5-3. These diets can be prepared by compression-pelleting in a manner similar to that used in making low-moisture commercial diets, or as a moist diet using a laboratory feed grinder. Moist diets may be prepared by mixing the dry ingredients, then adding the oil with further mixing, followed by the addition of water (30 to 40 percent) to give the mixture a plastic consistency. If gelatin is to be the primary binding agent, it is mixed with hot water along with the oil; this mixture is added to the dry mixture. The dough is then extruded through a feed grinder and the extruded strands are broken into smaller pieces and dried or frozen.

Purified diets are sometimes unpalatable to some species. Palatability can sometimes be improved by substituting a natural feedstuff, such as fish flour, for a chemically defined ingredient in the diet formula. The nutritional contribution of the commercial to the purified diet should be known and should not confound the experimental diet.

TABLE 5-2 Examples of Natural Ingredient Reference Diets for Salmonids and Channel Catfish

Ingredient or Composition	International Feed Number	Guelph Salmonid, C-203[a]	Abernathy Pacific Salmon, 19-2[b]	Channel Catfish[c]
Ingredient, percent				
Fishmeal				
Herring	5-02-000	30	50	—
Menhaden	5-02-000	—	—	8
Soybean meal	5-04-612	13	—	50
Corn gluten meal	5-28-242	17	—	—
Corn	4-02-935	—	—	34.1
Wheat middlings	4-28-220	16.5	12.2	5
Dried whey	4-01-182	10	5	—
Blood meal	5-00-381	—	10	—
Condensed milk solubles	—	—	3	—
Poultry by-product meal	5-03-798	—	1.5	—
Wheat germ meal	5-05-218	—	5	—
Dicalcium phosphate	6-01-612	—	—	1
Fish oil				
Marine	—	11.5	9	—
Catfish	—	—	—	1.5
Vitamin mixture[b]	[e]	1	2.2	0.2
Trace mineral mixture[f]	[e]	1	0.1	0.2
Pellet binder	[e]	—	2.0	—
Composition				
Crude protein (N × 6.25), percent	[e]	38	50[g]	32
Digestible energy, kcal/g	[e]	4,100	4,200[g]	3,000

NOTE: Dashes indicate data or information were not available.

[a] This diet has been used successfully for Atlantic salmon and rainbow, brook, and brown trout. Source: Cho, C.Y. 1990. Fish nutrition, feeds, and feeding: With special emphasis on salmonid aquaculture. Food Rev. Int. 6(3):333-357.

[b] An Abernathy salmon diet. Source: Hardy, R.W. 1991. Pacific salmon, *Oncorhynchus* spp. Pp. 105-121 in Handbook of Nutrient Requirements of Finfish, R.P. Wilson, ed. Boca Raton, Fla.: CRC Press.

[c] Robinson, E.H. 1991. Nutrition, feeds, and feeding of channel catfish. Miss. Agric. For. Exp. Sta. Bull. 979.

[d] Vitamin mix should meet the vitamin requirements for the species presented in Chapter 7 with an allowance for processing and storage losses.

[e] An International Feed Number is not assigned.

[f] Mineral mix should provide the following quantities in mg/kg of diet for the following diets:

•Guelph salmonid: copper (from cuprous sulfate), 6.25; iron (from ferrous sulfate), 13.2; manganese (from manganese sulfate), 21.5; iodine (from potassium iodide), 6; zinc (from zinc sulfate), 52; salt (NaCl), 3,000.

•Abernathy Pacific salmon: zinc (from zinc sulfate, 75; manganese (from manganese sulfate), 20; copper (from cuprous sulfate), 1.5; iodine (from potassium iodide), 10.

•Channel catfish: zinc, 100; iron, 30; copper, 5; iodine, 5; manganese, 2.5; selenium, 0.3; cobalt, 0.05.

[g] Estimated.

TABLE 5-3 Examples of Purified Reference Diets

Ingredient	Guelph Salmonid[a]	Pacific Salmon, Modified H-440[b]	Channel Catfish[c]
Ingredient, percent			
Casein	40	40.8	32
Gelatin	4	8.0	8
Starch	11.5	—	—
Dextrin	9	16.0	33
D-glucose	5	—	—
Alpha-cellulose	3	4.7	14
Carboxymethyl-cellulose	—	—	2
DL-methionine	0.5	—	—
L-arginine	1	—	—
Amino acid mixture	—	4.4	—
Vitamin premix[d]	3	3.1	1
Mineral premix[e]	8	8.0	4
Marine fish oil	15	15.0	3
Vegetable oil	—	—	3

NOTE: Dashes indicate data were not available.

[a] This diet has been used successfully for Atlantic salmon and rainbow, brook, and brown trout. Source: Cho, C.Y., C.B. Cowey, and T. Watanabe, eds. 1985. Finfish Nutrition in Asia: Methodological Approaches to Research and Development. Ottawa, Canada: International Development Research Center.

[b] Hardy, R.W. 1991. Pacific salmon, *Oncorhychus* spp. Pp. 105-121 in Handbook of Nutrient Requirements of Finfish, R.P. Wilson, ed. Boca Raton, Fla.: CRC Press.

[c] Li, Y., and R.T. Lovell. 1985. Elevated level of dietary vitamin C increases immune responses in channel catfish. J. Nutr. 115:123-131.

[d] Should meet the vitamin requirements for the species, as presented in Chapter 7.

[e] Should meet the mineral requirements for the species, as presented in Chapter 7.

6

Feeding Practices

Different sizes and species of fish and the diverse environmental and management conditions used in aquaculture require different feeding strategies. Diet characteristics, such as source (living or nonliving feed), particle size, texture, density, and palatability, must be carefully considered for size and species of fish. Feed allowance and frequency of feeding are important for growth rate and feed efficiency. Type of feed (floating or sinking) used and method of feeding will depend on the fish, the culture system, and the equipment and personnel available. These strategic factors are as important as meeting the nutrient requirements of the diets and have been addressed in a number of recent publications. This chapter will focus on feeding young fish and some key points on production feeding of several commercially important species; reference will be made in appropriate places to more detailed sources of information.

FEEDING LARVAL FISH

The larval stage is defined by the metamorphosis of external and physiological characters from hatch until the juvenile stage is attained. External characteristics and major organ functions of juveniles match those in adult fish. For practical purposes, larval fish can be divided into three groups according to alimentary tract morphology and the enzymes secreted into the gut (Dabrowski, 1984a). The first group includes such fishes as salmonids and channel catfish, which appear to have a functional stomach before changing from endogenous to external feed. The second group includes fish, such as striped bass and many marine species, that at the larval stage have a very rudimentary digestive tract with no functional stomach or gastric glands and which undergo complex metamorphosis of the digestive system. The third group of larval fish are those that develop a functional digestive tract but remain stomachless throughout life, such as carps. Species that at the time of first feeding have structurally and functionally differentiated alimentary tracts pose less of a problem with initial feeding. Those with immature digestive systems at first feeding are more difficult to feed and usually require live feeds as a part of their diet.

Larval fish undergo different phases of larval metamorphosis and at a certain phase can be weaned to dry, prepared diets. For example, striped bass, which complete metamorphosis in 21 to 42 days, cannot use dry diets at day 5, when initial feeding begins, but they can at day 15 (Baragi and Lovell, 1986). Common carp can be transferred to commercial dry diets at the size of 15 to 30 mg (Bryant and Matty, 1980), whereas larval whitefish must obtain a size of 50 mg to be weaned to dry diets (Dabrowski and Poczyczynski, 1988a). The transition from live to dry diet is a gradual process.

One reason for the poor ability of some larval fishes to utilize prepared diets at first feeding may be the low affinity of the proteolytic enzymes in the immature digestive tract for the proteins offered in formulated diets (Dabrowski, 1984b). Several fish species have been shown to be unable to digest the protein from prepared diets at larval stages (Bremer, 1980). Also, the relatively high volume of feed consumed contributes to increased passage speed and results in low digestive efficiency (Kaushik and Dabrowski, 1983). Larval fish ingest more feed per unit weight than adult fish, consuming 50 to 300 percent of their body weight daily compared to 2 to 10 percent of body weight for subadult fish fed to marketable size (Bryant and Matty, 1980, 1981). The inability of some larval fish to immediately use prepared diets may also be due to the absence of enzymes, hormones or their regulators, or growth factors that are provided in live feeds (Lauff and Hofer, 1984; Baragi and Lovell, 1986). A lipid-soluble growth factor extracted from zooplankton was shown to be active for coregonid larvae (Rembold and Fluchter, 1988); however, the same fish species were grown successfully on a prepared diet that did not contain the extract or the live zooplankton (Dabrowski et al.,

(1984). Other dietary factors in live feed might inhibit or stimulate hormone action in larvae. Thyroid hormones, which play an important role in larval fish metamorphosis and growth (Collie and Stevens, 1985) may be influenced by diet components (Miwa and Inui, 1987; Specker, 1988; Inui et al., 1989).

Live Foods

The preferred live food organisms for larval fish are those in their natural diets; however, rotifer (*Brachionus plicatilis*) and brine shrimp (*Artemia*) are the only zooplankters produced in mass quantities. Variations of nutritional quality, primarily (n-3) polyunsaturated fatty acid (PUFA) content, exist among sources of zooplankton from different geographical origins and culture conditions (Watanabe et al., 1983). Many fish larvae are very sensitive to a deficiency of n-3 PUFAs (Watanabe et al., 1983; Gatesoupe and Le Millinaire, 1985); thus, the composition and amounts of fatty acids in zooplanktonic food affects growth and survival. Enrichment of live zooplankton with essential fatty acids may be accomplished by two procedures (Sorgeloos, 1980): (1) the newly hatched zooplankton can be fed for a period of 24 hours on marine algae (*Chlorella* spp.) or yeast containing a high concentration of (n-3) PUFA, or (2) the zooplankton nauplii can be exposed to a suspension of lipid rich in (n-3) PUFA, such as fish oil, and an emulsifying compound for 3 to 12 hours before being offered to the larvae.

