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Nutrition in tropical aquaculture: Essentials of fish nutrition, feeds, and feeding of tropical aquatic species

Aquaculture Department, Southeast Asian Fisheries Development Center

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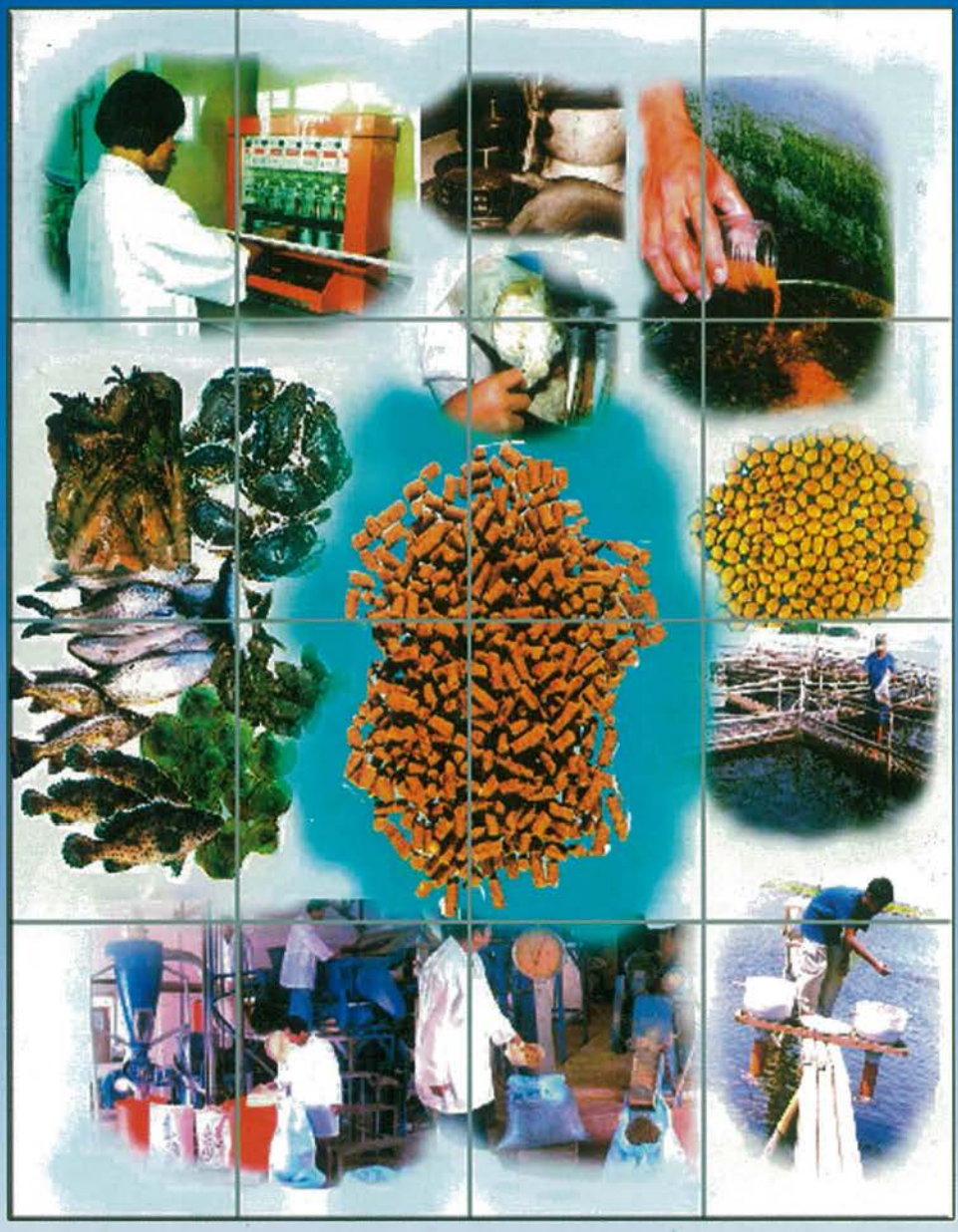
Edited by
Oseni M. Millamena, Relicardo M. Coloso, and Felicitas P. Pascual



Aquaculture Department
SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER
Tigbauan, Iloilo, Philippines
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Foreword

Aquaculture has been recognized as the fastest growing food producing sector, contributing significantly to national economic development, food supply, and food security. Alongside aquaculture development are the fisheries schools that supply the knowledge needs of the industry. These fisheries schools need current educational materials relevant to aquaculture in the tropics. This need prompted SEAFDEC Aquaculture Department (SEAFDEC AQD) to establish the textbook writing program. It was also established to fulfill SEAFDEC AQD's mandate of disseminating up-to-date knowledge in aquaculture technology. This textbook on Nutrition in Tropical Aquaculture is now the second in a series.

In developing countries, the growth of aquaculture is often hampered by a lack of expertise in aquaculture nutrition and knowhow on aquafeed formulation and preparation as well as evaluation of the nutritional and economic value of feeds. This book is intended to provide a foundation of scientific knowledge and understanding on nutrition, feeds, and feeding with emphasis on tropical aquaculture species. It is important that students in aquaculture realize the crucial role that proper nutrition, management, and economics of feeding plays in sustainable aquaculture development. The research-based information contained here are mostly products of several years of research experience in fish nutrition and feed development at SEAFDEC AQD.

While the textbook is intended for use primarily by fishery students, other aquaculture enthusiasts, like researchers, fishfarmers, feed manufacturers, farm managers, and technicians may also find it useful as a reference book. We welcome suggestions from students, instructors, and learners on how to improve this textbook.

It is our hope that, through this book, SEAFDEC AQD would be instrumental in advancing the frontiers of knowledge in aquaculture through education.

ROLANDO R. PLATON
Chief

Table of contents

LIST OF TABLES AND FIGURES

TABLES

Chapter 2

Proteins

Lipids and Fatty Acids

Carbohydrates

Vitamins

FIGURES

Chapter 1

Chapter 2

Proteins

Lipids and Fatty Acids

Carbohydrates

Energy

Vitamins

Minerals

Chapter 3

Chapter 4

Chapter 6

Chapter 7

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At SEAFDEC AQD, she works on various species such as milkfish, Nile tilapia, sea bass, anemone fish, grouper, tiger shrimp, white shrimp, and mud crab either in the hatchery, nursery, grow-out or broodstock stages. She has published a number of papers in international journals and presented papers in international conferences, including the Asian Fisheries Society (AFS) and World Aquaculture Society (WAS), of which she is a member. She is also a lecturer on topics pertaining to aquaculture nutrition and feed development in SEAFDEC AQD training courses.

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Her work has been focused on establishing nutrient requirements of some marine fishes and developing fish feeds. She is also involved in Japanese-funded projects on fish health management and biotechnology and is active in the training programs of SEAFDEC. She has authored or co-authored over 25 scientific papers in international journals and proceedings. Currently, she is a member of the board of directors of the Philippine Society for Biochemistry and Molecular Biology, Integrated Chemist of the Philippines-Panay Chapter, and is a member of the editorial board of the Philippine Journal of Science.

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She has conducted studies on the nutrition of important aquaculture species; shrimp, crab, sea bass, and snapper, on protein, vitamin C requirement, and nutrient digestibility. Her research results are published in refereed scientific international journals. She has also presented her work in local and international conferences. She is a member of the Integrated Chemists of the Philippines, the AFS and an associate member of the National Research Council of the Philippines (NRCP).

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His areas of specialization are on fish nutrition: nutritional biochemistry, amino acid and phosphorus metabolism, nutrition of milkfish, tiger shrimp, and Asian sea bass, molluscicides and environmental contamination, and toxicology. He was a recipient of a research grant from the International Foundation for Science (IFS) in 1991. He authored or co-authored over 30 scientific papers in international journals and proceedings. He is a member of Sigma-Xi Honor Society, AFS, Philippine Society for Biochemistry and Molecular Biology, NRCP, Integrated Chemists of the Philippines – Panay Chapter, and Philippine Fulbright Scholars' Association.

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His areas of specialization are varied: fish nutrition and feed development, water quality and soil chemistry, pond culture, feed milling, feed processing, and storage. He is a member of the Philippine Institute of Chemical Engineers, the Pollution Control Association of the Philippines-Region VI chapter, and the Philippine Association of Japanese Monbusho Exchange Scholars. He was a recipient of the Dr. Elvira O. Tan Award for best published research paper in Aquaculture in 1998. He is author and co-author of several papers published in refereed international journals.

Oseni M. Millamena is senior Scientist at SEAFDEC AQD. She completed her BS Chem Engineering *(cum laude)* at Central Philippine University; obtained a M. Environmental Engineering from Asian Institute of Technology, Thailand, as Southeast Asian Treaty Organization scholar; a Post-graduate Diploma in Chem Engineering from Tokyo Institute of Technology as a United Nations Educational, Scientific and Cultural Organization fellow; and a PhD in Fisheries Science (major in Aquatic Nutritional Chemistry), from Kagoshima University, Japan as recipient of a scholarship from Japan Society for the Promotion of Science. Her research interests are mainly on: nutritional requirements and developing cost-efficient feeds for tiger shrimp, mud crabs, grouper, abalone; and environmental engineering.

Singly and in collaboration with other scientists, she has undertaken several research studies, 40 of which are published in international journals and scientific proceedings. She is a recipient of national research awards for best published papers in Aquaculture, Aquaculture Engineering, and the "Fish for the People" 2001 award, given by PCAMRD, DA-BAR, and Marine Technological Foundation Inc., respectively. Her involvement in other activities are numerous: as lecturer, thesis adviser and critic, research evaluator, editor and contributor. She did collaborative research projects with the Australian Center for International Agricultural Research (ACIAR), and is a member of the AFS, the International Working Group in Crustacean Nutrition, the Society of Aquaculture Engineers in the Philippines, Philippine Aquaculture Society, and associate member of the NRCP. She was selected for inclusion in the 6th Edition of Who's Who in Science and Engineering.

Felicitas Piedad-Pascual is one of the technical advisers in fish nutrition of the International Foundation for Science, and consultant at Aquaculture Specialist, Guimbal, Iloilo. She was Scientist 1 when she retired from the Feed Development Section, SEAFDEC AQD in 1990. She was a fish nutrition consultant at the University of

Diponegoro, Semarang, Indonesia and of the Cuban government. She obtained her PhD in Nutrition from Iowa State University, and her MS in Food and Nutrition from Michigan State University. She finished her BS Pharmacy degree in UP Diliman. She is an associate member of the NRCP and a member of the Philippine Association of Nutrition, AFS, and others.

She is a recipient of numerous awards and honors, among them the Elvira O. Tan Memorial Award, Five Thousand Personalities of the World, and research grants from the International Atomic Energy Agency, American Soybean Association, San Miguel Corporation, and Tetra, among others. She also received training and study grants. She is author or co-author of more than 40 papers in international journals, proceedings, books, and extension manuals. She is co-author of the recently published Handbook of Ingredients for Aquaculture Feeds.

Myrna N. Bautista-Teruel is Scientist II at SEAFDEC AQD. She obtained her MS in Food Science through a PCAMRD-SEAFDEC Scholarship Grant from UP Diliman. She finished her BS Fisheries degree also in UP Diliman.

She has published several articles on various aspects of aquaculture nutrition in international and local journals.

She was a recipient of a research grant from the International Foundation for Science (IFS) from 1991 to 1997. She was a recipient of the Dr. Elvira O. Tan Award for best published paper in aquaculture in 1991. Her research interests are mainly on aquaculture nutrition, feed processing, and feed quality control. She is presently one of the technical advisers on Animal Production and Aquatic Resources for the IFS. She is a member of the Asian Fisheries Society, the International Working Group on Crustacean Nutrition, NRCP, and Philippine Aquaculture Society.

Preface

Increasing fish production through aquaculture to supply the demands of an ever increasing population is important because fish yield from the sea is unpredictable and many fish stocks are fast becoming depleted. Aquaculture is presently considered as the fastest expanding food producing sector in the world and is expected to assume a greater role in Philippine fish production in the new millennium. The recent advances in aquaculture technology are geared towards faster fish growth and higher yields. Proper fish nutrition and adequate feeding are crucial components of this technology. Feeds must be nutritious and economical for a given farming system to be competitive and sustainable and feeding must be properly managed to be effective.

The nutritive value of an aquaculture diet depends not only upon the proper balance of nutrients according to the nutrient requirements of the cultured species and nutrient digestibility but also on the use of proper techniques in the processing of feedstuffs and feeds to remove antinutritional factors. The optimal conditions during feed storage should be known so that feed nutrients do not deteriorate. Quality control and careful inspection of the feed during processing are necessary to ensure the production of high quality feeds. Good feed formulation and proper feeding management are necessary not only for making an aquaculture enterprise profitable but also for reducing metabolic wastes and preventing aquatic pollution. Environment-friendly feeds are critical in making fish farming sustainable.

This book is the product of more than 25 years of research experience in fish nutrition and feed development at the Southeast Asian Fisheries Development Center (SEAFDEC) Aquaculture Department (AQD). SEAFDEC AQD scientists have endeavored to develop cost-effective fish and crustacean diets for the aquaculture industry in the region. Most of the information presented are based on published papers on nutrition and feeding as well as unpublished researches from SEAFDEC AQD, those gathered from other research institutions, and from the literature published worldwide. Feeds and feeding management of tropical fish species like milkfish, Nile tilapia, rabbitfish, Asian sea bass; crustaceans like tiger shrimp and mud crab; and other local species are highlighted. This book is also a product of more than ten years of experience in the conduct of the International Fish Nutrition Training Course attended by international and local participants. A SEAFDEC AQD aquaculture extension manual entitled *Feeds and Feeding of Milkfish, Nile Tilapia, Asian Sea Bass, and Tiger Shrimp* preceded the publication of this textbook. In addition, our constant interaction with the trainees and their suggestions and comments were most beneficial in the improvement and upgrading of the extension manual into this textbook.

This book is intended to teach undergraduate students the essentials of aquaculture nutrition, feed formulation, and feeding management. It serves as a reference book for researchers in aquaculture, aquaculturists, fish farmers, and aquaculture nutritionists. For the basic understanding of the materials presented, it is essential that the students, teachers, and researchers have a good background in chemistry, fish biology, or fisheries. The book covers the subject areas of known nutrient requirements, effects of nutrient deficiencies on various aquatic species, nutrient sources, digestibility, and digestive physiology. Feed formulation, processing and storage, evaluation and quality control, feeding management, as well as the economics of feeding are included. Aspects on feeds and feeding related to the conservation of the aquatic environment are also dealt with.

Each chapter of this book has common features such as an introduction, basic concepts, and a summary. Both the basic and practical aspects of fish nutrition are included to give the students and allow the readers who are unfamiliar with the topics a clear understanding and knowledge of these concepts. Study questions at the end of each chapter serve as a guide to summarize and impress on the students the salient points of the subject matter in each chapter. To easily comprehend the subject matter, there is an appendix containing the analytical methods and a glossary of technical terms. The users particularly the students are encouraged to broaden their knowledge by referring to the list of references and suggested readings at the end of each chapter.

We wish to acknowledge the support of the following: the SEAFDEC Aquaculture Department and its Chief, Dr. Rolando R. Platon; Head of Training and Information Division, Engr. Pastor L. Torres, Jr.; and the President of Iloilo State College of Fisheries, Dr. Elpidio Locsin Jr. We also thank Marilyn Surtida, Edgardo Ledesma, Eric Gasataya, Josette Bangcaya, Elmer Mirasol, and Nathaniel Marte for technical and editing assistance.

The Editors

CHAPTER

Introduction to Nutrition in Tropical Aquaculture

OSENI M. MILLAMENA

1

Introduction

Fish is a vital component of food security especially in developing countries of the world. As the world population grows, the need for more food and more fish has correspondingly increased. Aquaculture, the farming and husbandry of fish and other aquatic organisms, is now a well-established industry worldwide and is the fastest growing food production sector. However, as aquaculture operations expand, the risk to the environment grows.

Fish nutrition and feeding play important roles in the sustainable development of aquaculture. The efficient conversion of feed to fish is important to fish farmers because feed is the largest component of the total cost of production. Improved feed composition and better feed efficiency will result in higher fish production, lower feed cost, and low waste production hence, decreased nutrient load from fish farming.

Figure 1.1
Fish nutrition, feeds, and feeding management play important roles in increasing the productivity of aquaculture farms.

Feeds in Aquaculture

Aquatic animals, like any other living organisms, need essential nutrients or substances for growth, tissue repair and maintenance, regulation of body functions, and to maintain health. As fish culture becomes more intensive, it also becomes less dependent on natural food and more on prepared feeds. A nutritionally-balanced feed and adequate feeding are important factors that help maximize fish production and profitability. Inappropriate feeds could result in disease outbreaks, poor growth, and high mortality of fishes in the farm. Good quality feed coupled with appropriate feeding management has been shown to result in improved feed conversion efficiency, lower costs of production, and reduced levels of environmental degradation.

An effective feeding program should consider the basic principles of fish nutrition and feeding. This requires an understanding of the nutrient requirements of cultured

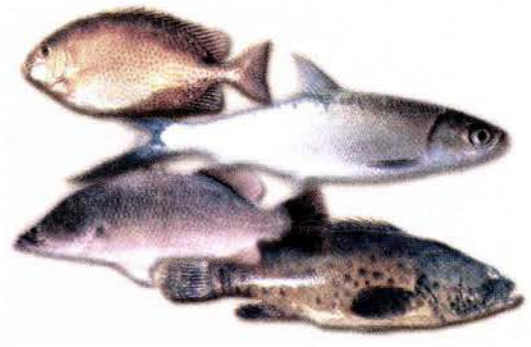

Figure 1.2
A nutritionally-balanced feed and adequate feeding are important factors that maximize fish production and profitability.

species, their feeding habits and behavior, and the ability of fish to digest and utilize essential nutrients.

Aquafeeds must satisfy the nutrient requirements of the cultured species in terms of protein and essential amino acids, lipid and essential fatty acids, energy, vitamins, and minerals. The quality of the feed will ultimately depend on the level of available nutrients for fish. Because fish eat to satisfy their energy requirement, the energy value of the feed will affect its efficiency.

The nutrient requirements should be known for a specific fish species intended for culture. For example, the protein requirements of an omnivore like milkfish and a herbivore like tilapia are generally lower than those of carnivores like grouper, sea bass, and snapper. Fish species also differ in their requirements for essential amino acids. Milkfish requires *n*-3 fatty acids while Asian sea bass and tiger shrimp need both *n*-3 and *n*-6 fatty acids. In contrast, tilapia requires *n*-6 fatty acids. Thus, feed formulations should be based on the requirement and levels of essential nutrients that are optimal for the cultured species.

Figure 1.3
Production of high quality feeds starts with good quality ingredients and proper quality control throughout processing until use in fish farms.

In feed development, there should be a continuous investigation on methods to improve the quality of raw materials, reduce feed cost, and improve feeding management in the farm. The nutrients in feeds have to be efficiently utilized by the fish. There are numerous new products in the market such as feed attractants, binders, and growth promoters. However, new feed additives are constantly being introduced without proper evaluation of their efficiency, thus caution must be exercised in using them in aquafeeds. Feed manufacturing should ensure that the nutrient composition is maintained and anti-nutrient factors are eliminated. Feed quality control must start from ingredient selection and continue through feed processing, storage, and use in fish farms.

A good quality and nutritionally-adequate feed can be ineffective unless proper feeding practices are used. Emphasis must also be given to good feeding management and improved feed performance. An effective feeding management requires answers to questions of what, how much, when, how often, and where, to feed the fish. The feeding regime used should match the feeding behavior and digestive cycle of the fish in order to maximize feed utilization. Any reduction in food wastage will have a significant impact on fish production costs and the quality of the culture environment.

Feeds and the Environment

The rapid development of aquaculture from extensive to intensive systems has posed some problems about its sustainability. Aquafeeds provide nutrients for fish, but can be major sources of pollutants in aquaculture production systems. Nitrogen, phosphorus, organic substances, and hydrogen sulfide are the main factors affecting environmental pollution of fish farms. As fish stocking density is increased, a proportionate amount of metabolic wastes is produced. Wastes that accumulate in the pond can slow down fish growth and are toxic to fish.

Fish farm wastes consist of: a) solid matter, mainly a mixture of uneaten food, feces, and colonising bacteria; b) dissolved matter, such as ammonia, urea, carbon dioxide, phosphorus and hydrogen sulfides. The wastes include amino acids, proteins, fats, carbohydrates, fiber, minerals and bacteria (Boyd 1989). Since the amount of waste increases with poor feed conversion, the less efficient fish and shrimp producers are at greatest risk. The amount of feed should be adjusted to mimimize unconsumed feed and prevent pollution of the pond, the surrounding areas, and the coastal zone. Poor feeding management can bring diseases, slow growth, low survival, environmental degradation, poor harvest, and large financial losses.

Aquafeeds have to be environment-friendly. Feed development should take into account new knowledge regarding nutrient requirements and digestibility, improved techniques to make more water stable feeds and greater utilization of alternative sources of protein. The major considerations in formulation of feeds for sustainable aquaculture are:

- ❑ Feeds should have a well-balanced nutrient profile; i.e., more precise amino acid profile, increased energy and balanced protein to energy ratio, so that more nitrogen will be assimilated and less nitrogenous wastes will be excreted by fish;
- ❑ Total phosphorus levels in feeds should be based on the requirement of cultured species and feed ingredients with highly available forms of phosphorus should be used. Phosphorus discharge in pond effluent is influenced by the level and bioavailability of phosphorus in the feed. A better understanding of factors involved in the partitioning of phosphorus in the various fractions of the pond effluent will result in better pond management;
- ❑ Digestible feed ingredients should be used to reduce organic wastes from feeds. Greater assimilation of feed nutrients by fish improves feed efficiency and reduces aquaculture wastes;
- ❑ Pellet water stability should be improved by using efficient diet binders and proper techniques for pelleting feeds. Uneaten feeds collect at the bottom of the pond and contribute to rapid water quality degradation;
- ❑ Greater use of alternative protein sources for fish meal in aquafeeds should be encouraged through continuous and improved research;
- ❑ Exotic feed ingredients which may contain unknown growth inhibitors should be avoided unless methods are available to minimize or eliminate these factors in the finished feed.

Sustainable Approaches to Aquaculture

In making aquaculture sustainable for future generations, the key issues relating to feed development and feeding are:

1. **the need to reduce feed cost.**
   Feed is the most costly single item in fish farming and the availability of a cost-effective feed remains as the bottleneck to aquaculture development. Feed formulas should be refined and feeds made cheaper. Readily available rather than expensive imported ingredients should

be used as feed ingredients. These alternative raw materials may differ from one country to another and their availability may be localized. Thus, feed surveys should be conducted to know the location of these resources and their availability, nutrient composition, and cost. Such an approach is necessary to maximize their utilization and reduce the reliance on imported feed ingredients.

2. **the search for alternatives to fish meal and fish biomass for aquaculture.**

Traditionally, fish meal has been the major component of fish feeds. Increased global aquaculture production and a reduction in fish meal production increases the need for alternative protein sources. Fish meal has become more expensive and scarce due to increased demand and stiff competition with other food production sectors. Increasing demand for fishery products due to a rapidly growing population, especially from Asian countries, will further decrease the availability of fish meal. Fish should be used to feed people rather than be converted into fish meal or be used to feed carnivorous fish.

Figure 1.4
A good quality feed and appropriate feeding management maximize feed efficiency, lower cost of production, and reduce environmental degradation.

Agricultural by-products, such as vegetable and animal meals, have been utilized to produce cheap and cost-effective diets for several aquaculture species. Some of these promising ingredients include high protein, low ash meat meals, poultry offal meals, and legumes such as beans and peas, modified wheat gluten products, oilseeds, and other grains. Biotechnology may also be used to produce novel ingredients with the desired nutritional characteristics.

3. **the greater use of supplementary feeds and supplemental feeding systems.**

Commercial aquafeeds are usually expensive because they contain nutrients in excess of what the fish needs. They are also formulated as complete diets irrespective of the intended fish stocking density and presence of natural food in the pond. Supplementary feeding practice is partly dependent on natural productivity to supply some nutrients. Feed is only used to supplement the endogenous food supply. Thus, the importance of natural productivity in the overall nutrition of pond-raised fishes should be given emphasis. Since feed is used only as a supplement to natural food, this feeding system is more cost-efficient if the pond ecosystem is well understood. Maximizing the use of natural food organisms will reduce the cost of fish production.

4. **integration of feed development and feeding management practices with environmental awareness.**

Uneaten feed and metabolic wastes of fish are major sources of pollutants from aquaculture production systems. There is a need to develop feeds that are environment-friendly. The nutrient composition, nutrient balance, digestibility, and water stability of fish feeds have a significant impact on water quality. Improved diets with better feed efficiencies and use of ecologically-sound feeding management will lead to significant reduction of wastes from aquaculture.

Summary

Fish nutrition, feeds, and feeding management are critical factors in the sustainable development of aquaculture. The choice of a suitable feed is an important task of an aquaculturist. A feeding strategy that utilizes cost-efficient, low fish meal, and low pollution aquafeeds in a supplemental feeding system will help ensure the long-term sustainability of aquaculture. It is also the responsibility of the aquaculturist to implement sound feeding strategies to increase fish production, profitability, and prevent the degradation of the culture environment.

Suggested Readings

Akiyama D, Dominy WG, and Lawrence AL 1991. Penaeid shrimp nutrition for the commercial feed industry. Revised. In: Akiyama DM and Tan RKH (eds). Proceedings of the Aquaculture Feed Processing and Nutrition Workshop. American Soybean Association, Singapore. 155 p.

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CHAPTER

2

The Essential Nutrients

OSENI M. MILLAMENA

This chapter discusses the essential food nutrients, their classification or types, chemical structures, general functions, and importance in the nutrition of aquatic animals. It is divided into six sections: proteins and amino acids, lipids and fatty acids, carbohydrates, energy, vitamins, and minerals. There are specific learning objectives for each section.

PROTEINS AND AMINO ACIDS

Introduction

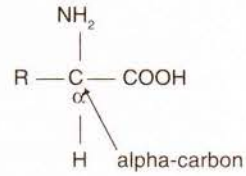
Proteins are macromolecules made up of carbon, hydrogen, oxygen, nitrogen, and may also contain sulfur. The nitrogen content of protein distinguishes it from fats and carbohydrates and other organic compounds. Proteins occur in every living cell as compounds of tissues and organs and are major components of fish tissues. They are needed for growth and tissue repair and maintenance. No other nutrient can take the place of protein in its major role of building and repairing worn out cells and tissues. In addition, proteins are also responsible for muscle contraction and are components of enzymes, hormones, and antibodies. Proteins may be complexed with heme, carbohydrate, lipid, or nucleic acids.

Aquatic animals must consume protein to provide a continuous supply necessary for replacing worn-out tissues (maintenance) and for the synthesis of new tissues (growth and reproduction). Inadequate dietary protein will result in retardation or cessation of growth or a loss of weight due to withdrawal of protein from less vital tissues in order to maintain the functions of more vital ones.

This section aims to teach the reader the ten essential amino acids required by fish and their chemical structures, distinguish between essential and non-essential amino acids; the fate of absorbed amino acids in fish; effects of deficiencies and excesses of dietary amino acids in fish diets; the procedure on how to determine the qualitative and quantitative amino acid requirements of fish; methods of evaluating protein quality; and how to determine protein requirements of some aquaculture species.

Amino Acids

Proteins can be broken down or hydrolyzed into a number of basic units called amino acids. These amino acids are called the building blocks of proteins. The term amino comes from the $-NH_2$ or an amino group which is "basic" in nature and the "acid" part comes from the -COOH or a carboxyl group, hence the term amino acid. In protein molecules, amino acids form peptide bonds (bonds between amino and carboxyl groups) in long strands called polypeptide chains. There are many amino acids in nature but only 20 are naturally occurring. These are also the 20 amino acids specified in the genetic code common to all life.



The components of the general structure of an amino acid are: 1) a carboxyl (-COOH) group, 2) an amino group ($-NH_2$) on the alpha (α) carbon and 3) an alkyl (R) group attached to the α C atom. The amino acids differ in their R group. The R group gives an amino acid its individual chemical characteristic. The formation of a peptide bond or amide linkage involves covalent bonding between an amino group of one amino acid and the carboxyl group of the adjoining amino acid. Proteins may consist of one or more polypeptide chains held together in the protein molecule.

Amino acids are commonly referred to by three-or one-letter abbreviations as listed in Table 2.1.

Table 2.1 Names and abbreviations of the common amino acids

Essential Amino Acids	Abbreviation Three-letter	One-letter
Arginine	Arg	R
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Threonine	Thr	T
Tryptophan	Trp	W
Valine	Val	V
Non-essential Amino Acids		
Alanine	Ala	A
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Proline	Pro	P
Serine	Ser	S
Tyrosine	Tyr	Y

The chemical structures of each of these essential amino acids are shown in Figure 2.1.

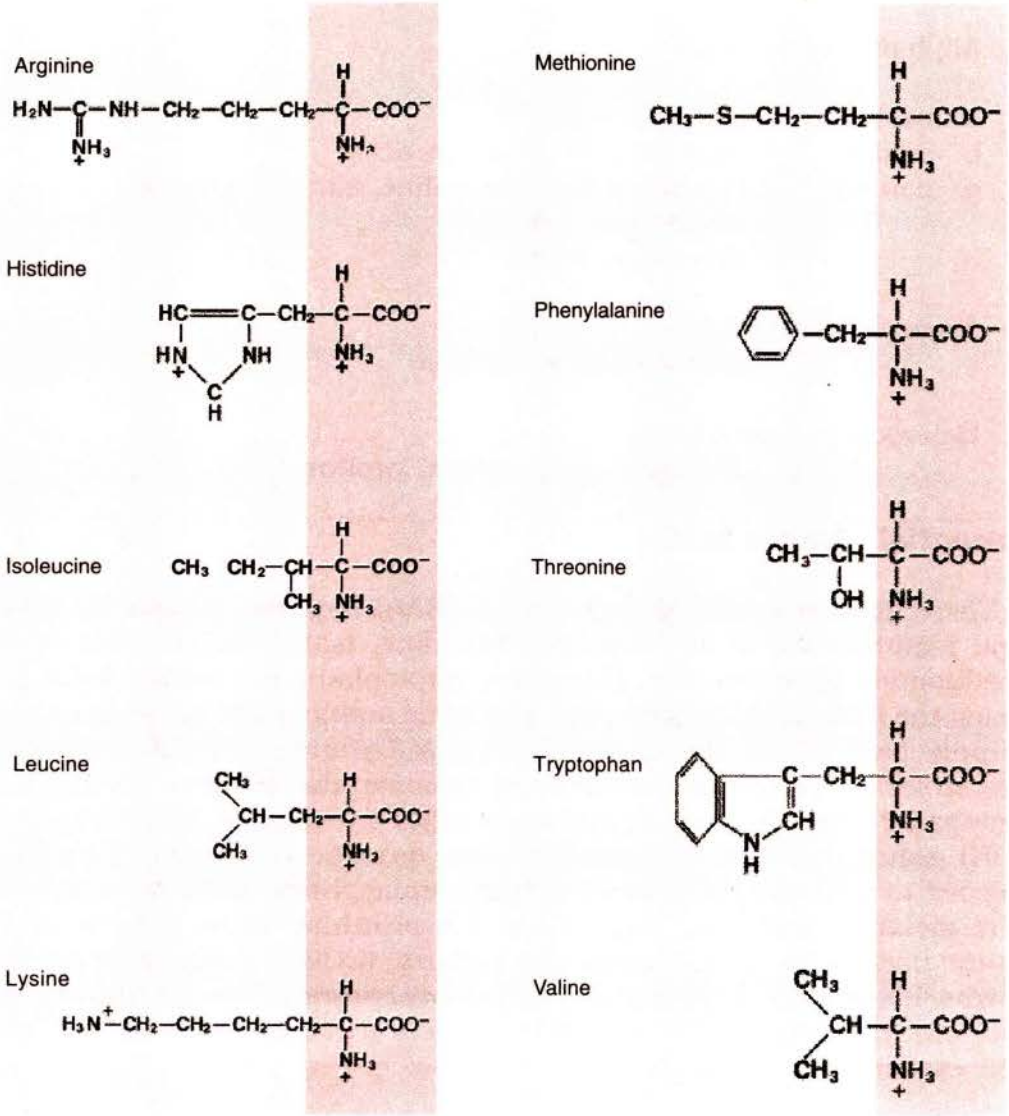


Figure 2.1
Chemical structures of the ten essential amino acids.

Classification of Amino Acids

Amino acids are classified as essential or indispensable, and non-essential or dispensable. Essential or indispensable amino acids cannot be made or synthesized by the animal or which are synthesized in amounts not enough to support maximum growth and have to be present in their diet. The capacity of different feed proteins to meet the amino acid needs of fish differs considerably. The essentiality of an amino acid will also depend on the animal being fed. For example, glycine is required by chicken but is not essential for fish. Non-essential or dispensable amino

acids can be adequately synthesized by the animal or formed from other amino acids in sufficient amounts in tissues and does not have to be present in their diet. Amino acids can also be classified according to the chemical composition of their side chain as listed below:

1. Aliphatic amino acids

   a. basic - arginine, lysine
   b. acidic - aspartic acid, glutamic acid
   c. neutral - leucine, isoleucine, valine, alanine, glycine
      methionine, cysteine
      threonine, serine

2. Aromatic amino acids
   phenylalanine, tyrosine

3. Heterocyclic amino acids
   histidine, tryptophan, proline

Essential Amino Acids

There are ten essential amino acids (EAA) required by fish for growth and maintenance of life: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Aside from being the building blocks of proteins, some amino acids are precursors or supply part of the structure of other substances. Methionine is the precursor of cysteine and cystine. Methionine also supplies methyl (CH_3) groups for creatine, choline, and many other substances. When a hydroxyl (OH) group is added to phenylalanine, tyrosine is formed. Tyrosine is needed to form the hormones thyroxine, epinephrine and norepinephrine, and melanin pigments. Arginine yields ornithine when urea is formed in the urea cycle. The removal of a carboxyl (COOH) group from histidine forms histamine. Tryptophan is the precursor of serotonin and the vitamin, nicotinic acid. All finfishes have a requirement for the same ten essential amino acids.

Non-essential Amino Acids

The non-essential amino acids for fish are: alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine. These amino acids have nutritional significance because their presence in the diet conserves energy that is required for protein synthesis. Some dispensable amino acids can partially replace or spare indispensable amino acids. Phenylalanine can be partially replaced by tyrosine and methionine by cystine. Thus, requirements for phenylalanine and methionine decrease when tyrosine and cystine, respectively, are present in the diet.

Classification of Proteins

Proteins are broadly classified according to their form, solubility, and physical properties.

- **Globular proteins** have compact spherical or globular shape. They are mostly soluble in aqueous systems. They include: enzymes, protein hormones, proteins of the serum fraction of the blood and antibodies.

- **Fibrous proteins** consist of a long chain of polypeptides. They are highly insoluble and resistant to action of the digestive enzymes. They include collagen, found in cartilage or soft bones, blood vessels, bone matrix, tendon, fins and skin; elastins, that are a component of arteries and ligaments; and keratins, in protective coverings such as skin and scales.

Based on physical properties, proteins are grouped into simple, conjugated, and derived proteins:

- **Simple proteins** yield only amino acids or their derivatives when hydrolysed. They include albumins (egg albumin, serum albumin from blood, lactoalbumin from milk, leucosin from wheat); albuminoids (keratin from hair, fingernails, feathers, wool, silk fibroin, elastin from connective tissue, collagen from cartilage and bones); globulins (edestin from hemp seed, serum globulin from blood, lactoglobulin from milk, legumin from peas); histones (globin from hemoglobin, scombrone from spermatozoa of mackerel); and protamins (salmine from salmon, scombrine from mackerel). These groups are differentiated by solubility in various solvents such as water, salt solution, alcohol, and by other characteristics.

- **Conjugated proteins** yield amino acids and non-protein components when broken down by hydrolysis. They include the nucleoproteins, glycoproteins, phosphoproteins, hemoglobins, and lecithoproteins. Nucleoproteins are compounds of one or more protein molecules with nucleic acid present in all cell nuclei. Glycoproteins are compounds of the protein molecule and substances containing a carbohydrate group other than nucleic acid (e.g., mucin). Phosphoproteins are compounds of the protein molecule with a phosphorus-containing substance other than nucleic acid or lecithin (e.g., casein). Hemoglobins are compounds of the protein molecule with hematin or a similar substance (e.g., hemoglobin). Lecithoproteins are compounds of the protein molecule with lecithin (e.g., tissue fibrinogen).

- **Derived proteins** consist of compounds representing altered and degraded products of naturally occurring proteins, produced by the action of heat, enzymes, or chemical agents.

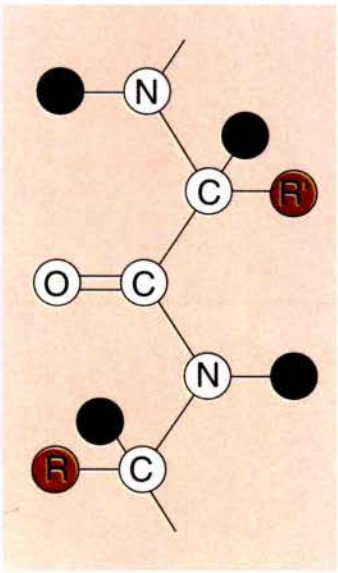


Figure 2.2A
Primary structure consists of amino acids joined in sequence by peptide bonds.

Protein Structure

The composition, organization and shape of proteins are directly related to their function. Because of the complicated structure of proteins, a new terminology has developed as an aid in describing the structural organization of a protein molecule. These are designated as primary, secondary, tertiary, and quaternary structure of proteins.

- **Primary structure** describes the specific sequence in which the amino acids are linked together by peptide bonds in a polypeptide chain (Figure 2.2A). For example, the peptide Leu-Gly-Thr-His-Arg-Asp-Val has a different primary structure from the peptide Val-Asp-His-Leu-Gly-Arg-Thr, even though both have the same number and kinds of amino acids.

  The primary structure determines the three-dimensional conformation of the protein molecule and its cellular role. It also shows the sequence of nucleotides in DNA or RNA and thus provides information on the genetic input to protein synthesis and cellular potential. Thus, a change in any one amino acid in the primary structure of a protein may produce a drastic effect in the animal.

- **Secondary structure** describes the manner in which the amino acids are arranged in the polypeptide chain. This develops through the interactions between adjacent amino acid residues in a polypeptide chain (Figure 2.2B).

  The secondary structures may exist in the form of coiled helices (alpha-helix) (Figure 2.2C) or in an extended form (beta-sheet) (Figure 2.2D).

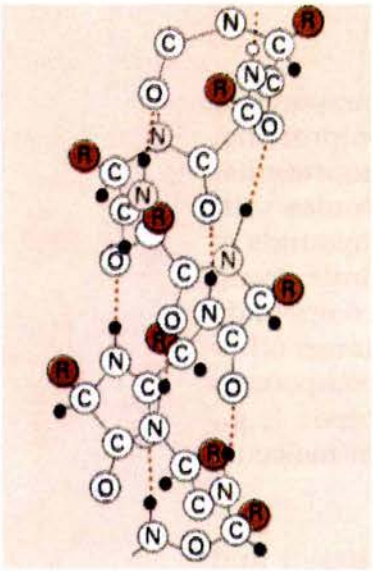


Figure 2.2B
Secondary structure develops through interactions between neighboring residues, as by hydrogen bonds between adjacent monomer units in an alpha-helix region.

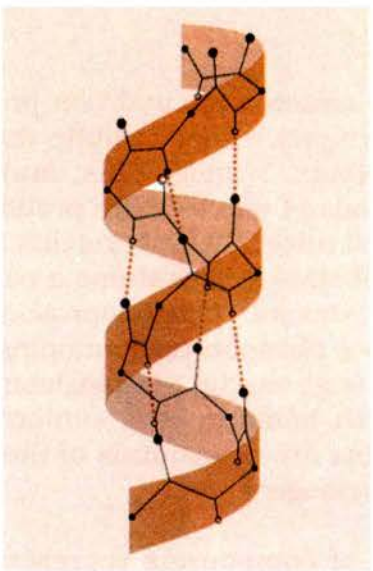


Figure 2.2C
The alpha-helix structure of a polypeptide chain, or part of the chain, is stabilized by hydrogen bonds (dotted lines) between the oxygen (color) of one peptide bond and the hydrogen of another.

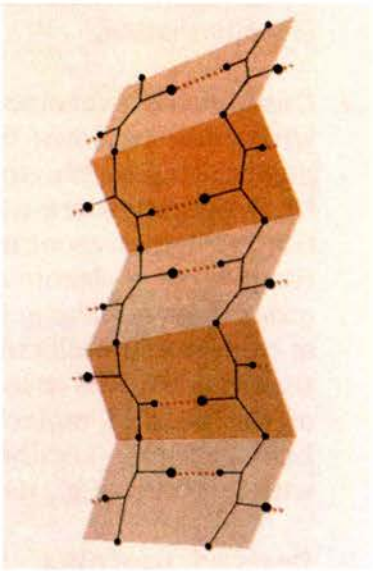


Figure 2.2D
The beta-sheet structure of all or part of a polypeptide chain is characterized by hydrogen bonds (dotted lines) between every peptide bond and its neighbor.

The alpha-helix structure is stabilized by hydrogen bonds between the oxygen of one and the hydrogen of another peptide bond while the beta-sheet structure is characterized by hydrogen bonds between adjacent peptide bonds. The hydrogen bonding between the peptide chains in the beta-sheet gives rise to a repeated zigzag structure hence, the name pleated sheet. Fibrous proteins exhibit a high degree of secondary structure forming sheets of molecules that are involved in cellular construction.

- **Tertiary structure** describes the three-dimensional arrangement or the actual conformation of all the atoms in the protein molecule. The interactions between distant amino acid residues of a polypeptide chain leads to the folding and a more globular conformation of the polypeptide chain assuming a three dimensional shape, for example, myoglobin (Figure 2.2E). Globular proteins usually have a considerable amount of unordered random coil regions, along with regions of ordered alpha-helix and other types of secondary structure. The secondary and tertiary structure of a protein can be determined simultaneously.

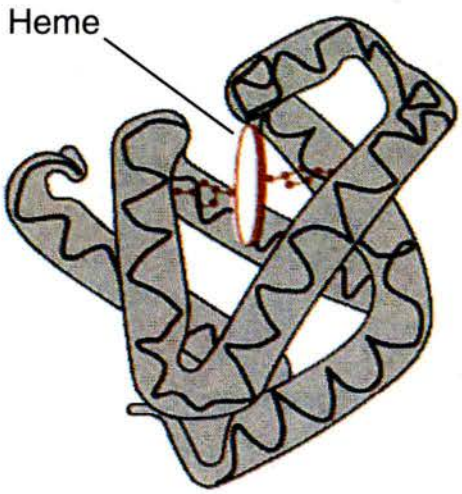


Figure 2.2E
Tertiary structure depends on the interaction between more distant residues, leading to folding and to a more globular conformation of the polypeptide chain.

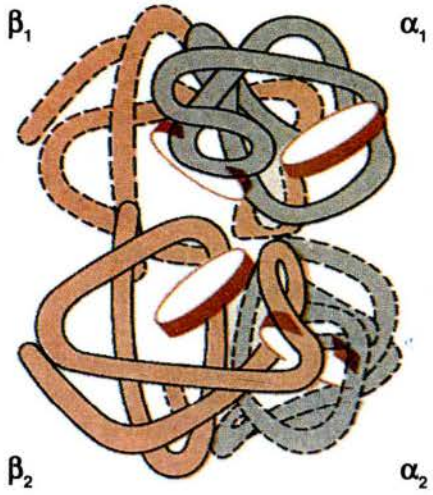


Figure 2.2F
Quaternary structure is the result of interactions between two or more polypeptide chains in a protein molecule (four chains in hemoglobin).

- **Quaternary structure** refers to the spatial organization when two or more polypeptide chains are part of a single protein molecule. Commonly occurring examples are dimers, trimers, tetramers, consisting of two, three, and four polypeptide chains, respectively. The polypeptides are held together by weak chemical bonds (Figure 2.2F). The absolute number of polypeptides and the number of different kinds of polypeptides in a protein varies. For example, hemoglobin molecules consist of two alpha- chains and two beta- chains. Each globin chain in hemoglobin is bonded to a heme group, which functions

in oxygen transport to body tissues. Disruption of any level of molecular organization, including quaternary structure, leads to malfunction of a protein.

Fate of Absorbed Amino Acids

Proteins are absorbed from the intestine, mainly as amino acids. The absorbed amino acids are used in one of the following ways: a) incorporated into the metabolic pool, mixed with free amino acids originating from various tissues and synthesized into tissue proteins; b) synthesized into nitrogen-containing tissue constituents such as nucleic acids, hormones and enzymes; c) deaminated (removal of nitrogen), resulting in a carbon chain (-C-C-) and an amino group ($-NH_2$).

The carbon chains are oxidized to produce energy or synthesized into sugars and fats, or again react with amino groups, forming amino acids. Amino groups separated from amino acids are excreted in the form of nitrogen compounds such as urea and ammonia in the urine or excreted through the gills.

Importance of Amino Acid Profiles in Fish Nutrition

Fish tissues contain about 65-75% protein on a dry weight basis. Dietary protein often constitutes the principal and most expensive item in fish diets. The free amino acids released from proteins by the action of digestive enzymes are absorbed in the intestinal tract and used by various cells to build and repair worn out tissues. Excess amino acids are used as energy source or converted to fat.

Information on gross protein requirement of fish is of limited value without data on essential amino acid requirements since protein quality depends largely on its amino acid composition and digestibility. The determination of essential amino acid profiles is helpful in the design of amino acid test diets used for research studies to determine the amino acid requirement of fish. It is also an important parameter in the evaluation of the protein quality of feedstuffs.

The nutritive value of a dietary protein is dependent on the extent to which the composition of its essential amino acids fulfills the requirement of the organism. The closer the profile to the requirement, the higher is the nutritional value. The amino acid lacking in the protein is known as the limiting amino acid. For example, lysine is limiting in corn while methionine is limiting in soybeans.

Qualitative Amino Acid Requirements

The two methods used to determine whether an amino acid is essential or non-essential are: 1) growth response method, and 2) the radioisotope method.

The growth response method as used by Halver (1957) uses a series of amino acid test diets containing crystalline L-amino acids as source of nitrogen. The diets are formulated based on the amino acid pattern of a reference protein such as whole chicken egg protein, chinook salmon egg protein, or chinook yolk-sac fry protein.

For each of the ten essential amino acids, a 10-week feeding trial is conducted using a basal diet containing all the amino acids and a diet deficient in the amino acid being tested. Fish are weighed every two weeks to measure growth response to the test diet. Fish fed the amino acid-deficient diet show poor growth but a substantial growth response is observed when fish is fed the complete amino acid diet. Subsequently, other investigators used a similar test diet for determining the essentiality of some amino acids in other fishes.

In the radioisotope method used by Cowey et al. (1970), fish are injected intraperitoneally with radioactively labeled ^{14}C glucose and fed on a natural diet for 7 days. Fish are then killed, homogenized, and protein was isolated. A sample of the isolated protein is then hydrolyzed and the constituent amino acids are separated by chromatography and counted for radioactivity. Significant radioactivity is incorporated into non-essential amino acids while the essential amino acids have very little or no radioactivity. This method was also used by Coloso and Cruz (1980) to determine the qualitative amino acid requirements of tiger shrimps.

Quantitative Amino Acid Requirements

The quantitative amino acid requirements of fish is determined using either purified or semi-purified test diets. A purified diet is made up of pure substances (casein, gelatin, crystalline amino acids) while a semi-purified diet may contain other ingredients such as fish meal or soybean meal. The test diet is formulated so that the amino acid profile is identical to that of a reference protein usually fish muscle protein. A series of experimental diets is then prepared containing graded levels of one amino acid for which the requirement is to be determined. Diets are fed to fish and the gain in weight are measured at weekly or biweekly intervals. The required dietary level is determined using the dose response curve (Figures 2.3A, B).

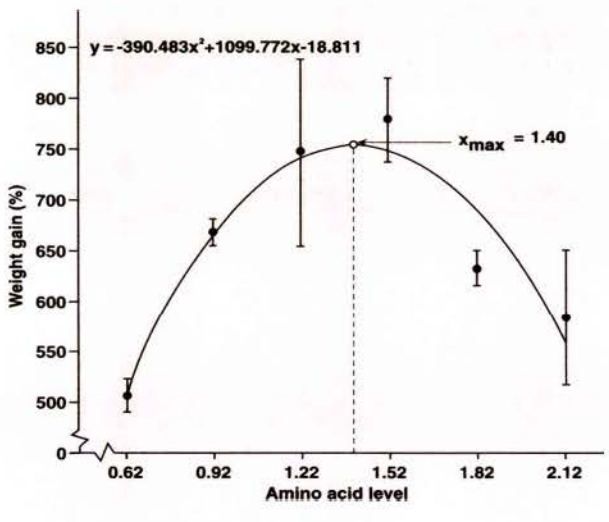


Figure 2.3A
Growth response of tiger shrimp fed graded levels of phenylalanine for 8 weeks as described by the quadratic regression model.
Source: Millamena et al. 1999

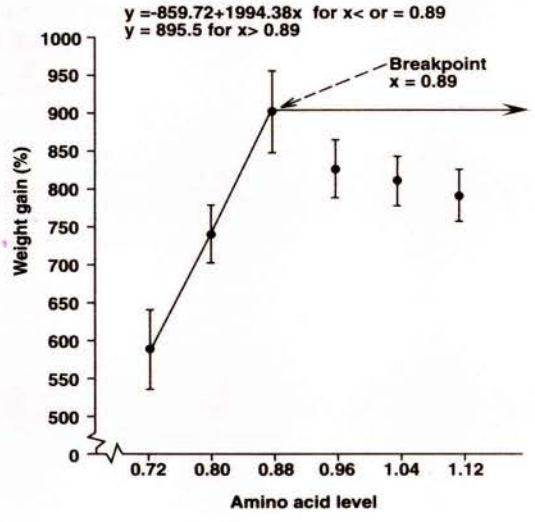


Figure 2.3B
Growth response of tiger shrimp fed graded levels of methionine for 8 weeks as described by the broken-line regression model.
Source: Millamena et al. 1996

A dose response curve indicates the growth response of the fish to graded levels of an essential nutrient in their diet. This method assumes that weight gain is linearly related to increasing dietary levels of the essential amino acid at or below the requirement level. When the requirement is met, weight gains abruptly plateau and then decline if the dietary concentration of the nutrient exceeds the animal's tolerance. Figure 2.3 (A) shows a quadratic curve with the requirement of phenylalanine at 1.4 percent of diet or 3.7 percent of dietary protein.

The broken line analysis has also been used to estimate the essential amino acid requirement. This method also assumes a linear relation between growth and dietary level of the essential amino acid at or below the requirement. At the requirement level, the linearly ascending line instantly breaks to horizontal. The point is known as breakpoint. Figure 2.3 (B) shows a linear curve with the requirement for methionine at 0.89 percent of diet or 2.4 percent of dietary protein.

In young growing animals, the greatest proportion of body weight is in the form of muscle. It is reasonable to infer that the dietary amino acid requirement will be closely related to the amino acid profile of muscle or whole body protein. Thus, when this hypothesis was examined for pigs, chicken, fish and shrimp, the amino acid requirement was closely correlated to the amino acid profile of the muscle protein.

There are several problems that occur in the accurate determination of amino acid requirements of fish when based on growth studies: a) growth rates that are commonly observed in the amino acid test diets are frequently inferior or lower than those observed with natural or intact proteins. b) some of the amino acids in test diets may leach out during feeding. c) the interpretation of the breakpoint in the growth response curve could be subjective if the appropriate statistical tool is not used. The requirements of some fishes and shrimp for essential amino acids are shown in Table 2.2.

Table 2.2 Amino acid requirements of some fishes and shrimp in percent of protein

	Eel	Carp	Rainbow trout	Chinook salmon	Milkfish	Nile tilapia	Sea bass	Tiger shrimp
Arginine	4.5	4.4	4.0	6.0	5.2	4.2	3.6	5.3
Histidine	2.1	1.5	1.8	1.8	2.0	1.7		2.2
Isoleucine	4.0	2.6	2.8	2.2	4.0	3.1		2.7
Leucine	5.3	4.8	5.0	3.9	5.1	3.4		4.3
Lysine	5.3	6.0	6.0	5.0	4.0	5.1	4.5	5.2
Methionine + Cys/2	5.0	2.7	3.3	4.0	2.5	3.2	2.9	2.4
Phenylalanine+ Tyr	5.8	5.7	6.0	5.1	4.2	5.5		3.7
Threonine	4.0	3.8	4.1	2.2	4.5	3.8		3.5
Tryptophan	1.1	0.8	0.6	0.5	0.6	1.0	0.5	0.5
Valine	4.0	3.4	3.6	3.2	3.6	2.8		3.4

Source: NRC 1993; FDS Manual 1994; Santiago and Lovell 1993; Borlongan and Coloso 1998; Coloso et al. 1999; Millamena et al. 1996-99

Deficiencies and Excesses of Dietary Amino Acids

Essential amino acid deficiency may be caused by the use of feed ingredients usually protein sources that are slightly or grossly deficient in at least one essential amino acid. Amino acid deficiency may also arise from the presence of chemicals that may affect some feed ingredients, excessive heat treatment during feed manufacture, and leaching out of nutrients.

Amino acid imbalance due to amino acid antagonism or amino acid toxicity may also cause reduced growth in animals. Amino acid antagonism occurs when some amino acids are fed in excess of the required levels, causing an increase in the requirement for another amino acid of similar structure. Some examples are the leucine-isoleucine antagonism and arginine-lysine antagonism observed in some fish species. Amino acid toxicity occurs when excess amounts of certain amino acids are fed to the animal and the negative effects cannot be improved by adding amino acids in the diet.

In practical diet formulation, the recommended dietary levels of essential amino acids can be met by carefully selecting and properly combining two or more protein sources. The limiting or deficient amino acid in one protein source can be supplemented by another protein source abundant in the same amino acid and hence make a better feed. Another way of meeting the EAA needs of an animal is by supplementing the practical diet with crystalline L-amino acids. Nutrient leaching can be minimized by using water stable diets through the use of efficient binders and employing appropriate feeding practices.

Evaluation of Protein Quality

Proteins are said to be of high quality when their amino acid composition closely resembles the amino acid pattern or approximates the essential amino acid requirements of the animal under consideration and when they are highly digested by the animal. The quality of proteins is usually evaluated by biological and chemical methods. The chemical method determines the quantity of protein or amino acid in a feedstuff whereas the biological method determines how the fish reacts to the protein in terms of growth and survival. In the biological method, body weight gain and nitrogen retention are used as criteria for protein quality which is considered as more accurate than the chemical method. Protein efficiency ratio, biological value, and net protein utilization are used to measure the biological value of a protein and can be calculated, by using the following formula:

1. Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Live weight gain (grams)}}{\text{Amount of protein fed (grams)}}$$

2. Biological value (BV)*

$$BV = \frac{\text{True nitrogen retained}}{\text{Nitrogen absorbed}} \times 100$$

where, R = true nitrogen retained
A = nitrogen absorbed

and $A = I - (F - Fo)$,
$R = A - (U - Uo)$

where, I = nitrogen intake
F = nitrogen excreted in the feces
Fo = metabolic faecal nitrogen
U = nitrogen excreted in the urine
Uo = endogenous nitrogen

$$\text{Thus, } BV = \frac{R}{A} \times 100 \; \frac{I - (F - Fo) - (U - Uo)}{I - (F - Fo)} \times 100$$

* Insufficient data on the biological value are available for fish due to difficulties in determining the metabolic faecal and endogenous nitrogen separately

3. Net protein utilization (NPU)

$$NPU = \frac{\text{True nitrogen retained}}{\text{Nitrogen intake}} \times 100$$

where, NPU is determined by the following formula:

$$NPU = \frac{\text{Nitrogen increase in fish fed the test protein diet} + \text{Nitrogen decrease in fish fed the protein free diet}}{\text{Nitrogen intake from the test protein diet}} \times 100$$

A detailed procedure for protein evaluation is discussed in Chapter 6.

Protein Requirement

The optimum protein requirement is the level of high quality dietary protein needed for maximum growth. To determine the protein requirement of a fish species, feeding trials have to be conducted using test diets containing graded levels of protein from sources of high biological value. Growth response, usually weight gain, is measured for each diet. The diet that gives the highest weight gain and survival is considered as the best diet. Maximum tissue protein retention may also be used as the criterion for determining protein requirement instead of weight gain. This is done by analyzing the amount of nitrogen in the tissues at certain intervals e.g. every two weeks until there is no more decrease in nitrogen retained in the tissues.

The minimum amount of dietary protein needed for optimum growth of most cultured aquatic species ranges from 27% to 60%. A summary of optimal protein levels and the protein sources used to determine these values are presented in Table 2.3.

Table 2.3 Optimal dietary protein levels (% of dry diet) for some aquaculture species

Species	Protein sources	Optimal protein level	References
Asian sea bass	fish meal, soybean meal	43	Catacutan & Coloso 1994
Common carp	fish meal, casein	31-38	Takeuchi 1979
Grouper	tuna, muscle meal	40-50	Teng et al. 1978
	fish meal, meat meal, shrimp meal	43	Shiau 1972
Japanese eel	casein + amino acids.	44	Nose & Arai 1972
Kuruma shrimp	squid meal	60	Deshimaru & Shigeno 1972
	casein + egg albumin	>55	Teshima & Kanazawa 1984
Milkfish	fish meal, casein	40	Lim et al. 1979
	casein, gelatin	30-40	Pascual 1989
	fish meal, soybean meal, cassava meal	24*	Sumagaysay & Borlongan 1995
Red sea bream	casein	55	Yone 1976
Snakehead	fish meal	52	Wee & Tacon 1982
Red Snapper	fish meal, soybean meal, squid meal	44	Catacutan & Pagador 2001
Tiger shrimp	casein	40	Alava & Lim 1983
	fish meal, soybean meal, shrimp meal	40	Millamena & Triño 1994
Nile tilapia	fish meal, casein	30	Wang et al. 1985
	fish meal	28	Santiago et al. 1986
White shrimp	mussel meal, fish meal, collagen,	34-42	Andrew & Sick 1972
	squid meal	28-32	Sedgwick 1979
Yellow tail	fish meal, casein	55	Takeda et al. 1975
Abalone	soybean meal, rice bran	27	Bautista-Teruel & Millamena 1999
	fish meal, squid meal		

* tested under pond conditions

Protein of high biological value such as whole egg protein, casein, combination of casein and gelatin, and fish meal are often used in determining protein requirements. Several factors influence protein requirements for maximum fish growth: species, fish size or age, water temperature, protein quality as reflected by the amino acid profile, dietary level of non protein energy and daily food allowance. Smaller or younger fish have higher protein requirements than older fish of the same species. Fish fed at feeding rates below satiation will require a higher protein level. Fish also respond better to a higher level of dietary protein when fed poor quality protein.

Guide Questions

1. What is a protein? What is the most important function of a protein?
2. How are proteins classified? Distinguish between simple, conjugated, and derived proteins.
3. What is the general structure of amino acids?
4. Why are amino acids called the building blocks of proteins?
5. Name the ten essential amino acids required by fish.
6. Differentiate between essential and non-essential amino acids.
7. Describe the fate of absorbed amino acids.
8. Why is the amino acid profile of a feed a valuable index in the assessment of the nutritive value of dietary proteins?
9. What is the difference between qualitative and quantitative amino acid requirement? Describe each briefly.
10. What are the two methods used to determine qualitative amino acid requirements?
11. Describe a dose-response curve used to determine the quantitative amino acid requirement.
12. What are the causes of dietary essential amino acid deficiency?
13. What are some of the problems that occur in determining essential amino acid requirements?
14. Explain briefly how the essential amino acid requirements can be met in a practical diet formulation.
15. In evaluating protein quality, why is the biological method considered more accurate than the chemical method?

LIPIDS AND FATTY ACIDS

Introduction

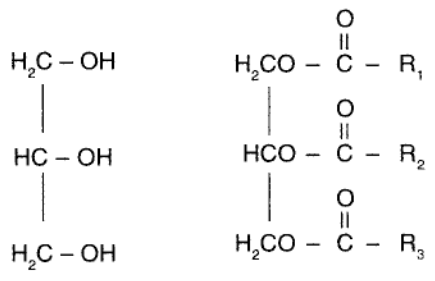
Lipids are a diverse group of organic compounds that are important components of plant, animal, and microbial membranes. They are insoluble in water but soluble in nonpolar, organic solvents such as ether and alcohol. Dietary lipid has two main functions: as source of metabolic energy and as source of essential fatty acids that have specific functions in the body such as for cellular structure and maintenance of the integrity of biomembranes.

Lipids are important components of fish diets because they supply a concentrated source of energy that is well utilized by fish. They also supply essential fatty acids which cannot be synthesized by fish. As a source of dietary energy, lipids have been shown to spare some protein for growth. Lipids are also important sources of sterols, phospholipids, and fat-soluble vitamins. Fatty acids from dietary lipids may also serve as precursors of steroid hormones and prostaglandins.

The objective of this section is to acquaint the reader about common fatty acids, their nomenclature and formulas, and differentiate between saturated and unsaturated fatty acids; to know how environmental factors (temperature, salinity, diet) influence the fatty acid composition of fish; the mechanisms of fatty acid biosynthesis and oxidation, and factors that favor fatty acid biosynthesis and oxidation; the effects of lipid peroxidation and the function of antioxidants; and to understand the importance of fatty acid profiles in fish nutrition, and differences in the essential fatty acid requirements of warmwater and coldwater fishes.

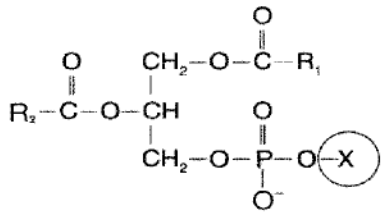
Types of Lipids

- **Triglycerides** or fats are formed by the reaction of glycerol with fatty acid molecules and hence called glycerides. Thus when a triglyceride is hydrolyzed, 3 molecules of fatty acid and one molecule of glycerol are formed. Triglycerides do not occur as components of biomembranes but they accumulate in the adipose or fat tissues. They are the primary means by which animals store energy.

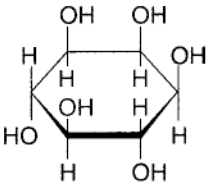
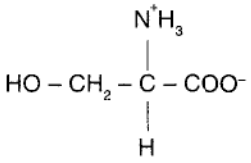


glycerol triglyceride

- **Phospholipids** are esters of fatty acids and phosphoric acid (H_3PO_4) and a nitrogenous base. The resulting compound is called a phosphatidic acid. Some of the important phospholipids are phosphatidyl choline (lecithin), phosphatidyl ethanolamine (cephalin), phosphatidyl serine, and phosphatidyl inositol. They are the main components of biological membranes.



phosphatidic acid

Name of X-OH	Formula of -X
choline	$HO-CH_2-CH_2-N^+(CH_3)_3$
ethanolamine	$HO-CH_2-CH_2-N^+H_3$
inositol	
serine	

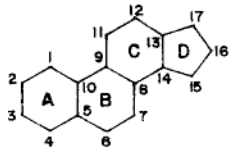
- **Waxes** are esters of long-chain fatty acids and of high molecular weight monohydric alcohols. Like triglycerides, they are sources of energy stored in plants and animals and serve as protective coating. They are solids at ambient temperature.

  a. some are esters of long-chain alcohols, R_1-CH_2OH and long chain fatty acids, R_2-COOH

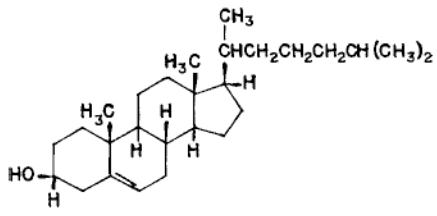
  for example, $R_2\text{-}\overset{\overset{O}{\|}}{C}\text{O-}CH_2\text{-}R_1$

  b. some are ethers, R_2-CH_2-O-CH_2-R_1

- **Steroids** are usually polycyclic long-chain alcohols. They are precursor of sex or other hormones in fish and shrimp and are biologically important in the reproductive processes. Steroids have the same general structure consisting of a fused-ring system. Cholesterol is a physiologically important sterol and is widespread in biological membranes, especially in animals.

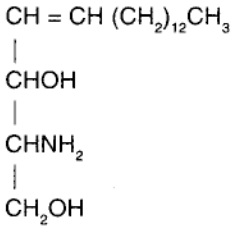


steroid nucleus

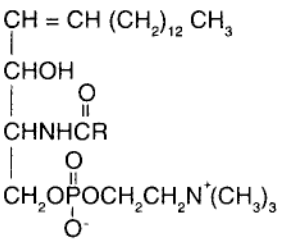


cholesterol

- **Sphingomyelins** do not contain glycerol, but are fatty acid esters of long-chain amino alcohol sphingosine. They are lipid components of the brain and nerve tissue of plants and animals.



sphingosine



sphingomyelin

General Function of Lipids

The general functions are:

1. Sources of metabolic energy, adenosine triphosphate (ATP). They contain approximately twice the energy of proteins and carbohydrates.
2. Sources of essential fatty acids (EFA) which are important for growth and survival. EFA cannot be synthesized by the animal itself or are synthesized in insufficient amounts for good growth and has to be provided for in the diet. Examples: linolenic, linoleic, arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are essential fatty acids in fish and crustaceans.
3. Essential components of cellular and sub-cellular membranes. Examples: phospholipids and polyunsaturated fatty acids.
4. Sources of steroids which perform important biological functions such as maintenance of membrane systems, lipid transport, and precursors of steroid hormones.

Fatty Acids

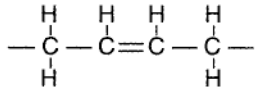
Structure and Classification

Fatty acids are an important constituent of lipids. Over 40 fatty acids are known to occur naturally. They can be represented by the general formula:

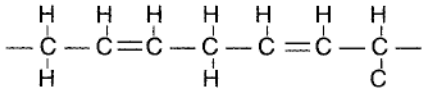
$$CH_3 (CH_2)_n COOH$$

where: n varies from 0 to 24 and is usually an even number

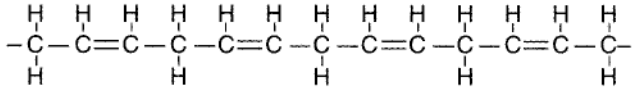
Most naturally occurring fatty acids contain a single carboxyl (COOH) group at one end and a straight unbranched hydrocarbon (C) chain. Fatty acids may be saturated (no double bond), where all carbon atoms are filled with hydrogen, or unsaturated, where one or more carbon atom lacks a hydrogen atom. Unsaturated fatty acids may either be monounsaturated (one double, bond), or polyunsaturated (PUFA) with two or more double bonds or highly unsaturated fatty acids (HUFA) containing four double bonds or more.



monounsaturated fatty acid



polyunsaturated fatty acid



highly unsaturated fatty acid

Polyunsaturated fatty acids normally have a methylene (-CH_2-) interrupted system of double bonds. The common fatty acids are shown in Table 2.4.

