

2002

# The essential nutrients: Vitamins

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# VITAMINS

## Introduction

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

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

## Classification of Vitamins

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

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

### ❑ Water-soluble vitamins

Among the group of water-soluble vitamins are included the B complex vitamins, thiamin or B<sub>1</sub>, riboflavin or B<sub>2</sub>, pantothenic acid or B<sub>5</sub>, pyridoxine or B<sub>6</sub>, and cyanocobalamin or B<sub>12</sub>, biotin, niacin, choline, inositol, folic acid, and ascorbic acid or vitamin C.

**Thiamin (Vitamin B<sub>1</sub>).** Thiamin is a complex substance that contains sulfur, which gives it a characteristic pungent odor. It plays an active role in carbohydrate metabolism as the coenzyme, thiamin pyrophosphate. Of particular importance is the role of thiamin in the conversion of

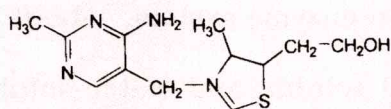
**Figure 2.11**

Thiamin (vitamin B<sub>1</sub>) deficiency in humpback grouper. Mechanical injuries with hemorrhages on the body surface, pectoral fins and abdomen, and erratic swimming behavior are signs of vitamin B<sub>1</sub>-deficient diet

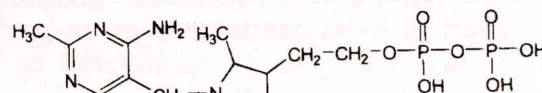
Source: Koesharyani et al. 2001

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

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

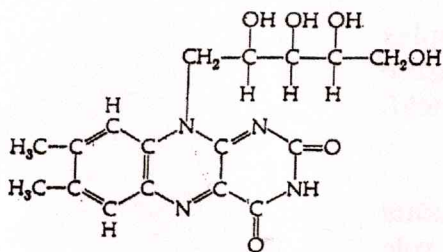


thiamin

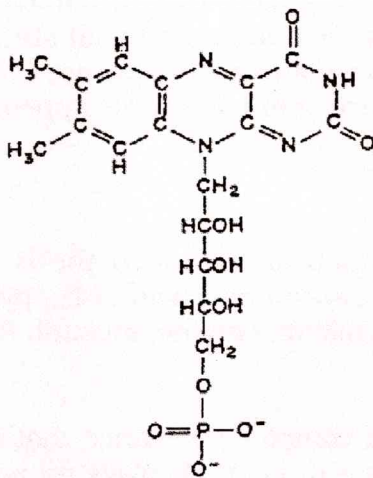


thiamin pyrophosphate

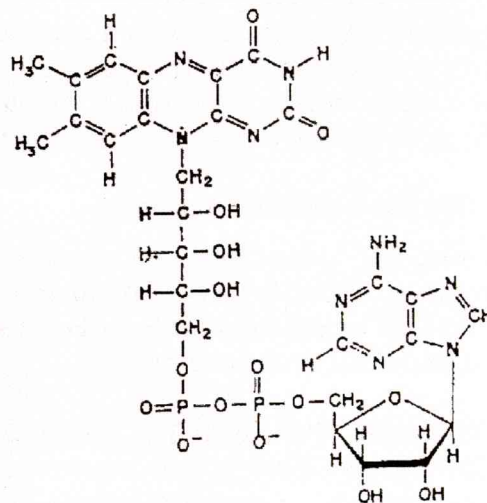
**Riboflavin (Vitamin B<sub>2</sub>).** Riboflavin is an orange pigmented molecule. It is involved in energy metabolism in the form of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes facilitate the breakdown of energy-yielding nutrients such as amino acids, fatty acids, and pyruvic acid. Riboflavin is relatively more stable to heat than thiamin but is destroyed by light and in strongly alkaline or acidic solutions. Ingredients and rations should be stored in dark bags or non-transparent tight containers and protected from



riboflavin



flavin mononucleotide (FMN)