Prepared Diets

Microparticulate diets for larval fish have to meet the nutritional requirements of the species, be of a size appropriate for ingestion; have the desired physical properties with regard to buoyancy, texture, and color; and, in many cases, simulate movement. Nutritional components of prepared diets for fish larvae should be determined on the basis of juvenile fish requirements, although outogenetic differences are possible. Scant information exists on the differences in quantitative nutrient requirements between larval fishes and juveniles, except for the common understanding that larval fish have a higher metabolic rate and thus benefit from a higher concentration of nutrients and energy in their diet (Dabrowski, 1986).

Optimum diet particle size increases in proportion to fish size and should not exceed 20 percent of the month opening (Dabrowski and Bardega, 1984). Frequent feeding is important in all larval fish; food can be offered 10 to 24 times per day or almost continuously and in excess (Charlon and Bergot, 1984; Charlon et al., 1986).

Diets containing 70 to 80 percent good-quality fishmeal support good growth in starter feeds for salmonids (Reinitz, 1983) and channel catfish (Winfree and Stickney, 1984); however, severe mortality occurred in larvae of sturgeon (Dabrowski et al., 1985), channel catfish (Winfree and Stickney, 1984), and common carp (Dabrowski et al., 1988), if casein was the major ingredient of the diet. Larval diet formulation based on single-cell protein and freeze-dried animal tissues (Dabrowski et al., 1984; Dabrowski and Poczyczynski, 1988b) proved successful with the stomachless larvae of common carp, grass carp, and silver carp. Microparticulate diets have been prepared for marine fish larvae composed of micropulverized meals from fish, crab, yeast, chicken egg yolk, short-necked clam, and krill (Kanazawa, 1986; Kanazawa et al., 1989).

CHANNEL CATFISH

Channel catfish, like salmonids, have a relatively well-developed digestive system and consume and assimilate prepared diets satisfactorily at the time the fish begin feeding. The time to initiate feeding is when the yolk sac reserves have been significantly reduced and the fish "swim up" to the water surface in search of feed. This phenomenon appears to be synchronized with histogenesis of the taste buds and mucous cells of the oropharyngeal region (Twango and MacCrimmon, 1977). Swim-up channel catfish are fed at hourly intervals by automatic feeders at rates of 25 percent of body weight per day. As fish size increases, these rates are reduced to four to two feedings of 5 to 10 percent of body weight daily while in the hatchery (Dupree and Huner, 1984). They usually begin on meal-type diets 0.5 mm in diameter and change to crumbles of 1 to 3 mm in size as they reach a fingerling size of about 10 g.

A popular commercial practice is to transfer the fish from the hatchery to prepared nursery ponds within a few days after feeding begins. The nursery ponds should have a good population of feed organisms and be free of predators (Dupree and Huner, 1984). The fish are fed prepared diets in the pond twice a day at the rate of 10 percent, decreasing to 3 percent, of body weight per day for the remainder of the growing season. Initially, nursery pond diets are 2- to 3-mm crumbles; later, small pelleted or extruded particles of 3 to 5 mm diameter are fed.

Most catfish produced in the United States are fed extruded diets in large ponds, 5 to 10 ha in size. Extruded diets float on the water surface and allow observation of the fish during feeding. Fish can, therefore, be fed closer to their maximum rate of consumption without overfeeding, and disease and water quality problems can be detected more easily. The use of pellets that sink can reduce feed costs by 10 to 20 percent when compared with feeding floating feeds; however, management skills must be better when feeding sinking pellets. Using sinking and floating feeds in combination (85 percent sinking and 15 percent floating) saves 10 to 15 percent in feed costs and still allows the management benefits of the floating feed (Mgbenka and

Lovell, 1985). Most commercial diets fed in production ponds contain 32 percent crude protein and 2.8 to 3.0 kcal of digestible energy/g. Li and Lovell (1991) showed that 24 percent crude protein diets allowed maximum weight gain in production ponds with satiation feeding, but 32 or 36 percent protein diets gave highest gains with restricted feeding. Most catfish farmers do not feed completely to satiation in large ponds to minimize wasted feed.

Traditionally, catfish are fed once daily, 6 or 7 days per week. Feeding twice daily when the water temperature is above 25°C will allow for a 20 percent higher rate of consumption and a comparably faster rate of growth (Andrews and Page, 1975). Feeding 7 days per week allows for 17 percent more feed to be consumed and 19 percent more growth than in a 6-day regimen (Lovell, 1979). Catfish should not be fed late at night or very early in the morning when dissolved oxygen (DO) in the pond water is low, because the increase in oxygen requirement of the fish should not coincide with the decrease in oxygen in the pond.

Table 6-1 shows daily feed allowances for channel catfish in the southern United States stocked in earthen ponds (8,800 fish/ha) in the spring and fed to near appetite capacity for a 6-month growing season (Stickney and Lovell, 1977). Factors such as temperature, water quality, fish density in the pond, and size of fish at different periods during the growing season will affect feed consumption by catfish. Therefore, the values in Table 6-1 are presented as a guide and other values may be appropriate for other conditions. Generally, catfish do not feed consistently in ponds when the water temperature drops below 21°C. A recommended guide for winter feeding of catfish in ponds is to provide feed-size fish at a daily rate of around 0.75 percent of their estimated weight when the water temperature at 1 m depth is equal to or greater than 13°C. Fingerling fish can be fed 1 percent of body weight three times per week or daily with extended periods of warm weather. Low-protein diets (25 percent) are recommended for winter feeding of marketable-size fish (>0.25 kg) (Robinette et al., 1982).

TABLE 6-1 Example of Daily Feeding Allowances for Channel Catfish Fed Once Daily from April until October in Ponds in the Southern United States

Water Temperature (°C)	Fish Size (kg)	Feeding Allowance (% of fish weight)
20.0	0.02	2.0
22.2	0.03	2.5
25.5	0.05	2.8
26.7	0.07	3.0
28.3	0.10	3.0
28.9	0.13	3.0
29.4	0.16	2.8
29.4	0.19	2.5
30.0	0.27	2.2
30.0	0.34	1.8
28.3	0.40	1.6
26.1	0.46	1.4
22.8	0.50	1.1

NOTE: Diet contains 3.0 kcal digestible energy/g and 32 percent crude protein.
SOURCE: Stickney, R. R., and R. T. Lovell, eds. 1977. Nutrition and Feeding of Channel Catfish. Southern Cooperative Series Bulletin 218. Auburn, Alabama: Auburn University.

More detailed information on feeding channel catfish is found in the comprehensive publications by Dupree and Huner (1984) on feeding practices for warm-water fishes and by Tucker and Robinson (1991) and Robinson (1991) on feeding channel catfish.

TILAPIA

Culture systems and husbandry methods used for producing tilapia (*Oreochromis* and *Tilapia* spp.) are diverse. Because tilapia are efficient feeders on natural aquatic feed organisms, they are often produced in ponds with low-cost supplemental diets. When natural feed constitutes an important source of nutrients, nutritionally balanced feeds are not necessary; impressive pond yields have been obtained by feeding only rice bran, brewery waste, copra meal, coffee pulp, or animal manures (Lim, 1989). Natural pond feed contributes a significant amount of protein, so 24 percent crude protein is sufficient for pond diets for tilapia (Lim, 1989). The importance of micronutrient supplementation of pond feeds for tilapia is not well known.

Intensive culture of tilapia has gained popularity in recent years. Nutritionally complete feeds are needed when the fish are stocked at high densities in tanks, raceways, net pens, and ponds, and natural feed is absent or insignificant. The nutrient requirements of tilapia appear to be similar to those of other warm-water fishes (Luquet, 1991). Commercial diets formulated for channel catfish and common carp have been fed successfully to tilapia (Lim, 1989).

Physical properties of pelleted tilapia feeds, especially size and water stability, are important. Tilapia prefer smaller pellets than channel catfish and salmonids of comparable size (Kubaryk, 1980). The most common pellet size for feeding tilapia to a marketable size of 0.5 kg is 3 to 5 mm in diameter. Tilapia respond to more frequent feeding than channel catfish and salmonids because of their continuous feeding behavior and smaller stomach capacity. Kubaryk (1980) found that Nile tilapia grew faster when fed four times daily rather than twice but did not grow faster when fed eight times. Suggested feeding rates and frequencies for various sizes of tilapia in commercial cultures are given in Table 6-2.

TABLE 6-2 Example of Daily Feeding Allowances and Frequencies for Various Sizes of Tilapias at 28°C

Size	Feeding Allowance (% 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

SOURCES: Jauncey, K., and B. Ross. 1982. A Guide to Tilapia Feeds and Feeding. Stirling, U.K.: University of Stirling, Institute of Aquaculture; Kubaryk, J. M. 1980. Effects of diet, feeding schedule, and sex on food consumption, growth and retention of protein and energy by tilapia. Ph.D. dissertation. Auburn University, Auburn, Alabama.

More details on feeding tilapia can be found in publications by Jauncey and Ross (1982), Hepher (1988), and Lim (1989).

STRIPED BASS AND HYBRID BASS

Striped bass and striped bass × white bass hybrids are important sport fish, and they are rapidly becoming a significant aquaculture fish in the United States. Larvae and juveniles must be supplied from hatcheries because inland waters do not provide essential spawning requirements (Stevens, 1966). These larvae have rudimentary digestive systems and are usually started on small brine shrimp nauplii or rotifers at day 4 to 5 posthatch. The concentration of nauplii in the rearing container is maintained at 10 to 100 nauplii per milliliter of water. Incorporation of dry larval diets of appropriate size may begin on day 5 to 8 and gradually replace all of the live food by days 14 to 28, depending on the acceptance of the dry diet and the size and condition of the larvae. A popular practice, where nursery ponds are available, is to release the larvae into prepared ponds with heavy zooplankton populations as early as 5 days after the larvae begin to feed. Clawson (1990) reported that the larvae of hybrid bass were 50 percent larger after 14 days in ponds than companion fish fed brine shrimp and a prepared diet in the hatchery.