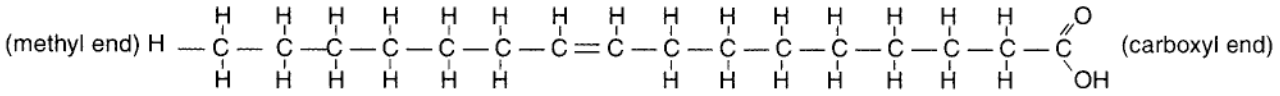
Table 2.4 The common fatty acids

Common Name	Chemical Name	Shorthand Notation*
Saturated		
Formic, Acetic, Propionic		1:0, 2:0, 3:0
Butyric, Valeric	Butanoic acid, Pentanoic acid	4:0, 5.0
Caproic	Hexanoic acid	6:0
Caprylic	Octanoic acid	8:0
Capric	Decanoic acid	10:0
Lauric	Dodecanoic acid	12:0
Myristic	Tetradecanoic acid	14:0
Palmitic	Hexadecanoic acid	16:0
Stearic	Octadecanoic acid	18:0
Arachidic		20:0
Behemic		22:0
Lignoceric		24:0
Unsaturated		
Palmitoleic acid	Hexadecenoic acid	16:1 *n*-7
Oleic acid	Octadecenoic acid	18:1 *n*-9
Polyunsaturated		
Linoleic acid	Octadecadienoic acid	18:2 *n*-6
Linolenic acid	Octadecatrienoic acid	18:3 *n*-3
Highly unsaturated		
Arachidonic acid	Eicosatetraenoic acid	20:4 *n*-6
Eicosapentaenoic acid		20:5 *n*-3
Docosahexaenoic acid		22:6 *n*-3

Nomenclature

Fatty acids are given a common name besides their chemical formula and shorthand abbreviation. In fatty acid nomenclature, a fatty acid is identified by the formula: A:B *n*-3, A:B *n*-6, A:B *n*-9, sometimes *n* is designated as ω (omega) where, A is the number of carbon atoms, B is the number of double bonds, *n*-3, *n*-6, *n*-9 is the position of the first double bond from the methyl end of the fatty acid. For example, the

numerical designation for palmitoleic or hexadecenoic acid is 16:1 *n*-7. This means that palmitoleic acid has 16 carbons and contains one double bond which appears on the seventh carbon, numbering from the methyl end of the fatty acid chain.



palmitoleic acid

Based upon the classification in Table 2.4, unsaturated fatty acids may be divided into three major families: the oleic or *n*-9 series, the linoleic or *n*-6 series, and the linolenic or *n*-3 series.

Each family name represents the shortest chain member of the group. The shorthand notation and their respective structural formulas are given in Table 2.5.

Table 2.5 Unsaturated fatty acids families

Class	Family	Shorthand* notation	Structural formula
n-9	Oleic	18:1 *n*-9 20:1 *n*-9	$CH_3 - (CH_2)_7 - CH = CH - (CH_2)_7 - COOH$
n-6	Linoleic	18:2 *n*-6 18:3 *n*-6 20:3 *n*-6 20:4 *n*-6 22:4 *n*-6	$CH_3 - (CH_2)_4 - CH = CH - CH_2 - CH = CH - (CH_2)_7 - COOH$
n-3	Linolenic	18:3 *n*-3 20:5 *n*-3 22:5 *n*-3	$CH_3 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - (CH_2)_7 - COOH$

*Number of carbon (C) atoms: number of double bonds and position of the first double bond, counting from the methyl (CH_3) end of the fatty acid

The degree of unsaturation of fatty acids influences the physical property of the fats. In general, unsaturated fatty acids are more reactive chemically and have lower melting points than the corresponding saturated fatty acids. Plant oils are liquid at room temperature because they have higher proportions of unsaturated fatty acids than do animal fats which tend to be solids.

Most fish are different from terrestrial animals in that their tissues contain fairly high amounts of *n*-3 highly unsaturated fatty acids (HUFA).

Fatty Acid Composition of Fish

The composition of fatty acids in fish is affected by a number of environmental factors like salinity, temperature, and diet.

1. Salinity

Fish live in environments of varying salinity. The major fatty acids in lipids of marine and freshwater fishes are shown in Table 2.6. The fatty acid composition of freshwater and marine fishes differ from each other:

a. marine species have higher content of long-chain (20 and 22 carbon) monoenoic acids while freshwater species have higher levels of medium-chain (16 and 18 carbons) monoenoic acids;
b. marine species contain more highly unsaturated fatty acids than freshwater species;
c. the ratio of *n*-3 fatty acids to *n*-6 fatty acids is greater for marine than freshwater species.

Differences in fatty acid composition is reflected also in fish that migrate from freshwater to marine environment. As fish migrate from freshwater to seawater, their fatty acid composition changes. The *n*-3/*n*-6 ratio is higher for migratory fishes like smelts and salmon.

Table 2.6 Major fatty acids in lipids of marine and freshwater fishes*

Fatty Acid	Percent Fatty Acids			
	Marine		Freshwater	
	A	B	C	D
14:0	3.7	2.2	2.8	6.7
14:1	0.1	0.2	1.0	0.7
16:0	12.6	17.0	16.6	14.6
16:1	9.3	4.1	17.7	14.7
18:0	2.3	3.2	3.3	1.5
18:1	22.7	21.4	26.1	18.2
18:2 *n*-6	1.5	2.0	4.3	3.7
18:2 *n*-3	0.6	1.0	3.6	3.6
20:1	7.5	5.4	2.4	1.6
20:4 *n*-6	1.4	0.9	2.6	2.4
20:5 *n*-3	12.9	6.7	2.7	8.2
22:1	6.2	9.4	0.3	0.4
22:4 *n*-6	0.1	0.6	0.4	0.4
22:5 *n*-3	1.7	2.3	2.0	1.5
22:6 *n*-3	12.7	16.1	2.0	6.0
Total saturated	18.6	22.4	22.7	22.8
Total monoenes				
medium	32.2	25.7	44.8	33.6
long-chain	13.7	14.8	2.7	2.0
Total *n*-3	27.9	26.1	10.3	19.3
Total *n*-6	4.1	3.5	7.3	6.5
Ratio $\frac{n\text{-}3}{n\text{-}6}$	6.8	7.5	1.4	3.0

* A = Atlantic cod
B = Chinook salmon
C = Sheepsherd
D = Alewife

Source: Ackman 1976

2. Temperature

Temperature is a major factor that causes differences in fatty acid composition. Fish that live in warmwaters contain more saturated fatty acids than fish that live in colder waters. Fish reared at higher temperatures accumulate more saturated fatty acids than the same fish acclimated at lower temperatures.

Fish and crustaceans are able to adjust their levels of polyunsaturated fatty acids to maintain membrane integrity and function in the cold. Most PUFAs remain in the liquid state even at low temperatures while saturated fatty acids congeal and solidify at colder temperatures, therefore, high levels of unsaturated fatty acids in coldwater fish is necessary for maintenance of membrane fluidity.

3. Diet

Diet is the largest single factor that affects the fatty acid composition. Under normal conditions, the fatty acid composition of fish comes from these sources:

a. fatty acids derived from the diet;
b. fatty acids derived from non-lipid sources by biosynthesis and
c. fatty acids derived from lipid sources by biosynthesis

The effect of diet on the fatty acid composition of fish and shrimp lipids has been demonstrated. When the shrimp *Penaeus setiferus* is fed a diet high in linoleic acid (18:2 *n*-6) and low in C20 and C22 PUFA for one month, the shrimp's content of *n*-3 and *n*-6 fatty acids is modified (increase in *n*-6 and decrease in *n*-3), hence the *n*-6/*n*-3 ratio increases. After three months, the *n*-6/*n*-3 ratio of shrimp continues to reflect that of the dietary lipid (Table 2.7). The seasonal variation of the fatty acid composition of fish may also be influenced by the changes in the composition of their natural food.

Table 2.7 Effect of diet on fatty acid composition of shrimp *Penaeus setiferus*

	Composition of *Penaeus setiferus* lipid			
Fatty acid	0 month	1 month	3 months	Diet
14:0	0.6	0.5	0.5	1.6
16:0	14.8	13.4	15.0	15.5
16:1	11.2	8.7	10.0	7.9
18:0	5.1	2.3	2.2	1.7
18:1	13.1	22.9	20.0	28.4
18:2 *n*-6	2.3	18.1	14.1	32.3
18:3 *n*-3	2.8	2.1	1.3	4.4
20:4 *n*-6	11.6	9.4	10.3	0.7
20:5 *n*-3	10.4	8.7	9.7	2.6
22:6 *n*-3	11.3	6.3	6.9	0.3
Total saturated	26.6	22.6	25.6	25.0
Total monoenes	18.2	25.2	22.2	30.1
Total *n*-6	13.9	27.5	24.4	33.0
Total *n*-3	24.5	17.1	17.9	7.3
Ratio $\frac{n\text{-}6}{n\text{-}3}$	0.57	1.61	1.36	4.5

Source: Castell 1981

Biosynthesis of Fatty Acids

Fish can biosynthesize fatty acids from a non-lipid source (acetate). The process begins from acetyl CoA and undergoes chain elongation by adding two carbon units at a time as it progresses through the biochemical pathway. The enzymes that catalyze the synthesis of fatty acids are: acetyl CoA carboxylase and FA synthetase. Acetate fragments are added to a fatty acid by the process of chain elongation. The reaction occurs in both the mitochondria and microsomes. Because fatty acids are synthesized from two-carbon acetyl units, they usually have an even number of carbon atoms. Palmitate is the major fatty acid produced by *de novo* biosynthesis.

De novo synthesis of saturated fatty acids

Acetate → C4 → C6 → C8 → C10 → C12 → C14 → C16:0 (chain elongation)
palmitic acid

In the synthesis of unsaturated fatty acids, desaturation or removal of a hydrogen molecule is an additional step that is required to make a double bond (-C=C-). Desaturation of fatty acids occurs in the microsomes. Of the monounsaturated fatty acids however, only those of the *n*-5, *n*-7, *n*-9, *n*-11 and *n*-13 series are formed from non-lipid sources. *De novo* biosynthesis of saturated and monoenoic fatty acids is further illustrated in Figure 2.4. The main sites for *de novo* fatty acid synthesis are the liver and the adipocytes. The process occurs when the cells have abundance of energy and have high ATP content.

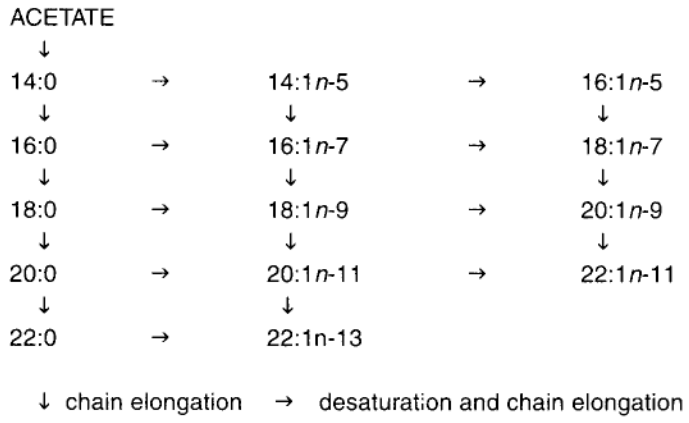


Figure 2.4
De novo synthesis of saturated monoenoic fatty acids

PUFAs are made by a series of chain elongation and desaturation steps. The chain elongation and desaturation of the *n*-3 and *n*-6 fatty acids are shown in Figure 2.5.

In common with other vertebrates, fish cannot directly synthesize *n*-3 and *n*-6 fatty acids *de novo* from non-lipid sources. Dietary precursors such as oleic, linoleic, and linolenic acids should be supplied in their diet.

Biosynthesis of fatty acids may be controlled by the competition between the different series of fatty acids for enzymes that desaturate and enzymes that chain elongate. High levels of *n*-3 inhibit the elongation and desaturation of the *n*-6 series. The potency of inhibition is ranked as follows: *n*-3> *n*-6> *n*-9. Thus, a proper balance of *n*-3 and *n*-6 essential fatty acids is needed in formulating the diet. After the biosynthesis of fatty acids, two major types of lipids are formed: the triglycerides and the phospholipids. The triglycerides are ultimately stored in fat deposits while the phospholipids are incorporated in the biomembranes.

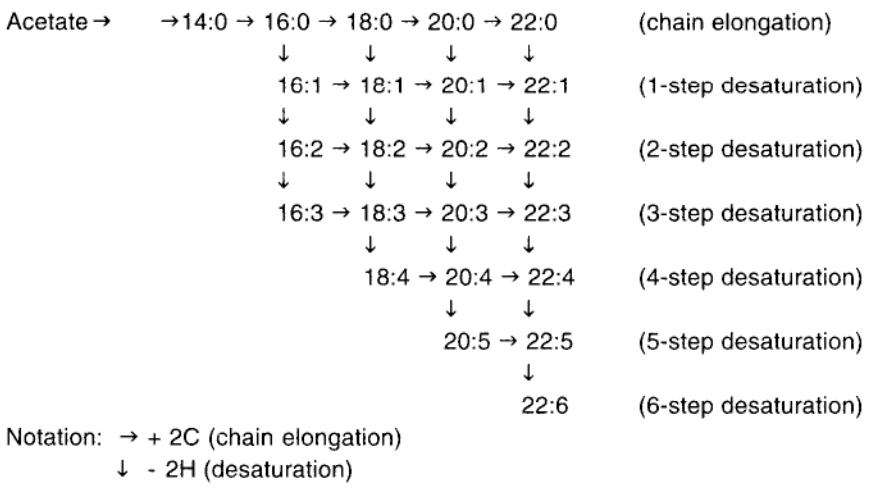


Figure 2.5
Biosynthesis of unsaturated fatty acids

Oxidation of Fatty Acids

Long chain fatty acids, combined as triglycerides, provide the long term storage form of energy in the fat or adipose tissue of the animal. When energy demands are great, fatty acids are broken down to yield energy. The most important mechanism by which these storage fatty acids are degraded in a step-wise manner to yield energy is known as β-oxidation.

According to the β-oxidation mechanism, the β-or 3rd carbon atom from the carboxyl end of the fatty acid chain is the site at which oxygen is introduced during oxidation. Catabolism begins with the carbonyl group by converting long-chain fatty acids to acetyl CoA, which is subsequently oxidized in the citric acid or Krebs cycle. In this process, two-carbon fragments are successively removed from the fatty acid in the form of acetyl CoA. Fatty acid oxidation occurs mainly in the mitochondria and this is followed by complete oxidation of the acetyl CoA in the citric acid cycle. It is one of the pathways through which cells derive energy for synthesis of ATP and ultimately produce energy.

For example, the β-oxidation of palmitic acid, C16:0, requires seven (7) cycles of β-oxidations and gives rise to eight (8) molecules of acetyl CoA which enters the citric acid cycle and produces ATP. The ATP yield for the complete oxidation of one molecule of palmitic acid is 106 ATP. The liver is the chief organ concerned with β-oxidation of fatty acids.

The oxidation of fatty acids is the chief source of energy in the catabolism of lipids. The pathways of lipid synthesis (anabolism) and catabolism occurs simultaneously but in the different parts of the cell.

Lipid Peroxidation

One of the characteristic reaction of lipids which are exposed to oxygen is the formation of peroxides. This reaction is of great practical importance as it leads to the deterioration of tissues and also causes spoilage of foods. This is due to the production of free radicals such as, ROO·, RO·, OH· during peroxide formation. These free radicals react by hydrogen removal and a variety of addition reactions that damage enzymes, proteins, vitamins and other lipids making other nutrients unavailable to fish. Antioxidants are substances that protect lipids from peroxidation. The use of lipids in fish diets requires the use of appropriate antioxidants (see Chapter 5).

Importance of Fatty Acid Profiles in Fish Nutrition

Both fish and vegetable oils have been found useful in fish feeds. The fatty acid composition of commonly used oil sources in fish diets is shown in Table 2.8. In comparison with other vegetable oils or fats, fish oils contain a greater variety of unsaturated fatty acids of longer carbon chain (20 or 22 carbon chain length), most of which belong to the *n*-3 family of fatty acids. The long-chain *n*-3 fatty acids generally make up one-fourth to one-third of all fatty acids in fish oils, whereas, long chain fatty acids in most vegetable oils seldom exceed 5% and is frequently less than 1%. The dietary lipid requirement of fish can be derived from their fatty acid profiles.

Table 2.8 Essential fatty acid composition of various lipid sources (g/100g fatty acid)

Lipid source	18:2*n*6	18:3*n*3	20:5*n*3	22:6*n*3
Plant sources				
Corn oil	58	1	0	0
Coconut oil	2	0	0	0
Cottonseed oil	53	1	0	0
Linseed oil	17	56	0	0
Palm oil	10	1	0	0
Palm kernel oil	2	0	0	0
Rapeseed oil	15	8	0	0
Peanut oil	30	0	0	0
Soybean oil	50	10	0	0
Sunflower oil	70	1	0	0
Marine animal sources				
Capelin oil	5	0	7	5
Cod liver oil	5	1	16	14
Cuttlefish liver oil	1	2	12	18
Herring oil	1	1	8	5
Pollack liver oil	2	0	12	7
Salmon oil	3	0	10	10
Sardine oil	3	1	13	10
Short-neck clam oil	1	1	19	14
Skipjack oil	5	3	7	12
Squid liver oil	3	3	12	10

Source: Tacon 1987

Essential Fatty Acid Requirements of Fish

Fish require *n*-3 and *n*-6 fatty acids in their diets. Linolenic acid (18:3*n*-3), linoleic acid (18:2*n*-6), eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) are needed by fish. Failure to provide these fatty acids in the diet can cause impairment of growth and prolonged lack of these fatty acids in the diet lead to death.

The essential fatty acid (EFA) requirements have been established for most cultured fishes. A summary of these values is presented in Table 2.9. Fish species differ in their EFA requirements.

Table 2.9 Essential fatty acid requirements of fish and shrimp

Fish species	Requirement	Reference
Asian sea bass	0.5% *n*-3 PUFA 0.5% *n*-6 PUFA	Borlongan and Parazo 1991
Ayu	1% 18:3*n*-3 1% 20:5*n*-3	Kanazawa et al. 1982
Carp	1% 18:2*n*-6 1% 18:3*n*-3	Watanabe et al. 1975 Takeuchi & Watanabe 1977
Chum salmon	1% 18:2*n*-6 1% 18:3*n*-3 0.5% *n*-3 HUFA	Takeuchi et al. 1979
Coho salmon	1-2.5% 18:3*n*-3	Yu & Sinnhuber 1979
Eel	0.5%18:2*n*-6 0.5%18:3*n*-3	Takeuchi et al. 1980
Grouper	1% *n*-3 HUFA	Millamena and Golez 1998
Milkfish	1-1.5% *n*-3 PUFA	Borlongan 1992
Nile tilapia	0.5% 18:2*n*-6 or 20:4*n*-6	Takeuchi et al. 1983
Rainbow trout	0.8%*n*-3 HUFA 0.8%*n*-3 HUFA 18:3*n*-3 20% of lipid *n*-3 HUFA 10% of lipid	Castell et al. 1972 Watanabe et al. 1974 Takeuchi & Watanabe 1977
Red seabream	0.5 *n*-3 HUFA 0.5% 20:5*n*-3	Yone et al. 1978
Turbot	0.8%*n*-3 HUFA	Gatesoupe et al. 1977
Yellow tail	2% *n*-3 HUFA	Deshimaru et al. 1984
Tiger shrimp	2.6% *n*-3 PUFA <5% *n*-6 PUFA 0.5% *n*-3 PUFA	Catacutan 1991
Kuruma shrimp	0.5-1% *n*-3 PUFA	Kanazawa et al. 1980

Rainbow trout (*Oncorhynchus mykiss*) requires about 1% 18:3*n*-3 in the diet. Combining 18:3*n*-3 and 18:2*n*-6 in various proportions did not improve growth rate or efficient feed conversion. In contrast, carp (*Cyprinus carpio*), one of the most important cultured fish in Japan requires both 18:2*n*-6 and 18:3*n*-3. The best weight gain and feed conversion are obtained in fish given a diet with a mixture of 1% 18:2*n*-6 and 1% 18:3*n*-3. The eel (*Anguilla*

japonica), another cultured warmwater fish, has a requirement for both 18:2*n*-6 and 18:3*n*-3, but at a level of 0.5%. A tropical herbivore, Nile tilapia (*Tilapia nilotica*) requires *n*-6 rather than *n*-3 fatty acids. Their dietary requirement for 18:2*n*-6 or 20:4*n*-6 is about 0.5% in the diet.

Highly unsaturated *n*-3 fatty acids (*n*-3 HUFA) are essential in the nutrition of some marine fish such as red sea bream (*Chrysophyrs major*), and yellow tail (*Seriola quinquerodiata*). The requirement for long-chain polyunsaturated fatty acids is due to the limited ability of some marine fishes to add more carbon atoms or to remove hydrogen from dietary precursors. Thus, most coldwater and marine fish requires *n*-3 fatty acids.

Studies on the essential fatty acid requirements of warmwater fish and shrimp species in the Philippines show that some species require both *n*-3 and *n*-6 fatty acids while others only *n*-3. Growth inhibition at certain levels of *n*-3 and *n*-6 has also been observed in warmwater fishes. Milkfish (*Chanos chanos*) cultured in seawater requires *n*-3 PUFA. Good growth and survival were obtained using either linolenic (18:3*n*-3) or *n*-3 HUFA as lipid sources. Asian sea bass (*Lates calcarifer*) juveniles require both *n*-3 and *n*-6 PUFA at 0.5% in the diet or an *n*-3/*n*-6 ratio of 1.0. Grouper (*Epinephelus coioides*) requires about 1% *n*-3 HUFA. In juvenile tiger shrimp (*Penaeus monodon*), about 2.6% dietary PUFA enhances growth while levels of 18:2*n*-6 greater than 5% have a negative effect on growth. Thus, different species require different EFAs and the differences are more marked in warmwater than in coldwater fishes.

Guide Questions

1. Define the term "lipid". Name the two major functions of lipids.
2. What are the important types of lipids? Briefly define each component.
3. Show the general formula for fatty acids. Explain briefly.
4. What are the three major families of polyunsaturated fatty acids
5. Name the environmental factors that affect the fatty acid composition of fish.
6. What are the two major types of lipids formed after biosynthesis of fatty acid?
7. Where are the biosynthesized fatty acids or triglycerides ultimately stored?
8. Under what conditions or nutritional state of the animal is fatty acid oxidation favored? Under what conditions is it slowed down?
9. What is βeta-oxidation of fatty acids?
10. Describe the phenomenon of lipid peroxidation. Why is it of great practical importance?
11. Why does the type of lipid required for warm water fish differ from cold water fish?
12. Which among the fish species studied requires more n-6 rather than n-3 fatty acids?
13. Differentiate between: a) saturated vs. unsaturated fatty acids b) polyunsaturated vs. highly unsaturated fatty acid.
14. Name some oils that are used in fish diets and explain why they are useful.

CARBOHYDRATES

Introduction

Carbohydrates (CHO) are a large group of organic compounds common in plants which include simple sugars, starches, celluloses, gums, and related substances. They contain C, H, and O with a ratio of hydrogen to oxygen of 2:1 which is similar to H_20 hence the name "carbohydrate". However the ratio of 2:1 is not always true. The general formula is $C_n(H_20)_n$. Carbohydrates are generally composed of carbon, hydrogen, and oxygen and sometimes nitrogen and sulfur. Chemically, carbohydrates on hydrolysis yield polyhydroxy aldehydes (the aldoses) and ketones (the ketoses). They form the largest part of the animal's food supply and make up 75 percent of the dry weight of plants.

Carbohydrates are a cheap source of energy and can spare the more expensive protein as an energy source. The protein sparing effects of carbohydrates and lipids should be maximized in order to reduce feed costs. Since carbohydrate is the most economical dietary energy source, as much digestible carbohydrate as the fish can utilize is used in fish diets. Carbohydrates such as starch, flour, alginates, agar, carrageenan, and guar gum are also used as feed binders to improve the water stability of fish and shrimp diets.

After studying this section, the reader should be able to differentiate among the various forms of carbohydrate and their significance in fish nutrition; distinguish between utilization of carbohydrates by warmwater and coldwater fishes and know how dietary carbohydrates are made available to fish.

Classification of Carbohydrates

Carbohydrates are classified as monosaccharides, disaccharides or oligosaccharides, and polysaccharides depending on whether the molecule is made up of one, two or many simple sugar units (Table 2.10). They are classified according to digestibility as digestible, partially digestible, and indigestible. Sugars, starches, dextrin, and glycogen are digestible carbohydrates whereas, cellulose, dietary fibers, and hemicellulose are indigestible carbohydrates. Galactogens, mannosans, inulin, and pentosans are considered partially digestible carbohydrates.

Table 2.10 Classification of carbohydrates

Monosaccharides (single glycose units)

Pentoses ($C_5H_{10}O_5$)	Hexoses ($C_6H_{12}O_6$)
Arabinose	Glucose
Ribose	Galactose
Xylose	Fructose
Xylulose	Mannose

Disaccharides (2 glycose units) and oligosaccharides (2 to 10 glycose units)

Sucrose	Raffinose
Maltose	Stachyose
Lactose	Verbascose

Polysaccharides (Glycan, >10 glycose units)

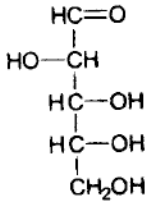
- Starch
- Dextrin
- Glycogen
- Cellulose
- Hemicellulose
- Lignin
- Chitin
- Pectin
- Gums and mucilages
- Alginates, agar, carrageenan (extracts from seaweeds)

A. Monosaccharides

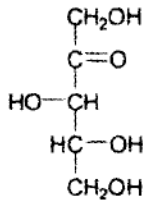
The monosaccharide is the fundamental unit from which all carbohydrates are derived. They are characterized by the number of carbon atoms they contain, C3, C4, C5, etc., and by their structural configuration either as aldose or ketose. The carbohydrate glucose is an aldose because of the presence of an aldehyde group while fructose is a ketose because of the presence of a ketone group. Most common sugars are aldoses rather than ketoses. Their structure includes a carbonyl group and several hydroxyl groups.

Most monosacharides are obtained by hydrolysis of more complex plant substances. Hydrolysis is a chemical reaction whereby a complex substance is broken into smaller units by the addition of water in the presence of a catalyst. The monosaccharides are often referred to as simple sugars. Two series of simple sugars are commercially important: the pentoses or five-carbon-atom sugars and the hexoses or six-carbon-atom sugars. The hexoses are abundant in nature, but the pentoses, ribose and deoxyribose, occur in the structures of RNA and DNA, respectively.

Pentoses The pentoses have the general formula $C_5H_{10}O_5$. Two pentoses are of commercial importance and both are aldopentoses: xylose and arabinose. Xylose is formed by the hydrolysis of pentosans. Considerable amount of xylose are formed in the pulping of wood through hydrolysis of hemicellulose. Arabinose is produced by the hydrolysis of gum arabic or wheat bran.



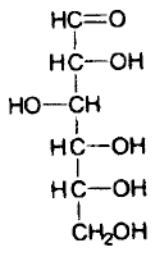
D-arabinose (an aldose)



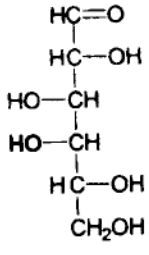
D-xylulose (a ketose)

Hexoses The hexoses have the general formula $C_6H_{12}O_6$. The hexose sugars: galactose, and glucose are aldoses, while fructose is a ketose. The most abundant carbohydrates in plants and animals are either hexoses or complex molecules which form hexoses on hydrolysis.

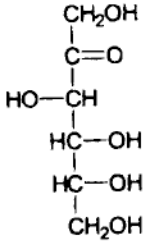
Glucose is the most common of the aldohexose sugars and is commercially produced from the hydrolysis of cornstarch. It is the basic molecule for the synthesis of starch and cellulose. Glucose is of great importance in nutrition, as it is the major end product of the digestion of carbohydrates by nonruminants. Glucose, oxidized to carbon dioxide and water, is the primary energy source of humans.



D-glucose (an aldose)



D-galactose (an aldose)



D-fructose (a ketose)

Fructose is the only important ketohexose in nature and is the sweetest of the carbohydrates. When cane or beet sugar (sucrose) is hydrolyzed, one molecule of fructose and one molecule of glucose are formed.

Galactose does not occur in the free form in nature.

The hydrolysis of lactose or milk sugar produces galactose and glucose.

Isomerism. Glucose, fructose, and galactose have the same molecular formula but their structural formulas differ in the arrangement of their atoms within a molecule. These sugars are optical isomers because when placed in a polariscope, glucose and galactose rotate the plane of polarized light to the right, while fructose rotates the light to the left. Thus, although they have the same molecular formulas they differ in their individual properties.

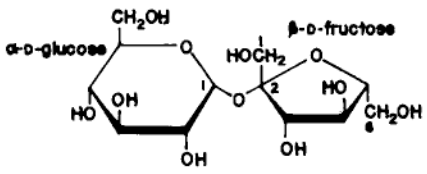
B. Disaccharides and Oligosaccharides

The oligomers of sugars are made up of two to ten monosaccharide units and frequently occur as disaccharides. Disaccharides are a combination of two molecules of monosaccharide. The formula, $C_{12}H_{22}O_{11}$, shows that one molecule of water was removed as two monosaccharides are combined. Hydrolysis results in cleavage of the molecule and formation of the hexoses. The three important disaccharides are sucrose, lactose and maltose.

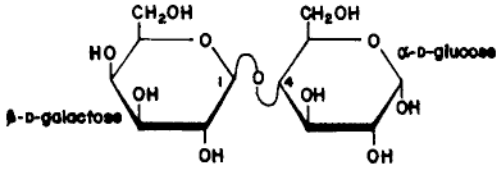
Sucrose, the common table sugar, is made up of a combination of one molecule of glucose and one molecule of fructose. It is derived largely from sugar cane and sugar beets which are sources of commercial sugar. When sucrose is consumed by animals, it is hydrolyzed to glucose and fructose, which are degraded by metabolic processes to provide energy.

Lactose, or milk sugar, is found only in the milk of all mammals. Nearly half of the milk solids is lactose hence hydrolysis of lactose will give a molecule of glucose and a molecule of galactose. Lactose is of special interest in human nutrition.

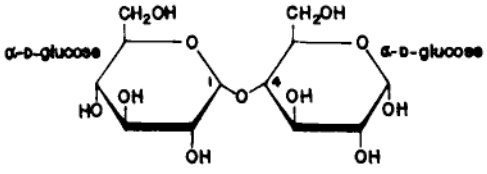
Maltose occurs naturally in seeds of starch-producing plants. It is formed by hydrolyzing starch with the enzyme α-amylase. When maltose is further hydrolyzed by the enzyme α-glucosidase, two molecules of glucose are produced.



Sucrose



Lactose (α form)



Maltose (α form)

C. Polysaccharides

The polysaccharides are formed by the combination of hexoses or other monosaccharides. They are of high molecular weight and mostly insoluble in water and are considered the most important nutrients of plant origin. Upon hydrolysis by acids or enzymes, they are broken down into various intermediate products and finally into their simple sugars (Figure 2.6). The polysaccharides have the general formula $(C_6H_{10}O_5)_n$.

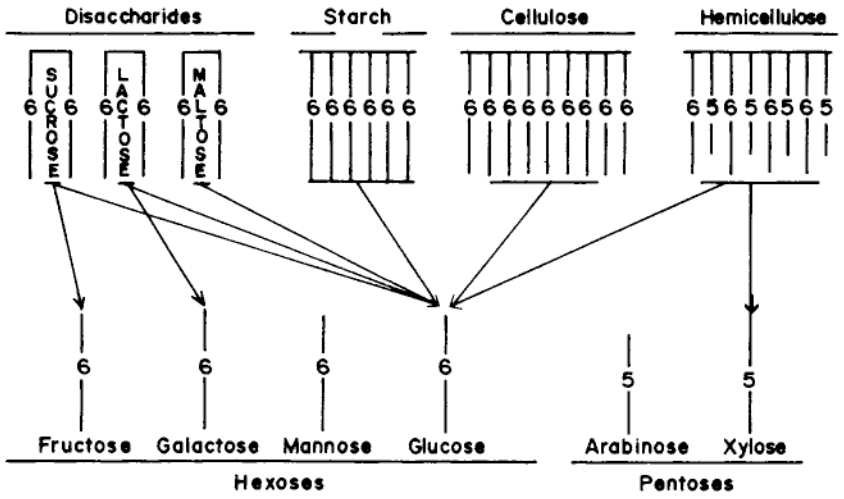
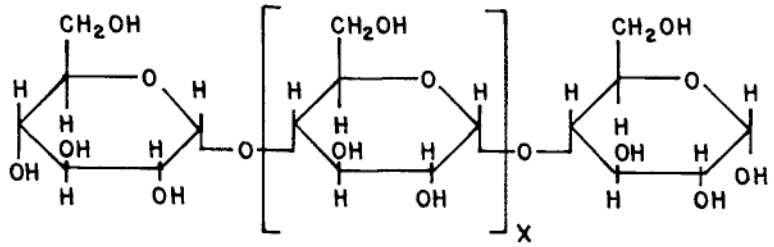


Figure 2.6
Summary of hydrolysis of carbohydrates.

Starch is the principal storage form of carbohydrates in plants and is found in the tuber, rhizomes, and seeds. It is the cheapest foodstuff and serves mainly in human and animal nutrition as a source of energy. Starch is hydrolyzed by either acids or enzymes into dextrin, maltose, and finally glucose. Both plants and animals contain enzymes, alpha- and beta- amylase, that hydrolyze starch. About 20 to 26 molecules of glucose are produced from each molecule of starch.



Starch (X = about 20 to 26)

Glycogen is manufactured by mammals and other animals from glucose in the blood and is found in the muscle tissue and liver. It is the storage form of carbohydrate in animals, as starch is in plants.

Cellulose is the major structural component of plant cell wall and is the most abundant substance in the plant kingdom. Cellulose is essentially insoluble and extremely resistant to degradation by enzyme action. It consists of several glucose units and can be hydrolyzed to glucose by strong acids. Animals lack enzymes called cellulase that hydrolyze cellulose to glucose.

Hemicelluloses are composed of a mixture of hexose and pentose units. Hydrolysis of hemicellulose yields glucose and a pentose, usually xylose. It is the principal component of plant cell wall. Unlike cellulose, it is less resistant to chemical degradation and can be hydrolyzed by a relatively mild acid treatment.

Lignin is found in the cobs and hulls and the fibrous portion of roots, stems, and leaves of plants. It has a complex structure consisting of carbon to carbon bonds and ether linkages which is resistant to acid and alkali.

Chitin is the major structural component of the rigid exoskeleton of invertebrates such as insects, crustacea, and also occurs in cell walls of algae, fungi, and yeasts. It is a polysaccharide with nitrogen atoms as well as C, H, and O and is composed of N-acetyl D-glucosamine. Like cellulose, chitin has a structural role and a fair amount of mechanical strength because the individual strands are held together by hydrogen bonds.

Pectin is found primarily on the spaces between plant cell walls and may also infiltrate the cell wall itself. Pectin cannot be hydrolyzed by mammalian enzyme pectinase but is digested by microbial action. It can be extracted with hot or cold water and forms a gel.

Plant gums are formed at the site of injury or by an incision on the bark. They are complex, highly branched residues containing D-glucuronic and D-galacturonic acids along with other simple sugars such as arabinose and shammose. They are viscous fluids which become hard when dry and are used commercially as thickening agents or stabilizers for emulsions. Gum arabic is a well-known commercial gum.

Alginates, agar, and carrageenan are extracts from seaweeds, the giant kelp, *Gracilaria* sp. and *Kappaphycus* sp., respectively. These are important binders in fish and crustacean diets.

Utilization of Carbohydrates

Aquatic animals do not have a specific dietary requirement for carbohydrates but their presence in diets may provide an inexpensive source of energy. The ability of fish to utilize dietary carbohydrates for energy varies considerably. Most carnivorous species have more limited ability compared with omnivorous and herbivorous species. Amylase is an enzyme that digests starches into sugars in the intestine of fishes. The activity of intestinal amylase is higher in omnivorous fishes, like milkfish, tilapia, and carp, than in carnivorous fishes, such as rainbow trout and yellow tail. Fibrous carbohydrates in the form of cellulose is essentially indigestible by some fish species and does not make a positive contribution to their nutrition. Thus, the level of crude fiber in fish feeds is typically restricted to less than 7% of the diet to limit the amount of undigested material that enters the culture system.

The availability of various forms of carbohydrates to fish and their nutritive value remains unclear. The highly digestible carbohydrates (carbohydrate with small molecular weight and shorter chain length such as glucose) have high nutritive value for coldwater fishes but are not effectively utilized by other fishes. Warmwater fishes such as red seabream and carp utilize starch more effectively than dextrin and glucose. Figure 2.7 shows the growth and feed efficiency of carp fed diets containing starch, dextrin or glucose at 42% level. The chain length of various carbohydrates and frequency of feeding affects their utilization by fingerling carp. The starch diet results in best weight gain and feed efficiency compared with other carbohydrates with shorter chain length such as dextrin and glucose.

The type and levels of carbohydrate in the diet have also been shown to affect the growth of penaeid shrimps. Tiger shrimp utilizes starch

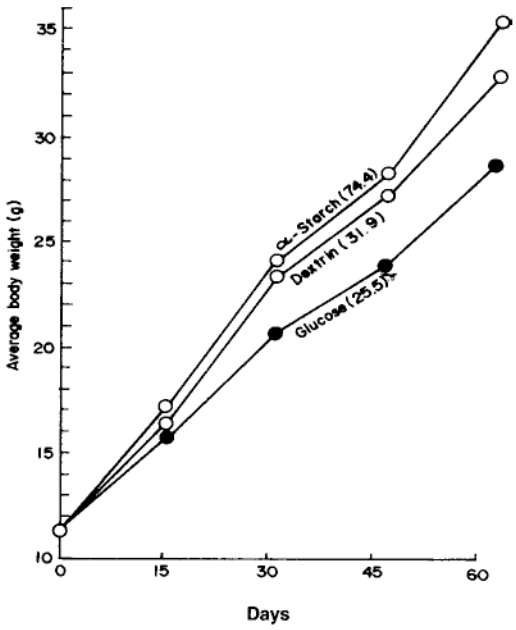


Figure 2.7
Growth and feed efficiency (in parenthesis) of carp fed diets containing 42% of α-starch, dextrin or glucose.
Source: Watanabe et al. 1988

better than dextrin and glucose. When the dietary starch level is increased from 20 to 30%, and the dietary protein is decreased from 40 to 30%, weight gain and feed efficiency are not affected but protein efficiency ratio is increased (Table 2.11). Other studies show that tiger shrimp can utilize trehalose and sucrose better than glucose. Glucose is also poorly utilized by other species of penaeids such as kuruma shrimp. Furthermore, different types and levels of carbohydrate in the diet significantly influences survival of juvenile tiger shrimp.

Table 2.11 Means for weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival rate of tiger shrimp fed diets with different carbohydrate sources and levels

Carbohydrate source	Weight gain (%)	FER	PER	Survival (%)
40% protein				
20% glucose	207.52^{d}	0.38^{d}	0.71^{c}	55.77^{c}
20% dextrin	370.99^{ab}	0.47^{bc}	1.05^{bc}	65.39^{b}
20% starch	408.17^{a}	0.50^{ab}	1.11^{b}	64.42^{b}
35% protein				
25% glucose	232.38^{d}	0.35^{d}	0.91^{cd}	47.29^{c}
25% dextrin	328.99^{bc}	0.44^{bc}	1.06^{b}	75.00^{ab}
25% starch	388.71^{ab}	0.48^{ab}	1.25^{b}	80.81^{a}
30% protein				
30% glucose	152.44^{c}	0.26^{c}	0.76^{cd}	55.94^{c}
30% dextrin	272.68^{cd}	0.40^{cd}	1.20^{b}	71.55^{ab}
30% starch	387.36^{ab}	0.54^{a}	1.51^{a}	74.36^{ab}

Column means having a common superscript are not significantly different (P>0.05).
Source: Shiau 1992

Guide Questions

1. What does the term "carbohydrate" mean?
2. What are the functions of carbohydrates?
3. Distinguish between lipid and carbohydrates.
4. What are carbohydrates chemically? Why is glucose called an aldose, fructose a ketose?
5. Give three classifications of carbohydrates and give one example of each.
6. What is isomerism? Why are glucose, fructose, and galactose optical isomers?
7. What is the major end product of carbohydrate digestion?
8. Show by means of chemical equation how glucose is made from starch.
9. What carbohydrate type is the principal component of the exoskeleton of insects and crustaceans?
10. Name the important binders for aquaculture feeds obtained from seaweeds.
11. If lipid gives twice as much energy as carbohydrates, why then should carbohydrates be included in the diet?
12. Name some good carbohydrate sources in fish diets.

ENERGY

Introduction

Energy is defined as the ability or capacity to do work. Energy may exist in different forms and do different kinds of work. Aquatic animals require food to supply the energy they need. The energy value of foods may come from carbohydrates, fats, and proteins and can be measured directly by means of a bomb calorimeter. Energy is required to do mechanical work (muscle activity), chemical work (chemical processes which take place in the body), electrical work (nerve activity), and osmotic work (maintaining the body fluids at equilibrium with each other and with the medium, whether fresh, brackish or sea water in which the animal lives). Free energy is that which is left available for biological activity and growth after the energy requirement is met.

The quantity and cost of energy which is available for the growth of the species being cultured is most important from the point of view of the aquaculturist. The energy requirements of the animal vary in quantity according to the species, feeding habits, size, environment and reproductive state. The energy supplied by food is one of the important considerations in determining its nutritional value. Energy is expressed in kilocalories (kcal) or kilojoule (kJ). A kilocalorie is the amount of heat necessary to raise the temperature of one kilogram of water 1°C. The Joule (J) is the unit of energy in the metric system and one kcal is equal to 4.186 kJ. For example, 70 kcal is equal to 293.02 kJ.

After studying this section, the reader should be able to differentiate the forms of energy and their measurement. understand dietary energy metabolism, the energy balance equation and factors that influence dietary energy requirement of fish; and understand the significance of optimal protein to non-protein energy in fish diets.

Utilization of Energy

The gross energy (GE) value of feed is the total energy contained in the feed. Not all of the GE is available to the animal. Digestible energy (DE) of a feed is the difference between the GE and the energy excreted in the feces. The energy available for growth is that which remains after the requirements for metabolism, reproduction, etc., have been supplied (Figure 2.8).

The use of digestible energy values is a more sensible and practical way of expressing the energy value of feedstuffs. Theoretically, metabolizable energy values are a more exact measure of the dietary energy used for metabolism by the tissues. However, direct measurement of metabolizable energy in fish is difficult and involves confining the fish in small volumes of water in metabolism chambers.

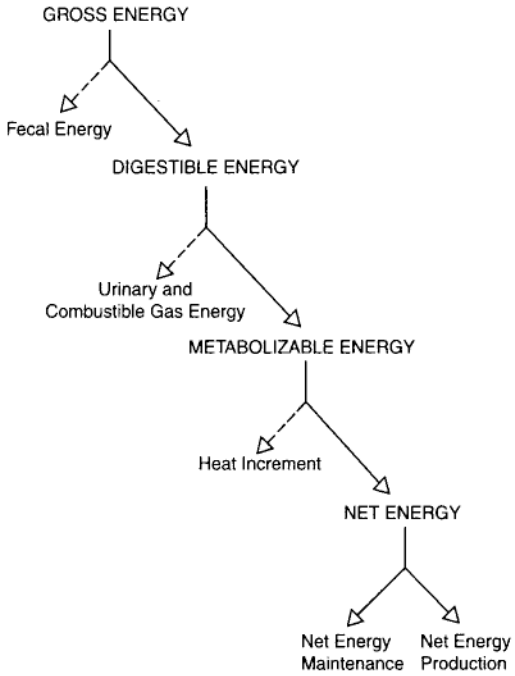


Figure 2.8
Utilization of energy. The energy available for growth is that which remains after the requirements for metabolism, reproduction, etc. have been supplied.

The metabolic rate or energy expenditure of small aquatic animals is greater than that of large animals. Small animals grow faster than large ones in terms of percentage increase in weight per day. Thus, the feed requirements of small animals are higher than those of large ones, when expressed as percentage of their weights.

Energy Metabolism

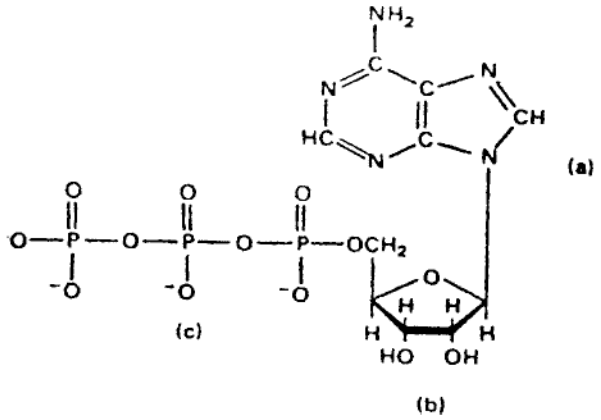


Figure 2.9
Chemical structure of ATP. Adenosine triphosphate contains adenine (a), ribose (b), and triphosphate unit (c) .

Energy is obtained after major nutrients carbohydrates, fats, and proteins undergo several chemical processes such as catabolism and oxidation within the animal body. The energy liberated is used for the maintenance of life processes such as cellular metabolism, growth, reproduction, and physical activity.

The free energy liberated from the catabolism and oxidation of major food nutrients is not utilized directly by the animal, but is trapped in the form of the energy-rich phosphorus bond of adenosine triphosphate or ATP (Figure 2.9). It is ATP which is the principal driving force in the energy requiring biochemical processes of life such as anabolism or synthesis, mechanical energy, electrical energy, osmotic work, and energy for transferring substances against concentration gradients. The role of ATP in cellular energetics is shown in Figure 2.10.

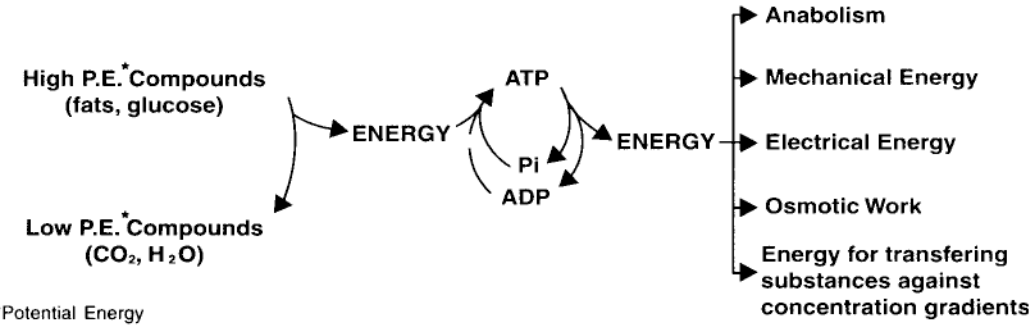


Figure 2.10
The role of ATP in cellular energetics. ATP is the principal driving force in the energy-requiring biochemical processes of life.

ATP is an energy-rich molecule because its triphosphate unit contains two phosphoanhydride bonds. A large amount of free energy is liberated when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (Pi) or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PPi), as shown below:

$$ATP + H_2O \longrightarrow ADP + Pi + H^+$$
$$ATP + H_2O \longrightarrow AMP + PPi + H^+$$

Under typical cellular conditions the ΔG for these reactions is -12 kcal per mol. ATP, ADP, and AMP are interconvertible. The number of moles of ATP that is liberated in the complete oxidation of 1 mole of a carbohydrate like glucose and 1 mole of a fatty acid like palmitate to CO_2 and H_20 are 30 ATP and 106 ATP, respectively.

Energy Balance and Dietary Requirement

Since all biological systems obey the laws of thermodynamics, the energy balance equation can be represented as follows:

C = P + R + U + F

where:

C (consumption) - the gross energy content of the food ingested
P (production) - energy utilized in growth materials
R (respiration) - net loss of energy as heat
U (urinary loss) - energy lost in nitrogenous excretory product
F (fecal loss) - energy lost in the feces

A generalized energy budget for young carnivorous and herbivorous fishes fed on natural food has been developed by Brett & Groves. An energy budget is the amount of energy in percent of ingested food that is utilized for each major process such as for growth and reproduction, digestion, respiration, urinary, and fecal production.

Carnivores : 100 C = 29P + 44R + 7U + 20F
Herbivores : 100 C = 20P + 37R + 2U + 41F

*Brett and Groves 1979

where the figures are expressed as percentage of ingested food energy or percentage of energy derived from eaten food. Metabolizable energy values for fish in kilocalories per gram of substance are: 4.5, 3.3, and 8.0 for protein, carbohydrate and fat, respectively. The energy needs for maintenance and voluntary swimming activity must first be satisfied before energy can be made available for growth.

Fish, like other animals, eat primarily to satisfy their energy requirements. When the feed has low energy density, fish are able to compensate by eating more of the feed but within certain limits. Therefore, it is essential that fish have easy access to feed or are given a palatable ration with sufficient energy to meet their energy needs.

Dietary Energy Requirement

Fish diets should have an optimal proportion of energy from dietary lipids, carbohydrates, and proteins. An excess or a deficiency of lipids and carbohydrates, which are nonprotein energy sources, may result in lower growth. If the diet is deficient in nonprotein energy, protein whose function is for growth and repair of tissues will be used as energy source

rather than for growth. Similarly, if the diet contains an excess of lipids and carbohydrates, fish appetite is met even before enough protein (and possibly other nutrients) is eaten to meet the demand for maximum protein synthesis and growth. Many factors influence the energy requirements of fish:

1. water temperature - metabolic rate and consequently energy requirements for maintenance increase with temperature.
2. animal size - metabolic rate and consequently maintenance energy requirements, decrease with increasing animal size.
3. physiological status - energy requirements increase during production of gonads and reproduction activity such as migration during spawning.
4. water flow rate - energy requirements for maintaining a certain position in water increase with an increase in water flow rate.
5. light exposure - energy requirements for voluntary activity decrease during rest periods at night.
6. water quality and stress - pollutants, increased salinity, low dissolved oxygen, crowding or overstocking increase energy requirements for maintenance.

Guide Questions

1. What is a kilocalorie? Give the equivalent of kilocalorie in kilojoules.
2. What is the difference between gross energy in feed and energy excreted in the feces?
3. Give three examples of energy-requiring biological processes.
4. Differentiate between digestible energy and metabolizable energy. Why is metabolizable energy more difficult to measure?
5. What is ATP? What is its function?
6. Write and explain briefly the energy balance equation.
7. What are the factors that influence the energy requirements of aquatic animals?
8. Why is it important to provide an optimal proportion of protein to non protein energy in fish diets?

VITAMINS

Introduction

Vitamins are organic compounds that are required in the diet in small amounts and unlike proteins, lipids, and carbohydrates, have unrelated chemical structure. This distinguishes vitamins from other organic macronutrients (proteins, carbohydrates, lipids). The chemical structure of vitamins is remarkably diverse and they are usually of small molecular size (molecular weight usually <1000). They have specific regulatory functions and are necessary for normal growth, maintenance of health, and reproduction. Insufficient amounts in fish diets may result in nutritional deficiency diseases, poor growth, and increased susceptibility to diseases and infections. They do not give energy but are needed in enzyme systems to hasten or catalyze reactions in energy utilization. Vitamins are essential for the regulation of metabolism in the cells and for the transformation of energy. Without vitamins, respiration, growth, muscle contraction, and other physical activities cannot occur. Many are used to form coenzymes or as part of an enzyme system. About 15 vitamins are known to be needed by fish.

This section describes the various lipid-soluble and water-soluble vitamins, their differences, physiological functions, and the symptoms of vitamin deficiencies in fish. It also shows a summary of nutritional deficiency signs and the requirements of various fish species for vitamins.

Classification of Vitamins

Vitamins are classified according to their solubility: water-soluble and lipid-soluble. The water-soluble vitamins, which are the B complex, and ascorbic acid or vitamin C and some cofactors, dissolve in water but not in oils and fats. On the contrary, the lipid-soluble vitamins, A, D, E and K dissolve in oils and fats.

Many of the water-soluble vitamins function either directly or in a modified form as a coenzyme. Coenzymes are organic molecules that help enzymes become biologically active. In certain instances, this functional role of vitamins has been used as a means to assess the nutritional status of an animal with respect to that vitamin. Water-soluble vitamins are excreted in the urine, while fat-soluble vitamins are more likely to appear in the bile and thus excreted in the feces.

❑ Water-soluble vitamins

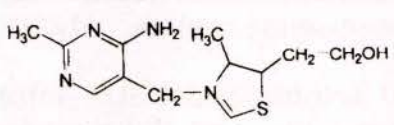
Among the group of water-soluble vitamins are included the B complex vitamins, thiamin or B_1, riboflavin or B_2, pantothenic acid or B_5, pyridoxine or B_6, and cyanocobalamin or B_{12}, biotin, niacin, choline, inositol, folic acid, and ascorbic acid or vitamin C.

Thiamin (Vitamin B_1). Thiamin is a complex substance that contains sulfur, which gives it a characteristic pungent odor. It plays an active role in carbohydrate metabolism as the coenzyme, thiamin pyrophosphate. Of particular importance is the role of thiamin in the conversion of

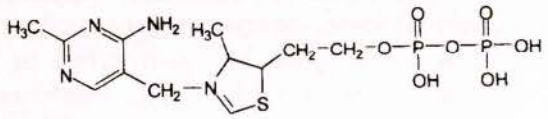
Figure 2.11
Thiamin (vitamin B_1) deficiency in humpback grouper. Mechanical injuries with hemorrhages on the body surface, pectoral fins and abdomen, and erratic swimming behavior are signs of vitamin B_1-deficient diet
Source: **Koesharyani et al. 2001**

pyruvate to acetyl CoA. Thiamin is destroyed by heat under slightly alkaline and moist conditions. However, the vitamin is relatively stable to dry heat, and is retained in feed pellets during the pelleting process and storage. Wet or moist fish diets should be used immediately to prevent loss of thiamin by hydrolysis.

Thiamin is essential for growth, reproduction and normal digestion. Lack of thiamin in fish results in loss of appetite, poor growth, muscle atrophy, instability and sensitivity to shock, hemorrhages and erratic swimming behavior (Figure 2.11).

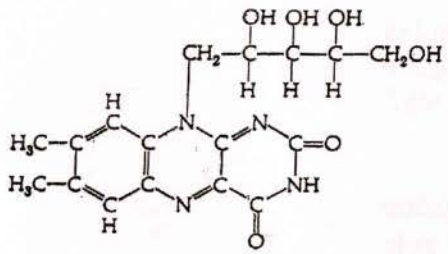


thiamin

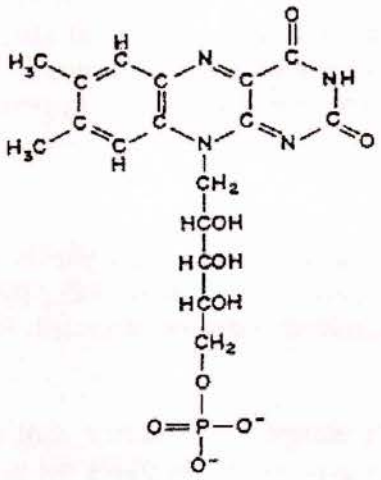


thiamin pyrophosphate

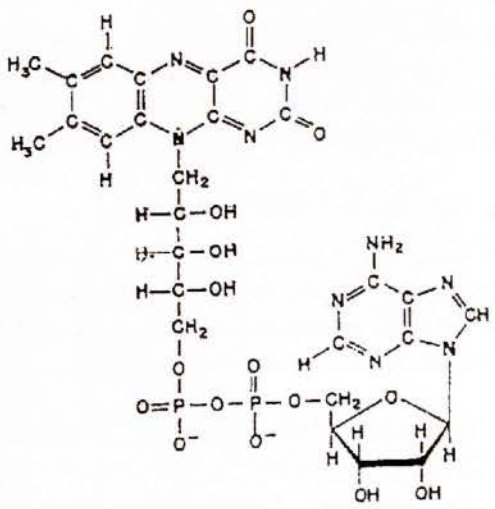
Riboflavin (Vitamin B_2). Riboflavin is an orange pigmented molecule. It is involved in energy metabolism in the form of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes facilitate the breakdown of energy-yielding nutrients such as amino acids, fatty acids, and pyruvic acid. Riboflavin is relatively more stable to heat than thiamin but is destroyed by light and in strongly alkaline or acidic solutions. Ingredients and rations should be stored in dark bags or non-transparent tight containers and protected from



riboflavin



flavin mononucleotide (FMN)



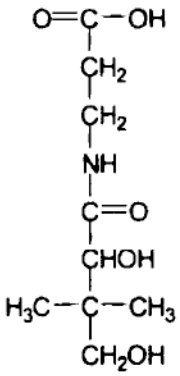
flavin adenine dinucleotide (FAD)

light to prevent loss of riboflavin activity in the feed.

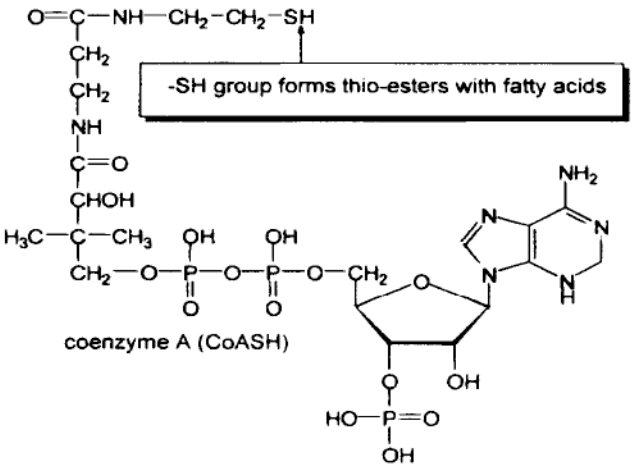
Riboflavin deficiency in fish results in formation of lens cataracts, photophobia, hemorrhagic eyes, anemia, loss of appetite, and poor growth.

Pantothenic Acid (Vitamin B_5). Pantothenic acid is an organic acid that contains nitrogen. It plays an essential role in protein, lipid, and carbohydrate metabolism as a component of the coenzymes, acetyl coenzyme A, and acyl carrier protein. These coenzymes are involved in the synthesis of fatty acids, cholesterol, steroid hormones, phospholipids, and hemoglobin. The sodium or calcium salt of pantothenic acid is relatively stable and can be incorporated into either dry or moist fish diets. It is stable in neutral solution but is destroyed by heat at either alkaline or acid pH. In diet preparation, some loss is incurred during autoclaving and excessive heat.

Pantothenic acid deficiency results in clubbed gills, loss of appetite, necrosis, cellular atrophy, sluggishness, and poor growth.



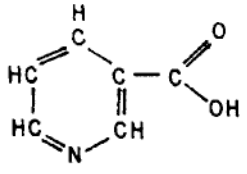
pantothenic acid



coenzyme A

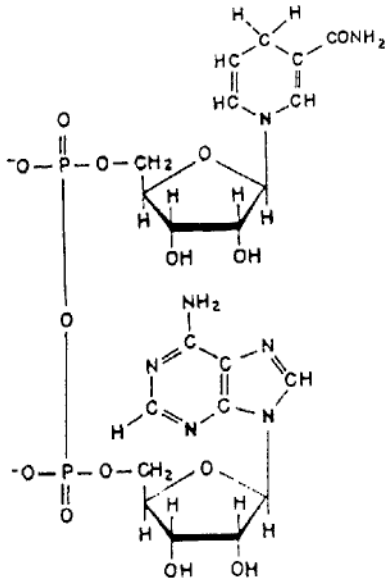
Niacin (Nicotinic acid). Niacin or nicotinic acid plays an essential role in the metabolism of carbohydrates, lipids, and proteins, as a component of two high energy molecules, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). NADH and NADPH are important in a number of oxidation and reduction reactions that occur within cells. These coenzymes are essential for the release of energy from food nutrients and are also involved in the synthesis of fatty acids and cholesterol, respectively.

Niacin deficiency results in loss of appetite, poor growth, lesions and edema of the colon, jerky motion, weakness, muscle spasms, and fin erosion.

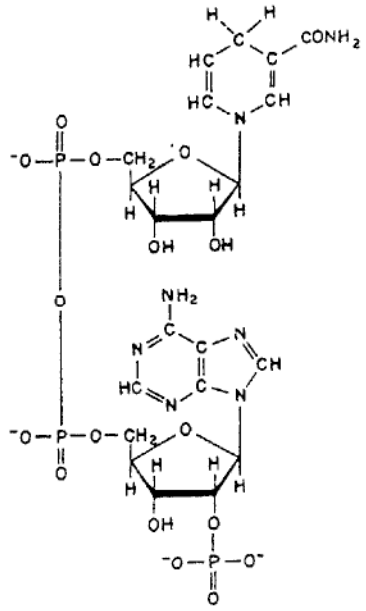


nicotinic acid

Pyridoxine (Vitamin B_6). Pyridoxine is essential in protein metabolism as the



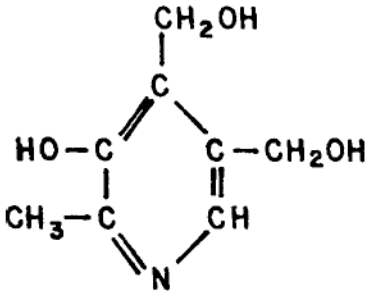
NADH



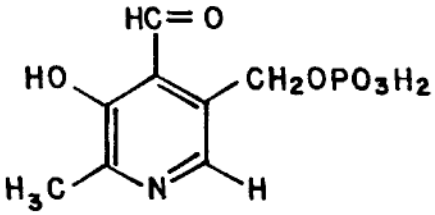
NADPH

coenzyme, pyridoxal phosphate, which is involved in the non-oxidative degradation of amino acids, including transamination, deamination and decarboxylation. The availability of vitamin B_6 in foods can be substantially reduced by processing. It is susceptible to destruction by light in neutral and alkaline solutions hence diet ingredients should be protected from exposure to sunlight.

Pyridoxine deficiency results in nervous disorders, hyperirritability, edema of the peritoneal cavity, anemia, rapid and gasping breathing, and loss of appetite.



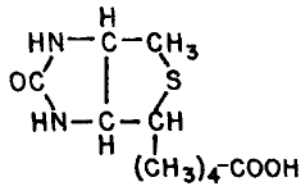
pyridoxine



pyridoxal phosphate

Biotin. Biotin plays an important role in the metabolism of carbohydrates, lipids and proteins, as a coenzyme for reactions involving transfer and removal of carbon dioxide from one compound to another. As such, it is essential for the synthesis of fatty acids and catabolism of certain amino acids. It is also important in cell immunity, as an activator of bacteria-destroying enzyme, lysozyme.

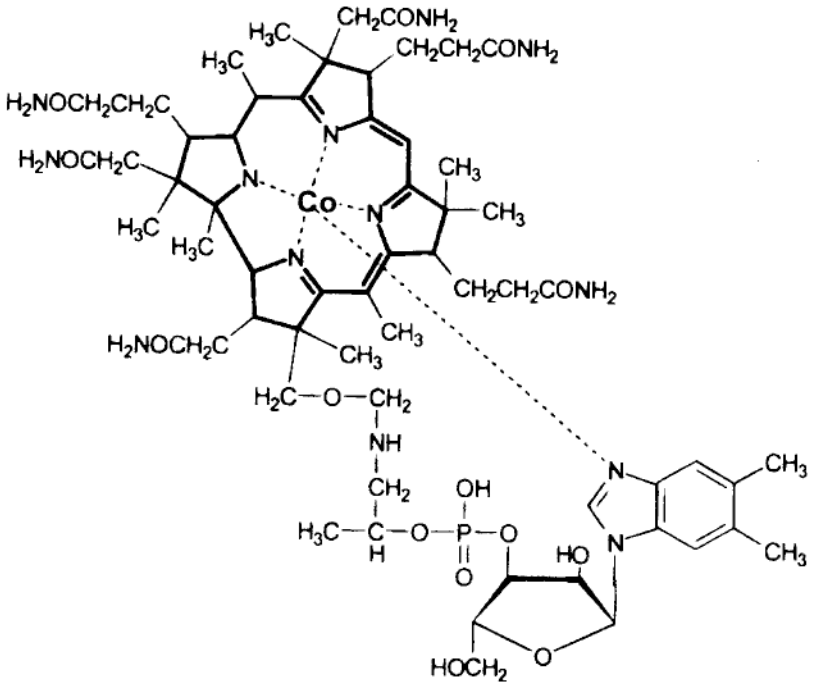
Biotin deficiency leads to reduced growth and activity, loss of appetite, skin disorders, muscle atrophy, lesions in the colon, spastic convulsions, skin lesions, and abnormal swimming behavior.



biotin

Cobalamin (Vitamin B_{12}). Cobalamin or B_{12} is essential for normal maturation and development, in the formation of red blood cells and maintenance of the nerve tissue as the coenzyme cobamide. The vitamin is destroyed by heating in a highly alkaline medium and in the presence of ascorbic acid. Stability of vitamin B_{12} is high in feeds stored at moderate temperatures.

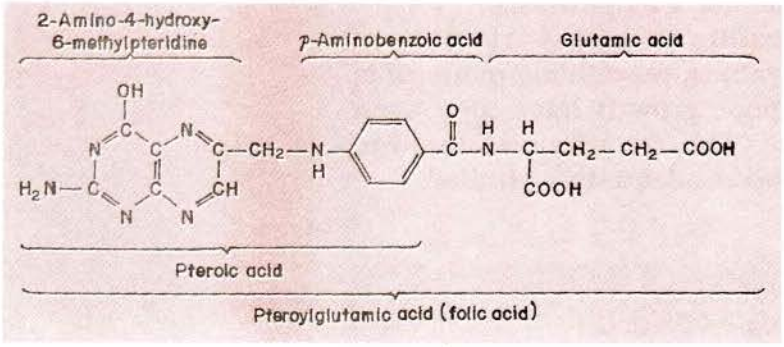
In salmonids, vitamin B_{12} deficiency results in high variability of fragmented erythrocytes and in hemoglobin values, resembling monocytic, hypochromic anemia. Poor appetite and poor growth have also been observed in cobalamin deficiency in fish. The requirement for and metabolism of vitamin B_{12} in fish has not been adequately studied.



