Introduction to Fish Nutrition

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Proteins and Amino Acids

Proteins are organic compounds comprised of conjugated amino acids in proportions that are characteristic of each protein. This nutrient always contains carbon, hydrogen, oxygen and nitrogen; however, some proteins also contain sulphur, phosphorus and iron. Since most proteins contain about 16% nitrogen, crude protein of a product can be obtained by determining the nitrogen content and then multiplying that value by 6.25 (100% ÷ 16% = 6.25). Plants generally contain their protein in actively growing portions, such as leaves and seeds, while animals tend to distribute protein in a wider variety of tissues, such as bones, skin, organs, feathers, scales and muscle. Body protein is constantly undergoing two processes: protein synthesis (anabolism) and protein breakdown (catabolism). Protein deposition (or loss) equals the amount of protein synthesis minus the amount of protein breakdown.

The basic structural component of proteins consists of amino acids. Ten amino acids cannot be synthesized by vertebrates, including fish, and must be supplied in the diet. These essential (indispensable) amino acids are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Fig. 1.1). There are other amino acids that fish can synthesize. These amino acids are termed non-essential (dispensable) amino acids and consist of: alanine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, hydroxyproline, proline, serine and tyrosine. Non-essential amino acids can be synthesized by transfer of an amino group to α-keto acids which can be derived from non-protein sources, such as glucose.

When a particular essential amino acid is deficient in a diet, it is referred to as a limiting amino acid because it limits the synthesis of protein. All of the amino acids needed to synthesize a protein must be available or no synthesis can occur. This is why protein quality is important in fish nutrition. Protein synthesis involves a series of reactions that are specific for each protein. Each protein is

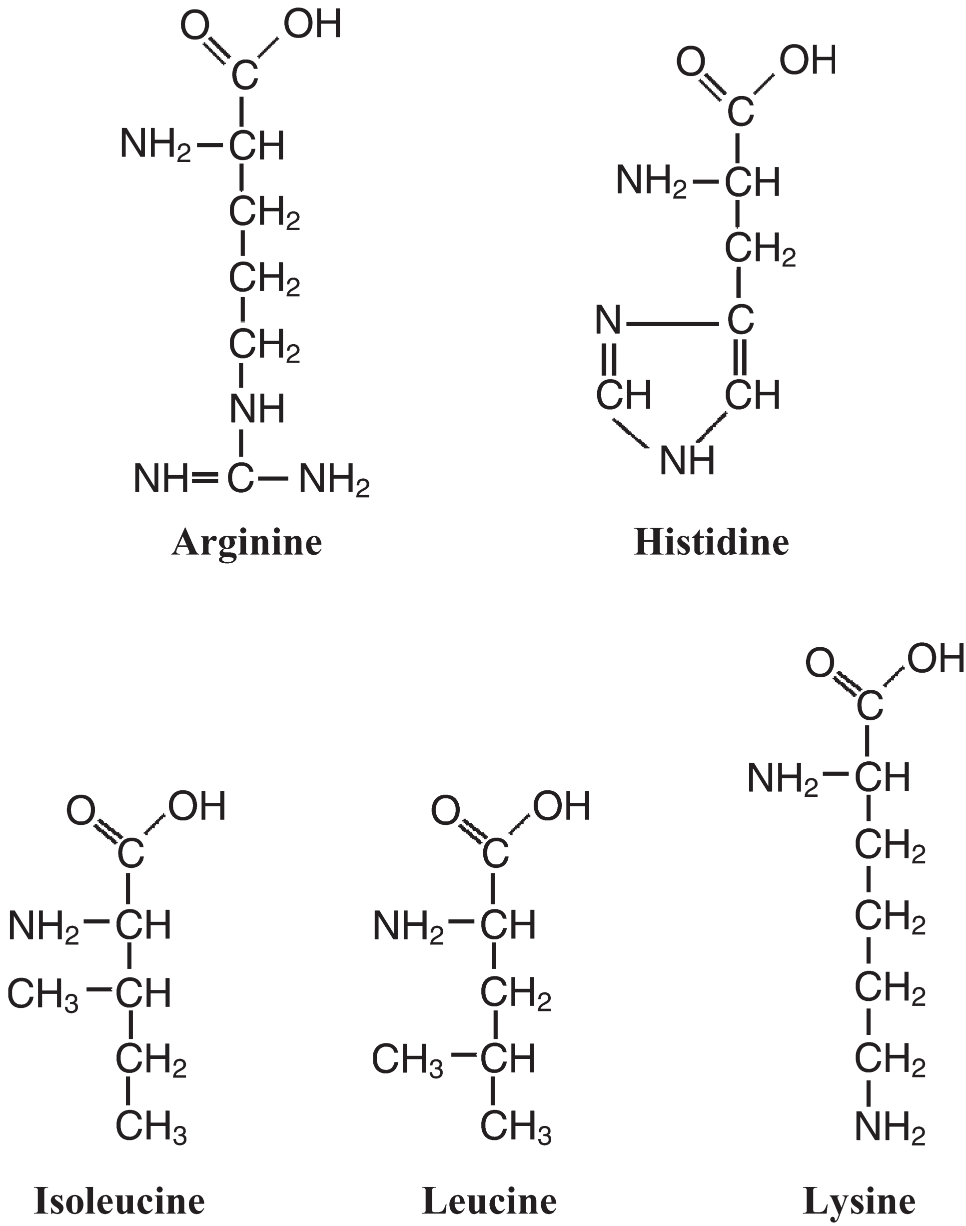
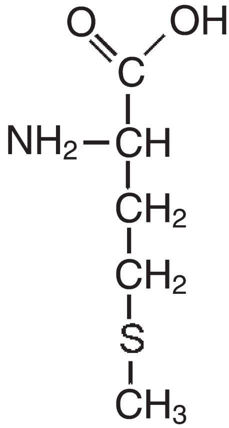
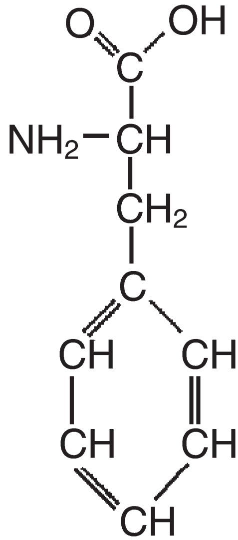


Fig. 1.1. Chemical structures of the ten essential amino acids (continued opposite).

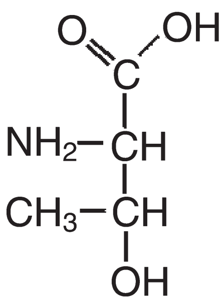
manufactured according to the code derived from deoxyribonucleic acid (DNA), a chromosomal component of the cell. Messenger ribonucleic acid (mRNA) transcribes the information from the DNA and brings it to the cytoplasm of the cell as a single strand; transfer RNA (tRNA) carries the specific amino acids to



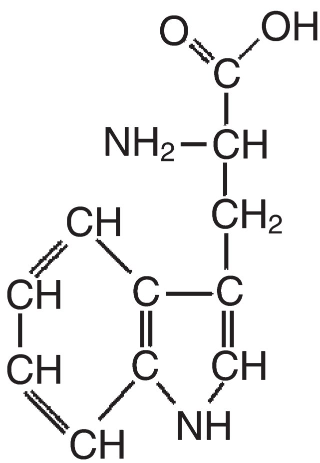
Methionine



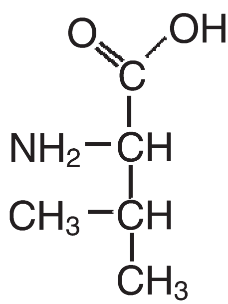
Phenylalanine



Threonine



Tryptophan



Valine

the ribosome, where they interact with mRNA; ribosomal RNA (rRNA) is a component of the ribosomes in the cell and is the site of protein synthesis. As the DNA message is decoded, tRNA brings the proper amino acids to be conjugated. The amino (NH_2) portion of one amino acid will combine with the carboxyl (COOH) portion of another amino acid, forming a peptide linkage and releasing water (H_2O). This process of joining amino acids is continued until the specific molecule (protein) encoded by the DNA is produced. Each sequence of amino acids is a different protein and these different proteins accomplish different functions in the body.

Proteins occur in nature in a number of forms that possess unique chemical properties and can be divided into simple proteins and conjugated proteins. Simple proteins consist of only amino acids or their derivatives, while conjugated proteins are joined to other non-protein substances. Simple proteins consist of the albuminoids, albumin, globulins, hormones and blood proteins, contractile proteins, such as actin–myosin, and keratins. Conjugated proteins include the chromoproteins (combination of a protein and a pigmented substance), lecithoproteins (combination of a protein and lecithin), lipoproteins (combination of a lipid and a protein), metalloproteins (combination of a metal and a protein), nucleoproteins (combination of a nucleic acid and a protein) and phosphoproteins (combination of phosphorus and a protein; the phosphorus is in a form other than phospholipid or nucleic acid).

