

Food Intake in Fish

Edited by

Dominic Houlihan
University of Aberdeen, UK

Thierry Boujard
Unité mixte INRA-IFREMER, Sainte Pee sur Nivelle, France

and

Malcolm Jobling
NFH, University of Tromsø, Norway



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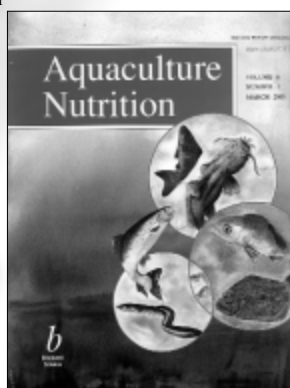
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10, rue Casimir Delavigne
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List of Contributors

Alanärä, Anders Department of Aquaculture, Swedish University of Agricultural Sciences, S-901 83 Umea, Sweden.

Azzaydi, Mezian Department of Physiology and Pharmacology, Faculty of Biology, University of Murcia, 3010 Murcia, Spain.

Baras, Etienne Laboratoire de Démographie des Poissons et d'Aquaculture, Université de Liège, 10 Chemin de la Justice, 4500 Tihange, Belgium.

Beauchaud, Marilyn Université Jean Monnet de St Etienne, 23 rue Pierre Michelon, 42023 St Etienne Cedex 2, France.

Bégout Anras, Marie-Laure CREMA L'Houmeau, BP 5, 17137 L'Houmeau, France.

Björnsson, Björn Thrandur Fish Endocrinology Laboratory, Department of Zoology, Göteborg University, PO Box 463, 40530 Göteborg, Sweden.

Bolliet, Valérie Unité de recherches en Hydrobiologie, Unité mixte INRA/IFREMER, Station d'Hydrobiologie INRA, BP. 3, 64310 Saint Pée sur Nivelle, France.

Boujard, Thierry Unité de recherches en Hydrobiologie, Unité mixte INRA/IFREMER, Station d'Hydrobiologie INRA, BP. 3, 64310 Saint Pée sur Nivelle, France.

Carter, Chris Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, P.O. Box 1214, 7250 Launceston, Tasmania, Australia.

Covès, Denis Laboratoire de Recherche en Pisciculture de Méditerranée, Station Expérimentale d'Aquaculture Ifremer, 34250 Palavas, France.

Damsgård, Børge Norwegian Institute of Fisheries and Aquaculture (FISKERIFORSKNING), 9005 Tromsø, Norway.

de la Higuera, Manuel Departamento de Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada, Avenida Fuente Nueva, 18001 Granada, Spain.

de Pedro, Nuria Departamento de Biología Animal II (Fisiología Animal), Facultad de Ciencias Biológicas, Universidad Complutense, 28040 Madrid, Spain.

Dias, Jorge Instituto de Ciencias biomedicas da Universidade do Porto, Aquatic Sciences Department, Largo Prof. Abel Salazar 2, 4050 Porto, Portugal.

Gomes, Emidio Instituto de Ciencias biomedicas da Universidade do Porto, Aquatic Sciences Department, Largo Prof. Abel Salazar 2, 4050 Porto, Portugal.

Gudmundsson, Olafur Agricultural Research Institute, Rala Building, Keldnaholt, 112 Reykjavik, Iceland

Houlihan, Dominic Faculty of Science and Engineering, University of Aberdeen, AB24 3FX Aberdeen, United Kingdom.

Jobling, Malcolm Norwegian College of Fishery Science (NFH), University of Tromsø, 9037 Tromsø, Norway.

Juell, Jon-Erik Agricultural University of Norway, P.O.Box N-5065, 1432 Aas, Norway.

Kadri, Sunil Division of Environmental & Evolutionary Biology, IBLS, University of Glasgow, G12 8QQ Glasgow, United Kingdom.

Kestemont, Patrick Unité de Recherches en Biologie des Organismes, Facultés Universitaires N.D. de la Paix, 61, rue de Bruxelles, 5000 Namur, Belgium.

Kettunen, Juhani Finnish Game and Fisheries Research Institute, PO Box 6, 00721 Helsinki, Finland.

Kiessling, Anders Department of Aquaculture, Swedish University of Agricultural Sciences, 901 83 Umea, Sweden.

King, Jonathan The Centre for Environment, Fisheries and Aquaculture Science, Conwy Laboratory, Benarth Toad, LL32 8UB Conwy, North Wales, United Kingdom.

Koskela, Juha Finish Game and Fisheries Research Institute, Laukaa Fisheries Research and Aquaculture, 41360 Valkola, Finland.