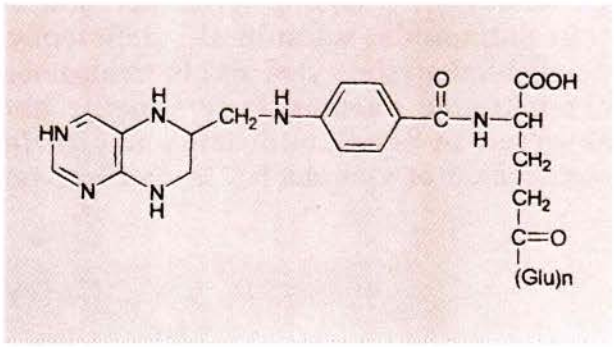
cobalamin

Folic Acid (Folacin, Pteroylglutamic acid). Folic acid is important in protein metabolism as a component of the coenzyme tetrahydrofolic acid. This coenzyme is needed for the synthesis of hemoglobin, glycine, methionine, choline, and purines. High temperature or prolonged heating as well as acid pH will destroy folate in feeds.

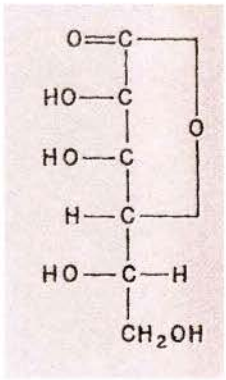
Macrocytic anemia occurs in the red blood cell-producing tissue of the anterior kidney in fish. Other signs that have been observed are poor growth, lethargy, blindness, melanism and dark skin coloration, and fragile fins.



folic acid



tetrahydrofolic acid



Ascorbic acid

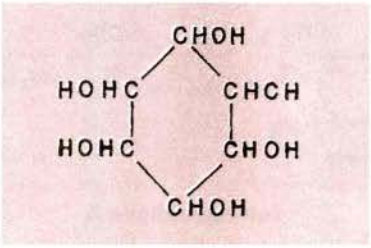
Ascorbic Acid (Vitamin C). Ascorbic acid is a white crystalline powder that is essential in maintaining the integrity of connective tissues, blood vessels, bone tissue, wound tissue and as a cofactor for numerous hydroxylation reactions. The vitamin also acts as a strong biological reducing agent and is required for the conversion of folic to tetrahydrofolic acid, tryptophan to serotonin, and in the synthesis of steroid hormones. Ascorbic acid is easily destroyed by heat and prolonged exposure to air and alkaline medium. Oxidation of vitamin C or destruction in the process of feed preparation for fish can be reduced by using coated or protected forms of L-ascorbic acid. Stable, biologically-equivalent derivatives are ascorbate-2-monophosphate (AMP) and ascorbate-2-polyphosphate (APP). Ascorbate-2-sulfate (C_2) is a stable storage form of vitamin C found in the thick dermal layer of fish tissue.

Scurvy is a specific syndrome caused by vitamin C deficiency in humans and other animals. It is characterized by impared collagen formation and widespread capillary hemorrhaging. In fish, vitamin C deficiency causes lordosis, scoliosis (Figure 2.12) impaired collagen formation, changes in the cartilage, exopthalmic eye, eye opacity, hemorrhagic skin, liver, kidney, intestine, and muscle.

Figure 2.12
Ascorbic acid (vitamin C) deficiency. Deformed spinal cord of humpback grouper as a result of vitamin C deficiency
Source: **Koesharyani et al. 2001**

Inositol. Myoinositol is an essential component of the inositol containing phospholipids and is an important structural component of skeletal, heart, and brain tissues. It maintains the integrity of cell membranes, prevents the accumulation of cholesterol in fatty liver disease, and is involved, with choline, in normal lipid metabolism. It also plays an important role in growth of liver cells, cholesterol transport, and in the synthesis of ribonucleic acid. The compound is stable and withstands normal feed processing and storage conditions.

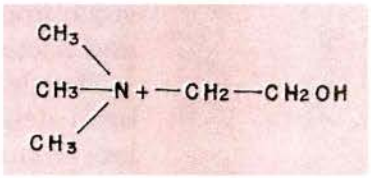
Deficiency symptoms are poor growth, distended stomach, skin lesions, and increased gastric emptying time.



myoinositol

Choline. Choline is important as component of acetylcholine and the phospholipids, lecithin, and sphingomyelin. As such, it plays a vital role in the maintenance of cell structure and transmission of nerve impulses. It is also important in lipid transport within the body. Choline is widely distributed in foods. It is usually incorporated in fish diets as choline hydrochloride.

Deficiency signs include impaired fat metabolism, fat cell necrosis syndrome, poor growth, and hemorrhagic kidneys and intestine.



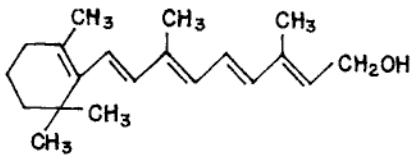
choline

❑ Lipid-soluble vitamins

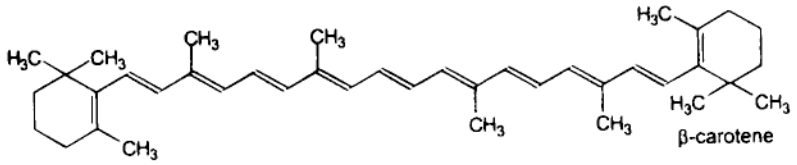
Retinol (Vitamin A). Vitamin A is a generic term of all compounds other than carotenoids that has retinol activity. It is an essential component of visual pigments and is required for the maintenance of epithelial cells. Vitamin A is also required for the release of proteolytic enzymes from lysosomes. Deficiency of vitamin A in fish leads to symptoms such as exophthalmia, eye lesion, anorexia, ascites, lens deformation and operculum deformation (Figure 2.13) in fish. Hypervitaminosis or excess vitamin A has been described in fish and involves enlargement of the liver and spleen, abnormal growth, skin lesions, epithelial keratinization, abnormal bone formation, and hyperplasia of head cartilage.

Cod liver oil and other liver oils contain vitamin A (retinol or retinyl esters). Synthetic vitamin A (retinyl palmitate) is often used to supplement rations low in fish meals or carotenes, a precursor of vitamin A. Carotenoids are found in phytoplankton and are changed to vitamin A in the liver of fish.

Figure 2.13
Operculum deformity in humpback grouper is generally caused by a deficiency in Vitamin A.
Source: **Koesharyani et al. 2001**



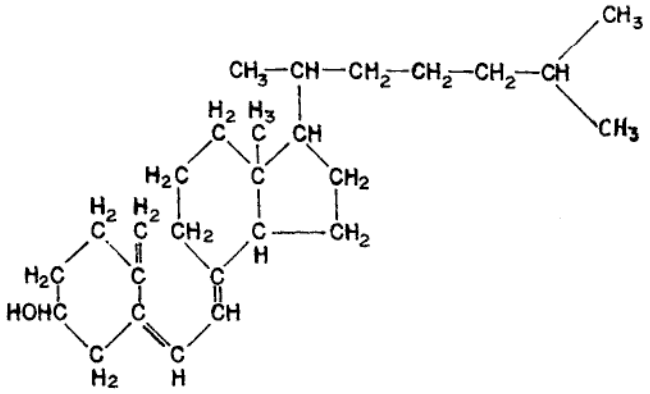
retinol, vitamin A



β-carotene

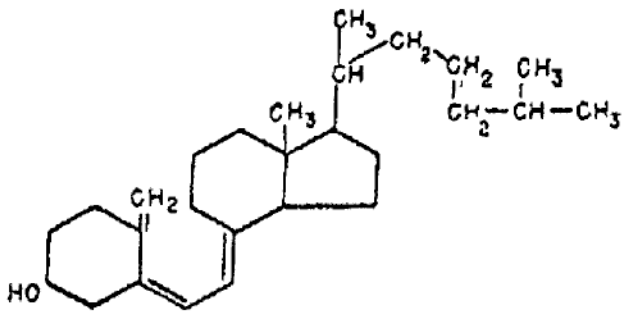
Cholecalciferol (Vitamin D). There are two active forms of vitamin D, ergocalciferol (vitamin D_2) and cholecalciferol or vitamin. D_3. Vitamin D plays an important role in the proper use of calcium and phosphorus to form bones and teeth. It promotes normal bone formation or mineralization. Cholecalciferol can be synthesized in the skin by sunlight irradiation of 7-dehydrocholesterol which is found under the skin. It is sometimes called the sunshine vitamin. Since the vitamin is fat soluble and accumulates in lipid stores, fish liver oil is a rich source of vitamin D.

The deficiency symptoms of rickets and abnormal bone formation has been described in fish fed a low vitamin D diet in water that contains low amounts of calcium. Poor growth, and tetany of white skeletal muscle have also been reported. Fish with hypervitaminosis D exhibit impaired growth, lethargy, and dark coloration. High doses of the vitamin mobilizes calcium and phosphate and may lead to fragile bones.



cholecalciferol, vitamin D

α-tocopherol (Vitamin E). Tocopherol is an important fat-soluble antioxidant within the animal body, protecting reactive compounds such as highly unsaturated fatty acids, and vitamins A and C from oxidative damage by trapping free radicals. The ester, α-tocopherol acetate or phosphate, is commonly used as a diet supplement because it is more stable than the free form, which is rapidly lost by air oxidation or in the presence of unstable reactive metabolites in fish oils. Vitamin E has physiologic antioxidant activity in growing animals including fish. An interaction exist between vitamin E and selenium, a metabolic antioxidant; hence vitamin E requirements are greater in selenium-depleted fish.



α-tocopherol, the most active form of vitamin E

Fish fed vitamin E-deficient diet containing rancid fat exhibit low survival, poor growth, fragile erythrocytes and fragmentation, emaciation and darkening of body color (Figure 2.14). An excess (hypervitaminosis) of vitamin E can cause poor growth, toxic liver reaction, and eventually, death.

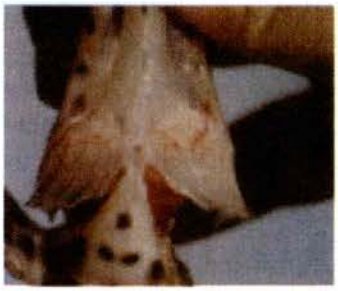
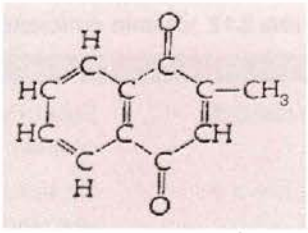


Figure 2.14
Picture of humpback grouper showing emaciation, darkening of body color, petechia at the base of operculum as a result of rancid dietary fat and vitamin E deficiency.
Source: Koesharyani et al. 2001

Menadione (Vitamin K). Vitamin K is essential for the maintenance of normal blood coagulation by facilitating the production of various plasma proteins. It may also play an important part in electron transport and oxidative phosphorylation. Synthetic menadione is a good supplement for adequate vitamin K intake. Vitamin K is labile to oxidation and exposure to ultraviolet radiation. Diets containing vitamin K must be kept dry, protected from air oxidation or oxidation by ultraviolet radiation.

Vitamin K deficiency signs include prolonged blood clotting, anemia, and hemorrhagic gills, eyes, and vascular tissues.



menadione, vitamin K

Vitamin Requirements of Fish

Vitamin requirements of fish have been studied by using vitamin test diets. The qualitative requirement is determined by feeding one group of fish a diet complete in vitamins, and another group, a diet from which the vitamin being studied has been deleted. Growth and typical deficiency signs are noted. If the vitamin is found to be essential, the quantitative requirement for that vitamin is then determined. The common approach is to feed groups of fish, test diets containing graded levels of one vitamin while all other vitamins and components of the diet are kept constant. The "broken line" analysis of the growth data is used to determine the minimum dietary requirement of fish. Qualitative and quantitative experiments on vitamin requirements have shown that the four fat-soluble and 11 water-soluble vitamins previously mentioned are required by fish.

The requirement for a vitamin may be affected by various factors such as species, size or age, dietary nutrient levels, diet composition, physical properties of diet, and culture conditions. For instance, the requirement for vitamin E may increase as the polyunsaturated fatty acid level in the diet increases. In low-density extensive culture in ponds or lakes, natural food are frequently abundant enough to provide essential vitamins. In high density intensive culture in ponds, cages, and raceways, natural food is limited, thus vitamins must be supplied in the diet to achieve normal growth of fish.

The vitamin requirements by different species vary greatly according to their usual feeding habit and capacity to synthesize them. A summary of the vitamin deficiency symptoms is given in Table 2.12. Some vitamins cannot be synthesized by most animals hence there is an absolute requirement for them to prevent the occurrence of specific deficiency diseases.

Certain vitamins may be destroyed during feed manufacture by heat, moisture, alterations in pH, the presence of some metals, lipid oxidation, etc. Destruction of vitamin C due to oxidation is a problem in feed manufacture. Some vitamins are also lost during storage, thus feed should be used soon after pelleting. In crustacean feed, allowance should be made for the leaching of vitamins from feed pellets. Only a few studies have been done on the vitamin requirement of aquatic animals. The supplementation levels of each vitamin in fish feeds are often higher than the required levels to provide a safety margin.

Table 2.12 Vitamin deficiency symptoms in fishes

Vitamins	Deficiency symptoms
Vitamin B_1	Equilibrium loss, irritability, lethargy, nerve disorder, anorexia, muscle atrophy, cataract, convulsions, skin discoloration, edema
Vitamin B_2	Fin erosion, fragile skin, eye lesion, photophobia, iris pigmentation, cornea vascularization, anemia, anorexia, ataxia, cataract. cloudy lens, dark skin coloration, poor feed efficiency
Vitamin B_5	Exudated gills, fragile skin, lethargy, liver necrosis, prostration, erratic swimming, ataxia, gill atrophy, clubbed gills, dark skin coloration, dermatitis
Vitamin B_6	Equilibrium loss, exophthalmus, irritability, nerve disorder, rapid rigor mortis, blue slime, erratic swimming, anemia, ataxia, convulsions, edema
Vitamin B_{12}	Anemia, anorexia
Vitamin C	Exophthalmus, fragile gill, kidney, liver and skin, eye lesion, lethargy, lordosis, prostration, scoliosis, anemia, anorexia, ascites, cartilage abnormality, low disease resistance
Inositol	Fatty liver, skin lesion, anemia, anorexia, distended stomach, poor feed efficiency
Biotin	Fatty liver, fragile erythrocytes, colon and skin lesions, blue slime, anorexia, muscle atrophy, dark skin coloration, convulsions, gill degeneration, poor feed efficiency
Choline	Fatty liver, fragile kidney, poor feed efficiency
Nicotinic acid	Anemia, anorexia, edema, fragile skin, colon lesion, lethargy, photophobia, muscle spasm, white muscle tetany, poor feed efficiency
Folic acid	Poor feed efficiency, fragile fins, lethargy, anemia, anorexia, dark skin coloration
Vitamin A	Erosion of fins, exophthalmus, fragile kidney and skin, eye lesion, anorexia, ascites, lens deformation, edema
Vitamin D	Scoliosis, white muscle tetany
Vitamin E	Exophthalmus, fatty liver, fragile erythrocytes, anemia, ascites, ceroid liver, muscular dystrophy, edema, epicarditis
Vitamin K	Anemia, slow clotting blood

Source: Halver 1989

The specific vitamin requirements of some fish species that have been studied are in Table 2.13.

Table 2.13 Summary of the vitamin requirements of various species of fish and shrimp

	Atlantic salmon	Ayu	Channel catfish	Common carp	Eel	Sea bass	Pacific salmon	Rainbow trout	Tilapia	Yellow tail	Shrimp
Thiamin		12	1	R	R	R	10-15	40		11.2	60
Riboflavin	R	40	9	7-14	R	R	20-25	9		11.0	25
Pyridoxine	5	12	3	5-6	R	5	15-20	9		11.7	
Pantothenic acid	R	50	15	30-50	R	R	40-50	40		35.9	75
Cobalamin			R		R		0.15-0.02	0.21	NR		0.2
Nicotinic acid		100	14	28	R		150-200	300		12.0	40
Biotin		0.3	R	1	R		1-1.5	0.4		0.67	
Inositol		400	NR	440	R	R	300-400	510		423	400
Choline		350	400	4000	R		600-800	11100		2920	600
Folic acid		3	R		R		6-10	21		1.2	10
Ascorbic acid	50	300	60	R	R	700	100-150	400	R	122	200
Vitamin A		10000 IU	1000-2000 IU	10000 IU			2000-2500 IU	7000 IU		5.68	5000 IU
Vitamin D		2000 IU	250-500 IU					3000 IU		NR	0.1
Vitamin E	35	100 IU	50 IU	1000 IU	200	R	30 IU	200	50-100	119.0	100
Vitamin K	R	10 IU	R					50		NR	5

Values are mg/kg diet unless stated otherwise.
R, required, NR, not required, IU, international units.
Source: **Halver 1989; Wilson 1991; D'Abramo et al. 1997.**

The table shows that not only do the vitamins required vary but there is also a variation in the dietary requirement level It is therefore very difficult to determine a recommended level of vitamin supplementation that will be satisfactory for all fish species. The essentiality and requirements for these vitamins generally have been determined based on weight gain, survival rate, tissue storage, and other specific deficiency signs.

Guide Questions

1. Define the term vitamin. What is the general function of vitamins?
2. Why must vitamins be included in the diet?
3. Distinguish between lipid-soluble and water-soluble vitamins? Name the vitamins that are lipid-soluble; name the vitamins that are water-soluble.
4. Give names and symptoms of 4 vitamin deficiency diseases. Tabulate as follows:

	Name of the deficient vitamin	Deficiency disease	Symptoms
a.	_____	_____	_____
b.	_____	_____	_____
c.	_____	_____	_____
d.	_____	_____	_____

5. What is the stable storage form of vitamin C in fish tissues?
6. What is the result of vitamin A deficiency in fish?
7. What vitamins are important biological antioxidants?
8. What are the consequences of hypervitaminosis or excess vitamin A in fish?
9. What vitamin is essential for maintenance of normal blood clotting? for bone formation?
10. What vitamin plays an important role in calcium and phosphorus metabolism?
11. What are the factors that affect the requirement for a vitamin?
12. Why is there hypervitaminosis for lipid-soluble vitamins?

MINERALS

Introduction

Minerals are inorganic substances that have many important functions in the animal body. They are required to maintain many metabolic processes and to provide material for major structures (e.g. bones, teeth, exoskeleton of crustaceans) of aquatic animals. They are also required for maintenance of osmotic pressure, acid-base balance (e.g., the regulation of blood pH, hemolymph, urine and other body fluids) and the proper functioning of muscles and nerves. Unlike carbohydrates, fats, and proteins, they do not provide energy but they may serve as components of enzymes, vitamins, and hormones. Fish, especially marine fish species, live in an environment that contains many of the minerals that they need for growth and survival.

This section discusses the macro, micro, and trace minerals; their physiologic functions; and deficiency signs and symptoms. It also gives a summary of the mineral functions and mineral requirements of fishes and shrimp.

Classification of Minerals

About 20 inorganic elements are required to meet the structural and metabolic functions of living organisms. They are grouped into macro, micro, and trace minerals.

The macrominerals calcium, phosphorus, magnesium, potassium, sodium and chlorine are required in the diet in relatively larger quantities than microminerals.

The microminerals are chromium, copper, cobalt, iron, iodine, manganese, molybdenum, selenium, and zinc. So far, trace minerals such as aluminum, arsenic, cadmium, lead, mercury, nickel, silicon, tin, and vanadium have no known function in fish.

General functions of minerals

The biochemical functions of minerals in aquatic animals are similar to those in land animals, with the exception of osmoregulation. Minerals may serve as components of hard-tissue matrices, soft tissues, metalloproteins, and as cofactors or activators of enzymes. The more soluble minerals (calcium, phosphorus, sodium, potassium, and chlorine) function in osmoregulation and in maintenance of acid-base balance and membrane potentials.

Unlike vertebrates, there are several physiological requirements unique to crustaceans, particularly in the molting cycle which is necessary in crustacean growth. Although some minerals are temporarily stored in the tissues, such as the hepatopancreas, a significant amount is lost during molting or ecdysis. A summary of functions of minerals is shown in Table 2.14.

Table 2.14 Summary of mineral functions

Mineral	Function and Essentiality
Calcium	Structural component of hard tissue; Co-factor for enzymatic processes; Muscle function and proper nerve impulse transmission; Osmoregulation
Phosphorus	Component of hard tissues and organic phosphates (e.g ATP, phospholipids, coenzymes, DNA and RNA); Buffer for the maintenance of normal pH of intra- and extra-cellular fluids
Potassium	Carbohydrate metabolism and protein synthesis; Osmoregulation; Acid-base balance; Phosphorylation reactions
Magnesium	Metabolism of fats, carbohydrates and proteins; Cellular respiration; Intra- and extracellular homeostasis; Phosphate transfer and thiamine pyrophosphate reactions; Osmoregulation
Copper	Functions in hematopoiesis and in numerous copper-dependent enzymes (e.g. cytochrome c oxidase, ferroxidase); Component of hemocyanin
Cobalt	Source for microbial synthesis of B_{12} in intestine
Iron	Heme containing enzymes (e.g. cytochromes, oxidases, peroxidase and catalases); Cofactor for enzymes; Hemoglobin
Iodine	Neuromuscular functions; Intermediary metabolism; Synthesis of thyroid hormones
Manganese	Cofactor of various enzyme synthesis
Selenium	Component of glutathione peroxidase
Zinc	Cofactor in several enzyme systems; Component of a large number of metalloenzymes

Source: Halver 1989

Mineral Availability

In general, bioavailability of minerals has been found to be positively correlated with their solubility in water. Highly soluble salts appear to be beneficial, but their leaching rates from diets have to be measured. In addition to inorganic sources, organic chelates and complexes of mineral elements are useful means of delivering minerals. Considering these limitations, water soluble inorganic salts or bioavailable organic salts are preferrable for use in mineral premixes.

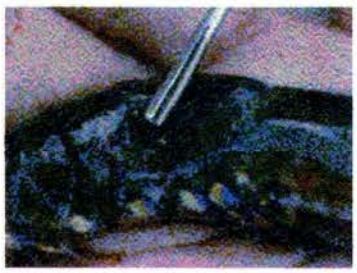


Figure 2.15
Calcium and phosphorus deficiencies. Soft-shelled shrimp mainly due to calcium and phosphorus deficiencies has caused losses in the shrimp industry.
Source: Lavilla 2001

Macrominerals

Calcium

Calcium is mostly found in the skeleton and scales of bony fish and in the exoskeleton of crustaceans. Fish scales are an important site of calcium metabolism and deposition. Aside from its structural function, calcium is important in physiological processes including metabolism, nerve and muscle contraction, nerve impulse transmission, maintenance of cell integrity, osmoregulation, and activation of important enzymes. Calcium and phosphorus deficiencies can cause soft-shelling in shrimps (Figure 2.15).

Fish may totally or partially meet their calcium requirement through absorption of calcium from the water via their gills, fins and oral epithelia. The gills are the most important site of calcium regulation. Generally,

calcium from feed ingredients for example, fish meal, may meet the requirements of most fish, however, it is a common practice to supplement the feed with calcium.

Phosphorus

Phosphorus is another major mineral required by fish and is an important constituent of nucleic acids and cell membranes. It is directly involved in the energy-producing cellular reactions and is a component of essential molecules such as adenosine triphosphate (ATP). Feed is the main source of phosphorus for fish because the concentration of phosphorus in natural waters is low. Consequently, the need for a dietary source of phosphorus is more critical than for calcium because fish must effectively absorb, store, mobilize, and conserve phosphate in both freshwater and seawater environments.

Most fish species require a dietary inclusion of 0.5% to 1.0% available phosphorus. Generally, phosphorus requirements are not affected by dietary calcium levels. In controlled experiments, the growth of both common carp and trout have been shown to be positively correlated with dietary phosphorus levels but not with calcium levels. The apparent phosphorus availability values for several phosphorus sources have been estimated. They are: calcium phosphate monobasic, 46%; calcium phosphate dibasic, 14%; calcium phosphate tribasic, 5.7%; sodium phosphate monobasic, 70%; and potassium phosphate monobasic, 68%. Dietary calcium and phosphorus are important in the prevention of soft-shelling in shrimps.

Sodium, potassium, and chlorine

Sodium, potassium, and chlorine are the most common inorganic elements found in fish. They are abundant electrolytes in the body and are essential for a number of physiological processes. Appropriate levels of these ions are required for proper functioning of cells and for maintaining nerve function. Fish readily absorb these elements from the aquatic medium, especially in marine waters, and the tissue levels of these ions are maintained as a result of osmoregulation. They are abundant in sea water and in common feedstuffs used in preparation of fish diets. Thus, dietary supplementation of these elements is normally not required.

Magnesium

A large proportion of magnesium in fish is contained in the skeletal tissue. Magnesium is an essential cofactor in many metabolic reactions. These enzymes include the phosphokinases, thiokinases, phosphatases, pyrophosphatases, and amino acyl synthetases. It is also needed in skeletal tissue metabolism, osmoregulation, and in maintaining muscle tone.

General symptoms of magnesium deficiency include reduced weight gain and poor feed conversion. In rainbow trout, magnesium deficiency leads to renal calcinosis and flexibility of the muscle, partly due to an increase in extracellular fluid volume. Most feed ingredients, especially those of plant origin, are abundant in magnesium thus, magnesium supplementation in practical diets is generally not required.

Microminerals

Copper

Copper is an important component of a number of metalloenzymes that are involved in a wide variety of metabolic processes. It is associated with cytochrome c oxidase of the electron transport chain in cells. It is the primary oxygen carrier in hemocyanin of crustaceans and molluscs. Fish appears to tolerate copper in the diet than dissolved copper from the water. Concentrations of 0.8-1.0 mg copper per liter in water are toxic to many fish species. Feed ingredients such as fish solubles, krill meal, and yeast contain relatively high levels of copper. Shrimp in general cannot meet their physiological requirement for copper from seawater. In white shrimp *Penaeus vannamei*, symptoms of deficiency include poor growth, reduced copper levels in the carapace, hepatopancreas and hemolymph, and enlargement of the heart.

Iron

Dietary iron is essential in fish for blood formation, maintaining normal hemoglobin content, hematocrit value, and cell size. A minimum dietary concentration of 150*ug* per gram diet is required to prevent iron deficiency resulting in hypochromic, microcytic anemia in red sea bream and common carp. Dietary iron deficiency has not been observed in shrimp. In contrast, dietary supplementation of shrimp feed with iron greater than 150*ug* per gram has been found to give poor growth. Since crustacean diets generally contain polyunsaturated fatty acids, excessive supplementation of ferrous iron in the diet may affect diet stability through increased lipid oxidation.

Manganese

Manganese is important either as a cofactor that activates metal-enzyme complexes or as an integral part of metalloenzymes in protein, carbohydrate, and lipid metabolism. The uptake of manganese from water by fish has been demonstrated but it is more efficiently absorbed from feed. However, due to the potential inhibitory effects of phytic acid on the bioavailability of manganese, feed supplementation may be desirable. Dietary deficiencies in fish have resulted in poor growth, skeletal abnormalities, embryo mortalities, and poor hatching rates.

Selenium

Selenium is an integral component of the enzyme glutathione peroxidase that protects cells and membranes from deleterious effects of peroxides. In conjunction with vitamin E, this enzyme functions as a biological antioxidant which protects biological membranes against lipid peroxidation. Selenium imparts a protective effect against the toxicity of heavy metals such as cadmium and mercury. The selenium requirement of fish varies with the polyunsaturated fatty acid and vitamin E content of the diet. Both selenium and vitamin E are required to prevent muscular dystrophy in Atlantic salmon. In general, practical diets containing fish meal contains adequate amount of selenium and does not require supplementation.

Zinc

Zinc is an integral component of metalloenzymes including dehydrogenases, aldolases, peptidases, and phosphatases. Approximately 20 different enzymes has been found to contain zinc. Many metabolic functions are affected by zinc deficiency. Fish can accumulate zinc from both water and feed but dietary zinc is more efficiently absorbed. Practical diets contain feedstuffs that are good sources of zinc; for example, fish meal. However, zinc bioavailability is generally very low, thus supplementation is essential. Feedstuffs that are relatively high in phytate may further reduce zinc bioavailability. Cataract is a common symptom of zinc deficiency in fish (Figure 2.16).

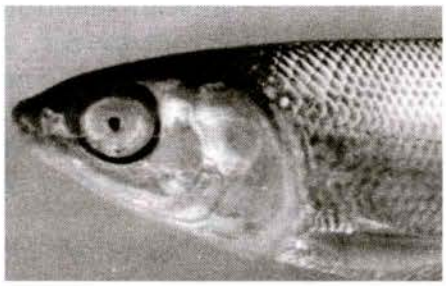


Figure 2.16
Zinc deficiency cataract: non-availability of zinc due to high levels of calcium and phosphorus in fish feed has caused losses in the fish farming industry.

Trace minerals

Information on the dietary requirements of other microminerals is limited. A number of trace elements are required for adequate fish nutrition but their dietary deficiencies have not been reported. Cobalt acts as a component of vitamin B_{12}. Sulfur is required for the synthesis of cysteine. Chromium is important in normal glucose and lipid metabolism.

Mineral Supplementation of Practical Fish Diets

Although the dietary mineral requirements of fish have not been well established, practical diets are usually supplemented with a mineral premix. Practical diets may normally contain substantial amounts of endogenous minerals, thus a complete mineral premix may not be necessary. Excessive mineral supplementation increases the cost of feed and may reduce the bioavailability of other minerals and increase phosphorus pollution. Many commercial feed binders may also contain high levels of calcium and magnesium that may reach undesirable levels of these minerals in the feed.

Most studies on the mineral requirements of fish have been conducted using semipurified diets. Dietary requirements are determined by feeding graded levels of the element being studied and measuring the physiological response of the test animal. Minerals required by fish are calcium, magnesium, phosphorus, and micro elements such as copper, iodine, iron, manganese, selenium and zinc. In general, the dietary requirements for minerals are poorly understood because of the difficulty in devising mineral-deficient diets and the need to deplete tissue mineral stores.

Mineral Requirements of Fish

Although inorganic elements are required for the normal life processes of fish, knowledge of their mineral nutrition is still one of the least known areas of fish nutrition. Unlike other nutrients, significant amounts of minerals can be absorbed from their external environment thus it is

difficult to control the dietary intake of the mineral being studied. Calcium, sodium, potassium, iron, zinc, copper and selenium are generally derived by fish from the rearing water. The exchange of ions from the surrounding water across the gills and skin of fish complicates the measurements of mineral requirements. Interaction between minerals further complicates the assessment of dietary requirements. So far, there is very limited information on the dietary requirements for minerals by fish.

A summary of the known dietary mineral requirements of various fish species is presented in Table 2.15 and their deficiency symptoms are summarized in Table 2.16.

Table 2.15 Summary of the mineral requirements of various fish and shrimp species

Mineral	Channel catfish	Common carp	Japanese eel	Nile tilapia	Rainbow trout	Kuruma shrimp	Tiger Shrimp
Ca			0.27%			1.0-2.0%	1.0%
P	0.33-0.45%	0.6 - 0.7 %	0.58 %	0.8-1.0 %	0.7-0.8 %	1.0-2.0%	1.0%
Mg	0.04 %	0.04-0.05%	0.04 %	0.05-0.07 %		0.30%	
Cu	3µg/g			3 - 4µg/g	3µg/g		
Fe		150µg/g	170µg/g				
Mn		13µg/g	13µg/g	12µg/g			
Zn	20µg/g	15-30µg/g		10µg/g	15-30µg/g		

Source: Watanabe et al. 1988; Bautista and Baticados 1988

Table 2.16 Mineral deficiency symptoms in fish and shrimp

Minerals	Deficiency symptoms
Ca	Poor growth in channel catfish; Soft-shell syndrome in crustaceans
P	Poor growth & skeletal abnormality in common carp & rainbow trout; Low feed efficiency and high lipid content in common carp; Low ash in whole body and vertebrate in common carp, rainbow trout and channel catfish
Mg	Poor growth and high mortality in common carp and rainbow trout; Sluggishness and convulsion in common carp, rainbow trout and channel catfish; High Ca content in bone of carp and rainbow trout; Anorexia in channel catfish; Skeletal abnormalities and renal calcinosis in rainbow trout
Cu	Poor growth in common carp and rainbow trout; Dwarfism in Japanese eel
Co	Poor growth in common carp
Fe	Anemia in common carp
I	Dwarfism in Japanese eel
Al	Dwarfism in Japanese eel
Zn	Poor growth, high mortality, erosion of fins and skin, low Zn and Mn content in bone of common carp and rainbow trout; Dwarfism in rainbow trout and Japanese eel; Cataract in rainbow trout; Low Ca, Mg and P and high mortality in common carp; Low Zn and Mn in common carp and rainbow trout

Source: Watanabe et al. 1988

Guide Questions

1. What are minerals?
2. What are the 3 groups of minerals? Give examples of each group.
3. What are the general functions of minerals?
4. What mineral is needed for normal formation of bones, scales and teeth in fish?
5. What element is a component of thyroid hormones? of metalloenzymes?
6. What mineral is a component of vitamin B_{12}?
7. What mineral protects biological membranes against lipid oxidation?
8. What minerals are important to prevent soft-shelling in shrimps?
9. Why is the need for a dietary source of phosphorus more critical than for calcium?
10. What mineral is a component of glutathione peroxidase?
11. What minerals are needed for blood formation in fish? hemocyanin formation in mollusks and crustaceans?
12. What is the consequence of iron deficiency in fish?
13. Why is mineral nutrition of fish one of the least known areas of fish nutrition?

Summary

Proteins and amino acids

Amino acids are the basic unit of proteins and have a general structure with an amino group (-NH_2) and a carboxyl group (-COOH) bonded to the alpha-carbon atom. The nature of the side chain, referred to as the R groups, are the basic differences among amino acids. The amino acids are divided into two groups: the dispensable or non-essential amino acids and the indispensable or essential amino acids. Of the 20 naturally occurring amino acids, ten are essential because they cannot be made by the fish or cannot be made in amounts that satisfy the requirement. These are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The non-essential amino acids can be made by the animal from carbon and nitrogen precursor compounds. Cysteine (which can replace part of methionine) and tyrosine (which can replace part of phenylalanine), glycine, glutamic acid, glutamine, aspartic acid, asparagine, alanine, proline, and serine are non-essential amino acids.

Proteins are polymers of amino acids joined by peptide bonds. The structure of proteins is complex, with no obvious regular structure. To facilitate structure determination, it is customary to define four levels of organization. Primary structure is the order in which the amino acids are covalently linked together. Secondary structure is the hydrogen bonded arrangement of the polypeptide chain. Tertiary structure includes the three dimensional arrangement of all the atoms in the protein.

Quaternary structure is the arrangement of subunits in multi-subunit proteins. Protein is digested and broken down to its component amino acids, which are used by the organs and tissues to make new proteins (growth) or replace existing proteins (maintenance). Excess can be converted to energy to fuel the activity of the fish.

Since protein quality is determined largely by its amino acid composition and digestibility, information on the protein requirement of fish is limited without data on the essential amino acid requirement. The nutritive value of a dietary protein is dependent on the extent to which its essential amino acid composition fulfills the requirement of the organism. 'A deficiency in any essential amino acid will result in poor growth. The limiting amino acid of a protein is the essential amino acid that is present in lowest amount relative to the requirement. Proteins from animal sources are more digestible than protein from plant sources. Proteins from plant sources are deficient in some essential amino acids; such as, methionine in soybean meal, and lysine in corn meal. Proper combination of proteins from both animal and plant sources provide diets with balanced amino acid profiles.

Lipids and fatty acids

Lipids are compounds that are insoluble in water and soluble in nonpolar organic solvent. Lipids are components of cellular membranes, provide essential fatty acids and are good sources of energy. Triglycerides and waxes are the storage forms of fatty acids, while phospholipids are important components of biological membranes such as sphingolipids. Steroids are precursors of sex hormones in fish and are important in the reproductive processes.

Fatty acids are aliphatic carboxylic acids with a –COOH group. The important fatty acids have between 2 and 24 carbon atoms and may be completely saturated or have one (mono-unsaturated), two or more (polyunsaturated, PUFA) or more (highly unsaturated, HUFA) double bonds in the carbon chain. Essential fatty acids cannot be synthesized in the body and must be provided in the diet. The essential fatty acids for fish are either the polyunsaturated or the highly unsaturated fatty acids.

The composition of fatty acid in fish is affected by a number of environmental factors: salinity, temperature, and diet. Diet is the largest single factor that affects the fatty acid composition. The fatty acid requirement varies among fish species. Marine fishes need more *n*-3 PUFAs. Linolenic, linoleic, arachidonic, eicosapentaenoic and docosahexaenoic acids are essential fatty acids for fish. Lipids such as cholesterol and lecithin are also needed by shrimps. Fish lipids have more *n*-3 fatty acids while plant lipids contain more *n*-6 fatty acids. Some fish require *n*-3 fatty acids; some need both *n*-3 and *n*-6 fatty acids, while others require *n*-6 fatty acids. The dietary lipid requirement of fish can be derived from their fatty acid profiles.

Carbohydrates

Carbohydrates are either aldehydes or ketones with two or more hydroxyl groups. A carbohydrate like glucose is called an aldose because of the presence of an aldehyde group, while fructose is called a ketose because

of its ketone group. The simplest form of carbohydrates are monosaccharides, compounds that contain a single carbonyl group and two or more hydroxyl groups. Monosaccharides can undergo various reactions which give rise to oligosaccharides and polysaccharides. Three important oligosaccharides are the disaccharides sucrose, lactose and maltose. Sucrose is a common table sugar, lactose occurs in milk, and maltose is obtained from the hydrolysis of starch, an important polysaccharide. Polysaccharides are formed by the combination of hexoses or other monosaccharides. Starch which is found in plants while glycogen which occurs in animals are energy storage polymers of glucose. Cellulose and chitin are polysaccharides based on single monomer units of glucose and N-acetylglucosamine, respectively.

Carbohydrates are a cheap source of energy and, like lipids, can spare protein as an energy source. The ability of fish to utilize dietary carbohydrates for energy varies. Carnivorous species have more limited ability than omnivorous and herbivorous species. Diets for carnivorous fishes contain lower carbohydrate levels than those of herbivores. Since carbohydrate is the least expensive energy source, it is advantageous to use as much carbohydrate as the fish can utilize.

Energy

Energy is defined as the capacity to do work. It may exist in different forms and do different kinds of work. Energy is obtained from major food nutrients: carbohydrates, lipids, and proteins and undergo several chemical processes such as catabolism and oxidation within the animal body. Adenosine triphosphate (ATP) is the principal driving force in the energy-requiring biochemical processes of life. An energy budget is the amount of energy in ingested food that is utilized for each major processes such as growth and reproduction. A generalized energy budget for carnivores and herbivores has been developed by Brett and Groves (1979).

Fish like other animals, eat primarily to satisfy their energy requirements. When fed low energy feed, fish are able to compensate by eating more of the feed within certain limits. It is essential that fish are given a ration with sufficient energy to meet their energy needs.

From the point of view of an aquaculturist, the quantity and cost of energy which is available for growth of culture species is most important.

Vitamins

Vitamins are complex compounds whose presence in the diet is necessary for normal growth and maintenance of health and whose insufficiency in the diet results in the development of specific pathologic conditions. Vitamins are essential for the regulation of cell metabolism and for transformation of energy. They may act as co-enzymes or part of an enzyme system. They occur in trace amounts in most natural food and cannot be synthesized by most fishes hence, must be provided in the diet.

Vitamins are classified into water-soluble and lipid-soluble. Water-soluble vitamins are hydrophilic while lipid-soluble vitamins are hydrophobic. The water-soluble vitamins are thiamin (vitamin B_1), riboflavin (B_2), pyridoxine (B_5), pantothenic acid (B_6), cobalamin (B_{12}),

ascorbic acid (vitamin C), niacin, folic acid, inositol, choline, and biotin. Lipid-soluble vitamins are retinol (vitamin A), cholecalciferol or ergocalciferol (D), α-tocopherol (E) and menadione (K). Water-soluble vitamins may be excreted in the urine and rarely build up to toxic levels. In contrast, lipid-soluble vitamins may be excreted in the feces or accumulate in the tissues. A derivative of vitamin A plays a crucial role in vision. Vitamin D controls calcium and phosphorus metabolism, affecting the structural integrity of bone. Vitamin E is an antioxidant, and the presence of vitamin K is required in the blood clotting process. Toxicity may occur from an overdose of vitamins A and D. Most vitamin requirements are known for juveniles of coldwater, but not for warm water fishes.

Minerals

Minerals are required in the formation of bone, scales, and teeth, in osmoregulation, in the regulation of nerve function and in maintenance of blood pH. Some minerals are constituents of soft tissues, enzymes, vitamins, and hormones. The macro minerals: calcium, phosphorus, magnesium, sodium, potassium and chlorine, are required in the diet in large amounts. The micro minerals: chromium, cobalt, copper, iodine, iron, manganese, molybdemum, selenium and zinc, are required in much smaller amounts. The trace minerals have no known function in fishes and are mostly toxic. In general, the bioavailability of minerals has been found to correlate with their solubility in water. Water-soluble inorganic salts or bioavailable organic salts are preferred for use in mineral premixes.

Mineral nutrition of fish is one of the least known areas of fish nutrition. Unlike other nutrients, fish can derive significant amounts of minerals from their external environment, thus it is difficult to control their intake of the mineral being studied. Interaction between minerals further complicates the studies on dietary mineral requirements of fish. However, although fishes can derive many minerals from the water, mineral supplements in the diet improve growth, survival and health.

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CHAPTER

Feeding Habits and Digestive Physiology of Fishes

3

ILDA G. BORLONGAN, RELICARDO M. COLOSO, and NELSON V. GOLEZ

Introduction

This chapter provides basic information on the feeding habits and behavior, and physiology of fishes and crustaceans. The mechanisms that control the movement and digestion of food, methods of assessing digestibility of feed, factors affecting digestion and absorption of food nutrients, and feeding processes in fish are discussed. An understanding of the feeding habits, feeding mechanisms, and the digestion and absorption processes can help fish farmers and nutritionists maximize the use of feed. The rate at which fish digest their food is of primary importance in determining feeding rates, frequency, and ration size. Knowledge of the digestive physiology of fish is also necessary for an effective feed formulation and in choosing a proper feeding regime.

This chapter aims to teach the reader: the feeding habits and behavior of fishes and crustaceans; the structural adaptation in the anatomy of the digestive tract; the various organs of the digestive systems of fishes and crustaceans and their functions; nutrient digestion and absorption by fishes and the fate of digested and undigested food; the factors that affect the rate of digestion and absorption; and the feeding process in fish.

Feeding Habits and Behavior

The feeding habits and behavior of fishes refer to the process of the search for and ingestion of food. This also includes the manner and the stimuli for feeding.

Fishes can be classified according to their food and diet, which refer to the materials they habitually eat as:

Herbivores – those that feed exclusively on plant materials
Carnivores – those that feed exclusively on animal matter
Omnivores – those that derive their nutrients from both plants and animals
Planktivores – those that feed on plankton, the microscopic plant and animal life in water including bacteria
Detritivores – those that feed on decaying matter

Food availability is a key factor in determining what the fish will eat. Most fishes are highly adaptable in their feeding habits and utilize the most readily available foods. Table 3.1 summarizes the natural food and feeding habits of commonly cultured species of fish and shrimp.

Table 3.1. Feeding habits and natural food of some juvenile fish and shrimp

Fish	Feeding Habit	Natural Food
Milkfish	microphagous planktivore	Microplankton (lab-lab), benthic algae (filamentous green algae)
Seabass	carnivore	Fish of same species or other fish
Grouper	carnivore	Fish of same species or other fish
Snapper	omnivore	Fish, crabs, stomatopods, mollusks, crustaceans and other bottom dwellers
Rainbow trout	carnivore	Fish
Channel catfish	omnivore	Insects, snails, worms, plants and general organic debris in muddy bottom
Common carp	omnivore	Plants and other organic debris in muddy bottoms
Siganid	herbivore	Macroalgae
Mullet	omnivore	Small algal cells and other organic debris
Shrimp	omnivore	Small crabs, shrimps, mollusks, fish, polychaetes, algal matter, and debris

Another classification of feeding behavior of fishes according to the manner of feeding are:

1. **Predators** are fishes that feed on macroscopic animals. They may either be constantly on the move, hunting and pursuing their prey, or lie-on-wait to catch prey that stray into their territory. Some predators feed upon small fishes or insects found at or near the water surface. Predators mainly use vision to hunt for prey, although sharks, eels, and other predatory fishes that feed at night may also rely on smell, taste, and lateral line sense organs to locate their prey.
2. **Grazers** feed on bottom organisms or planktons that are selectively consumed. Some grazers feed on algae or nibble on coral reefs to eat polyps. The actual taking of food is by bites while browsing continuously.
3. **Strainers** are those which filter organisms, mainly diatoms and crustaceans from water. These fishes swim through rich plankton beds, filter the water, and swallow the soup-like concentrate. Strainers normally have numerous, fine, and elongated gill rakers.
4. **Suckers** are those which suck in mud or food-containing material to obtain their food. Sometimes, food items are separated from the sediments before being swallowed, although in some catfishes, food is ingested together with flocculent bottom deposits.
5. **Parasites** such as lampreys and hagfishes, are very different from other finfishes in their behavior. They obtain nutrients by sucking body fluids of host fish.

Anatomy and Physiology of the Digestive System

In nature, there is a wide variety of food available on which fish and crustaceans depend. Fish adapt to their food differences by anatomic as well as behavioral means. Thus, there are many differences in the anatomy and physiology of digestion in fish. There is a strong correlation between the anatomical structure of the digestive tract and the feeding habits of the fish. Herbivorous fish that depend on fibrous foods such as phytoplankton and macrophytes differ anatomically and behaviorally from carnivorous fish that consume meat and other more digestible feeds. Carnivorous fishes have a relatively simple and short gut, with thick mucosa for absorption. Herbivorous fishes have an accessory masticatory apparatus or other physiological adaptation to help in breaking down plant cell walls before the digestion process starts, and a long, thin gut to increase gut retention time and enhance digestion and absorption.

A. Fishes

The digestive system of fish includes the mouth, esophagus, stomach, pylorus, intestine, liver, and pancreas. An illustration of the digestive tract of four commonly cultured fishes that differ in their food preferences is shown in Figure 3.1. The digestive tract is tubular in structure. The whole digestive tract is often referred to as the gut and in fish, the gut usually has four divisions: these are the headgut, foregut, midgut, and hindgut. The **headgut,** which is the most anterior part includes the mouth (oral or buccal cavity)

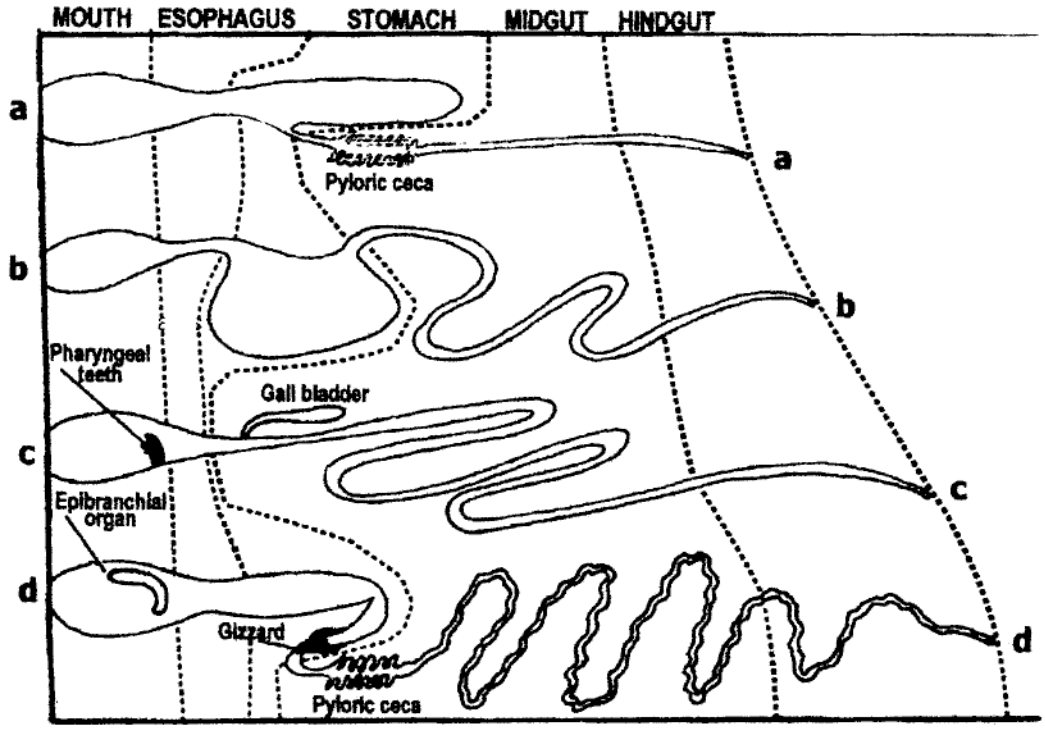


Figure 3.1
Diagrammatic representation of the digestive systems of four fishes, arranged in order of increasing gut length. a) Rainbow trout (carnivore); b) Catfish (omnivore, eating more of animal source); c) Carp (omnivore, eating more of plant source); d) Milkfish (omnivore, microphagus planktivore).
Source: Smith 1989

and gills (branchial or pharyngeal cavity). The **foregut** begins at the posterior edge of the gills and includes the esophagus and stomach. The **midgut** consists of the intestines and pyloric caeca, if present. The midgut is the longest portion of the gut and may be coiled into complicated loops. The **hindgut** includes the enlarged portion of the intestines and the rectum or anus. Each portion of the gut has a very variable structure for adaptation. The liver and pancreas are organs involved in digestion but are found outside the tubular structure.

1. Headgut

Mouth and various ingestion mechanisms

The first phase of digestion is the ingestion of food into the mouth. The mouth has a variety of adaptations for capturing, handling, and sorting of food before entry into the stomach. Figure 3.2A shows the different shapes of mouth in response to their food adaptations. Fish have teeth that vary in type, number, and arrangement. They serve to catch and hold the prey. The arrangement and structure of the teeth are related to the kind of food that the fish normally eat. There is a strong correlation among kind of teeth, feeding habits, and food eaten.

Generally, the more active feeders have strong jaws with sharp teeth to bite and shred the food. Some major kinds of jaw-teeth are the following: cardiform, villiform, canine, incisor and molariform (Figure 3.2B). Those feeding on mollusks and crustaceans have short heavy teeth, strong enough to crush the mollusk shell. Zooplankton feeders and most planktivores have practically no teeth. The shredding of food is most often done in the throat or pharynx. Here, another set of specialized teeth may be found. Again, the structure, size and shape of the pharyngeal teeth are also variable. Plankton feeders have fine rows of pharyngeal teeth, while mollusk eaters have large but flat crowned teeth, which is better adapted to crushing their food.

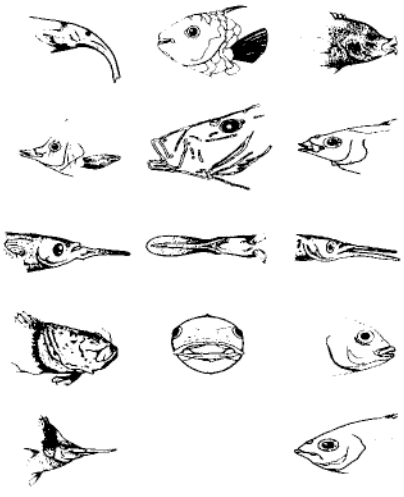


Figure 3.2A
Variations of the mouth structure in fishes.

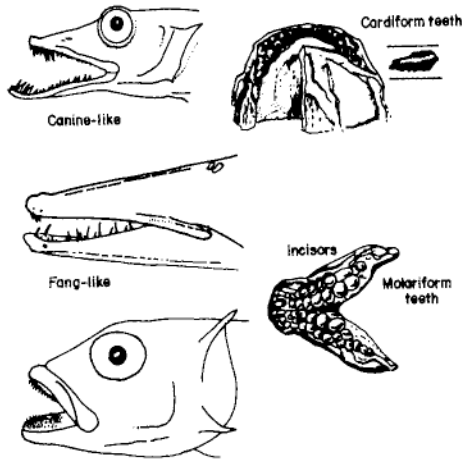


Figure 3.2B
Some major kinds of jaw teeth.

2. Foregut

Esophagus

Most fish have short, wide esophagus that serves as a transitional area between the striated muscles of the mouth and the smooth muscles of the gut. Mucus producing cells are present in the esophagus. In general, the esophagus serves only as a passage way, however, enzyme activity has been detected in the esophagus of some fishes indicating a more active role of the esophagus in the digestion process in these species.

Other fishes with long, slim body shapes like the seawater-adapted eels have a long esophagus. Osmoregulation may take place in the esophagus if mucus is present. The mucus is much thicker anteriorly than posteriorly and is electrically charged. Some reports have suggested that both passive and active transport of ions into the blood may also take place in the esophagus without addition of water such as the dilution of ingested seawater in freshwater eels.

Stomach

The stomachs of fishes vary greatly in their anatomical structure due to adaptations to specific foods. There are four general configurations or shapes of fish stomachs. These include:

a. a straight stomach with an enlarged portion
b. a U- or J-shaped stomach
c. a stomach shaped like a Y on its side where the stem faces the caudal portion
d. stomachless fish, such as in carps and other cyprinids

The stomach has a configuration or shape which is convenient for containing food in the shape in which it is ingested. Food is temporarily stored in the stomach while the rest is gradually being processed through the other portion of the digestive tract. The size or capacity of the stomach in relation to the body weight varies between species and is usually related to the interval between feedings and to the size of food particles. Generally, fish that eat relatively small, soft particles have small stomach whereas fish that eat large food particles, e.g. whole fish, or eat at infrequent intervals have larger stomach.

The cecum of the Y-shaped stomach is adapted to stretch posteriorly to accommodate large food particles or prey. In contrast, the absence of a stomach has been suggested to benefit fish adapted to freshwater (low chloride concentration) where stomach acids impose added osmoregulatory pressure. This is to avoid acidifying large amounts of alkaline food, as in omnivorous fish that eat plant sources, corals, shells, and others.

In milkfish, the stomach can be divided into cardiac and pyloric portion. The cardiac portion is often more enlarged while the pyloric stomach is highly muscular (Figure 3.3). The pyloric stomach intensely grind the food particles resulting in chyme (a paste like mass).

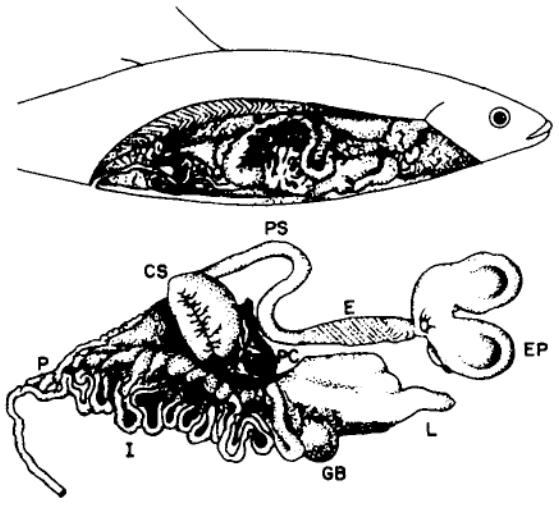


Figure 3.3
Regions of the digestive tract of milkfish *Chanos chanos*. EP-epibranchial organ; E-esophagus; CS-cardiac stomach; PS-pyloric stomach; PC-pyloric caeca; I-intestine; L-liver; GB-gall bladder; P-pancreas.

The capacity or volume of the stomach in relation to the body weight varies between species and reflects the size of the meal that can be taken voluntarily. It can vary from as small as 10% of body weight and as large as 50% of body weight in a single feeding.

3. Midgut

The digestion process actively continues into the intestines after preliminary digestion in the stomach. All fishes have intestines. The length of the intestines varies from as low as 1/5 to as high as 20 times the body length. In some fish, the intestines may be short and straight while it can be long, folded, and looped in others. In general, herbivores have longer intestines than carnivores. Although there are some cases of overlaps, some general statements can be made on gut length in relation to feeding habits of fish (Table 3.2). Within the same fish species, the relative gut length can change as feeding habit of the fish changes. Gut length is directly more related to the amount of indigestible material in

Table 3.2 General observations on feeding habits and relative gut lengths (ratio of intestine to body length) in fish

Feeding habits	Relative gut length
Carnivores	0.2 - 2.5
Omnivores	0.6 - 8.0
Herbivores	0.8 - 20.0

Feeding habit and relative gut length in some fishes

Species	Food or feeding habit	Relative gut length
Gobio gobio	Invertebrates	0.80
Chelethiops elongatus	Zooplanktons	0.75
Elopichthys bambusa	Carnivores	0.63
Borilius meorei	Carnivores	0.65- 0.80
Catla catla	Plants, insect larvae	4.7
Garra dembensis	Algae, invertebrates	4.5
Cirrhina mrighala	Algae, detritus	8.0
Gadus morhua	Carnivores	1.05 - 1.50
Labeo calbasu	Herbivores	3.75 - 10.0
Labeo horie	Detritivores	15.0 - 21.0
L. niloticus	Algae, detritus	16.9
L. variegatus	Algae, detritus	16.9
L. lineatus	Algae, detritus	16.1
Ctenopharyngodon idella	Plants	2.5
Doras grypus	Plants	2.8
Hypophthalmichthys molotrix	Herbivores	4.6 - 7.1
Micropterus salmoides	Carnivores	0.7 - 0.9
Salmo salar	Carnivores	0.73 - 0.80

the food rather than whether the food source is of plant or animal origin. Fish that ingest large amounts of detritus have gut lengths similar to those of herbivores.

Some fishes possess pyloric caeca. There are conflicting reports on the functions of the **pyloric caeca** in fish. Histologically, the pyloric caeca resembles the intestines. Most studies indicate that it serves as an extension of the intestines thus increasing the effective surface area for digestion and absorption. Other studies show that it acts as an accessory food reservoir, for temporary storage, possibly a devise for saving space. It is clear, however, that rainbow trout caeca takes up amino acids and sugars across the apical membrane of the epithelial cells. Electron microscopy has shown that both intestinal and caecal cells are involved in lipid absorption, with caecal cells being more active.

The structure of the absorptive cell of the intestines reflects its specialized function in digestion (Figure 3.4). The cell contains many mitochondria, which provide energy for metabolic processes; endoplasmic reticulum, where proteins (including digestive enzymes) are assembled, and golgi bodies where carbohydrate side chains are attached to proteins. Tight junctions and desmosomes bind the absorptive cells into a single sheet on the surface of the mucosa. The nucleus lies deep in the cell. The most striking feature of the cell is the presence of a brush border, a prominent structure on the surface facing the lumen of the intestine. The brush border is composed of minute projections called microvilli. On the membrane of the microvillus are found several kinds of digestive enzymes and transport proteins.

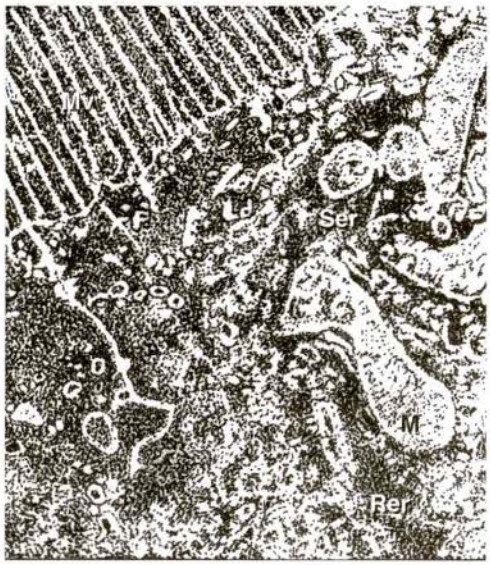


Figure 3.4
Schematic representation of a portion of an absorptive cell from the intestine, Mv-microvilli, M-mitochondrion, Ser- smooth endoplasmic reticulum, Rer-rough endoplasmic reticulum.

4. Hindgut

The hindgut is an extension of the midgut. Digestion has been shown to continue in the hindgut although with a gradually diminishing digestive or absorptive function, an increased secretion of mucus and a pH near neutral. Histological sections show a sudden change from columnar secretory and absorptive to a squamous epithelium that produces mucus.

5. Liver

The liver is an important metabolic organ. It aids in digestion by secreting bile, a greenish fluid with strong emulsifying properties. The bile is stored in the gall bladder and is composed of a mixture of bile salts, taurocholate, glycocholate. Bile acids are derived from metabolism of cholesterol, and degradation products of hemoglobin, bilirubin and biliverdin. Bile serves to emulsify lipids in the gut and may contain other waste products. The bile duct opens into the anterior intestines or into the pyloric caeca if present. Fish can reabsorb bile in the hindgut even though most lipid uptake occurs in the anterior intestine. The liver is also a storage organ for lipids and glycogen or stored starch. In some fishes, large amounts of lipid is stored in the liver to help maintain buoyancy. In other fishes, glycogen is the major stored nutrient.

6. Pancreas

The pancreas is involved in many important functions in digestion. Pancreatic morphology is variable in many bony fishes. In most fishes, unlike in land animals, there is no discrete pancreas. The pancreas is diffused, scattered, and embedded in the mesenteries, in the liver, and clustered around the bile duct, or in combinations of sites. In a diffused pancreas, several small ducts open into the intestine and the pyloric caeca. In other cases where the pancreas is found inside the liver, the pancreas delivers its secretions directly into the gallbladder. The pancreas produces insulin and digestive secretions, principally proteases and bicarbonates. Insulin stimulates uptake of amino acids from the intestine and may stimulate growth. In the northern pike, insulin decreases blood amino acids with uptake into skeletal muscles, while in cod, insulin decreases blood glucose.

B. Crustaceans

The crustacean digestive tract is mostly straight and consists of the foregut, the midgut, and the hindgut (Figure 3.5). The foregut and the hindgut are lined with chitin. The midgut arises from the endoderm and the innermost layer adjacent to the lumen is the mucosa lined with epithelial cells which is composed of simple columnar cells. These are supported by a basement membrane, a layer of circular muscles and then by a layer of longitudinal muscles. These muscle layers are surrounded by a layer of fibrous connective tissue or serosa and contain a network of arterial hemolymph vessels.

In the anterior portion of the midgut epithelium are simple columnar cells with medial nuclei. The surface adjacent to the lumen also contains microvilli. Many mitochondria, secretory granules and golgi bodies are present in epithelial cells. In contrast, the posterior portion of the midgut epithelium contains many squamous cells which secrete mucus. The cell surface facing the lumen also contains microvilli.

In almost all crustaceans, the midgut is made up of one or more pairs of glandular appendages called the **hepatopancreas**. The hepatopancreas contains a wide range of digestive enzymes which hydrolyze food nutrients and aid in breaking down by the gastric mill. The hepatopancreas is composed of simple blind-ending tubules or diverticula which open into secondary secretion ducts. These ducts, in turn, open into the primary secretion or collecting duct, through which the secretion is poured into the midgut, behind the stomach. The lumen of the hepatopancreatic tubule contains granular material and cells lining the mucosa are covered with a microvillus brush border. The tubules are lined with an epithelium in which different cell types are present. The apex of the tubule contain undifferentiated embryonic or **E-cells**. Farther away from the apex, cells begin to differentiate into developing absorptive, storage or **R-cells**. In the proximal region of the

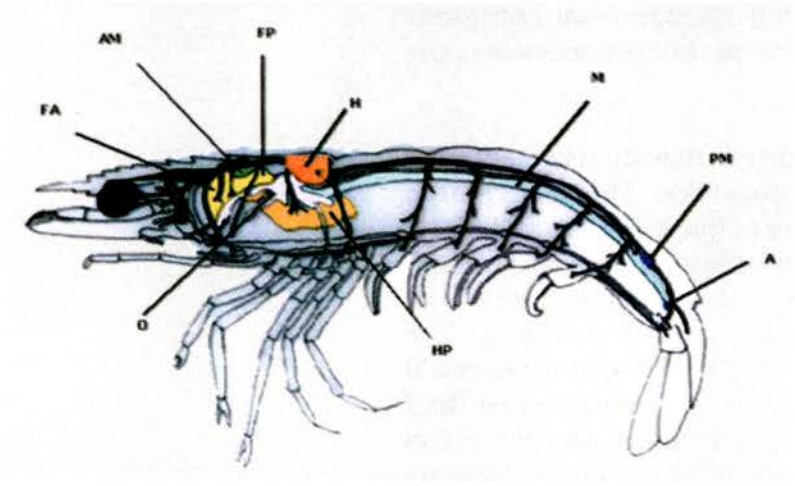


Figure 3.5
Diagram of the digestive system of shrimp with gills and musculature removed to show major organ systems. FA-foregut (anterior chamber); AM-anterior midgut cecum; FP- Foregut (posterior chamber); H- heart; O- ovary; HP- hepatopancreas; M-midgut; PM-posterior midgut cecum; A-anus

hepatopancreatic tubule, in addition to the E and R cell types, are found large distinctive secretory or **B-cells** (Figure 3.6).

The contents of the gut of crustaceans are moved forward by peristaltic or contractile movements of the longitudinal muscles. Peristalsis occurs in the esophagus, midgut, and hindgut. Absorption occurs in the anterior part of the midgut and digestion in the posterior part. Therefore, it is necessary that strong anti-peristaltic movements also take place in the midgut from the posterior end to the anterior portion. In decapods, if mandibles armed with sharp teeth for chewing food are absent, there is a gastric mill with movable teeth fixed to platelike ossicles that serve as substitute for grinding the food. The gastric mill is an adaptation to a sedentary existence. It allows crustaceans to swallow their food first, then chew at leisure while hidden from other animals.