Striped bass and hybrids are voracious feeders. They respond to multiple daily feeding and can grow rapidly (Hodson et al., 1987). They respond to diets high in crude protein (36 to 45 percent) that contain a high percentage of fishmeal (Klar and Parker, 1989). Little is known about their preferences for energy sources. Young striped bass and hybrid bass require eicosapentaenoic or docosahenaenoic acid in the diet for normal growth (Clawson, 1990; Webster and Lovell, 1990). Commercial trout and salmon diets can be successfully used for the rearing of these fish from juveniles to marketable size.

More information on the practical feeding of striped bass and hybrid bass can be obtained from publications by Hodson et al. (1987) and Brandt (1991).

RAINBOW TROUT

The feeding of rainbow trout fry must start as soon as fish deplete their yolk sac and begin to swim up. At this time the fish are capable of consuming dry, prepared diets. Swim-up fry should be fed at least once every hour during the normal light hours, and may even be slightly overfed to ensure adaptation to dry feed as long as uneaten feed is removed regularly. Water temperature should be kept above 6°C for swim-up fry. Fish must be fed with appropriately sized granules or pellets, and, when necessary, the feed should be screened to remove particles that are too small for the fish to consume and to prevent fouling of the water. Optimum size of diet particles are 0.5 to 1.5 mm granules for 1 to 10 g fish, 2 to 3 mm granules for 20 to 40 g fish, 3 to 4 mm pellets for 50 to 100 g fish, and 5 to 7 mm pellets for fish over 200 g (Cho, 1990). Fish should be fed carefully in production units to allow all fish to have the opportunity to obtain sufficient feed for maximum growth but to avoid overfeeding. Overfeeding reduces feed efficiency and increases the concentration of nutrients in the discharge from the culture operation. Close observation by the feeder or use of an appropriate feeding guide compatible with the energy requirement of the fish without feed wastage is required to prevent overfeeding.

Daily feed allowance for rainbow trout varies with fish size, strain, water temperature, feeding frequency, and energy concentration of the diet. A number of feeding guides have been developed for rainbow trout under various management and environment conditions. In some areas of North America there is a trend toward higher concentrations of energy and nutrients in salmonid diets for economic and environmental reasons. For these diets of higher nutrient density, less feed is recommended than some of the early feeding charts recommend. Cho (1992) prepared feeding guides based on energy requirements for weight gain of fish of various sizes at different temperatures. This approach allows for an adjustment of the feed allowance for dietary energy and nutrient density. Table 6-3 shows an example of a feeding guide calculated from energy requirements of fish of various sizes at different water temperatures and to be used with a contemporary, high performance trout diet (4.06

TABLE 6-3 Example of Daily Feeding Guide for Rainbow Trout Calculated from Energy Requirements of Fish of Various Sizes

5°C			10°C			15°C		
Fish Weight (g)	Digestible Energy (kcal/ fish)	Feed (g/100 g of fish)	Fish Weight (g)	Digestible Energy (kcal/ fish)	Feed (g/100 g of fish)	Fish Weight (g)	Digestible Energy (kcal/ fish)	Feed (g/100 g of fish)
1.0	—	—	1.0	—	—	1.0	—	—
1.2	0.08	2.01	1.4	0.17	4.19	1.7	0.27	6.52
1.4	0.09	1.90	1.9	0.22	3.72	2.5	0.37	5.45
1.7	0.10	1.80	2.5	0.26	3.36	3.7	0.49	4.69
1.9	0.12	1.71	3.3	0.32	3.06	5.2	0.63	4.12
2.2	0.13	1.63	4.2	0.38	2.81	7.0	0.78	3.69
2.5	0.14	1.56	5.2	0.45	2.61	9.2	0.96	3.34
2.9	0.16	1.49	6.4	0.52	2.43	11.8	1.15	3.05
3.3	0.17	1.43	7.7	0.59	2.28	14.9	1.37	2.81
3.7	0.19	1.38	9.2	0.68	2.14	18.5	1.60	2.61
4.2	0.20	1.33	10.9	0.76	2.02	22.6	1.85	2.44
4.7	0.22	1.28	12.8	0.86	1.92	27.3	2.12	2.29
5.2	0.24	1.23	14.9	0.96	1.83	32.6	2.42	2.16
5.8	0.25	1.19	17.3	1.07	1.74	38.5	2.73	2.04
6.4	0.27	1.16	19.8	1.18	1.66	45.1	3.06	1.94
7.0	0.29	1.12	22.6	1.30	1.60	52.4	3.42	1.85
7.7	0.31	1.09	25.7	1.42	1.53	60.5	3.79	1.76
8.4	0.33	1.06	29.0	1.55	1.47	69.3	4.19	1.69
9.2	0.36	1.03	32.6	1.69	1.42	79.0	4.61	1.62
10.0	0.38	1.00	36.4	1.83	1.37	89.5	5.04	1.56
10.9	0.40	0.98	40.6	1.98	1.33	100.9	5.51	1.50
11.8	0.43	0.95	45.1	2.14	1.28	113.3	5.99	1.45
12.8	0.45	0.93	49.9	2.30	1.24	126.6	6.49	1.40
13.9	0.48	0.91	55.0	2.47	1.21	141.0	7.02	1.35
14.9	0.50	0.89	60.5	2.64	1.17	156.4	7.57	1.31
16.1	0.53	0.87	66.3	2.82	1.14	172.8	8.14	1.27
17.3	0.56	0.85	72.4	3.01	1.11	190.4	8.74	1.23
18.5	0.59	0.83	79.0	3.21	1.08	209.1	9.35	1.20
19.8	0.62	0.81	85.9	3.41	1.05	229.1	9.99	1.17
21.2	0.65	0.79	93.2	3.61	1.03	250.2	10.66	1.13
22.6	0.68	0.78	100.9	3.83	1.00	272.6	11.34	1.11
24.1	0.71	0.76	109.1	4.05	0.98	296.3	12.05	1.08
25.7	0.74	0.75	117.6	4.28	0.96	321.4	12.79	1.05
27.3	0.77	0.74	126.6	4.51	0.94	347.8	13.55	1.03
29.0	0.81	0.72	136.1	4.75	0.92	375.6	14.33	1.00
30.7	0.84	0.71	146.0	5.00	0.90	404.9	15.13	0.98
32.6	0.88	0.70	156.4	5.26	0.88	435.7	15.96	0.96
34.5	0.92	0.69	167.2	5.52	0.86	468.0	16.82	0.94
36.4	0.95	0.67	178.6	5.79	0.84	501.8	17.70	0.92
38.5	0.99	0.66	190.4	6.07	0.83	537.2	18.60	0.90
40.6	1.03	0.65	202.8	6.35	0.81	574.3	19.53	0.89

NOTE: Feed contains 4.06 kcal digestible energy/g and 92 mg digestible protein/kcal of digestible energy.
SOURCE: Adapted from Cho, C. Y. 1990. Fish nutrition, feeds and feeding: With special emphasis on salmonid aquaculture. Food Rev. Int. 6:333-357.

kcal of digestible energy/g and 92 mg of digestible protein/kcal of digestible energy).

Feed may be dispensed by hand, usually twice daily, or by mechanical devices at predetermined amounts with appropriate feeding frequencies. Hand feeding offers human contact with the fish. However, mechanical feeders have the advantages that they can reduce labor costs and allow fish to feed many times during the day. These feeding devices must be carefully designed for the culture system and diet, and adjusted and serviced frequently for optimum feeding allowance.

More detailed information on the practical feeding of trout is given in publications by Hardy (1989, 1991) and Cho (1990, 1992).

PACIFIC SALMON

Most of the Pacific salmon culture in the United States involves rearing the fish to smolt stage in freshwater hatcheries and then releasing them to migrate to the Pacific Ocean where they grow to adulthood and return to near-shore areas where most enter capture fisheries. Some Pacific salmon are grown from postjuvenile to marketable size in net pens on the Pacific coast of North America, in South America (Chile), South Australia, and Japan.

The fry are started on a meal diet (<0.6 mm), and as size increases they go to crumbles (0.8-2.0 mm), then to pellets (>2.0 mm). Fry should be fed frequently; automatic feeders that dispense feed at approximately hourly intervals are often used. Starter diets contain at least 40 percent crude protein with whole fishmeal being at least one-half of the formula. Many hatcheries feed moist pellets instead of dry pellets for better feed consumption. The Oregon Moist Pellet (OMP), the standard for moist diets, contains 30 percent pasteurized wet fish or fish hydrolysate and 28 to 32 percent moisture (Hardy, 1991). Dry compressed (pelleted) or extruded diets have replaced moist diets in many hatcheries because of reduced cost, and it eliminates the need for frozen storage. The Abernathy salmon diets have served as the basis for many commercial pelleted and extruded formulations. The Abernathy salmon diet 19-2, which is presented in Chapter 5 (Table 5-2) as a natural ingredient reference diet, contains 50 percent fishmeal. This formula has been modified to replace some of the fishmeal with soybean meal, cottonseed meal, or poultry by-product meal for a cost reduction.

Extruded feeds that float or sink slowly are frequently fed in net pens. These porous, low-density feeds will absorb more oil on the surface than compressed pellets, they will give the fish more time to consume the feed before it sinks through the net, and they will give the feeder a better opportunity to see how much feed the fish consume. Extruded feeds, however, are more expensive than compressed pellets. Semimoist diets (18 to 22 percent moisture) that do not require frozen storage are also used to feed Pacific salmon. Processing of these diets is discussed in Chapter 5. Many commercial feeders and hatchery managers claim that intermediate moisture diets, like the OMP, are also more palatable than dry diets to young salmon.

Salmon diets must contain carotenoid pigments to give the flesh a pink-red color. Traditional commercial feedstuffs do not contain these pigments, so sources of astaxanthin and canthaxanthin must be included in feeds for salmon that are grown for human food. Crustacean meals or oils, dried *Phoffia* yeast, and certain algae contain astaxanthin. Astaxanthin and canthaxanthin are also available as synthetic products, but are restricted-use compounds in the United States. A concentration of 40 to 50 mg of carotenoid per kg of diet fed for about 6 months is required to obtain satisfactory flesh color.