Protein requirement is somewhat of a misnomer in that it is actually the requirement for essential amino acids that is important for the proper growth, development and health of fish. While crude protein values are important to a nutritionist formulating a fish diet, they may not allow the nutritionist to determine the quality of the protein. Gelatin is a high-protein source; however, it is virtually devoid of tryptophan. Feather meal, while high in protein, is very poorly digested and utilized by fish, resulting in reduced growth if incorporated at high levels in the diet. Thus, it is important to know the amino acid composition and digestibility of a protein source, as well as its total protein content, when formulating a diet. There are some measurements and mathematical equations that one can utilize to quantify protein quality. One such method is to determine the apparent protein utilization (APU). APU is the amount of protein gain of fish fed an experimental diet, divided by the amount of protein fed.

A second means of determining protein quality is the protein efficiency ratio (PER), which is defined as the total weight gain of fish divided by the total protein intake over the period of the feeding trial. PER assumes that all protein is used for growth and no allowance for maintenance (turnover) is made.

A third method, net protein utilization (NPU) (or net protein value), attempts to take into account protein used for maintenance. This is accomplished by the inclusion of a treatment group of fish that receive diets containing no dietary protein. The NPU is the protein gain of a group of fish fed the experimental diet minus the protein loss of a similar group fed a protein-free diet, divided by the weight of the protein consumed.

Most proteins are highly digestible. In fish possessing a stomach, pepsins and hydrochloric acid (HCl) initiate proteolysis. Once in the intestine, luminal enzymes, such as trypsins, chymotrypsins and carboxypeptidase A and B, further break down the protein into amino acids. Amino acids are absorbed across the brush border of the intestines in fish. In agastric fish (fish without stomachs) proteolysis begins directly in the anterior intestine.

There are a number of factors that affect the protein requirement of fish. One is the size of the fish; small fish require more protein than larger fish. As mentioned previously, protein quality is important. A third factor is water temperature; when water temperature is at or near a fish's optimum temperature for growth, a higher dietary protein level is needed for optimum growth. A fourth factor is feeding rate; if fish are fed all they consume, the lower the protein level required compared with fish fed a restricted (not all the diet a fish will consume) amount of diet. A fifth factor is the presence of natural food items in the culture system. Fish stocked in ponds at low density may have access to more natural foods than those stocked at high density or in a raceway. Lower dietary protein level or poorer-quality dietary protein may be used for fish with access to natural foods as compared with fish cultured where natural foods are absent or limiting. Lastly, the dietary energy level affects the dietary protein requirements of fish. If the non-protein energy level of a diet is low, the fish will utilize protein to meet metabolic energy needs. This is inefficient and financially wasteful. If the energy level is too high, it may suppress food intake and the fish will not consume enough diet to meet protein requirements. This may reduce fish growth.

Energy

Energy is not a nutrient but is a property of nutrients that is released during metabolic oxidation of protein, lipids and carbohydrates. Thus, energy is an abstraction; it can only be measured as it is converted from one form to another. Energy is defined as the capacity to do work, but in a biological sense it is muscle activity and energy needed to run chemical reactions in the body, to move molecules against a concentration gradient and for other bodily processes. Fish, like other animals, must obtain their energy from oxidizing chemical bonds. Energy is measured in heat units called calories and is defined as the amount of heat needed to raise the temperature of 1 g of water by 1°C (from 14.5°C to 15.5°C).

The energy of ingested diets is partitioned into various fractions in terms of animal utilization (Fig. 1.2). Gross energy (GE) is the energy released as heat when an organic substance is completely oxidized to carbon dioxide (CO_2) and water using a bomb calorimeter. Intake energy (IE) is the gross energy in the diet (food) consumed and can be calculated as: IE = GE (amount of food consumed). Faecal energy (FE) is the gross energy of the faeces. Digestible energy (DE) is the intake energy of the diet (food) consumed that is not excreted in faeces and is calculated as: DE = IE – FE. Urinary energy (UE) and gill excretion energy (GEE) can be measured but are generally negligible in fish. Metabolizable energy (ME) is the intake energy that is not lost in the faeces or urine or through the gills, and can be calculated from the equation: ME = IE – (FE + UE + GEE). Net energy (NE) is the intake energy that is left after FE, UE, GEE and heat energy (HE) are deducted. The NE is the portion of IE that is available for maintenance (NEM) and various productive purposes (NEP), such as growth and reproduction.

Fish, like all animals, need energy to live. The citric acid (Krebs) cycle is the primary producer of adenosine triphosphate (ATP) and hydrolysis of ATP is the

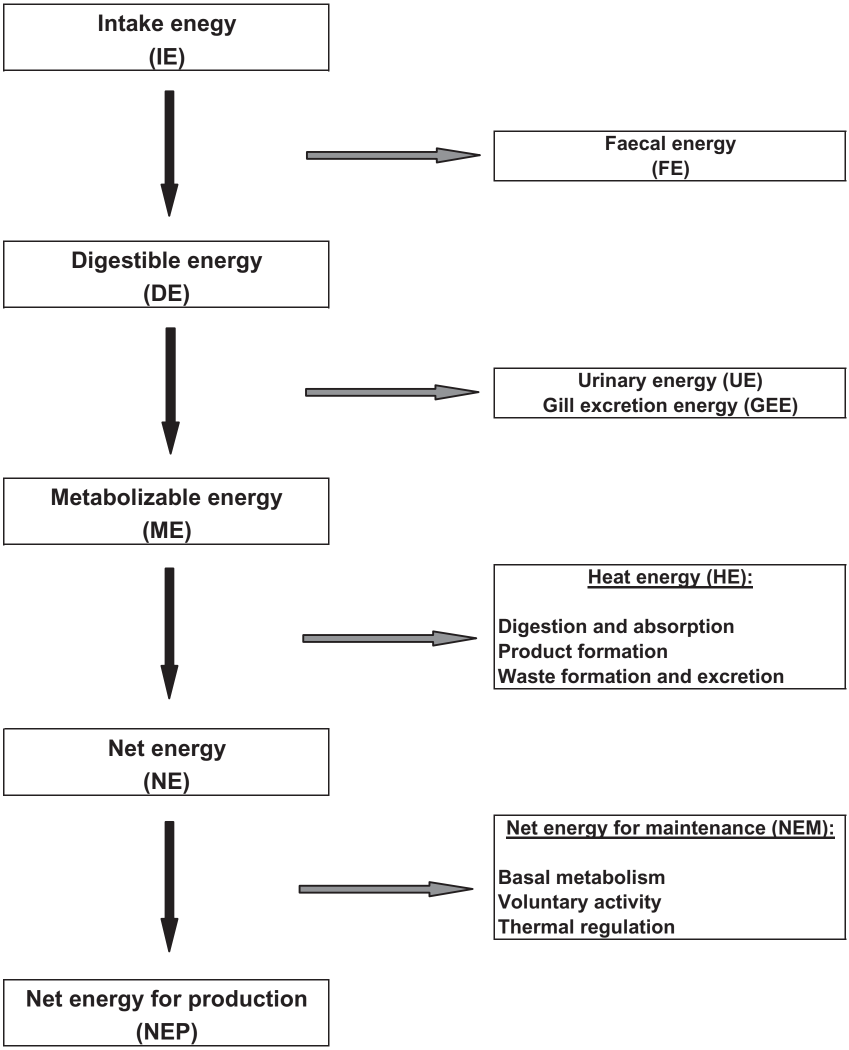


Fig. 1.2. The energy of ingested food is comprised of various fractions. The breakdown of the energy intake occurs in the course of the metabolic process.

source of energy at the cellular level. Complete oxidation of 1 mol of glucose yields 686 kcal and hydrolysis of ATP yields 8 kcal mol^{-1}. Thus, theoretically, the hydrolysis of 1 mol of glucose could produce 85 mol of ATP (686 ÷ 8 = 85); however, in reality, only about 39 mol of ATP are produced, the rest being lost as heat to the surrounding water.

While it appears that fish may have a high protein requirement compared with other animals, in reality, fish have a low requirement for energy. One reason for the lower energy requirement of fish is that they do not need energy for maintenance of body temperature as do mammals. A second reason is that fish live in water and are neutrally buoyant. This minimizes the energy required by the fish's muscles to maintain their position in space. Land animals must maintain their body position against the force of gravity. Since many fish have swim-bladders to assist in maintaining their position in the water column, they require less muscle activity to maintain their position in space. A third reason for the lower energy needs of fish is that fish excrete approximately 85% of metabolic wastes as ammonia (NH_3) directly through the gills into the surrounding water with little energy cost. In contrast, mammals must expend energy to produce urea, while birds expend energy to produce uric acid.