The digestive juice is produced chiefly by the cells of the hepatopancreas and transported to the stomach. In some species, the pH is about 5 during hunger and rises to pH 6.6 on feeding. In others species, the pH is neutral

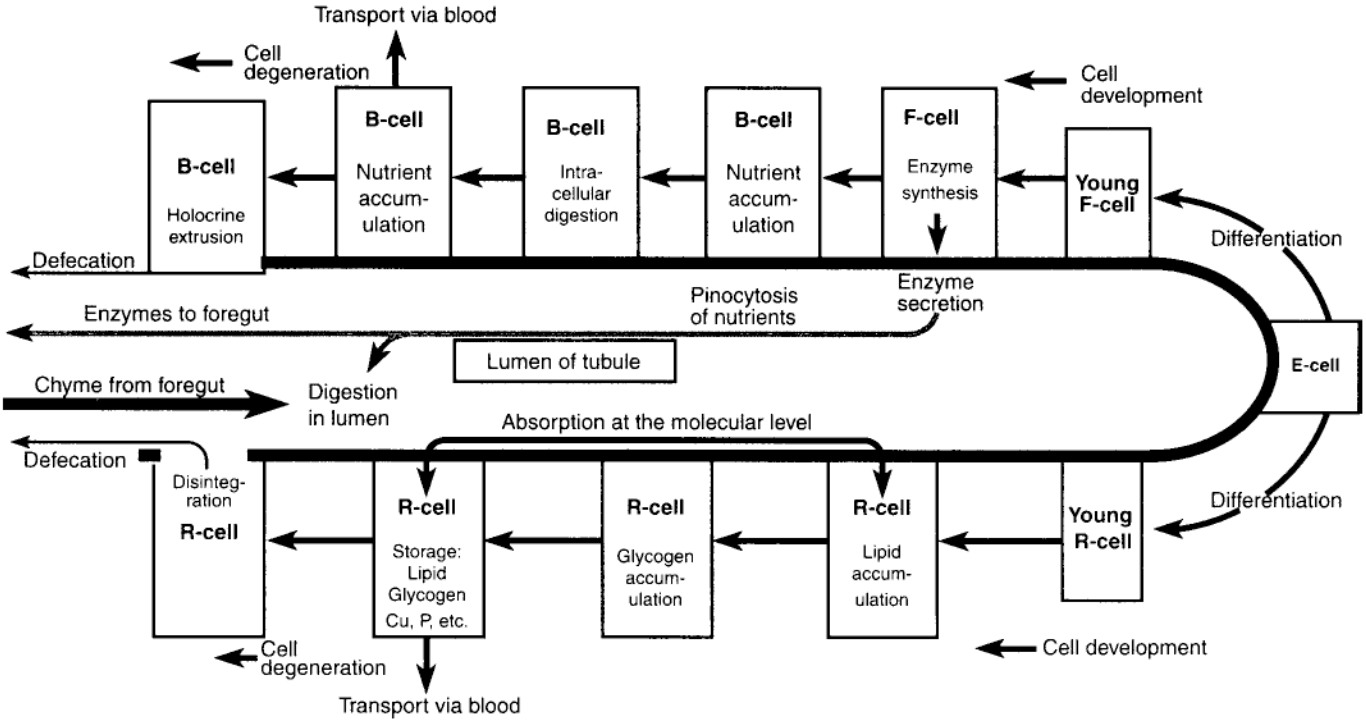


Figure 3.6
Scheme of differentiation and function of the digestive gland tubule. The E-cells differentiate into F-cells (fibrillar) or R-cells (storage). F-cells secrete enzymes and differentiate into B-cells. B-cells absorb small particles by pinocytosis. R-cells absorb nutrients and store lipids, glycogen, copper, phosphorus and other substances.
Source: Dall, 1992

in the hungry animals and increases to pH 7.5 after feeding. The digestive juice contains various digestive enzymes and bile salts that act in emulsifying fats.

Absorption occurs mainly in the hepatopancreas and in the anterior part of the midgut. The R-cells of the hepatopancreas have the capacity to absorb nutrients.

Digestion and Absorption

Digestion is a process whereby ingested food nutrients such as proteins, lipids and complex carbohydrates are broken down into units that are small enough to be absorbed across the gut wall. The process is accomplished through the action of digestive enzymes. The ability of fish to digest feed depends on the secretion of adequate quantities of the appropriate types of enzymes. Many of the enzymes are stored in an inactive or proenzyme form. Once secreted into a favorable environment for digestion, usually influenced by pH, these inactive enzymes are converted to the active form ready to perform their specific digestive function. When various nutrients have been adequately digested, they are then absorbed primarily in the midgut.

There are several mechanisms of nutrient absorption in fishes-**simple diffusion, active transport, and pinocytosis.** In simple diffusion, solutes pass through the membrane from an environment of high to low solute concentration without using energy. Active transport differs from simple diffusion as it requires a continuous supply of energy and transports solutes only in one direction from low to high solute concentration. A carrier system that utilizes Na^+ and ATPase activity is needed in the active transport of glucose and some amino acids. Pinocytosis (cell drinking) is the process by which materials are taken into the cell through an invagination and subsequent dissolution of a part of the cell membrane. This process enables the cell to absorb some proteins and lipids in intact form. Absorptive cells of the midgut are capable of undergoing pinocytosis.

A. Digestion and absorption of proteins

Proteins are hydrolyzed into amino acids or polypeptide chains of a few amino acids with the help of enzymes known as **proteases**. The process can occur in acidic or basic pH.

1. Pepsin

Pepsin and hydrochloric acid play important roles in protein digestion in fish stomachs. The pH optimum for pepsin is about pH 2.0. Pepsin is synthesized in the gastric gland in the inactive form called **pepsinogen**. Hydrochloric acid converts the inactive pepsinogen to the active pepsin. Pepsin is an endopeptidase and cleaves or cuts most peptide bonds in the interior region particularly where linkages are formed by aromatic amino acids (phenylalanine, tyrosine, and tryptophan), and acidic amino acids (aspartic and glutamic acid).

2. Trypsin and Chymotrypsin

Trypsin and chymotrypsin are involved in the alkaline digestion of proteins. These enzymes are synthesized, stored and secreted in an inactive form by the pancreas and transported to the midgut and the pyloric caeca. **Trypsinogen** is activated in the intestine by enterokinase, an enzyme secreted from the intestinal mucosa. When activated, trypsinogen becomes **trypsin.** In turn, trypsin, activates **chymotrypsinogen** to **chymotrypsin**. Both trypsin and chymotrypsin are endopeptidases but cleave different linkages in a protein. Trypsin cleaves peptide linkages which are formed by basic amino acids, arginine, lysine, and histidine. Chymotrypsin cleaves linkages with aromatic amino acids, phenylalanine, tyrosine, and tryptophan.

In milkfish, both tryptic and chymotryptic activities are higher in the posterior intestines than in the anterior part. The pyloric caeca also contains high activities of both enzymes. Milkfish intestinal protease activity appear to have two pH optima, one at pH 7.0 to 7.6 and another at pH 9.5 to 10.0. It also has a temperature optimum of about 50° to 60°C.

PEPSINOGEN	TRYPSINOGEN	CHYMOTRYPSINOGEN
⇓ HCl	⇓ enterokinase	⇓ trypsin
PEPSIN	TRYPSIN	CHYMOTRYPSIN

Conversion of pepsin, trypsin, and chymotrypsin from their inactive forms

3. Carboxypeptidases

Carboxypeptidases are also secreted from the pancreas in the inactive form. These are exopeptidases which cleave the C-terminal amino acid of peptides or proteins. There are two types: carboxypeptidase A and B. **Carboxypeptidase A** is active towards proteins with aromatic C-terminal amino acids (phenylalanine, tyrosine, and tryptophan) while **carboxypeptidase B** acts preferentially on those peptides with basic amino acids (lysine and arginine).

4. Aminopeptidases

Aminopeptidases are exopeptidases that act on N-terminal peptide of proteins.

In summary, because of the complexity of proteins, their complete digestion has to proceed in a number of steps (Figure 3.7). The protein molecule is first hydrolyzed into relatively large polypeptide fragments by endopeptidases. These fragments are then hydrolyzed by enzymes acting on the amino and carboxyl bonds and finally dipeptidases reduce the protein to its constituent amino acids.

Proteins can be absorbed as whole proteins, peptides, or free amino acids. Protein macromolecules may be absorbed in the gut epithelium via pinocytosis. Rainbow trout have granule cells in the lamina propria of the intestine just under the mucosa which could be part of this uptake system.

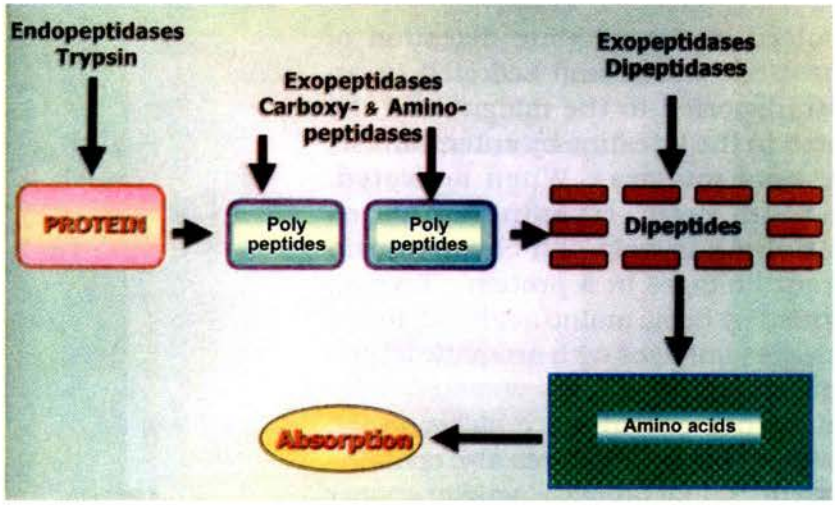


Figure 3.7
Sequence of protein digestion. The protein molecule is first hydrolyzed into polypeptides by endopeptidases. Polypeptides are further hydrolyzed to smaller dipeptides and finally into constituent amino acids.

For completely digested proteins, each amino acid has its own uptake characteristics. In rainbow trout, L-leucine uptake is shown to be specific for the L-isomer, active and sodium-dependent and could be inhibited by other neutral amino acids such as L-valine and L-methionine. Lysine uptake is slowed down by the presence of glucose and hastened by ATP. Some amino acids may be taken up by simple diffusion; others may be transported by carriers in a sodium-independent system which usually occur in the basolateral membrane.

B. Digestion and absorption of carbohydrates

Carbohydrases digest complex carbohydrates and are found in fish intestines. They are very important in herbivorous fish because of the high levels of carbohydrate in plants. The enzyme **amylase** catalyses the digestion of starch. There are two forms: **α-amylase** which acts randomly cleaving the chain from within and **β-amylase** which cuts the chain at every two glucose units. At the branched point, another enzyme, **dextrinase** does the work. The amylase and dextrinase produce maltose. Maltase hydrolyses maltose to give glucose, the final product of starch digestion (Figure 3.8). Most fish have amylase; in herbivorous fish, such as tilapia, it may be present in all parts of the digestive tract, whereas in carnivorous fish it may be found only in the pancreas, pyloric caeca and intestines. In milkfish (an omnivore), extracts from the intestines, pancreas, pyloric ceca and liver showed high levels of amylase activity. In addition, maltose, trehalose, dextrin, starch, and glycogen are rapidly hydrolyzed in the presence of crude extracts from the intestines and pyloric caeca of milkfish.

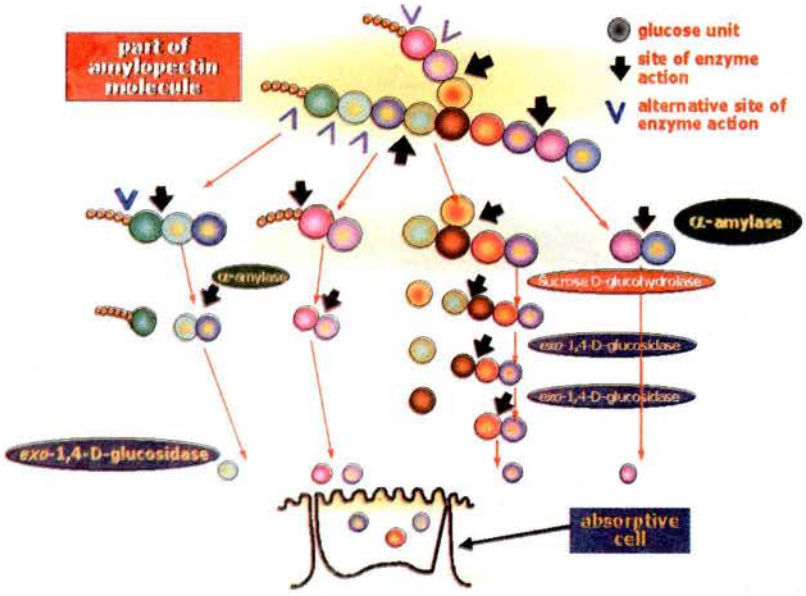


Figure 3.8
Representation of digestion and absorption of carbohydrates.

Other complex carbohydrates that are potential sources of energy but are not readily digested are cellulose, a constituent of plant cell wall, and chitin, a component of crustacean exoskeleton. The complete digestion of cellulose is mediated by two enzymes: **cellulase** and **cellobiase**. **Cellulase** hydrolyses cellulose to

disaccharide cellobiose, which is then acted upon by **cellobiase** producing the final breakdown product, glucose. Very few fish have cellulase activity, most likely the intestinal microflora actively provide the cellulase. Cellulytic bacteria are widely distributed in nature.

Carbohydrate absorption has been tested by measuring the uptake of glucose, the usual final end product of carbohydrate digestion. Glucose transport appears to be lowest in carnivores (e.g. catfish) and highest in herbivores (e.g. carp).

C. Digestion and absorption of lipids

Lipases hydrolyze ester linkages in triglycerides. The end products of lipase activity are glycerol and fatty acids. Lipases are detected in the pancreas, pyloric ceca, intestine, and liver. In milkfish, lipase has also been detected in the esophagus. Milkfish intestinal and pancreatic lipases appear to have two pH optima- pH 6.8 to 8.0; and pH 6.4 to 8.6, respectively. The detection of two well-defined pH optima, one at slightly acidic and the other at alkaline pH for both the intestinal and pancreatic lipases suggests a physiological versatility for lipid digestion in milkfish. **Phospholipases** are enzymes that hydrolyze phospholipids.

Lipids are taken up by the intestinal and cecal epithelium, partly as fatty acids, mostly as mono-glycerides and partly as droplets, and transferred into blood and lymph vessels (Figure 3.9). In the intestinal cell, fatty acids are re-esterified to triglycerides and are transported as very low density lipid particles into lymph vessels. In the blood, triglycerides are transported as chylomicra. The lipid absorption process in fish, although much slower than in mammals, does not differ fundamentally.

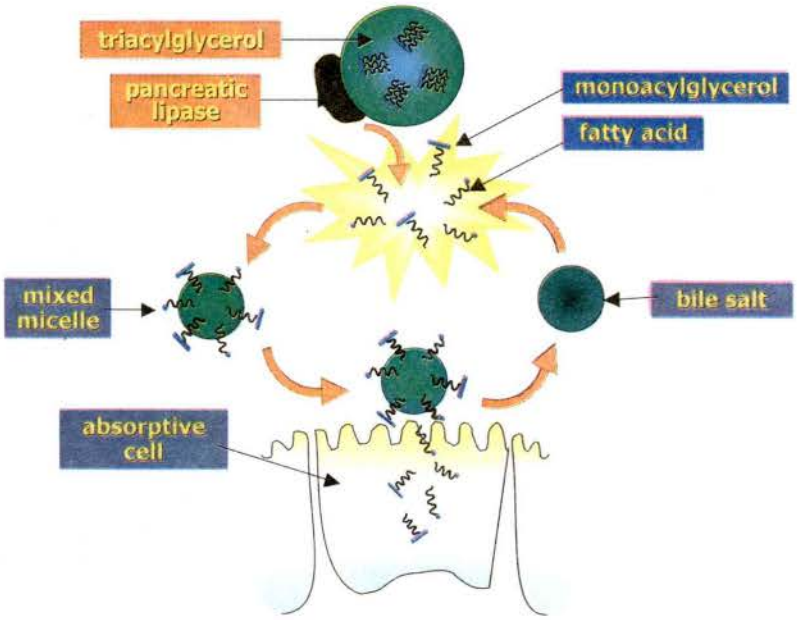


Figure 3.9
Diagrammatic representation of digestion and absorption of lipids.

Measurements and Analysis used in Digestion Studies

A. Measurement of Stomach Contents

Analysis and measurement of stomach contents can give information on the feeding preferences of a particular species as well as its frequency of feeding. Several methods are used to measure stomach contents. however, all of these methods have their limitations.

1. The occurrence of a particular type of food in the fish stomach can be determined qualitatively as in web studies but the quantity of the food present cannot be obtained by this method.

2. Each kind of individual food organism in the stomach can be counted to show the choice of the fish for certain food types from a general population of potential food organisms. The method can not be used in cases where the food is chewed or when the food is not in distinct units as with detritus.
3. The use of stomach flushing methods measures the displacement volume of a food type or of the total food volume and is most applicable if the food is in liquid form.
4. Measurement of the wet weight or the dry weight of food as a percentage of the body weight is another procedure for determining stomach contents. This method may give inaccurate results when heavy items such as mollusk shells, sand or mud are present.
5. Estimate of fullness- half, third, quarters, etc.- of the stomach by appearance is an inexact method but useful under practical conditions.

Gastric emptying rate and food passage rate may be determined in a number of ways. This can be done by killing or not killing the fish to obtain stomach contents. In many cases, a certain number of fish is fed a known amount of food. Samples are obtained periodically by serial slaughter to determine gastric emptying rate. One limitation of this method is that it assumes that all the fish ate exactly the same amount. An alternative procedure is to feed an individual fish a known amount of food, and then after a certain period stomach contents are removed from the fish under anesthesia. The stomach contents may be flushed out with water using a variety of gadgets. Other methods of measuring the amount of food in the stomach or intestines without removing food involve feeding fish a diet with a marker like barium sulfate or iron powder and using an x-ray to view the labelled food at various times. This is useful in fish that are too small for other methods.

B. Measurement of digestibility

The first step in evaluating the potential of a feedstuff for inclusion in a diet is to determine or measure its digestibility. Digestibility can be measured either by *in vivo* or *in vitro* methods.

1. In vivo methods

The most common method of measuring digestibility is to add to the diet a marker, such as chromic oxide or iron powder that is neither digested nor absorbed. The diet is fed to the fish and the concentration of the marker is followed through the digestive tract as the indigestible components are excreted. Feces from the fish is collected by one of the following methods: netting, fecal settling, and stripping or dissection of the hindmost part of the digestive tract (Figure 3.10).

Some technical problems arise in each of these methods. Vigorous stripping may remove parts of the digestive tract. Fecal collection is difficult if the excreta is soft and cannot be collected as a solid. The amount that leaches out from the feces as they stay in the tank bottom or in a collecting device is quite difficult to determine. Therefore, care should be taken that the best fecal collection method for a particular species is used.

Endogenous markers such as cellulose, hydrolysis resistant organic matter, and acid-insoluble ash may also be used.

The apparent protein digestibility (%APD) of a feed is defined as:

$$\textbf{APD (\%)} = 1 - \left[\frac{(\%F_p)}{(\%D_p)} \times \frac{(\%D_i)}{(\%F_i)} \right] \times 100$$

where:

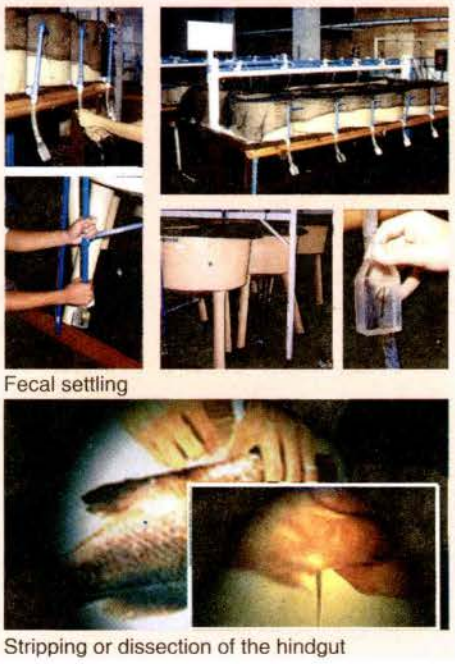
F_p = protein in feces or intestinal content
F_i = indicator in feces or intestinal content
D_p = protein in diet
D_i = indicator in the diet

True protein digestibility (%TPD) of a feed is obtained by correcting the amount of protein in intestinal contents or feces with protein of endogenous origin with the use of the formula:

$$\textbf{TPD (\%)} = 1 - \left[\frac{(\%F_p)}{(\%\ D_p)} \times \frac{(\%D_i)}{(\%F_i)} + \frac{(\%D_i)}{(\%F_{ci})} \times \frac{(\%F_{cp})}{(\%D_p)} \right] \times 100$$

where:

F_{cp} = protein in feces or in intestinal content of control fish (fish fed a non-protein diet)
F_{ci} = indicator in feces or intestinal content of control fish


Fecal settling

Stripping or dissection of the hindgut

Figure 3.10
Experimental set-up for *in-vivo* digestibility measurement.

2. *In vitro* assays

In estimating protein digestibility by *in vitro* assays, test rations are incubated with intestinal extracts of fish at an optimum temperature for a specified length of time (e.g. 24 h). The proteolytic activity in the intestinal extract will digest the protein component of the diet. The *in vitro* digestibility is computed using the formula:

$$\textbf{\textit{In vitro} protein digestibility (\%)} = \frac{(Nr - Ni)}{Nr} \times 100$$

where:

Nr = protein in ration
Ni = indigestible protein

The conditions under which *in vitro* techniques are carried out are highly unphysiologic, thus caution must be exercised in extrapolating the results of such experiments to the *in vivo* condition. At best, the value of digestibility obtained using this technique is only an estimate of true digestibility.

Factors Affecting Digestion and Absorption

In fish, as in all animals, both extrinsic and intrinsic factors can alter the efficiency of digestion and absorption. These are those involving the feed itself, feeding practices, as well as the prevailing condition of the digestive tract.

Feed composition, digestibility, and preparation influence digestion and absorption. Dietary fiber is highly indigestible in non-herbivores due to the absence of the enzymes necessary to breakdown the complex cell walls found in the feed. Roughage are high in fiber, thus their use is limited in practical feed for non-herbivores. Plant proteins are known to vary in their digestibility in fish because of differing amino acid composition and secondary as well as tertiary structures of the proteins. In addition, if a certain type of feed is known to pass quickly through the digestive tract of an animal, the feed will not be adequately digested because of inadequate exposure to the digestive enzymes.

The presence of antinutritional factors in feed such as trypsin inhibitors and tannins will also decrease the efficiency of protein digestion and absorption. Processing the feed such as cooking soybean to destroy the trypsin inhibitor is necessary before the feed can be utilized by fish. Dehulling or removal of the seed coat of some legumes will increase the digestibility of the feed because unbroken seed coats are not easily digested and in some cases contain high levels of tannin. Furthermore, excessive amounts of feed given to the fish decrease digestibility.

The efficiency of digestion and absorption is also affected by the condition of the digestive tract, pancreas, and the liver in fishes or hepatopancreas in crustaceans. Nutritional diseases and bacterial infections of the digestive system can diminish the digestive and absorptive functions in fishes and crustaceans.

Feeding Process in Fish

The cycle of feeding process in fish is shown in Figure 3.11.

Appetite and Satiation

Appetite is the state that initiates arousal and feeding behavior. Knowledge on the factors that trigger appetite and satiation are very important to fish farmers who want to maximize feed consumption, growth, and conversion efficiencies of their fish stock by adjusting feeding schemes. Appetite, which is controlled by the hypothalamus, is stimulated by the gut fullness and or other metabolic changes which affect food consumption. In general, stomach expansion after feeding inhibits appetite and gastric evacuation stimulates appetite. The optimal interval between meals has been estimated to correspond to gastric evacuation.

Several factors both biotic and abiotic tend to affect the appetite of the fish and shrimp.

The biotic factors include:

1. Food availability and food distribution, which may be seasonal in nature;
2. Competition. More aggressive fish in a stock affect the appetite and or feeding and hence the growth of subordinates. Generally, size differences

of the stock and dominance cause increased variation in body sizes. To avoid such occurrence, periodic size grading, to ensure that no fish is 1.5 times larger than another, should be done to prevent cannibalism. In addition, increasing the frequency of feeding or better dispersal of feed may help the inferior fish to obtain food. The presence of competing species may entail a shift to other "less desirable food";

3. The presence of predators may inhibit feeding;
4. Physiological condition such as starvation or motivation level, and circadian rhythm of the animal may dictate feeding time and amount of food ingested;
5. Selection of prey
   a) Choice of prey or food eaten, in terms of size and form, is limited by the mouth gape of the fish.
   b) The "optimal foraging theory" states that the natural selection favors those fish that maximize efficiency of prey capture. Since energy is spent in searching, pursuing, handling and digesting prey, an optimal foraging strategy must balance these factors such that the total energy expenditure is minimized and the net energy gain from the captured prey is maximized.
6. Handling causes stress in fish. Brown trout refuse to feed for three days after handling while rainbow trout, milkfish, or sea bass resume feeding the next day.

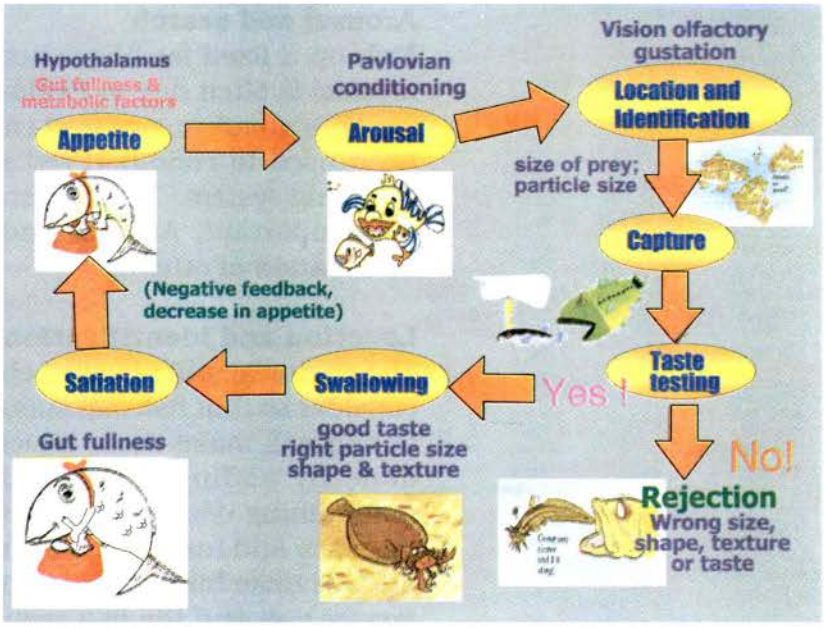


Figure 3.11
Feeding process in fish.
Source: Modified from Knight 1985

The abiotic factors include:

1. Dissolved oxygen (DO) is one of the most important abiotic factors affecting feeding behavior of fishes. Low DO levels lead to decreased feeding activity. Milkfish and shrimps raised in ponds cease feeding when DO levels drop below 1.5 and 3.0 ppm, respectively.
2. In most fish species, daily and seasonal water temperature fluctuation affect food intake. For milkfish, a tropical species, ambient temperature does not affect feeding activity in ponds, however, extreme temperature fluctuations affect feeding activity of milkfish. Shrimps are also affected by extreme temperature fluctuations.
3. Light intensity also influences feeding activity of most fish, especially larval fish, which rely on vision to recognize food particles.
4. Unionized ammonia (NH_3) levels of about 0.1 ppm inhibits growth in most species. However, appetite could be inhibited at ammonia levels high enough to cause serious physiological and morphological damage.

Arousal and search

Fish on a fixed feeding schedule can learn to anticipate feeding times but arousal is often due to **Pavlovian** conditioning. The sight or sound of farm workers about to give feed can stimulate feeding. Sound (200-700 Hz) has been used to condition red sea bream to come to a feeding spot in a sea ranching system. The influence of social interaction among fish in a stock is also important. Arousal and feeding of one or a few usually lead to the stimulation of others.

Location and identification

The chemical and physical characteristics of food particles are important to consider so that fish can successfully locate food. Making food more obvious to fish will make them easier to locate in the water column. This can be done by adding chemical attractants, providing color and contrast, maintaining desirable size and shape of particles, and manipulating light intensity and turbidity. Vision, olfaction, and gustation (sense of taste using surface taste bud) are all important in finding and locating food items. The farmer can feed the fish regularly in a set area of the farm or the tank and encourage fish to aggregate. It is a usual practice in shrimp farms to provide feeding trays at several places where shrimp can come to eat.

Capture

In carnivores, small particles can be ingested whole by being sucked into the mouth from a distance. Capture success in the wild depends on prey size and avoidance behavior, but this is not applicable in culture using complete or supplementary feed. However, movement in water currents may cause problems in getting at the food. Food particle size is a factor in capture success. Big particles not eaten immediately will disintegrate and be wasted. However, in general, some fish and shrimp will continue to nibble big food particles until ingestion is possible.

Taste Testing

Once the food is captured inside the mouth, the fish or shrimp tastes the food leading to swallowing or rejection. Not all materials taken into the mouth are swallowed, they are tested for suitability by taste receptors inside the mouth, gill arches, gill rakers, and in the tissue surrounding the pharyngeal teeth.

Swallowing or Rejection

Food which tastes good and have optimal particle size, shape, and texture will be swallowed by fish. More rejections and disintegration occur in pellets that are hard, abrasive and are much longer than their diameter. Large particles are usually ejected through the mouth with a "coughing" action. Therefore, feeds should have the correct size and texture for the species in culture. Delays in the ingestion of food due to repeated spitting out of food particles by the fish lead to increased energy expenditure and food wastage.

The overall practical implications of the knowledge on feeding process to fish culture are: encounter rates should be maximized by concentrating food availability in time and space; food supply must be matched to appetite; and chemical and physical characteristics of food particles need to be related to fish species, size, and sensory abilities to aid location, identification, and capture.

Summary

The rate at which fish digest their food is of primary importance in determining frequency, feeding rates, and ration size. Fishes can be classified according to their diet or food they habitually eat as herbivores, carnivores, omnivores, planktivores, and detritivores. A classification based on the manner of feeding or food getting includes predators, grazers, strainers, suckers, and parasites.

Fish adapt to their food differences by anatomic as well as behavioral means. There is a strong correlation between anatomical structure of the digestive tract and the feeding habits of the fish and the food eaten. The mouth has a variety of adaptations for capturing, handling and sorting of food before entry into the stomach. Fish have teeth that vary in type, number, and arrangement. The arrangement and structure of the teeth are related to the kind of food that the fish normally eat.

The stomach of fishes has a configuration or shape which is convenient for containing food. The size or capacity of the stomach in relation to the body weight varies between species and is usually related to the interval between feedings and to the size of food particles. All fishes have intestines. The length of the intestines varies from as low as 1/5 to as high as 20 times the body length. Carnivores have a relatively simple and short gut, with thick mucosa for absorption while herbivores have a long and thin gut to increase gut retention time and enhance digestion and absorption.

Digestion is a process whereby ingested food nutrients such as proteins, lipids, and carbohydrates are broken down into units that are small enough to be absorbed across the gut wall. The process is accomplished through the action of digestive enzymes. Enzymes that aid in digestion of proteins are known as proteases. For carbohydrate digestion, the enzymes involved are carbohydrases. Lipid digestion is facilitated by enzymes lipases and phospholipases.

The main difference between fish and crustacean digestion is that, in the latter, enzymes are secreted by the hepatopancreas. The hepatopancreas also acts as storage organ for glycogen, fat, and contains enzymes for secretion of bile acids. The crustacean hepatopancreas to a great extent fulfills the role of the liver in vertebrates.

Analysis and measurement of stomach contents gives information on the feeding preferences as well as frequency of feeding of a particular species. Digestibility measurements can be done through *in vivo* and *in vitro* methods.

Knowledge of feeding process in fish and shrimp is useful to maximize food location, capture, and ingestion.

Guide Questions

1. What correlation exists between anatomy of the digestive tract and feeding habits of fishes?
2. What are the four types of feeding behavior of fish in their natural habitat?
3. What are the different parts and functions of the digestive system of:
   a) fish
   b) crustaceans

4. What are the four general configurations or shapes of the fish stomach?
5. Discuss the relationship between gut length and feeding habits of fishes?
6. Differentiate the four types of epithelial cells found in the hepatopancreatic tubule of the crustaceans.
7. Define and explain digestion.
8. Describe the steps and enzymes involved in:
   a) protein digestion
   b) lipid digestion
   c) carbohydrate digestion in fishes and crustaceans
9. Discuss the methods used to measure stomach contents of fishes and digestibility of feeds.
10. Discuss the factors that may affect digestion and absorption in fishes.
11. Explain the different stages in the feeding process in fish

Suggested Readings

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CHAPTER

Formulation of Aquafeeds

4

MAE R. CATACUTAN

Introduction

The development of a feed that is both effective and economical for an aquaculture species in all its life stages is a continuous effort. Aquafeed development started when natural food sources in culture systems became inadequate and had to be supplemented with prepared feed. As fish stocking densities in culture increase, supplemental feeding is no longer sufficient. A complete feed that contains all the necessary nutrients in sufficient amounts to bring about good growth, survival, and reproduction is needed. Feed ingredients generally come from animal or plant sources and some are by-products of the food industry. There is no single feed ingredient or feedstuff that contains all the nutrients in adequate amounts. Thus, different feed ingredients are combined to make a feed that has the desired composition and nutrient levels. In combining various feed ingredients, it is important to know how much of each feed ingredient should be used to produce a cost-effective aquafeed.

With the growth and expansion of aquaculture into a major industry, several fish species are being cultured; thus, the development of more efficient aquafeed formulations should continue. In developing cost–effective formulated diets, many important factors have to be considered. This chapter discusses these factors and the mathematical calculations in formulating a feed. It aims to enable students to formulate diets using purified and practical feed ingredients, and also to formulate effective supplemental and complete diets for aquaculture species.

Feed Ingredients for Aquaculture

Feed ingredients or feedstuffs for aquaculture come from many different sources and are used because oftentimes they are not utilized for human consumption. The choice of a feed ingredient in aquafeed formulation depends on: content of essential nutrients; digestibility and bioavailability; absence of antinutritional factors and toxic substances; commercial availability; and cost.

Animal and plant materials are the most common feed ingredients. Some wastes and by-products of the food industry are also utilized. There

Table 4.1 Some sources of protein, lipid, and carbohydrate in aquaculture feeds

Protein	Lipid	Carbohydrate
Blood meal	Beef tallow	Bread flour
Copra meal	Corn oil	Cassava starch
Defatted soybean meal	Cod liver oil	Corn meal
Fish meal	Coconut oil	Corn starch
Meat and bone meal	Cottonseed oil	Fine rice bran
Shrimp head meal	Menhaden oil	Sago palm starch
Shrimp meal	Pollack liver oil	Seaweeds
Squid meal	Tuna liver oil	
Trash fish	Squid liver oil	
Yeast	Soybean oil	

are feed ingredients that are indigenous in some areas and may not be available in commercial quantities. Some feed ingredients for aquaculture feeds are listed in Table 4.1 and shown in Figure 4.1.

Feedstuffs of animal origin usually contain high amounts of protein with good amino acid profile (Table 4.2). A protein source is considered good if the amino acid profile is close to that of the species being fed. Some plant protein sources have high protein content but they often have inferior amino acid profiles compared with protein from animal sources. Feedstuffs from plants are good sources of carbohydrate, and some are of good protein quality such as soybean meal. Some protein sources like legumes and nuts are also sources of energy but are more expensive than the common carbohydrate sources with high levels of digestible carbohydrate such as sago palm starch and bread flour. Generally, carbohydrates are cheaper sources of energy (Figure 4.2) than lipid or fat sources. They are also used as feed

Figure 4.1
Some feedstuffs for aquafeeds, fish offal (A) shrimp meal (B) animal meat waste (C) meat and bone meal (D) soybean meal (E) and yeast (F).

Table 4.2 Amino acid composition of some fish meals (FM), leaf meals (LM) and other protein sources

	Amount (g/100 g protein)												
Amino acid	White FM	Peruvian FM	Sapsap FM	Tuna FM	Tabagak FM	Shrimp meal	Squid meal	Soybean meal	Kangkong LM	Camote LM	Ipil-ipil LM	Acacia LM	Tamarind LM
Alanine	6.0	6.1	6.7	5.0	5.5	5.5	5.8	4.0	5.0	5.2	6.2	3.2	3.6
Arginine	6.2	5.5	4.0	5.4	4.7	7.1	7.5	6.0	3.3	3.7	5.2	2.2	2.4
Aspartic acid	9.4	8.7	9.5	8.5	8.1	8.3	10.1	11.1	8.7	10.2	11.4	6.3	7.6
Cystine	0.7	0.9	0.8	0.7	0.9	0.6	0.9	1.2	0.5	0.3	0.6	0.5	0.5
Glutamic acid	13.8	13.2	14.9	11.2	11.3	12.4	15.8	18.7	8.8	10.2	11.2	6.6	7.6
Glycine	7.0	5.4	6.4	5.2	5.3	4.9	5.7	4.0	4.3	4.7	6.0	3.2	4.0
Histidine	2.2	1.8	3.9	5.6	2.3	2.1	2.2	3.2	2.7	2.8	1.4	3.9	4.0
Isoleucine	3.9	4.1	4.5	3.9	4.2	3.8	4.2	4.5	3.4	3.7	6.6	2.4	3.0
Leucine	7.4	7.1	7.5	6.5	6.6	6.7	7.7	7.2	6.5	7.9	6.6	4.8	5.7
Lysine	7.7	7.4	7.3	6.2	7.3	6.3	7.8	5.8	4.6	4.4	6.1	2.9	3.4
Methionine	3.0	2.8	2.9	2.3	2.8	2.4	2.8	1.4	1.5	1.8	1.2	0.9	0.9
Phenylalanine	4.3	3.8	3.8	3.8	3.5	3.8	3.4	5.2	5.7	6.5	3.9	3.8	4.2
Proline	4.3	4.2	3.5	3.7	3.0	3.2	3.8	5.5	3.9	3.8	5.5	3.6	3.8
Serine	4.4	3.6	3.4	3.3	3.1	3.1	3.9	5.2	3.7	4.3	4.4	3.2	3.8
Threonine	4.2	3.9	3.9	3.6	3.7	3.6	4.1	3.8	3.9	4.4	5.1	2.7	3.6
Tryptophan	1.0	0.7	1.2	0.8	0.8	1.1	1.0	0.4	NA	NA	NA	NA	NA
Tyrosine	3.8	3.2	4.4	2.6	3.3	3.3	3.1	2.7	4.1	6.5	3.4	3.5	3.0
Valine	5.1	5.2	5.2	4.5	4.9	4.3	4.3	4.4	5.3	5.8	6.3	3.5	3.9
% Protein	70.5	70.5	66.0	77.2	78.4	71.8	78.7	43.8	31.9	30.8	27.8	25.3	15.1

Values given are means; NA, not analyzed
Source: Peñaflorida 1989

Figure 4.2
Some sources of dietary energy are corn (A), rice bran (B), cassava (C).

binders. Aside from carbohydrates, lipids are also used as sources of energy. They also provide essential fatty acids in fish diets. Examples of commonly used dietary lipid sources are fish liver oils and plant oils such as soybean oil and corn oil.

The choice of a feed ingredient is mainly dependent on the amount of essential nutrients that it contains. Table 4.3 gives the proximate composition (crude protein, crude fat, digestible carbohydrate or NFE, crude fiber, and ash) of some feed ingredients. Feedstuffs containing high amounts of protein with good amino acid profile are usually expensive and their use is constrained by cost. The bioavailability of nutrients present in a feedstuff varies for different aquaculture species and will influence the level of inclusion of a feedstuff in the feed formula. Although feedstuffs may contain the same amount of nutrient, for example protein, the feedstuff with more digestible protein should be chosen over that with

Table 4.3 Proximate composition of some feed ingredients analyzed by the Centralized Analytical Laboratory at Southeast Asian Fisheries Development Center, Aquaculture Department *

	Amount (% dry matter)					
	Moisture	Crude Protein	Crude Fat	Crude Fiber	NFE**	Ash
Animal:						
Fish meal (FM, local) (6)	10.3	64.1	6.5	0.8	8.5	20.1
FM, Chilean (27)	8.4	70.1	8.5	0.5	4.1	16.8
FM, Danish (2)	9.5	73.9	9.4	0.3	2.4	14.0
FM, Peruvian (30)	8.3	68.3	5.9	0.8	7.7	17.3
FM, Peruvian (26)	7.1	67.9	10.0	1.3	4.1	16.7
FM, tuna (9)	9.4	65.4	8.0	0.8	8.8	17.0
FM, white (11)	7.2	69.0	7.6	0.6	4.8	18.0
Prawn head meal (35)	6.5	51.2	5.2	13.3	5.3	25.0
Shrimp meal, *Acetes* sp. (60)	8.2	68.6	3.9	3.6	7.6	16.3
Squid meal (60)	6.9	78.5	5.5	1.3	6.7	8.0
Squid meal, scrap (4)	5.5	74.1	7.1	0.9	8.1	9.8
Frog meal (2)	7.6	62.5	1.7	1.2	4.7	29.9
Blood meal (2)	6.3	87.7	3.0	0.4	3.3	5.6
Meat and bone meal (19)	5.6	46.8	9.6	2.0	7.5	34.1
Plant:						
Acacia Leaf Meal (LM) (2)	4.4	25.7	5.6	21.2	41.7	5.8
Alfalfa LM	7.2	17.2	3.0	27.7	42.9	9.2
Camote LM, (7)	4.5	29.7	4.9	10.0	43.2	12.2
Cassava LM, (8)	5.9	22.1	9.3	12.4	49.2	7.0
Ipil-ipil LM, giant (14)	7.8	25.1	6.8	10.6	44.0	13.5
Ipil-ipil LM, native (6)	10.3	29.3	8.8	11.5	43.5	6.9
Kang-kong LM (6)	5.7	28.5	5.4	10.5	43.6	12.0
Malunggay LM (7)	3.5	30.4	8.4	8.3	43.7	9.2
Papaya LM (10)	5.4	20.7	11.6	11.2	42.6	13.9
Copra meal (10)	7.9	22.0	6.7	17.3	44.3	9.7
Cowpea (7)	8.0	23.0	1.3	4.1	67.5	4.1
Cowpea, dehulled (2)	7.7	25.4	0.9	1.4	68.3	4.0
Mungbean, green (5)	7.1	23.2	1.2	3.1	68.7	3.8
Mungbean, yellow (5)	7.7	24.1	1.1	3.8	67.1	3.9
Rice bean (2)	5.0	26.5	0.8	4.0	64.6	4.1
Corn meal (10)	8.4	7.8	4.7	2.6	83.1	1.8
Cornstarch (5)	11.9	0.4	0.2	1.1	98.2	0.1
Flour, bread (40)	12.1	12.9	1.2	0.3	84.9	0.7
Flour, whole wheat (15)	11.3	15.3	1.7	0.8	81.1	1.1
Wheat, Pollard (4)	9.5	15.4	4.5	10.3	64.0	5.8
Germ, wheat (2)	6.0	27.8	4.3	3.4	59.6	4.9
Gluten, corn (5)	7.3	62.6	7.7	2.2	25.9	1.6
Gluten, wheat (6)	8.9	80.7	1.4	0.4	16.4	1.1
Rice bran (78)	9.2	13.3	14.1	8.5	53.4	10.7
Rice bran, tiki-tiki (5)	10.7	18.0	2.0	8.0	62.4	9.6
Rice hull (7)	7.0	3.3	2.0	32.4	41.6	20.7
Soybean meal, as is (21)	5.6	35.8	19.8	4.9	33.9	5.6
Soybean meal, defatted (108)	8.4	43.6	1.5	5.5	41.7	7.7
Other sources:						
Casein (11)	7.2	89.7	0.1	0.3	8.9	1.0
Crab meal (2)	4.2	37.9	4.1	10.7	8.9	38.4
Gelatin (6)	7.9	94.4	0.0	0.1	5.1	0.4
Mussel meal, green (30)	5.9	64.6	8.6	3.0	12.5	11.8
Oyster meal (6)	4.4	54.6	9.4	4.0	20.1	11.9
Scallop meal (2)	7.3	65.2	10.9	1.4	8.8	13.7
Snail meal, kuhol (5)	4.0	52.1	1.8	2.1	15.7	28.3
Yeast, Brewers (2)	7.2	49.4	1.6	2.4	34.5	12.1

Table 4.3 *(continued)*

	Amount (% dry matter)					
	Moisture	Crude Protein	Crude Fat	Crude Fiber	NFE**	Ash
Yeast, *Candida* (3)	8.3	55.2	0.8	1.7	35.1	7.4
Natural Food:						
Acartia sp. (copepods)	7.8	71.2	8.3	5.4	9.9	5.2
Artemia (37)	8.0	55.5	6.8	11.3	15.0	11.4
Azolla (2)	8.0	27.2	3.4	12.9	36.5	20.0
Brachionus sp.(5)	8.1	51.9	10.4	3.5	15.3	18.9
Chaetoceros calcitrans (7)	7.6	24.4	7.1	2.5	26.7	39.3
Chlorella, marine (3)	10.1	35.1	4.2	5.6	27.7	27.4
Isochrysis galbana (2)	10.4	33.6	18.1	4.4	23.0	20.9
Moina macrocopa (3)	8.5	57.8	7.6	8.4	17.2	9.0
Sargassum (2)	10.4	9.0	0.8	9.6	46.4	34.2
Skeletonema sp. (4)	10.4	24.7	2.6	0.7	20.2	51.8
Spirulina (2)	8.0	56.7	2.8	0.6	28.1	11.8
Tetraselmis sp. (4)	5.5	49.1	10.7	2.1	19.0	19.1
Digman (4)	9.8	20.6	3.3	16.4	35.9	23.8
Enteromorpha (lumot) (15)	15.2	13.8	1.9	9.3	36.9	38.1
Gracilaria sp. (18)	7.0	10.2	0.4	5.8	44.8	38.8
Kappaphycus sp. (10)	6.1	5.4	0.8	6.1	57.3	30.4

* Values are means for the number of samples given in parentheses. Not all feed ingredients are available in commercial quantities but may be used where they are commonly found in large amounts.

**NFE - Nitrogen-free extract

poor protein availability. The digestibility of protein (expressed in percentage) in some feedstuffs for some aquaculture species are listed in Table 4.4.

Table 4.4 Apparent protein digestibility coefficients (APDC) in % of some feedstuffs for aquaculture species

Aquaculture Species	Feedstuffs	APDC %	References
Shrimp			
Tiger shrimp	Fish meal	61	Catacutan 1997
	Soybean meal, defatted	93	"
	Squid meal	96	"
	Shrimp meal	95	"
	Shrimp head meal	89	"
	Meat and bone meal	74	"
	Yeast Candida sp.	93	"
	Copra meal	75	"
Fish			
Milkfish	Fish meal	45-81*	Ferraris et al. 1986
	Defatted soybean meal	45-94*	"
Carp	White fish meal, mechanical extracted	95	NRC 1977
	Soybean seed meal, solvent extracted	81-96	"
Red sea bream	White fish meal, mechanical extracted	61-87	"
Channel catfish	Soybean seed meal, solvent extracted	72-84	"

* tested at different salinities

Many other components are added in the feed formula aside from the major sources of nutrients. In complete feed formulations, micronutrients are added in small amounts in the form of vitamin and mineral mixtures. Examples of these mixes for crustaceans and fishes are shown in Tables 4.5, 4.6, and 4.7. For economic reasons, other substances are also

Table 4.5 Vitamin and mineral mixtures for crustaceans (A) and tiger shrimp juvenile (B).

	mg/100 g dry diet	
	A*	B**
Thiamine HCl (B_1)	1.26	4.0
Pyridoxine HCl (B_2)	3.70	12.0
Riboflavin (B_6)	2.57	8.0
Cyanocobalamin (B_{12})	0.025	0.08
Nicotinic acid	12.62	40.0
Folic acid	0.25	0.8
Biotin	0.13	0.4
Para aminobenzoic acid	3.16	10.0
Calcium pantothenate	18.93	60.0
Inositol	126.18	400.0
Na-ascorbate (C)	630.92	2000.0
Choline chloride	189.27	600.0
β-carotene (A)	3.03	9.6
Calciferol (D)	0.38	1.2
α-tocopherol (E)	6.31	20.0
Menadione (K)	1.26	4.0
TOTAL	1000.00	3170.08
MINERALS	g/100 g dry diet	
	A*	B**
K_2HPO_4	2.339	2.000
$Ca_3(PO_4)_2$	3.181	2.720
$MgSO_4.7H_2O$	3.556	3.041
$NaH_2PO_4.2H_2O$	0.924	0.790
TOTAL	10.000	8.551

* *Source:* Teshima and Kanazawa 1982

included in the feed formula to reduce fines during feed manufacture, and storage losses due to feed degradation and spoilage, and improve feed durability during handling and water stability. These substances include synthetic binders, antioxidants, and mold inhibitors. To make feed more attractive to fish, pigments and attractants may be added (Table 4.8).

Feed Formulation

Feed formulation is a process that involves combining various feed ingredients, which contain different amounts of nutrients, so that the resulting composition would meet the specific requirement of the cultured species. The nutrient levels of feed ingredients to be used are balanced mathematically in order to come up with the desired final composition.

Table 4.6 Recommended vitamin mixture for warmwater fishes such as milkfish, sea bass, and catfish *

Vitamin	Amount (per kg) in dry diet[a] Supplemental	Complete
Vitamin A activity	2,000 IU	5,500 IU
Vitamin D_3 activity	220 IU	1,000 IU
Vitamin E	11 IU	50 IU
Vitamin K	5 mg	10 mg
Choline	440 mg	550 mg
Niacin	17-28[b] mg	100 mg
Riboflavin	2-7[b] mg	20 mg
Pyridoxine	11 mg	20 mg
Thiamin	0	20 mg
Calcium pantothenate	7-11[b] mg	50 mg
Biotin	0	0.1 mg
Folacin	0	5 mg
Vitamin B_{12}	2-10 mg	20 mg
Ascorbic acid	0-100[b] mg	30-100[b] mg
Inositol	0	100 mg

[a] These amounts do not allow for processing or storage losses.
[b] Highest amounts are appropriate when "standing crop" of fish exceeds 500 kg/hectare.
* *Source:* NRC 1977.

Table 4.7 Mineral mixtures for purified and practical warmwater fish diets*

Mineral	Dry Diet (g/100g)
	Practical Diets
$CaCO_3$	0.750
$MnSO_4.H_2O$	0.030
$ZnSO_4.7H_2O$	0.070
$CuSO_4.5H_2O$	0.006
$FeSO_4.7H_2O$	0.050
NaCl	0.750
KIO_3	0.0002
$CaHPO_4.2H_2O$	2.00
	Purified Diets
$CaHPO_4.2H_2O$	2.07
$CaCO_3$	1.48
KH_2PO_4	1.00
KCl	0.10
NaCl	0.60
$MnSO_4.H_2O$	0.035
$FeSO_4.7H_2O$	0.05
$MgSO_4$	0.30
KIO_3	0.001
$CuSO_4.5H_2O$	0.003
$ZnCO_3$	0.015
$CoCl_2$	0.00017
$NaMoO_4.2H_2O$	0.00083
Na_2SeO_3	0.00002

Source: NRC 1977

Table 4.8 Other feed additives

Feed Binders
- agar
- alginic acid
- α starch
- bentonites
- carboxymethy cellulose (CMC)
- carrageenan
- gelatin
- gracilaria (dried and ground)
- hemicellulose
- lignosulfates

Antioxidants
- vitamin C
- butylated hydroxyanisole (BHA)
- butylated hydroxytoluene (BHT)
- ethoxyquin (1,2 dihydro-6-ethoxy, -2,2,4-trimethylquinoline)

Mold inhibitors
- citric acid
- sodium, calcium, or potassium sorbate

Attractants:

Color
- carotenoids
- xanthoterin (red and yellow xanthophylls)

Feed:
- betaine
- glutamic acid
- taurine

It also takes into account the cost of materials, acceptability, and ease of preparation.

The two most important factors to consider in formulating a feed for any aquaculture species are nutrient requirements and feeding behavior. Other factors to consider are the stages in the life cycle (larval, grow-out or broodstock) and the type of culture system. Like any other animal, fish and crustaceans need enough protein, lipid, energy, vitamins, and minerals. These nutrients should be present in the formulated feed in adequate amounts. Excessive amounts of nutrients is wasteful, while insufficient levels will result in slow growth. An allowance is made in the formula for nutrient losses during feed manufacture.

The feeding habit and behavior of the animal is also an important aspect to consider in feed formulation. For example, crustaceans such as shrimps and crabs are slow eaters and they take some time to consume the pelleted feed. In contrast, fish consume the feed immediately and, oftentimes, the feed is eaten before it touches the pond bottom. Thus, crustaceans require a more water stable feed than fish. A feed binder, usually a carbohydrate source, is added to the formulation to make the pelleted feed stay intact much longer in the water. Seaweed extracts such as kappa-carrageenan and alginates are commonly used as binders in microbound larval diet preparation. Larval diets may be prepared in suspension form, colloid form, in solution, or in dry form enclosed within a microcapsule or microcoated with a binder. Sometimes an attractant is added to the feed so that the pellet can easily be located and quickly consumed by the fish. Most aquatic species in the larval stage stay in the water column and gradually become bottom dwellers. Thus, the preferred feed at the early life stages is a feed that does not settle in the tank bottom but stays in the water column for a long time. Some aquatic species may prefer a specific feed form, type, or size that should be considered in feed formulation.

The requirement for nutrients and energy varies for broodstock, grow out, and larval stages; therefore, feed formulation would also vary in nutrient content depending on the growth stages. Although feed has been formulated and tested for all the life stages of milkfish, tilapia, and shrimp and for various stages in some species (see Chapter 7), these are continuously being refined based on recent research findings. Recent data on nutrient requirements, and availability of new, cheap but good protein sources are important information in refining a feed formula.

The processed feed is influenced by the quality of the feed ingredients. Some feedstuffs show wide fluctuations in their nutrient content due to seasonal and geographical variations. Feedstuffs should first be analyzed for their proximate composition, but if this is not possible, feed composition tables (FCT) can be used as guide in formulating feeds. In some FCTs, the moisture content is included and the amounts of nutrients are expressed either on a dry matter basis or on as received basis. In formulating feeds, a uniform set of values should be consistently used. Values are expressed either on a dry matter basis, or as received basis. To convert the amount of nutrients from dry matter basis to as received basis, use the formula:

$$\%\ \text{nutrient (as received)} = \%\ \text{nutrient (dry matter basis)} \times \frac{(100 - \%\ \text{moisture})}{100}$$

There is no definite feed formula for any species because of the many environmental and physiological factors involved as well as differences in the availability of feed ingredients from one locality to another. Substitution of a feedstuff or feedstuffs in the formula is possible provided that the final formulation is similar in nutrient content and there are no negative effects on growth and survival and increase in feed cost. Feeding experiments have shown that favorable results are obtained as long as the amounts of nutrients present do not exceed the recommended nutrient levels. Excess of nutrients can be expensive, and can cause deterioration of the culture system. Maximum levels of incorporation of some feedstuffs in the formula for carnivores, omnivores, and herbivores are listed in Table 4.9

Table 4.9 Recommended maximum inclusion levels (%) of some major feed ingredients in a practical diet for fish and shrimp

Feedstuffs	Fish		Shrimp	
	Carnivore	Omnivore/ Herbivore	Carnivore	Omnivore/ Herbivore
Alfalfa meal	5	10	5	10
Blood meal, spray dried	10	10	10	10
Cassava/Tapioca meal	15	35	15	25
Coconut meal	15	25	15	25
Corn grain meal	20	35	15	
Corn gluten meal	15	20	15	20
Cottonseed meal, solvent extracted	15	20	10	15
Corn distillers	10	15	10	15
Dicalcium phosphate	3	3	3	3
Hydrolyzed feather meal	10	10	10	10
Fish meal	no limit	no limit	20	35
Fish protein concentrate	15	10	15	15
Ground meal, solvent extracted	15	25	15	25
Liver meal	50	50	25	20
Meat and bone meal,	20	25	15	20
Poultry by-product meal	15	20	15	20
Rapeseed meal, solvent extracted	20	25	15	20
Rice bran, solvent extracted	15	35	15	35
Shrimp meal	25	25	no limit	no limit
Squid meal	no limit	no limit	no limit	no limit
Sorghum meal	20	35	15	35
Soybean meal, solvent extracted	25	35	20	30
Soybean meal, full fat	35	40	20	30
Wheat grain meal	20	35	20	35
Wheat bran	15	30	15	30
Wheat gluten meal	15	15	20	20
Wheat middlings	25	40	20	35
Whey	10	10	10	10
Yeast (Brewers), dried	15	15	15	15

Source: Tacon 1988

There are several methods of formulating feeds. These are Pearson's Square Method, Algebraic Equation, Trial and Error, and Linear Programming. The Pearson's Square method is recommended in formulating a supplemental feed with only two to four ingredients. The Trial and Error method is generally used in calculating a formula for a complete diet with many ingredients. In commercial feed production where cost is a principal consideration, a computer program (Linear Programming) is used to combine feed ingredients that will give an effective formulation with the lowest cost.

Mathematical calculations using these methods, except for Linear Programming, are given in the following examples.

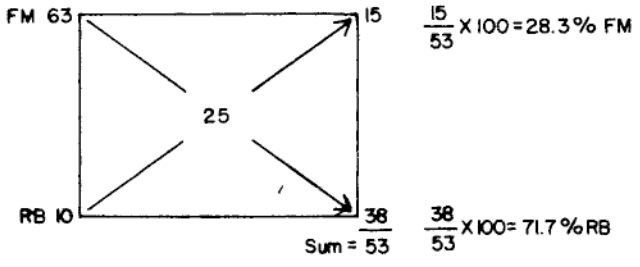
I. Pearson's Square and Algebraic Equation Methods

Example 1. When only two feed ingredients are to be combined.

To determine the amount of each ingredient in a supplemental feed that would contain 25% protein using Fish Meal (FM) and Rice Bran (RB), with protein contents of 63% and 10%, respectively.

❑ Pearson's Square Method:

a) First draw a square and write the desired protein level of the feed at the center of the square.
b) Write the two ingredients with their respective protein contents on each corner of the left side of the square, the ingredient with higher protein on the upper and with lower protein on the lower left hand corner.
c) Subtract the desired level of protein from each ingredient and write the difference on the right corner of the square that is diagonally opposite the protein level of each ingredient.
d) Get the sum of the numbers at the right side of the square.
e) Determine the percentage of each ingredient needed for the feed formula by dividing the numbers written on the right hand side by the sum of the difference multiplied by 100.



About 283 g FM and 717 g of RB are required to make a kg of the supplemental feed that will contain 25% protein. To check for the amount of protein in the feed, the protein content (in percent) of each ingredient

is multiplied by the amount of FM and RB (283 g and 717 g respectively) to be combined. The amount of protein coming from each ingredient is added to get the total amount. The total amount is divided by 1000 and multiplied by 100 to give 25% protein.

FM	=	283 g x 63%	=	178.3 g protein
RB	=	717 g x 10%	=	71.7 g protein
		Total protein		250.0 g /kg or 25% protein

or (250 / 1000) x 100 = 25%

❑ **Algebraic Equation Method:**

Let: x = g FM/ kg feed
y = g RB/ kg feed

so that :

$x + y = 1000$ g feed (Equation I)

$0.63x + 0.10y =$ 250 g protein/1000 g feed (Equation II)

Multiply Equation I by 0.10:

$0.10x + 0.10y = 100$ g (Equation III)

Subtract Equation III from Equation II:

$0.63x + 0.10y =$ 250 g protein/1000 g feed
$-(0.10x + 0.10y = 100\text{ g})$
$0.53x + 0 = 150$
$x = 150/0.53 =$ 283 g FM

Substitute in Equation I:

$283 + y = 1000$
$y = 1000 - 283 =$ 717 g RB

Example 2: When three or more feed ingredients are available for use.

Formulate a fish diet to contain 35% protein by combining the following ingredients with their respective protein content:

FM	-	60% protein
Soybean Meal (SBM)`	-	45% protein
RB	-	8% protein
Corn Meal (CM)	-	12% protein

Assume the proportion of 1 part FM to 2 parts SBM or 1:2 and equal parts of RB and CM or 1:1. FM and SBM are the main sources of protein while RB and CM are the main sources of carbohydrate or energy but also contain protein.

❑ Pearson's Square Method:

a) Draw a square and write at the center the desired protein level as in the first example.

b) Calculate the protein level from the protein sources FM and SBM, according to the specified ratio: 1:2

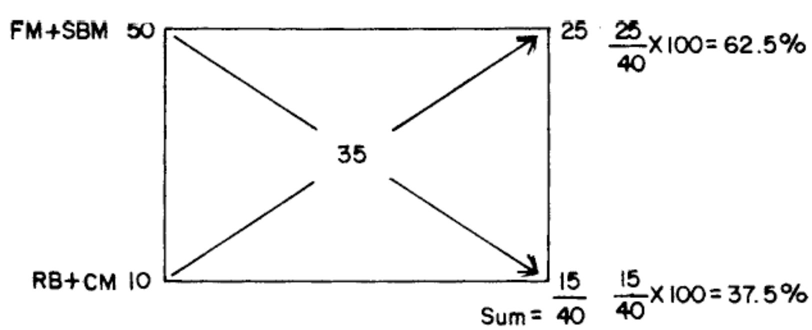
FM	:	1 x 60 = 60
SBM	:	2 x 45 = 90
Average	:	150 / 3 = 50

c) Calculate the energy sources, RB and CM also according to the ratio 1:1

RB	:	1 x 8 = 8
CM	:	1 x 12 = 12
Average	:	20 / 2 = 10

d) Write the calculated average protein content on the upper left hand corner for the protein sources and the protein content of the energy sources on the lower left corner of the square.

e) Write the desired protein level at the center and substract this value from the protein content of FM and SBM and protein content of RB and CM. Write the number diagonally opposite the ingredients or on the lower right hand corner for protein sources and upper right hand corner for energy sources. Proceed as in d) and e) of Example 1.



f) Multiply the final percentage derived for the protein sources by 1/3 for FM and 2/3 for SBM. For the energy sources, multiply by 1/2 each of the RB and CM to find out the exact amount of each ingredient to be used in the formula with the desired level of 35% protein.

Protein sources	=	62.5%				
				In %		In g/kg feed
FM	=	62.5 x 1/3	=	20.83%		208.3
SBM	=	62.5 x 2/3	=	41.67%		416.7
Energy sources	=	37.5%				
RB	=	37.5% x 1/2	=	18.75%		187.5
CM	=	37.5% x 1/2	=	18.75%		187.5
Total				100.00%		1,000

g) To check that a kilo of feed contains 35% protein, proceed as follows:

FM	=	208.3 g x	60%	protein	=	125.0 g protein
SBM	=	416.7 g x	45%	protein	=	187.5 g protein
RB	=	187.5 g x	8%	protein	=	15.0 g protein
CM	=	187.5 g x	12%	protein	=	22.5 g protein
Total per 1000 g feed						350.0 g protein/kg or 35%

❑ Algebraic Equation Method:

Separate ingredients into protein and energy sources and calculate average protein contribution of each group according to specified proportions as in Example 2 letters a), b) and c) Pearson's Square method. Then:

Let: x = g of FM and SBM of protein sources/ kg feed
y = g of RB and CM as energy sources/ kg feed

$$x + y = 1000 \text{ g feed} \quad \text{(Equation I)}$$
$$0.50x + 0.10y = 350 \text{ g protein/ kg feed} \quad \text{(Equation II)}$$

Multiply (I) by 0.10 : $0.10x + 0.10y = 100$ (Equation III)

Subtract (III) from (II):

$$\begin{array}{rrrrr} 0.50x & + 0.10y & = & 350 \\ -(0.10x & + 0.10y & = & 100) \\ \hline 0.40x & + 0 & = & 250 \\ & x & = & 625 \end{array}$$

FM : 625 x 1/3 = 208.3 g
SBM : 625 x 2/3 = 416.7 g

From Equation (I):

$$y = 1000 - x$$
$$y = 1000 - 625 = 375$$

RB = 375/2 = 187.5 g
CM = 375/2 = 187.5 g

Example 3: When some feed components are fixed.

Prepare a fish diet to contain 32 % protein using FM (60% protein) and Copra meal or CpM (20% protein) as protein sources to be included in the diet:

Rice bran (10% protein)	=	22%
Mineral mix	=	2%
Vitamin mix	=	1%

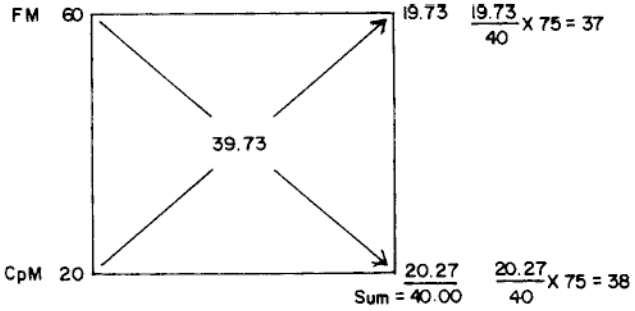
❑ **Pearson's Square Method:**

a) Determine the amount of FM and CpM per 1 kg feed mixture, by subtracting the amounts of RB, mineral mix and vitamin mix from 1000.
1000 - (220 + 20 + 10) = 750 g/ kg feed

b) Vitamin and mineral mixes do not contain protein, but 220 g RB supplies 22 g protein. Therefore, subtracting 22 g protein from RB from the 320 g desired protein level equals 298 g protein, which must come from 750 g of FM and CpM.

c) Convert to percentage the amount of protein (298 g/kg) and solve by the Pearson's Square method the amount that should come from the combined levels of FM and CpM (750 g):
298/750 x 100 = 39.73%

d) Follow steps a) to e) in Example 1 and check for final protein in one kg diet:



e) A total of 370 g FM (222 g protein) and 380 g CpM (76 g protein) gives 298 kg protein. This amount of protein plus the 22 g protein from 220 g RB makes a total of 320 g protein or 32% of one kg diet.

❑ **Algebraic Equation Method:**

Before proceeding to formulate the necessary equations, first do steps a) and b) described in the Pearson's Square method.