Kristiansen, Henrik Mobile Nutrients ApS, Forskerparken Foulum, P.O. Box 10, 8830 Tjele, Denmark.

Lagardère, Jean-Paul CREMA L'Houmeau, BP 5, 17137 L'Houmeau, France.

Lamb, Charles F. Department of Biology Black Hills State University 1200 University
Spearfish, SD 57799-9095 U.S.A.

Madrid, Juan Antonio Department of Physiology and Pharmacology, Faculty of Biology,
University of Murcia, 3010 Murcia, Spain.

Médale, Françoise Unité de recherches en Hydrobiologie, Unité mixte INRA/IFREMER,
Station d'Hydrobiologie INRA, BP. 3, 64310 Saint Pée sur Nivelle, France.

Paspatis, Mihaelis Aquaculture Department, Institute of Marine Biology of Crete, PO
Box 2214, 71003 Heraclion, Crete, Greece.

Petursdottir, Thuridur E Agricultural Research Institute, Rala Building, Keldnaholt, 112
Reykjavik, Iceland.

Ruohonen, Kari Finnish Game and Fisheries Research Institute, Evo Fisheries Research
Station, 16970 Evo, Finland.

Sánchez-Vázquez, F. Javier Department of Physiology and Pharmacology, Faculty of Bi-
ology, University of Murcia, 3010 Murcia, Spain.

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Preface

This book arose from collaboration among participants in a European Union COST programme: regulation of voluntary feed intake in fish. COST is a framework for research and development co-operation in Europe and the objective of the programme was to promote, co-ordinate and harmonise European research on feed intake in fish at a crucial moment for the development of new management techniques in fish feeding. Indeed, food intake in fish is an area of study that is of great importance to the applied science of fisheries and aquaculture, as well as being of basic biological interest. For example, the more we know about the various factors that influence the ingestion of feed the better able we will be to manipulate the rearing environment to ensure improved growth performance and feed utilisation of cultured fish, and also to decrease the amount of waste per unit of fish produced.

The book seeks to illustrate how insights into the biological and environmental factors that underlie the feeding responses of fish may be used to address practical issues of feed management. The main focus is on the feeding of fish held in captivity, and there is an emphasis on the application of the results of feeding studies in the practical context of fish culture. Feeding in fish, as in all animals, encompasses a wide range of factors. The animals encounter the food and capture it, make decisions concerning palatability and nutritional value, and then either ingest or reject it. Ingested food is then digested and absorbed, after which the animal's physiological systems deal with the influx of nutrients.

Palatability of feed is a major factor determining feed acceptance. Animals of several taxonomic groups produce, or concentrate, noxious substances, which reduce their attractiveness as prey, and many plants produce and store secondary metabolites that act as feeding deterrents. Feeds based on plant, rather than on animal, materials may lead to palatability problems in aquaculture, particularly when carnivorous fish species are being reared. In addition to containing substances that influence whether or not a fish will ingest them, plants may contain a range of antinutritional factors that have effects on digestion, absorption and nutrient metabolism. However, there will be a need to make greater use of plant materials in feed formulations as aquacultural production increases. The inclusion of plant proteins in formulated feeds reduces production costs, so there are important economic incentives to improve the palatability of plant feedstuffs, and for the investigation of potential feeding stimulants. The first two chapters of the book present basic information about feed composition and manufacture.

Chapter 1 deals with feed composition and the methods used in the analysis of feeds. As the farming of fish is often reliant upon formulated feeds we need to have a good knowledge

of the nutrients present, and how we should analyse for them. Although chemical analyses provide useful information about the nutrients present in feeds and feedstuffs, it is essential to carry out growth trials on live animals to obtain a complete assessment of the biological value of feeds. Such trials will usually incorporate digestibility studies to provide an assessment of nutrient bioavailabilities and feed evaluation will also involve an assessment of how efficiently the various nutrients have been used to support growth. The chapter describes the methods used for carrying out digestibility studies, and provides information about the biological evaluation of feeds and feed ingredients.

In Chapter 2, the question of feed formulation is addressed. Feed fed to aquatic organisms may be subject to leaching of nutrients while it remains in the water column or at the bottom of a tank. This creates a special set of problems for the manufacturer of feeds for fish and other aquatic animals. Dry feeds are most commonly used in present-day fish culture, and both the manufacturer and the farmer need to know how ingredient selection and processing can affect the availability of the nutrients present in the finished feed. These are topics considered in this chapter, as is the important question of the transition from feed formulations based upon fish meal to those based upon plant proteins.