There are a number of factors that affect the energy requirements of fish, including: (i) physical activity; (ii) temperature; (iii) fish size; (iv) growth rate; (v) species; and (vi) food consumption. Physical activity includes swimming, fleeing from a predator and stress. Water temperature has the greatest effect of any of the factors that influence the energy requirement of fish. Since the body temperature of the fish is similar to that of the surrounding water, water temperature near the optimal temperature of a particular fish species leads to an increase in its metabolic rate and increases in appetite, growth rate and activity. Conversely, as the water temperature declines below the optimal range, metabolic rate is reduced. The size of the fish influences energy requirement because small fish tend to have higher energy requirements per unit weight than do larger fish. Similarly, fast-growing fish will require more energy than slow-growing fish.

For most land animals, carbohydrates are the major sources of energy; however, fish generally do not utilize carbohydrates as well as land animals. Pigs can utilize (digest) carbohydrates at about 90%, while the digestibility of carbohydrates for carps can range from 55 to 60%. Carbohydrates contain 4.1 kcal of GE g^{-1}, and are the least expensive dietary energy source. Thus, they are important constituents in the diets of herbivorous and omnivorous fish species. Further, carbohydrates allow for pellet expansion during extrusion of pellets, which controls pellet density and buoyancy. Proteins contain 5.6 kcal of GE g^{-1} of energy and are readily utilized by fish. However, as protein is the most expensive component in a diet, it is uneconomical to use protein as an energy source. Thus, lipid and carbohydrate sources are added to diets to increase the necessary energy. Lipids are the most concentrated sources of energy added to aquaculture diets; lipids contain approximately 9.4 kcal of GE g^{-1}. Lipids contain more energy because they contain more carbon and hydrogen. Oxidation of 1 g of hydrogen produces 34.5 kcal, while oxidation of 1 g of carbon produces 8 kcal. Carbohydrates have their hydrogens oxidized and only the carbons are not yet oxidized. However, there are many unoxidized hydrogens and carbons in a lipid molecule.

Fish must be fed diets containing appropriate amounts of energy. Since fish eat to satisfy their energy requirement, too much energy in the diet may result in fatty fish, which may decrease dressing percentage or the fish will not consume enough diet to satisfy their protein and other nutrient requirements, and growth rate will be reduced. If there is too little energy in the diet, fish will utilize part of the protein as an energy source, which is economically wasteful.

Lipids and Fatty Acids

Lipids include fats, oils and waxes. Fats are esters of glycerol and fatty acids that are solid at room temperature. Oils are glycerol esters that are liquid at room temperature. Waxes are esters of fatty acids with alcohols other than glycerol. Lipids are essential nutrients in fish diets and contain the three elements carbon, hydrogen and oxygen; however, unlike carbohydrates, a larger proportion of carbon and hydrogen comprise lipid molecules. Because of the larger proportion of carbon and hydrogen atoms, lipids liberate approximately 9.4 kcal of GE g^{-1} and are the best sources of energy in terms of kcal g^{-1} compared with carbohydrates (4.1 kcal of GE g^{-1}) and proteins (5.6 kcal of GE g^{-1}).

Biosynthesis of lipids involves the combining of two carbon units, called acetyl coenzyme A (CoA), into long chains and then adding hydrogen. Most lipid formation occurs in the microsomes of adipose tissue, the liver and the mitochondria of the cell (to a lesser degree). Approximately 98% of the lipids in diet ingredients (foods) and 90% of the lipid in the body occur as triacylglycerols (triglycerides). Triglycerides are a combination of three fatty acid molecules (chains) attached to a glycerol backbone.

Fatty acids are the key components of all lipids. The amount of hydrogen on each carbon (degree of saturation) and the length of the carbon chain determine the physical and nutritional characteristics of lipids. Saturation refers to the number of hydrogen atoms on carbon atoms. When a single bond joins two carbon atoms together, the carbon atoms within the chain have two hydrogen atoms associated with each carbon while the carbon at one of the ends of the fatty acid chain has three hydrogens. The other terminal carbon has an acid group (COOH) with a double bond between the carbon (C) and the oxygen (O), and a single bond between the C and the hydroxyl group (OH). If all the bonds connecting carbon atoms together in the fatty acid chain are single bonds, the fatty acid is said to be saturated with hydrogen (Table 1.1). When carbon atoms within the chain are joined by one or more double bonds, those carbon atoms are only able to have one hydrogen atom bonded to them. Fatty acids that have one or more double bonds are said to be unsaturated. A fatty acid with one double bond is called a monoenoic fatty acid (monounsaturated); a fatty acid which has two double bonds is a dienoic fatty acid; a fatty acid with three or more double bonds is called a polyenoic (polyunsaturated) fatty acid.

The second factor that influences the characteristics of a fatty acid is the length of the chain or number of carbon atoms in the fatty acid molecule. Fatty acid chains are formed by the addition of acetyl CoA (two-carbon) units. Thus, the majority of fatty acids in nature have an even number of carbons and, of these, the most biologically important to fish are those having between 16 and 22 carbon atoms.

There are other important lipids, including phospholipids, lipoproteins and cholesterol. Phospholipids are the structural components of cell membranes. Lipoproteins are the principal vehicle for lipid transport in the blood and there are four main types: chylomicrons, very low-density lipoproteins (VLDL), low-density

Table 1.1. Structure of some common fatty acids.

Common name	No. of carbons (class)	Structure
Saturated		
Caproic	6	$CH_3(CH_2)_4$ COOH
Caprylic	8	$CH_3(CH_2)_6$ COOH
Capric	10	$CH_3(CH_2)_8$ COOH
Lauric	12	$CH_3(CH_2)_{10}$ COOH
Myristic	14	$CH_3(CH_2)_{12}$ COOH
Palmitic	16	$CH_3(CH_2)_{14}$ COOH
Stearic	18	$CH_3(CH_2)_{16}$ COOH
Arachidic	20	$CH_3(CH_2)_{18}$ COOH
Lignoceric	24	$CH_3(CH_2)_{22}$ COOH
Monoenoic		
Palmitoleic	16 (n-7)	$CH_3(CH_2)_5CH{=}CH(CH_2)_7$ COOH
Oleic	18 (n-9)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$ COOH
Dienoic		
Linoleic	18 (n-6)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7$ COOH
Polyenoic		
Linolenic	18 (n-3)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7$ COOH
Arachidonic	20 (n-6)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2$ $CH{=}CH(CH_2)_3$ COOH
Eicosapentaenoic	20 (n-3)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}$ $CHCH_2CH{=}CH(CH_2)_3$ COOH
Docosahexaenoic	22 (n-3)	$CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}$ $CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_2$ COOH

lipoproteins (LDL) and high-density lipoproteins (HDL). Cholesterol is synthesized in the body, but some species, notably crustaceans, require cholesterol in the diet.

Digestion of lipids differs from digestion of carbohydrates and proteins in that lipids are not water-soluble. Thus, before enzymes can begin to break down lipids, they are first solubilized by bile secreted from the gall-bladder. Bile emulsifies the lipid and allows for digestion to occur. When the emulsified lipids come into contact with lipases (enzymes that hydrolyse lipids), the lipids are broken down into monoacylglycerols (monoglycerides), diglycerides, free fatty acids and glycerol. Micelles, emulsified mixtures of monoglycerides, fatty acids and bile salts, attach to the surface of the intestinal epithelial cells and are absorbed. Once inside the cells, long-chain fatty acids are re-esterified (joined to glycerol) to form triglycerides. The triglycerides combine with cholesterol, phospholipids and lipoproteins to form chylomicrons, which are small lipid droplets. Chylomicrons pass into the lymphatic system and eventually enter the blood, where they are transported to body tissues. Adipose tissue is the major site for the removal and storage of chylomicron triglycerides. However, fatty acids in phospholipids are vital for maintaining cell and subcellular membrane integrity, and fatty acids play a role in cholesterol metabolism and are the precursors of prostaglandins.

Lipids perform four main functions in the body: (i) provide energy; (ii) provide essential fatty acids; (iii) serve as structural components; and (iv) serve in regulatory functions. As sources of energy, lipids are the most concentrated source, with approximately 9.4 kcal of GE g^{-1}. An excess of energy, whether derived from carbohydrates, protein or lipid, is stored as triglycerides within the adipose tissue (cells) of the body. Adipose cells are found beneath the skin, between the muscle fibres and around the abdominal (visceral) organs and the membranes that support the organs. Free fatty acids (FFA) are the sources of immediate energy for fish. As they are oxidized from the fatty acid chains into two carbon segments, they form acetyl CoA, which releases energy during the Krebs cycle (tricarboxylic acid (TCA) or citric acid cycle).