Let : x = g of FM required
y = g of CpM required

$x + y = 750$ g (Equation I)
$0.60x + 0.20y = 298$ g protein (Equation II)

Multiply (I) by 0.2 :
$0.20x + 0.2y = 150$ (Equation III)

Subtract (III) from (II):

$$\begin{array}{rl} 0.60x + 0.20y &= 298 \\ -(0.20x + 0.2y &= 150) \\ \hline 0.40x + 0 &= 148 \end{array}$$

$x = 148 / 0.4 = 370$g FM

Substitute (I):

$370 + y = 750$ g
$y = 380$ g CpM

II. Trial and Error Method

For the Trial and Error method, a worksheet and a table of proximate composition are necessary.

Example 1: Formulate a diet that will contain fish meal (FM), defatted soybean meal (DSBM), meat and bone meal (MBM), rice bran (RB), and vitamin and mineral mixes. Cod liver oil (CLO) is the lipid source, bread flour (BF) is the source of carbohydrate and binder, and lime is used as filler. The finished diet should contain 35% crude protein, 12% crude fat, with a dietary energy of 340 to 400 kcal/100 g diet.

The proximate composition of these feed ingredients are:

Ingredients	g/100 g				
	Protein	Fat	Fiber	Ash	NFE
FM	66.70	9.11	0.59	13.36	10.24
DSBM	50.34	1.45	8.59	7.64	31.98
MBM	46.91	10.90	1.11	36.10	4.98
RB	14.80	12.66	4.17	8.92	59.45
BF	14.17	1.54	0.56	0.68	83.05

The following ingredients are incorporated in fixed amounts:

FM	=	10%
SBM	=	18%
BF	=	5%
Vit/min mix	=	3%
Total	=	36%

The remaining percentage, which is 64%, will be supplied by MBM, RB, CLO, and the filler (lime). About 58 g will come from MBM and RB and 6 g from lipid and filler. The closest estimates to the required values are obtained by trial and error.

1. Fill in the required ingredients and the corresponding amounts in Columns 1 and 2 in the Worksheet (Appendix A).

2. Fill in the respective nutrient content based on the proximate composition of each ingredient on the upper left hand corner of each box (Worksheet 1).

3. Calculate the amount of nutrient from each ingredient by multiplying the specified amount of the ingredient (column 2) by the percentages of the nutrient (column 3) to obtain the amount in grams. Start with the protein sources.

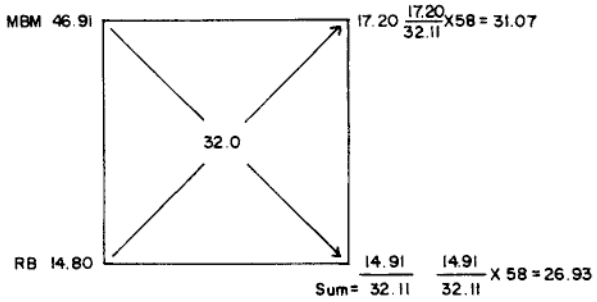
   For example, 10 g FM with 66.7% protein contributes 6.67 g protein to the diet. Add up the protein from FM, SBM, and BF (Worksheet 1, column 3). Do the same for the rest.

4. Calculate the amount of protein that should come from MBM and RB by subtracting the known protein contribution of the other ingredients (FM+DSBM+BF) from the total protein required (35%).

   35 g protein – (6.67 + 9.06 + 0.71) = 18.56 g protein to come from MBM +RB

   This value should come from 58 g of MBM + RB, which is 18.56/58 = 32% protein

5. Use Pearson's Square to calculate the exact amount of MBM and RB to be used in the feed formulation:



6. Fill-up Worksheet 2 with calculated amounts of MBM (31.07 g) and RB (26.93 g).

7. Calculate nutrient contribution from each feed ingredient. After the amounts of protein sources have been calculated, proceed to calculate the lipid sources and lipid levels.

   For lipids, the partial amount from other ingredients is equal to 8.05%, therefore, to meet the requirement of 12%, CLO will be added at 3.95% (column 4). To make the formulation 100%, calculate all the other nutrients and determine how much filler or lime to add by subtracting 97.95 from 100 equals 2.05.

8. Calculate the sum of fiber, ash and NFE to have a complete record of the major nutrients. Indicate these values in columns 5, 6, and 7, respectively. These can be compared to the analyzed proximate composition of processed feed.

9. To determine the dietary energy/100 g diet use the physiological values of 4.5, 8, 3.3 as follows:

Protein	= 35	g	x 4.5 kcal/g	=	157.5	kcal
Fat	= 12	g	x 8.0 kcal/g	=	96	kcal
Carbohydrate	= 28.5	g	x 3.3 kcal/g	=	94	kcal
					347.5	kcal/100 g diet

Worksheet 1
Calculated nutrient composition of feed

Feed formulation code: M-1
Date computed: March 8, 2000
Formulated by: Mary Cruz

Ingredients (1)	g/100g (2)	Protein% (3)	Fat% (4)	Fiber% (5)	Ash% (6)	NFE% (7)
Fish Meal	10	66.7 6.67	9.11	0.59	13.36	10.24
Meat and Bone Meal						
Soybean meal (defatted)	18	50.34 9.06	1.45	8.59	7.64	31.98
Rice bran						
Bread flour	5	14.71 0.71	1.54	0.56	0.68	83.05
Vitamin/mineral mix	3					
Cod liver oil						
Lime						
		35.00 16.44 18.56				
TOTAL	36					

Worksheet 2
Calculated nutrient composition of feed

Feed formulation code: M-1
Date computed: March 8, 2000
Formulated by: Mary Cruz

Ingredients (1)	g/100g (2)	Protein% (3)	Fat% (4)	Fiber% (5)	Ash% (6)	NFE% (7)
Fish Meal	10	66.70 / 6.67	9.11 / 0.91	.59 / 0.06	13.36 / 1.34	10.24 / 1.02
Meat and Bone Meal	31.07	46.91 / 14.57	10.90 / 3.39	1.11 / 3.34	36.1 / 11.22	4.98 / 1.55
Soybean meal (defatted)	18	50.34 / 9.06	1.45 / 0.26	8.59 / 1.55	7.64 / 1.38	31.98 / 5.76
Rice bran	26.93	14.8 / 3.98	12.66 / 3.41	4.17 / 1.12	8.92 / 2.40	59.45 / 16.01
Bread flour	5	14.17 / 0.71	1.54 / 0.08	0.56 / 0.03	0.68 / 0.03	83.05 / 4.15
Vitamin/mineral mix	3					
Cod liver oil	3.95		100 / 3.95			
Lime	2.05				100	
TOTAL	100	35.0	12			

Example 2: Formulate a diet that would contain not less than 40% protein with dietary energy content of not less than 340 kcal/100 g diet with the following feed ingredients. Consider also the digestibility coefficients of the major nutrients, protein, lipid, and carbohydrate

Use FM and SM in a 1:1 ratio
Lipid source is cod liver oil, with total dietary fat not to exceed 15%
Carbohydrate source is bread flour
Vitamin mix 2%, Mineral mix 1%, and Lecithin 0.5%
Filler is cellulose

The proximate analysis and digestibility coefficients of protein, lipid, and carbohydrate sources on a dry matter basis:

	% Nutrients					% Digestibility		
	Protein	Lipid	Carbo-hydrate	Crude fiber	Crude ssh	Protein	Lipid	Carbo-hydrate
Fish meal	65.8	5.9	7.8	0.8	19.7	80	93	94
Shrimp meal	68.6	3.7	7.5	3.6	16.6	75	96	97
Squid meal	78.5	5.5	6.8	1.2	8.0	88	91	89
Bread flour	13.9	1.2	83.9	0.3	0.7	75	90	95

The fixed amount from vitamin and mineral mixes and lecithin, is 3.5%. The remaining amount is equal to 96.5% and will be supplied by the other ingredients. In Worksheet 3:

1. Fill in the fix ingredients and the corresponding amounts in Worksheet 3. Then, try a 1:1 ratio of 25 g each of FM and SM and write these numbers on the worksheet.

2. Fill in the nutrient composition of FM and SM in Worksheet 3, columns 3 to 7 on the left hand box for each column corresponding to each nutrient. Calculate the nutrient contribution based on the proximate composition and digestibility coefficients and fill in the respective box.

   Compute the protein, lipid, and carbohydrate contributed by a feedstuff using the formula:

$$\text{Weight of feedstuff} \times \frac{\%\ \text{Protein}}{100} \times \frac{\%\ \text{Digestibility}}{100}$$

   From the proximate composition table,

   FM: 25 g x 0.658 x 0.80 = 13.16 g protein
   25 g x 0.059 x 0.93 = 1.37 g lipid
   25 g x 0.078 x 0.94 = 1.83 g carbohydrate

   Do the same for SM.

3. Calculate the amount of protein to be contributed by SqM as:

   13.6 g (FM) + 12.7 g (SM) = 26.3 g

   40g (total protein) – 26.3g (FM + SM) = 13.7 g SqM protein

   To calculate weight of SqM to be used in the formula:

$$\text{Weight of SqM} = \left(\frac{\text{SqM protein}}{\%\text{Protein}/100}\right) \times \left(\frac{\%\ \text{Digestibility}}{100}\right)$$

$$= \frac{13.7\ \text{g}}{(0.785 \times 0.88)} = 19.8\ \text{g SqM}$$

4. Fill in the calculated amounts of protein, lipid, and carbohydrate from 19.8 g SqM (using the formula in step 2).

5. Sum up the dietary lipid from all the protein sources and subtract the value to calculate the amount of CLO to be added. Assume dietary lipid to be about 12% [12 – (1.37 + 0.89 + 0.99) = 8.75].

6. Sum up the dietary energy (use energy values for each nutrient in the previous example) at this point to be able to calculate the amount of energy to be contributed by bread flour (340 kcal – 292.5 kcal = 47.5 kcal):

Protein	: 40 g x 4.5 kcal/g	=	180
Lipid	: 12 g x 8.0 kcal/g	=	96
NFE	: 5g x 3.3 kcal/g	=	16.3
			292.5 kcal

7. Bread flour is about 90% carbohydrate with a digestibility of about 95%, so that 47.5 kcal divided be 3.3 is about 14.4 g. Since the digestibility is less than 100%, the amount of bread flour maybe increased to 16 g. Calculate the nutrients contributed by 16 g bread flour (as in step 2).

8. Determine the sum of ingredients used (25+25+19.8+16+3+0.5+8.75 = 98.05) and subtract the value from 100. The difference is the amount of the filler, cellulose (1.95) to make the total equal to 100.

9. Add up the nutrients and calculate the total dietary energy /100g diet.

Protein	: 41.5 g x 4.5 kcal/g	=	186.8
Lipid	: 12.67 g x 8.0 kcal/g	=	101.4
Carbohydrate	: 17.83 g x 3.3 kcal/g	=	58.8
			347.8 kcal/100g

10. Compute the total ash and fiber content in the feed formula (column 5 and 6). The minimum dietary level for ash or fiber is about 10% because higher levels can cause poor growth and survival. Compare the computed values to the actual results of the chemical analysis to detect discrepancies.

Worksheet 3
Calculated nutrient composition of feed

Feed formulation code: M-1
Date computed: March 10, 2001
Formulated by: Mary Cruz

Ingredients (1)	g/100g (2)	Protein% (3)	Fat% (4)	Fiber% (5)	Ash% (6)	NFE% (7)
Fish Meal	25	65.8 / 13.2	5.9 / 1.37	0.8 / 0.20	19.7 / 4.92	7.8 / 1.83
Shrimp meal	25	68.6 / 12.9	3.7 / 0.89	3.6 / 0.90	16.6 / 4.15	7.5 / 2.0
Squid meal	19.8	78.5 / 13.7	5.5 / 0.99	1.2 / 0.24	8.0 / 1.58	6.8 / 1.2
Bread flour	16.0	13.9 / 1.7	1.2 / 0.17	0.3 / 0.05	0.7 / 0.11	83.9 / 12.8
Vitamin/mineral mix	3.0					
Lecithin	0.5		100 / 0.5			
Cod liver oil	8.75		100 / 7.5			
Cellulose	1.95					
TOTAL	100	41.5	12.67	1.39	10.76	17.83

III. Linear Program for Least-Cost Formulation

Linear programming is used when many ingredients are to be combined for a least cost feed formula. This method is especially useful in commercial feed manufacture wherein large quantities of feed ingredients are used. A computer is necessary in this type of formulation. When one or more ingredients are not available, other feed ingredients are utilized as substitutes in order to come up with the same feed quality. The quantities of substitute ingredients are determined using the linear programming method. Information on the amounts of nutrients in each of the feed ingredients to be used is essential in this method. This can be obtained from a feed composition table or chemical analysis. A computer program will list several combinations to come up with almost similar feed quality at a lesser cost. Examples of computer program for least cost formulation are the simple linear programming spreadsheet and the sophisticated Brill Formulation Package.

Purified Diet Formulation

In nutritional requirement studies, purified ingredients are used in the formulation of diets. This is necessary to study the optimum level of one specific nutrient, e.g. essential amino acid. The use of purified ingredients enables one to vary the level of the nutrient whose dietary requirement is being studied while keeping the other nutrient levels constant. Examples of purified ingredients include casein and gelatin as protein sources, dextrin and sucrose as carbohydrate sources, and cellulose for the non-nutritive filler.

Example: Formulate a purified diet to contain about 50% protein using casein and gelatin at 1:1 ratio, cod liver oil level at 12%. and vitamin and mineral mixtures at 3% each, with a dietary energy of 380 kcal/100 g diet. Assume casein and gelatin to contain 93% protein with apparent protein digestibility of 96%, while dextrin is 100% digestible. Include feed additives such as attractant at 1% and binder at 2%.

Feed ingredients	Percentage
Gelatin	
Casein	
Dextrin	
Cod liver oil	12
Vitamin mix	3
Mineral mix	3
Feed binder	2
Attractant	1

Following the computation in Example 2 in the Trial and Error Method section, the amount of casein and gelatin would be 28 g each. The total dietary energy content of the above diet is:

50 g protein	x	4.5 kcal/g protein	=	225
12 g lipid	x	8.0 kcal/g lipid	=	96
		Total		321 kcal/100 g diet

The amount of dextrin will make up the remaining energy requirement. If the total dietary energy content is 380 kcal/100 g diet, then the amount of dextrin to be added should supply the energy difference which is 380 kcal – 321 kcal = 59 kcal. Since a gram of carbohydrate has an energy content of 3.3 kcal, therefore, 59 kcal divided by 3.3 kcal/g dextrin is 18 grams. To make the formula 100%, a filler, such as cellulose, can be used. The final composition would be:

Feed ingredients	%
Gelatin	28
Casein	28
Dextrin	18
Cod liver oil	12
Vitamin mix	3
Mineral mix	3
Feed binder	2
Attractant	1
Cellulose (filler)	5
Total	100

Summary

Feed is a very important component in the success of an aquaculture venture. With increased stocking densities, natural food in culture systems has to be supplemented with formulated feeds. It is important to know the nutrients required by the aquaculture species as these would be the starting point in feed formulation. A single feedstuff does not have all the required nutrients for growth, survival, and reproduction. Hence, it is necessary to know the nutrient content and levels in commonly used feedstuffs so that these can be combined to come up with the desired formulation by mathematical calculations.

Several mathematical methods can be used in feed formulation: the Pearson's square technique, the algebraic equation method, the trial and error method, and linear programming. The first two are simple, the third uses a worksheet, and the fourth requires a computer software. In balancing a ration, protein which is the major component of the diet is computed first, the energy (lipid and carbohydrate) levels of the diet are then adjusted to the desired dietary level.

Guide Questions

1. Name some methods in formulating diets. Give the advantages and disadvantages of each method.
2. Why is it important to know the nutrient content of feedstuffs and their levels before one can start formulating a feed?
3. Why is protein the first nutrient to be considered in formulating a diet?
4. Discuss factors to be considered and their importance in formulating a feed for a specific species.
5. Give at least 3 feedstuffs that are good sources of:
   a) protein
   b) lipids
   c) carbohydrate
6. Calculate the amount of fish meal and rice bran in a diet that contains 30% protein. The protein contents of fish meal and rice bran are, 60% and 15%, respectively.
7. Use the ingredients in Example 1 in the Trial and Error Method section in formulating a diet that will contain not less than 35% protein, dietary lipid of not more than 10% and dietary energy not less than 350 kcal/100g diet.

Suggested Readings

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CHAPTER

Processing of Feedstuffs and Aquafeeds

5

NELSON V. GOLEZ

Introduction

Feed manufacturing and quality control of the finished feed are important to successful aquaculture. The technology of feed processing has undergone considerable improvements through the years. Processing of feedstuffs and aquafeeds has progressed from simple mixing of several ingredients by hand to mechanical mixing, to continuous mixing, and recently to computer-controlled processing. In spite of this, the basic concept of mixing the ingredients together to obtain a nutritionally-balanced feed remains unchanged. In feed processing, several factors have to be considered.

The production of good quality feeds requires the use of ingredients of high quality. There is also a need to avoid the conditions that could cause deterioration of the feedstuffs from harvesting to processing and storage. Many feedstuffs contain antinutritional factors that prevent utilization of nutrients (particularly proteins) by fish and other cultured animals. For example, the seeds of many legumes contain substances that inactivate trypsin and chymotrypsin, the enzymes for protein digestion in animals. The nutritional quality of feedstuffs depends in part on the processing they undergo to remove the antinutritional factors. Some of these factors are easily destroyed by heat while others are resistant to heat.

Growth and productivity of the aquatic animals greatly rely on the quality of feed given to them. Feeds represent a major portion of total costs in animal production. Thus, processing of feed ingredients, feed preparation, and storage are major considerations in making feeds. However, proper methods applied during processing and preparation will be useless if the end product is not carefully handled and properly stored.

In feed preparation, the main objective is not just to mix whatever ingredients are available but to prepare the feed using properly processed ingredients. Oftentimes, before these raw materials can be used, they have to be processed to remove substances that may prevent their proper utilization by the fish. The feed preparation process is more concerned with the physical conversion of a given formulation into compounded, nutritionally-effective diet. Feed storage must also be given equal importance as in feed preparation and processing because it affects the shelf-life of the finished product.

This chapter will help the reader understand and appreciate the basic principles of processing, preparation, storage, and quality control in the preparation of aquafeeds. The material in this section is presented in sequence beginning with the processing of basic ingredients to remove antinutritional factors, followed by steps in feed preparation, from the easiest to the more complex processes, and storage. This chapter presents methods and equipment that are useful not only for feed millers, but also for extension workers and fish farmers.

Feedstuff Processing

Feedstuff processing refers to all operations necessary to obtain the highest nutritional value of a feedstuff and the best economic returns from their use. There are various ways of processing raw materials that can destroy some antinutritional factors present in feedstuffs. They are soaking, heating, chemical treatment such as extraction with organic solvent, and dehulling. The kind of treatment will depend on the substances present in the feed ingredient. Table 5.1 lists some antinutritional substances and their removal or inactivation.

Some are natural component of feedstuffs such as gossypol in cotton seed and thiaminase in raw fish. Others may originate from natural contamination of the raw materials such as aflatoxins produced by the mold *Aspergillus flavus*, or man-made contaminants such as polychlorinated biphenyls (PCBs), pesticides, herbicides, and hydrocarbons.

Table 5.1 Various antinutritional substances in some feedstuffs and their removal or inactivation

Substances	Adverse actions	Occurrence	Removal or remedy
Heat Labile substances			
Trypsin inhibitor	Binds trypsin to form an inactive compound	Soybean and other legumes	Dry heating at 175-195°C, or cooking for 10 min.
Lectins	Destroy the red blood cells	Soybean and other legumes	Boiling in water or autoclaving for 30 min.
Goitrogens	Inhibit the uptake of iodine by the thyroid gland	Soybean and other legumes	Steam and/or autoclave for 10 to 30 min
Anti-vitamin D	Binds Vit. D, making it unavailable	Soybean and other legumes	Autoclaving or boiling for 30 min.
Anti-vitamin E	Contributes to Vit. E deficiency	Soybean and other legumes	Autoclaving
Thiaminase	Promotes destruction of thiamin (Vit. B_1)	Raw and spoiled fish, mussels, clams, and soybean	Autoclaving, heating, and cooking
Heat resistant substances			
Estrogens (isoflavones)	Interfere with reproductive performance	Plant glycosides	Solvent extraction
Gossypol	Binds phosphorus and some proteins	Cottonseed meal	Addition of iron salts or phytase
Tannin	Binds protein and inhibit trypsin digestion	Beans and other legumes	Dehulling
Cyanogens	Release poisonous hydrocyanic acid	Cassava leaves	Soaking in water for 12 h
Mimosine	Interferes with enzyme synthesis in the liver; destroys hepato-pancreatic cells of shrimps	Ipil-ipil leaves	Soaking leaves in water for 24 h
Peroxides	Bind proteins and vitamins	Poorly stored and unprotected oils	Proper storage
Phytates	Bind proteins and minerals and reduces their availability	Cottonseed meal, cereal hulls soybean, and other legumes	Dehulling

Sources: Liener 1962, 1980; Eusebio and Eusebio 1984; Eusebio 1991

Feedstuffs are processed to:

- remove antinutritional factors and toxins
- increase palatability, digestibility, and nutrient availability
- adjust feed particle size to suit a given species and size
- reduce feed wastage
- maximize profit through optimum processing of feeds
- lower the moisture content of feedstuff to 10% or less

Ipil-ipil leaf

Cassava leaf

Different methods of feedstuff processing

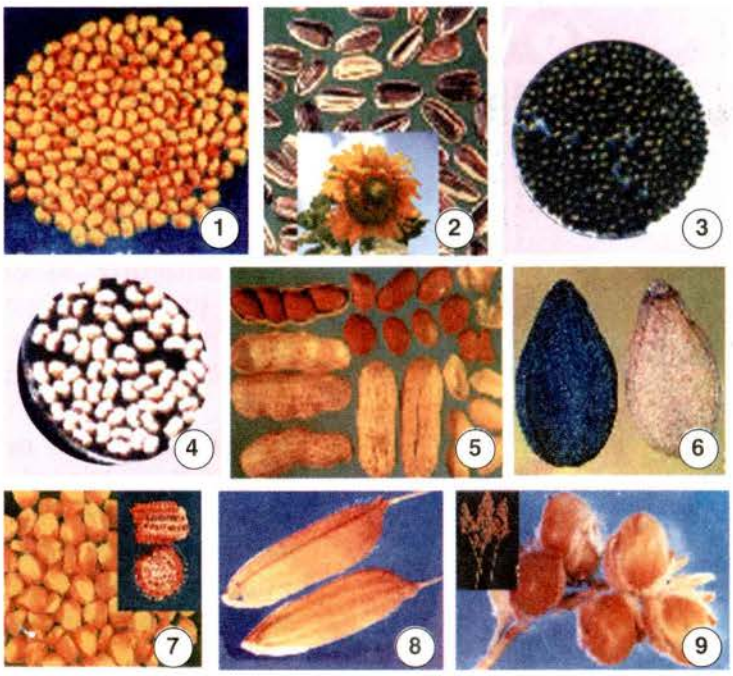
Soaking

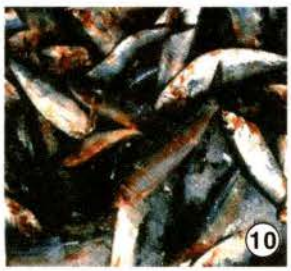
Some feedstuffs have to be soaked in water for 6-24 h at room temperature. Soaking is sometimes done with heat to soften the grains that swell during the process and facilitate removal of some toxins (antinutritional factors) in some feed ingredients such as ipil-ipil *Leucaena leucocephala* leaf meal, cassava leaf meal, etc. Soaking *Leucaena* leaves which are rich in protein and some minerals releases mimosine, a non-protein amino acid that is stable to heat. Mimosine interferes with enzyme production and destroys the hepatopancreas of shrimps and liver of fish. Soaking the *Leucaena* leaves for 24 h reduces the mimosine content to an acceptable level. Incorporation of *Leucaena* leaf meal in shrimp feed should not be more than 5% to avoid toxicity. Untreated cassava leaf meal contains poisonous hydrocyanic acid. Cassava leaf meal when soaked for 6-12 h or blanched with boiling water releases its cyanogens and produces a safe cassava leaf meal.

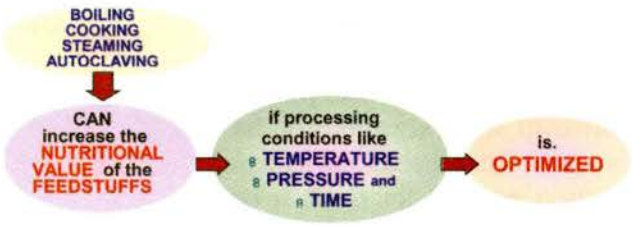
Heating and Cooking

Heat treatment is applied to dry feedstuffs to inactivate some antinutritional factors and increase utilization of nutrients. These antinutritional factors present in raw soybeans and other legumes can markedly affect the intestinal tract of the animals and prevent digestion and utilization of many nutrients particularly proteins and vitamins. The time and temperature of heating a feedstuff is of great importance in obtaining a good quality feed. In moist heat treatment, the sample is boiled for 30 min at 100°C, pressure cooked for 10 min at 120°C, or steamed for 30 min. In dry heat treatment, the sample is sun-dried for 6-12 h, oven-dried for 12 h at 60°C, or roasted for 2-5 min at 250°C

Soybean ① is a very good source of protein but it contains an antinutritive factor called trypsin inhibitor. Trypsin, an enzyme that digests protein is destroyed by the trypsin inhibitor in raw soybean meal. Sunflower meal ② is also a potential protein source but contains protease inhibitor that is destroyed by heat. Legumes, like mung beans ③, and cowpea ④, peanuts ⑤ and sesame seeds ⑥, and cereals such as corn kernel ⑦, rice grains ⑧, and sorghum ⑨ are potential ingredients but may contain non-







nutritive components if not properly processed.

Protease or trypsin inhibitors bind trypsin to form an inactive compound. Soaking and dry heating at 175-195°C for 15 min or pressure cooking for 10 min at 120°C inactivates these inhibitors. Lectins in raw soybean meals are destroyed or inactivated by boiling in water or autoclaving for 10 minutes at 120°C resulting in a digestible soybean meal. There are anti-vitamin D and E substances in raw soybeans that bind these vitamins. Autoclaving or boiling for 10 to 30 minutes destroys these factors making the vitamins available to fish.

Thiaminase, an enzyme in raw and spoiled fish ⑩, mussels ⑪, oysters ⑫, and clams ⑬ destroys the vitamin, thiamin and is inactivated by boiling in water or autoclaving for 30 minutes. This method makes raw and spoiled fish, mussels, and clams safe for use.

Moist heat is more effective than dry heat for legume seeds. Moist heat treatment also improves the digestibility and nutritional values of grains. It is also used to extract oil from oilseeds. Likewise, dry heat treatment increases the nutritional value of feedstuff but such treatment makes handling and pelleting easier than in the moist treatment

Dehulling

Tannins are found mostly in seed coats or hulls of colored beans ①. Tannins and phytates bind proteins, minerals, and vitamins thus reducing the availability of these nutrients. Dehulling removes most of the tannins by splitting or peeling the soft seed coat using a dehuller to separate the hulls ②. This method is done by use of a splitting machine or by soaking 1 part of the seeds by volume in 3 parts of water for 4 to 6 h, followed by sundrying for 12 h or oven drying at 80°C for 12 h. Subsequently, splitting is done by using an ordinary corn mill or by hand pressing. Another way is by soaking the seeds in water or 12 to 48 h and allowing them to germinate. The germinated seeds are then dehulled and sundried.

Extraction with organic solvent and chemical treatment

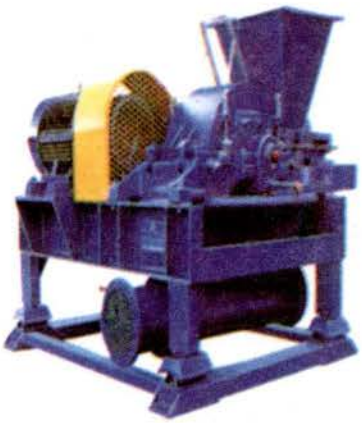
Organic solvents are also used to remove the antinutritional components of various feedstuffs. Extraction of lipid from leguminous seeds such as rape seed ①, and black beans ② with alcohol and water eliminates the beany flavor. Extraction of oil from cottonseed meal ③ with hexane decreases the toxin gossypol.

Feed preparation techniques

To achieve good feed characteristics, feed must be prepared by the following procedures:

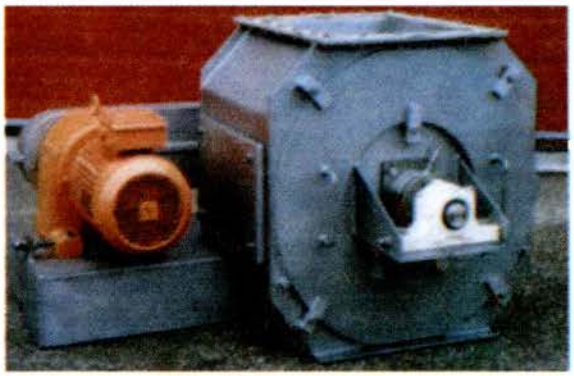
1. **Grinding** increases the surface area of ingredients. This improves mixing, digestibility, pelletability, and water stability of feedstuffs. The grinding equipment varies with the nature and texture of materials and the desired particle size. Feed components normally come in different particulate sizes and should be uniformly ground. Materials with a wide range of particle sizes do not mix well and produce structurally weak pellets.

   There are a number of grinding equipment used for size reduction of feedstuffs. The hammer mill is a commonly used machine. Basically, hammer mills are impact disintegration machines composed of a high speed rotating shaft with free swinging hammers. The size of the ground material is controlled by the size of the screens or metal bars mounted on the exit opening usually found at the bottom of the machine. Other grinding machines that can be used for grinding and size reduction of feed components are:



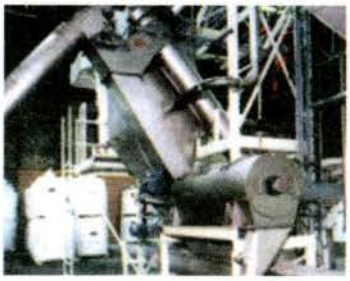
Hammer or grinder shattering

   a. **Swing type hammer mill** - an impact grinder with swinging or stationary steel bars forcing ingredients against a circular screen or solid serrated section designated as a striking plate. Materials are held in the grinding chamber until they are reduced to the size of the screen openings. This type of hammer mill efficiently grinds dry and low-fat ingredients.
   b. **Attrition mill** - grinding principle is through shattering by impact. However, it also imparts a shearing and cutting action. Grinding is accomplished between two discs equipped with replaceable wearing surfaces. This type of grinder is used for blending and smoothing out ingredients or a mixture (containing liquid) that have clumps.

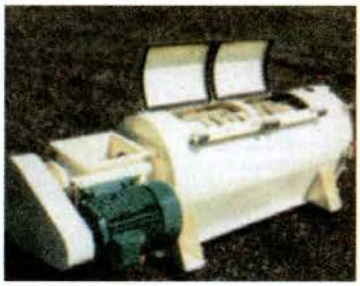


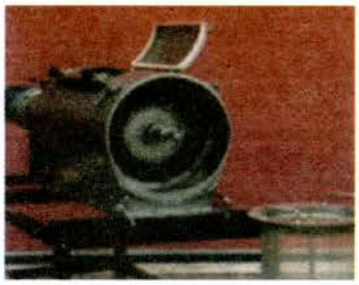
Swing type hammer mill

   c. **Roller mill** - combine cutting, attrition, and crushing processes. It has smooth or corrugated rollers set at a pre-determined distance apart rotating at the same speed and with the material passing between the rollers. An additional tearing action may be provided by the bottom roll lateral corrugations or by operating the rolls at different speeds.



Attrition mill





Roller mill

Cutter mill

Sieving

d. Cutters - reduce dry particle solids mainly by shearing with knife edges against a striking plate. The mill consists of a rotating shaft with four parallel knives attached and a screen occupying one fourth of the 360° rotation. The mill is best suited for cracking whole grains with a minimum amount of fines. Cutters should not be used as a final process for reducing the size of ingredients used in aquafeeds.

2. **Size grading or sieving** screens ground materials to obtain a specific particle size of the feedstuffs. The size of the screen opening will determine the largest particle size that will pass through the screen which is usually expressed in mesh number. A No.40 mesh sieve (425 microns) is often used. Vibrating screens operate with high frequency and low amplitude. Some machines built with gas or airtight casings and suction fans are used for screening dusty materials like rice bran. Coarse materials (big particles) that do not pass thru the sieve are returned to the grinder and reground until all materials pass through the sieve.

3. **Weighing** of all ingredients. Weighing is a very important step in feed preparation. The feed components as prescribed in the feed formulation must be accurately weighed. An accurate balance with a taring device is best because it can be adjusted to zero and allows weighing with the container. The type of weighing machines depends on the amount and kind of feed ingredients to be weighed.

4. **Mixing** of weighed ingredients. Mixing is the process of scattering dissimilar parts into a blend. Feed ingredients are sequentially added and mixed for at least 5 min to produce a well blended mixture. Mixing of the feed components to form the meal are done in batches. There are two mixing operations involved in the process: premixing of micronutrients, and blending of the bulk diet components. Feed components are sequentially added a little at a time or by batch. The three basic mechanisms in mixing are: transfer of adjacent particles from one location in the mass to another, distribution of particles over a freshly developed surface, and slipping of particles in the mass.

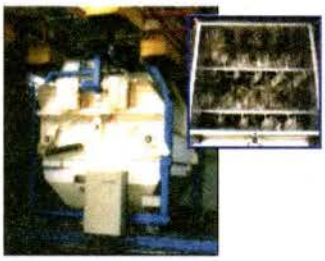
Weighing of feed ingredients

There are three general types of mixers used in the feed milling industry:

- **Horizontal ribbon type mixers** (batch and continuous). Normally, mixing is done through a horizontal ribbon type mixer when mixing ingredients of different particle size with some liquid. The design and configuration of the mixing ribbon in relation to the kind of material being mixed is essential.

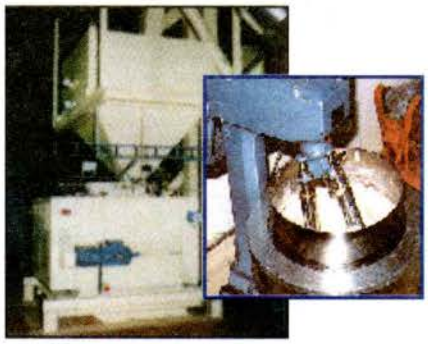
Horizontal mixer

- **Vertical boot loading mixer**. Mixers should be constructed for easy loading, meal discharge as well as cleaning of the mixer unit after every mixing operation. Residual meal can cause quality problems in the next batch or load.



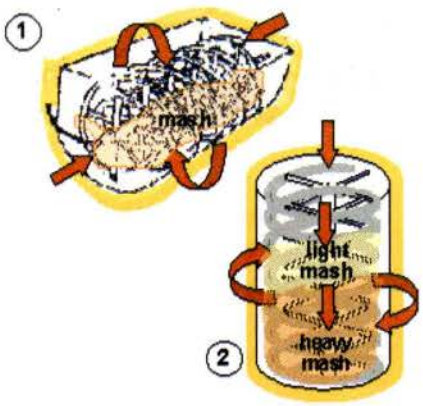
Vertical mixer

- **Oscillating screw mixer**. Mixing, particularly when working with dry powders in the absence of dosing liquids, can cause fine feed particles to escape from the mixer unless proper covers are provided for in the mixing vessel. The power and speed of the mixer drives should be designed to meet the needs of the mixer when loaded at a maximum material density.

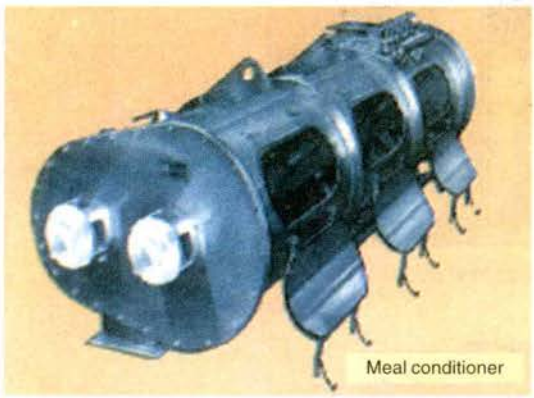


Oscillating screw mixer

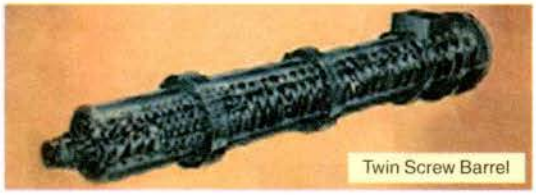
Horizontal mixers ① are preferred in the industry because handling and mixing of bulk and high density materials are efficiently achieved. In vertical mixers ② high density ingredients tend to settle at the bottom leaving light weight (powdery) ingredients to escape during mixing. After the feed ingredients are mixed, the meal requires conditioning before pelletizing.



5. Conditioning is the process of adding liquids to the meal, kneading of the meal and steaming to allow some of the starch and other binders to gelatinize. The meal is usually steamed at a pressure of about 10 to 25 pounds/inch^2 (psi) with enough time (approximately 1-2 min) allowing a starchy material to form a gel. Control of steam temperature and pressure is very essential in order not to affect some of the heat-sensitive ingredients.


Meal conditioner


Single Screw Barrel


Twin Screw Barrel

Normally, pelleting machines are provided with the necessary source of steam, metering and dosing pump for the liquid additives, and a variable speed drive. The speed drive allows for the adjustment of the meal conditioning time to obtain optimum pellet quality. In conventional conditioning, about 30-35% of starch is gelatinized. However, the amount of starch and type of ingredients used may not be sufficient to produce a water stable pellet. Therefore, for pelleted shrimp feed it is recommended that at least 50% of the starch should be gelatinized. This is done by increasing the steam pressure to 28 psi.

Most pellet mills are equipped with one to three direct steam conditioner barrels where steam can be injected into the mash or into the conditioner jackets to partially gelatinize starch or diet binders (see Chapter 4). Increasing the retention time or using multiple conditioners and control of steam pressure can increase starch gelatinization. The feed mixture in the third conditioner reaches a temperature higher than 90°C before entering the pellet die. Steaming improves the water stability and digestibility of the feed and kills most harmful bacteria. In small-scale feed preparation, steaming is necessary for shrimp feeds but may not be necessary for fish diets. Steamed pellets are stable in water for 4-12 h depending on the effectiveness of the binder. Unsteamed pellets ① break up within 30 min ②.


1


2

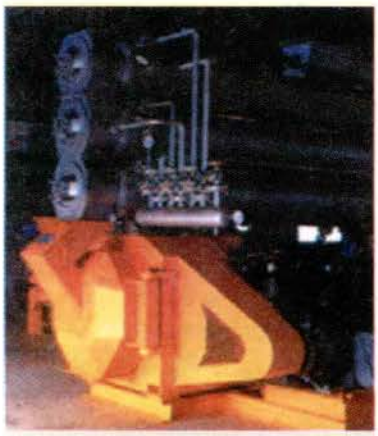
6. Pelleting and Extrusion. Pelleting involves forcing the mixture through holes of a metal die plate to transform a soft feed into a hard pellet. The process is accomplished by compression, extrusion, and adhesion. The primary objective of pelleting is to compact the feed from the mixed powder form or meal. For shrimp feed, pelleting prevents the feed from immediate dispersion in the pond water which results in feed loss and water pollution. The loss of not more than 10% of the pellet weight after a 10 min immersion in water is considered acceptable.

The more common types of pelleting machines are:

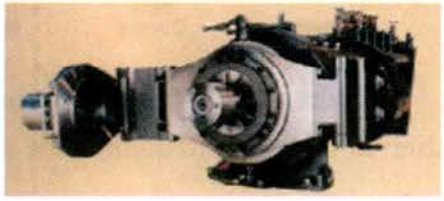
- ❑ Ring die pellet mill ①
- ❑ Flat die pellet mill ②
- ❑ Scheuler type pellet
- ❑ Dimpled rolls pelletizer
- ❑ Screw type pellet extruder press

The ring and flat die pellet mills are generally employed in aquaculture feed mills. Both are capable of producing fairly small pellets suitable for aquatic feeds.

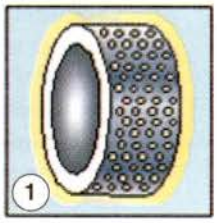
In the extrusion process the meal is heated, kneaded, and mixed. A pressure is increased to 20 to 70 bars depending on the product formulation. The sudden pressure drop at the outlet of the die results in material expansion. The length of the die channel plus the number of

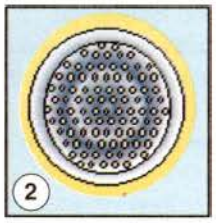


Pelleting machine

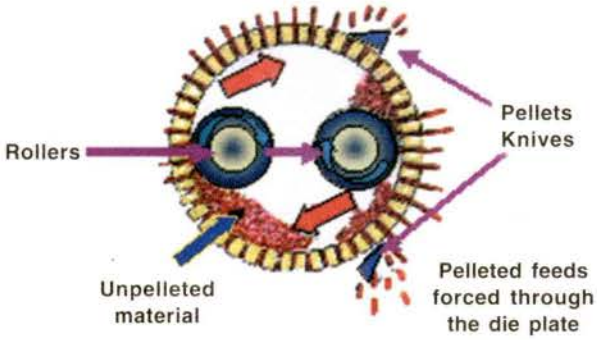


Pelleting machine drum with built-in rollers





Internal parts of a ring die pelleting machine

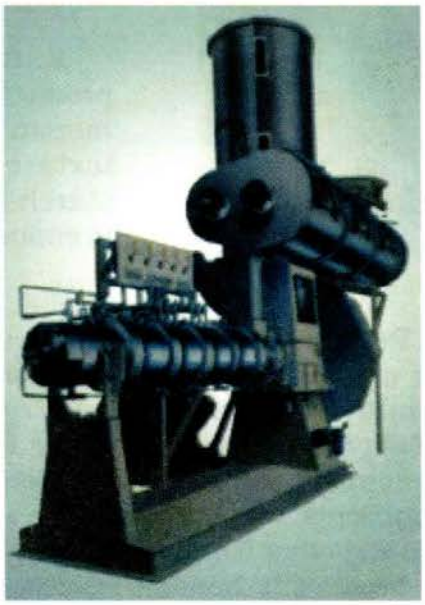


dies greatly influence the outcome of the final product. Unlike a pellet mill, the extruder can shape the material in almost any form.

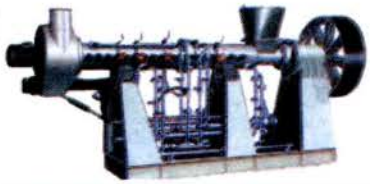
The two types of extrusion are:

Dry extrusion - the meal is extruded with water only. Usually the pellets are of burned taste and low bulk density. About 30-35% of the vitamins are damaged. The final product need not be dried but cooling is necessary.

Wet extrusion - the meal is extruded with steam and cooked at lower temperature than in dry extrusion. Vitamin losses are only about 10%.



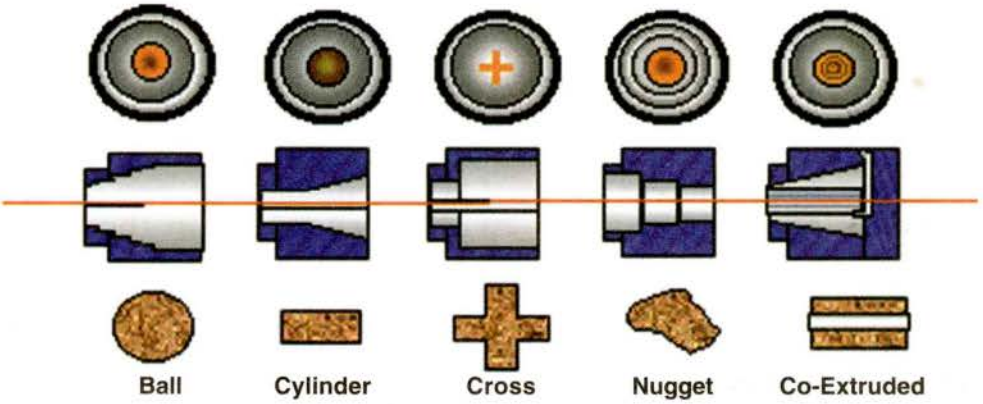
Extrusion machine



Expansion pellet mill

Internal parts of an extruder

The product is paste-like before it enters the die plate. Some of the possible shapes are:



Extrusion vs Pelleting

The decision whether to use an extrusion or a pelleting machine depends on many factors.

Extrusion	Pelleting
1. Highly flexible in feed formulation.	1. Less flexible to feed formulation.
2. Can process meal with 55% moisture.	2. Moisture content up to 17% maximum.
3. Cooking of the meal is achieved at 90% or higher.	3. Cooking of the meal is achieved at 50% only
4. Can handle fat levels up to 22% lipid in the formulation.	4. Can handle fat levels up to 8% lipid only in the formulation.
5. Can bind coarsely ground ingredients.	5. Requires finely ground ingredients.
6. Bacteria are eliminated in the process.	6. Bacteria may still be present in the final product.
7. High product durability and water stability.	7. Low product durability.
8. Higher acceptability of finished product.	8. Product are reworked through system to reduce fines.
9. Produces versatile (floating, sinking and slow sinking) feeds.	9. Produces only sinking feeds.
10. Higher capital and production costs.	10. Lower capital and production cost.

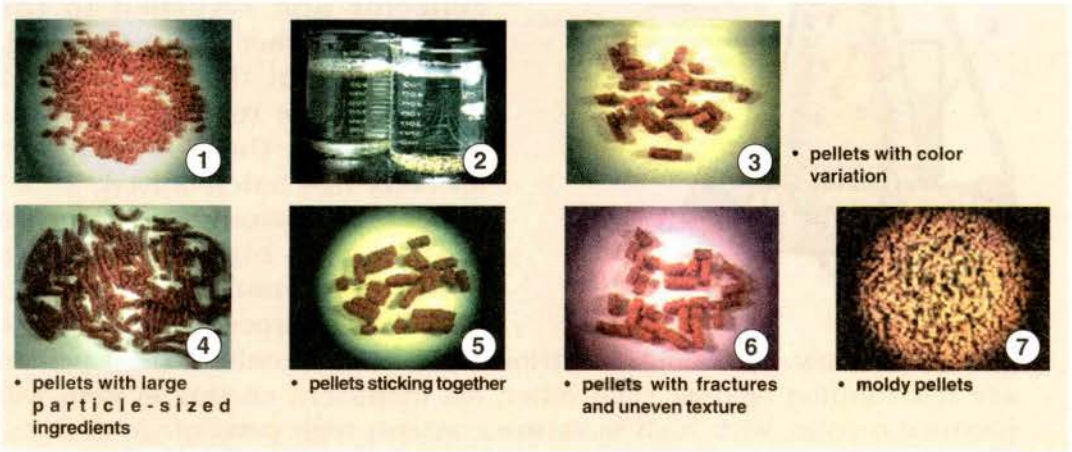
Extrusion is more efficient in producing varied forms of aquafeeds ①, human (breakfast cereals, snack foods, textured vegetable proteins (TVP) and starch-based industrial products) and premium pet foods ②.

Benefits of Pelleted Feeds

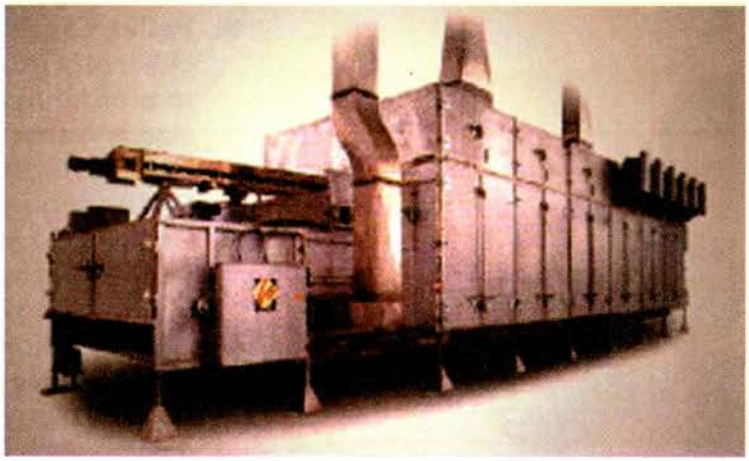
- ❑ Feeds are more stable and can be stored for at most three months with minimal nutrient degradation.
- ❑ Pellet stability is maintained during feeding.
- ❑ Feed consumption is increased thereby reducing the leaching of water-soluble nutrients.
- ❑ *Salmonella* and other harmful and disease causing organisms are destroyed.
- ❑ Finished product is handled easily.

Physical characteristics of a good pellet

The ideal pellet is smooth, has uniform length and size ①, water stable ②, without color ③ and size variation ④ within a batch, does not clump ⑤, without fractures ⑥, and not wet and moldy ⑦.



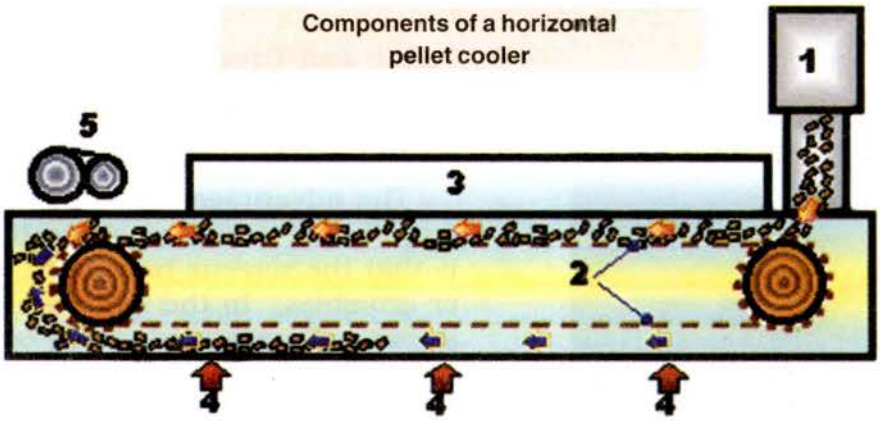
7. **Pellet Cooling and Drying.** The pellets leaving the die are still hot and must be cooled to allow the binders to set and harden. The cooling process is accomplished by blowing cool air in a counter current direction allowing the binder to set as the pellets are cooled. The vertical or horizontal type pellet coolers are commonly used.



Horizontal cooler

Horizontal cooler uses a number of horizontal moving perforated trays with the hot pellets moving countercurrent to the cooling air. One disadvantage of a horizontal pellet cooler is that it occupies a larger floor area than a vertical cooler of similar size. Of the two pellet coolers, the vertical type is commonly used for small capacity feed plants together with the pellet crumbler, installed at the lower exit end of the pellet cooler. A cyclone collector is also necessary to recover fine feed particles that may be carried

Components of a horizontal pellet cooler

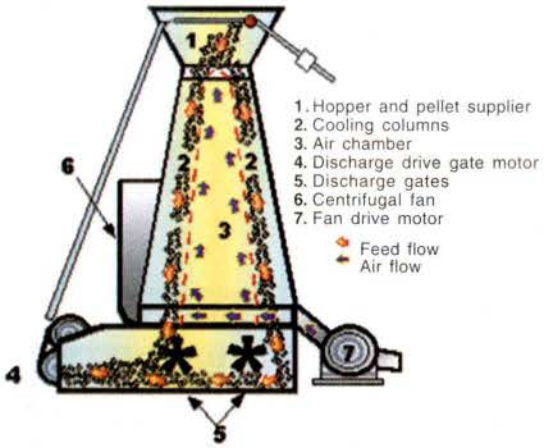


1. Feeder device
2. Product carrying belt
3. Air chamber
4. Air inlet
5. Cooling belt drive

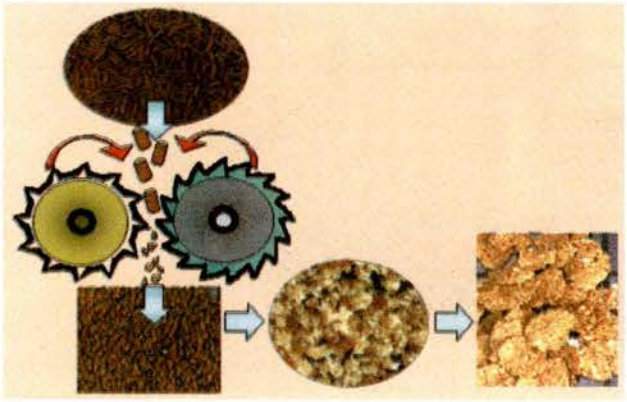
Feed flow
Air flow

Vertical cooler

Components of a vertical pellet cooler



by the cooling air as it passes through the pellets. Coolers extract moisture and heat and separate the fine particles from the pellets. Fines are removed from the cooling air by a cyclone collector and returned to the meal conditioner for re-pelleting. It is important that the recycled meal fines are removed from the system when there is a change for every new batch of feed. Pellet temperature should not be more than 4-5°C higher than the ambient or room temperature. The cooling process should have a high air flow, and long retention time in the cooler where pellets are not rubbing against each other. An inefficient cooling system will produce a pellet with high moisture content, high percentage of fines, and poor water stability.



Breaking of cylinder pellets into crumbled feeds using rotating rollers

8. **Pellet Crumbler**. The size of the pellets that can be efficiently produced by a pelleting machine is limited to a minimum diameter of about 2.5 mm. This feed size is rather large for fry of up to 3 g size to handle. The most effective way to produce small size of feed for the young fry is to reduce the pellet by crumbling. Crumbled feeds are rhombic and irregular in shape because they are produced by breaking the cylindrical pellets.

   The crumbler is composed of two closely spaced rotating steel rollers that crush the pellets as they pass through the rollers. Most crumblers are designed so that the surfaces of the two rollers run at slightly different speeds. This gives a shearing action on the pellets as they are crushed between the rollers to yield a 0.5 to 1.0 mm crumble size.

9. **Pellet and Crumbled Feed Cleaner.** Fines produced after pelleting must be removed by sifting or by using a fine separator in order to produce fine-free feeds. To separate fines from the pellets, multi-decked vibrating screens or rotary type sifters are used.

   The advantages of the rotary sifter are its compact size, higher output, and effective separation of fines. However, the disadvantage is that the screens have to be changed for each desired size of pellets or crumbles. In the multi-decked vibrating screen a number of pellet sizes can be obtained in one operation due to the different sizes of the screens installed in the machine.

Sifter/ Fines separator

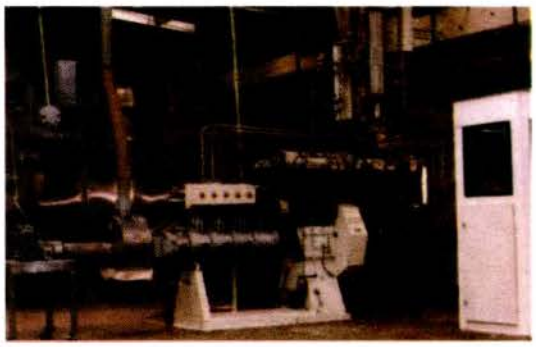
10. Product Packaging and Storage. Packaging and storage of a pelleted feed play very important roles in maintaining good feed quality. Feeds usually have a limited shelf life (3 - 4 months without antioxidants). Packaging materials such as porous sacks and loosely sewed openings can shorten shelf-life.

Freshness of the feed can be maintained when packed in bags made of laminated polypropylene (PLP) or paper bags with liners. The PLP is light, very strong, inexpensive, and provides good water proofing. Paper bags are prone to tearing during handling and are not recommended when transporting feeds to far places. Unlike feeds for livestock, aquafeeds are packed in smaller sizes of 5, 10, and 25 kilograms to avoid tearing of the bags.

Packing of pelleted feeds requires care and attention to prevent breaking of the pellets and production of fines. The very essential components of feeds such as vitamins, minerals, and lipids may be adversely affected by prolonged exposure to light, excessive moisture, poor ventilation or high temperature. In the farms, pellets are stored in tightly covered plastic buckets or jars after dispensing from feed bags.

The steps to be followed in large-scale, small-scale, and larval feed preparation and a guide to the type and kind of feed at various sizes and ages of aquatic animals are presented.

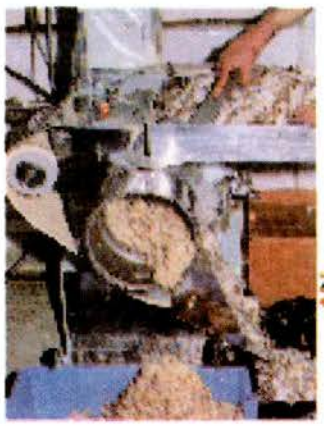
A. Steps in Large-Scale Feed Preparation

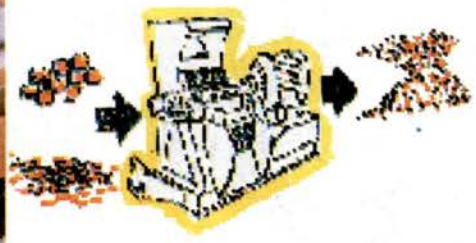


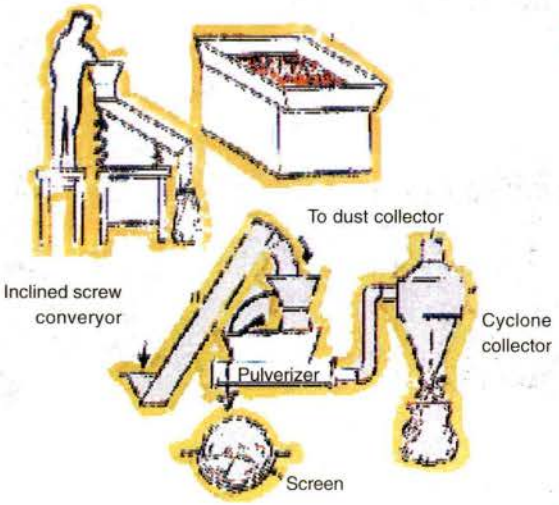
Sophisticated and large equipment are used in a feed mill. Pelleted feeds can be made much better and in larger amounts (as much as 1-20 tons of feed a day). The steps involved are:

Grinding ❶

All dry ingredients are ground separately with a hammer mill or crusher







2 Sifting and sieving

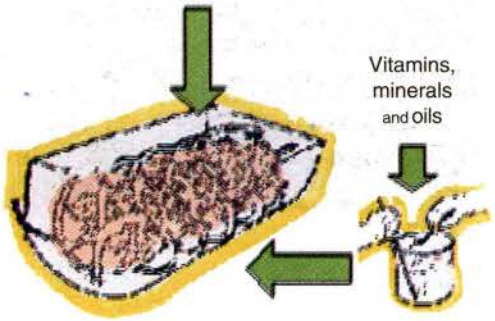
Coarse materials are separated by sieving and returned to the grinder until all pass through the sieve. By means of a screw conveyor, sifted materials pass to the pulverizer and ground to uniform size. The sieved ingredients are blended for 5 to 10 min to obtain a homogenous product. The ground ingredient should be placed in tightly covered containers, and stored in an adequately ventilated room.

3 Weighing

All ingredients are individually weighed as accurately as possible by using a top loading weighing balance with a taring device.

4 Mixing

The formulation is then mixed by batch in a large mixer. The major ingredients (protein sources like fish meals, leaf meals, etc.) are poured into the mixer one at a time and mixed for 5 min. The other dry ingredients are added into the mixer one at a time and mixed for another 5 min.

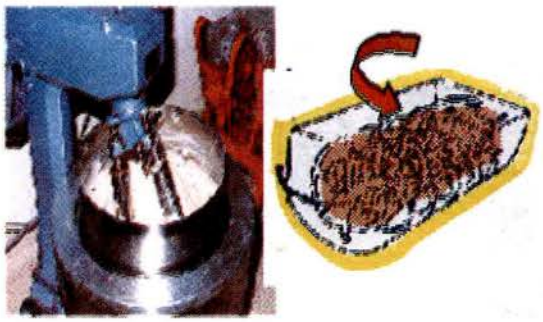


5 Batch mixing

In a separate container, the micronutrients like vitamins and minerals are combined with a small amount of dry mixed ingredients and part of the lipid sources in the formula is added to the dry ingredients, thoroughly mixed, and poured into the mixer. This ensures good mixing of small feed components with the major ones.

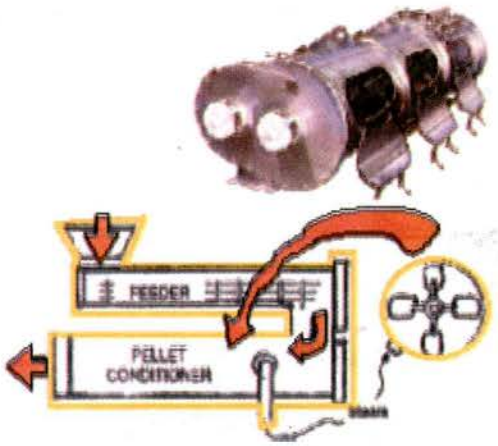
Note:
Never place micronurients in an empty mixer

When all dry ingredients including the micronutrients have been mixed, add the rest of the oil and mix for 15 min.



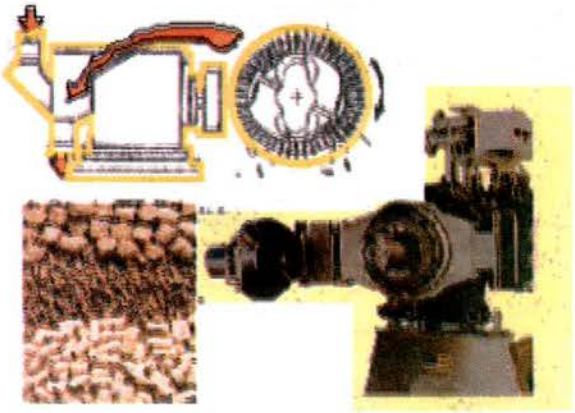
Conditioning and gelatinization 6

The starch or binder is gelatinized by introducing steam into the pellet conditioner. The amount of water and heat required to cook the dried mixed ingredient is equivalent to the amount of steam injected. This is determined by water stability and palatability tests.



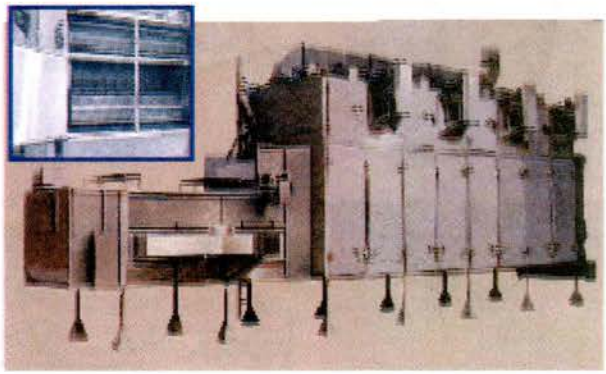
Pelleting and extrusion 7

The conditioned feed mix is pelletized to the desired size. As the mixture passes through the die hole (2-22 mm in diameter), a rotating blade cuts it to the desired length (1.5 – 2.5 times the diameter).



Drying and cooling 8

The pellets drop automatically into a cooling chamber with air at ambient temperature.



Packaging and storage 9

Special feed finishing such as coating and glazing may be adopted to improve the quality and strength of the pellets.

The finished feed are packed or bagged and labeled properly including the date of manufacture. Feeds are normally good only for three months. Bags should be piled (not more than 5 bags high) on a platform 12-15 cm from the floor in a well-ventilated room. The first bags that come in should be the first ones to go out.

B. Steps in Small-Scale Feed Preparation

Mixing of locally available feed ingredients at home is a practical way of producing feeds for shrimps and fishes.

1 Grinding

Dry ingredients are finely ground until fine particles of similar size are obtained.

2 Screening

The ground ingredients are sieved or sifted using a No.40 sieve or a nylon net with mesh size of 425 mm.

3 Weighing

All ingredients are weighed or measured accurately in a bowl or basin.

4 Mixing

All dry ingredients are mixed thoroughly. The vitamins and minerals are mixed separately with the lipid source (oil) and added to the dry mixture. The mixture is thoroughly mixed for another 5 min.

5 Gelatinization or cooking

To cook corn starch, bread flour, and binders, 1 part starch is added into 4 parts of water (50 g in 200 ml water for 1 kg of feed) in a saucepan. The starch is cooked into a jelly-like consistency in a double boiler.

Kneading

6

The gelatinized binder is removed from heat and allowed to cool. The cooled gelatinized starch or binder is added into the dry mixture and mixed for 5 minutes until a stiff dough is formed.

Pelleting

7

The dough is squeezed and passed through a meat grinder. The appropriate dye is used for the size of fish or shrimp to be fed (Table 5.2) for various sizes.

Steaming

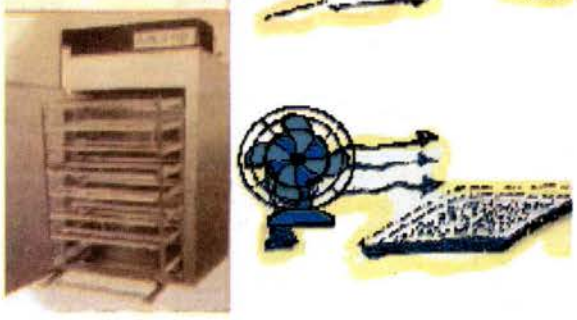
8

The pelleted feed is spread evenly on wire nets that fit inside the metal holders of the steamer. Water is boiled (5-8 cm deep) in a big pot and the pellets are steamed for 5 min. Overcooking or steaming can cause loss of nutrients and makes pellet stick to each other.

Drying and cooling

9

The steamed pellets are air dried for a few minutes with an electric fan, transferred to an oven and left overnight or for 8-12 h at 50-60°C. Pellets should not be dried under the sun to prevent destruction of some vitamins and other light-sensitive feed components.



Cutting, packaging, and storage

10

After the pellets are removed from the oven, they are cooled for 30-60 min. The dried extruded or pelleted feeds are cut to the desired lengths, placed in covered plastic jars, and stored in a cool dry place.

C. Steps in Larval Feed Preparation

1 Weighing

All ground ingredients are measured or weighed accurately.

2 Mixing

All dry ingredients are mixed thoroughly. If large batches are to be prepared, the dry ingredients can be mixed in a large cake or cement mixer.

3 Blending of oil and lecithin

The oil and lecithin are blended separately and added to the dry ingredients gradually with continuous mixing. When all the oil and lecithin have been added, mixing is continued for another 5 min.

4 Cooking of carrageenan

The carrageenan is cooked to gelatin-like consistency in a water bath at 80° to 100°C then slightly cooled.