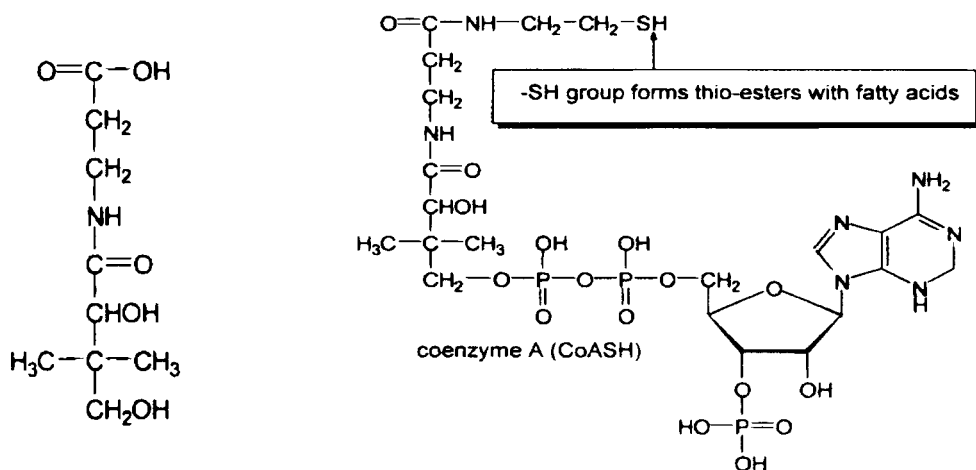
flavin adenine dinucleotide (FAD)

light to prevent loss of riboflavin activity in the feed.

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

**Pantothenic Acid (Vitamin B<sub>5</sub>).** Pantothenic acid is an organic acid that contains nitrogen. It plays an essential role in protein, lipid, and carbohydrate metabolism as a component of the coenzymes, acetyl coenzyme A, and acyl carrier protein. These coenzymes are involved in the synthesis of fatty acids, cholesterol, steroid hormones, phospholipids, and hemoglobin. The sodium or calcium salt of pantothenic acid is relatively stable and can be incorporated into either dry or moist fish diets. It is stable in neutral solution but is destroyed by heat at either alkaline or acid pH. In diet preparation, some loss is incurred during autoclaving and excessive heat.

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

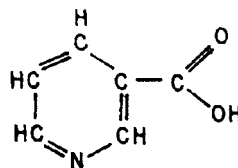


pantothenic acid

coenzyme A

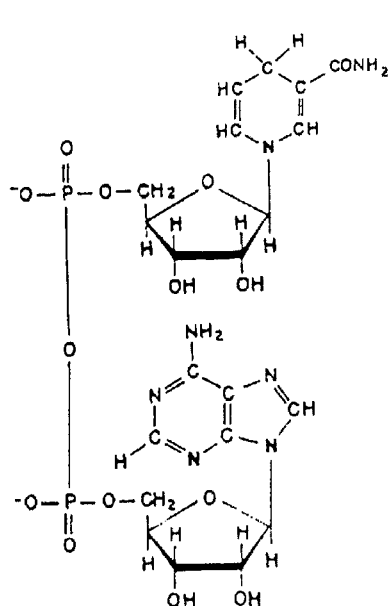
**Niacin (Nicotinic acid).** Niacin or nicotinic acid plays an essential role in the metabolism of carbohydrates, lipids, and proteins, as a component of two high energy molecules, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). NADH and NADPH are important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