Fish require dietary lipids to meet their essential fatty acid requirements. Each species of fish may have a specific fatty acid requirement, but, in general, it appears that cold-water fish require highly unsaturated fatty acids (HUFA) of the n-3 class of lipids, while warm-water fish require HUFA from either the n-3 or n-6 classes, or a mixture of both. Some fish, such as rainbow-trout (*Oncorhynchus mykiss*) can chain-elongate and desaturate fatty acids with 18 carbons, specifically linolenic acid (18:3n-3), to HUFA with 20 and 22 carbons of the n-3 class, specifically eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). This ability to synthesize EPA and DHA from linolenic acid allows nutritionists to formulate diets containing less expensive plant oils that contain linolenic acid (such as linseed oil) instead of using more expensive marine fish oils, which are rich sources of EPA and DHA (Table 1.2). However,

Table 1.2. Table of fatty acids of various fats and oils.

Common name	Numerical name	A	B	C	D	E	F	G	H	I	J	K
Myristic	14:0				1					8.0	7.6	3.0
Palmitic acid	16:0	13	26	13	48	12	3	12	8	29	18	27
Palmitoleic acid	16:1 (n-7)		1	1						8	8	
Stearic acid	18:0	4	3	2	4	6	2	2	5	4	2	21
Oleic acid	18:1 (n-9)	29	18	75	38	55	58	24	21	13	17	40
Linoleic acid	18:2 (n-6)	54	48	9	9	26	19	54	66	1	2	2
Linolenic acid	18:3 (n-3)						10	8		1	1	0.5
Eicosapentaenoic acid	20:5 (n-3)									10	9	
Docosahexaenoic acid	22:6 (n-3)									13	8	

A, maize oil; B, cottonseed oil; C, olive oil; D, palm oil; E, groundnut oil; F, canola oil; G, soybean oil; H, sunflower oil; I, menhaden oil; J, herring oil; K, beef tallow.

most marine fish have a limited ability to convert linolenic acid into EPA and DHA, and thus the dietary sources of these essential fatty acids must be supplied, usually through the addition of marine fish oil (such as menhaden fish oil, cod-liver oil or herring oil) to the diet. The striped bass (*Morone saxatilis*) and its hybrids are examples of fish that require a dietary source of essential EPA and DHA.

Carbohydrates

Carbohydrates are organic compounds composed of carbon, hydrogen and oxygen. They are one of the major classes of nutrients besides proteins and lipids. Carbohydrates are abundant in plants because they are the storage form of energy in plants, in contrast to animals, which store energy as lipid (fat). Carbohydrates typically fall into three main groups: monosaccharides, oligosaccharides and polysaccharides.

Monosaccharides

Monosaccharides are simple sugars and are rarely found in the natural world. Rather, they occur as components of complex carbohydrate molecules. Simple sugars are categorized by the number of carbon atoms in the molecule. A sugar containing three carbons is a triose, four a tetrose, five a pentose and six a hexose. Pentoses and hexoses are the most abundant groups of monosaccharides.

Pentoses

All pentoses have the same chemical formula, $C_5H_{10}O_5$, but each has a slightly different structure. There are three pentose sugars: arabinose, ribose and xylose. Arabinose is found in gums and, when several arabinose molecules are joined together, a pentosan (pentose sugars polymerized into large molecules) is formed. Ribose is a very important pentose sugar because, when it is joined with pyrimidines and purines, nucleosides are formed. Ribose is also a component of riboflavin (a vitamin). Xylose is produced from the breakdown of woody substances and, when several xylose molecules are joined together, the pentosan xylan is formed.

Hexoses

As with the pentoses, all hexoses have the same chemical formula, $C_6H_{12}O_6$, but slightly different structures. There are four hexoses: fructose, galactose, glucose and mannose. In nature, only fructose and glucose occur in free form. Galactose joined with glucose forms the disaccharide lactose (also called milk sugar). Mannose is found in the polysaccharide mannan.

Derivatives of monosaccharides

Monosaccharides that have been reduced by having a hydroxyl group (OH) replace the ketone or aldehyde group (C-O) are known as sugar alcohols. One such sugar alcohol, glycerol, is important in the metabolic pathway, while other sugar alcohols, such as galactitol, inositol, mannitol, sorbitol and xylitol, are present in some foods. Amino sugars have an amino (NH_2) group substituted for a hydroxyl group (OH). Galactosamine and glucosamine are two important members of this group of amino sugars. Sugar acids are a third group of monosaccharide derivatives and these compounds contain an acid group (COOH) in their chemical structure. Some familiar sugar acids are gluconolactone, glucuronic acid and ascorbic acid (vitamin C).

Oligosaccharides

Oligosaccharides contain between two and ten monosaccharides that are chemically bonded together. Most oligosaccharides are the result of catabolism (breakdown) of polysaccharides. There are three categories of oligosaccharides: disaccharides ($C_{12}H_{22}O_{11}$), trisaccharides ($C_{18}H_{32}O_{16}$) and tetrasaccharides ($C_{24}H_{42}O_{21}$).

Disaccharides

Disaccharides are compound sugars comprised of two monosaccharides and include lactose, maltose, sucrose and trehalose. Lactose (milk sugar) and sucrose (table sugar) are two of the most important carbohydrates in human foods; however, they are not important in fish diets as most fish tend to utilize monosaccharides and oligosaccharides poorly.

Trisaccharides

There are three trisaccharides: maltotriose, melezitose and raffinose; however, these are found only in limited quantities in some plants. Maltotriose is a trisaccharide formed during starch digestion and is comprised of three glucose molecules. Melezitose is found in sap from some coniferous plants and contains two molecules of glucose and one fructose molecule. Raffinose is found in sugar beets, molasses, beans and cottonseed meal, and consists of one molecule each of glucose, fructose and galactose.

Tetrasaccharides

There are two sugars in this group: stachyose and maltotetrose. Stachyose is composed of two molecules of galactose, one molecule of glucose and one molecule of fructose. Stachyose and raffinose, two sugars found in beans, cannot be split into monosaccharides by enzymes in the digestive tract of some monogastric animals and must be broken down by microbial action. This results in fermentation, which produces gas in the digestive tract and results in flatulence in many mammals, including humans. Maltotetrose is formed during the digestion of starch and consists of four glucose molecules.

Polysaccharides

Polysaccharides are large sugar complexes that contain repeating chains of monosaccharides. There are three categories of polysaccharides: pentosans $(C_5H_8O_4)_n$, hexosans $(C_6H_{10}O_5)_n$ and mixed polysaccharides.

Pentosans

There are two pentosans, araban and xylan, which yield pentose sugars when hydrolysed. Araban is a chain of arabinose molecules while xylan is a chain of xylose molecules. Both are widely found in plant polysaccharides.

Hexosans

Hexosans are polysaccharide sugars that contain hexose as their repeating sugar units. There are six hexosans: cellulose, starch (amylose and anylopectin), dextrins, glycogen, inulin and mannan. For fish, these are the most important carbohydrate sources, except for cellulose, which is indigestible.

Mixed polysaccharides

There are several mixed polysaccharides found in nature, many of which serve structural or protective roles. These include agar, carrageenan, chitin, hemicelluloses, pectins and gums.

Carbohydrates have several important roles in fish nutrition and are useful in fish diets for numerous contributions. Carbohydrates, like proteins, provide approximately 4.1 kcal of GE g^{-1}. Following digestion and absorption, carbohydrates can be used to meet the immediate energy needs of cells, converted to glycogen and stored in the liver and muscle for later energy needs or converted to fat and stored in adipose tissue for energy reserves. Carbohydrates can spare protein in some fish species by supplying metabolic energy, which spares protein for anabolic purposes, such as tissue synthesis. However, not all carbohydrates yield nutrients that can be utilized by fish. Cellulose, gums, pectins and hemicelluluses are commonly referred to as fibre. Generally, fibre is undesirable in fish diets since it may hinder nutrient absorption and results in the production of diet pellets that have low binding properties and water stability.

Energy is derived from the catabolism of glycogen (or glucose) to pyruvate with the generation of ATP. In the glycolytic pathway, glycogen is converted to glucose-1-phosphate; glucose-6-phosphate; fructose-6-phosphate; fructose-1, 6-diphosphate; glyceraldehyde-3-phosphate; 1, 3-diphosphoglycerate; 3-phosphoglycerate; 2-phosphoglycerate; and ultimately pyruvate. The pyruvate is then converted to ATP in the citric acid (TCA) cycle. Steps in the TCA cycle are pyruvate to citrate, isocitrate, oxalosuccinate, α-ketoglutarate, succinyl-CoA, succinate, fumarate, malate and oxaloacetate. When 2 mol of pyruvate are oxidized, 30 mol of ATP are produced. These 30 mol of ATP are added to the 6–8 mol of ATP produced in the glycolytic pathway. Since each mole of ATP yields 8 kcal, approximately 300 kcal are produced from oxidation of each mole of glucose.

Some carbohydrates and their derivatives have special functions. Glucuronic acid detoxifies chemicals and bacterial by-products; heparin prevents blood clotting; and carbohydrates that bind to lipids occur in nerve tissues.

