Asist.Prof.Dr. Measem Hassan
Advanced nutrition
Master degree

Vitamins and biochemistry

Vitamins are usually defined as organic compounds that are required in small amounts for normal growth and maintenance of animal life. But this definition ignores the important part that these substances play in plants and their importance generally in the metabolism of all living organisms. For example, many of the B vitamins act as cofactors in enzyme systems but it is not always clear how the symptoms of deficiency are related to the failure of the metabolic pathway.

In addition to avoiding explicit vitamin deficiency symptoms (see below) or a general depression in production due to a subclinical deficiency, some vitamins are added to the diet at higher levels in order to :

(1) enhance the quality of the animal product, e.g. vitamin D for eggshell strength and vitamin E for prolonging the shelf life of carcasses.

(2) improve health, e.g. vitamin A to improve the health status of the mammary gland in dairy cows.

Vitamins are required by animals in very small amounts compared with other nutrients; for example, the vitamin B1 (thiamin) requirement of a 50 kg pig is only about 3 mg/day. Nevertheless, a continuous deficiency in the diet results in disordered metabolism and eventually disease. Some compounds function as vitamins only after undergoing a chemical change; such compounds, which include _-carotene and certain sterols, are described as provitamins or vitamin precursors Many vitamins are destroyed by oxidation, a process speeded up by the action of heat, light and certain metals such as iron.This fact is important since the conditions under which a food is stored will affect the final vitamin potency. Some commercial vitamin preparations are dispersed in wax or gelatin, which act as a protective layer against oxidation .

The system of naming the vitamins by letters of the alphabet was most convenient and was generally accepted before the discovery of their chemical nature. Although this system of nomenclature is still widely used with some vitamins, the modern tendency is to use the chemical name, particularly in describing members of the B complex.

At least 14 vitamins have been accepted as essential food factors, and a few others have been proposed. It is convenient to divide the vitamins into two main groups: fat-soluble and water-soluble. Table 5.1 lists the important members of these two groups.

Table 5.1 Vitamins important in animal nutrition

Vitamin	
Fat-soluble vitamins	
A	Retinol
D2	Ergocalciferol
D3	Cholecalciferol
E	Tocopherola
K	Phylloquinone
Water-soluble vitamins	
B complex	
B1	**Thiamin**
B2	**Riboflavin**
	Nicotinamide
B6	**Pyridoxine**
	Pantothenic acid
	Biotin
	Folic acid
	Choline
B12	**Cyanocobalamin**
C	**Ascorbic acid**

aA number of tocopherols have vitamin E activity.

bSeveral naphthoquinone derivatives possessing vitamin K activity are known.

<u>FAT-SOLUBLE VITAMINS</u>

1-Vitamin A

The vitamin is a pale yellow crystalline solid, insoluble in water but soluble in fat and various fat solvents. It is readily destroyed by oxidation on exposure to air and light. A related compound with the formula C20H27OH, originally found in fish, has been designated dehydroretinol or vitamin A2.

Sources

Vitamin A accumulates in the liver and this organ is likely to be a good source; the amount present varies with species of animal and diet. The oils from livers of certain fish, especially cod and halibut, have long been used as an important dietary source of the vitamin. Egg yolk and milk fat also are usually rich sources, although the vitamin content of these depends, to a large extent, upon the diet of the animal from which it has been produced. Vitamin A is manufactured synthetically and can be obtained in a pure form.

Provitamins

Vitamin A does not exist as such in plants, but it is present as precursors or provitamins in the form of certain carotenoids, which can be converted into the vitamin. At least 600 naturally occurring carotenoids are known, but only a few of these are precursors of the vitamin. In plants, carotenoids have yellow, orange or red colours but their colours are frequently masked by the green colour of chlorophyll.When ingested, they are responsible for many of the varied and natural colours that occur in crustaceans, insects, birds and fish. They are also found in egg yolk, butterfat and the body fat of cattle and horses, but not in sheep or pigs. Carotenoids may be divided into two main categories: carotenes and xanthophylls. The latter include a wide range of compounds, for example lutein, cryptoxanthin and zeaxanthin, most of which cannot be converted into vitaminA. Of the carotenes, _-carotene is the most important member and this compound forms the main source of vitamin A in the diets of farm animals.