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

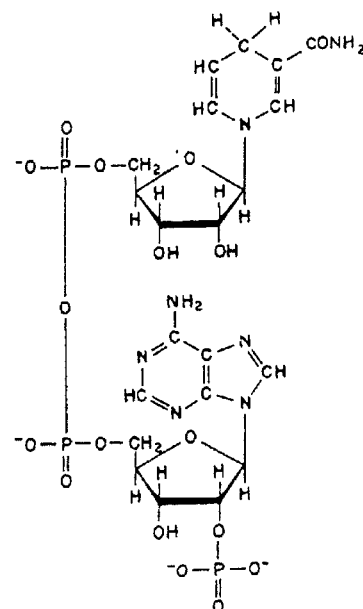


nicotinic acid

**Pyridoxine (Vitamin B<sub>6</sub>).** Pyridoxine is essential in protein metabolism as the



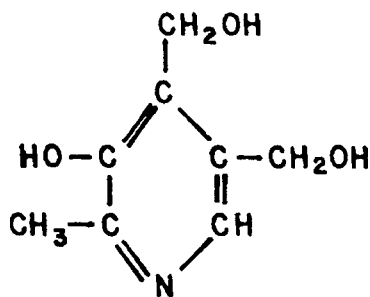
NADH



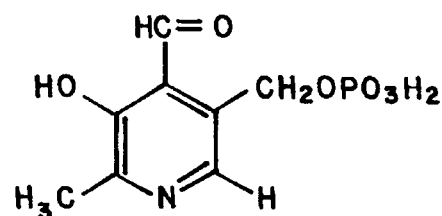
NADPH

coenzyme, pyridoxal phosphate, which is involved in the non-oxidative degradation of amino acids, including transamination, deamination and decarboxylation. The availability of vitamin B<sub>6</sub> in foods can be substantially reduced by processing. It is susceptible to destruction by light in neutral and alkaline solutions hence diet ingredients should be protected from exposure to sunlight.

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



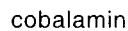
pyridoxine



pyridoxal phosphate

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

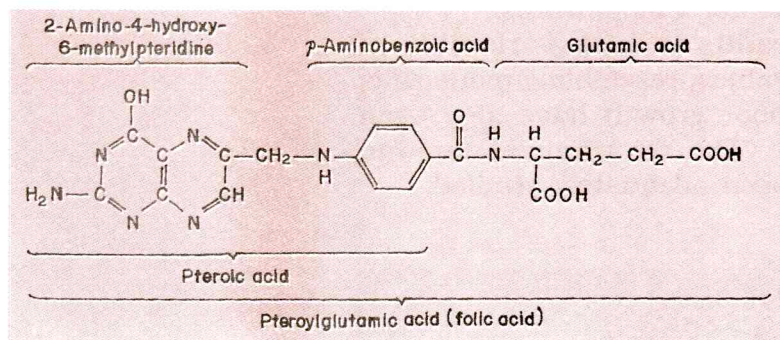
In salmonids, vitamin B<sub>12</sub> deficiency results in high variability of fragmented erythrocytes and in hemoglobin values, resembling monocytic, hypochromic anemia. Poor appetite and poor growth have also been observed in cobalamin deficiency in fish. The requirement for and metabolism of vitamin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



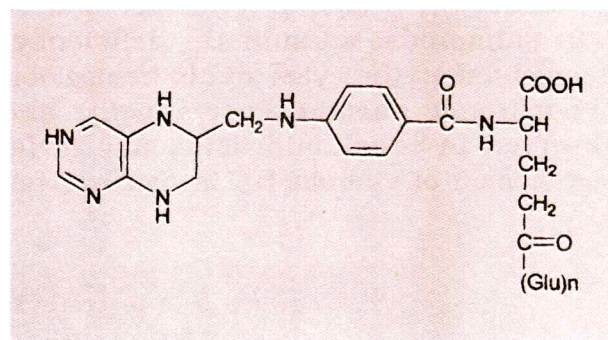


**Folic Acid (Folacin, Pteroylglutamic acid).** Folic acid is important in protein metabolism as a component of the coenzyme tetrahydrofolic acid. This coenzyme is needed for the synthesis of hemoglobin, glycine, methionine, choline, and purines. High temperature or prolonged heating as well as acid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

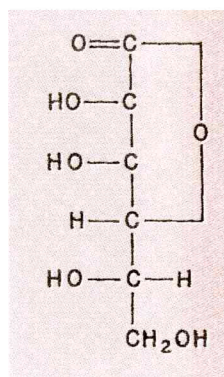
Macrocytic anemia occurs in the red blood cell-producing tissue of the anterior kidney in fish. Other signs that have been observed are poor growth, lethargy, blindness, melanism and dark skin coloration, and fragile fins.