Measuring feed consumption of animals held in water is not easy, and this is probably the major reason for the shortage of information on feed intake in fish, relative to terrestrial animals. In the 1950s some researchers trained fish to hit a rod to obtain food; this approach resulted in several short-term studies into feed preferences and feeding behaviour of fish. These first self-feeding devices were rather crude in construction, but the results obtained were very promising. The devices became gradually more refined as time progressed, and the introduction of computerised systems added a new dimension of sophistication. Self-feeders represent one type of on-demand feeding system, of which there are currently two main forms: self-feeders and interactive feedback feeders. When the former are used the fish must act to trigger the feed delivery system and feed themselves, whereas the latter type of system monitors how much feed is being consumed and controls delivery in response; the fish feed and the delivery of additional food is automatically regulated accordingly.

In addition to these methods, where the fish determine the amount of food delivered, a number of techniques have also been developed to measure the amount of food consumed when the experimenter or fish farmer controls the delivery of feed. The various techniques are considered in Chapter 3. There is no single method that is suitable for use under all conditions; different techniques are best for individual fish, small groups of fish held under experimental conditions, and larger groups in a cultured context. In addition to considering the measurement of feed intake of fish held in captivity the authors also present some information about the measurement of food consumption of wild fish.

Chapter 4 considers how we do experiments involving the measurement of food consumption, and how we analyse the data obtained. In a novel approach the authors pose the question: how should we design experiments to obtain results that reduce the amount of uncertainty? This question is of central importance because a lower level of uncertainty means increased reliability of scientific results. In the context of the aquaculture industry, reductions in uncertainty mean fewer risks of making mistakes when estimating economic returns for investment. The authors emphasise the need to decide upon the method to be used for statistical analysis in advance, as the method chosen will determine the best experimental approach.

There are lessons for all in this chapter; adoption of the principles would improve experimentation, not only in fish biology but also in the life sciences generally.

Fish may use inputs from various combinations of sensory systems during the different phases of feeding behaviour, but the ultimate acceptance or rejection of a food item is almost invariably dependent upon inputs from chemoreceptors. Fish possess several chemosensory systems, including gustation (taste), olfaction (smell) and a common chemical sense, but attributing specific roles to each system is difficult because each system responds to aqueous chemical stimuli, some of which may be common across the different systems. Nevertheless, it seems that the gustatory sense is the most important in the acquisition and ingestion of food, and in the rejection of potentially harmful or toxic substances. The gustatory system, which is very highly developed in some fish species, is the subject of Chapter 5. The emphasis is on the adaptations shown by the 'taste specialists', species that possess taste buds in epithelia outwith the oropharynx and have substantial enlargements of the brain regions responsible for processing gustatory information.

Chapters 6 and 7 consider the environmental factors that can influence fish feeding. Light, temperature, water velocity, social factors, predators and disturbance by humans can all influence the distribution of the fish in the water and their chances of gaining access to food. Thus there may be numerous combinations of biotic and abiotic factors that can have an important influence on feeding by fish. Most people who have fed fish have experienced that the fish may suddenly stop feeding for no apparent reason, and have observed that rates of feeding may decrease or increase over a period of days. If we have a better understanding of the factors that can influence feeding we may be better able to manage the allocation of food, and to reduce the variability in feeding that is usually evident both among individuals and among groups of fish.

Behavioural factors influence feed intake, and may even lead to feed refusal. Examples of factors that can influence feeding behaviour are stocking density, the sex ratio and the reproductive status of the fish, and biological rhythms. Endogenous clock mechanisms seem to control some of these rhythms, but environmental factors, such as day length or temperature, may either control others, or act as time setters or zeitgebers. There are three chapters that consider the rhythmic activity of feeding. Chapter 8 concentrates upon a discussion of whether the rhythms are based around 24 hours, days, weeks or months. There are many questions concerning whether these rhythms are generated within the fish and the extent to which external factors can influence the rhythms. Chapter 9 deals with anticipatory behaviour. When faced with the periodic delivery of food animals may develop some sort of activity in anticipation of the forthcoming meal. For example, the animals may become active some time prior to feeding, and such behaviour may be coupled to the cycles of feed delivery. The anticipatory behaviour is often seen to persist in fasting animals, so the suggestion is that there is also an internal timing mechanism in fish, as there seems to be in birds and mammals. The practical implications are that fish may adjust to the arrival of meals at set times, and that there may be optimal feeding times for each species. One very important topic concerns whether the presence of rhythms can influence not only the amount of food consumed but also the efficiency with which the food is converted into growth. This is discussed in Chapter 10. It is an intriguing possibility that if we knew more about the physiological rhythms then we could time feeding in order to maximise growth, improve efficiency, reduce waste and decrease pollution.