Vitamins and Minerals

Vitamins

Vitamins are organic substances that are essential for growth, health, reproduction and maintenance, but required in small amounts. Since fish cannot synthesize vitamins at all or can only synthesize in insufficient quantity for normal development, growth and maintenance, they must be supplied in the diet. Each vitamin performs a specific function in the body and one vitamin cannot substitute for or replace another vitamin. Vitamins can be classified into two groups: fat-soluble and water-soluble. The fat-soluble vitamins include vitamin A, vitamin D, vitamin E and vitamin K; water-soluble vitamins consist of biotin, choline, folic acid, niacin, pantothenic acid (B_3), riboflavin (B_2), thiamine (B_1), pyridoxine (B_6), cyanocobalamin (B_{12}), and vitamin C (ascorbic acid). The fat-soluble vitamins contain carbon, hydrogen and oxygen, while water-soluble vitamins contain these three elements plus nitrogen, and cyanocobalamin contains cobalt. Fat-soluble vitamins are absorbed from the intestinal tract along with lipids, and any condition or factor that increases lipid absorption will increase the absorption of fat-soluble vitamins. Absorption of water-soluble vitamins is simpler because water is constantly absorbed from the intestine into the bloodstream.

Water-soluble vitamins are generally not stored in the body and excess vitamins are excreted; however, excess fat-soluble vitamins can be stored in the body. Excesses of fat-soluble vitamins in fish diets may cause physiological or health problems. Thus, adding excess amounts of vitamins not only may be financially wasteful, but may compromise the well-being of the fish.

Not all the vitamins present in diet ingredients are in available forms. For instance, niacin in many cereal grains is bound to protein and cannot be absorbed unless the ingredient is treated with an alkali. Further, vitamins are destroyed during diet processing and storage, due to heating, oxidation, sunlight or mould growth. Thus, some vitamins may need to be added to the diet in excess of requirements due to the anticipated losses during diet processing, production and storage. The requirements and deficiency signs for various vitamins will not be presented in this chapter as this information is provided in detail in the following chapters.

Vitamin A

Vitamin A can exist in several forms as vitamin A alcohol, vitamin A aldehyde and vitamin A acid. The alcohol is the most common form and is generally referred to as retinol; the aldehyde is called retinal or retinene; the acid is called retinoic acid. Retinol (traditionally called vitamin A) is an ester (retinyl palmitate) and is biologially active as an alcohol, or aldehyde, and an acid (Fig. 1.3). Dehydroretinol, also called vitamin A_2, differs from retinol in that it has an extra double bond and has only 40% of the biological activity.

Vitamin A is a fairly colourless fat-soluble vitamin. It is insoluble in water and thus almost no absorption of vitamin A occurs in the stomach. In the intestine, vitamin A is emulsified with bile salts and absorbed into the intestinal mucosa. Vitamin A is essential for a number of physiological processes: vision, growth, reproduction, and coenzyme and hormone roles. The best understood role of vitamin A is related to vision. When light hits the retina, the pigment rhodopsin (which contains vitamin A) is converted to another pigment, called retinaldehyde. As a result of this change, images are sent to the brain via the optic nerve. Rhodopsin is reconverted in the dark, but some vitamin A is lost in the reaction and vision would be impaired if sufficient vitamin A were not supplied in the diet or by body stores to replace that which is lost.

Vitamin D

Vitamin D consists of approximately ten different sterol compounds with vitamin activity; however, only two are of practical importance: ergocalciferol (vitamin D_2 or calciferol) (Fig. 1.4) and cholecalciferol (vitamin D_3) (Fig. 1.5). Cholecalciferol is the form added to fish diets. This is transported by a specific vitamin D carrier protein to the liver, where it is converted to 25-hydroxycholecalciferol (25-OH-D_3) and is eventually converted to 1, 25-(OH_2)-D_3. This compound is then transported and stored in fatty tissues, skeletal muscles and bones. Vitamin D is associated with calcium and phosphorus metabolism, influencing the absorption of these minerals and their deposition in bones; vitamin D increases calcium and phosphorus from the intestine. However, excessive dietary amounts of vitamin D can lead to hypercalcaemia and can cause reduced growth and abnormal deposition of calcium in soft tissues (such as the heart and blood-vessels) which

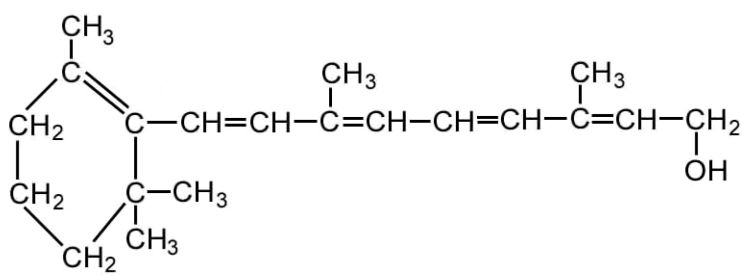


Fig. 1.3. Retinol (vitamin A_1).

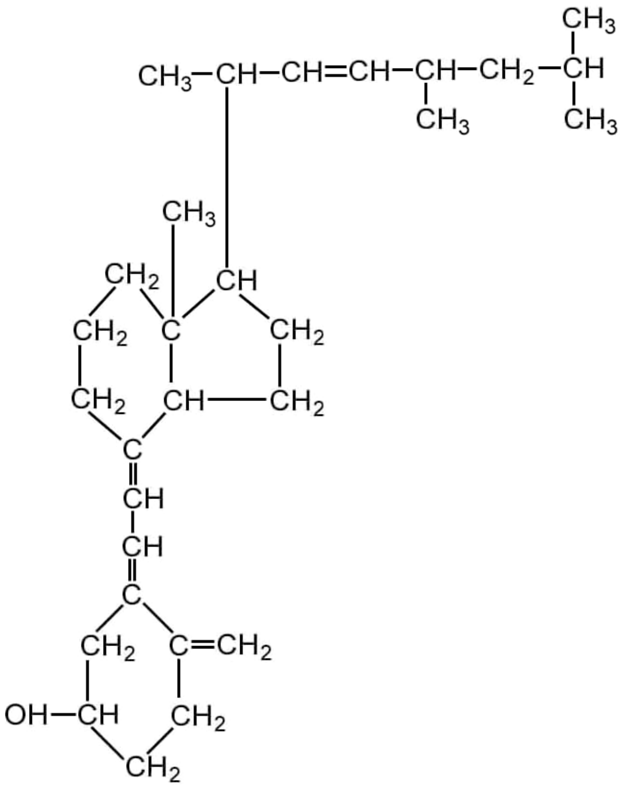


Fig. 1.4. Vitamin D_2.

may result in these tissues calcifying so that their normal functioning is impaired or adversely affected.

Vitamin E

There are eight tocopherols and tocotrienols that have been identified as having vitamin E activity and all are called vitamin E. The molecule with the most vitamin E activity is α-tocopherol (Fig. 1.6). Absorption takes place in the intestine and the presence of bile and lipid is required for proper absorption. Once absorbed, vitamin E is transported to and stored in adipose tissue, liver and muscle. The tocopherols and tocotrienols are soluble in alcohol and lipid solvents, but, as with the other fat-soluble vitamins, are insoluble in water. Vitamin E is stable to heat, but destroyed by exposure to oxygen, iron, copper and ultraviolet light. The primary function of vitamin E is to help protect cell structure, intracellular components and enzymes from degradation. It is a powerful antioxidant that not only prevents the rancidity of lipids in the diet and the fish's digestive tract, but also interrupts the oxidation of highly unsaturated fatty acids in tissue membranes, especially cellular membranes. In an animal's body, vitamin E also protects vitamin A, carotene, vitamin C and ATP from being oxidized. Vitamin E also works with selenium to protect cell membranes.

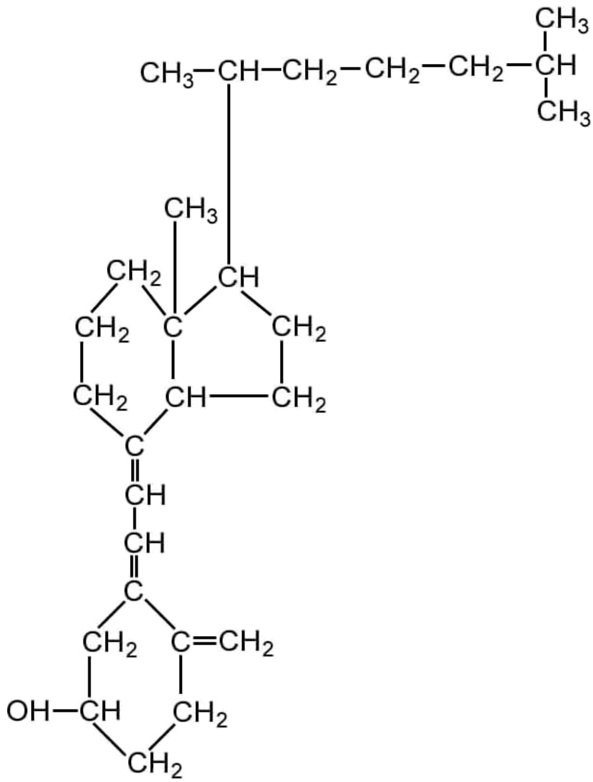


Fig. 1.5. Vitamin D_3.