folic acid



tetrahydrofolic acid



Ascorbic acid

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

**Figure 2.12**

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

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



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

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

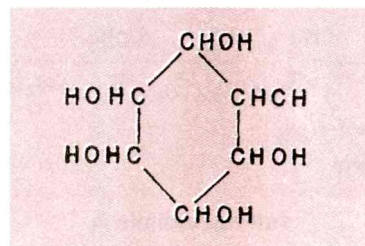
**Choline.** Choline is important as component of acetylcholine and the phospholipids, lecithin, and sphingomyelin. As such, it plays a vital role in the maintenance of cell structure and transmission of nerve impulses. It is also important in lipid transport within the body. Choline is widely distributed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

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

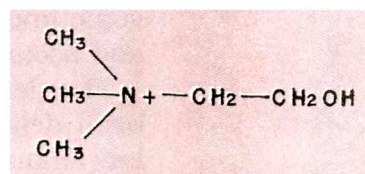
### □ Lipid-soluble vitamins

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

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



myoinositol



choline

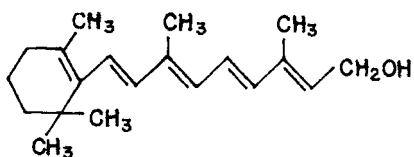


**Figure 2.13**

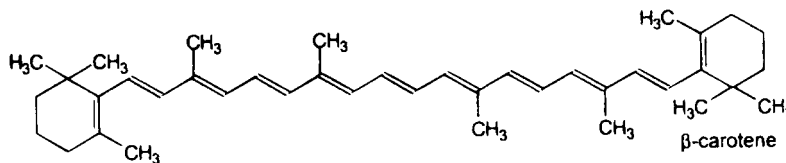
Operculum deformity in humpback grouper is generally caused by a deficiency in Vitamin A.

Source: Koesharyani et al. 2001





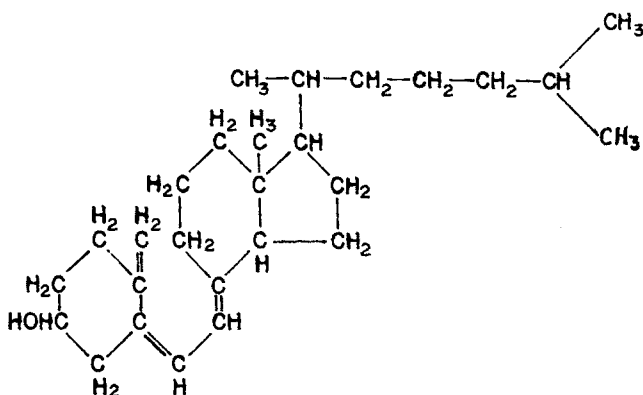
retinol, vitamin A

 $\beta$ -carotene

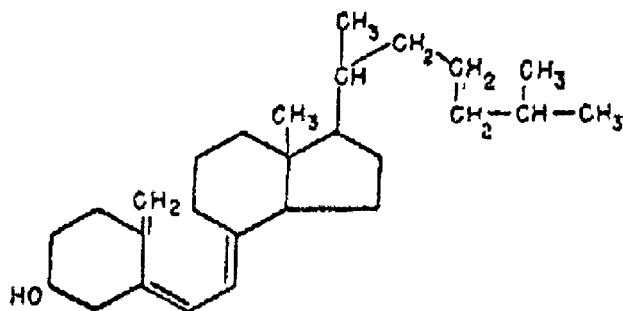
**Cholecalciferol (Vitamin D).** There are two active forms of vitamin D, ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol or vitamin D<sub>3</sub>. Vitamin D plays an important role in the proper use of calcium and phosphorus to form bones and teeth. It promotes normal bone formation or mineralization. Cholecalciferol can be synthesized in the skin by sunlight irradiation of 7-dehydrocholesterol which is found under the skin. It is sometimes called the sunshine vitamin. Since the vitamin is fat soluble and accumulates in lipid stores, fish liver oil is a rich source of vitamin D.