The diet of an animal should meet requirements for essential nutrients, and food should also be consumed in sufficient quantity to enable growth and reproduction to be maintained. Thus, nutrient composition is expected to influence whether or not a feed is ingested preferentially, but selection of feeds is also influenced by the sensitivity of the fish to other components, such as chemical deterrents and antinutritional factors. The topic of feed selection is dealt with in Chapter 11, in which it is suggested that fish recognise and discriminate between feeds having different chemical patterns. It is proposed that this enables them to create a diet that meets their needs, via the acceptance of the feeds that most closely match their nutritional preference and by rejection of others.

Chapter 12 deals with the hormones that are involved in the regulation of feeding in fish. Even in mammals the interactions of the factors that influence satiety are not completely understood, although it appears that feeding is under the control of a central feeding system in combination with a peripheral satiation system. The same general principles also probably apply to fish, and many of the chemical factors involved in the regulation of feeding appear to have similar structures, and roles, in the different groups of vertebrates.

Chapter 13 is concerned with how animals deal with a nutrient influx in terms of their physiology. Feed intake represents a challenge to the animal's physiology in that, following digestion and absorption, the nutrients provide the energy for maintenance and are incorporated into tissues as growth. After the ingestion of a meal there is an increase in oxygen consumption. This is partly a reflection of the energy demand for the synthesis of proteins. The proportion of ingested amino acids synthesised into proteins is central to the efficiency with which food is converted into growth. The more we understand about the balance between amino acid oxidation and protein retention, the better will be our ability to optimise feed composition and the timing of meal delivery.

Chapter 14 discusses ways of calculating the amount of feed to be distributed to farmed fish. The efficiency with which fish utilise the feed supply is a major factor that determines the economic returns of a fish farm, so the farmer should aim to feed the fish in a manner that ensures both rapid growth and minimal waste. The approach adopted by the authors involves a consideration of how knowledge about energy requirements can be converted into practical advice about feeding under given sets of conditions. The chapter also contains examples illustrating the use of on-demand feeding systems in a practical setting. These devices allow a continuous record of feed demand to be kept, so may reveal both day-to-day and seasonal variations in feeding, and also give information about the diel timing of feeding at different times of the year.

Fish are an important part of the diet of human populations in many countries. They are an excellent source of protein, contain unsaturated fatty acids that have a beneficial effect on human health, and are also valuable sources of some vitamins and minerals. Ultimately, the value of cultured fish as a source of nutrients for humans will depend upon the conditions under which they have been reared, and the types of feed with which they have been fed. Usually it is fish muscle, or fillet, that is used as a source of food by humans, but internal organs, such as gonads and livers, may also be consumed. The final chapter of the book addresses the question of the partitioning of nutrients among the different organs and tissues of fish species, and also considers how fish body composition may be influenced by the chemical composition of the feed.

Although much of the research into feeding behaviour and food intake in fish incorporates studies of basic principles, its purpose is often applied. For this reason we conclude this preface with a statement of the hope that this book will prove to be of interest not only to students and researchers within fish biology, but also to those directly involved in the aquaculture industry. It is certain that aquaculture will continue to expand, and will provide an increasing proportion of the world supply of fish products. This expansion will be dependent upon continued developments within feed formulation, further studies of feeding behaviour and feed management, and research aimed at the improvement of feed efficiency and the reduction of environmental loads from fish farms.

*Dominic Houlihan
Thierry Boujard
Malcolm Jobling*

Chapter 1

Feed Composition and Analysis

Malcolm Jobling

1.1 Introduction

Farmed fish and livestock do not usually consume natural prey organisms and forages, but are provided with feed that has been treated or preserved in some way prior to presentation. The feed is usually manufactured from a range of raw ingredients, or feedstuffs, and is formulated to contain an array of essential and non-essential nutrients. A nutrient can be considered to be a chemical element or compound that an animal can utilise in metabolism or growth, an essential nutrient being one that the animal cannot synthesise *de novo*. Thus, an animal's requirements for essential nutrients must be met via the diet. Feedstuffs may vary from simple compounds, such as common salt (NaCl), to meals of plant and animal origin that are made up of complex mixtures of compounds from several nutrient classes.