Daily feed allowance and feeding frequency vary with fish species size, temperature, economics, and moisture content of the diet. Several feeding charts have been developed for various conditions. These feeding guides are based on air-dry weight of the feed and should be adjusted when feeding moist or semimoist diets. Hand feeding twice daily is commonly used in growing Pacific salmon from postjuvenile to food-size. This offers human contact with the fish, and it also provides for more growth than once-daily feeding. Automatic and demand feeders have advantages in that they can be labor saving and they allow the fish to be fed many times throughout the day. However, these feeding devices must be carefully designed and managed because malfunction can result in wasted feed or the fish being underfed and frequent servicing can negate the economics.

Hardy (1991) and Halver (1989) present more detailed information on feeding strategies for Pacific salmon.

ATLANTIC SALMON

Atlantic salmon farming is a relatively new industry, but it provides for a major portion of the salmon cultured for human food in the world. It involves two phases: the juvenile freshwater stage and the finishing saltwater stage. The freshwater stage, during which the fish grows from fry to smoltified postjuvenile, lasts approximately 1.5 years. The saltwater phase may last for 2 years when the targeted market size is 4 to 6 kg.

Atlantic salmon fry can use prepared, dry diets as their first feed. They begin on a finely ground (<0.6 mm) mash diet and are changed to crumbles and then to small compressed pellets during the freshwater stage. The starter diets contain over 50 percent high-quality fishmeal and 10 to 12 percent marine fish oil (Storebakken and Austreng, 1987).

In spite of the commercial importance of Atlantic salmon, relatively little information is available on the nutrient

requirements for this species. Functional commercial diets have been formulated from nutrient requirement data for rainbow trout and Pacific salmon and from feeding trials with natural-ingredient diets. Both moist and dry grower diets are used. Moist diets, containing dry ingredients supplemented with raw fish parts or fish ensilage, are highly palatable but are generally more expensive to feed. Atlantic salmon accept dry, compressed, or extruded diets satisfactorily. Slowly sinking extruded diets have become popular because they absorb more oil than compressed pellets, and they give the feeder a better opportunity to observe feeding activity of the fish. Fishmeal has traditionally been the primary source of protein; however, as much as 20 percent soybean meal is used successfully in the diet formula (Helland et al., 1991). Over 20 percent lipid, as fish oil, is commonly used in commercial grower diets that contain 40 to 45 percent crude protein. This is more lipid than is used in production diets for other fish species, and it produces fish with a high concentration of body fat. The effect of such a high amount of fat on the consumer-quality attributes of the processed fish has been questioned. Approximately 50 mg of carotenoid, as astaxanthin or canthaxanthin, is added per kg of diet and fed for 1 year for satisfactory flesh pigmentation.

Austreng et al. (1988) contend that Atlantic salmon have a smaller stomach than rainbow trout and must be fed more often. They recommend 5- to 10-minute feeding intervals for fry and 30-minute intervals for parr. Automatic feeders are often used in hatcheries. Fish in net pens are usually hand fed at least twice daily.

More information on feed preparation and feeding practices for Atlantic salmon is presented by Helland et al. (1991).

7

Nutrient Requirements Table

The values in the nutrient requirement table (Table 7-1) represent minimum requirements for maximum performance of the fish under experimental conditions. With few exceptions, the data were obtained with small fish and under optimum conditions. Where several values appear in the literature, the value presented in the table represents a consensus of the subcommittee for the most reasonable requirement.

The stated requirements do not include any surpluses. In practice, however, a margin of safety is commonly added to compensate for processing and storage losses, variation in composition and bioavailability of nutrients in feed ingredients, and variation in requirements caused by environmental effects. If higher or lower concentrations of dietary energy or protein than the values listed in the table are used, the other nutrient concentrations should be changed appropriately.

The requirements for amino acids, fatty acids, vitamins, and minerals were determined with diets containing purified and chemically defined ingredients that are highly digestible to fish; therefore, the values in the table represent near 100 percent bioavailability to the fish. This fact should be considered when formulating diets from natural feedstuffs in which the bioavailability of the nutrients is markedly less than that in the laboratory diets.

TABLE 7-1 Nutrient Requirements for Channel Catfish, Rainbow Trout, Pacific Salmon, Common Carp, and Tilapia as Percentages of Diet, Milligrams per Kilogram of Diet, or International Units (IU) per Kilogram of Diet (as-fed basis)[a]

	Channel Catfish	Rainbow Trout	Pacific Salmon	Common Carp	Tilapia
Energy Base[b] (kcal DE/kg diet)->	3,000	3,600	3,600	3,200	3,000
Protein, crude (digestible), percent	32 (28)	38 (34)	38 (34)	35 (30.5)	32 (28)
Amino acids					
Arginine, percent	1.20	1.5	2.04	1.31	1.18
Histidine, percent	0.42	0.7	0.61	0.64	0.48
Isoleucine, percent	0.73	0.9	0.75	0.76	0.87
Leucine, percent	0.98	1.4	1.33	1.00	0.95
Lysine, percent	1.43	1.8	1.70	1.74	1.43
Methionine + cystine, percent	0.64	1.0	1.36	0.94	0.90
Phenylalanine + tyrosine, percent	1.40	1.8	1.73	1.98	1.55
Threonine, percent	0.56	0.8	0.75	1.19	1.05
Tryptophan, percent	0.14	0.2	0.17	0.24	0.28
Valine, percent	0.84	1.2	1.09	1.10	0.78
n-3 fatty acids, percent	0.5-1	1	1-2	1	—
n-6 fatty acids, percent	—	1	—	1	0.5-1
Macrominerals					
Calcium, percent	R	1E	NT	NT	R
Chlorine, percent	R	0.9E	NT	NT	NT
Magnesium, percent	0.04	0.05	NT	0.05	0.06
Phosphorus, percent	0.45	0.6	0.6	0.6	0.5
Potassium, percent	R	0.7	0.8	NT	NT
Sodium, percent	R	0.6E	NT	NT	NT
Microminerals					
Copper, mg/kg	5	3	NT	3	R
Iodine, mg/kg	1.1E	1.1	0.6-1.1	NT	NT
Iron, mg/kg	30	60	NT	150	NT
Manganese, mg/kg	2.4	13	R	13	R
Zinc, mg/kg	20	30	R	30	20
Selenium, mg/kg	0.25	0.3	R	NT	NT
Fat-soluble vitamins					
A, IU/kg	1,000-2,000	2,500	2,500	4,000	NT
D, IU/kg	500	2,400	NT	NT	NT
E, IU/kg	50	50	50	100	50
K, mg/kg	R	R	R	NT	NT
Water-soluble vitamins					
Ribolfavin, mg/kg	9	4	7	7	6
Pantothenic acid, mg/kg	15	20	20	30	10
Niacin, mg/kg	14	10	R	28	NT
Vitamin B_{12}, mg/kg	R	0.01E	R	NR	NR
Choline, mg/kg	400	1,000	800	500	NT
Biotin, mg/kg	R	0.15	R	1	NT
Folate, mg/kg	1.5	1.0	2	NR	NT
Thiamin, mg/kg	1	1	R	0.5	NT
Vitamin B_6, mg/kg	3	3	6	6NT	
Myoinsitol, mg/kg	NR	300	300	440	NT
Vitamin C, mg/kg	25-50	50	50	R	50

NOTE: These requirements have been determined with highly purified ingredients in which the nutrients are highly digestible, therefore the values presented represent near 100 percent bioavailability.

[a] R, required in diet but quantity not determined; NR, no dietary requirement demonstrated under experimental conditions; NT, not tested; and E, estimated.

[b] Typical energy concentrations in commercial diets.

8

Composition of Feed Ingredients

The tables of feed ingredient composition (Tables 8-1 to 8-5) provide information that make it possible to define fish feeds nutritionally and prepare them economically for both research and commercial use. Data in the tables come from the National Research Council (1979, 1982, 1984), the Feed Composition Data Bank (U.S. Department of Agriculture), and published sources considered to be reliable by this committee. Feed ingredients are identified by their commonly used name and assigned an International Feed Number. Values in the tables are presented on an "as-fed" basis, with dry matter content stipulated.

The composition data represent chemical analyses with no correction for availability to the animal. The values must therefore 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 chemically defined ingredients in which the nutrients are highly digestible, and the nutrient requirement data are presented on the basis of being nearly 100 percent available.

Digestion coefficients for protein and for individual essential amino acids are presented for several feed ingredients in Chapter 4. The amino acid data are based on digestibility to channel catfish and Atlantic salmon; however, similar amino acid digestibility in these feeds for other fish species is probably a safe assumption. Availability of minerals varies among sources and fish species. Phosphorus digestibility in some feeds by the stomachless carp is much lower than that by channel catfish or rainbow trout. Availability of minerals from technical-or reagent-grade compounds will be higher and more consistent than from feedstuffs. Availability of phosphorus in several feed ingredients for three fish species is presented in Chapter 4.

Energy values for feedstuffs are presented on a digestible energy basis for channel catfish and Chinook salmon and on digestible and metabolizable energy bases for rainbow trout. Most fish digest protein and fats well, and the energy in them is available to the fish. The digestibility of carbohydrate varies more. Thus the available energy values presented for grains, starch, and dextrin for channel catfish, Chinook salmon, and rainbow trout should be used with discretion for other species.