5 Microbounding

The cooked carrageenan is added to the dry mixture and blended well until a completely homogenized mixture is obtained.

Flaking

6

The soft mass is passed through a drum dryer or flaking machine and the brittle flakes are collected.

Grading/Sieving

7

The flakes are ground gently using a mortar and pestle or meat grinder and sieved to uniform particulate size using varied mesh size sieves.

Different particle sizes for larval feeds are 25, 50-60, 125, and 250 µm.

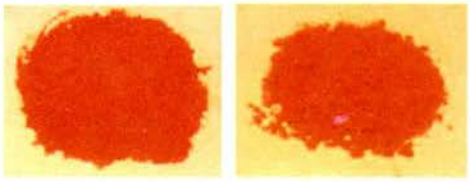


Table 5.2 Guide to types and kinds of feed for aquatic animals at various sizes and ages

Fish size approx wt (g)	Type of feed	Feed size, dia (mm)	Length (mm)
0.35 g and less	Starter	1.0	-
2 – 5	Grower	2.0	-
5 – 12	Grower	3.0	2–3
12 – 20	Finisher	5.0	3–5
20 – 30	Finsher	7.0	5–7

Shrimp age (days)	Shrimp size (g)	Feed type	Feed form	Feed size (mm)	Feed length (mm)
$PL_{15} - PL_{35}$	< 1.0	Starter	Fine crumble	0.6 – 1.0	-
$PL_{36} - PL_{55}$	2 – 3	Starter	Coarse crumble	1.0 – 2.0	-
$PL_{56} - PL_{75}$	4 – 7	Grower	Pellet	2.0 – 2.2	1.2 – 3.0
$PL_{76} - PL_{95}$	8 – 14	Finisher	Pellet	2.0 – 2.5	2.2 – 5.0
$PL_{96} - PL_{115}$	14 – 22	Finisher	Pellet	2.2 – 2.5	2.2 – 5.0
$PL_{116} - PL_{135}$	23 – >30	Finisher	Pellet	2.5 – 3.0	4.0 – 8.0

Stocking of 10 bags per pallet is practiced when feeds are stored for short periods (usually 3 to 5 days).

Indiscriminate storage of feed can trigger rapid deterioration of nutrients.

Quality Control

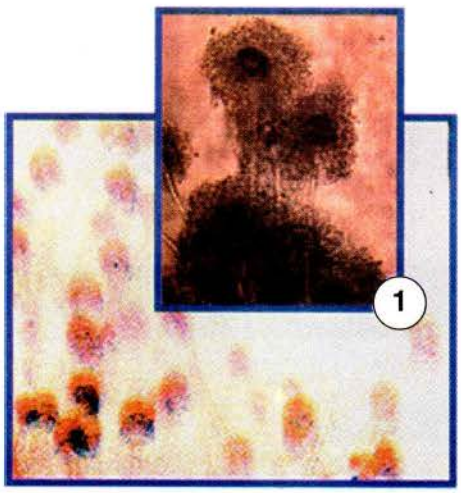
Feed quality control is one of the major keys to a successful culture by selecting, evaluating, and monitoring feed ingredients during processing, manufacture, and until storage of aquafeeds. Storage conditions should be optimal so that the nutrients in feed ingredients and feeds do not deteriorate and economic losses of feed millers and fish culturists are avoided. The following pointers should be followed to avoid rapid deterioration of feeds.

- ❑ moisture content should not be more than 10% and relative humidity not greater than 65% to prevent fungal growth and insect infestation that grows best at 26°-37°C.
- ❑ temperature should not be too high to prevent destruction and reduction of nutrient.
- ❑ exposure to oxygen should be limited to prevent oxidative rancidity particularly in feeds of high lipid content. The peroxides formed from lipid peroxidation may bind with proteins or vitamins and reduce their availability.

Feed should be:

- ❑ free from insects and rodents and spoiled feeds should not be used or recycled.
- ❑ stored in dry, cool, and well ventilated area and not under direct sunlight. Spoilage will occur immediately if feeds become wet.
- ❑ placed on wooden pallets and not directly on floors with not more than 5 bags per pallet if intended for long storage. Feeds should be stored away from walls to avoid moisture accumulation due to heat transfer from the wall to the feed during sudden temperature changes.
- ❑ used within three months from the time of processing and production. The first one that is stored should be the first to be used. The first-in first-out policy of storage should be strictly observed.

Feed mill sanitation and maintenance



Aspergillus sp. on contaminated feed particles.

Effective management and proper maintenance of the storehouses and feed mill help ensure good quality feeds. Sanitation prevents infestation of insects, fungi, and bacteria. Infested materials should be removed and the infested areas should be disinfected. Only after disinfection can a new batch of feedstuff and feeds be stored. To prevent and control insect infestation and fungal and bacterial contamination particularly *Aspergillus flavus* ① and *Salmonella* in feed mills, several measures should be strictly followed.

- ❑ feeds and feedstuffs should be dried to a moisture content of 10% or less.
- ❑ feedstuffs should be processed to ensure complete destruction of bacteria, fungi, and viruses, or reduction to acceptable levels.
- ❑ whenever possible, varieties of feedstuffs that are resistant to fungi and toxins should be used. Feedstuffs such as maize, copra, and peanuts are easily contaminated with aflatoxin.

- ❑ environment-friendly chemicals such as vinegar, ammonia, and lysol should be used to disinfect the mill.
- ❑ moving parts such as gears, roller chains, etc. should be lubricated at least once every 4 h for every continuously mixed batch.
- ❑ at the end of each milling period, the feeder, conditioner, and the main drive of the pelleting machine should be always cleaned with a bag of rice bran (ordinary grade) or sawdust with cooking oil (100 ml of oil for every 2 kg of rice bran).

Summary

In processing feeds, the ingredients to be used should be free from molds and/or other harmful substances. Thus, the preparation of feedstuffs will depend on the presence or absence of harmful substances in the ingredients to be used in making feeds. Quality pellets can be produced when proper manufacturing practices are followed. Among the basic points to consider to produce good and quality pellets with minimal fines are the use of: a) satisfactory mixing practices with the right kind of mixer for the type of meal to be produced, b) the correct kind and quality of dosing liquid or binders, for the size of pellets and feed ingredients used, c) use of correct die size and thickness, d) use of proper pelleting pressure and temperature, e) correct type of pellet cooling rate and cooling air flow rates, f) correct crumbling roll setting in relation to the desired crumbled feed size, and g) post-pelleting, crumbling, and finishing treatments to remove fines and fractures from the finished product. A technically trained and skilled feed mill operator should oversee the manufacturing process.

The steps to be followed in large scale, small-scale, and larval feed preparation will guide the reader as to the type and kind of aquafeeds for fish and crustaceans at various stages and ages.

Guide questions

1. What are the important things to consider in the preparation of aquafeeds?
2. Why do we need to process feed and feed ingredients?
3. Why is grinding of ingredients a necessary step in feed preparation?
4. Explain the importance of knowing the moisture content of feedstuff and feed especially prior to storage?
5. What are some typical examples of antinutritional factors in feed ingredients and their effects on aquatic animals? How are these factors eliminated or minimized?
6. What is the purpose of pelleting and how does one make floating and sinking feeds?
7. Explain briefly the basic steps in small-scale feed preparation and compare these to steps in commercial feed preparation (milling).
8. What are the deleterious effects of prolonged and improper storage of feeds?

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CHAPTER 6

Evaluation of Feedstuffs and Aquafeeds

MYRNA B. TERUEL

Introduction

The use of quality feeds is important in the success of any aquaculture venture. Feed quality is highly dependent on the quality of raw material and the processing technique. A formulated feed which makes use of low quality raw materials will not give the fish farmer any significant benefit. Feedstuffs and finished feeds should, therefore, undergo the process of evaluation and quality control in order to produce high quality feed. Systematic evaluation of feedstuffs and feed using physical, chemical, microbiological, and biological methods is necessary to assure their effectiveness when fed to fish. This procedure starts from procurement of feedstuffs and continues to feed processing until manufacture and storage of the finished product. The finished feed must contain all the nutrients required by the fish in adequate amounts and proper proportions.

Different methods are used to evaluate feedstuffs and feed quality. In carrying out all these methods, a standard sampling procedure is necessary to obtain a representative sample. A spear probe is inserted diagonally and horizontally from one corner of the feed bag to the other. Samples are taken from all bags in case of smaller lots while only 10% are considered in bigger lots. There are four methods of feedstuff and feed evaluation: physical, chemical, microbiological, and biological. Physical method involves senses of smell, taste or sight to detect the presence of adulterants in feedstuffs and feeds. Chemical method quantifies the amount of given compound present in the feed. Microbiological method involves the use of microorganisms in the evaluation of nutrients. Biological method involves actual feeding experiment. This method is more tedious and expensive than the first three methods but gives a more accurate estimate of feed utilization.

This chapter discusses how to evaluate feedstuffs and feeds. The results of feed evaluation will be used to ensure the production of high quality feeds for fish, crustaceans, and shellfish.

Physical Evaluation

A. Use of the Senses

Rancidity and off-odors in the feed can be detected by sense of smell. Off-flavors of the main ingredients contained in the ration can be detected by sense of taste. The presence of extraneous materials like small stones, scrap metal, dirt, pieces of wood, and seeds that are added to increase the weight of the product, as well as presence of insects and molds can be detected by sight. Finally, the wetness, dryness or hardness of a feed or feed ingredient can be detected by touch. Feedstuffs and feeds of acceptable quality must be dry, free flowing, and uniform in appearance.

B. Feed Microscopy

The microscope identifies the physical composition of a feedstuff or feed ingredient that either confirms or denies the presence of unwanted materials. A high-powered compound microscope can detect even the finest ground adulterants in a sample. This method is more accurate than the use of the senses in checking adulteration in feeds and feed ingredients.

There are two types of microscope used in feed microscopy: the compound microscope, to identify the internal structure of feed components; and the stereomicroscope, to identify the external structure.

Techniques in sample preparation for feed microscopy

1. Screening. Feedstuffs or mixed feed of different particle sizes are separated by hand screen using sieves of no. 10, 20, and 30 meshes. Sieving separates fine starchy dust from the larger particles in the feed for better identification.
2. Flotation. Feedstuff or feed sample is soaked in a solvent (either carbon tetrachloride or chloroform), stirred, and allowed to settle to separate the organic portion (top fraction) from the inorganic portion (lower fraction). Each fraction is removed and placed in a petri-dish and allowed to dry at room temperature.

C. Measurement of Feedstuffs Bulk Density

The bulk density of the sample is compared with that of a pure feedstuff. If contaminants or adulterants are present, the bulk density will either be higher or lower than the values of the pure feedstuffs. Bulky feeds are less efficient in producing fish flesh. The use of bulky feed ingredients in a feed mixture instead of heavier ones lowers the total digestible nitrogen of the mixture. Bulky feed yields low biologically available energy. The bulk density is computed as weight of samples in gram per liter after the sample has been placed and poured off in a 1 l cylinder.

D. Attractability

Attractants are important components in any feed formulation since they determine how fast and how much of a feed will be taken in by the aquatic animal. A well formulated feed will be useless if the animal does not accept it. Attractability tests are carried out with a single animal and effectiveness is measured usually with a stopwatch based on how fast the animal is attracted to the pellet.

E. Water Stability

Feeds should be stable in water for a certain period to increase their availability to aquatic animals. The feed has to maintain its integrity in the water so that the entire feed is consumed. This is especially true if the species to be fed are slow feeders such as crustaceans. If feeds are not water stable, they disintegrate rapidly losing much of the nutrient content and resulting in feed wastage and degradation of water quality. The use of efficient binders and processing techniques, such as steaming and extrusion, produces a more stable pellet. A simple test to determine whether a feed is water stable or not is by crumbling the pellet by hand or checking for rough edges. Another method for testing water stability is by determining weight loss of the pellet after it is placed in water for a specific period. Correct interpretation of the results is necessary in testing other binders to improve water stability. Proper processing condition for the formulated diet and the binders used are equally important in achieving a water stable pellet. The higher the percentage of pellet disintegration at a given time, the higher the weight loss, and the lower the pellet stability.

A simple method of determining water stability is as follows:

1. Wire baskets are totally oven-dried at 100°C (1-3 h), cooled in a dessicator, and weighed to constant weight.
2. A certain amount of feed (about 5 g) with known moisture content is then placed in the wire basket.
3. The wire baskets with feed are then allowed to stay in the water under conditions similar to those of the experimental tanks at designated times (2, 4, 6, and 8 h).
4. The wire baskets are then oven-dried, cooled in a dessicator, and weighed to constant weight.
5. Percent dry weight loss is calculated after subtracting the basket weight.
6. Percent water stability is then computed as:

$$\% \text{ Water Stability} = \frac{Fo}{Io} \times 100$$

where: Io = Initial dry weight of feed
Fo = final dry weight of feed

Example: Compute the water stability of a feed

$$\begin{aligned} \text{wt feed (as is)} &= 5.26\ \text{g} \\ \text{dry matter (DM)} &= 95\% \\ \text{wt feed (dry basis)} &= \text{wt feed (as is)} \times \frac{\%DM}{100} \end{aligned}$$

$$\text{Io, initial dry wt of feed} = 5.26 \times \frac{95}{100}$$

$$\text{Io} = 5.0\ \text{g}$$

After the indicated immersion time:

$$\begin{aligned} \text{wt feed + basket} &= 12.5\ \text{g} \\ \text{wt empty basket} &= 8.0\ \text{g} \end{aligned}$$

$$\text{Fo, final dry wt of feed} = \text{wt basket + feed} - \text{wt basket}$$

$$\begin{aligned} \text{Fo} &= 12.5 - 8.0 \\ &= 4.5\ \text{g} \end{aligned}$$

$$\%\ \text{water stability} = \frac{\text{Fo}}{\text{Io}} \times 100 = \frac{4.5}{5.0} \times 100 = 90\%$$

Chemical evaluation

A. Proximate analysis

The proximate composition is an index to the nutritive value of feeds and feedstuffs. This analytical technique is designed to differentiate between nutritive and non-nutritive components and analyze moisture/dry matter content, crude protein, crude fat or ether-extract, crude fiber, and ash. Nitrogen–free extract (NFE) is obtained by subtracting the sum of these constituents from 100. Although proximate analysis gives a general indication of the feed value, it does not deal with specific nutrients. This method establishes the category in which a feedstuff belongs and is a useful descriptive device in establishing the characteristics of feeds (Figure 6.1).

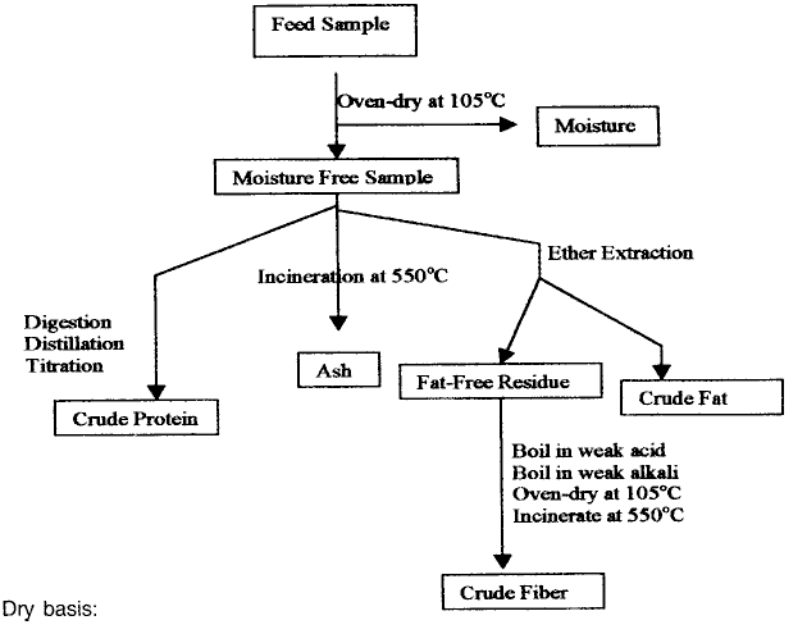

Figure 6.1
Flow diagram for the proximate analysis of feedstuffs and feeds.

1. **Moisture/dry matter content.** It is important to determine how much of the feed sample weight is actually made up of dry matter especially in formulating rations using ingredients with high moisture content. Moisture is a diluent of the nutrient in feedstuffs and feed and has an effect on their stability and shelf life. Feedstuffs and feeds should be dried to contain less than 10% moisture prior to storage especially in tropical countries. High moisture content can lead to growth of molds during storage of feedstuffs and feeds.

   Moisture content determination involves drying the feed sample to constant weight using a drying oven or a moisture balance (Figure 6.2). It is important to interconvert feed analysis data from dry-matter basis to as-received basis for a more accurate feeding management using the following formulae:

Figure 6.2
A moisture balance used to determine moisture content of feedstuffs and aquafeeds.

$$\% \text{ nutrient (as received)} = \% \text{ nutrient (dry basis)} \times (\% \text{ dry matter})/100$$

$$\% \text{ nutrient (dry basis)} = \% \frac{\text{nutrient (as received)}}{\% \text{ dry matter}} \times 100$$

2. **Crude Protein**. Crude protein determination is done using the Kjeldahl method. The sample is digested in concentrated sulfuric acid, resulting in the complete oxidation of all organic materials and the nitrogen is converted to ammonium sulfate. Excess sodium hydroxide is then added to liberate ammonia, which is absorbed in boric acid and titrated with standard hydrochloric acid. The procedure for protein analysis using Kjeldahl method is in Appendix B1. A picture of the Kjeldahl distillation-titration (Kjeltec™) instrument used to analyze the crude protein content of feedstuffs and feeds is shown in Figure 6.3. The term crude protein means that other nitrogenous materials, which are not true proteins, such as urea, amides. nucleic acids, and amino sugars may be present. Since fish has very limited ability to utilize non-protein nitrogen (NPN), the measurement of the true amino acid protein should be done on feedstuffs.

Figure 6.3
The Kjeldahl distillation-titration (Kjeltec™) apparatus used to analyze crude protein.

3. **Crude fat or ether extract.** The crude fat content of feedstuffs and feeds is determined by extraction of ground samples with ether. Ether-soluble materials include a variety of organic compounds but only a few have nutritional significance such as the true fats, fatty acid esters, compound lipids, and fat soluble vitamins or pro-vitamins such as the carotenoids. This method does not remove all lipids, especially the phospholipids and other fats bound to proteins.

Figure 6.4
The Soxtec™ apparatus used for crude fat analysis.

Figure 6.5
A muffle furnace used to analyze the ash content of feedstuffs and aquafeeds.

Figure 6.6
The Fibertec™ used for crude fiber determination.

If the ether extract contains a large percentage of fats and fatty acid esters, this method of evaluation is valid because it aims to isolate a fraction of feedstuff which has a high caloric value. However, if the extract contains a large percentage of plant waxes, essential oils, resins or similar compounds, this analysis has little meaning, as these compounds have little value to fish. A procedure for fat analysis is shown in Appendix B2. Soxtec™ apparatus used in crude fat analysis of feedstuffs and feed is shown in Figure 6.4.

4. **Ash or mineral matter.** Ash or the total mineral content is measured by burning the feed sample in a muffle furnace at 550-600°C. This burns all organic matter, leaving a residue of ash or inorganic mineral salts. Excessively high ash values may indicate contamination, or dilution of feedstuffs with such substances as limestone and salt. The content of calcium, phosphorus, magnesium, and other minerals in feeds and feedstuffs are determined from the ashed sample. Figure 6.5 shows a picture of a muffle furnace used for ash determination.

5. **Crude fiber.** In crude fiber determination the ether-extracted sample is boiled in dilute acid, then in dilute base, dried, and burned in a furnace. The difference in weight before and after burning is the crude fiber fraction. This method simulates digestion occurring in the gastric stomach and in the small intestines of the fish. Crude fiber is made up primarily of plant structural carbohydrates, such as cellulose and hemicellulose, and it also contains some lignin, a highly indigestible material associated with the fibrous portion of plant tissues. Figure 6.6 shows a picture of the Fibertec™, an instrument used for crude fiber determination.

6. **Nitrogen-free extract (NFE).** NFE is derived by subtracting the sum of the other proximate components, crude protein, crude fat, ash, crude fiber on a dry weight basis from 100. It represents mainly starch, sugars, and other readily soluble carbohydrates. It may also include hemicelluloses and some of the more soluble lignin.

B. Methods of Protein Evaluation

1. **True protein value determination** (Spectrophotometric methods).
   a. Biuret method. This method is applicable to extracted liquid fish protein aliquots, with a protein concentration of between 0.1 to 0.5 mg N/ml. The method is based on the reaction of Cu^{+2} with peptides in alkaline solution to yield a purple complex that has a peak absorption at 545 nm.
   b. Lowry method. This method estimates total protein of feedstuffs and feeds. It uses Folin-Ciocalteu reagent and has a blue end color with extinction at 660 nm. The principle behind this method is the reduction of the phosphomolybdic acid-phosphotungstic acid (Folin-Ciocalteu) reagent by tyrosine and tryptophan residues in the protein.

2. **Measurement of protein quality.** The usefulness of feeds as sources of protein depends primarily on the total concentration of protein and the composition of amino acids making up the protein. Imbalance among amino acids in a formulated feed results in inadequate protein nutrition. The imbalance decreases growth and feed efficiency or may result in fish mortality. The relative usefulness of the protein of a particular feed in meeting the needs of the fish is known as its quality.

   a. Amino acid composition. Amino acid analysis is done using either an amino acid analyzer (AAA) or high performance liquid chromatograph (HPLC) with fluorescence detector. Figure 6.7 shows a picture of HPLC, an instrument used to analyze amino acid composition of a protein. HPLC utilizes o-phthalaldehyde/N-acetylcystine (OPA/AcCys) and Na hypochlorite reagent. Amino acids react with OPA/AcCys to form a fluorescent substance with an excitation wavelength of 350 nm and fluoresces at 450 nm. Protein samples are hydrolyzed and injected into a column with cation-exchange resin. Buffers of different pH and ionic strengths are pumped through the column to bring about the separation of various amino acids. The amount of fluorescent compound is directly proportional to the amino acid concentration in the eluate. The amino acid is identified in the resulting chromatogram by the retention time of the peak and quantified by the area under such peak. In the amino acid analysis by AAA, ninhydrin solution is used for color development instead of OPA/AcCys.

Figure 6.7
The high performance liquid chromatograph (HPLC) used for analysis of amino acid composition of a protein.

   b. Chemical scores for protein. This method considers the relative amounts of amino acids present in the protein as determined by chemical analysis. The quality of protein is affected by a relative deficiency of one or more essential amino acids. Whole egg protein is considered as standard or ideal in terms of amino acid composition and is commonly used as a "reference protein". The

amino acid usually in greatest deficit in a protein is called the "limiting" amino acid. The percentage of amino acid of greatest deficit is subtracted from 100 to give the chemical score for specific protein source. For example: A protein with lysine content of 40% of that in egg protein will have a chemical score of 60, provided that this is the amino acid in greatest deficit.

Table 6.1
Essential amino acid indices (EAAI) of some common feedstuffs for shrimp

Feedstuffs	EAAI values
White fish meal	0.96
Peruvian fish meal	0.92
Slipmouth fish meal	0.94
Tuna fish meal	0.92
Herring fish meal	0.95
Shrimp meal	0.98
Squid meal	0.96
Soybean meal	0.87
Ipil-ipil leaf meal	0.54
Sweet potato meal	0.53

Source: Penaflorida 1989

c. Essential amino acid index (EAAI). The EAAI method of chemically evaluating a protein considers all essential amino acids rather than the most limiting amino acid with respect to some standard. EAAI is the geometric mean of the amino acid found by comparing the content of the ten essential amino acids in a feed protein with that found in fish tissue protein. The closer the EAAI value to 1.0 the better is the protein quality. This equation is expressed algebraically as:

$$\text{EAAI} = 10\sqrt{\frac{100a}{a_e} \times \frac{100b}{b_e} \cdots \frac{100j.}{j_e}}$$

in which a, b, ... j are the percent of essential amino acids in the food protein and a_e, b_e,.... j_e are the percent of the respective amino acids in whole egg protein. For computation, it is convenient to express the equation in logarithmic form as:

$$\log \text{EAAI} = \frac{1}{10} \log\left(\frac{100a}{a_e} + \log \frac{100b}{b_e} + \cdots \log \frac{100j.}{j_e}\right)$$

Examples of essential amino acid indices of feedstuffs are in Table 6.1. Thus protein from ipil-ipil leaf meal and sweet potato meal are of poor quality compared to fish meals, shrimp meal, squid meal, and soybean meal.

Figure 6.8
The gas chromatograph (GC), an instrument used for analysis of fatty acid composition.

C. Methods of Lipid Evaluation

1. Measurement of lipid quality. The quality of lipid contained in feedstuffs and feeds is determined by its fatty acid composition which is obtained thru gas chromatographic (GC) analysis (Figure 6.8). It is a technique of separating sample into its constituents and then measuring or identifying components in same way. The separation technique involves two phases, the stationary and mobile.

The sample is carried by the mobile phase, usually an inert gas, through the stationary phase (column), where

separation takes place. Since the technique requires that samples should be volatile, lipids are converted into fatty acid methyl esters (FAME) before injection.

The measurement of fatty acids uses detectors and the most popular is the Flame Ionization Detector. Sample components eluting from the column in the gas stream are ionized creating a current that is measured by an electrometer. The peaks indicate a measure of the fatty acid concentration in the sample. The peak retentions times are used to identify the component fatty acids based on a standard fatty acid mixture.

Sample preparation involves lipid extraction from the feeds or feedstuffs (Appendix B3) followed by saponification and esterification processes, as described in Appendix B4. The output from this GC is represented by a chromatogram shown in Figure 6.9.

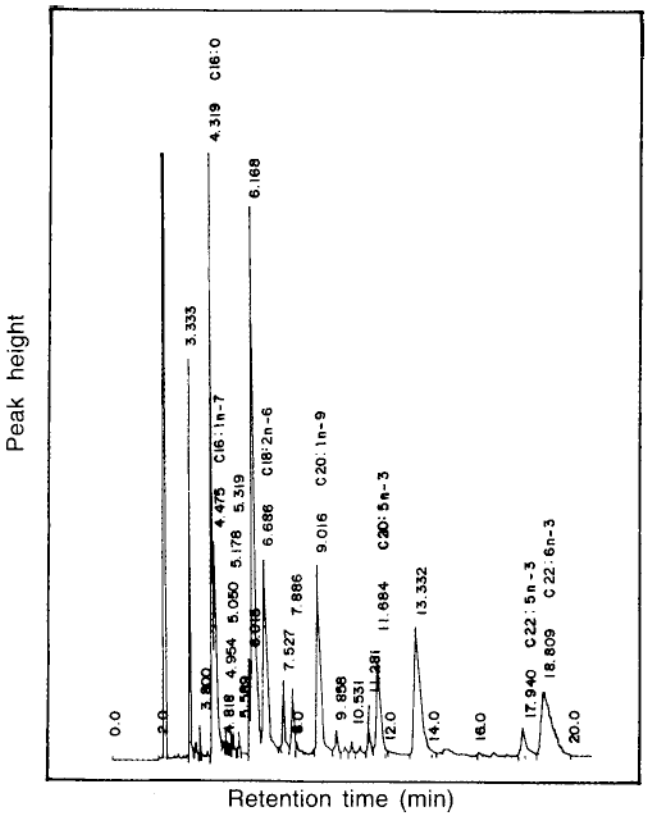

Figure 6.9
A sample gas chromatographic analysis showing retention times of various fatty acids in a feed sample.

2. Tests for lipid rancidity. Oxidative deterioration of lipids has been shown to cause rancid flavor in stored feedstuffs and feeds. Lipid sources such as fish and vegetable oils which are commonly used in the preparation of feeds are rich in polyunsaturated fatty acids and are susceptible to oxidation. The degree of oxidation in feedstuffs and feeds can be determined through the following methods:

a. Peroxide value (PV). Peroxide value is defined as the reactive oxygen content expressed in terms of milliequivalents (meq) of free iodine per kg of fat. Peroxides are precursors of breakdown products that cause rancid flavors in fat. The concentration of peroxides indicates oxidation during early stages of lipid deterioration. This index becomes less reliable during the later stage of deterioration, because peroxide degradation increases. PV is determined by titrating the iodine liberated from potassium iodide with sodium thiosulfate solution. Details of the method of peroxide value determination is shown in Appendix B5.

b. Free fatty acid value (FFA). Free fatty acid values may be determined using the titrimetric method. The free fatty acid value is usually calculated as oleic acid by dividing the acid value by 2. The presence of these free fatty acids can speed up oxidative deterioration. Oils with high content of free fatty acids will develop undesirable color and flavor. For details of the procedures, see Appendix B6.

c. **Thiobarbituric acid number (TBA)**. This method involves heating the oil with thiobarbituric acid in the presence of a strong acid. A red coloration is produced as a result of a reaction between the thiobarbituric acid and malonaldehyde. The latter may be present in the oil or it may be produced from an oxidation product during the course of the reaction. The intensity of the red color is proportional to the concentration of malonaldehyde in the mixture and can be measured in a spectrophotometer. Results may be expressed as mg of malonaldehyde per kg of oil. It is necessary to carry out the reaction in the absence of oxygen in order to obtain reliable results with marine oils. This is a sensitive test and can be correlated with the development of off-odors and flavors. It is especially suited for the detection of oxidative rancidity in lipids which are unsaturated and contain 3 or more double bonds. For details of the procedure, see Appendix B7.

The absorbance of a 1 g sample in 100 ml reagent multiplied by the factor 46 is the TBA number, or the mg of malonaldehyde per 1000 g of sample. As the amount of reagent used is only 20 ml, the result must be multiplied by 0.2 to give the absorbance of the sample in 100 ml reagent as specified by the definition.

D. Method of Vitamin Evaluation

High Performance Liquid Chromatography (HPLC). The HPLC is a powerful analytical method or tool with a wide range of applications. It provides high resolution, sensitivity, and automatic operation, and is efficient in determining the amount of vitamins contained in feedstuffs and feeds (Figure 6.10).

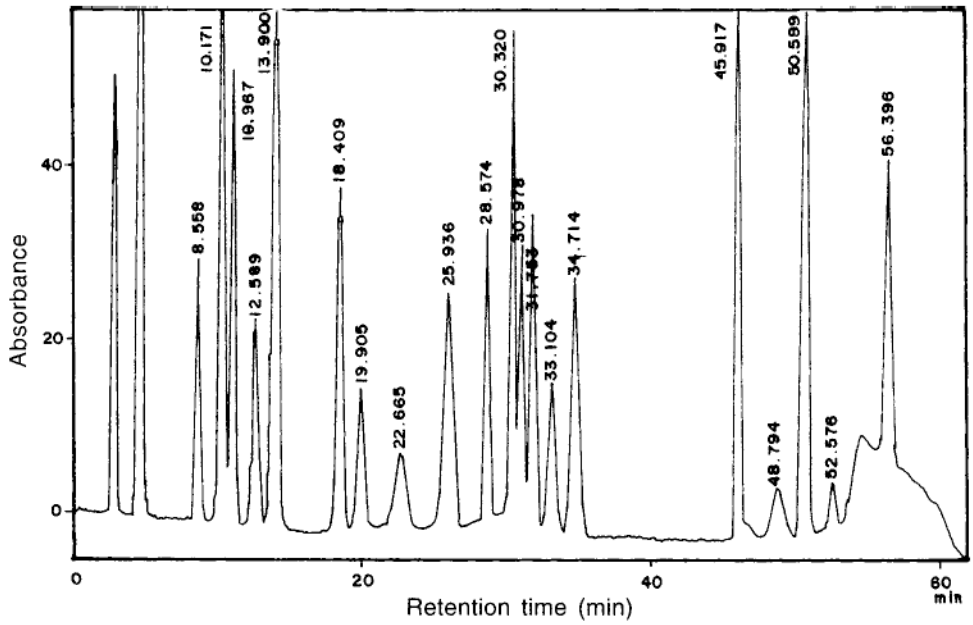

Figure 6.10
A sample HPLC chromatogram of a vitamin mixture.

E. Methods of Mineral Evaluation

There are qualitative and quantitative methods for determining minerals in feedstuffs. Some of the qualitative methods are:

1. **Spot tests for presence of some minerals.** The minerals or inorganic compounds used in animal feed either occur in nature or are chemically compounded. To test for the presence of minerals qualitatively, a spot test is usually applied. The minerals in mixed feed are powdered and sieved. The fine portion is placed in chloroform in a beaker. The floating material is poured off and the residue is sprinkled in the filter paper with a small spatula for spot test of the following minerals.

   a. **Cobalt, Copper, and Iron**. To test for the presence of these minerals, a filter paper is moistened with solution A (100 g sodium potassium tartrate dissolved in 500 ml distilled water). The sample is sprinkled in the filter paper and 2-3 drops of solution B (1 g of 1-nitroso-2-hydroxynaphthalene-3,6-disulfonic acid) are added. The filter paper is then dried. Cobalt gives a pink color; copper gives a light brown colored ring; and iron gives a deep green color.

   b. **Manganese.** To test for the presence of manganese oxide, sulfate, and carbonate, a filter paper is moistened with solution A (2 N NaOH). The test sample is sprinkled in the paper and 2-3 drops of solution B (0.07 g benzidine dihydrochloride in 10 ml of glacial acetic acid is diluted with 100 ml distilled water). Manganese oxide gives a dark blue color with black center and manganese sulfate gives a larger spot of lighter blue which appears quickly.

   c. **Iodine, Magnesium, and Zinc**. To test for the presence of iodine, magnesium, and zinc, the same procedure as above is followed, except that starch paper moistened with bromine solution (1 ml of saturated bromine water made up to 20 ml with distilled water) is the one sprinkled with sample for the iodine test. Iodine then gives a blue-purple color.

      In the case of magnesium, solution A (1 N KOH) and B (12.7 g iodine and 40 g KI dissolved in 25 ml distilled water and then diluted to 100 ml) are mixed to give a very dark brown color. A small part of this is taken and again 2-3 drops of solution A is added until it turns pale yellow and this is sprinkled in the filter paper together with the sample. Magnesium gives yellow brown spots.

      For zinc, the filter paper is moistened with solution A (2 N NaOH). The sample is sprinkled in the filter paper and 2-3 drops of solution B (0.1 g dithizone in 100 ml of carbon tetrachloride) is added. Zinc gives a raspberry red color.

2. **Mineral composition.** The resulting ash from the crude ash analysis is used for mineral analysis of the sample. The quantitative methods for mineral evaluation are:

   a. **Titrimetric method.** In the titrimetric method, permanganate titration is done. In calcium analysis, calcium is precipitated as oxalate at pH 2.5-3, dissolved in sulfuric acid, and the liberated oxalic acid is titrated with standard potassium permanganate solution. The level of phosphorus in the feed is determined based on Misson's reaction.

   b. **Spectrophotometric method**. The phosphorus present as orthophosphate reacts with vanadate-molybdate reagent to produce a yellow-orange complex which is measured spectrophotometrically at 400 nm. Calcium and phosphorus determinations are conducted on feeds such as fish meals, bone meal, calcium phosphate, and calcium carbonate sources.

   c. **Atomic emission spectroscopy.** This method utilizes high-temperature atomization sources to determine the concentration of about 70 elements in feedstuffs. This operates on the principle that when a substance is excited by a plasma or electric discharges, elements present emit light at wavelengths that are specific for each element. The light emitted is dispersed by a prism monochromator. The spectral lines produced are recorded on a photographic plate that is linked directly to a computer-driven data processing system. The samples are pre-concentrated and most elements can be determined at the low parts per billion level.

   d. **Flame spectrometric methods.** The qualitative and quantitative means of these methods can be applied to plant materials, plant nutrients, soils, and other biological fluids. The specific frequency of radiation, emitted or absorbed, identifies the element. The intensity of emitted or absorbed radiation at the specific frequency is proportional to the amount of the element present.

   e. **Atomic absorption spectrometry (AAS).** This method uses combustion and is used to observe the atomic vapor that is produced when a sample solution is nebulized and passed into the flame in an atomic absorption spectrometer. This instrument consists of a centralized hooded area in which a large flame, often 6 in wide by 6 in high, is located. This can be used to identify mineral elements present in feeds and feed ingredients.

F. Methods of Energy Determination

The caloric content of the feed sample is determined through the use of a bomb calorimeter. The principle involved in bomb calorimetry is that the heat of combustion is measured, when the heat exchange process takes place in the water contained inside the calorimeter jacket. Bomb

calorimetry generally measures BTU's, calories, or joules in solid or liquid fuels and combustible samples as well. It is a system that determines directly the caloric value or gross energy of the sample.

To determine the fraction of the gross energy that the fish can actually utilize, a metabolism trial is conducted to determine, digestible, metabolizable, and net energy values. The use of a bomb calorimeter is necessary to avoid difficulties and inaccuracies inherent in determining the NFE. Subsequently, similar determinations on the fecal residue from such diets make it possible to correct the gross caloric value for the apparent loss in digestion and obtain values for digestible energy. This is a better measure than gross energy for expressing the useful energy of feedstuffs.

1. **Digestible energy (DE).** An inert material, chromium oxide, is added to the food at a level of 0.5-1%. The fish are fed the diet for several days for fecal collection, and the chromium oxide levels of both the feed and feces are determined. The digestible energy (DE) is defined as follows:

$$\text{DE (kcal/100 g diet)} = \text{energy of diet} - \left(\text{energy of feces} \times \frac{\text{mg } Cr_2O_3\text{/g dry diet}}{\text{mg } Cr_2O_3\text{/g dry feces}}\right)$$

2. **Metabolizable energy (ME).** The ME is the portion of the gross energy consumed by the fish for growth, fattening, or heat production. It does not appear in the feces or in the urine of fish. The digestible energy minus the energy in the urine and feces is the ME, which provides a better measure of the energy value of the feed than the digestible energy. The large differences in the efficiency of utilization of ME are due to wide variations in heat losses.

3. **Net energy (NE).** This represents the most precise measure of the energy needs of fish and the capacity of different feeds to meet these needs. Not only do NE values allow for the energy lost in the urine and feces, but they also take into consideration the energy lost as heat during nutrient utilization. However, actual NE values have been determined for only a limited number of feeds. Thus, most available NE values are only estimates.

G. Analysis of Toxins in Feeds

Some feed ingredients contain natural toxins that are growth inhibitory at high levels and may be deleterious to fish. Methods of determining these toxins are:

1. **Urease activity**. The enzyme urease usually found in raw soybeans produces toxicity through the hydrolysis of urea to ammonia. Heat treatment of soybean meal at 120°C for 20 min is adequate to remove this enzyme. Quantitative analysis to determine urease activity level in feedstuffs is patterned after Chow (1980).

2. **Gossypol.** This is an endogenous toxin present in the gland of cottonseed which persists during meal production unless removed by a special process, or, unless the cottonseed is a glandless variety. The gossypol level in a feed ingredient is determined spectrophotometrically.

3. **Aflatoxin**. This is a class of potent toxins produced by the mold *Aspergillus flavus* and is usually present in feed materials such as groundnut cake, copra, peanuts, corn, rice, and legumes produced and stored under hot and humid conditions. The chromatographic methods most widely and routinely used for aflatoxin analysis are one- and two-dimensional thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC). In view of the potential deleterious effects of these antinutritional factors on the growth of fish, analysis should be done on feedstuffs known or suspected to contain these materials.

Microbiological Evaluation

Amino acid composition. Another method of determining amino acid composition is through the microbiological method. This method is valuable in analyzing mixtures of amino acids because of the speed and reproducibility of results obtained. A nutrient medium which contains all of the essential compounds needed for the growth of a particular microorganism except the amino acid to be assayed is prepared. Addition of this amino acid results in growth of the microorganism in proportion to the amount of amino acid added. Culture tubes are set up and graded amounts of the unknown are added to a series of tubes. Standards are set up at the same time with graded amounts of the pure amino acid. The unknown can be compared with standards by measuring the rate of growth of the microorganism. With organisms which form acid such as *Lactobacilli*, titration of the acid formed can be used as a measure of the number of cells present. Pure cultures need to be used in this kind of evaluation.

Biological evaluation

A feeding experiment is conducted to test the efficacy of formulated feeds. It is usually done in tanks, ponds, or cages. In a laboratory experiment, environmental conditions are easily kept constant. In carrying out a feeding experiment, the following factors have to be considered:

a. The objective of the study has to be clearly defined.

b. Experimental treatments and statistical design appropriate to the objective of the experiment have to be carefully selected. A completely randomized design (CRD) or randomized complete block design (RCBD) is usually applied in most feeding experiments. However, when there are more variables, a factorial design is used. Prior to a feeding experiment the number of replications per

treatment and the number of fish per replicate should be chosen such that a meaningful statistical analysis of data can be accomplished at a set level of significance. It is best that the data to be gathered, sampling frequency, number of samples per replicate, and statistical methods to be used in the data analysis are known beforehand. A statistician should be consulted for the design of the experiment.

c. The experimental fish species have to be identified with its scientific and common names, together with its strain, source, size, age, and previous nutritional history. Acclimation of the test animal is usually done for at least a week to enable the fish to adjust to its new environment. Initial body weight and length have to be measured during stocking. It is best to use an equal number of fish of approximately the same size and age per replicate. In some studies, body composition (initial and final) has to be known. This is usually determined in three groups of fish in three replicates. Fish are usually weighed, sacrificed, chopped, dried in a freeze drier, and oven-dried a 60°C to constant weight. Results of proximate composition is then related to the initial weights of the fish to obtain absolute weights of the nutrient components. The same procedure is followed to determine the final body composition of the fish. The data is used to determine nutrient retention.

d. The experimental tanks should be large enough to allow substantial fish growth (Figure 6.11). Experimental tanks, dimensions, water source, volume, and depth should be clearly defined. Water may be static but replaced regularly, recirculating, or flow-through depending on the available resources. Filtration system should be adequate and efficient in removing particulate matter and metabolites that may influence the response of fish to treatments. Water temperature is usually ambient and sometimes controlled. Water quality (temperature, dissolved oxygen, pH, ammonia, etc.) should be monitored daily and should be favorable for maximum growth.

e. The duration of a feeding experiment should be long enough, at least 8 weeks, to allow fish to manifest definite growth trends and significant differences in response parameters as affected by the dietary treatments.

Figure 6.11
A laboratory set-up for a feeding experiment using 250 l tanks.

f. Growth, the most widely accepted response parameter for evaluating treatments in feeding, is a sensitive and practical indicator of the adequacy of essential nutrients in the diets. Other parameters such as survival, feed efficiency, etc. should also be taken into account.

Parameters to be monitored in a feeding experiment:

1. Growth

a) Absolute growth

wt gain = wf – wi,
where: wf = final wt; wi = initial wt

Absolute growth rate

$$\text{wt gain/day} = \frac{\text{wf} - \text{wi}}{\text{days of culture}}$$

b) Relative growth

$$\% \text{ wt gain} = \frac{\text{wf} - \text{wi}}{\text{wi}} \times 100$$

Relative growth rate

$$\% \text{ wt gain/day} = \frac{\text{wf} - \text{wi}}{(\text{wi})\ (\text{days of culture})} \times 100$$

Specific Growth Rate (SGR)

$$\text{SGR} = \frac{\ln w2 - \ln w1}{t_2 - t_1}$$

where: w1 and w2 are weights at periods 1 (t_1) and 2 (t_2), respectively.

2. Efficiency of feed utilization

$$\text{Feed conversion ratio (FCR)} = \frac{\text{dry feed consumed}}{\text{wt gain}}$$

$$\text{Feed efficiency (\%)} = \frac{\text{wt gain}}{\text{dry feed consumed}} \times 100$$

3. Digestibility of nutrients

$$\% \text{ Digestibility} = \frac{\text{nutrient absorbed}}{\text{nutrient consumed}} \times 100$$

4. Efficiency of protein utilization

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{wt gain}}{\text{protein intake}}$$

$$\text{Biological Value (BV)} = \frac{\text{N retained}}{\text{N absorbed}}$$

Net Protein Value (NPV) or Productive Protein Value (PPV)

$$= \frac{\text{N retained}}{\text{N consumed}} \quad \text{or} \quad = \text{BV x digestibility}$$

Net Protein Utilization (NPU)

$$= \frac{\text{nitrogen increase in fish fed the test protein diet} + \text{nitrogen decrease in fish fed the protein free diet}}{\text{nitrogen intake from the test protein diet}} \times 100$$

5. Survival rate

$$\% \text{ Survival} = \frac{\text{final count}}{\text{initial count}} \times 100$$

6. Proximate composition of fish samples (initial and final)

7. Biological parameters

a) amino acid and fatty acid composition
b) stored nutrient levels in tissues, serum, or plasma
c) enzyme activity
d) oxidation of radioactively-labelled nutrients

8. Histological changes in tissues (e.g. gills, skin, liver, muscle)

Summary

The choice of high quality feedstuffs for incorporation into aquafeeds is crucial to the success of an aquaculture venture. Proper methods of feedstuff and feed evaluation should be learned and applied to attain an effective feed. The systematic evaluation of feedstuffs and feed includes physical, chemical, and biological methods. In the application of these methods, standard sampling procedures should be followed in order to effectively carry out the evaluation process. Among these methods, the biological method which involves actual feeding experiments gives a more accurate estimate of feed utilization.

Guide Questions

1. What are the different methods of feed evaluation? Differentiate one from the other.
2. What does proximate analysis measure in feed evaluation? What are the limitations of this procedure?
3. What is the principle behind the "Kjeldahl method" of protein determination?
4. How is the protein quality evaluated?
5. Describe briefly how to calculate the essential amino acid index (EAAI) of a protein.

6. Explain the principle behind gas chromatography.
7. What are the tests used to detect lipid rancidity?
8. What are peroxides? Differentiate between peroxide value and thiobarbituric acid number.
9. What is the implication of having a high free fatty acid value in feed materials?
10. What is the significance of the thiobarbituric acid number or TBA number?
11. Explain the principle behind atomic emission spectroscopy?
12. What are the characteristics of a high quality feed?
13. How is aflatoxin produced in feed/feed ingredient?
14. What are the antinutritional factors that may be present in the feed ingredient and explain how they are minimized or removed from feedstuffs.

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CHAPTER

Management of Feeding Aquaculture Species

7

VERONICA R. ALAVA

Introduction

Production targets, environmental conditions, and socio-economic considerations determine the level of management in aquaculture production systems. The contribution of natural food as a source of nourishment is important in producing fish with few inputs. However, more inputs such as complete high quality feeds are required to increase fish yield per unit area.

The use of cost-effective feeds, proper feeding management, and maintenance of good water quality are critical for a successful aquaculture enterprise. As the stocking density increases, feed requirement and metabolic wastes also increase. Feeding must be regularly monitored and continuously adjusted so as not to overfeed or underfeed the fish. The success of an artificial diet is dependent on the management of the feed on the farm. Feeding practices must therefore be directed towards optimizing feed utilization to prevent pollution of the culture environment and the coastal vicinity.

This chapter teaches the reader to: differentiate the different feeding strategies in pond culture; learn feeding management methods such as stock sampling and record keeping, calculating daily feed ration, choosing appropriate feed size, and methods of applying feeds; understand the impact of feeding management on water quality and environment and on the cultured animal's growth, survival, and feed conversion ratio; and describe the different feeding schemes used to culture fishes (milkfish, tilapia, rabbitfish, bighead carp, native catfish, sea bass, orange-spotted grouper, and mangrove red snapper; and crustaceans (tiger shrimp and mud crab). Other species for aquaculture stock enhancement (donkey's ear abalone, seahorses, window-pane oyster) are also discussed.

Feeding strategies in pond culture

Fish production depends on the stocking density and feeding strategy used. At lower stocking density as in the extensive culture system, natural pond productivity is important. As stocking density increases as in the semi-intensive and intensive systems, artificial feed becomes more essential (Figure 7.1).

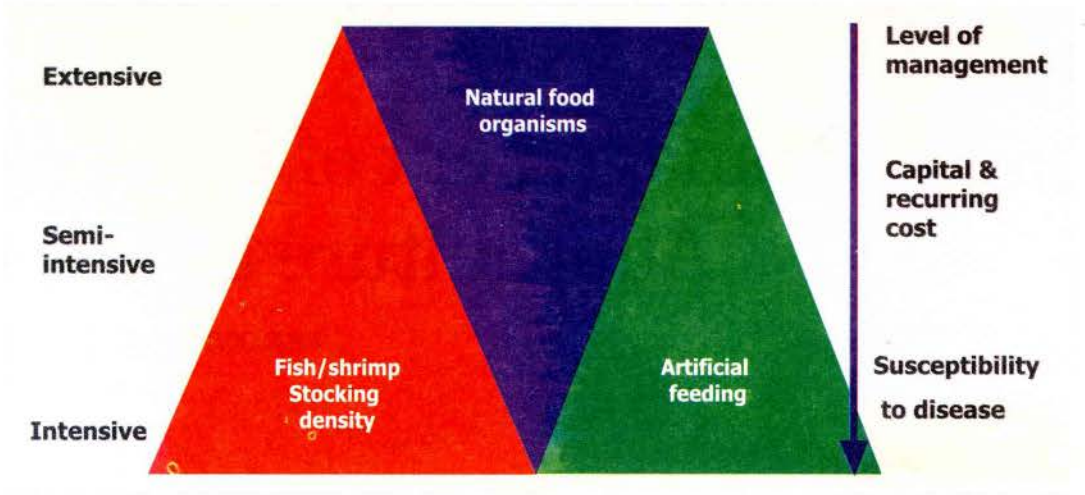


Figure 7.1
Range of aquaculture practices in relation to inputs.
Source: Tacon 1998

In pond aquaculture, the feeding strategies used are:

A. Production of natural aquatic food

During the early growth stages of the cultured animal and at low stocking density, the role of natural aquatic food, as the only source of nutrition is very important. The natural food bases in ponds are: *lablab* (a complex of blue-green and green algae, diatoms, rotifers, crustaceans, insects, roundworms, detritus, plankton (Figure 7.2A), and *lumut* (fibrous filamentous green algae) (Figure 7.2B). Organic or inorganic fertilizers are periodically applied as sources of carbon, nitrogen, and essential minerals. With sunlight, fertilizers enhance the growth of phytoplankton thereby increasing the natural productivity and hence fish production. An integrated system using various species in different trophic levels such as seaweed, shellfish, and herbivorous and omnivorous fish species such as milkfish and tilapia are also used to efficiently utilize various forms of nutrients and to obtain high productivity. In extensive culture systems, inputs and production costs are generally lower than in semi-intensive and intensive systems, but yields are also lower.

Figure 7.2
Natural aquatic food in ponds: lablab (A) and lumut (B).

B. Feeding a supplementary diet

When stocking density and standing crop of cultured animals is increased, the natural productivity can no longer support adequate

growth. Supplemental feeds become necessary. The commonly used supplemental feeds are rice bran, chicken feed, breadcrumbs, boiled corn, cooked cassava, and chicken entrails. These are nutritionally incomplete and would be inadequate if used as the only source of food. When supplemental feeding is applied, higher stocking densities are possible resulting in higher production per unit area. The most cost-effective way of producing aquatic animal is to grow natural food and to provide supplemental feed when natural food becomes inadequate. As natural food decreases with increasing standing crop, the quality of the feed should be improved to sustain fish growth.

C. Feeding a complete diet

At higher stocking densities typical of intensive culture, a complete diet is necessary to provide the nutrients required by the animal for growth and survival. Production does not rely on natural food. Since the aim of high level of production is to maximize yield per unit area in a shorter period, highly effective artificial feeds are used.

Feeding management

Sampling and record keeping

Regular sampling of cultured stocks is essential in order to assess the effectivity of feeding management. Sampling involves weighing or measuring a representative group (sub-sample) of animals. These data can then be used to determine the total biomass, and the changes in weight or length from previous sampling data. All sampling methods should be carefully designed to avoid bias, and to minimize any stress on the animals. A work schedule should be set prior to sampling, and all equipment and materials should be on hand before animals are collected and measured. It is important that the fish handling and measuring procedures are consistent and repeatable.

To estimate biomass in the pond, tank or cage, fish are sampled by using a cast or lift net (Figure 7.3 A,B). When fish in large ponds are

Figure 7.3
Sampling by cast net (A) and lift net (B).

sampled, the nets are cast in several areas of the pond in order to get a better sample of biomass. For example, in a one-hectare pond, a net may be cast in the middle, and one or two in each side of the pond. The samples are then weighed individually or in bulk. A good estimate of survival rate is necessary to calculate total fish biomass but this is often difficult to obtain in the ponds. In practice, most farmers assume a certain value for survival based on data from previous culture operations in the same pond or other ponds with similar conditions. This value is modified by observations of actual mortalities, water quality, and occurrence of diseases.

Data on fertilizers, animal stock, feed, water quality, and other useful parameters listed in Table 7.1 must be recorded. Accurate records are necessary to enable the farmer to assess the efficiency of feeding and farm management during the past and current culture runs.

Table 7.1 Some useful parameters that must be recorded in the farm

	Parameters
Pond	Pond number, area, depth
Fertilizer	Source, date of fertilizer applications, type and quantity of fertilizer used
Animal stock	Date of stocking, source of stock, species, behavior, stocking density, average initial weight or length, date of sampling, date of harvest, average final weight or length, kg harvested, weight gain or growth, survival at harvest, size distribution at harvest
Feed	Date of purchase of feeds, feeding rate and amount, type of feed used, date each feed type is given, feeding frequency, feeding time, feeding location in the pond, presence of left-over feeds, feed conversion ratio
Water quality	Secchi disk reading (water transparency), water exchange rate, salinity, dissolved oxygen level, date of water change, water temperature, water color, time and duration of paddlewheel operation if used
Others	Disease or abnormalities of stock, predators in pond, weather conditions during the growing cycle, unusual events, costs of inputs, investment returns

Feeding Ration

The amount of the daily feed ration, and the frequency and timing of feeding are important factors affecting growth and feed conversion. Each cultured species has a dietary feeding rate optimal for growth and feed efficiency. The optimal feeding level varies with age, fish biomass, dietary nutrient requirement, water quality, and natural food availability. Fish lose weight when their feed intake falls below the required level for maintenance. As ration size and feed intake increase, the growth rate increases up to a certain limit. The larval stages of aquatic animals have very high metabolic activity and rapid gastro-intestinal evacuation rates. Thus, it is critical that their high energy requirements are met by continuous feeding to satiation. Frequent feeding is recommended when fish are small, when natural food is inadequate, and when the feeds are less water-stable.

Optimal growth and feed efficiency can only be attained if sufficient amount of feed is provided, completely ingested, and digested. Pond-reared shrimps must be fed frequently on small amounts, with the bulk of the feed given during the night when the feeding activity is highest. Increasing the frequency of feeding reduces leaching and feed loss, and improvement of growth and feed efficiency. Normal feeding patterns in crustaceans are interrupted during molting. Immediately before and during actual molting, crustaceans stop feeding. This usually lasts for two days after which feeding levels increase. Thus, an adjustment in feeding levels is needed.

Various methods are used to estimate feed ration. These include calculations based on the use of feeding charts, feed equations, and growth predictions. Feeding tables are calculated from estimates of growth, survival, and feed conversion. Tabulated feeding levels for various species are commonly provided by feed manufacturing companies. Daily feeding rate is expressed as percent of body weight per day. The percentage of feed consumed in relation to body weight decreases as the fish grow and their metabolic rates decline. Feeding charts provide only a general guide to feed intake. These do not take into account the short- or long-term fluctuations in appetite in response to many physiological and environmental factors. However, they are useful in estimating feed requirements. These baseline values, later modified as feeding and growth data from the various culture batches, are collected. Clearly, optimal feeding rate and frequency must be determined for each feed and pond by careful monitoring of feed consumption, growth, and feed efficiency over several growing cycles.

In order to calculate the daily feed ration of the cultured animal, data on average body weight and the estimated survival rate at a given time are needed, and these two are obtained by sampling the stock. The feeding rates recommended for the different species at various stages of culture are given in the section on Feeding Schemes. The formulas to compute for average body weight, survival rate, and daily feed ration are:

$$\text{Average body wt (g)} = \frac{\text{total wt of sampled stock (g)}}{\text{number of sampled stock (pcs)}}$$

$$\text{Survival rate (\%)} = \frac{\text{number of fish at a given time (pcs)}}{\text{original number of fish stocked (pcs)}} \times 100$$

$$\text{Daily feed ration (kg/day)} = \frac{\text{original number stocked (pcs)} \times \text{average body wt (g)} \times \text{estimated survival rate (\%)} \times \text{feeding rate (\%)}}{1{,}000}$$

Feed Particle Size

Feed particles must be of a size that can be readily ingested and this increases as fish grow. Large fish can ingest small particles but requires more energy to capture an adequate amount resulting in reduced feed efficiency. Unlike fish feed, the particle size of crustacean pellet is not related to the mouth size because crustaceans physically break down pellets prior to ingestion. However, the pellets must be of an appropriate size that crustaceans can carry and nibble on using their feeding appendages.

Feed application methods

Feeding fish is the most important daily activity at fish farms requiring a considerable amount of time. The choice of feeding methods will depend on the following factors: labor costs, scale of farm operation, species being farmed, type of holding system (ponds, cages, or tanks), hatchery and grow-out operation. Manual feeding includes broadcasting the feeds into the pond as well as placing feeds into floating frames (for floating feeds) or feeding trays (for sinking feeds). Feeding trays are used either to monitor actual feeding or to serve as feeding areas (Figure 7.4). Manually broadcasting feeds in the middle of a big pond requires the use of a small banca. Mechanical means of partially replacing hand feeding include: automatic feeders which can be set to release controlled amounts of feed when activated, and demand feeders which can release a few pellets each time a triggering mechanism is bumped by the fish (Figure 7.5). These mechanical feeders can save time, however, these must be calibrated and adjusted regularly.

Figure 7.4
Use of feeding tray to monitor feeding.

Figure 7.5
A demand feeder.

Whether manual or mechanical, the feeding method used should be economical and allow some control and reliability in dispensing feeds. The method should minimize feed waste, provide a way to monitor fish appetite and feed consumption, and should distribute feeds widely in order to promote uniform feeding and growth. If carried out carefully, manual feeding can closely monitor the appetite, feeding behavior, and abnormalities if any, of the animal. However, it is labor intensive and time consuming.

Mechanized feeding requires high capital and operating costs but can be offset by the reduction of labor costs associated with manual feeding. With timing controls, automated systems can be set to deliver feeds at any time, at any given frequency in accurate amount, and spread feed more evenly and over large areas, than is possible when broadcasting by hand. Manual feeding may be combined with the use of mechanical feeding systems to effectively control feeding routine, thereby reducing waste.

Feeding, Water Quality, and the Environment

Nutritional and economic success of an aquafeed is based upon a variety of interlinked factors and that no one factor can be considered by itself. These are presented in Figure 7.6. Feeds that are uneaten within a certain period can deteriorate the water quality of the culture system. Hence, proper feeding strategies and good water quality management are very important factors.

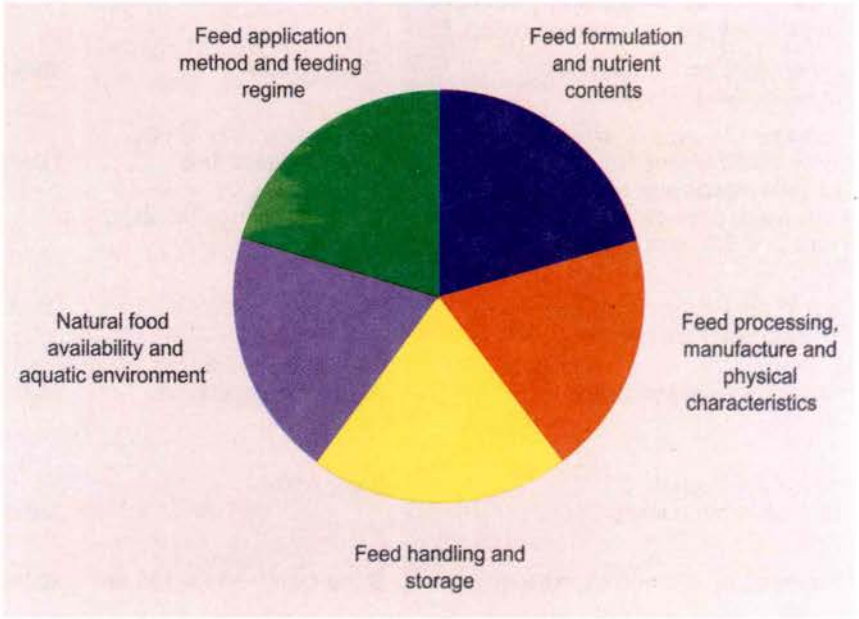


Figure 7.6
Interlinked factors that are critical for the success of an aquafeed.
Source: Tacon 1993

A. Feeding, oxygen requirements, and water quality

Oxygen, together with an organic substrate, is required for all oxidative metabolic processes. The rate of oxygen consumption rises to a peak after feeding, and then slowly falls back to the pre-feeding level.

Low water exchange rates, photosynthesis in plants, and the respiratory activity of the pond biomass create fluctuations in dissolved oxygen (DO) levels. Low DO depresses the feeding activity of aquatic animals. When DO level is low, feeding should not be done. Giving less feed at each feeding while increasing the frequency of feedings helps prevent low DO problems. Careful monitoring of DO levels, combined with observations of the behavior of the aquatic animal, is

essential and should be routinely done. Oxygen depletion can be alleviated by increasing water exchange (at least 30% of total volume) and the use of mechanical aerators such as a paddlewheel aerator. Other water quality parameters should be carefully monitored so that conditions that adversely affect growth and survival can be minimized. Some water quality problems in brackishwater ponds and methods to manage them are given in Table 7.2.

Table 7.2 Water quality parameters, method of measurement, water quality problems and possible causes, scheme/ method of management, and target water conditions in brackishwater pond culture

Parameter	Method of measurement	Water quality problem	Possible causes of problems	Scheme/method of management	Target water condition
Dissolved oxygen (DO)	DO meter, titration	Low DO	Increased DO demands due to: over-abundance of plankton and benthic organisms, and subsequent die-offs of plankton or animal, waste accumulation in pond bottom Slow O_2 diffusion from atmosphere Limited photosynthesis during overcast days or the algal population collapses	Aeration if DO is < 4 ppm Water change 30-50%, flushing of bottom wastes and detritus	> 4 ppm
		DO stratification	Limited light penetration due to high plankton density	Algal control	No stratification
pH	pH meter, pH paper	Low pH	Production of organic acids by anaerobic bacteria from uneaten feeds and metabolic wastes Acids leach from dikes of acid sulfate soil Excessive CO_2 production	Application of dolomitic or agricultural lime Water exchange/ flushing	7.0 to 8.5 pH
		pH stratification	Limited light penetration due to high plankton density	Algal control	No stratification
Salinity	Refractometer, hydrometer	Salinity stratification	Rain and freshwater runoff	Aeration / circulation	15 to 30 ppt
Temperature	Thermometer	Temperature	Limited penetration due to high plankton density	Algal control	No temperature stratification
		Unstable water temperature	Influenced by climatic conditions	Deep water, about 150 cm	Stable, 26 to 33°C
Ammonia-N	Ammonia test kit	High NH_3-N	Excess feed, metabolic wastes and decaying matter; phytoplankton die-off	Water change/ flushing Aeration	< 1.0 ppm
Nitrite-N	Nitrite test kit	High NO_2-N	Excess feed, metabolic wastes and decaying matter	Water change/ flushing Aeration	< 0.5 ppm
Alkalinity	Alkalinity test kit, titration	Low alkalinity	Excess feed, metabolic wastes and decaying matter	Water change/ flushing Aeration	> 20 ppm
Hydrogen Sulfide	Sulfide test kit	High H_2S	Excess feed, metabolic waste and decaying matter	Water change/flushing Aeration	< 0.003 ppm
Transparency	Secchi disk visibility	Turbid	Increased suspended solids, erosion of dikes, turbid water source, excessive and rapid plankton bloom	Coagulants Water change	30 to 45 cm
		Clear	Absence of good plankton bloom, plankton die-off	Fertilizers: urea, chicken manure	Golden brown, light to brownish green color

Source: Modified from Chiu 1988

B. Fish farm wastes

The impact of aquaculture on the environment is primarily related to feed management. The composition of feeds and feed conversion affect both the physical and chemical nature of waste materials and the amounts produced. Wastes produced during the culture of aquatic animals fall into two groups: solid wastes resulting from uneaten feed, dust and feces, and soluble excretory products, primarily ammonia and urine, dissolved organic materials, and carbon dioxide. These materials are potentially harmful to both the immediate farm environment and water bodies receiving the farm effluents.

If organic materials accumulate to high levels on the bottom of the ponds or in the sediment below floating cages, the sediment will become anaerobic, releasing toxic hydrogen sulfide and methane gas. Suspended solid materials have a harmful effect on the gills of aquatic animals, affect respiratory function, and provide potential sites for bacterial and fungal infection. Long-term effects may result from the release of phosphates and nitrates from fish farm effluents leading to eutrophication or enrichment of the receiving water. In extreme cases, these may lead to the occurrence of potentially harmful plankton blooms.

Performance Measures

The success of feeding operations is reflected in overall production and profit. Growth, survival, and feed conversion ratio, the major indicators commonly used by farmers to measure performance are computed at the end of each culture cycle (See Chapter 6).

Biomass. This refers to the total weight of species being cultured, expressed in terms of a given area or volume of the habitat, e.g., per hectare.

$$\text{Biomass (kg/ha)} = \frac{\text{original no of stock (pcs) x ave body wt (g) x survival (\%)}}{1{,}000}$$

Feed conversion ratio (FCR). Many farmers use FCR as the indicator of the effectiveness of both feeding practices and overall husbandry. FCR can only be accurately determined if the amount of feed consumed is accurately monitored and recorded. To compare the FCRs of feeds, moisture values must be corrected when calculating the weights of feed fed. Poor or variable FCRs may reflect problems with feed or feeding methods, or may be indicators of problems such as the occurrence of disease in stocks or worsening water quality. Low FCR of 1.1 means that it only takes 1.1 kg of feed to produce 1 kg of fish. High FCR of 2.5 is considered as poor since it takes 2.5 kg of feeds to produce 1 kg of fish. The lower the FCR value, the better is the feed utilization.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{amount in dry wt of feed given (kg)}}{\text{wet wt gained within a feeding period (kg)}}$$

Feeding Schemes

A. Milkfish

Milkfish (*Chanos chanos*) (Figure 7.7) is a major protein source in the Philippines. It is omnivorous and grows in a wide range of salinity. The traditional low-density culture in brackishwater ponds has expanded to high-density culture in freshwater lakes, brackishwater ponds, marine pens, and offshore cages. High quality feeds and good feeding management become more critical in these culture systems. Milkfish are harvested and marketed as fresh, chilled, or processed into value-added products, such as deboned, marinated, 'choice cuts' packs, smoked, and canned.