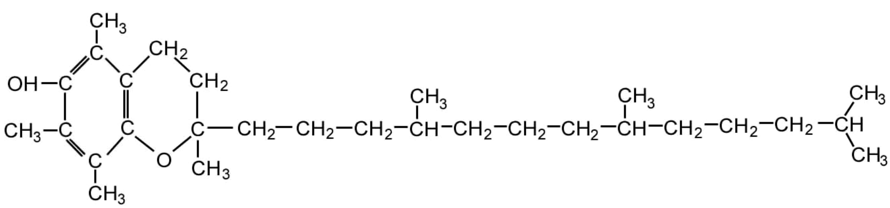


Fig. 1.6. Vitamin E (α-tocopherol).

Vitamin K

There are two naturally occurring forms of vitamin K: vitamin K_1 (phylloquinone), which occurs in green plants, and K_2 (menaquinone), which is synthesized by microorganisms. There are also several synthetic compounds that possess vitamin K activity; the best known and commonly utilized vitamin K is menadione (zimethyl-1, 1, 4-naphthoquinone), also known as K_3 (Fig. 1.7). Menadione is converted to vitamin K_2 in the body and is two to three times more potent than either K_1 or K_2. Vitamin K is absorbed from the intestine and, since it is fat-soluble, it requires bile for maximum absorption. Vitamins K_1 and K_2 are stored only in small amounts in the liver and, unlike the other fat-soluble vitamins, any excess can be excreted.

Biotin

Biotin is a sulphur-containing vitamin and is a cyclic derivative of urea with an attached thiophene ring (Fig. 1.8). Biotin is absorbed from the intestine, but, if avidin, a protein in raw egg-white, is present, absorption is prevented because avidin binds the biotin. Heating egg-whites inactivates the avidin, thus eliminating its ability to bind to biotin. Some fish, such as tilapia, have bacteria in the

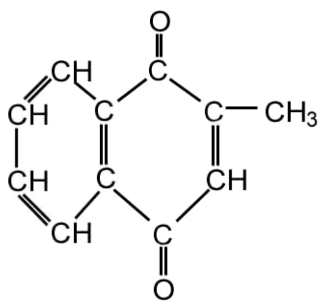


Fig. 1.7. Menadione (vitamin K_3).

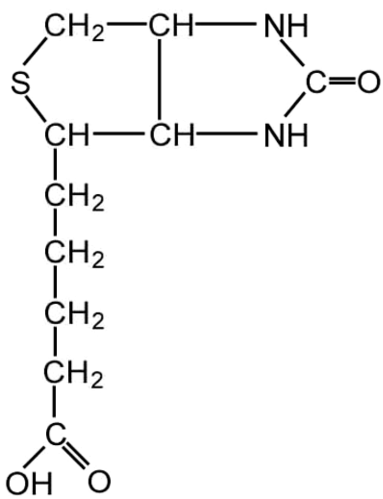


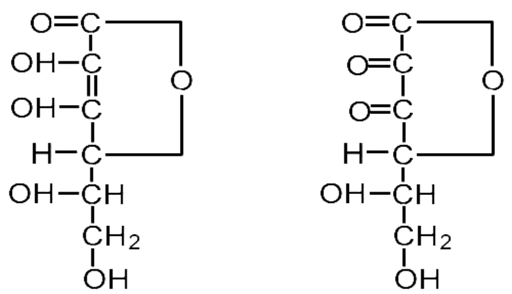
Fig. 1.8. Biotin.

intestine that can synthesize biotin, but even this synthesis may occur too late in the intestine for it to be maximally absorbed. Thus, a dietary source of biotin should be added to fish diets to meet their dietary requirements. Biotin is a coenzyme for transferring CO_2 from one compound to another (decarboxylation and carboxylation). These reactions are involved in carbohydrate, lipid and protein metabolism and include the interconversion of pyruvate and oxaloacetate, the interconversion of succinate and propionate and the conversion of malate to pyruvate.

Vitamin C

Vitamin C, also known as ascorbic acid, is closely related in structure to the monosaccharide sugars. It is synthesized from glucose and other simple sugars by plants and many animal species. However, humans, monkeys, guinea-pigs, fruit-eating bats and teleost fish cannot convert glucose to ascorbic acid because they lack the enzyme L-gulonolactone oxidase. Thus, a dietary source of vitamin C must be provided to meet their requirement. Two forms of vitamin C occur in the body: ascorbic acid and dehydroascorbic acid (Fig. 1.9). While most of the vitamin C exists as ascorbic acid, it can be oxidized to dehydroascorbic acid. Vitamin C is readily absorbed in the intestine and is taken up by several tissues, including the retina of the eye, liver, spleen, brain and kidney. Oxidation of vitamin C is accelerated by air, light, heat, copper and iron. Thus, when adding ascorbic acid to fish diets, processing losses must be factored in so that extra vitamin C is added to compensate for processing losses.

Vitamin C is important in the formation and maintenance of collagen; metabolism of the amino acids proline, lysine, tyrosine and tryptophan; absorption and transport of iron; metabolism of lipids and cholesterol; as an antioxidant in the protection of vitamins A and E and highly unsaturated fatty acids; in the development of strong bones; and in the metabolism of folic acid.



Ascorbic acid **Dehydroascorbic acid**

Fig. 1.9. Ascorbic acid (vitamin C).

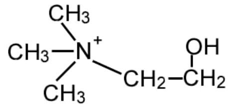


Fig. 1.10. Choline.

Choline

Choline ($C_5H_{15}NO_2$) is a simple molecule with three methyl (CH_3) groups joined to a nitrogen (Fig. 1.10). Choline is absorbed in the small intestine and is water-soluble. It is extremely hygroscopic (absorbs water when exposed to air) and forms stable crystalline salts, such as choline chloride. It is fairly stable to heat, but unstable in strong alkali. Choline is a constituent of phospholipids (primarily lecithin) and prevents fatty livers through the transport of lipids. It is also a component of acetylcholine and is important in nerve transmission, and it acts as a methyl donor.

Cyanocobalamin

Cyanocobalamin, also called vitamin B_{12}, is the largest and most complex of all the vitamins in terms of molecular structure (Fig. 1.11). The main component of the molecule is a porphyrin ring containing cobalt. A cyanide (CN) group may be attached to the cobalt, producing the compound cyanocobalamin, which is the commercially available form of the vitamin. The cyanide group can be replaced by a hydroxyl group (OH), giving hydroxycobalamin (the naturally occurring form of the vitamin) and can be replaced by a nitrite group (NO_2) producing nitritocobalamin. Vitamin B_{12} is absorbed in the intestine and is stable to heat but destroyed by light and strong acid or alkaline solutions.

In fish, vitamin B_{12} is converted to one of two active coenzyme forms: coenzyme B_{12} (adenosylcobalamin) or methyl B_{12} (methylcobalamin). Coenzyme B_{12} has an adenosine ribonucleoside attached to the cobalt atom in place of the cyanide group. Vitamin B_{12} is essential for blood formation and the synthesis of myelin (a lipoprotein of the nervous system), is required for normal carbohydrate and lipid metabolism and serves as a coenzyme in the biosynthesis of methyl groups (CH_3).