TABLE 8-1 Proximate Composition and Digestible and Metabolizable Energy Values for Natural and Chemically Defined Ingredients Commonly Used in Fish Feeds (as-fed basis)

Ingredient	International Feed Number	Typical Dry Matter (%)	Digestible Energy (kcal/kg)			Metabolizable Energy (kcal/kg)	Proximate Composition (%)			
			Rainbow Trout	Channel Catfish	Chinook Salmon	Rainbow Trout	Crude Protein	Crude Fat	Crude Fiber	Ash
Alfalfa meal, dehydrated, 17 percent protein	1-00-023	92	513	730	—	859	17.1	2.8	24.1	9.8
Blood meal, spray dehydrated	5-00-381	93	4,289	3,410	1,864	3,410	89.2	0.74	1.0	2.3
Brewers grains, dehydrated	5-02-141	92	—	—	—	—	23.1	6.4	13.7	3.7
Canola meal, prepress solvent extracted	5-06-145	93	2,725	—	2,373	—	38.0	3.8	11.1	6.8
Corn distillers grains with solubles, dehydrated	5-28-236	91	—	—	—	—	27.0	9.3	9.1	6.4
Corn distillers solubles, dehydrated	5-28-237	90	2,265	—	—	2,283	27.6	8.5	4.6	7.5
Corn gluten meal, 60 percent	5-28-242	91	4,260	—	—	3,554	60.4	1.8	1.5	2.1
Corn	4-02-935	88	—	2,200	—	—	8.5	3.6	2.3	1.3
Corn, extrusion cooked	4-02-935	88	—	3,060	—	—	8.5	3.6	2.3	1.3
Cotton seed meal, solvent extracted	5-01-619	92	2,474	3,110	—	2,468	41.7	1.8	11.3	6.4
Crab meal, process residue	5-01-663	92	—	—	—	3,214	32.0	2.5	10.6	41.0
Fish solubles, condensed	5-01-969	50	—	—	—	—	31.5	6.1	0.5	9.6
Fish solubles, dehydrated	5-01-971	93	3,426	—	—	3,345	64.3	8.2	1.3	2.5
Fishmeal, anchovy, mechanically extracted	5-01-985	92	4,204	—	4,828	4,328	65.4	7.6	1.0	14.3
Fishmeal, catfish by-product, mechanically extracted	5-09-835	92	—	—	—	—	50.8	9.6	0.5	18.0
Fishmeal, herring, mechanically extracted	5-02-000	92	4,340	—	4,876	—	72.0	8.4	0.6	10.4
Fishmeal, menhaden, mechanically extracted	5-02-009	92	—	4,060	4,063	—	64.5	9.6	0.7	19.0
Fishmeal, tuna, mechanically extracted	5-02-023	93	—	—	—	—	59.9	6.8	0.8	21.9
Fishmeal, white, mechanically extracted	5-02-025	92	3,211	—	—	2,974	62.3	5.0	0.5	21.3
Meat meal	5-09-323	93	—	—	—	—	55.6	8.7	2.3	27.0
Meat and bone meal	5-09-322	94	3,186	2,930	—	—	50.9	9.7	2.4	29.2
Molasses, sugar cane, dehydrated	4-04-695	94	—	—	—	—	9.6	0.8	6.2	12.5
Peanut meal, solvent extracted	5-03-650	92	—	3,370	—	—	49.0	1.3	9.9	5.9
Poultry by-product meal	5-03-798	93	3,459	—	3,633	2,982	59.7	13.6	2.1	14.5
Poultry feather meal	5-03-795	93	—	3,400	3,250	—	83.3	5.4	1.2	2.9
Rice bran with polishings	4-03-928	91	—	2,110	—	—	12.8	13.7	11.1	11.6
Rice bran, with germ, solvent extracted	4-03-930	91	—	—	—	—	14.0	1.5	12.9	10.8
Rice polishings	4-03-943	90	—	—	—	—	12.8	14.6	5.3	7.4

Ingredient	International Feed Number	Typical Dry Matter (%)	Digestible Energy (kcal/kg)			Metabolizable Energy (kcal/kg)		Proximate Composition (%)			
			Rainbow Trout	Channel Catfish	Chinook Salmon	Rainbow Trout		Crude Protein	Crude Fat	Crude Fiber	Ash
Shrimp meal, process residue	5-04-226	88	—	—	—	—		39.5	3.2	12.8	27.2
Sorghum (milo)	4-04-444	89	—	—	—	—		9.9	2.8	2.3	1.8
Soybean seed, steam cooked, full fat	5-04-597	90	3,809	—	—	3,641		38.0	18.0	5.0	4.5
Soybean meal, solvent extracted	5-04-604	90	—	3,010	—	—		44.0	1.1	7.3	6.3
Soybean meal, solvent extracted without hulls	5-04-612	—	2,934	—	3,250	2,566		48.5	0.9	3.4	5.8
Sunflower meal, solvent extracted	5-04-739	93	—	—	—	—		45.5	2.9	11.7	7.5
Wheat	4-05-268	88	—	2,400	—	—		12.9	1.7	2.5	1.6
Wheat bran	4-05-190	89	—	2,790	—	—		16.4	4.0	9.9	5.3
Wheat flour	4-05-199	88	1,588	—	—	—		11.7	1.2	1.3	0.4
Wheat middlings	4-05-205	89	2,173	—	2,032	2,237		17.0	4.3	8.0	4.6
Yeast, brewers, dehydrated	7-05-527	93	3,522	—	—	2,922		42.6	1.0	3.2	6.6
Yeast, torula, dehydrated	7-05-534	93	3,421	—	—	—		49.0	1.5	2.2	7.7
Casein[a]	5-01-162	91	—	4,400	—	—		84.3	0.6	Trace	2.1
Cellulose powder[a]	[b]	96	—	—	—	—		0	0	92.6	0
Corn starch[a]	[b]	88	—	2,700	—	—		0.2	Trace	0.08	0.08
Corn starch, cooked[a]	[b]	88	—	—	—	—		0.2	Trace	0.08	0.08
20 percent of diet			—	3,400	—	—	—	—	—		—
40 percent of diet			—	2,800	—	—	—	—	—		—
Dextrin[a]	4-08-023	90									
30 percent of diet			—	2,920	—	—	—	—	—		—
60 percent of diet			—	1,920	—	—	—	—	—		—
Gelatin[a]	5-14-503	90	—	4,700	—	—	87.6	0.1	Trace		Trace

NOTE: Dash indicates data were not available.

[a]Purified ingredients.
[b]An International Feed Number is not assigned.

TABLE 8-2 Amino Acid Composition of Ingredients Commonly Used in Fish Feeds (as-fed basis)

Ingredient	International Feed Number	Dry Matter (%)	Typical Crude Protein (%)	Arginine (%)	Histidine (%)	Isoleucine (%)	Leucine (%)	Lysine (%)	Methionine (%)	Cystine (%)	Phenylalanine (%)	Tyrosine (%)	Threonine (%)	Tryptophan (%)	Valine (%)
Alfalfa meal, dehydrated 17 percent protein	1-00-023	92	17.1	0.77	0.33	0.81	1.28	0.85	0.27	0.29	0.80	0.54	0.71	0.34	0.88
Blood meal, spray dehydrated	5-00-381	93	89.2	3.75	5.14	0.97	10.82	7.45	1.08	1.24	5.92	2.55	3.76	1.04	7.48
Brewers grains, dehydrated	5-02-141	92	23.1	1.27	0.52	1.54	2.54	0.88	0.46	0.35	1.44	1.15	0.93	0.37	1.61
Canola meal, solvent extracted	5-06-145	93	38.0	2.32	1.07	1.51	2.65	2.27	0.70	0.47	1.52	0.93	1.71	0.44	1.94
Casein, dehydrated	5-01-162	93	87.3	3.40	2.59	5.00	8.46	6.92	2.67	0.31	4.52	4.60	3.81	1.21	6.71
Corn, yellow	4-02-935	88	8.5	0.43	0.26	0.35	1.21	0.25	0.17	0.22	0.48	0.31	0.35	0.08	0.44
Corn distillers grains with solubles, dehydrated	5-28-236	91	27.0	1.12	0.64	1.09	2.89	0.65	0.50	0.46	1.39	0.99	0.98	0.10	1.50
Corn distillers solubles, dehydrated	5-28-237	90	27.6	0.97	0.68	1.28	2.24	1.07	0.56	0.45	1.49	0.87	1.02	0.24	1.55
Corn gluten meal, dehydrated, 60 percent	5-28-242	91	60.7	2.02	1.31	2.54	10.2	1.11	1.63	1.20	3.96	3.32	2.07	0.43	3.09
Cotton seed meal, solvent extracted	5-01-619	92	41.2	3.97	0.83	1.15	1.80	1.89	0.50	0.45	2.10	0.80	1.02	0.42	1.68
Crab meal, process residue	5-01-663	92	31.4	1.66	0.49	1.17	1.54	1.38	0.53	0.24	1.16	1.17	1.00	0.29	1.47
Fish solubles, condensed	5-01-969	50	32.7	1.58	1.62	0.77	1.55	1.86	0.63	0.27	0.88	0.44	0.87	0.22	1.22
Fishmeal, anchovy	5-01-985	92	65.5	3.85	1.61	3.17	5.05	5.04	1.99	0.60	2.78	2.24	2.82	0.75	3.50
Fishmeal, catfish, processing by-product	5-09-835	92	51.1	3.18	0.80	1.95	3.17	3.10	1.09	0.38	1.58	1.55	1.96	0.41	2.31
Fishmeal, herring	5-02-000	92	72.0	4.54	1.65	3.13	5.19	5.57	2.08	0.74	2.71	2.20	2.90	0.77	4.30
Fishmeal, menhaden	5-02-009	92	64.5	3.82	1.45	2.66	4.48	4.72	1.75	0.56	2.41	1.94	2.50	0.65	3.22
Fishmeal, tuna	5-02-023	93	59.9	3.43	1.75	2.45	3.79	4.06	1.47	0.47	2.15	1.69	2.31	0.57	2.77
Fishmeal, white	5-02-025	92	62.2	4.21	1.34	2.67	4.52	4.53	1.68	0.75	2.34	1.94	2.57	0.60	3.02
Gelatin	5-14-503	—	87.6	6.97	0.71	1.38	2.74	3.55	0.73	0.13	1.71	0.47	1.81	0.01	2.09
Meat meal, rendered	5-09-323	93	55.6	3.60	0.89	1.64	2.85	2.93	0.66	0.59	1.72	1.17	1.64	0.34	2.52
Meat with bone meal	5-09-322	94	50.0	3.37	0.96	1.43	3.00	2.67	0.65	0.50	1.70	1.09	1.65	0.30	2.45
Peanut meal, solvent extracted	5-03-650	92	48.1	5.89	1.33	1.76	3.33	1.71	0.49	0.59	2.49	2.23	1.67	0.48	1.88
Poultry by-product meal	5-03-798	93	59.7	4.06	1.09	2.30	4.11	3.06	1.10	0.84	2.10	1.87	0.94	0.46	2.86
Poultry feather meal	5-03-795	93	83.3	5.65	0.62	3.65	6.64	1.83	0.55	3.70	3.78	2.40	3.79	0.52	6.48
Rice bran with germ, solvent extracted	4-03-930	91	15.7	0.85	0.29	0.51	1.01	0.54	0.21	0.20	0.56	0.54	0.45	0.21	0.65
Rice polishings	4-03-943	90	12.8	0.63	0.17	0.35	0.70	0.52	0.20	0.13	0.43	0.42	0.34	0.10	0.72
Shrimp meal, process residue	5-04-226	88	39.9	2.35	0.90	1.46	2.60	2.17	0.82	0.59	1.59	1.45	1.42	0.42	1.83
Sorghum (milo)	4-04-444	89	9.9	0.37	0.22	0.41	1.28	0.28	0.24	0.16	0.48	0.35	0.34	0.10	0.52
Soybean seeds, steam cooked	5-04-597	90	31.2	2.53	0.86	1.60	2.63	2.24	0.46	0.34	1.72	1.25	1.41	0.52	2.02
Soybean meal, solvent extracted	5-04-604	90	44.8	3.39	1.19	2.03	3.49	2.85	0.57	0.70	2.22	1.57	1.78	0.64	2.02
Soybean meal, solvent extracted, without hulls	5-04-612	93	50.0	3.67	1.22	2.14	3.63	3.08	0.68	0.75	2.44	1.76	1.89	0.69	2.55
Sunflower meal, solvent extracted	5-04-739	93	40.7	3.60	0.96	1.96	2.73	1.66	0.83	0.74	2.09	0.75	1.61	0.61	2.60
Wheat	4-05-268	88	12.9	0.64	0.30	0.51	0.89	0.36	0.21	0.27	0.63	0.43	0.37	0.17	0.59
Wheat bran	4-05-190	89	16.4	0.86	0.39	0.51	0.92	0.58	0.19	0.26	0.55	0.38	0.46	0.25	0.69
Wheat midlings	4-05-205	89	17.0	0.98	0.41	0.67	1.08	0.67	0.18	0.22	0.64	0.40	0.54	0.20	0.75
Yeast, brewers, dehydrated	7-05-527	93	42.6	2.25	1.09	1.98	2.85	2.97	0.67	0.49	1.62	1.50	2.04	0.52	2.36