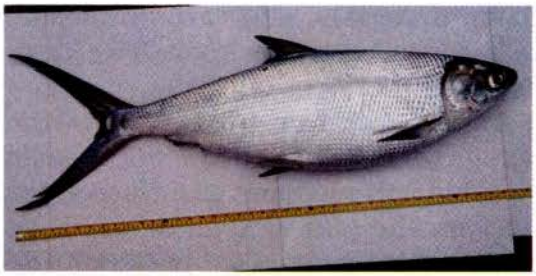


Figure 7.7
Milkfish *Chanos chanos*.

1. Feeding milkfish broodstock in floating cages and tanks

The milkfish broodstock rearing facilities used at SEAFDEC AQD are floating marine net cages and concrete tanks (Figure 7.8A, B). A formulated broodstock diet (Table 7.3) is broadcast twice daily (0800 to 0900 h and 1400 to 1500 h) at 2 to 4% of biomass. Milkfish anticipates feeding time and can swallow whole pellets measuring 1 cm diameter x 2 cm long. Mature milkfish can spontaneously spawn during the season with nutritionally adequate feeds and proper feeding management.

Figure 7.8
Floating marine net cages (A), and concrete tanks (B), for broodstock.

Table 7.3 Practical diet formulas (g/kg dry diet) for milkfish at various stages of culture

Ingredient	Broodstock diet [1]	Larval diet [2]	Fry freshwater diet [3]	Fry seawater diet [4]	Grow-out diet [5]
Fish meal	200	330.0	566.0	300	110
Soybean meal	430	180.0	114.0	200	308
Squid meal	-	100.0	-	-	-
Shrimp meal (*Acetes* sp.)	-	120.0	90.0	-	-
Shrimp head meal	-	-	-	160	-
Rice bran	255	-	87.0	115	492
Bread flour	40	66.9	-	150	50
Sago palm starch	-	-	50.0	-	-
κ-carrageenan	-	50.0	-	-	-
Cod liver oil	20	80.0	25.0	30	20
Soybean oil	-	-	25.0	-	20
Lecithin	-	10.0	-	-	-
Vitamin mix	15	30.0	6.9	10	-
β-carotene	-	2.5	-	-	-
DL-α-tocopherol acetate	-	0.1	-	-	-
Mineral mix	-	30.0	36.0	35	-
Dicalcium phosphate	40	-	-	-	-
Butylated hydroxytoluene	-	0.5	0.4	-	-
Proximate composition (% DM):					
Crude protein	37.6	46.3	43.9*	37.6	26.7
Crude fat	8.7	11.4	10.3*	8.7	10.9
Crude fiber	3.9	5.6	3.6*	3.9	8.4
Nitrogen-free extract	36.4	27.3	16.8*	36.4	45.1
Ash	13.4	9.4	16.2*	13.4	8.9

Sources: [1] Marte & Borlongan in FDS Manual 1994; [2] Borlongan et al. 2000; [3] Santiago et al. 1983; [4] Alava and Lim 1988; [5] Sumagaysay 1998
*as-fed basis

2. Feeding milkfish larvae in hatcheries

In intensive production, milkfish larvae are reared in concrete tanks (3 to 10 m^3 capacity, Figure 7.9) at high stocking rates (30,000 to 50,000 larvae per m^3). Milkfish larvae are pelagic feeders, thus their feed should be buoyant and remain suspended in the water column until eaten. A feeding scheme is developed with the use of artificial diets to reduce the need for natural food production in hatcheries (Figure 7.10). A nutritionally-balanced, flaked microbound diet or MBD (Table 7.3) may be fed to milkfish larvae in combination with *Brachionus* starting day 2 or day 7, and used solely as feed from day 15 onwards. The natural foods are given once daily (0900 h), while formulated larval diets are dispensed three times a day at 0800, 1100, and 1300 h.

For a semi-intensive hatchery system, milkfish larvae are fed initially with *Brachionus*. At later stages, copepod *Acartia* and *Pseudodiaptomus* nauplii and MBD are provided. MBD is given at 2 g/m^3/day, four times daily (0800, 1100, 1400, and 1700 h) until harvest.

FIGURE 7.9
Intensive larval rearing tanks.

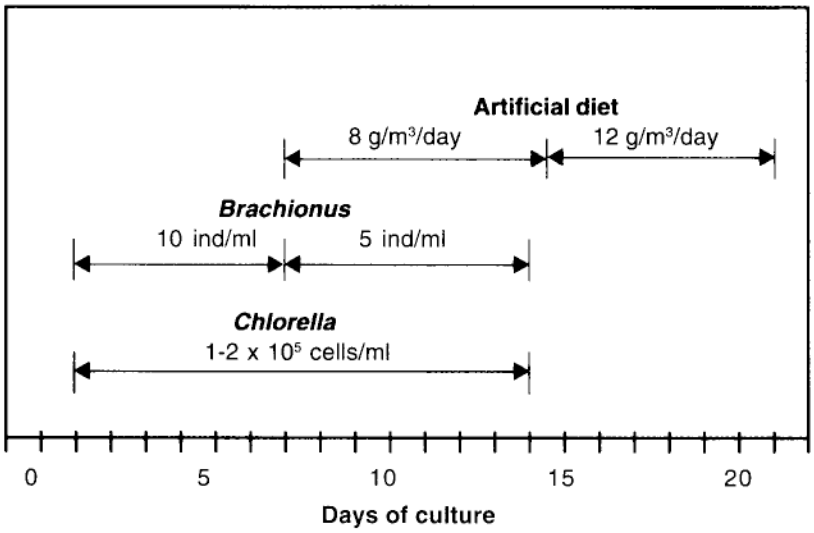


Figure 7.10
Feeding management scheme for larval rearing of milkfish.
Source: Borlongan et al. 2000

3. Feeding milkfish fry in nursery tanks and ponds

Traditionally, milkfish fry obtained from the wild are reared in fertile nursery ponds with abundant natural food. Hatchery-bred fry are also stocked into nursery ponds. The natural food bases for milkfish in ponds are *lablab*, plankton, and *lumut*. *Lablab* is cultivated during the dry season and plankton during the wet season in deep-water ponds (70 to 100 cm).

Wild-sourced fry metamorphose and has good growth and survival in freshwater when fed on an artificial diet (Table 7.3) at 15 to 10% of biomass four times daily (0900, 1200, 1500, and 1800 h) for 35 days in tanks. Likewise, wild-sourced fry reared in seawater tanks and fed artificial diet (Table 7.3) three times daily (0900, 1300, and 1700 h) at 20 to 15% of the body weight per day attain good growth and excellent survival in one month.

4. Feeding milkfish in grow-out ponds

Milkfish grow-out culture started from the traditional shallow water straight culture of uniform-sized fish stock in ponds to multi-size stocking and harvesting using modular, and deep-water plankton methods.

Milkfish depends on natural food grown in the ponds during the first and second months of culture (Figure 7.2). Artificial feeds (Table 7.3) can be fed at 3 to 4% of body weight per day when natural food is low. There is no common feeding table for supplemental feeding; feeding depends on total fish biomass, the amount of natural food, and on water quality. Feed can be broadcast always in the same area of the cage or pond (Figure 7.11A, B). Before feeding, the fish are conditioned to sound to make feeding easier and more effective because the fish gather in one area.

Figure 7.11
Feed can be broadcast in the same area of the cage or pond since fish gather in same area for feeding (A,B).

Milkfish are daytime feeders. Feeding is done twice a day, at mid-morning (when DO is not less than 3 ppm) and late afternoon. At mid-day, milkfish prefer to feed on natural food. Thus, high plankton density should be maintained through periodic application of fertilizers. For example, 25 kg of 16-20-0 monoammonium phosphate may be applied per hectare every 15 days.

When feed is the major source of nutrients, feeding is increased to three times a day; 0800 to 0900, 1200, and 1600 to 1700 h. The feed ration could be higher at noon and in the afternoon (3/4 of the daily ration) than in the morning (1/4 of the daily ration).

Milkfish feeding activity is affected by abrupt changes in environmental factors such as temperature, salinity, and DO. The daily feed ration is reduced by 25% when the temperature drops from 32°C to 25-28°C, and by 50% when the temperature falls from 26-33°C to 21-24°C, when salinity is more than 36 ppt, or when DO is less than 3 ppm.

B. Tilapias

In the Philippines, tilapias (*Tilapia nilotica* or *Oreochromis niloticus*, *T. mossambica* or *O. mossambicus*, *T. aurea* or *O. aureus*, red tilapia *Oreochromis* spp. (Figure 7.12A, B, C, D) rank second to milkfish in economic importance. Tilapias are omnivorous; they feed on phytoplankton, zooplankton, bottom organisms, and detritus. Tilapias are ideal for culture because of their flexible feeding habits. They can readily take a variety of feeds, in meal form, and in sinking and floating pellet form. Tilapias feed continuously and they respond well to frequent feeding. They prefer small pellets, and chew rather than swallow them whole. For high survival and efficient feed conversion, dry pellet crumbles are better utilized than the non-pelleted mixture of the same diet.

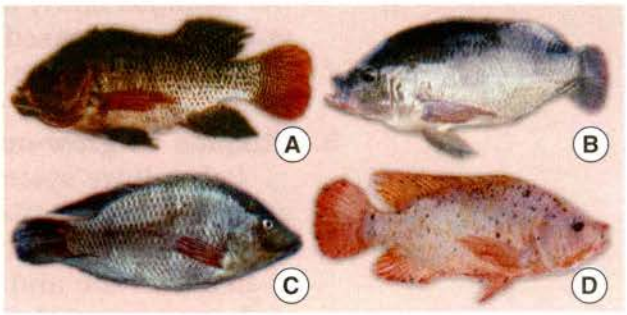


Figure 7.12
Tilapia nilotica (A), *mossambica* (B), *aurea* (C), red tilapia (D).

1. Broodstock in tanks, hapas or ponds

Sexually mature tilapias stocked in fertilized ponds are usually given a supplement of rice bran or a 3:1 combination of rice bran and fish meal. Broodstock in tanks and in hapas are fed complete diets to obtain high fry production. A diet for tilapia broodstock is shown in Table 7.4. This diet is given at a feeding rate of 1% of the body weight twice daily at 0900 h and 1500 h and is sufficient to increase growth and fry production.

Figure 7.13
Larvae released from mouth-brooding female.

Tilapias spawn asynchronously. The females deposit their eggs at the bottom of the pond, tank or hapa where the males fertilize them. The females incubate their eggs and shelter the larvae in their mouths until the fry are free-swimming (Figure 7.13). During this time they eat very little or none at all.

Restricted feeding is suitable for broodstock. Tilapia that have been fed to satiety at temperatures 28 to 30°C can be stimulated to reproduce by reducing the feed ration by 25 to 50%. It is important not to overfeed the broodstock. Frequent cleaning of tanks and hapas disturbs the brooders, causing them to spit out or swallow the eggs and larvae.

2. Larvae or fry in nursery ponds, tanks, and hapas

Nursery ponds are fertilized to grow plankton. Tilapia fry are stocked in ponds or in hapas inside the pond. Natural foods differ in nutritional value for tilapia fry. The diatom *Navicula* and cyanobacterium *Chroococcus* are more acceptable and are better assimilated than the green alga *Chlorella* and the phytoflagellate *Euglena.*

When natural food starts to decline in ponds, the nursery operator introduces feed which may be fine rice bran or a 7:3 combination of fine rice bran and fish meal. In nursery tanks and hapas where natural food is scarce, a formulated diet (Table 7.4) is given to fry as soon as they start feeding. Fry are fed to satiety, or at about 30% of the biomass daily, four times a day. As they grow, the feeding rate is gradually decreased to 15%. The feed is broadcast over areas in the nursery so that the fry do not have to swim far to search for food.

3. Tilapias in grow-out tanks, ponds, and cages

Polyculture systems of tilapias with fish of different food preference are used to maximize the utilization of natural foods. The manures of chicken, ducks, or pigs are used to fertilize the ponds. Locally available crude and inexpensive feedstuffs such as rice bran, pollard, and copra meal, leftover bread and biscuits, and commercial poultry feeds are given as supplemental feeds twice a day at 2 to 3% of biomass.

Table 7.4 Practical diet formulas (g/kg dry diet) for tilapia at various stages of culture

Ingredient	Broodstock diet [1]	Larvae/fry diet [2]	Grow-out diet [3]
Fish meal	362	301.7	182.5
Corn gluten meal	204	-	-
Soybean meal	177	259.5	250.0
Copra meal	118	114.8	100.0
Ipil-ipil meal	-	81.0	-
Cassava flour	-	-	364.2
Rice bran	75	149.7	60.0
Starch	32	30.0	-
Cod liver oil	5	10.0	-
Vegetable oil	5	10.0	-
Vitamin-mineral mix	22	43.3	43.3
Proximate composition (% DM):			
Crude protein	44.0	38.1	28.1
Crude fat	5.5	8.7	3.8
Crude fiber	9.1	5.6	3.6
Nitrogen-free extract	29.6	30.8	54.6
Ash	11.8	16.8	9.9

Sources: [1,2,3] Santiago et al. 1985,1986,1987, respectively

Broadcasting by using a cup or a ladle is the most common feeding method. The feed is distributed evenly or in several sites so that all fish have easy access to feed. Water temperature, fish size, the quality of the feed, and the presence of natural food affect the feed consumption of tilapias. Thus, the daily feeding ration is adjusted, depending on fish appetite.

Intensive culture of tilapia has gained popularity in recent years. This method of farming requires the use of high quality feeds and modern management tools. Fish are stocked at high densities in concrete tanks, earthen ponds or floating net cages (50 to 74 fingerlings per m^3). For grow-out cages in lakes (Figure 7.14), feeding trays or a second fine-mesh net at the bottom of the cage are installed to help minimize the loss of feeds. A diet with 28% protein (Table 7.4) is given three times daily at 5 to 6% of biomass. Daily feeding rate can be decreased to about 2 to 3% as the fish size increases from 30 g to more than 100 g. The daily ration is given in three feedings 3 to 4 h apart, at 0900, 1200, and 1600 h. During cool months, when metabolic rate is low, feeding frequency can be reduced to twice a day at 0900 and 1500 h. The appetite is generally poor when water temperature is low, and highest in the afternoon, when the water is warmer.

Figure 7.14
Grow-out floating net cages in a lake.

Figure 7.15
Rabbitfish *Siganus guttatus* juveniles.

C. Rabbitfish

Rabbitfishes are widely distributed in the Indo-Pacific region. The local species *Siganus guttatus* (Figure 7.15) is an economically important food fish in the Philippines. It breeds in captivity, spawns regularly, and grows in brackishwater ponds feeding only on algae.

1. Broodstock in tanks

Rabbitfish spawns naturally every month the whole year round. Broodstock are fed with an artificial diet (Table 7.5) at 3% of the body weight per day, morning and afternoon. This diet enhances reproductive performance, and egg and larval qualities of rabbitfish.

Table 7.5 Practical diet formulas (g/kg dry diet) for rabbitfish broodstock and fry

Ingredients	Broodstock diet [1]	Nursery diet [2]
Fish meal	100.0	145.0
Shrimp meal (*Acetes* sp.)	-	145.0
Squid meal	100.0	134.0
Shrimp meal	100.0	-
Soybean meal	80.0	-
Corn gluten meal	80.0	-
Wheat germ	80.0	-
Brewer's yeast	60.0	-
Cellulose	187.3	28.5
Bread flour	22.5	376.0
κ-carrageenan	-	50.0
Cod liver oil	100.0	71.0
Soybean oil	15.0	-
Soybean lecithin	45.0	-
Vitamin mix	20.0	30.0
Mineral mix	10.0	20.0
Butylated hydroxytoluene	0.2	0.5
Proximate composition (% DM):		
Crude protein	38.4	39.2
Crude fat	17.4	9.9
Crude fiber	13.1	5.5
Nitrogen-free extract	23.6	37.8
Ash	7.5	7.6

Sources: [1] Duray et al. 1994; [2] Parazo 1991.

2. Larval rearing and nursery

In the hatchery, rabbifish larvae (day 1 to 21) can grow and survive well with feeding either highly unsaturated fatty acids (HUFA)-enriched rotifer at 15-20 ind/ml, HUFA-enriched rotifer supplemented with artificial diet (0.5 g/m^3/day), or *Chlorella*-fed rotifers supplemented with artificial diet. Good growth and survival are also obtained when larvae (day 25 to 45) are fed an artificial diet (Table 7.5). Feeding rate is at 23% of initial biomass daily, given at 0900, 1030,1200,1300, 1500, and 1700 h. A feeding scheme from day 1 to 45 outlined in Figure 7.16 yields consistently fair survival.

3. Culture in ponds and floating netcages

The natural food recommended in the rearing pond are filamentous algae such as *Cladophora linum*, *Chaetomorpha* spp., and *Enteromorpha tubulosa*. These algae are planted uniformly in small clumps in the entire pond, done during the late afternoon to prevent them from floating the next day. Rabbitfishes reared in floating cages and in ponds are also fed supplementary feeds or commercial tilapia feeds.

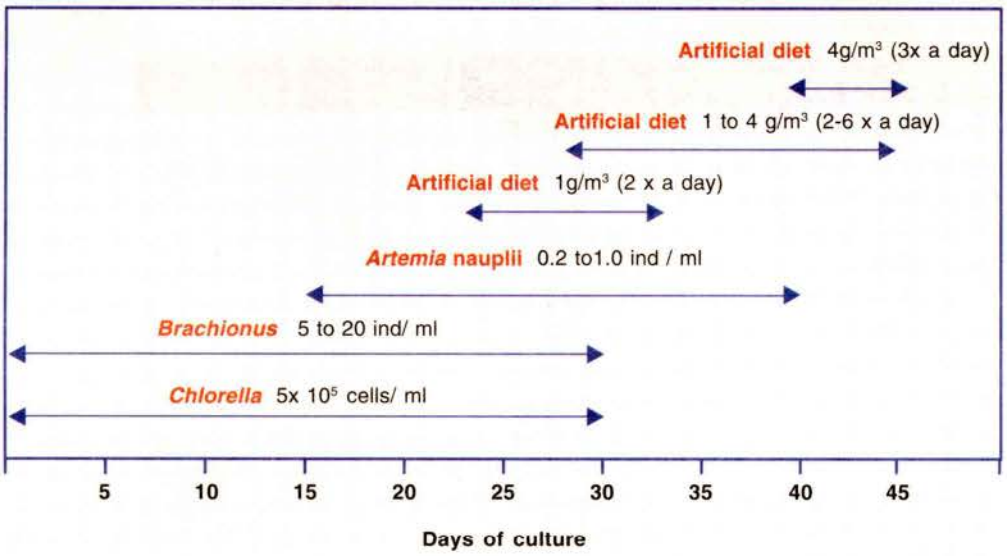


Figure 7.16
Feeding scheme for rabbitfish larvae.
Source: Hara et al. 1986

D. Bighead Carp

Carps are among the most commercially desirable freshwater fish species in Asia and the Indo-Pacific region. Bighead carp (*Aristichthys nobilis*) (Figure 7.17A) has a high reproductive capacity and fast growth rate, feeds low on the food chain, and can be grown in monoculture in ponds, cages and fish pens or in polyculture with milkfish and tilapia in pens.

1. Broodstock in fish pens and cages

Bighead carp mature in cages in the lake without supplemental feeding, but fry production and survival are enhanced when the broodstock are fed supplemental diets. Broodstock diet (Table 7.6) is fed at 2 to 0.8% of body weight per day at 0930 and 1500 h. Artificial diets positively influence the onset of gonad maturation and enhance the hatchability of eggs.

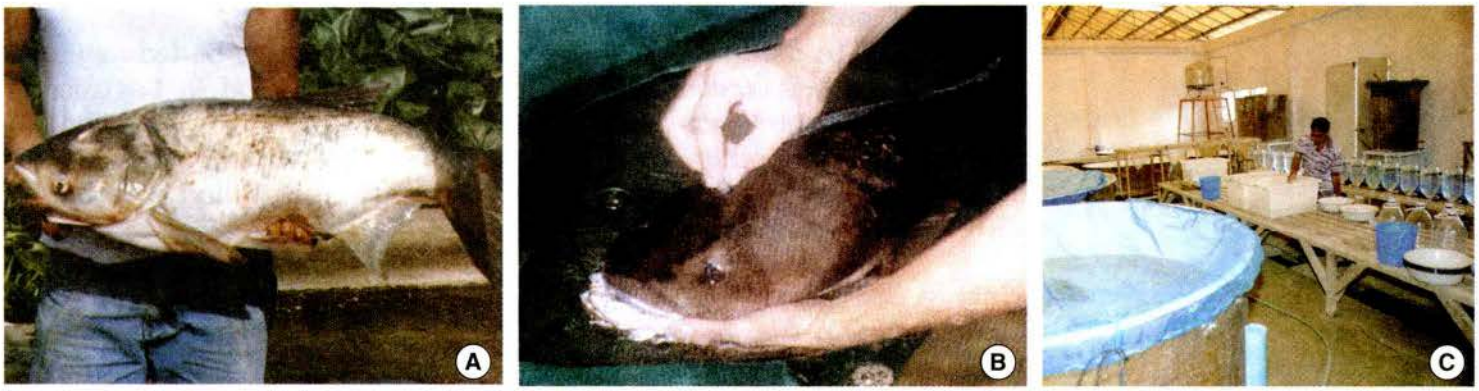


Figure 7.17
Bighead carp *Aristichthys nobilis* (A), induced to spawn using hormonal injection (B), and indoor larval rearing facilities (C).

Table 7.6 Practical diet formulas (g/kg dry diet) for bighead carp at various stages of culture

Ingredients	Broodstock diet [1]	Larval diet A [2]	Larval diet B [3]
Copra meal	200.0	-	-
Fish meal	150.0	406.0	566.0
Shrimp meal (*Acetes* sp.)	-	-	90.0
Rice bran	25.0	150.0	127.0
Soybean meal	320.0	361.3	114.0
Corn gluten meal	-	-	-
Meat and bone meal	224.0	-	-
L-lysine	1.0	-	-
DL-methionine	5.0	-	-
Starch	26.9	-	10.0
Cod liver oil	5.0	32.7	25.0
Corn oil	5.0	-	25.0
Vitamin-mineral premix	38.1	50.0	43.0
Proximate composition (% as-fed):			
Crude protein	41.5	40.2	41.5
Crude fat	5.1	6.8	11.9
Crude fiber	3.9	no data	no data
Nitrogen-free extract	30.4	26.5	24.5
Ash	14.6	no data	12.5

Sources: [1] Santiago and Gonzal 2000; [2] Fermin and Recometa 1988; [3] Santiago and Reyes 1989

2. Larval and nursery rearing

Induced spawning of the broodstock using hormones (Figure 7.17B) produces bighead carp larvae. In the nursery (Figure 7.17C), the combination of live food organism and artificial diet enhances the growth of bighead carp larvae. The combination of *Moina* and larval diet A (Table 7.6) fed daily to satiation at 0900, 1100, 1300, and 1600 h gives the best growth of bighead carp larvae (day 5 to 12 weeks). Similarly, the combination of *Brachionus* and larval diet B (Table 7.6) has a positive effect on growth. Bighead carp larvae can grow well on artificial diet given initially at 100% of the body weight per day, and finally at 30% of the fish biomass daily. These larvae are reared in indoor nursery tanks for 30-45 days before they are harvested for stocking in grow-out cages.

3. Grow-out culture

Cultured in fish pens, bighead carps do not have to be fed artificial feeds, since they thrive well on abundant natural food in Laguna de Bay. Carp farming has already expanded to upland and landlocked areas in the Philippines. Bighead carps are reared together with tilapia in fertilized ponds where they rely mainly on natural food organisms, with occasional supplemental feeds.

E. Native Catfish

The native catfish *Clarias macrocephalus* (Figure 7.18A) is an important indigenous freshwater food fish in Malaysia, Thailand, and the Philippines. At SEAFDEC AQD, catfish is induced to breed in captivity in order to arrest the decline of the natural population, and ensure a sufficient supply of fry and fingerlings for stocking.

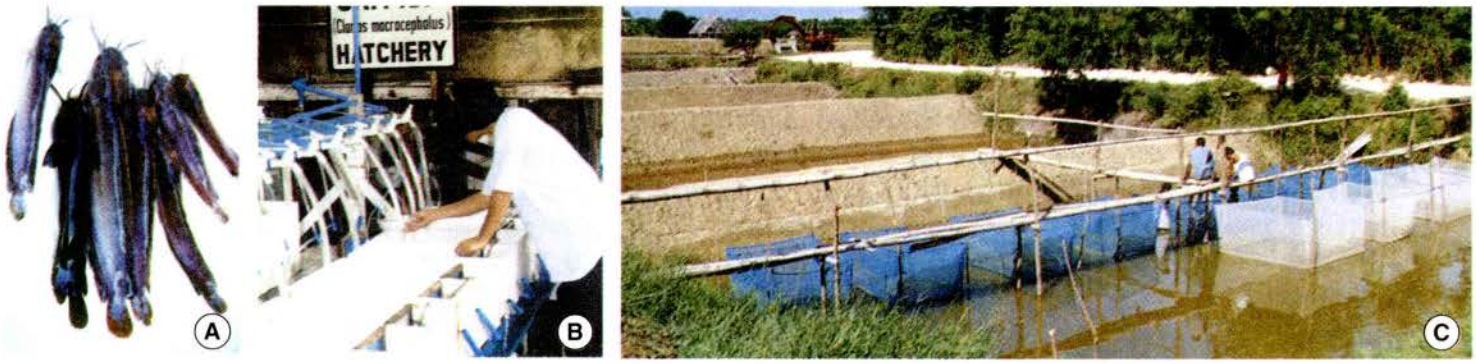


Figure 7. 18
Native catfish *Clarias macrocephalus* (A), reared in hatchery (B), and in net-cages in pond (C).

1. Broodstock

Traditionally, raw fish alone or in combination with artificial diet is used as feed to condition the broodstock for induced spawning. Live foods like *Tubifex* worms are also used to supplement the dry broodstock diet. Without supplementation of natural food, two artificial diet formulations enhance the reproductive performance of catfish and can be used in production and maintenance of broodstock (Table 7.7). Broodstock are fed two times a day to satiation or about 8 to 10% of the fish biomass daily.

Table 7.7 Practical diet formulas (g/kg dry diet) for Asian catfish at various stages of culture

Ingredient	Broodstock diet A[1]	Broodstock diet B[1]	Larval diet [2]	Grow-out diet [3]
Fish meal	250.0	150.0	73.8	200
Soybean meal	350.0	350.0	380.0	300
Shrimp meal (*Acetes* sp.)	-	-	77.5	-
Meat and bone meal	53.3	224.0	-	-
Squid meal	-	-	71.3	-
Rice bran	-	156.1		310
Copra meal	261.2	34.4		-
Ipil-ipil leaf meal	-	35.5		-
Bread flour	-	-	191.4	90
Starch	20.0	-		-
κ-carrageenan	-	-	10.5	-
Cellulose	-	-	14.7	-
Cod liver oil	-	-	80.8	-
Soybean oil	30.0	20.0		50
Vitamin mix	35.5	30.0	50.0	-
Vitamin-mineral mix	-	-		10
Mineral mix	-	-		10
Dicalcium phosphate	-	-	50.0	30
Proximate composition (% DM):				
Crude protein	42.5	43.1	39.9	34.2
Crude fat	7.2	7.9	10.6	9.5
Crude fiber	4.2	3.4	1.5	5.8
NFE	33.4	30.2	40.2	36.3
Ash	12.7	15.4	7.5	14.2

Sources: [1]Santiago and Gonzal 1997; [2] Fermin and Bolivar 1996; [3]Coniza et al. 2001

2. Larval and nursery rearing

Live zooplankton feed (*Artemia* or *Moina*) is required for successful larval rearing (Figure 7.19B) of catfish. During the first week of rearing, newly hatched *Artemia* are fed to fish at 10 individuals per day and artificial diet at 50% of body weight. At the second week of culture, *Artemia* is given at 20 individuals per day and artificial diet at 25% of body weight per day. The daily ration is divided into four feedings given between 0900 and 1500 h. Weaning of catfish larvae to dry artificial diet can be started four days after feeding live zooplankton. A combined diet of *Artemia* and a dry artificial feed (Table 7.7) improves the growth and survival of catfish larvae.

Hatchery-bred catfish fry stocked in net cages installed in tanks and in ponds (Figure 7.18C) grow well on artificial diet. In ponds, natural food (copepods and cladocerans) also serves as food source for catfish juveniles.

3. Grow-out culture

Traditionally, catfish feeds on decaying organic matter in the pond with kitchen refuse as supplement. Feeding catfish blanched chicken entrails and rice bran (80 : 20) at 10% of the fish body weight per day is also practised.

Cultured in pens inside a pond (Figure 7.18C), catfish fry are fed an artificial diet (Table 7.7) at 5, 4.5, 4, and 3.5% of fish body weight per day for the 1st, 2nd, 3rd and 4th month of culture, respectively. Feeds are given at 0800 and 1600 h with the crumble diet for the first two months and about 2.5 mm diameter pellets thereafter until harvest.

F. Asian sea bass

Figure 7.19
Asian sea bass *Lates calcarifer*.

Sea bass (*Lates calcarifer*) (Figure 7.19) is an important aquaculture species in Thailand, Hong Kong, Singapore, and Malaysia. Called "barramundi" in Australia and Papua New Guinea, sea bass is an important resource, forming the basis of their commercial and recreational fisheries. Sea bass is an opportunistic predator throughout its life cycle.

1. Broodstock in seacages and tanks

Sea bass reared to maturity in floating net cages and tanks spawn naturally. Sea bass are fed raw fish at 3 to 5% body weight once daily and reduced to 1 to 2% body weight during the peak of the spawning season.

2. Larval and nursery rearing

The phytoplankton *Chlorella*, *Tetraselmis* or *Isochrysis* are added to larval rearing tanks as water conditioners and as food for rotifers. Sea bass begin to feed 50 h after hatching but feed is given earlier at 36 h. Larvae are weaned gradually to each new feed type by increasing

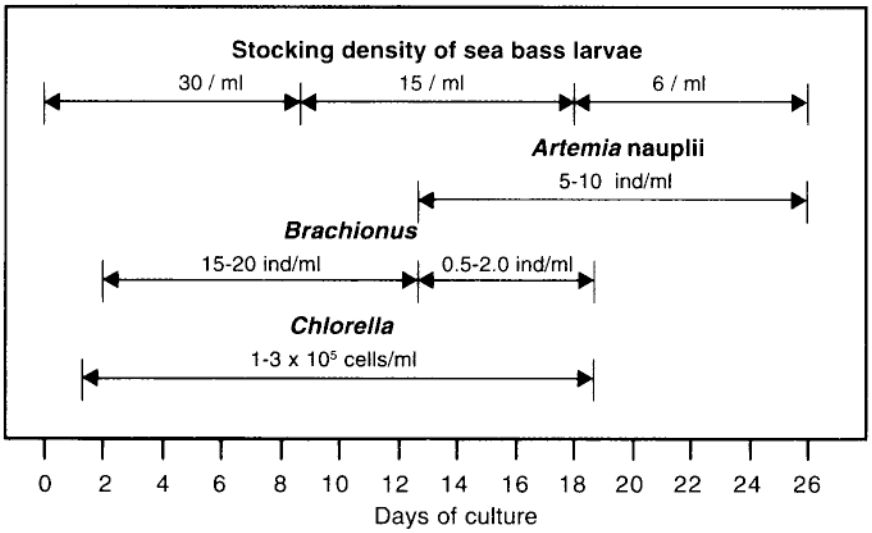


Figure 7.20
Feeding management scheme for the larval rearing of sea bass.
Source: Parazo et al. 1998

the daily proportion of the new feed type while gradually decreasing that of the preceeding feed. (Figure 7.20)

As a partial or complete replacement of *Artemia*, the freshwater cladoceran *Moina* may be fed to sea bass larvae starting with 17 to 20 day old larvae at a feeding rate of not less than 1 ind/ml. *Moina* are first sieved to obtain small adults and neonates before feeding to 17-day old larvae. Unsieved *Moina* can be fed directly to 20-day old larvae. When feeding with *Moina*, the salinity of rearing water is gradually lowered to about 10 ppt within a 24-h period. Larvae are fed at least four times daily. The brackish water cladoceran, *Diaphanosoma*, is also a potential live food for sea bass larvae. It can be fed once a day to 15-day old and older larvae at 2 ind/ml at 32 to 35 ppt.

Sea bass larvae and juveniles can be reared in nursery cages installed in open waters using night-light to attract zooplankton (mostly copepods) as food. A stocking density of 600/m^2 can be used when fish feed on zooplankton alone but minced fish flesh given *ad libitum* during daytime is needed when stocking density is increased to 1200/m^2.

Table 7.8 Practical diet formula (g/kg dry diet) for juvenile sea bass

Ingredients	Amount [1]
Fish meal	420.0
Soybean meal	90.0
Shrimp meal (*Acetes* sp.)	100.0
Squid meal	50.0
Bread flour	77.5
Cellufil	145.0
Cod liver oil / soybean oil (1:1)	57.5
Vitamin mix	40.0
Mineral mix	20.0
Proximate composition (% DM):	
Crude protein	43.2
Crude fat	9.3

Source: [1]Catacutan and Coloso 1995.

3. Grow-out culture

Sea bass juveniles respond well to dry artificial diet as long as they are trained to feed an artificial diet are early in the nursery. In tanks, juveniles fed an artificial diet (Table 7.8) at 17 to 14%, 12 to 8%, and 8 to 5% of the body weight for the first 20

days, next 20 days, and last 14 days, respectively. Daily ration is given in three equal portions at 0930, 1300, and 1600 h. In ponds and in sea cages, sea bass grow well on raw fish alone or in combination with artificial feeds.

G. Orange-spotted grouper

Groupers are among the most valuable species for export markets. Their demand is mostly in countries with high seafood consumption or with high economic growth. Groupers spawn readily in captivity but larval survival is still low. At present, the grow-out industry relies on fry taken from the wild.

1. Broodstock in sea cages and tanks

Orange-spotted grouper (*Epinephelus coioides*) (Figure 7.21) first mature as females at around 3 to 4 kg body weight, some of the fastest growing females change into males when they reach more than 6 kg. Broodstock held in tanks or cages spawn monthly, usually within a week before or after the last quarter moon phase. Broodstock maintained in sea cages and in tanks are fed a variety of species of raw fish every other morning at satiation level.

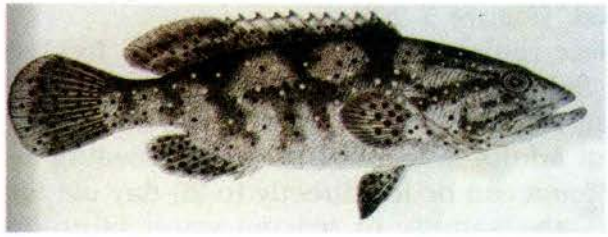


Figure 7. 21
Orange-spotted grouper *Epinephelus coioides*.

2. Larval rearing

In intensive larval rearing, the rearing protocol involves feeding young larvae (day 2 to day 15) with screened rotifers previously enriched with high *n*-3 HUFA boosters. Older larvae (day 20 to 50), are fed enriched *Artemia* nauplii or *Artemia* meta-nauplii until metamorphosis to the juvenile phase (Figure 7.22).

In semi-intensive larval rearing, grouper larvae stocked at low densities (10 larvae/l) with minimal water exchange feed on copepod nauplii during the early rearing phase. Copepods are collected from brackish water ponds and inoculated in the larval tanks 2 to 3 days before stocking of larvae. First-feeding larvae prefer copepod nauplii than rotifer.

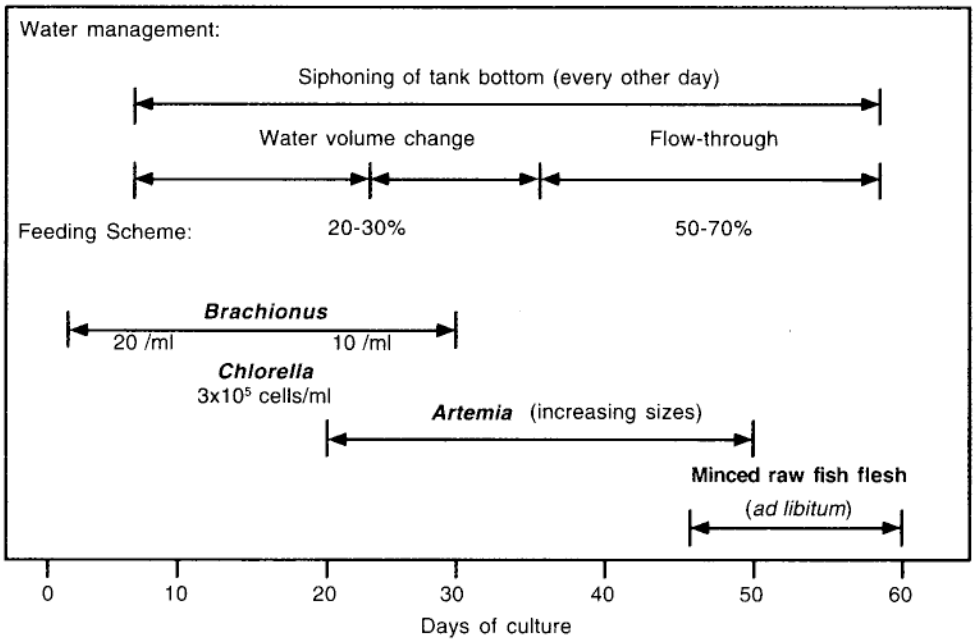


Figure 7.22
Feeding and water management scheme for intensive rearing of grouper larvae.
Source: Duray et al. 1997

3. Grow-out culture

Generally, raw fish is used as feed for groupers farmed in sea cages, ponds, and net-cages in ponds. Another feeding strategy is to initially stock adult tilapias (5,000 to 10,000 per hectare) in ponds and allow them to reproduce. Tilapia fingerlings are then used as food for grouper juveniles. In addition, chopped raw fish is given every other day at 5% of grouper biomass. Half of the ration is given in the morning and half in the afternoon, some are placed in feeding trays for monitoring purposes and the rest are broadcasted. If raw fish is the sole feed, groupers may be given up to 10% of biomass daily.

Hatchery-bred grouper juveniles accept dry artificial pellets as long as they are trained to feed on the pellets early in the nursery. Artificial feed (Table 7.9) is given to groupers cultured in offshore cages and in cages installed inside the pond at 4 to 6% of biomass daily.

Table 7.9 Practical diet formulas (g/kg dry diet) for grow-out culture of grouper

Ingredients	Grow-out diet	Grow-out diet
Chilean fish meal	80	200
Meat and bone meal, local	-	200
Meat and bone meal, imported	320	-
Blood meal	80	80
Shrimp meal	100	100
Soybean meal	60	60
Squid meal	10	10
Wheat flour	150	150
Rice bran	70	70
DHA-Selco		
Cod liver oil	60	60
Vitamin mix	40	40
Mineral mix	30	30
Vitamin-mineral mix	-	-
Butylated hydroxytoluene	-	-
Proximate composition (% DM):		
Crude protein	44.0	44.0
Crude fat	11.5	11.5
Crude fiber	1.8	1.8
NFE	25.8	25.8
Ash	16.9	16.9

Sources: Millamena et al. 2001

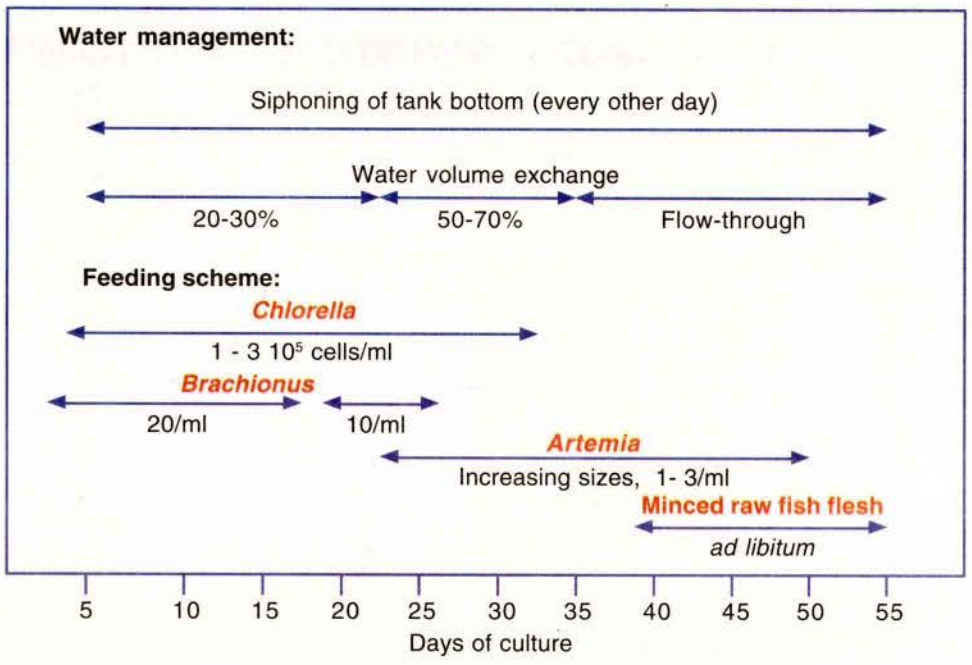
H. Mangrove red snapper

Mangrove red snapper *Lutjanus argentimaculatus* (Figure 7.23) is an important food fish in Southeast Asia and is popularly cultured in brackish water ponds and marine net cages. Snappers are opportunistic carnivores and feed mainly on fish and crustaceans.

Figure 7. 23
Mangrove red snapper *Lutjanus argentimaculatus.*

1. Breeding

Wild-caught or hatchery produced fry are sexually mature after 5 (males) and 6 years (females) in floating net-cages or in tanks. Broodstock are fed raw fish every other morning at 5% of body weight. They are sexually mature from April to October and they spawn naturally for up to four consecutive days.



2. Larval rearing

The feeding regime for the red snapper larvae consists of *Chlorella*, rotifers, *Artemia*, and minced fish flesh (Figure 7.24). Growth and survival of red snapper larvae are best when fed screened rotifers (<90 μm) during the first 14 days and *Artemia* nauplii (2 per ml) given four times a day starting on day 22. Day 21 to 35 larvae have similar survival with *Artemia* alone, artificial diet alone, or combination *Artemia* and artificial diet.

Figure 7.24
Feeding and water management during larval rearing of the mangrove red snapper.
Source: Duray et al. 1994

3. Grow-out culture

Snappers are usually fed raw fish in floating net-cages but they also respond well to artificial diet. In tests done in 250 l tanks the feed (Table 7.10) given twice a day at 0930 and 1600 h at 3.5 to 4.5% of body weight per day enhances growth and survival.

Table 7.10 Practical diet formula (g/kg dry diet) for red snapper

Ingredients	Amount[1]
Peruvian fish meal	430.0
Squid meal	80.0
Shrimp meal (*Acetes* sp.)	54.0
Defatted soybean meal	80.0
Bread flour	200.0
Rice bran	58.0
Cod liver oil	38.0
Soybean lecithin	5.0
Vitamin and Mineral mix	50.0
Ascorbyl monophosphate	0.2
Dicalphos	5.0
Proximate composition (% DM):	
Crude protein	44.0
Crude fat	10.0
Carbohydrate	26.5
Energy (MJ/kg diet)	18.9

Source: [1]Catacutan et al. 2001

I. Tiger shrimp

The tiger shrimp *Penaeus monodon* (Figure 7.25A,B) is the most widely cultured shrimp species and accounts for over one third of globally farmed shrimp. High-density production is supported by greater use of formulated feeds, where production may yield up to 10 tons/ha/year. Tiger shrimp culture has evolved from traditional extensive to semi-intensive and intensive systems using modern equipment and techniques. The aim is to produce significantly high yield per unit area while minimizing farm waste discharges to the environment.

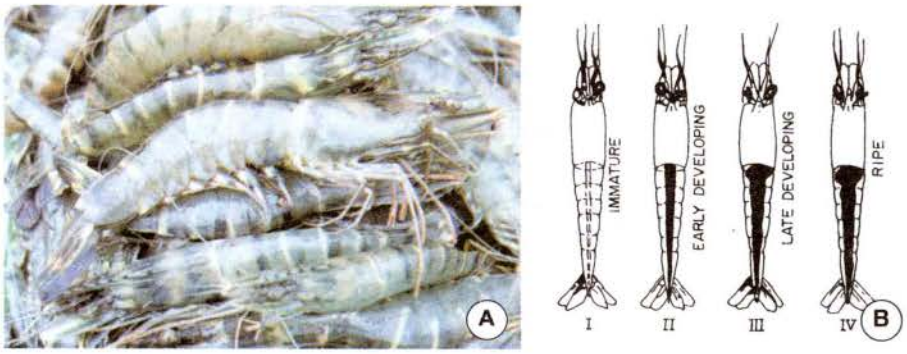


Figure 7. 25
Tiger shrimp *Penaeus monodon* (A), and stages of ovarian maturity (B).

1. Broodstock in tanks

Shrimp broodstock are fed on a variety of live, fresh, or fresh-frozen natural food, such as marine annelids, mussel meat, squid, clam meat, and other meat of mollusks and can be given alternately. These natural food items contain essential nutrients particularly *n*-3 HUFA that are needed for shrimp maturation (Figure 7.25B). Brown mussel meat and squid are chopped into small pieces. Marine worms are given to shrimps preferably alive. For convenience, they are bought in bulk, weighed, packed, frozen, and thawed as needed for feeding. An artificial diet has been formulated using the fatty acid and amino acid profiles of mature shrimp ovaries as guide. The diet is supplemented with fresh wet feeds in an attempt to ensure an optimal nutrient balance and a full range of essential nutrients. Reproductive performance of pond-reared shrimp broodstock is markedly improved when fed a combination diet of natural food and pellets. A mixture of various feedstuffs is better than a single item in the diet. Most of the females given natural food alone undergo ovarian regression and do not spawn.

The standard feeding regime for shrimp broodstock at SEAFDEC AQD consists of fresh food and pellets (Table 7.11) given alternately. Fresh or frozen natural foods (squid, mussel meat, or marine worms) are given daily at 0800 to 0900 h at 5 to 10% of biomass, and pellets at 1600 to 1700 h at 2 to 3% of biomass. Natural foods are broadcasted into the maturation tank while pellets are placed in feeding trays.

Table 7.11 Practical diet formulas (g/kg dry diet) for tiger shrimp at various stages of culture

Ingredient	Broodstock diet [1]	Larval diet [2]	Grow-out diet [3]
Squid meal	300.0	300.0	-
Shrimp meal	-	-	150.0
Shrimp head meal	200.0	350.0	-
Fish meal	200.0	-	250.0
Soybean meal	-	-	250.0
Bread flour	55.0	110.0	130.0
Rice bran	22.5	-	69.5
Seaweed (*Gracilaria*)	40.0	-	50.0
κ-carrageenan	-	5.0	-
Celufil	-	22.0	-
Cod liver oil	60.0	80.0	25.0
Soybean oil	-	-	25.0
Soybean lecithin	30.0	25.0	-
Cholesterol	5.0	10.0	-
Vitamin mix	27.0	60.0	20.0
β-carotene	-	2.5	-
Mineral mix	60.0	40.0	10.0
Dicalcium phosphate	-	-	20.0
Butylated hydroxytoluene / Ethoxyquin	0.5	0.5	0.5
Proximate composition (% DM):			
Crude protein	52.8	50.3	41.7
Crude fat	12.1	14.2	8.8
Crude fiber	3.8	20.4	5.9
Nitrogen-free extract	13.4	10.1	29.2
Ash	17.9	5.0	14.4

Sources: [1] Millamena et al. 1986; [2] Bautista et al. 1989; [3] Millamena and Triño 1994

2. Larvae in hatchery and nursery tanks

In the hatcheries, the food of shrimp protozoeal larvae up to postlarvae PL_5 consists mostly of planktonic microalgae *(Skeletonema, Tetraselmis, Chaetoceros)* and *Artemia* nauplii. At PL_6 onwards, postlarvae are gradually introduced to minced mussel meat, raw fish, and shrimp meal *Acetes* sp. or pellet crumbles.

Artificial diets that partially replace live food help alleviate the need for large tanks necessary for phytoplankton production. Shrimp larval diets are readily available and are easy to use. Many commercial microparticulate diets can partially replace the natural food requirements of shrimp larvae. At SEAFDEC AQD, a microbound diet (Table 7.11, Figure 7.26) is used to partially replace diatoms and *Artemia* as food for shrimp larvae. However, it needs to be more buoyant and should remain suspended in water longer to be more available to the larvae. Seawater is replaced at the rate of 30% per day from Z_2 to Z_3 stage and 50% per day during mysis and postlarval stages.

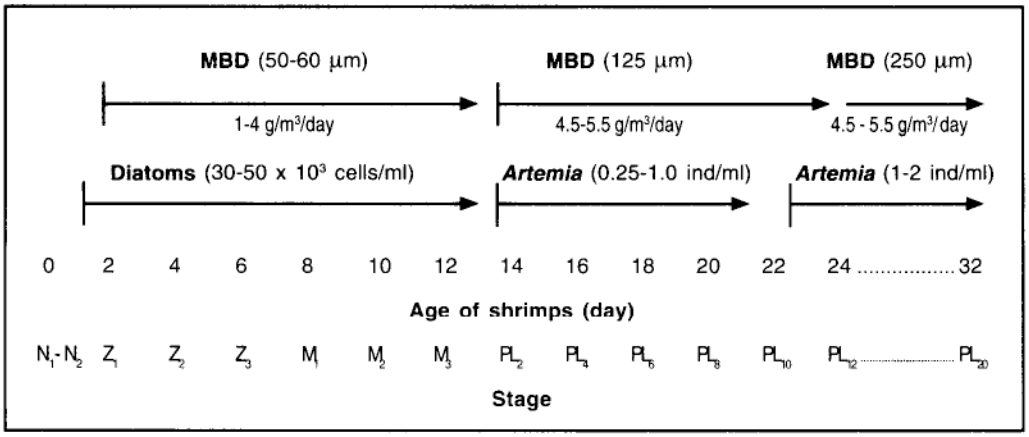


Figure 7.26
Feeding scheme for tiger shrimp larvae Z-zoea, M-mysis, PL-postlarvae at days 1 to 20.
Source: Bautista et al. 1991

3. Shrimps in grow-out ponds

Shrimp postlarvae are released directly into nursery ponds. Farmers propagate natural food organisms such as copepods in the ponds by applying organic (chicken manure) and/or inorganic (monoammonium phosphate 16-20-0, diammonium phosphate 18-46-0) fertilizers. In extensively managed ponds, shrimp feed mostly on detritus, animal remains, diatoms, cyanobacteria, and green algae. The most cost-effective way of producing shrimp is to grow natural food in the ponds and provide artificial feed when necessary. A suggested feeding formula for shrimp grow-out culture is shown in Table 7.11. At 50,000/ha, this formulated diet may be given starting 1-1.5 months after stocking depending on the amount of natural food in the pond. At 100,000/ha, this diet is given soon after the shrimps are stocked.

Early juvenile stages prefer to feed in the shallower water along the walls of the dike. As development proceeds through late juvenile into the adult stages, the shrimp feed freely on the benthos of the whole pond. Shrimp are slow bottom feeders and feed continuously and more actively at night. The ingested feed takes about four hours to leave the midgut. Daily feeding is usually done 4 to 5 times (0600, 1000, 1400, 1800, and 2200 h) with about 30% of the total feed given in the morning and 70% in the late afternoon and evening.

Metabolic rates of animals decrease with size. The feeding rate is not fixed, but is lowered as the shrimp grow. Dedicated management and appropriate feeding practices represent a major labor component but is crucial to the success of the culture. Feeding based on biomass alone without adjustment over time may result in underfeeding when shrimp are small, and overfeeding when they are larger.

The most efficient way to feed shrimp is "by demand" rather than by following a feeding table. Daily rate can be adjusted according to how much of the previous day's ration is consumed. Feeding trays are used to observe the size and health condition of shrimp as well as to monitor feeding activity to avoid overfeeding and underfeeding.

Figure 7. 27
A feeding tray for shrimp.

Trays measuring 1 x 1 m can be made from nylon screen and wood or bamboo strips and stone sinkers (Figure 7.27). Usually 1 to 3% of the daily ration allotted to the pond is spread between several feeding trays. Five to twenty feeding trays are usually distributed over one hectare, positioned not too near the dikes or the pond bottom. Feed is placed on trays to monitor feeding activity. Feeding trays should be installed immediately after stocking to permit observation of the condition and growth of the shrimp, and to detect the presence of any pests or predators. The feed allowance can be adjusted based on the amount of feed left on the trays after one to two hours. The next feeding ration is then adjusted accordingly based on the average amount of feed remaining on the trays.

At the end of the grow-out cycle, shrimps avoid very shallow areas during the day to avoid light and those areas where anaerobic sediments and sludge accumulate. Thus, feed is distributed only in deeper areas during daytime and none in areas where sludge deposits are high because shrimp will not consume it.

Figure 7.28
Mangrove crabs *Scylla* spp.

J. Mud Crabs

Various species of mud crabs occur throughout tropical to warm and temperate zones. They are highly valued and are important sources of income for fishers throughout the Asia-Pacific region. Extensive mud crab (*Scylla serrata, S. tranquebarica, S. olivacea*) (Figure 7.28) farming has been an on-going industry for many decades but the technology for broodstock, hatchery, nursery, and semi-intensive grow-out culture have been developed only recently.

1. Broodstock in tanks

Mud crabs are provided with flow-through aerated seawater in tanks. They are fed a combination of natural food consisting of mussel meat, raw fish, and formulated diet (Table 7.12). Feeding rate is 3 to 5% of biomass for natural food and 1 to 1.5% for formulated diet. Feed is given twice daily with formulated diet given in the morning at 0800 h, and natural food in the afternoon at 1700 h. Reproductive performance and larval quality are better with this combination diet than on either natural food or formulated diet alone.

2. Larval rearing and nursery

Spawned eggs hatch and are raised to zoea then to megalopa, the highest larval stage. Initially, zoea are fed *Brachionus* at 10 to 15 ind/ml are fed then *Artemia* nauplii are introduced at $zoea_3$ and are gradually increased from 1 to 5 ind/ml as the larvae develop to megalopa (Figure 7.29). As a supplement, a commercial larval diet for shrimp may be given at 1.5 to 2.0 g/m^3 water per day.

Megalopas are nursed until crab stage either in tanks or in net cages installed in ponds. In tanks, food consist of newly hatched Artemia at 3 to 5 ind/ml or adult Artemia, then to minced fish flesh, green mussel meat, or small shrimps given *ad libitum* twice a day as soon as the megalopa metamorphose to crab stage. In net cages

Table 7.12 Practical diet formulas (g/kg dry diet) for mud crab broodstock and grow-out

Ingredients	Broodstock diet [1]	Grow-out diet in pond [2]	Grow-out diet in tank [3]
Chilean fish meal	200	250	335
Shrimp meal (*Acetes* sp.)	-	-	75
Soybean meal, defatted	-	-	110
Shrimp head meal	200	-	-
Brown mussel meat	-	250	-
Squid meal	200	-	65
Wheat flour	170	170	118
Seaweed (*Gracilaria* sp.)	40	50	50
Rice bran	-	125	-
Corn bran	-	100	-
Cellufil -	-	-	162
Carboxy methyl cellulose	-	-	25
Cod liver oil	50	25	14
Soybean oil	-	25	-
Lecithin	30		5
Cholesterol	10	-	1
Vitamin mix	30	-	15
Mineral mix	40	-	5
Dicalcium phosphate	30	-	20
Ethoxyquin	-	5	-
Proximate composition (% DM):			
Crude protein	46.0	40.1	43.3
Crude fat	11.6	11.9	4.7
Crude fiber	4.2	1.4	12.0
Nitrogen-free extract	23.2	38.0	27.1
Ash	15.0	8.6	12.9

Sources: [1] Millamena and Quinitio 2000; [2] Triño and Millamena 2001; [3] Catacutan and Teshima 2001

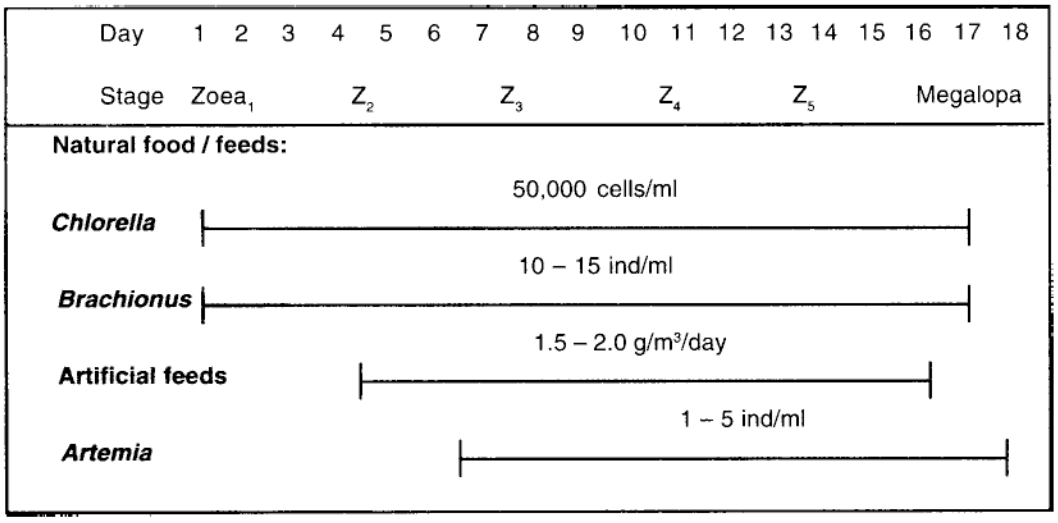


Figure 7.29
Feeding scheme used in the culture of mud crab larvae.
Source: Quinitio et al. 2001

installed in brackish water nursery ponds, crab megalopas are fed macerated brown mussel meat or fish flesh at 30 to 20% of biomass per day. Feed ration is given at 0800, 1300, and 1700 h daily. Stocking 30 megalopas/m^2 in net cages is feasible.

3. Pond culture

Culture of either male, female, or both sexes of mud crab in earthen ponds (Figure 7.30) or in pens installed in tidal flats with reforested mangrove are economically viable. Stocking density of 1/m^2 gives high survival. Raw fish and fresh brown mussel meat are used as feed. Crabs are fed at 10% of their biomass daily when carapace length (CL) is <6 cm and 5% when CL is ≥6 cm. The daily feed ration is broadcasted over areas in between mangrove trees of each pen, 40% at 0700 h and 60% at 1700 h. Feed ration is determined by placing 1% of the scheduled feeding ration on feeding trays. When feed on the tray is consumed after four hours, the ration for the next feeding is increased by 5%. Feeding rate is reduced by 5% when 0.5% or more of the feed is left uneaten on the tray while no feed adjustment is made if less than 0.5% feed remains on the trays. Dry artificial diet can also be used as grow-out feed for mud crabs (Table 7.12). This diet is given at 5% of biomass daily when CL is <6 cm and 2% when CL is >6 cm. The daily feed ration is given at 0730 and 1700 h.

Mud crab can also be fed *Acetes*, green filamentous algae, animal hides and entrails, and snails. Crabs are fed at 10% then 6% of body weight as culture progresses. Daily feed allowance is placed on a feeding tray or broadcast twice a day, half of the feed in the morning, half in the afternoon.

A mud crab artificial diet (Table 7.12) has been developed in the laboratory. It is presently used as maintenance feed for mud crab under indoor conditions.

Figure 7.30
Grow-out culture in pond (A) and in pens installed in reforested mangroves (B).

Other species for aquaculture stock enhancement

A. Donkey's ear abalone

Donkey's ear abalone (*Haliotis asinina*) (Figure 7.31A) is a gastropod mollusk that live around algal stands in rocky and shallow sea waters. Abalones are popular in specialty restaurants in the Philippines and other Southeast Asian countries but their declining yield from the wild necessitates intensive aquaculture. Research at SEAFDEC AQD aims to develop seed production and culture techniques in order to produce seed for stock enhancement in the wild. Abalones are herbivores and feed mainly on seaweeds. Among the three naturally occurring abalone species in the Philippines, donkey's ear abalone grows to a maximum size of 100 to 110 mm in shell length (SL).

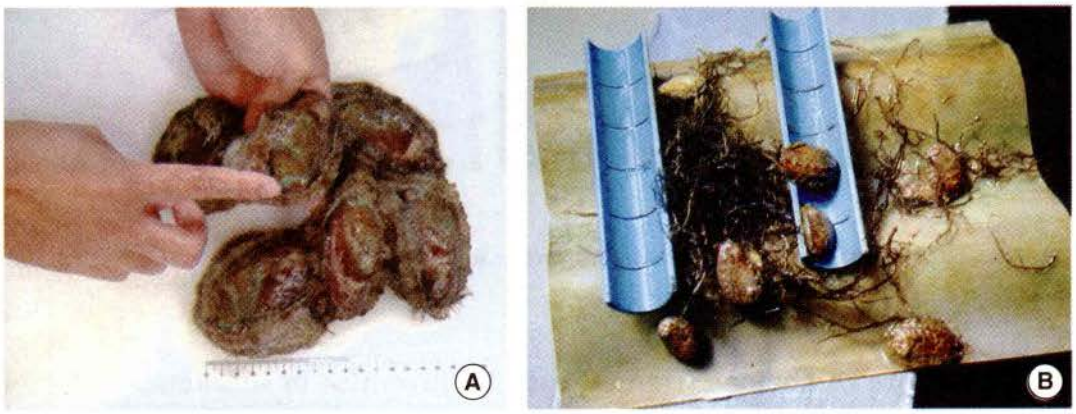


Figure 7.31
Donkey's ear abalone *Haliotis asinina* (A), feeding on red alga (B).

1. Breeding

Abalone breeders are kept in perforated plastic baskets and fed the red alga, *Gracilariopsis* (Figure 7.31B), to satiation replenished at weekly intervals. Captive abalone attains sexual maturity within 6 to 8 months of culture at shell size range of 35 to 40 mm. Artificial diet (Table 7.13) in combination with *Gracilariopsis* improves fertilization rate and spontaneous fecundity in abalones. Abalones are fed 2 to 5% of their body weight per day using the dry diet while the red alga is given *ad libitum*. The dry ration is given once at 1600 h.

Table 7.13 Practical diet formula (g/kg dry diet) for abalone broodstock

Ingredients	Amount
Fish meal	100.0
Shrimp meal	150.0
Defatted soybean meal	200.0
Rice bran	149.5
Wheat flour	200.0
Seaweed	70.0
Cod liver oil	15.0
Soybean oil	15.0
Vitamin mix	30.0
Mineral mix	40.0
Dicalcium phosphate	30.0
BHT	0.5
Proximate composition (% DM):	
Crude protein	27.9
Crude fat	5.8
Carbohydrate	40.4

Source: [1]Bautista-Teruel and Millamena 1999

2. Postlarval settlement and nursery rearing

Settlement tanks are prepared one week before stocking to allow the growth of epiphytic diatoms on the plate substrates (Figure 7.32A, B). Veliger larvae and early juveniles feed on epiphytic diatoms such as *Navicula* and *Nitzschia*, then gradually shift to red alga *Gracilariopsis* when they reach >5 mm SL.

Feeding larvae a commercial feed or the SEAFDEC formulated diet (Table 7.13) is feasible. These feeds are given at 3 to 5% of the body weight twice daily.

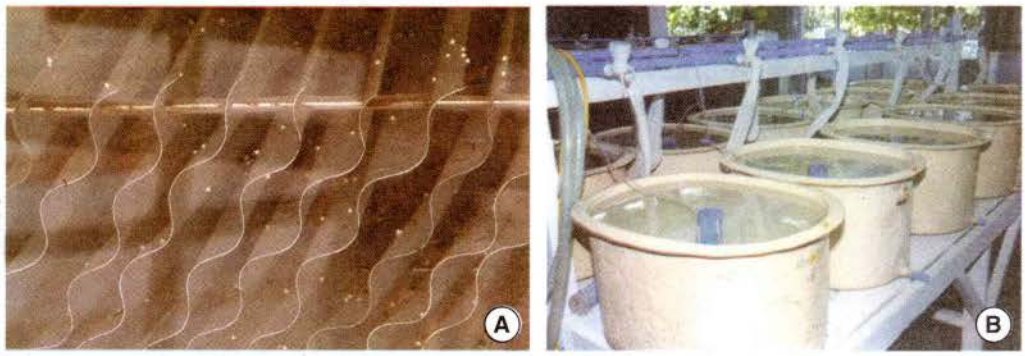


Figure 7.32
Plate substrates (A) for epiphytic diatoms, live food for larvae installed in tanks (B).

3. Grow-out

Culture of abalone from 30 mm SL juveniles to marketable size of 55 to 60 mm is carried out in flow-through tanks and in sea cages for 8 to 10 months. Abalones are fed fresh seaweeds to satiation given at weekly intervals.

In cages, feeding rates as well as growth and survival are higher at lower stocking densities (17 to 35 per cage). The rapidly growing small juveniles (16-20 mm) feed at 35 to 40% of body weight while the bigger abalones (> 50 mm) feed less, only 5 to 10% of body weight.

B. Seahorses

Live seahorses (Figure 7.33A) are traded on the aquarium fish market while the dried ones are highly valued in traditional Chinese medicine. Seahorses came near to extinction due to destruction of their natural habitat and overexploitation. At SEAFDEC AQD, breeding and seed production techniques are being undertaken on *Hippocampus kuda* and *H. barbouri* ultimately for stock enhancement in the wild.

Live food organisms are used as food for seahorses to breed and produce seeds in tanks (Figure 7.33B). The combination diet of HUFA-enriched *Artemia* adults, mysids, or tilapia fry promotes frequent parturition events and great brood size per female in tanks. *Artemia*, mysids, and tilapia fry are given at 15, 6, and 5% of the body weight per day, respectively. A combination diet of rotifers and copepods enhances survival of *H. kuda* larvae.

Figure 7.33
Seahorses (A) and a rearing tank (B).

C. Window-pane oyster

Window-pane oyster or *kapis* shell (*Placuna placenta*) (Figure 7.34A) is a bivalve mollusk that is commercially and economically important in the Philippines. Their translucent shells are used as raw materials for the fabrication of beautiful handicrafts like window sills, wall panels, lamp shades, and chandeliers (Figure 7.34B,C). With very high world market demand for these products, they become overexploited and their natural population is nearly decimated. Artificial propagation is carried out at SEAFDEC AQD in order to reseed and enhance the declining natural population in the wild, thus rehabilitating the *kapis* industry.