This chapter provides a brief introduction to the major nutrients found in feeds and feedstuffs, and methods for the evaluation of feeds and feedstuffs are also considered. Methods described include those for analysis of the chemical composition of feeds, and the biological methods used to assess the utilisation of feeds by animals.

1.2 Nutrient classes

Feeds and feedstuffs contain a range of chemical substances that can be grouped into classes according to constitution, properties and function (Fig. 1.1). All feeds will contain a certain proportion of water, so an initial division of feed components into moisture and dry matter can be made. The dry matter is conveniently divided into organic and inorganic material.

Carbohydrates, lipids and proteins make up the major part of the dry matter of feeds; these organic macronutrients can be used directly as metabolic fuels, can be stored within the body for utilisation at a later date, or be deposited in the structural materials that represent the somatic growth of the animal. The main component of the dry matter of feedstuffs of plant origin is carbohydrate, exceptions being oilseeds (e.g. peanut, sunflower, soybean) which contain large amounts of protein and lipid. In contrast to plants, the carbohydrate content of the animal body is low. One of the main reasons for the difference in carbohydrate content between plants and animals is that plant cell walls are made up of carbohydrate, largely cellulose, whereas the walls of animal cells are composed of protein and lipid. Furthermore,

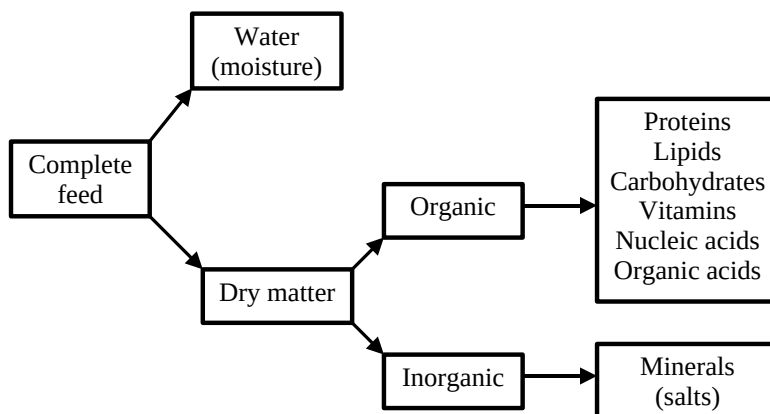


Fig. 1.1 Hierarchical subdivision of a feed showing the major chemical components.

plants store most of their energy as carbohydrates, such as starch, whereas lipids are an animal's main energy store.

Proteins are the major nitrogen-containing compounds present in both plant and animal feedstuffs. Like proteins, nucleic acids are also nitrogen-containing compounds, but these are present in small quantity. Organic acids, such as citric, malic, succinic and pyruvic, will also be present in most feedstuffs in small amounts, as will members of the heterogeneous group of essential organic micronutrients known as vitamins.

Inorganic matter will usually make up a small proportion of a feed or feedstuff. Calcium and phosphorus are generally the major mineral components in feedstuffs of animal origin, whereas potassium and silicon are the main inorganic elements in plants.

1.2.1 Proteins

Proteins are large organic, nitrogen-containing compounds comprising long chains of amino acids. The elemental compositions of proteins tend to be similar, with approximate percentages being C = 50–55%, H = 6–8%, O = 20–23%, N = 15–18% and S = 0–4%. The majority of the amino acids, of which 20 or so may be incorporated into proteins, have a chemical structure $\text{RCH}(\text{NH}_2)\text{COOH}$, with a carboxyl ($-\text{COOH}$) and an amino ($-\text{NH}_2$) group attached to the α carbon atom. R represents the side chain, which differs in configuration depending upon the amino acid. The amino acids can be divided into a number of series depending upon their structure and side chain configurations (Table 1.1):

- (1) *Aliphatic series* – glycine, alanine, serine, threonine, valine, leucine, isoleucine.
- (2) *Aromatic series* – phenylalanine, tyrosine.
- (3) *Sulphur amino acid series* – cysteine, cystine, methionine.
- (4) *Heterocyclic series* – tryptophan, proline, hydroxyproline.
- (5) *Acidic series* – aspartic acid, glutamic acid.
- (6) *Basic series* – arginine, histidine, lysine.

Two amide derivatives of amino acids are also found as constituents of proteins; these are the

Table 1.1 Characteristics of the different series of amino acids. Indispensable (essential) amino acids are indicated in bold type, and conditionally indispensable amino acids are shown in italics.