The deficiency symptoms of rickets and abnormal bone formation has been described in fish fed a low vitamin D diet in water that contains low amounts of calcium. Poor growth, and tetany of white skeletal muscle have also been reported. Fish with hypervitaminosis D exhibit impaired

growth, lethargy, and dark coloration. High doses of the vitamin mobilizes calcium and phosphate and may lead to fragile bones.



cholecalciferol, vitamin D

 $\alpha$ -tocopherol, the most active form of vitamin E

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

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

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

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

#### **Vitamin Requirements of Fish**

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

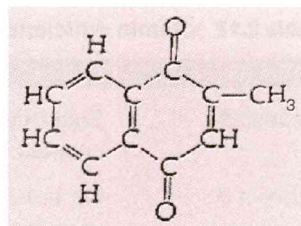
The requirement for a vitamin may be affected by various factors such as species, size or age, dietary nutrient levels, diet composition, physical properties of diet, and culture conditions. For instance, the requirement for vitamin E may increase as the polyunsaturated fatty acid level in the diet increases.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



**Figure 2.14**

Picture of humpback grouper showing emaciation, darkening of body color, petechia at the base of operculum as a result of rancid dietary fat and vitamin E deficiency.

Source: Koesharyani et al. 2001



menadione, vitamin K

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

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

**Table 2.12 Vitamin deficiency symptoms in fishes**

Vitamins	Deficiency symptoms
Vitamin B <sub>1</sub>	Equilibrium loss, irritability, lethargy, nerve disorder, anorexia, muscle atrophy, cataract, convulsions, skin discoloration, edema
Vitamin B <sub>2</sub>	Fin erosion, fragile skin, eye lesion, photophobia, iris pigmentation, cornea vascularization, anemia, anorexia, ataxia, cataract, cloudy lens, dark skin coloration, poor feed efficiency
Vitamin B <sub>5</sub>	Exudated gills, fragile skin, lethargy, liver necrosis, prostration, erratic swimming, ataxia, gill atrophy, clubbed gills, dark skin coloration, dermatitis
Vitamin B <sub>6</sub>	Equilibrium loss, exophthalmus, irritability, nerve disorder, rapid rigor mortis, blue slime, erratic swimming, anemia, ataxia, convulsions, edema
Vitamin B <sub>12</sub>	Anemia, anorexia
Vitamin C	Exophthalmus, fragile gill, kidney, liver and skin, eye lesion, lethargy, lordosis, prostration, scoliosis, anemia, anorexia, ascites, cartilage abnormality, low disease resistance
Inositol	Fatty liver, skin lesion, anemia, anorexia, distended stomach, poor feed efficiency
Biotin	Fatty liver, fragile erythrocytes, colon and skin lesions, blue slime, anorexia, muscle atrophy, dark skin coloration, convulsions, gill degeneration, poor feed efficiency
Choline	Fatty liver, fragile kidney, poor feed efficiency
Nicotinic acid	Anemia, anorexia, edema, fragile skin, colon lesion, lethargy, photophobia, muscle spasm, white muscle tetany, poor feed efficiency
Folic acid	Poor feed efficiency, fragile fins, lethargy, anemia, anorexia, dark skin coloration
Vitamin A	Erosion of fins, exophthalmus, fragile kidney and skin, eye lesion, anorexia, ascites, lens deformation, edema
Vitamin D	Scoliosis, white muscle tetany
Vitamin E	Exophthalmus, fatty liver, fragile erythrocytes, anemia, ascites, ceroid liver, muscular dystrophy, edema, epicarditis
Vitamin K	Anemia, slow clotting blood

Source: Halver 1989

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

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

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

Values are mg/kg diet unless stated otherwise.

R, required, NR, not required, IU, international units.

Source: Halver 1989; Wilson 1991; D'Abramo et al. 1997.

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



### Guide Questions

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

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

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