NOTE: Dash indicates data were not available.

TABLE 8-3 Mineral Composition of Ingredients Commonly Used in Fish Feeds (as-fed basis)

Ingredient	International Feed Number	Minerals												
		Dry Matter (%)	Calcium (%)	Phosphorus (%)	Potassium (%)	Chlorine (%)	Magnesium (%)	Sodium (%)	Sulfur (%)	Copper (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Selenium (mg/kg)	Zinc (mg/kg)
Alfalfa meal, dehydrated, 17 percent protein	1-00-023	92	1.40	0.23	2.38	0.47	0.29	0.10	0.23	10.0	404	31.0	0.33	19
Blood meal, spray dehydrated	5-00-381	93	0.41	0.30	0.15	0.25	0.15	0.38	0.34	8.2	2769	6.4	—	306
Brewers grains, dehydrated	5-02-141	92	0.29	0.51	0.09	0.13	0.15	0.20	0.29	21.7	233	37.2	0.7	27
Casein, dehydrated	5-01-162	91	0.61	0.82	0.01	—	.01	0.01	—	3.8	14	4.3	0.13	27
Corn	4-02-935	88	0.03	0.28	0.33	0.05	0.11	0.01	0.11	3.5	33	5.7	0.07	19
Corn distillers grains with solubles, dehydrated	5-28-236	91	0.14	0.66	0.40	0.16	0.16	0.52	0.35	52.8	236	22.8	0.35	80
Corn distillers solubles, dehydrated	5-28-237	91	0.30	1.44	1.64	0.26	0.59	0.23	0.36	81.0	555	72.8	0.36	84
Corn gluten meal, 60 percent	5-28-242	90	0.07	0.44	0.19	0.07	0.07	0.05	0.57	26.1	229	6.3	0.83	31
Cotton seed meal, solvent extracted	5-01-619	92	0.17	1.17	1.39	0.04	0.41	0.04	0.3	19.0	208	21.0	0.06	61
Crab meal, process residue	5-01-663	92	14.56	1.59	0.45	1.51	0.94	0.88	0.25	32.73	4356	133.0	—	—
Fish solubles, condensed	5-01-969	50	0.16	0.57	1.64	2.93	0.03	2.45	0.12	46.6	276	13.2	1.97	43
Fishmeal, anchovy	5-01-985	92	3.73	2.43	0.90	1.00	0.24	1.10	0.54	9.03	220	9.5	1.36	103
Fishmeal, herring	5-02-000	92	2.20	1.67	1.08	0.99	0.14	0.59	0.46	5.6	114	4.8	1.95	125
Fishmeal, menhaden	5-02-009	92	5.19	2.88	0.70	0.55	0.15	0.41	0.56	10.3	544	37.0	2.15	144
Fishmeal, tuna	5-02-023	93	7.86	4.21	0.72	1.01	0.23	0.74	0.68	10.31	355	8.4	4.30	211
Fishmeal, white	5-02-025	91	7.31	3.81	0.83	0.50	0.18	0.78	0.48	5.90	181	12.4	1.62	90
Gelatin	5-14-503	90	0.49	0.06	—	—	0.05	—	—	—	—	—	—	—
Meat meal	5-09-323	93	8.27	4.10	0.55	1.15	0.27	1.15	0.50	9.70	441	9.5	0.40	80
Meat with bone meal	5-09-322	94	9.4	4.58	1.13	0.74	1.13	0.73	0.26	1.50	508	12.5	0.25	89
Molasses, sugarcane	4-04-696	74	0.77	0.08	2.98	2.26	0.31	0.16	0.35	59.0	196	43.7	—	16
Peanut meal, solvent extracted	5-03-650	93	0.27	0.61	1.16	0.03	0.27	0.07	0.31	15.0	142	26.7	—	20
Poultry by-product meal	5-03-798	93	3.51	1.83	0.39	0.54	0.18	0.82	0.52	14.12	442	11.0	0.78	121
Poultry feathers, hydrolyzed	5-03-795	93	0.25	0.66	0.28	0.28	0.20	0.69	1.47	6.4	74	12.5	0.82	68
Rice bran with germ, solvent extracted	4-03-930	91	0.11	1.37	1.48	0.07	0.95	0.10	0.18	13.0	187	232.2	—	30
Rice polishings	4-03-943	89	0.03	0.27	0.13	0.08	0.11	0.07	0.04	—	—	18.0	0.27	17
Safflower meal, solvent extracted	5-04-110	92	0.34	0.76	0.75	—	0.34	0.05	0.13	9.9	497	18.2	—	41
Sorghum, grain (milo)	4-04-444	89	0.03	0.28	0.31	0.08	0.13	0.04	0.08	10.0	48	15.8	0.20	17
Soybean meal, dehulled, solvent extracted	5-04-612	90	0.26	0.64	2.13	0.04	0.30	0.01	0.44	20.3	131	37.2	0.10	57
Soybean meal, solvent extracted	5-04-604	90	0.30	0.65	2.11	0.04	0.29	0.04	0.42	23.0	140	30.6	0.10	52
Sunflower meal, dehulled, solvent extracted	5-04-739	93	0.42	0.94	1.19	0.16	0.69	0.22	0.21	4.0	31	18.9	2.13	98
Wheat	4-05-268	88	0.04	0.37	0.43	0.05	0.12	0.02	0.14	5.1	35	30.4	0.29	35
Wheat bran	4-05-190	87	0.13	1.16	1.22	0.05	0.57	0.05	0.21	11.0	145	115.0	0.64	95
Wheat middlings	4-05-205	89	0.13	0.89	0.98	0.04	0.34	0.12	0.17	15.9	60	114.1	0.74	97
Yeast, brewers, dried	7-05-527	93	0.14	1.36	1.69	0.07	0.24	0.07	0.43	38.4	109	6.7	0.91	39

NOTE: Dash indicates data were not available.