Figure 7.34
Window-pane oyster (A), and handicrafts using kapis shells (B,C).

1. Broodstock and seed production in tanks

Broodstock fed daily with *Isochrysis* and *Tetraselmis* at 3:1 ratio at 200,000 cells/ml promotes rapid gonad development. Sexual maturity is reached after 4 months, and then induced spawning is done to produce viable gametes. The larvae are reared in fiberglass tanks at a density of 900 larvae per liter. Larvae grow and survive best with *Isochrysis* as food at initial concentration of 10,000 cells/ml, and are progressively increased to 30,000 cells/ml as the larvae grow. Settling is reached after 14 days.

2. Stock enhancement

Kapis shells are filter-feeders and their food consist of plankton, detritus and other suspended materials in the estuary. The natural beds of *kapis* shells are the bluish-soft mud or slightly sandy-muddy substrates in areas with high primary productivity. To rehabilitate the natural beds of *kapis* shell at Tigbauan, Iloilo coastline (Figure 7.35A,B), immature broodstock (72 mm SL, 14.5 g body wt) are stocked at 75 ind/m^2. The broodstock feed on the available natural food in the estuary. Two months after reseeding, they spawn naturally.

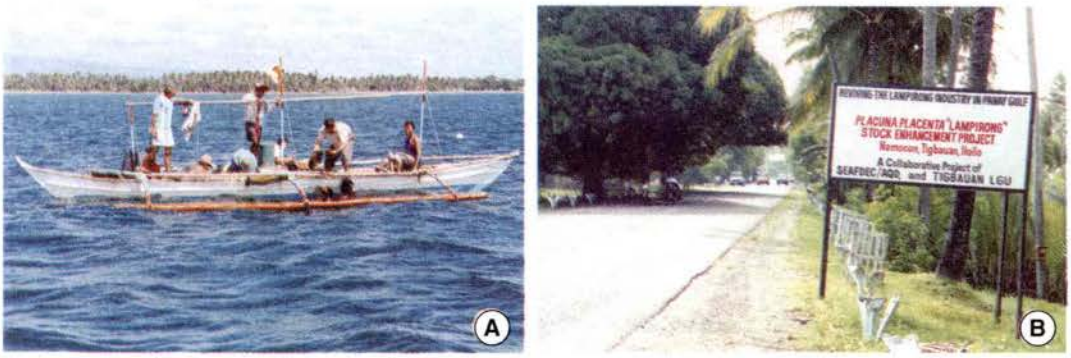


Figure 7.35
Rehabilitation of Iloilo coastline (A,B).

Summary

The objective of feed management is to make available to the animals the most cost-effective, formulated feed in the proper amounts and at the right times and locations. Feeding practices must be continually modified and adapted to account for changes in feeding activity and preferences as the animals grow and as environmental conditions change. Feed management practices are specifically adapted to species, culture system, and area in order to increase production efficiency and minimize environmental impact.

The farmer wants the cultured aquatic animal to grow maximally with a minimum amount of feed waste. Thus, one should have a good knowledge of how much the fish should be fed and then monitor their feeding activity carefully. Feed consumption can vary by species, size/age of animals, intensity level of culture, water temperature, dissolved oxygen, feed attractability and water stability, frequency and times of feeding, and other factors. Feeding rations are based on feed consumption from feeding trays, or survival estimates, average size, and feeding tables developed by the farmer himself using data from several culture cycles. Many biotic and abiotic factors are used to adjust feed quantity, and these criteria include low DO, extreme water temperatures, excessive plankton blooms, feed consumption, molting/lunar cycle, animal size and weather.

How often to apply feed is an important decision the culturist has to evaluate and determine based on experience, season, changing environmental conditions, species, age and size, stocking densities, production system used, stress, available resources, and other considerations. Constant feeding of newly hatched larvae is important for good survival. Particle size should be increased in close relation with growth.

Hand feeding is commonly used and has the advantage of close observation of the fish. There is then an assurance of feeding to maximum effectiveness and minimum waste since the fish culturist is more aware of the conditions and problems if they develop. Automatic and demand feeders have advantages in that they can be labor-saving and can allow fish to be fed many times throughout the day. Automatic feeders can be used as a useful alternative to manual broadcasting, but should not

replace regular observation and visual assessment by trained personnel.

Considering the diversity of species and production systems, best management practices, particularly those on feeding management, are important steps towards assuring that aquaculture will grow in a responsible manner and can be sustained.

Guide Questions

1. Compare the different feeding strategies in pond culture.
2. Why is supplementary feeding considered as the most cost-effective feeding strategy?
3. Discuss the importance of sampling and record keeping, adjustments of feeding rations and use of appropriate feed size to optimize the use of feeds.
4. How are feed rations and total feed requirement determined?
5. What are the significant contributing factors in the choice of feeding methods?
6. What are the advantages and disadvantages involved in manual vs. mechanical feed application methods?
7. Why does the high value for FCR indicate poor feed conversion efficiency?
8. What are the main waste products associated with feeding and how are they minimized?
9. What feed nutrients are agents of "eutrophication" in receiving waters for fish farm effluents? Discuss their harmful consequences in the environments.
10. How do we determine the appropriate time and frequency of feeding for fish and crustaceans?
11. Discuss the factors that influence feed consumption of aquatic animals.
12. What are the do's and dont's in feeding management?

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CHAPTER

8 Economics of Feeding

RENATO F. AGBAYANI

Introduction

The number of aquaculture farmers who adopt higher stocking densities and provide supplementary feeds to fish stocks is increasing because of increased production and improved profits. For example, in shrimp culture, the natural productivity of the water may generate from 100 to 300 kg/ha/yr and fertilization may further increase production to 600-1,000 kg/ha/yr in the Philippines. The use of feeds can raise production up to 20,000 kg/ha/yr in Taiwan and 30,000 kg/ha/yr in Japan.

In shrimp farming in the Philippines, although there is an increase in production, the cost of feeds takes up 54-63% of operating costs when stocking density is increased such as in the semi-intensive and intensive farming methods. In mudcrab culture, costs of feeds is 50-57% of operating expenses while in milkfish culture, the costs of feeds comprise 10-77% of total costs. Hence, an evaluation of feed quality and economic efficiency is a very important undertaking to determine the profitability of an aquaculture venture.

This chapter aims to introduce concepts and methods in doing economic analysis applicable in aquaculture in general with emphasis in feed production and feeding in aquaculture farms. This chapter discusses the following topics: cost of producing feeds; simple single-input (feeds) and single output (fish) production function; indices for measuring economic efficiency of feeds; the least-cost combination; and linear programming as used in the allocation of limited resources such as feed ingredients that will meet the nutritional requirements of the fish.

Cost of producing feeds

The cost items in producing aquaculture feeds can be classified into direct costs and indirect costs. Direct costs include the raw materials such as fishmeal, mineral and vitamin mixes, and other ingredients, and direct labor used in the manufacture of the feeds. The direct materials become part of the final products in the form of feeds. Direct labor cost items include wages and salaries of the workers that manufacture the feeds.

Indirect costs include (1) supervisory and management overhead expenses; (2) electricity; (3) supplies other than feed ingredients such as gasoline and machine oil, if any; (4) repair and maintenance; (5)

depreciation of fixed assets such as equipment, buildings; and (6) other incidental expenses required in manufacturing the feed. The cost of packaging and storage of the feeds is not included in the computation.

Depreciation is the allocation of the original cost of all fixed assets over their economic or useful life. For example, if a feed mill costs P1,000,000 and will be economically useful for ten years, the depreciation is computed by dividing P1,000,000 over 10 years, which is equal to P100,000. It is a non-cash expense because the money for constructing or purchasing a fixed asset such as a feed mill has been used up but is being allocated over the period of its useful life.

Table 8.1 shows a list of cost items in producing shrimp diet. The direct cost of ingredients comprises 92% of the total cost of producing feeds.

The cost of direct labor used in grinding, pulverizing, mixing, pelletizing, and oven drying is computed by multiplying the number (2) of workers with the wage rate (P200 per day) for example which is equal to P400. The cost of electricity is computed by multiplying the number of hours used (200-kilowatt hour) with the cost (P2.50 per kwh) which is equal to P500.

The total cost of producing one ton of shrimp feeds which amounted to P25,293.83 is the sum of the direct cost (ingredients and direct labor) and the indirect cost or P25.29 per kg.

Table 8.1 Production cost of shrimp diet

Item	Quantity	Unit cost per kg	Cost/kg	Cost/ton
A Ingredients	Composition (%)	(in Philippine Peso)		
Fish meal	25.00	25.00	6.2500	
Soybean meal	25.00	11.25	2.8125	
Shrimp head meal	15.00	21.00	3.1500	
Bread flour	16.00	8.60	1.3760	
Rice bran	3.95	4.96	0.1959	
Seaweeds	5.00	12.00	0.6000	
Cod liver oil	2.50	84.79	2.1198	
Soybean oil	2.50	45.00	1.1250	
Vitamin mix	2.00	200.00	4.0000	
Mineral mix	1.00	130.00	1.3000	
Dicalphos	2.00	14.00	0.2800	
Ethoxyquin	0.05	8.00	0.0040	
Subtotal	100.00	232.132	23.2132	
B. Direct labor (grinding, pulverizing, mixing, pelletizing, oven drying)		2 aides	200.00	400.00
C. Overhead				
Electricity (200 kwh)			2.5	500.00
Miscellaneous (5% of Items A & B)				1,180.66
Subtotal				1,680.66
D. Cost per ton				25,293.83
E. Cost per kg				25.29

Source: Millamena 1994

Single-input and single-output production function

The previous section discussed simple methods of computing the production cost of feeds using several inputs. Feeds, in turn, are one of the inputs in producing fish. This chapter describes an economic method of evaluating the relationship between the inputs used to produce a particular output. This method is called production function. Production function is defined as the technical relationship between the farm inputs and the output at a given time using a technology. Simply stated, the total yield or output varies with the quantities and combination of inputs used in the production process. The fish grower or the management of corporate farms decides what, how, when, and how much to produce from the limited resources of the company.

In aquaculture, farm inputs are generally comprised of fry or fingerlings, feed, fertilizers, chemicals, labor, as well as technical and management services. The desired output is the marketable-size fish.

For example, the relationship between the various inputs, denoted as $X_1,...X_n$, and an output, denoted as Y, is expressed in the following equation:

$$Y = f(X_1,X_2,X_3,X_4,X_5,...X_n)$$

where:

Y = total fish yield (output)
X_1 = amount of feed
X_2 = stocking size of fingerlings
X_3 = amount of fertilizer
X_4 = stocking density
X_5 = amount of labor
X_n = other variables related to growth of fish and total yield

The mathematical expression shows that the fish yield (Y) is related or is a "function" of the variables or production inputs ($X_1,...X_n$) in some particular way. The purpose of the production function analysis is to estimate the physical and marginal relationships between output (dependent variable) and a number of inputs (independent variables). The inputs or independent variables that significantly influence the yield are included in the production function. There may be other inputs that are used but their marginal influence on fish production is not significant.

A simple input (feed) and single output (fish) production function (Shang 1990) is shown in Table 8.2. The level of feeding given to the fish is represented by Y_1 and the output or the level of the total physical product (TPP) is represented by Y. The Y or TPP increases up to a certain level as the level of X_1 (feed) increases. The highest level of output of production is 69 units. This output level is attained when feeding level is 11 units. This production level is called the maximum sustainable yield (MSY). The yield or TPP starts to decrease beyond this feeding level. Therefore, there is no reason or logic in increasing the level of input when MSY has been attained. There is no additional benefit in giving additional feeds to the fish beyond the MSY.

The average physical product (APP) is the amount of fish produced per unit of input (feed) given to the fish. It is computed by dividing the total physical product (TPP) over the level of X_1 (feed). The marginal physical product (MPP) is defined as the increment or the change in output (fish) resulting from one additional unit of input (feed) given the fish.

Table 8. 2. Relationship among total physical product, average physical product, and marginal product

Level of X_1 combined with mixed resources	Level of total physical product (Y) (TPP)	Average physical product (APP)	Marginal physical product (MPP)
0	0		
			0.5
1	0.5	0.5	
			1.5
2	2	1	
			3
3	5	1.67	
			7
4	12	3	
			11
5	23	4.6	
			16
6	39	6.5	
			13
7	52	7.43	
			8
8	60	7.5	
			5
9	65	7.22	
			3
10	68	6.8	
			1
11	69	6.27	
			-1
12	68	5.67	
			-3
13	65	5	
			-5
14	60	4.29	

Source: Shang 1990

Graphically, the relationship among TPP, APP and MPP to the yield or level of output Y, and level of input (X_1,...X_n) is shown in Figure 8.1. The graph can be divided into three stages. In Stage I, TPP, APP and MPP exhibit increasing returns. Furthermore, the TPP increases at an increasing rate. MPP increases until marginal increment per unit is at its peak. APP continues to increase. Stage II is the phase where diminishing returns occurs. MPP decreases for every additional unit of input (feed) given to the fish. At this stage, TPP is still increasing although at a declining rate. The peak of the TPP is the maximum sustainable yield (MSY) or the point where there is no longer an increase of production in spite of the introduction of an additional unit of input (feed) into the fish culture system. At this point the MPP is zero. APP is still increasing but begins to decline. Beyond this point is Stage III or the declining

phase. There is no longer an advantage of providing an additional input because there will be a decrease in the output or production yield. This means that feeding the fish at this stage will result to decrease in fish production.

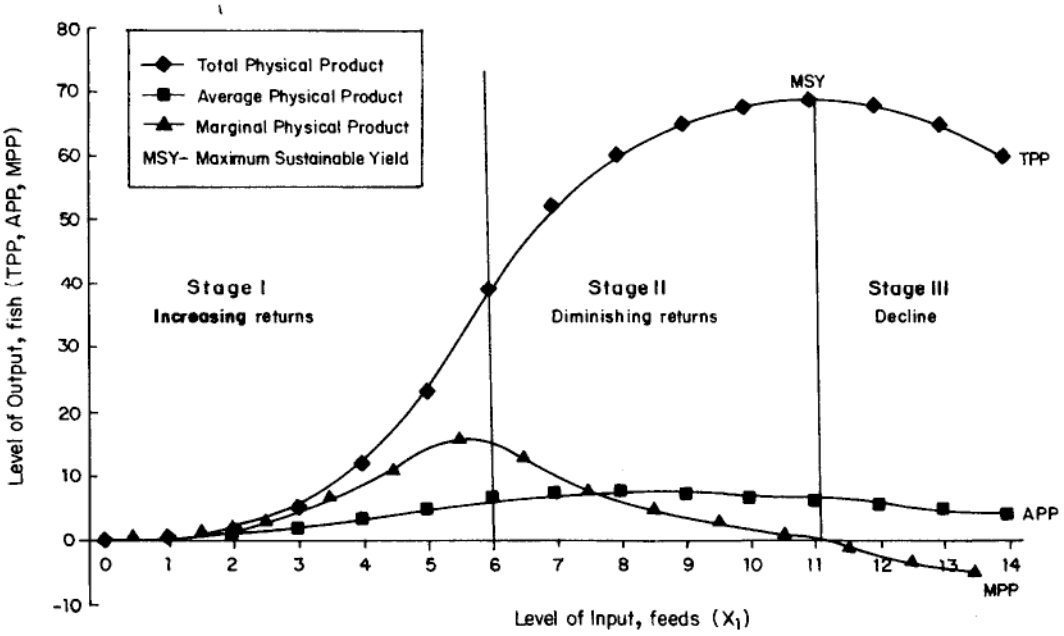

Figure 8.1
Relationship between TPP, APP, and MPP.

The production function and the cost of production

The introduction of cost in production analysis is an economic tool that can guide the fish farmer in his decisions related to production. The main objective in production analysis is to maximize profit. What level of production will result to maximum profit given a level of resources such as feed? If a fish farmer will base his decision on the production function discussed earlier, he may be tempted to maximize production up to the MSY (Table 8. 3 and Figure 8.2) or where the production output is at the maximum. However, this section will show that the fish farmer should consider the economic profit that could be attained at various levels of input.

Let us define some new terms at this point.

- Value of TPP (VTP) is the monetary value of the output (fish) based on the farm gate prices or prices of the produce sold in the farm. As an example, Table 8.3 assumed that the price of one unit of output is equivalent to one peso (P1.00).
- Value of APP (VAP) is APP multiplied by the farm gate price.
- Value of MPP (VMP) is the additional revenue or sales resulting from an increase in additional unit of input.

- Total variable input cost (TVIC) is the sum of the cost of the variable input (feed) at the different levels of input.
- Profit is the difference between the VTP or gross revenue and the TVIC or input cost.

The last column of Table 8.3 shows the highest profit level is 20 units which is attained at the feeding level of 8 units and the corresponding yield is 60 units. This point is the maximum economic yield (MEY). Figure 8.2 illustrates the relationship between MEY and other cost and revenue variables. VTP or the value of the total product is the revenue curve. The TIC is the cost curve. The shaded area is the profit area where the difference between the revenue and the cost are positive. The biggest difference between the two curves is at the MEY. The MEY is reached before the point where maximum yield or the MSY is attained. Therefore, with profit as the main objective, the desired feeding level should be at the MEY where it is most cost efficient. At feeding levels beyond the MEY, there is no additional or marginal economic benefit in inputting additional unit of feed since the additional cost of feed will be greater than the additional income derived from the additional output.

There are two conclusions that can be derived from Table 8.3 and Figure 8.2;
1. maximizing farm production does not always maximize profits;
2. decision on maximizing profit is based on marginal analysis or on whether there is additional benefit from additional input (feed).

Economic Efficiency of Feeds

The usual practice in measuring the efficiency of formulated feeds is to measure the feed conversion ratio (FCR). FCR is defined as the total amount of feeds by weight given over a certain period divided by the yield in kilograms. The lower the ratio, the more efficient the feed, regardless of the cost.

FCR, however, does not take into account the cost of feeds and the economic efficiency of using feeds. A method of measuring cost efficiency of feeds called the incidence cost, indicates the cost of producing a unit (by weight) of fish.

$$\text{Incidence cost} = \frac{\text{total cost of feed used}}{\text{wt of the fish produced}}$$

In comparing two feed formulations available to a fish farmer, the feed with a lower incidence cost is more economically efficient and, therefore, more beneficial to the farmer.

Another method of measuring economic efficiency of feeds is to look at the value of the fish produced and the cost of feeding. This is called the profit index, which indicates the profit for every unit cost of feeds incurred. This is computed using the following formula;

$$\text{Profit index} = \frac{\text{total value of the fish produced - total cost of feeds}}{\text{total cost of feeds}}$$

Table 8.3 Relationship among total physical product, average physical product, marginal physical product, value of total physical product, value of average physical product, value of marginal physical product, total variable input cost, and profit.

Level of X_1 combined with mixed resources	Level of total physical product (Y) (TPP)	Average physical product (APP)	Marginal Physical Product (MPP)	Value of TPP (TPPx1)	Value of APP (APPx1)	Value of MPP (MPPx1)	Total variable input cost (X_1x5)	Average variable input cost (APPx5)	Marginal variable input cost (MPPx5)	Profit (TVP-TVIC)
0	0			0			0			0
			0.5			0.5			0	
1	0.5	0.5		0.5	0.5		5	2.5		-4.5
			1.5			1.5			7.5	
2	2	1		2	1		10	5		-8
			3			3			15	
3	5	1.67		5	1.67		15	8.35		-10
			7			7			35	
4	12	3		12	3		20	15		-8
			11			11			55	
5	23	4.6		23	4.6		25	23		-2
			16			16			80	
6	39	6.5		39	6.5		30	32.5		9
			13			13			65	
7	52	7.43		52	7.43		35	37.15		17
			8			8			40	
8	60	7.5		60	7.5		40	37.5		20
			5			5			25	
9	65	7.22		65	7.22		45	36.1		20
			3			3			15	
10	68	6.8		68	6.8		50	34		18
			1			1			5	
11	69	6.27		69	6.27		55	31.35		14
			-1			-1			-5	
12	68	5.67		68	5.67		60	28.35		8
			-3			-3			-15	
13	65	5		65	5		65	25		0
			-5			-5			-25	
14	60	4.29		60	4.29					60

Source: Shang 1990

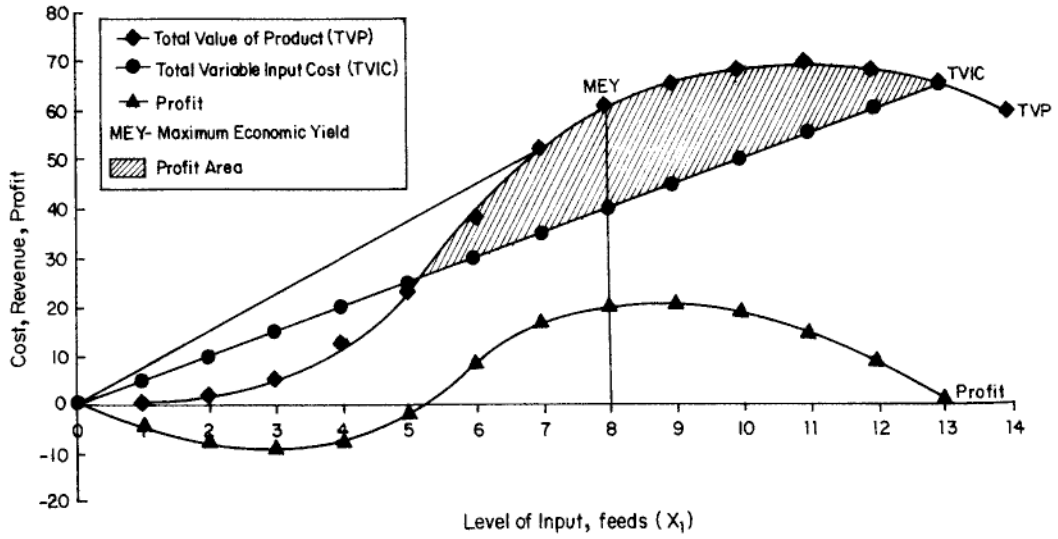

Figure 8.2
Relationship between TVP, TVIC and profit.

The total value of the fish does not only consider the weight but also the overall quality including freshness of the fish. The value attributed to quality is accounted in the market price of the fish. These factors are included in the profit index. The farmer can then compare the profit index of various feeds when choosing feeds for his stock.

A third indicator of determining the economic efficiency of feeds is the returns on feeds. This indicator shows the rate of return on investment on feeds. This is measured by the following formula;

$$\text{Returns on feeds} = \frac{\text{net profit}}{\text{cost of feeds}}$$

Table 8.4 is a cost and returns computations that shows the comparative economic efficiencies based on the three indicators (incidence cost, profit index, and return on feeds) in the pen culture of Asian catfish (*Clarias macrocephalus*) fed three different types of diets. Using the aforementioned formulas, the summary of the economic indicators using the three diets is:

Diet 3 and Diet 2 have lower incidence costs, higher profit indexes, and returns on feeds compared to Diet 1. We conclude that the use of Diets 3 and 2 are economically viable. The use of Diet 1 is a losing proposition.

Table 8.4 Cost and returns of the pen culture of *Clarias macrocephalus* at a stocking density of 10 fish/m² and fed three different diets for 120 days. Values are on a per ha per crop basis in Philippine Peso (PhP)*

Items	Quantity (kg)	Unit Price	Diet 1	Diet 2	Diet 3
Revenue					
Diet 1 (30.2 g MBW)	2208	120	264960		
Diet 2 (58.3 g MBW)	4696	120		563520	
Diet 3 (67.35 g MBW)	4808	120			576960
Less:					
Variable costs					
Fingerlings (pcs)	100000	1.5	150000	150000	150000
Feeds (kg) Diet 1	6308	14.69	92665		
Diet 2	9979	15.4		153677	
Diet 3	21964	7			153748
Lime (ton)	1	1000	1000	1000	1000
chicken manure (ton)	1	800	800	800	800
45-0-0 (bag)	0.5	415	207.5	207.5	207.5
16-20-0 (bag)	1	410	410	410	410
Diesel fuel (l)	100	15.15	1515	1515	1515
Miscellaneous expenses			5299.2	11270.4	11539.2
Repair and maintenance			598.8	598.8	598.8
Caretaker's salary (mo)	5	1000	5000	5000	5000
Pond rent (yr)	0.5	8000	4000	4000	4000
Subtotal			**261495**	**328478**	**328819**
Fixed cost					
Depreciation			2649	12849	12849
Interest expenses			12849	15595	15598
Subtotal			**15498**	**28444**	**28447**
Total costs			**276993**	**356922**	**357266**
Net profit before tax/crop			**-12033**	**206598**	**219694**
Incidence cost = $\frac{\text{total cost of feed used}}{\text{wt of the fish produced}}$ =			**41.97**	**32.73**	**31.98**
Profit index = $\frac{\text{total value of fish - total cost of feeds}}{\text{total cost of feeds}}$ =			**1.86**	**2.67**	**2.75**
Returns on feeds = $\frac{\text{net profit}}{\text{cost of feeds}}$ =			**-12.99%**	**134.44%**	**142.89%**

Source: Coniza et al. 2001

Least-cost Combination of Feeds

Considering the sensitivity of the cost of feeds in the profitability of fish farming, keeping the cost down is crucial to ensure the economic viability of the fish farming enterprise. Least-cost combination techniques are used in determining the lowest cost from different feed combinations that would result in the same production.

For example, two types of feeds (F_1 and F_2) are sold at different prices and are available to the fish farmer. If the two types of feeds can be substituted, the rate of substitution indicates the amount by which one feed must be changed in order to offset the change in the amount of the other feed. This is called marginal rate of substitution (MRS) between feeds, the value of which is negative.

$$\text{MRS} = \frac{\text{amount of feed replaced } (F_1)}{\text{amount of feed added } (F_2)}$$

The MRS between inputs (feeds) is a physical relationship and cannot determine the least-cost combination of feeds. Feed prices (prices of PF_1 and PF_2) are needed and the ratio is compared with MRS.

$$\text{Price ratio} = \frac{\text{price of feed added } (PF_2)}{\text{price of feed replaced } (PF_1)}$$

The least-cost combination of feeds F_1 and F_2 occurs when the MRS is equal to the inverse of the price ratio.

$$\frac{F_1}{F_2} = \frac{PF_2}{PF_1} \quad \text{or} \quad F_1 (PF_1) = F_2 (PF_2)$$

The cost of change in one feed is equal to the cost of change in the other feed. In Table 8.5, the least cost combination of feeds is at P180, which corresponds with the use of 10 parts of F_2 and 15 parts of F_1. In this connection, the cost of change in F_1 is equal to the cost of change in F_2.

Table 8.5 Hypothetical relationship for combining feeds to produce a given level of output (P_1 = P9, P_2 = P6)

X_2	X_1	F_1/F_2 (MRS*)	P_2/P_1	Cost ($F_1xP_1 + F_2xP_2$)
0	40		240	
4	24	0.25	0.67	180
10	15	0.67	0.67	180
15	8	0.71	0.67	183
20	3	1.00	0.67	198
25	0	1.67	0.67	225

*Marginal rate of substitution
X_1 is the feed being replaced
X_2 is the feed being added
F_1 is the change in the quantity of X_1
F_2 is the change in the quantity of X_2

Source: Shang 1990

Minimum Cost of Feed Formulation using Linear Programming

Linear programming is a computational method used to allocate scarce resources to maximize profit or minimize cost. In aquaculture, it is often used in the minimization model of computing the least-cost feed combinations or ration and still meet the required nutrients for the fish. The concepts involved in linear programming include:

- Objective – usually to maximize profits or minimize cost;
- Constraints – resources such as raw feed ingredients are restricted or limited;
- Alternative ways of attaining the objective are determined;
- Relationship between input and output is assumed to be linear;
- Prices paid or received are assumed constant; and
- Quantity of inputs used should be equal to or less than the quantity available.

Table 8.6 presents a hypothetical example that illustrates the data on nutrient availability, cost, requirements, objectives, and constraints to consider in computing for least cost combination using linear programming as adopted from Shang 1990.

Table 8.6 Data on nutrient availability and requirements, feed cost, objective, and constraints in linear programming

	Nutrient		Feed A	Feed B	Minimum daily requirement
A.	Nutrient availability and requirements (Unit of nutrient per unit of feed)				
	Calcium		2	1	18
	Protein		2	2	20
	Calories		1	5	25
B.	Cost per unit of feed		1.00	2.00	
C.	Objective: Minimize cost =	$P_1A + P_2B$			
D.	Subject to constraints such as:	2A + 1B => 18 2A + 2B => 20 1A + 5B => 25 A=>0, B=>0			

where: A, is the amount of Feed A, and
B, is the amount of Feed B.

Source: Shang 1990

The constraints in the equations in Table 8.5 insure that the minimum requirement for ingredient is met at the least cost. For example, the minimum units of calcium, protein and calories are 18, 20, and 25 respectively. Simple linear programming can be solved graphically. But as the problem becomes more complicated, systematic computational techniques using computers are required.

Summary

The concepts and approaches on the evaluation and computation of selected indicators of the economic efficiency of feeding in aquaculture operations are discussed. The discussion started with the identification of cost items involved in producing aquaculture feeds. These cost items are classified into direct costs and indirect costs. Total costs and per unit costs are useful economic indicators to guide the aquaculture farmers in their operations.

This chapter introduced the production function approach for evaluating the relationship between farm inputs such as feeds, and output (fish). Quantitative examples demonstrated marginal analysis that provided basis for understanding the various stages in production systems. These are the stages where profit is increasing, maximum, or decreasing. The concepts of maximum sustainable yield (MSY) and maximum economic yield (MEY) were discussed in order to highlight the optimum feeding level that will result to the highest profit level.

This chapter also presented indices for measuring the economic efficiency in producing feeds. These indices include incidence cost and profit index. The least-cost approach was discussed to demonstrate the method of determining combinations of ingredients of different feed formulations given the prevailing prices.

Finally, the chapter introduced the concept of linear programming in determining the minimum feed cost that would yield the desired levels of nutrients required by the fish.

Guide Questions

1. What items comprise the direct costs of producing shrimp feeds?
2. What items comprise the indirect costs of producing shrimp feeds?
3. In terms of yield levels, at what point is additional feeding no longer beneficial to the fish farmer? What is the most profitable level of feeding the fish? Explain using concepts of marginal analysis.
4. How do you measure the economic efficiencies of different feeds available to the fish farmer?
5. What is linear programming?
6. What are the concepts and requirements in linear programming and analysis of feed formulations?

Suggested Readings

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Sample Worksheet for
Calculating the Nutrient Composition of Feed

Feed formulation code: _____
Date computed: _____
Formulated by: _____

Ingredients (1)	g/100g (2)	Protein% (3)	Fat% (4)	Fiber% (5)	Ash% (6)	NFE% (7)

1. Method of protein analysis (Kjeldahl method)

Procedure:

1. Pre-heat digestor to 400°C inside a hood.
2. Weigh 60-70 mg dry sample in a tared filter paper and place in digestion tube.
3. Record the weight of the sample. Make 2 replicates/sample.
4. Add catalyst and 5 ml concentrated sulfuric acid to each of the digestion tubes.
5. Digest the sample until the solution is clear (1 h).
6. Cool the digest and add 25 ml ammonia-free distilled water.
7. Distill and titrate.
8. Compute for % crude protein.

Computation:

% crude protein = % nitrogen x factor (6.25)

Specific conversion factor for percent crude protein varies according to types of products as follows:

Meat (animal or fish)	6.25
Milk and dairy products	6.38
Cereal proteins	5.90
Flour	5.70
Oil seed proteins	5.40

2. Crude fat analysis (Soxhtec method)

Procedure:

1. Weigh out two (2) replicates (about 1 g) for each dry sample in crude filter paper.
2. Place into crucibles. Place crucibles inside the pre-weighed extraction cups with 5 pieces of beads.
3. Position the crucibles in the extraction unit.
4. Place about 50 ml of ether inside each cup. Insert each cup into the unit and lock firmly.
5. Extract for 30 minutes (extraction knobs in " boiling" position).
6. Rinse for 30 minutes (extraction knobs in "rinsing" position).
7. Recover solvent (15-30 minutes). Condenser valves are closed.
8. Remove extraction cups from extraction unit. Reserve defatted samples for crude fiber analysis.
9. Dry the cups (with crude fat) in an oven for 10 minutes.
10. Place the cups in a dessicator and cool for 30 minutes.
11. Weigh to constant weight.

12. Compute for % crude fat

Computation:

$$\% \text{ crude fat} = \frac{\text{wt of fat (corrected)}}{\text{wt of sample}} \times 100$$

3. Lipid extraction (Bligh and Dyer method)

Procedure:

1. Weigh 1 g wet samples or 0.5 g dry sample.
2. Homogenize for 2 min in a solvent mix: (40 ml CH_3OH, 20 ml $CHCl_3$, 16 ml H_2O).
3. Add 20 ml $CHCl_3$ and homogenize for 2 min.
4. Filter through celite-R filter and collect the filtrate, scrape off the upper layer of celite paper and transfer into the homogenizing tube.
5. Mix with new quantity of solvent mix and homogenize for 2 min and filter through celite filter.
6. Rinse the celite filter with 40 ml $CHCl_3$ and transfer the combined filtered extract in a separatory funnel.
7. To break the emulsion, add 1 ml of a 40 ml H_2O in which a pinch of NaCl was previously dissolved.
8. Shake the separatory funnel for about 2 min and let stand for 10 min to allow separation.
   Upper layer = CH_3OH & H_2O
   Lower layer = $CHCl_3$ & lipid
9. Drain off the lower layer into a flask passing through the water free Na_2SO_4 filter.
10. Rinse the Na_2SO_4 filter with $CHCl_3$ and evaporate the collected lipid fraction using a rotary evaporator until nearly dry and flush out the remaining solvents with nitrogen (temperature of evaporation should not exceed 40°C).
11. Determine the quantity of the total lipids in g/100g dry wt.

4. Saponification and transesterification

Saponification

Procedure:

1. Transfer the lipids (4 ml benzene) in a pyrex centrifuge glass-tube with teflon lined cap; rinse the pear-shaped flask with 1 ml more benzene.
2. Add 1 ml distilled water and 10 ml 0.5 N KOH/CH_3OH to the lipid extract in the centrifuge glass tube.
3. Flush the tube with nitrogen.
4. Close well and shake.
5. Saponify in a boiling water bath (100°C) and shake well every 10 min (total of 40 min). Cool down the centrifuge glass tube.
6. Add 5 ml 2M HCl and 5 ml petroleum benzene. Shake well.
7. Let stand for 5 minutes or until two distinct layers are formed.
8. Transfer the benzene phase to a pear-shaped flask with a pipette (get the upper part).
9. Distill off the solvent using vacuum rotary evaporator.

Esterification

1. Add 2 x 1 ml CH_3OH and transfer the fatty acids into a screw-capped glass tube.
2. Add 2 x 1 ml benzene and transfer the remaining fatty acids to the glass tube.
3. Add 3 ml of 14% BF_3-CH_3OH, flush with nitrogen, close well, and shake.
3. Place the screw-capped glass tube in a boiling water bath for 8 min and shake after 4 minutes.
5. Cool down the tube, add 5 ml H_2O (to stop the reaction) and 5 ml petroleum benzene.
6. Let stand for 5 min and transfer the petroleum benzene phase (upper layer) to a pear-shaped flask.
7. Distill off the solvent using rotary evaporator.
8. Dissolve the fatty acid mehyl ester (FAME) in 1 ml isooctane and transfer to an amber vial with a screw cap and teflon-faced silicone septa liner.
9. Sample is ready for GLC (gas liquid chromatography) injection.

5. Method of peroxide value determination

Procedure:

1. Place 0.3 g fat sample in 250 ml flask with stopper; add 10 ml of $CHCl_3$-CH_3COOH mixture, shake.
2. Add 1 ml of saturated KI solution.
3. Stopper and allow to stand in the dark for 5 min.
4. Add 20 ml distilled water, shake.
5. Titrate with 0.01N $Na_2S_2O_3$ solution (until light yellow color appears).
6. Add 1 ml of 1.5% starch solution (until colorless).

Calculation:

PV = (Vs – Vb) x f x 10/w

where; Vs = titration volume of sample

Vb = titration volume of blank

F = factor of 0.01N $Na_2S_2O_3$ soln.

W = weight of fat in volume of extract used (g)

N = normality of $Na_2S_2O_3$ solution (in this case N/100)

6. Procedure of fatty acid value determination

Free fatty acid value (FFA)

Calculation:

FFA (%) = acid value x mol wt of oleic acid/mol wt of KOH x 100/1000
= acid value x 282.27/56.11 x 1/10
= acid value x ½

7. Thiobarbituric acid (TBA) value determination

Procedure

1. Place 0.2-0.4 g fat sample in a test tube with a screw cap.
2. Add 3 drops of antioxidant solution.
3. Remove the solvent using the rotary evaporator under reduced pressure at 35-40°C.
4. Add 3 ml TBA solution. And 17 ml of trichloroacetic acid solution (TCA).
5. Flush N_2 gas into the test tube and immediately stopper.
6. Heat at 100°C in a boiling water bath for 30 min until the colour appears.

7. Cool to room temperature with tap water.

8. Add about 5 ml chloroform and mix for a few seconds with a Vortex mixer.

9. Transfer about 15 ml of the color solution to a glass centrifuging tube.

10. Centrifuge for 10 min at 3,000 rpm.

11. If the aqueous solution is not clear, centrifuge again at 10,000 rpm for 10 min.

12. Transfer a part of the clear aqueous solution and read absorbance at 532 nm.

13. Blank test should be carried out in the same manner without fats.

Calculation:

TBA (mg malonaldehyde/kg fat) = abs x f x 0.2/w

where: abs = absorbance at 532 nm
w = weight of fat in volume of extract (g)
f = factor = 46

Glossary

A

Acclimatization: adaptation or increased tolerance of a species to a changed environment

Acid: a compound that when dissolved in water, dissociates to yield hydrogen ions (H^+)

Active transport: movement of a molecule across a membrane or other barrier driven by energy other than that stored in the concentration gradient or electrochemical gradient of the transported molecule

Ad libitum feeding: providing unlimited amount of feed until satiation

Additive: an ingredient or combination of ingredients, other than premix, added to the basic feed mix or parts thereof to fulfill a specific need; usually used in micro-quantities and requires careful handling and mixing

Adipose tissue: connective tissue specialized for the storage of large amounts of triglycerides

Aeration: a mechanical process usually by bubbling air through water or spraying water into air by which the level of dissolved oxygen in the water is raised and/or the level of dissolved noxious volatile gases is reduced

Aldose: a simple sugar that contains an aldehyde group at one end of the chain as part of its structure

Aldehyde: a compound with an HC=O group attached to an aliphatic carbon chain

Algal bloom: an exponential increase in the population of unicellular algae caused by high nutrient load in the water

Algal die-off: an abrupt massive mortality of unicellular algae resulting from natural or man-made cause

Algebraic equation: a mathematical method used in formulating feeds using only few ingredients

Alimentary canal: a tubular passage that extends from the mouth to anus and function in digestion and absorption of food and elimination of residual waste

Alkalinity: (total) the total concentration of bases in water expressed as milligrams per liter of equivalent calcium carbonate ($CaCO_3$)

Amino acid: a carboxylic acid that includes an amino group as part of its structure; any one class of organic compounds which contain both the amino (NH_2) group and the carboxyl (COOH) group

Amino acid antagonism: occurs when some amino acids are fed in excess of required levels causing an increase in requirement for another amino acid of similar structure, e.g. arginine-lysine antagonism

Ammonia (NH_3): a product of fish protein metabolism and decomposition of organic matter by bacteria

Anabolism: metabolic reactions resulting in the synthesis of more complex compounds from simple precursors; commonly linked to the hydrolysis of ATP to ADP and phosphate

Anaerobic: a condition or chemical reaction where gaseous oxygen is not present or not required, e.g. decomposition of organic wastes by microorganisms, releasing toxic hydrogen sulfide and methane gas

Anoxic: devoid of oxygen

Antinutritional factors: substances in the feedstuff which can reduce nutritional value

Antioxidant: a strong reducing agent, which is easily oxidized and thus prevents the oxidation of other substances

Appetite: natural desire to satisfy hunger

Aquaculture: the farming of aquatic organisms, including fish, crustaceans, mollusks, and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as, regular stocking, feeding, and protection from predators

Aquafarm: natural and/or reconstructed body of water intended for aquaculture

Aquafeeds: feeds that are intended for aquaculture species

Arachidonic acid: a 20-carbon unsaturated fatty acid having four double bonds

Adenosine triphosphate (ATP): a universal energy carrier in cells; the principal driving force in energy requiring biochemical processes of life

ATPase: one of a large class of enzymes that catalyze a process that involves the hydrolysis of ATP

Attractant: substances added to feeds for fast consumption especially for crustacean species

Automatic feeder: a device, usually electronically operated, that dispenses feed at pre-selected times

Autotrophic: organisms that require carbon dioxide or carbohydrates or simple inorganic nitrogen compounds for metabolic synthesis; these organisms can manufacture their own food (e.g. plants)

Average physical product (APP): the amount of fish produced or product per unit of input (feed) given to the fish

B

Benthic: pertaining to bottom terrain of aquatic regions; the part of the aquatic environment inhabited by organisms that live on or in the sediment

Benthos: organisms that live on or in the sediment of aquatic environments

Binder: substances added to feeds to make it stable in the water, usually a carbohydrate

Biomass: in aquaculture, this normally refers only to the total weight of species being cultured, expressed in terms of a given area or volume of the habitat

Biosynthesis: the formation of chemical substances from other chemical substances in a living organism

Broodstock: captive parent stock is allowed to reach sexual maturity and spawning readiness to make eggs and sperm available

Brush border: dense covering of microvilli on the apical surface of epithelial cells in the intestine and kidney; the microvilli aid in absorption by increasing the surface area of the cell

C

Canine teeth: dog-tooth like, often quite fang-like; they are elongated and subconical, straight curved and are adapted for piercing and holding

Calorie: a unit of heat or energy; the amount of heat required to raise 1 g of water to 1°C. Nutritionally, the kcal is sometimes used; 1 kcal=1000 cal, 1 cal=4.186 joules, 1 joule=0.239 cal

Cannibalism: eating flesh of one's own kind

Carbohydrate: a large group of organic compounds common in plants which include simple sugars, starches, celluloses, gums and related substances

Carbon dioxide: (CO_2) a colorless, odorless gas, resulting from the oxidation of carbon-containing substances, highly soluble in water, toxic to fish at levels higher than 20 ppm; toxicity increases with low levels of oxygen

Cardiform teeth: teeth that are numerous, short, fine and pointed

Carnivores: animals that feed exclusively on animal matter

Carrying capacity: maximum biomass that a pond can support and maintain given a certain level of management, e.g. no fertilization, with fertilization, fertilization plus feeding

Casein: the colloidal protein in milk

Catabolism: metabolic reactions resulting in breakdown of complex molecules to simple products, commonly oxidation to carbon dioxide and water

Cellulose: a polymer of glucose, an important structural material in plants; major structural component of plant cell wall

Chitin: major structural component of the rigid exoskeleton of invertebrates

Cholesterol: a physiologically important sterol and is widespread in the biomembrane

Coenzyme: a nonprotein substance that takes part in an enzymatic reaction and is regenerated at the end of the reaction; a partner required by some enzymes to produce enzymatic activity

Cofactor: an inorganic ion or coenzyme required for enzymatic activity

Complete diet: feed that contains all the essential nutrients (protein, lipid, carbohydrate, vitamins, minerals) required by the animal for maintenance and growth

Compound feed: A feed composed of several ingredients

Concentrate: a feed used with another to improve the nutritive balance and intended to be further diluted and mixed to produce a supplement or complete feed

Critical standing crop: the standing crop or biomass by which the increase in nutrient requirement could no longer be supplied by the natural food, thus growth rate is less than the potential maximum. This is the point where feeding can effectively increase yield

Cytosol: the portion of the cell that lies outside the nucleus and other membrane-bounded organelles

D

De novo biosynthesis: synthesis of biomolecules that occurs within the animal body

Depreciation: the decline in value of fixed assets in a production process with the passage of time

Depreciation cost: the allocation of the original cost of all fixed assets over their economic lifespan

Desmosome: specialized cell-cell junction, usually formed between two epithelial cells, characterized by dense plaques of protein into which intermediate filaments in the two adjoining cells insert

Deterioration: the action of gradual impairment

Detritivores: animals that feed on decaying matter

Detritus: fragments or remains of organic matter or other disintegrated material moved about by water

Docosahexaenoic acid: (DHA) a 22-carbon unsaturated fatty acid having six double bonds, an essential fatty acid in fish

Diatom: a single-celled plant (phytoplankton) covered with two overlapping porous shells of silica

Diel: 24-hour period

Diet: food regularly provided and consumed

Digestibility: (apparent) the percentage of the feedstuff taken into the digestive tract that is absorbed into the body. It is based on the difference between the amount of feed ingested and the amount in the feces

Digestibility: (true) describes the portion of the feed that is absorbed minus the materials that are lost by the gut in the process of ingestion and digestion

Digestibility coefficient: values expressed in percentage that gives information as to the availability of specific nutrients in the feed or feed ingredients

Digestible energy: that part of the gross energy of a feed which does not appear in the feces

Digestion: process of breaking down nutrients into simple chemical compounds through the action of enzymes; the breakdown, in the alimentary tract, of complex organic substances into simplier substances so that they may be used in metabolism

Diluent: a volatile liquid used along with solvents in coating materials especially to reduce cost

Disaccharides: a sugar consisting of two monosaccharides linked by a glycoside bond. The common dietary disaccharides are sucrose (cane or beet sugar), lactose, and maltose

Dissolved oxygen (DO): the amount of elemental oxygen, O_2, in solution under existing atmospheric pressure and temperature. Oxygen is added to water by photosynthesis and diffusion from the atmosphere

DNA: deoxyribonucleic acid

Double bond: a covalent bond in which two pairs of electrons are shared between the participating atoms

E

Ecdysis: the act of molting a cuticular layer, e.g. in crustaceans and insects

Economic efficiency: a financial or monetary-based criteria for evaluating the desirability of a production process or the utility of an input. It is often measured as a ratio of the cost and the yield associated to a particular production process or input

Energy: the ability or capacity to do work

Effluent: water that is discharged from a tank, pond, aquaculture farm or power station

Endogenous: originating within the body

Endoplasmic reticulum: extensive netlike labyrinth of branching tubules and flattened sacs in the cytosol and serves as a factory for the production of almost all of the cell's lipid and trans-membrane proteins

Enzyme: complex proteinaceous substances that are produced by living cells and bring about or accelerate reactions or processes in living cells

Eicosapentaenoic acid (EPA): a 20-carbon unsaturated fatty acid having five double bonds, an essential fatty acid in fish

Essential amino acids: amino acids required for protein synthesis that cannot be synthesized in the body and must be provided in the diet

Essential fatty acids: polyunsaturated fatty acids that cannot be synthesized by the body and must be provided from dietary sources originating outside the organism

Eutrophication: enrichment of the receiving water and oxygen deficiency in the surrounding water resulting from aquaculture discharges of nitrogen and phosphorus, and oxygen-consuming substances into lakes, rivers, and seas

Extrusion: to shape by forcing through a specially designated opening after a previous heating of the material or of the opening

F

Factors of production: the main categories of inputs used in a production process, namely: land, labor, capital and management

Fat: triacylglycerols, esters of glycerol with three fatty acids; fats are generally considered to be those triacylglycerols that are solid at room temperature, whereas oils are triacylglycerols that are liquid at room temperature

Fatty acid: aliphatic carboxylic acids (i.e. with a -COOH group); the metabolically important fatty acids have between 2 and 24 carbon atoms (always an even number) and may be completely saturated or have one (mono-unsaturated) or more (polyunsaturated) C=C double bonds in the carbon chain

Fecundity: the number of eggs per unit body weight of female broodstock

Feed composition table: a list of food materials with respective nutrient compositions expressed in percentage

Feed conversion efficiency (FCE): expressed as percent wet weight gained divided by the amount of dry weight of feed given within a feeding period. The higher the FCE value, the better the feed is utilized

Feed conversion ratio (FCR): the amount of dry weight of feed given over the wet weight gained within a feeding period. The lower the FCR value, the better the feed is utilized

Feeding charts: these provide only a general guide to feed intake. Tabulated feeding levels for various species are commonly provided by feed manufacturing companies

Feeding frequency: the number of times feed is given in a day

Feeding rate: the amount of feed given per day and is based on a certain percentage of the body weight

Feeding tray: usually nylon screen and wood or bamboo strips and stone sinkers, used to monitor feed consumption

Feedstuff: one or a mixture of substances which form the nutrients; protein, carbohydrate, fat, vitamins, minerals, and water that are eaten by an animal as part of its daily ration

Filler: a substance added in the feed to complete the feed formula

Fish meal: dried fish or fish waste prepared from numerous species of fish that are used as animal food and generally not directly used for human consumption

Floating feed: produced by an extrusion process through which feed materials are moistened, pre-cooked, expanded (higher moisture, temperature and pressure than ordinary pelleting) and dried, resulting in low density feed particles. This is most applicable for surface and column feeders and its being visible avoids overfeeding

Formulated feed: two or more ingredients proportioned, mixed, and processed according to specifications

Free energy: energy which is left available for biological activity and growth after the energy requirement is met

Free radicals: formed during lipid peroxidation, such as $ROO^{\cdot}$,$RO^{\cdot}$,$OH^{\cdot}$, these react by hydrogen removal and a variety of addition reactions that damage other food nutrients making them unavailable to fish

G

Glucose: a monosaccharide; a hexose (six-carbon) sugar, of empirical formula $C_6H_{12}O_6$; basic molecule for the synthesis of starch and cellulose

Glycerol: a trihydric alcohol to which three fatty acid molecules are esterified in the formation of triacyglycerols (fats and oils)

Glycogen: a branched chain polymer of glucose, linked by alpha 1-6 links; the storage form of carbohydrate in animals, as starch is in plants

Golgi bodies: membrane-bounded organelle in eukaryotic cells where the proteins and lipids made in the endoplasmic reticulum are modified and sorted

Growth: a normal process of increase in size of a tissue, organ, or organism. The total weight of sampled animals are determined by batch weighing, usually in a tared container with water on a weighing scale

H

Hemicellulose: composed of a mixture of hexose and pentose units; any of various polysaccharides that accompany cellulose and lignin in the skeletal substances of wood and green plants. Unlike cellulose, it can be hydrolyzed in relatively mild acids

Herbivores: animals that feed exclusively on plant materials

Hexose: a monosaccharide with six carbon atoms, and hence the empirical formula $C_6H_{12}O_6$. The nutritionally important hexoses are glucose, galactose and fructose

Highly unsaturated fatty acids (HUFA): fatty acids that contain four or more double bonds

Hydrogen sulfide (H_2S): a gas produced by microbial decomposition of organic matter (e.g. excess feed and fecal wastes) deposited in the pond bottom in the absence of oxygen

Hydrolysis: a chemical reaction of water in which the reagent other than water is decomposed and hydrogen and hydroxyl are added

Hydrophilic: a compound which is soluble in water, or a region of a macromolecule which can interact with water molecules

Hydrophobic: a compound which is insoluble in water, but soluble in lipids, or a region of a macromolecule which cannot interact with water, although it does interact with lipids

Hypervitaminosis: an abnormal condition resulting from the intake of an excess of one or more vitamins

I

Immune-response: relating to the physiological reaction characteristic of the immune state

Incidence cost: the ratio of the cost incurred to the output produced

Incisor: sharply edged cutting teeth

Inorganic: chemical compounds which do not contain carbon as the principal element (except carbonates, cyanides, and cyanates)

Inputs: the resources used to produce an output using a technology (e.g. fry or fingerlings, feeds, land built into ponds, fish cages, labor and managerial skills)

Insulin: polypeptide hormone that is secreted by B-cells in the pancreas and helps regulate glucose metabolism in animals

Integrated aquaculture system: system using various species in different trophic levels such as seaweed, shellfish, and herbivorous and omnivorous fish species such as milkfish and tilapia

Intraperitoneal: the region between the skin and muscle layer and organs

Isocaloric: having similar caloric value

Isomerism: have the same molecular formula but their structural formula differ in the arrangement of their atoms within a molecule

J

Joule: unit of energy in the metric system and one kcal is equal to 4.186kJ

K

Keratin: a sulfur-containing protein which is the primary component of epidermis, hair, wool, hoof, horn, and the organic matrix of the teeth

Ketose: a sugar that contains a ketone group as part of its structure

Kilocalorie: amount of heat necessary to raise the temperature of one kilogram of water 1°C

L

Lablab: natural food in ponds, composed of complex of blue-green and green algae, diatoms, rotifers, crustaceans, insects, roundworms, detritus, and plankton

Lactose: the sugar of milk; a disaccharide composed of glucose and galactose

Lecithin: a common source of phospholipids

Lignin: a polymer of coniferyl alcohol; a structural material found in woody plants

Liming: the application of neutralizing agents specifically calcium containing compounds: CaO (quicklime or burnt lime); $Ca(OH)_2$ (calcium hydrate or slaked lime); $CaCO_3$ (agricultural or dolomitic lime), to either pond bottom or water

Linear programming: computational method aimed at allocating scarce resources to maximize profit or minimize cost (e.g. computing for the least-cost feed combinations or ration that still meet the nutrients required by the cultured fish)

Linolenic acid: an 18-carbon unsaturated fatty acid having three double bonds

Lipids: a broad term for fats and fat-like substances including phospholipids, waxes, steroids, and sphingomyelins

Lumut: fibrous filamentous green algae grown in ponds

M

Macronutrients: nutrients needed in large amounts such as proteins, carbohydrates, or lipids

Maltose: a disaccharide composed of two molecules of glucose

Manual feeding: includes broadcasting the feeds into the pond as well as placing feeds into floating frames (for floating feeds) or feeding trays (for sinking feeds)

Marginal physical product (MPP): the change in output per unit change in input or the increment in the units of the product or fish resulting from one additional unit of input (feed) given the fish

Maximum economic yield (MEY): the production level where profit is highest since no additional or marginal benefit can be gained from any additional unit of input

Maximum sustainable yield (MSY): the production level where output per unit of input is highest but there is no longer any increase in production beyond this level in spite of any additional unit of feeds or input

Mechanical feeding: means of partially replacing hand feeding to include: automatic feeders which can be set to release controlled amounts of feed when activated, and demand feeders which can release a few pellets each time a triggering mechanism is bumped by the fish

Metabolic rate: the amount of oxygen used for total metabolism per unit of time per unit of body weight

Metabolic wastes/metabolites: by-product of metabolism discharged from the body of an organism

Metabolism: the physical and chemical processes by which feedstuffs are synthesized into complex elements (anabolism), complex substances are transformed into simple ones (catabolism), and energy is made available for use by an organism

metabolizable energy: a more exact measure of the dietary energy used for metabolism by the tissues

Metamorphosis: the marked change off shape or structure, particularly in the transition of one developmental stage into another

Methane gas: an odorless, colorless, inflammable hydrocarbon, CH_4, which forms explosive mixtures with air. It results from the decay of organic matter

Methionine: one of the essential amino acids; it is sulfur containing and maybe replaced in part by cystine

Microencapsulated feed: a larval feed made by encapsulating a solution, colloid or suspension of feed ingredient mixture within a membrane or capsule; these particles can be designed to have a slow release of the material inside the capsule, or to totally prevent leaching of the water-soluble nutrients

Micronutrients: nutrients needed in small amounts, such as some vitamins and minerals

Micro-organism: any organism of microscopic or submicroscopic size

Microphagous: referring to organisms that feed on relatively small food items

Microscope: an optical instrument used to examine minute objects by giving an enlarged, well resolved image of them

Microvilli: small slender vascular, finger-shaped processes of the mucous membrane of the small intestines that serve in the absorption of nutrients

Mitochondria: membrane-bounded organelle that carries out oxidative phosphorylation and produces most of the ATP

Mobile: capable of moving or being moved from one place to another; capable of moving or being moved about readily

Moist pellet ration: a diet with about 30% moisture prepared from dry ingredients and ground fish biomass, formed into balls or pellets, and fed fresh or unfrozen before feeding

Molariform teeth: have flattened, often broadly occlusal surfaces; used for crushing and grinding

Mold inhibitor: substances added to feeds that inhibit mold growth

Monosaccharide: a simple sugar, the basic units from which disaccharides and polysaccharides are composed. The nutritionally important monosaccharides are the pentoses (five-carbon sugars) and the hexoses (six-carbon sugars)

Monounsaturated fatty acids: any one of the fatty acids containing one double bond

N

Natural aquatic food: in ponds, these are lablab and lumut

Nebulize: to reduce to a fine spray

Neutral lipids: the major form of storage lipids that are comprised mainly of triglycerides

Nitrate (NO_3^-): the end product of the aerobic stabilization of organic nitrogen, its presence in water indicates organic enrichment

Nitrification: the aerobic bacterial conversion of ammonia and organic nitrogen to stable salts (nitrates): commonly by *Nitrosomonas* sp and *Nitrobacter* sp

Nitrite (NO_2^-): an intermediate product in the biological oxidation of ammonia (NH_3) to nitrate (NO_3^-)

Non-essential amino acids: those amino acids required for protein synthesis that can be synthesized in the body in adequate amounts to meet requirements, and therefore do not have to be provided in the diet

Nutrient: any substance that is physiologically useful or necessary for growth and development

Nutrition: the science of nourishing an organism; the sum of the processes by which an animal or plant absorbs and utilize food substances. It involves the ingestion, digestion, absorption and transport of food nutrients into body cells and release of waste products of metabolism

O

Oligosaccharides: a general term for polymers containing about 3-10 monosaccharides

Omnivores: animals that derive their nutrients from both plants and animals

Organic materials: relating to the compounds of carbon (other than some of its simpler compounds)

Output: the product of combining various inputs using a technology (e.g. in aquaculture, the usual outputs are the fish, shellfish and other valuable by-products)

Oxidation: a reaction in which oxygen combines chemically with another substance; a reaction which involves the loss or transfer of electrons

P

Partial budgeting: a quantitative procedure that involves a marginal analysis to determine the profitability of a change in the production system

Particulate organic matter: particles of living or dead organic matter that are suspended in water; plankton is a form of particulate organic matter

Pearson square method: a mathematical method used in formulating feeds applicable only to few ingredients and with few nutrients to balance

Pectin: found primarily on the spaces between plant cell walls and may also infiltrate the cell wall itself

Peptide bond: the link between amino acids in a protein; formed by condensation between the carboxylic acid group (-COOH) of one amino acid and the amino group (NH_2) of another to give a -CO - NH- link between the amino acids

Peristalsis: successive waves of involuntary contraction passing along the walls of the intestine on other hallow muscular structure and forcing the contents onward

pH: the pH is defined as the negative logarithm of the hydrogen ion activity: $pH = -Log(H^+)$

Phenylalanine: one of the essential amino acids; it may be replaced by tyrosine

Phosphates: any of several substances containing any salt or ester of phosphoric acid (e.g. phosphate of calcium), used as fertilizer to supply phosphorus

Phosphatidic acid: a compound in which two fatty acids and phosphoric acid are esterified to the three hydroxyl groups of glycerol

Phosphatidyl choline: a major form of phospholipid containing choline in the molecule

Phospholipid: a lipid in which glycerol is esterified to two fatty acids, but the third hydroxyl group is esterified to phosphate, and through the phosphate to one of a variety of other componds; esters of fatty acid, glycerol, and phosphatidic acid

Photosynthesis: the synthesis of chemical substances with the aid of light, such as in the formation of carbohydrates (e.g. in green plants) from carbon dioxide and water with the liberation of oxygen, in the presence of chlorophyll

Phytoplankton: microscopic aquatic plants suspended in the water column; major oxygen-producing organisms in a pond

Pinocytosis: a property of cells that leads to uptake of macromolecules

Planktivores: animals that feed on plankton, the microscopic plant and animal life in water including bacteria

Plankton: the microscopic plant and animal life in the water including bacteria

Plant gums: complex, highly branched residues containing D-glucoronic and D-galacturonic acids along with other simple sugars such as arabinose and shammose

Polysaccharides: formed by the combination of hexoses or other monosaccharides

Predator: any organism that catches another for food

Premix: a uniform mix of micro-ingredients with a diluent or carrier

Primary production: it is the total increase in biomass of green plants observed over a period of time, expressed as production divided by the period of time

Production cost: the cost or monetary value of the inputs used in the production process

Production function: an expression of the technical relationship between outputs and inputs at a given time using a technology

Profit: the difference between the value of total product (VTP) or gross revenue and the total value of variable cost (TVC); or simply revenue minus cost

Profit index: the ratio of the net revenue to total cost

Protein: a polymer of amino acids joined by peptide bonds

Polyunsaturated fatty acids (PUFA): fatty acids with two or more carbon-carbon double bonds in the molecule, separated by a methylene (-CH_2) group

Purified diet: a diet composed of purified ingredients e.g. casein, gelatin, etc.

Pyrophosphate: a coenzyme involved in the transfer of two-carbon units

Q

Qualitative: concerned with, relating to or involving quality

Quality control: system for ensuring quality of output involving inspection, analysis, and action to make required changes

Quantitative: relating to or concerned with quantity

R

Rancidity: smelling or tasting foul because of chemical change, especially due to age

Ration: the food allowance of one person or one animal per day

Recirculating: system of using recycled material

Residue: something that remains after a part is taken, separated, removed, or designated

RNA: ribonucleic acid

S

Salinity: the concentration of mineral salts in the water, often expressed in ppt (parts per thousand)

Sampling method: a specific procedure for sampling, the details of setting up the instrument and use, and the protocol for sample preparation

Satiation: the quality or state of being satiated; the act or process of achieving qualification

Secchi disk: a disk 20 cm in diameter painted in alternate quadrants black and white (attached to a pole with graduations) and used to measure light penetration, transparency, or turbidity in the water column

Semi-purified: diet composed partly of highly purified and practical ingredients, e.g. fish meal

Sinking feed: prepared through extrusion under fairly low temperature and pressure such that pellets produced sink when placed in water. Prawns are bottom feeders and are given this type of feed

Soy lecithin: lecithin made from soya beans

Sphingomyelins: fatty acid esters of long-chain alcohol and sphingosine

Starch: a polymer of glucose units; are usually polycyclic long-chain alcohols; principal storage form of carbohydrates in plants

Static: relating to, or used in weighing; exerting force by reason of weight alone apart from effects of inertia

Steroids: compounds derived from cholesterol (itself a steroid), most of which are hormones

Stocking density: refers to the number of cultured animals stocked per unit area (e.g. 25,000 juveniles per hectare). Natural pond productivity is important in extensive culture system where stocking density is low

Substantial: consisting of; relating to, sharing the nature of, or constituting substance

Sucrose: a disaccharide having the formula $C_{12}H_{22}O_{11}$. It hydrolyzes to glucose and fructose

Sugar: chemically, a monosaccharide or small oligosaccharide. Cane or beet sugar is sucrose, a disaccharide of glucose and fructose

Supplement: a feed used with another to improve the nutritive balance or performance of the total and intended to be (I) fed undiluted as a supplement to other feeds; (II) offered free choice with other parts of the ration separately available; or (III) further diluted and mixed to produce a complete feed

Supplemental feed: feed supplied to meet the nutrient requirement of fish for maintenance and growth when natural food is inadequate

Survival: the final number of animals left is divided by the original number of animals stocked

T

Technical efficiency: a technology-based criteria for evaluating the desirability of a production process or the utility of an input

Temperature: the quantitative statement concerning heat usually expressed in degrees Celsius (°C)

Tetrahydrofolate: the metabolically active form of the vitamin folic acid, a carrier of one-carbon groups

Tight junction: cell-cell junction that seals adjacent epithelial cells together, preventing the passage of most dissolved molecules from one side of the epithelial sheet to the other

Total value of physical product (TVP): the monetary value of the output (fish) that is obtained by multiplying the unit price of the product to the total quantity of the product

Total variable input cost (TVIC): the sum of the cost of the variable input (feed) at different levels of input

Toxin: any of several intensely poisonous substances produced by certain bacteria

Trash fish: any of various sea fishes that have low market value as human food

Trial and error method: a method used in calculating feed formulation using many ingredients by trial and error

Triglycerides: esters of fatty acid and glycerol, the major form of storage lipids

U

Unsaturated: an organic compound containing one or more carbon-carbon double bonds, and therefore less than the possible proportion of hydrogen

Unsaturated fat: a fat formed from the reaction of glycerol with any one of several unsaturated fatty acids, e.g. olein & linolein

Unsaturated fatty acid: any one of several fatty acids containing one or more double bonds, e.g. oleic, linoleic, linolenic & arachidonic

Urea: the main excretory end-product of amino acid metabolism

V

Villiform teeth: are more or less elongated cardiform in which the length to diameter ratio resembles that of intestinal villi

Vitamin: an organic compound required in small amounts for the maintenance of normal growth, health, and metabolic integrity. Deficiency of a vitamin results in the development of a specific deficiency disease, which can be cured or prevented only by that vitamin

Volatile: a substance which can be easily liberated

W

Water quality: the limiting concentration of a water constituent (pollutant) or degree of intensity of some other adverse condition which is permitted in a body of water

Z

Zooplankton: small animals in water making up the secondary production level which depend on the water movement for locomotion

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Chapter 2

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Chapter 5

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Index

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in December 1967 for the purpose of promoting fisheries development in the region. Its member countries are Japan, Malaysia, the Philippines, Singapore, Thailand, Brunei Darussalam, the Socialist Republic of Vietnam, Union of Myanmar, and Indonesia.

Representing the Member Countries is the Council of Directors, the policy-making body of SEAFDEC. The chief administrator of SEAFDEC is the Secretary-General whose office, the Secretariat, is based in Bangkok, Thailand.

Created to develop fishery potentials in the region in response to the global food crises, SEAFDEC undertakes research on appropriate fishery technologies, trains fisheries and aquaculture technicians, and disseminates fisheries and aquaculture information. Four departments were established to pursue the objectives of SEAFDEC.

- The Training Department (TD) in Samut Prakan, Thailand, established in 1967 for marine capture fisheries training
- The Marine Fisheries Research Department (MFRD) in Singapore, established in 1967 for fishery post-harvest technology
- The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines, established in July 1973 for aquaculture research and development
- The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia, established in 1992 for the development and management of the marine fishery resources in the exclusive economic zones (EEZs) of SEAFDEC Member Countries.

SEAFDEC/AQD is mandated to:

- promote and undertake aquaculture research that is relevant and appropriate for the region
- develop human resources for the region
- disseminate and exchange information on aquaculture

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