Folic acid (folacin)

There is no single vitamin with the name ‘folacin’, but the term is used to describe a group of closely related substances having the biological activity of folic acid (Fig. 1.12). Folic acid is absorbed by active transport and by diffusion in the intestine and is slightly soluble in water, unstable to heat and destroyed by exposure to light. After it has been absorbed, folic acid is changed into several active coenzyme forms, including tetrahydrofolic acid. These coenzymes transfer single carbon units from one compound to another and are involved in the formation of haem (the protein containing iron in haemoglobin), formation of tyrosine from

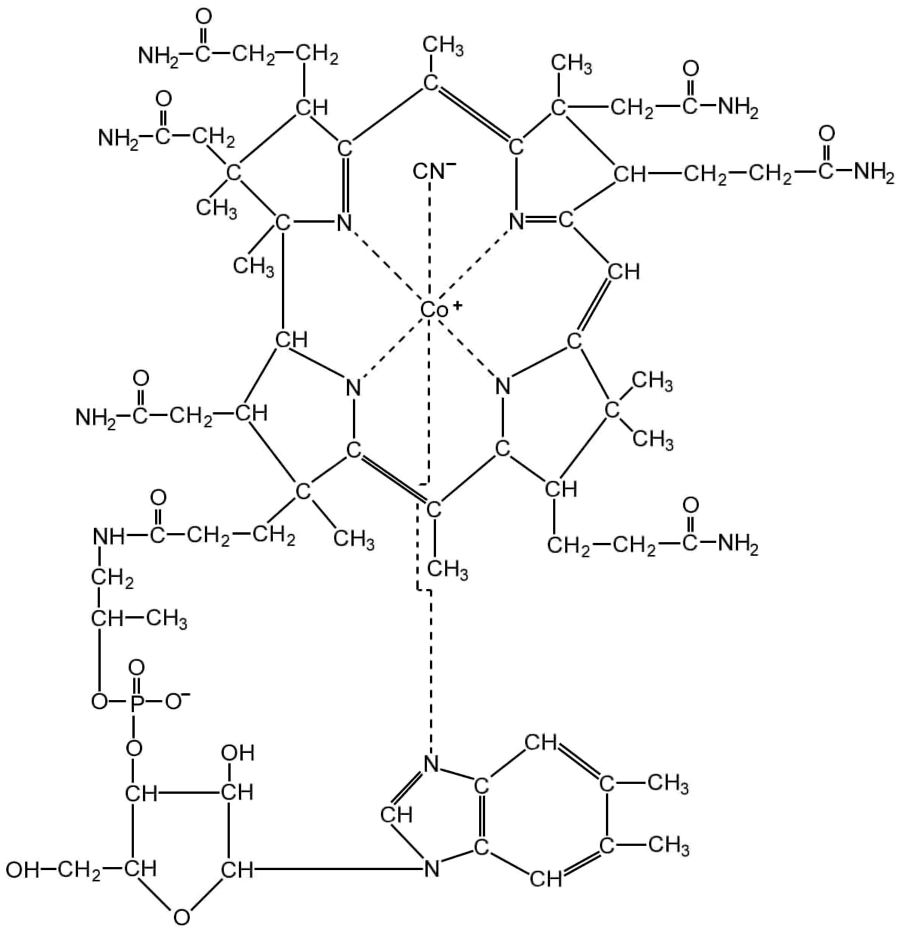


Fig. 1.11. Vitamin B_{12}.

phenylalanine and glutamic acid from histidine, synthesis of choline from ethanolamine and formation of methionine from homocysteine.

Niacin

Niacin is a term used to include several B-complex vitamins, including nicotinic acid and nicotinamide (Fig. 1.13). Both of these natural forms have equal niacin activity. Both nicotinic acid and nicotinamide are derivatives of pyrimidine. Niacin is readily absorbed from the intestine and transported to the liver, where it is converted to nicotinamide adenine dinucleotide (NAD). Niacin is found in the body tissues as either NAD or NAD phosphate (NADP). Nicotinic acid and nicotinamide are soluble in water and are not destroyed by light, oxidation or heat. The principal role of niacin is as a component of NAD and NADP. These coenzymes function in systems necessary for cell respiration. Along with the riboflavin and thiamine-containing coenzymes, NAD and NADP serve as hydrogen acceptors and donors in oxidation–reduction reactions that release energy. Both NAD and NADP are also included in fatty acid synthesis, protein synthesis and DNA synthesis.

Pantothenic acid

Pantothenic acid, also known as vitamin B_3, is composed of pantoic acid and β-alanine (Fig. 1.14). Like other B vitamins, pantothenic acid is readily absorbed from the intestine and is transported to body tissues, with high concentrations in the liver. Calcium pantothenate, the commercially available form of the vitamin, is water-soluble and very stable. Pantothenic acid is a component of two enzymes: CoA and acyl carrier protein (ACP). CoA and ACP are required by cells to synthesize fatty acids. Pantothenic acid (as a part of CoA) is also required in the citric acid cycle (TCA cycle, also called the Krebs cycle), for the formation of acetylcholine and in the metabolism of proteins, lipids and carbohydrates.

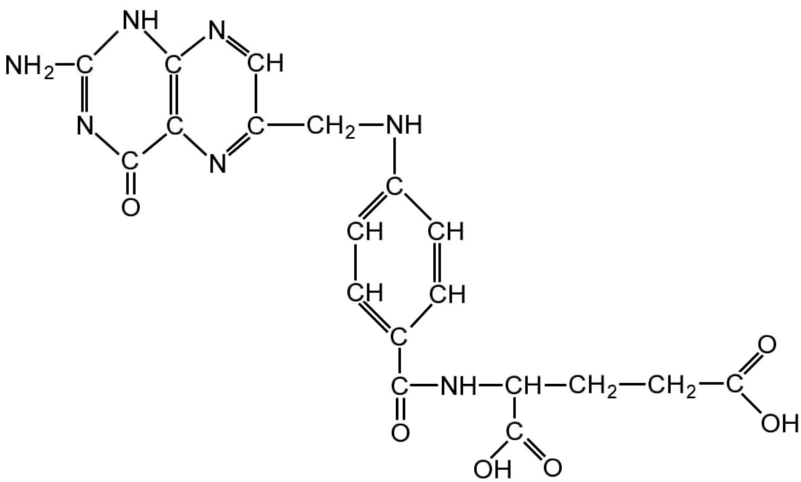


Fig. 1.12. Folic acid.

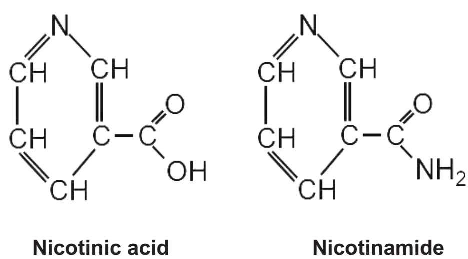


Fig. 1.13. Niacin.

Pyridoxine

Pyridoxine, also known as vitamin B_6, is found in three readily interconvertible forms: pyridoxine (Fig. 1.15), pyridoxal phosphate and pyridoxamine phosphate. In the free form, pyridoxine is absorbed from the intestine and is readily soluble in water and stable to heat, but is destroyed by oxidation and ultraviolet light. In its coenzyme forms (usually as pyridoxal phosphate or pyridoxamine phosphate), it is involved in a large number of physiological processes, especially protein metabolism, but also lipid and carbohydrate metabolism. In protein metabolism, pyridoxine is involved in: (i) shifting an amino group (NH_2) from a donor amino acid to an acceptor acid so that another amino acid can be formed; this process is called transamination; (ii) removal of the carboxyl groups (COOH) (decarboxylation) from certain amino acids to form other compounds, such as serotonin, noradrenalin and histamine; and (iii) assisting in the removal of amino groups from amino acids not needed for growth (deamination) so that carbon residues can be utilized for energy.

Riboflavin

Riboflavin, also called vitamin B_2, is composed of an alloxine ring that is linked to an alcohol derivative of ribose (Fig. 1.16). Riboflavin is absorbed in the intestine by tissue diffusion. It is not very soluble in water and is heat-stable in neutral and acid solutions, but is destroyed by light (especially ultraviolet light). Riboflavin functions as a part of the enzyme group called flavoproteins. Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are two coenzymes in several important oxidation–reduction reactions by which energy is released to the cell. Riboflavin is involved in fatty acid, amino acid and carbohydrate metabolism. Riboflavin and pyridoxine are also required to produce niacin from tryptophan.

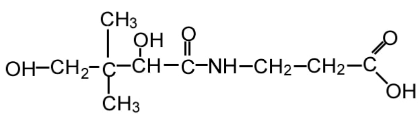


Fig. 1.15. Pantothenic acid.

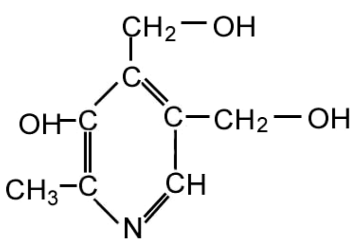


Fig. 1.14. Pyridoxine.

Thiamine

Thiamine, also called vitamin B_1, was the first of the B-complex vitamins to be isolated in pure form (hence the designation B_1). Thiamine (Fig. 1.17) is composed of carbon, hydrogen, oxygen, nitrogen and sulphur and consists of a molecule of pyrimidine (pyrimidine ring) and a molecule of thiazole (thiazole ring) connected by a methylene (CH_2) bridge. Thiamine is water-soluble and heat-stable in acid solutions, but auto-oxidation and ultraviolet light destroy it. Thiaminase, an enzyme present in the raw flesh of some species of fish, also destroys thiamine, so fish diets should not be formulated to contain large amounts of raw fish unless extra thiamine is added. Thiamine is essential as a coenzyme (thiamine diphosphate) in the conversion of pyruvic acid and the formulation of acetyl CoA. Thiamine diphosphate is also a coenzyme in the conversion of glucose to lipid (transketolation).