TABLE 8-4 Vitamin Composition of Ingredients Commonly Used in Fish Feeds (as-fed basis)

		Vitamins										
Ingredient	International Feed Number	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Pantothenic Acid (mg/kg)	Pyridoxine (mg/kg)	Riboflavin (mg/kg)	Thiamin (mg/kg)	Vitamin B_{12} (ug/kg)	Vitamin E (mg/kg)	Vitamin K (mg/kg)
Alfalfa meal, dehydrated, 17 percent protein	1-00-023	0.33	1,369	4.34	37	29.7	7.10	12.9	3.4	—	111.0	9.0
Blood meal, spray dehydrated	5-00-381	0.28	600	0.40	22	3.2	4.45	2.9	0.3	13.0	—	—
Brewers grains, dehydrated	5-02-141	0.63	1,652	7.1	44	8.2	0.68	1.5	0.6	4.0	26.7	—
Casein, dehydrated	5-01-162	0.04	208	0.47	1	2.7	0.42	1.5	0.4	—	—	—
Corn	4-02-935	0.07	504	0.30	23	5.1	4.69	1.1	3.7	—	20.9	0.22
Corn distillers grains with solubles, dehydrated	5-28-236	0.77	2,551	0.90	72	13.9	5.00	8.3	2.8	—	39.1	—
Corn distillers solubles, dehydrated	5-28-237	1.63	4,687	1.30	122	22.9	8.85	17.0	6.6	3.0	50.5	—
Corn gluten meal, 60 percent	5-28-242	0.19	352	0.30	60	3.5	6.90	2.0	0.3	—	23.4	—
Cotton seed meal, solvent extracted	5-01-619	0.97	2,764	1.4	41	13.7	7.00	3.3	6.6	0	16.0	
Crab meal process residue	5-01-663	0.07	2,011	0.11	45	6.5	6.63	6.1	0.4	438.3	—	—
Fish solubles, condensed	5-01-969	0.14	3,370	0.20	176	35.7	12.14	12.9	5.5	507.0	—	—
Fishmeal, anchovy	5-01-985	0.23	4,408	0.20	100	15.0	4.64	7.1	0.1	352.0	5.0	—
Fishmeal, herring	5-02-000	0.49	5,266	0.30	85	17.3	4.77	9.7	0.4	430.0	22.1	2.2
Fishmeal, menhaden	5-02-009	0.18	3,112	0.12	55	8.6	4.66	4.8	0.6	123.0	12.0	—
Fishmeal, tuna	5-02-023	0.20	2,994	—	144	7.7	—	6.8	1.5	300.1	5.6	—
Fishmeal, white	5-02-025	0.08	3,099	0.35	59	9.9	5.92	9.1	1.7	89.5	8.9	—
Gelatin	5-14-503	—	—	—	—	—	—	—	—	—	—	—
Meat meal	5-09-323	0.11	1,922	0.50	53	4.9	4.6	5.3	0.2	91.0	1.0	—
Meat with bone meal	5-09-322	0.14	2,136	0.50	51	4.4	8.74	4.5	0.2	217	1.1	—
Molasses, sugarcane	4-04-696	0.67	704	0.10	36	37.5	4.22	2.8	0.9	—	5.4	—
Peanut meal, solvent extracted	5-03-650	0.33	1,896	0.70	178	46.6	6.38	9.1	5.7	0	2.9	—
Poultry by-product meal	5-03-798	0.09	6,029	0.51	47	11.1	4.41	10.5	0.2	301.2	2.2	—
Poultry feathers, hydrolyzed	5-03-795	0.04	895	0.22	21	8.9	2.98	2.0	0.1	83.3	—	—
Rice bran with germ, solvent extracted	4-03-930	0.42	1,128	2.20	284	23.0	29.12	2.9	22.6	—	60.7	—
Rice polishings	4-03-943	0.08	878	0.2	23	3.3	—	0.4	1.4	—	14.5	—
Safflower meal, solvent extracted	5-04-110	1.43	816	0.4	11	37.3	—	2.0	-	—	0.9	—
Sorghum, grain (milo)	4-04-444	0.23	638	0.2	37	11.0	4.7	1.1	4.1	—	12.1	—
Soybean meal dehulled, solvent extracted	5-04-612	0.32	2,753	0.7	22	14.8	4.9	2.9	3.1	—	3.3	—
Soybean meal, solvent extracted	5-04-604	0.32	2,609	0.6	28	16.3	6.0	2.9	6.0	—	2.4	—
Sunflower meal, dehulled, solvent extracted	5-04-739	—	3,632	—	242	40.6	13.7	3.5	3.1	—	11.1	—
Wheat	4-05-268	0.11	1,004	0.4	53	10.1	3.0	1.3	4.5	—	11.1	—
Wheat bran	4-05-190	0.38	1,232	1.8	197	28.0	8.5	3.6	8.4	—	14.3	—
Wheat middlings	4-05-205	0.24	1,247	1.2	95	17.8	8.0	2.0	14.2	—	23.9	—
Yeast, brewers, dried	7-05-527	1.04	3,847	9.7	443	110.7	37.1	34.1	85.2	1.0	2.1	—

NOTE: Dash indicates data were not available.

TABLE 8-5 Fatty Acid Composition of Common Animal Fats, Fish Oils, and Vegetable Oils

		Percentage of Total Fatty Acids						
Lipid Source	International Feed Number	14:0	16:0	16:1	18:0	18:1	18:2 n-6	18:3 n-3
Animal fat								
Beef tallow	4-08-127	3.7	24.9	4.2	18.9	36.0	3.1	0.6
Pork fat	4-04-790	1.3	23.8	2.7	13.5	41.2	10.2	1.0
Poultry fat	4-09-319	0.9	21.6	5.7	6.0	37.3	19.5	1.0
Fish oils								
Anchovy		7.4	17.4	10.5	4.0	11.6	1.2	0.8
Cod liver	7-01-994	3.2	13.5	9.8	2.7	23.7	1.4	0.6
Capelin	7-16-709	7.9	11.1	11.1	1.0	17.0	1.7	0.4
Channel catfish, cultured		1.4	17.4	2.9	6.1	49.1	10.5	1.0
Herring, Atlantic	7-08-048	6.4	12.7	8.8	0.9	12.7	1.1	0.6
Herring, Pacific		5.7	16.6	7.6	1.8	22.7	0.6	0.4
Menhaden	7-08-049	7.3	19.0	9.0	4.2	13.2	1.3	0.3
Redfish		4.9	13.2	13.2	2.2	13.3	0.9	0.5
Salmon, sea caught		3.7	10.2	8.7	4.7	18.6	1.2	0.6
Vegetable oil								
Canola	4-06-144	—	3.1	—	1.5	60.0	20.2	12.0
Coconut	4-09-320	16.8	8.2	—	2.8	5.8	1.8	—
Corn	4-07-882	—	10.9	—	1.8	24.2	58.0	0.7
Cottonseed	4-20-836	0.8	22.7	0.8	2.3	17.0	51.5	0.2
Linseed	4-14-502	—	5.3	—	4.1	20.2	12.7	53.3
Palm		1.0	43.5	0.3	4.3	36.6	9.1	0.2
Peanut	4-03-658	0.1	9.5	0.1	2.2	44.8	32.0	—
Safflower	4-20-526	0.1	6.2	0.4	2.2	11.7	74.1	0.4
Soybean	4-07-983	0.1	10.3	0.2	3.8	22.8	51.0	6.8
Sunflower	4-20-833	—	5.9	—	4.5	19.5	65.7	—

NOTE: Dash indicates that measurements were taken but no values were detected.

18:4 n-3	20:1	20:4 n-6	20:5 n-3	22:1	22:5 n-3	22:6 n-3	Σn-6	Σn-3	n^3:n^6 Ratio
—	0.3	—	—	—	—	—	3.1	0.6	0.19
—	1.0	—	—	—	—	—	10.2	1.0	0.10
1.1	0.1	—	—	—	—	—	19.6	1.0	0.05
3.0	1.6	0.1	17.0	1.2	1.6	8.8	1.3	31.2	24.0
0.9	7.4	1.6	11.2	5.1	1.7	12.6	3.0	27.0	9.0
2.1	18.9	0.1	4.6	14.7	0.3	3.0	1.8	12.2	6.78
0.2	1.4	0.3	0.4	—	0.3	1.3	12.7	3.2	0.25
1.7	14.1	0.3	8.4	20.8	0.8	4.9	1.4	17.8	12.71
1.6	10.7	0.4	8.1	12.0	0.8	4.8	1.0	15.7	15.7
2.8	2.0	0.2	11.0	0.6	1.9	9.1	1.5	25.1	16.73
1.1	17.2	0.3	8.0	18.9	0.6	8.9	1.2	19.1	15.92
2.1	8.4	0.9	12.0	5.5	2.9	13.8	2.1	31.4	15.00
—	1.3	—	—	1.0	—	—	20.2	12.0	5.94
—	—	—	—	—	—	—	1.8	0.0	0.0
—	—	—	—	—	—	—	58.0	0.7	0.01
—	—	—	—	—	—	—	51.5	0.2	0.0
—	—	—	—	—	—	—	12.7	53.3	4.2
—	0.1	—	—	—	—	—	9.1	0.2	0.02
—	1.3	—	—	—	—	—	32.0	0.0	0.0
—	—	—	—	—	—	—	74.1	0.4	0.0
—	0.2	—	—	—	—	—	51.0	6.8	0.13
—	—	—	—	—	—	—	65.7	0.0	0.0