Series	Characteristics	Amino acid
Aliphatic	Aliphatic amino acids containing one carboxyl group and one amino group	Gly, Ala, Ser, Val, Thr, Leu, Ile
Sulphur amino acids	Aliphatic, monoamino-monocarboxylic amino acids containing sulphur	Met , Cys
Acidic	Aliphatic, dicarboxylic amino acids; aqueous solutions are acidic	Asp, Glu
Basic	Aliphatic amino acids giving basic aqueous solutions	Arg, His, Lys
Aromatic	Amino acids with the aromatic, or benzenoid, ring	Phe , Tyr
Heterocyclic	Heterocyclic structure incorporating nitrogen; proline and hydroxyproline have an imino (NH) group, but no amino group	Trp , Pro, Hyp

amides of aspartic and glutamic acids. Asparagine, the β -amide of aspartic acid, is a constituent of many proteins, and it is hydrolysed to aspartic acid and NH_3 by acid. Glutamine, the γ -amide of glutamic acid, occurs in biological materials both in free form and as a constituent of proteins. β -Alanine, an isomer of alanine, is not a constituent of proteins, but it occurs as the free amino acid, and as a component of several biological molecules: the vitamin pantothenic acid, coenzyme A, and the peptides carnosine and anserine found in muscle. The two amino acids citrulline and ornithine are part of the metabolic sequence leading to the formation of urea; they are not constituents of proteins, but do occur in the free form in animal tissues.

The structure of a protein is dependent upon the amino acid sequence (the primary structure) which determines the molecular conformation (secondary and tertiary structures). Proteins sometimes occur as molecular aggregates which are arranged in an orderly geometric fashion (quaternary structure). Constituents other than amino acids may be covalently bound and incorporated into proteins. For example, phosphoproteins such as milk casein and egg phosphovitin contain phosphoric esters of serine, and glycoproteins such as collagen and some fish serum proteins contain monosaccharide or oligosaccharide units bound to serine, threonine or asparagine.

Proteins – or more correctly some of the amino acids they contain – are an essential component of the diet for all animals. The amino acids may, therefore, be classified according to whether or not protein synthesis and growth can proceed in the absence of a dietary supply (Macrae *et al.* 1993; Friedman 1996). Essential amino acids are those that animals cannot synthesise, or cannot synthesise in sufficient quantity to enable the maintenance of good rates of growth, whereas non-essential, or dispensable, amino acids can be synthesised *de novo* from other compounds (Table 1.1):

- *essential or indispensable* – arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine.
- *conditionally indispensable* – cystine, tyrosine.
- *non-essential or dispensable* – alanine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, proline, serine.

Some of the 10 essential amino acids can be replaced by their α -hydroxy or β -keto analogues, illustrating that it is the carbon skeleton of the essential amino acid that the animal is unable to synthesise. Cystine and tyrosine are classified as being conditionally indispensable because

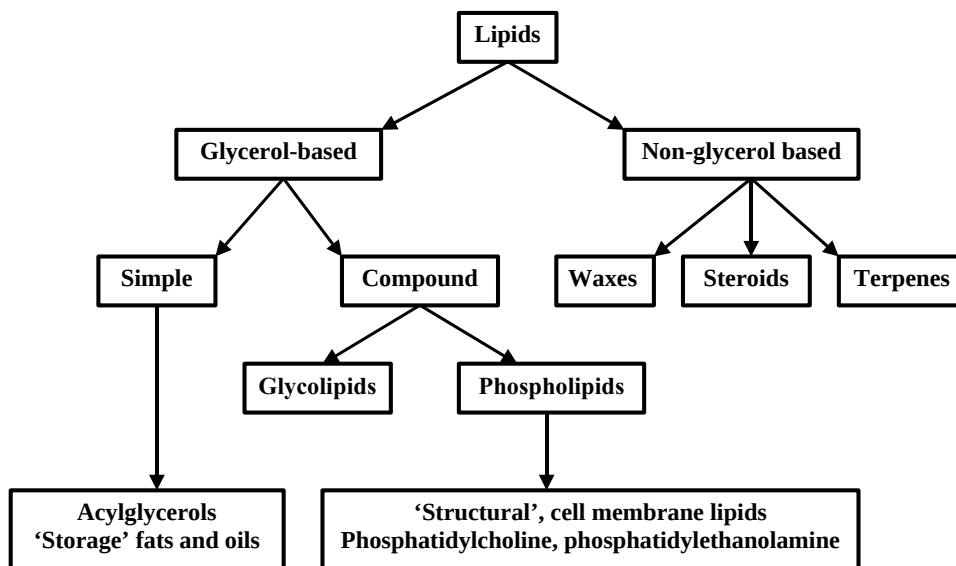


Fig. 1.2 Hierarchical diagram illustrating the classification of lipids.