Minerals

A mineral is an element found in ash when a food or body tissue is burnt. Minerals are classified into two groups based on the relative amounts needed in the diet: macrominerals, elements that are required in large amounts, and microminerals or trace minerals, elements that are required in very small amounts. The general functions of minerals include: structural components of the skeletal system (such as calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), and potassium

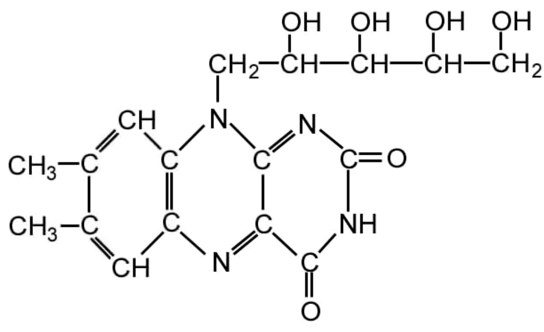


Fig. 1.16. Riboflavin.

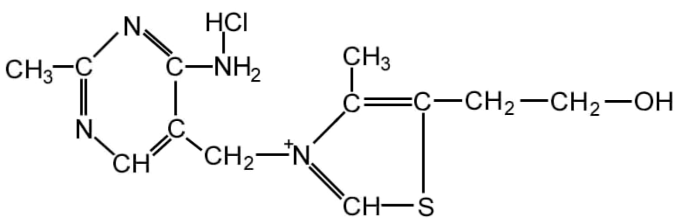


Fig. 1.17. Thiamine.

(K)), components of organic compounds (such as proteins and lipids), enzyme-system activators (such as zinc (Zn) and copper (Cu)) and maintaining acid–base and osmotic balances (such as Na, K and chloride (Cl)).

Macrominerals

CALCIUM The major function of calcium is to give strength to bones, but it is also important in controlling the heartbeat, used in the transmission of nerve impulses and necessary for blood clotting, and calcium controls various enzymes. Calcium is absorbed in the intestine and several factors enhance calcium uptake by the body, including vitamin D, dietary protein and an acid medium. Conversely, a vitamin D deficiency reduces the absorption of calcium, as does an excess of dietary phosphorus, excessive lipid or fibre in the diet and the presence of phytic acid. Phytic acid, which is found in cereal grains and oil-seed meals, prevents the absorption of calcium by forming an insoluble salt (calcium phytate).

CHLORIDE Chloride is an essential mineral because it is an electrolyte and is found in extracellular fluids in the body. The chloride content of the blood is higher than that of any other mineral and the negatively charged chloride ion plays a major role in the regulation of osmotic pressure and acid–base balance.

MAGNESIUM Nearly 70% of magnesium in the body is located in bones, while approximately 30% is found in various fluids and soft tissues (such as the liver and muscle). Magnesium is a constituent of bones and teeth; it is essential for cellular metabolism, often as an enzyme activator involved in high-energy phosphate transfer of adenosine diphosphate (ADP) and ATP; and it is involved in certain peptidase activations for protein digestion. If not enough magnesium is added to a diet, calcium could be deposited in soft tissues, forming calcified lesions, while excess of magnesium may disrupt calcium and phosphorus metabolism.

PHOSPHORUS Phosphorus is readily absorbed by fish from the water via the gills so a dietary supply of phosphorus is theoretically not needed. However, since phosphorus is limiting in aquatic systems, being taken up by plants and algae, a dietary source is needed for proper fish growth and well-being. Phosphorus is essential for bone formation and maintenance, for muscle-tissue function, as a component of nucleic acids, maintaining osmotic and acid–base balances, phospholipid synthesis, protein formation and enzyme systems. Phosphorus is involved in several chemical relationships. If the diet contains an excess of calcium relative to phosphorus, free calcium will be present and form insoluble tricalcium phosphate. An excess of dietary phosphorus over calcium will decrease the absorption of both calcium and phosphorus. Further, excesses of iron, aluminium and magnesium may bind phosphorus to form insoluble salts, which inhibit phosphorus absorption.

Much of the phosphorus in many plant-protein sources (such as soybean meal) and plant ingredients is in the form of phytate, which is poorly utilized and may depress the absorption of iron and calcium. Highly digestible sources of phosphorus, such as monocalcium and dicalcium phosphate, are often added to fish diets to meet the phosphorus requirement of fish.

POTASSIUM Potassium is the third most abundant element in the body, after calcium and phosphorus. Potassium and sodium are closely interrelated in the maintenance of proper osmotic pressure within cells, and these minerals are involved in the maintenance of proper acid–base balance. Potassium ions relax muscles and are used in enzyme reactions. Excessive levels of potassium could interfere with magnesium absorption.

SODIUM Sodium is the major positively charged ion (cation) in the fluid outside the cell (extracellular fluid), where it assists in the maintenance of osmotic and acid–base balance. Sodium is also associated with muscle contraction, nerve function and carbohydrate absorption. Sodium, potassium and chlorine (chloride) all control osmotic pressure and the acid–base equilibrium.

Microminerals

CHROMIUM Chromium is an essential element, but it does not work alone; instead it acts with other substances to control metabolism, such as insulin, several enzymes and DNA and RNA. Chromium, in the form of glucose tolerance factor (GTF), is released into the blood whenever there is a dramatic increase in the glucose and/or decrease in insulin levels in the blood. GTF and insulin both act to allow amino acids, fatty acids and sugars to pass more easily from the blood into the cells of tissues. Chromium, as well as other minerals, can also activate the digestive protease trypsin.

COBALT Cobalt is a component of vitamin B_{12} (cyanocobalamin), which has an essential function in red blood cell formation.

COPPER Copper is a cofactor for several enzyme systems used in energy metabolism, and is required for normal functioning of the brain, skeleton and spinal cord. Copper is also involved in iron metabolism, as it facilitates the absorption of iron from the intestine and releases iron from storage in the liver. However, excesses of cadmium, iron, lead and zinc reduce the utilization of copper. Copper in the diet is relatively non-toxic to most monogastric species; however, fish are very susceptible to copper toxicity if exposed to copper in the water.

IODINE Iodine is an essential component of the thyroid hormones, thyroxine and tri-iodothyronine, which regulate the rate of oxidation with cells. In so doing, the thyroid influences physical growth, nerve- and muscle-tissue functions, circulatory activity and metabolism. Some aquaculture diet ingredients, such as canola meal and mustard seed, can contain goitrogens, which interfere with the production of thyroxine and may produce goitre.

IRON Iron is an essential mineral in the formation of red blood cells. Iron combines with protein to make haemoglobin, the iron-containing compound of red blood cells. Iron is involved in transporting oxygen via red blood cells and is also a component of enzymes that are utilized in energy metabolism. A deficiency of iron may cause microcytic anaemia, characterized by small, pale red blood cells.

An excess of iron in the diet can bind with phosphorus to form an insoluble iron–phosphate complex, which could lead to a phosphorus deficiency. Since free iron is toxic, the iron molecule is transported along with a protein. Two atoms of ferric iron are bound to one molecule of transferrin, a β-globulin protein. If the level of iron ions exceeds the binding capacity of transferrin, iron toxaemia may occur.

MANGANESE Manganese is involved in the formation of bone, blood clotting, insulin function and cholesterol synthesis, and is an activator in several enzymes in the metabolism of proteins, lipids, carbohydrates and nucleic acids.

SELENIUM Selenium is a rare, non-metallic element that appears to be very important in maintaining the health of animals under stressful conditions. Vitamin E and the amino acids cysteine and methionine may act as partial substitutes for selenium in some of its functions. The biological availability of selenium from different sources varies. Selenium in wheat appears to be 100% available; however, only about 33–50% is available in fish-meal, possibly due to selenium being bound to mercury and other heavy metals. Fish diets with high percentages of polyunsaturated fatty acids (PUFA) but reduced levels of vitamin E might increase the requirement for selenium, because PUFA may be converted to toxic peroxides unless there is sufficient vitamin E to prevent this process, and selenium is required to activate the enzyme glutathione peroxidase, which destroys peroxides. Thus, like vitamin E, selenium acts as an antioxidant to protect the cell against oxidative damage. However, these antioxidants act differently; selenium functions throughout the cytoplasm to destroy peroxides, while vitamin E is present in the membrane constituents of the cell and prevents peroxide formation. Thus, the requirements for each nutrient can only be partially offset by addition of the other. Further, when diets are deficient in the amino acids methionine and cysteine, the selenium requirement may increase. This is due to two sulphur amino acids being converted to glutathione, which has a limited ability to carry out the functions of glutathione peroxidase.

ZINC Zinc is poorly absorbed, with approximately 10% of zinc being taken up by body tissues. Further, excesses of calcium, phytic acid or copper inhibit zinc absorption. Zinc is required for normal bone calcification, in the transfer of carbon dioxide in red blood cells, and for the synthesis and metabolism of proteins and nucleic acids.