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Appendix

TABLE A-1 Common and Scientific Names of Species Discussed in This Report

Common Name	Scientific Name	Common Name	Scientific Name
Abalone	*Haliotis* spp.	Largemouth bass	*Micropterus salmoides*
Atlantic salmon	*Salmo salar*	Milkfish	*Chanos chanos*
Atlantic silverside	*Menidia menidia*	Mossambique tilapia	*Oreochromis mossambica*
Ayu	*Plecoglossus altivelis*	Nile tilapia	*Oreochromis nilotica*
Bighead carp	*Hypophthalmichthys nobilis*	Oriental weatherfish	*Misgurnus anguillcaudatus*
Black sea bream	*Mylio macrocephalus*	Pacific salmon	*Oncorhynchus* spp.
Blue tilapia	*Oreochromis aurea*	Pike	*Esox lucius*
Bluegill	*Lepomis machrochirus*	Pink salmon	*Oncorhynchus gorbuscha*
Brook trout	*Salvelinus fontinalis*	Plaice (American)	*Hippoglossoides platessoides*
Brown trout	*Salmo trutta linnaeus*	Platyfish (southern)	*Xiphophorus maculatus*
Catfish	*Schilbeades mollis*	Pollock	*Pollachius virens*
Channel catfish	*Ictalurus punctatus*	Puffer (marbled)	*Sphoeroides dorsalis*
Chinook salmon	*Oncorhynchus tshawytscha*	Rainbow trout	*Oncorhynchus mykiss*
Chum salmon	*Oncorhynchus keta*	Red sea bream	*Pagellus bogaraveo*
Cod	*Gadus morhua*	Roach	*Rutilus rutilus*
Coho salmon	*Oncorhynchus kisutch*	Sablefish	*Anoplopoma fimbria*
Common carp	*Cyprinus carpio*	Sea bass (European bass)	*Morone labrax*
Estuary grouper	*Epinephelus striatus*	Silver carp	*Hypophthalmichthys molitrix*
Fancy red carp	*Cyprinus carpio*	Skate	*Raja* spp.
Giant sea perch	*Archoplites interruptus*	Skipjack tuna	*Katsuwonus pelamis*
Gilthead (sea) bream	*Sparus auratus*	Smallmouth bass	*Micropterus dolomieu*
Goldfish	*Carassius auratus*	Snakehead	*Channa striata*
Grass carp	*Ctenopharyngodon idella*	Sockeye salmon	*Oncorhynchus nerka*
Grey mullet	*Mugil cephalus*	Striped bass	*Morone saxatilis*
Guppy	*Poecilia reticulata*	Striped jack	*Longirostris delicatissimus*
Haddock	*Melanogrammus aeglefinus*	Sturgeon	*Acipenser* spp.
Hake	*Merluccius bilinearis*	Sunfish(redbreast)	*Lepomis auritus*
Herring	*Clupea harengus*	Swordtail (green)	*Xiphophorus helleri*
Indian catfish	*Heteropneustes fossilis*	Turbot	*Scophthalmus maximus*
Indian major carp		White bass	*Morone chrysops*
Catla	*Catla catla*	Whitefish	*Coregonus* spp.
Rohu	*Labeo rohita*	White sturgeon	*Acipenser transmontanus*
Japanese (blue) parrotfish	*Scarus coeruleus*	Winter flounder	*Pseudopleuronectes americanus*
Japanese eel	*Anguilla japonica*	Yellowtail	*Seriola lalandi*
Lake trout	*Salvelinus namaycush*	Zillii's tilapia	*Tilapia zillii*

TABLE A-2 Amino Acid and Mineral Deficiency Signs Reported in Fish

Nutrient	Salmonids	Channel Catfish	Japanese Eel	Common Carp	Tilapia	Red Sea Bream
Amino acids						
Methionine	Cataracts					
Tryptophan	Scoliosis, cataracts, fin erosion, deranged mineral metabolism					
Lysine	Fin erosion, mortality					
Macrominerals						
Calcium	Reduced growth		Reduced growth, anorexia		Reduced growth, poor feed conversion and bone mineralization	Reduced growth, anorexia, poor feed conversion
Magnesium (Mg)	Reduced growth, anorexia, sluggishness, nephrocalcinosis, cataracts, degeneration of muscle fibers and epithelial cells of pyloric caeca and gill filaments, skeletal deformity, reduced bone mineralization, reduced bone Mg concentration	Reduced growth, anorexia, sluggishness, mortality, reduced bone Mg concentration	Reduced growth, anorexia	Reduced growth, anorexia, sluggishness, convulsions, cataracts, mortality, reduced bone Mg concentration		
Phosphorus	Reduced growth, poor food conversion and bone mineralization	Reduced growth, poor food conversion and bone mineralization	Reduced growth, anorexia	Reduced growth, poor bone mineralization, skeletal and cranial deformity, increased visceral fat		Poor feed conversion and mineralization, curved and enlarged spongy vertebrae
Potassium	Anorexia, convulsion, tetany, mortality					

Nutrient	Salmonids	Channel Catfish	Japanese Eel	Common Carp	Tilapia	Red Sea Bream
Microminerals						
Copper (Cu)	Reduced liver Cu-Zn-superoxide dismutase and heart cytochrome *c*oxidase activity	Reduced growth and reduced heart cytochrome *c*, oxidase activity				
Iodine	Thyroid hyperplasia					
Iron	Hypochromic microcytic anemia	Reduced growth, poor feed conversion	Hypochromic microcytic anemia	Hypochromic microcytic anemia		Hypochromic microcytic anemia
Selenium	Muscular dystrophy, exudative diathesis, reduced glutathione peroxidase activity	Reduced growth, reduced glutathione peroxidase activity		Reduced growth, anemia, cataracts		
Zinc (Zn)	Reduced growth, short-body dwarfism, cataracts, fin erosion, mortality	Reduced growth, anorexia, reduced bone Ca and Zn concentration, low serum Zn		Reduced growth, anorexia, cataracts, fin and skin erosion, mortality		Reduced growth, anorexia, loss of equilibrium, mortality

TABLE A-3 Major Vitamin Deficiency Signs Reported in Fisha

Vitamin	Salmonids	Channel Catfish	Japanese Eel	Common Carp	Yellowtail	Red Sea Bream
A	Skin depigmentation, exophthalmia, eyes and lens displacement, corneal thinning, retinal degeneration, edema, ascites	Exophthalmia, edema, hemorrhagic kidney, skin depigmentation	Not tested	Skin depigmentation exophthalmia, twisted opercula, hemorrhagic fin and skin	Arrested growth of opercula; dark skin coloration; anemia; hemorrhagic fins, eyes, and liver	Not tested
D	Impaired calcium homeostasis, tetany of skeletal muscle, increased liver lipid	Low bone ash, potassium, and calcium contents	Not tested	Not tested	Not tested	Not tested
E	Skin depigmentation, anemia, ascites, abnormal erythrocytes, muscular dystrophy, edema	Skin depigmentation, exudative diathesis, muscular dystrophy, fatty liver, pancreatic atrophy, ceroid deposition	Not tested	Exophthalmia, lordosis, muscular dystrophy, kidney degeneration, pancreatic degeneration	Dark skin coloration, congested fins and bronchial mantles, spasms	Not tested
K	Prolonged blood clotting, anemia, hemorrhagic gills and eyes	Hemorrhagic skin	Not tested	Not tested	Not tested	Not tested
Thiamin	Nervous disorders, loss of equilibrium, hyperirritability, convulsions, low transketolase activity in erythrocytes and kidney	Dark skin coloration, loss of equilibrium, nervousness	Trunk-winding activity, subcutaneous hemorrhage, congested fins	Nervousness, skin depigmentation, subcutaneous hemorrhage	Dark skin coloration, congested fins and bronchial mantles	Subcutaneous hemorrhage, congested fins
Riboflavin	Lethargy, dark pigmentation, spinal deformities, fin hemorrhage and erosion, photophobia, corneal vascularization, eye hemorrhage, reduced activity of erythrocyte glutathione reductase	Short-body dwarfism	Lethargy, dermatitis, photophobia, fin hemorrhage, abdominal hemorrhage	Emaciation, photophobia, nervousness, hemorrhage of skin and fins, anterior kidney necrosis	Dark skin coloration, cloudy cornea	Poor growth

Vitamin	Salmonids	Channel Catfish	Japanese Eel	Common Carp	Yellowtail	Red Sea Bream
Pyridoxine	Nervous disorders, anemia, eleptiform convulsions, hyperirritability, erratic spiral swimming, low resistance to handling	Nervous disorders, tetany, erratic swimming, greenish-blue coloration	Nervous disorders, eleptiform convulsions	Nervous disorders, anemia, low hepatopancreatic transferase	Lethargy, eleptiform spasms and convulsions	Poor growth
Pantothenic acid	Clubbed gills, distended operculum, atrophied pancreatic acinar cells, mortality	Emaciation, clubbed gills, eroded epidermis, anemia, mortality	Dermatitis, congested skin, hemorrhagic skin, abnormal swimming	Poor growth, lethargy, exophthalmia, skin hemorrhage	Clubbed gills, mortality	Poor growth, mortality
Niacin	Skin, fin, and colon lesions; photosensitivity; sunburn; abdominal edema; muscular weakness; anemia	Skin and fin lesions, exophthalmia, deformed jaws, anemia, mortality	Abnormal swimming, skin hemorrhage	Skin hemorrhage, mortality	Caudal fin erosion, skin hemorrhage, mortality	Poor growth
Biotin	Degenerative gill lamellae; skin lesions; muscle atrophy; spastic convulsion; reduced hepatic acetyl CoA, carboxylase, and pyruvate carboxylase; lipid infiltration of liver; degeneration of pancreatic acinar cells	Skin depigmentation, hypersensitivity, reduced hepatic pyruvate carboxylase	Abnormal swimming, dark skin coloration	Lethargy, increased number of dermal mucous cells	Lethargy, hemorrhage in gastrointestinal tracts	Not tested
Folate	Lethargy, slow growth, dark skin coloration, anemia	Lethargy, anemia, increased sensitivity to bacterial infection	Dark skin coloration	None detected	Congested fins and bronchial mantles	None detected

Vitamin	Salmonids	Channel Catfish	Japanese Eel	Common Carp	Yellowtail	Red Sea Bream
B_{12}	Microcytic hypochromic anemia, fragmented erythrocytes	Reduced growth, low hematocrit	Anorexia, poor growth	None detected	Congested fins and bronchial mantles, anemia, morality	Poor growth
Choline	Fatty liver, exopthalmia, extended abdomen, hemorrhagic kidney and intestine	Enlarged liver, hemorrhagic kidney and intestine	White-grey intestine	Fatty liver, vacuolization of hepatic cells	Dark skin	Poor growth, mortality
Inositol	Dark skin coloration, distended abdomen, anemia, fin erosion, reduced activity of cholinesterase and transaminase	None detected	White-grey intestine	Loss of skin mucosa	Lethargy, dark skin coloration	Poor growth
Ascorbic acid	Lethargy, hemorrhagic exophthalmia, intramuscular hemorrhage, distorted gill filaments, lordosis, scoliosis, ascites, anemia	Internal and external hemorrhages, fin erosion, reduced bone collagen, lordosis, scoliosis	Fin and dermal hemorrhages, lower jaw erosion	Poor growth	Skin hemorrhage, dark skin coloration, lordosis, scoliosis, anemia, mortality	Poor growth

[a] Major deficiency signs exclude anorexia and poor growth unless those are the only signs observed. Mortality means high and rapid mortality and thus might be useful for distinguishing some vitamin deficiencies.

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