they can be synthesised from methionine and phenylalanine, respectively. Thus, a dietary supply of cystine and tyrosine is not required if sufficient quantities of their respective precursors are available. In addition to being components of proteins, several of the amino acids are precursors for biologically active molecules: for example, histidine is decarboxylated to form histamine, tyrosine is the precursor for thyroxine and catecholamines, and tryptophan is the precursor of serotonin (5-HT) and melatonin. Estimates of quantitative amino acid requirements have been made for a range of fish species, and the data have been summarised in several overviews (Wilson 1989, 1991; NRC 1993; Cowey 1994; Jobling 1994; Mambrini & Kaushik 1995; Akiyama *et al.* 1998; Kaushik 1998).

1.2.2 Lipids

The lipids are a heterogeneous class of water-insoluble organic compounds that are readily soluble in organic solvents such as chloroform, hexane and diethyl ether (Fig. 1.2). More specifically, the term lipids may be used to describe substances which conform to these solubility characteristics and which contain esterified fatty acids. The glycerol-based lipids can be broadly subdivided into simple, neutral lipids, which are involved in storage, and more complex polar lipids, several of which form the structural components of cell membranes (Sargent *et al.* 1989; Macrae *et al.* 1993; Bell 1998).

Acylglycerols, the major neutral lipids, are formed by esterification of fatty acids with the trihydric alcohol, glycerol. Fatty acids are long-chain organic acids having the general formula $\text{CH}_3(\text{C}_x\text{H}_y)\text{COOH}$. The hydrocarbon chain is either saturated ($Y = 2X$), or there are double bonds between some of the adjacent carbon atoms (unsaturated fatty acid). Triacylglycerols make up the great bulk of neutral lipids found in nature, but mono- and diacylglycerols are also found. The wax esters are a group of compounds closely related to the

acylglycerols. Wax esters, which are formed by the esterification of fatty acids with long-chain alcohols, are found as water-proofing agents on the outer surfaces of fruits and leaves of plants, and on the exoskeleton of insects. Wax esters are also a major lipid storage reserve in some zooplanktonic crustacea and, hence, may provide an important energy source for a number of fish species.

Although the majority of the lipid extracted from animal tissues will be made up of neutral, storage triacylglycerols, the total lipids will also contain smaller quantities of other lipid classes. Among the polar lipids it is the phospholipids that are important components of cell membranes, where they form lipoprotein complexes. Phospholipids are esters of glycerol, in which two of the alcohol groups are esterified with fatty acids. The third alcohol group is esterified with phosphoric acid which, in turn, is esterified by a nitrogenous base, the amino acid serine, the sugar alcohol inositol or by glycerol phosphate. The nature of the esterification compound provides the basis of the name of the specific families of phospholipids, e.g. phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. The major cell membrane phospholipids are phosphatidylcholine and phosphatidylethanolamine, whereas phosphatidylinositol appears to have a number of important roles in signal transduction over biomembranes.

The fatty acids that are esterified to the glycerol backbone of the acylglycerols and phospholipids differ in the numbers of carbon atoms in the molecule, and in the number and positioning of the double bonds between the carbon atoms. These differences impart different physical and chemical properties to the various lipids. The fatty acids form distinct series, and shorthand systems have been devised for classifying these fatty acid series (Table 1.2). For example, palmitic acid may be represented by the shorthand formula 16:0, indicating that palmitic acid is a fatty acid with 16 carbon atoms and no double bonds. A fatty acid that lacks double bonds between the carbons is known as a saturated fatty acid. The monounsaturated

Table 1.2 Classification and naming of some representative fatty acids. Note that in the scientific designation anioic refers to a fatty acid without double bonds in the carbon chain, enoic to a fatty acid with one double bond, dienoic to two double bonds, trienoic to three, tetraenoic to four, etc. The shorthand notation gives the number of carbons, the number of double bonds, and the position of the first double bond counting from the methyl end.

Trivial name (scientific designation)	Number of carbon atoms	Number of double bonds	Fatty acid series	Shorthand notation
Saturated fatty acids (SFAs)				
Lauric (dodecanoic)	12	0		12:0
Palmitic (hexadecanoic)	16	0		16:0
Stearic (octadecanoic)	18	0		18:0
Monounsaturated fatty acids (MUFAs)				
Palmitoleic (hexadecenoic)	16	1	(n-7)	16:1 (n-7)
Oleic (octadecenoic)	18	1	(n-9)	18:1 (n-9)
Erucic (docosenoic)	22	1	(n-9)	22:1 (n-9)
Polyunsaturated fatty acids (PUFAs)				
Linoleic (octadecadienoic)	18	2	(n-6)	18:2 (n-6)
γ -Linolenic (octadecatrienoic)	18	3	(n-6)	18:3 (n-6)
α -Linolenic (octadecatrienoic)	18	3	(n-3)	18:3 (n-3)
Highly-unsaturated fatty acids (HUFAs)				
Arachidonic (eicosatetraenoic)	20	4	(n-6)	20:4 (n-6)
EPA (eicosapentaenoic)	20	5	(n-3)	20:5 (n-3)
DHA (docosahexaenoic)	22	6	(n-3)	22:6 (n-3)

fatty acid oleic acid is designated as 18:1(n-9), showing that oleic acid is an 18 carbon fatty acid with one double bond, the (n-9) denoting that the double bond occurs between the ninth and tenth carbon atoms from the methyl end. The shorthand formula 18:3(n-3) represents α -linolenic acid, an 18 carbon fatty acid with three double bonds, the first double bond being found in the link between the third and fourth carbon atoms from the methyl end. Fatty acids with one or more double bonds are unsaturated fatty acids. Those having two to four double bonds in the molecule are often termed polyunsaturated fatty acids (PUFAs), and those with more than four double bonds may be described as being highly-unsaturated fatty acids (HUFAs).

Lipid nutrition and metabolism have been extensively studied in those fish species, such as salmonids, which are farmed intensively (Henderson & Tocher 1987; Sargent *et al.* 1989, 1995; Kanazawa 1993; Bell 1998). Fish are not able to synthesise PUFAs of the (n-3) and (n-6) series *de novo*, so fatty acids of these two series are essential nutrients that must be supplied in the diet. The fatty acids may be supplied as 18:2(n-6) and 18:3(n-3) provided that the fish are able to convert and chain-elongate these fatty acids into the longer-chain HUFAs, e.g. 20:5(n-3), 22:6(n-3), 20:4(n-6), that are essential components of cell membranes. In those fish that are incapable of converting the 18C fatty acids to their 20C and 22C homologues, 18:2(n-6) and 18:3(n-3) will not be effective as essential fatty acids (EFAs). In species that are able to perform the conversion reactions, the 18C, 20C and 22C fatty acids will all have EFA properties, although the 20C and 22C fatty acids are the most efficacious. It appears that the EFA requirements of many freshwater fish species can be met via a dietary supply of 18:2(n-6) and 18:3(n-3), although the pike, *Esox lucius*, a piscivorous freshwater species, seems incapable of converting 18C fatty acids to the longer-chain HUFAs. Consequently, the pike has a dietary requirement for long-chain HUFAs. The marine fish species investigated thus far also seem to have very limited ability to convert 18C fatty acids to their long-chain HUFA metabolites, so these fish also require a dietary supply of the HUFAs. The broad differences in EFA requirements observed for marine and freshwater fish may depend upon the composition of the natural diet, i.e. whether the species is herbivorous, omnivorous or carnivorous, and upon the relative abundance of the particular fatty acids within freshwater and marine food chains. The (n-6) series fatty acids are typically found in terrestrial and freshwater environments, whilst in marine ecosystems fatty acids of the (n-3) series tend to dominate. The food chain in fresh water is characterised by 18:2(n-6), 18:3(n-3) and 20:5(n-3) fatty acids, whereas the fatty acid profiles of marine phyto- and zooplankton tend to be dominated by 18:3(n-3), 20:5(n-3) and 22:6(n-3) fatty acids, and this pattern is generally reflected at higher levels in the food chain.

1.2.3 Carbohydrates

The term carbohydrate expresses the originally determined empirical formula $C_x(H_2O)_y$, but some compounds not showing the 2:1 ratio of hydrogen to oxygen also have many of the chemical properties of carbohydrates, e.g. deoxyribose ($C_5H_{10}O_4$). Further, a number of compounds that contain small proportions of nitrogen and sulphur in addition to carbon, hydrogen and oxygen have characteristics considered typical for this class of nutrients (Macrae *et al.* 1993). Classification of the carbohydrates may be made according to the size of the molecule (Fig. 1.3). Monosaccharides are simple sugars that cannot be hydrolysed into smaller units,