The long unsaturated hydrocarbon chains in carotenes (and vitamin A) are easily oxidised to by-products that have no vitamin potency. Oxidation is increased by heat, light, moisture and the presence of heavy metals. Consequently, foods exposed to air and sunlight rapidly lose their vitaminA potency, so that large losses can occur during the sun-drying of crops. For example, Fresh grass is an excellent source (250 mg/kg DM), but this is halved during ensilage.

Deficiency symptoms

Ability to see in dim light depends upon the rate of resynthesis of rhodopsin; when vitamin A is deficient, rhodopsin formation is impaired. One of the earliest symptoms of a deficiency of vitamin A in all animals is a lessened ability to see in dim light, commonly known as 'night blindness'. In adult cattle, a mild deficiency of vitamin A is associated with roughened hair and scaly skin. If it is prolonged the eyes are affected, leading to excessive watering, softening and cloudiness of the cornea and development of xerophthalmia, which is characterised by a drying of the conjunctiva. Constriction of the optic nerve canal may cause blindness in calves. In breeding animals a deficiency may lead to infertility, and in pregnant animals deficiency may lead to failure of embryo growth, disrupted organ development, abortion, short gestation, retained placenta or the production of dead, weak or blind calves. Less severe deficiencies may result in metritis and dermatitis and calves born with low reserves of the vitamin; it is then imperative that colostrum, rich in antibodies and vitamin A, should be given at birth, otherwise the susceptibility of such animals to infection leads to scours and, if the deficiency is not rectified, they frequently die of pneumonia.

Vitamin D

Chemical nature

Anumberof forms of vitaminDareknown,although not all of these are naturallyoccurring compounds.The two most important forms are ergocalciferol (D2) and cholecalciferol (D3).The termD1was originally suggested by earlierworkers for an activated sterol, which was found later to be impure and to consist mainly of ergocalciferol, which had already been designatedD2.The result of this confusion is that in the group of D vitamins the term vitaminD1 has been abolished. The D vitamins are insoluble in water but soluble in fats and fat solvents. The sulphate derivative of vitamin D present in milk is a water-soluble form of the vitamin. Both D2 and D3 are more resistant to oxidation than vitamin A, D3 being more stable than D2.

Sources

The D vitamins are limited in distribution.They rarely occur in plants except in sundried roughages and the dead leaves of growing plants. In the animal kingdom vitamin D3 occurs in small amounts in certain tissues and is abundant only in some fishes. Halibut-liver and cod-liver oils are rich sources of vitamin D3. Egg yolk is also a good source, but cow's milk is normally a poor source, although summer milk tends to be richer than winter milk. Colostrum usually contains six to ten times the amount present in ordinary milk. Clinical manifestations of avitaminosis D, and other vitamin deficiencies, are frequently treated by injection of the vitamin into the animal.

Provitamins

Reference has been made (p. 49) to two sterols, ergosterol and 7-dehydrocholesterol, as being precursors of vitamins D2 and D3, respectively.The provitamins, as such, have no vitamin value and must be converted into calciferols before they are of any use to the animal. For this conversion it is necessary to impart a definite quantity of energy to the sterol molecule, and this can be brought about by the ultraviolet light present in sunlight, by artificially produced radiant energy or by certain kinds of physical treatment. Under natural conditions activation is brought about by irradiation from the sun. The activation occurs most efficiently with light of wavelength 290–315 nm, so that the range capable of vitamin formation is small. The amount of ultraviolet radiation that reaches the earth's surface depends upon latitude and atmospheric conditions: the presence of clouds, smoke and dust reduces the radiation. Ultraviolet radiation is greater in the tropics than in the temperate regions, and the amount reaching the more northern areas in winter may be slight. Since ultraviolet light cannot pass through ordinary window glass, animals housed indoors receive little, if any, suitable radiation for the production of the vitamin. Irradiation is apparently more effective in animals with light-coloured skins. If irradiation is continued for a prolonged period, then the vitamin may be altered to compounds that can be toxic. The chemical transformation occurs in the skin and also in the skin secretions, which are known to contain the precursor. Absorption of the vitamin can take place from the skin, since deficiency can be treated successfully by rubbing cod-liver oil into the skin. Vitamin D requirements are often expressed in terms of international units (iu). One iu of vitamin D is defined as the vitamin D activity of 0.025 μg of crystalline vitamin D3.

Deficiency symptoms

A deficiency of vitamin D in the young animal results in rickets, a disease of growing bone in which the deposition of calcium and phosphorus is disturbed; as a result the bones are weak and easily broken and the legs may be bowed. In young cattle the symptoms include swollen knees and hocks and arching of the back. In pigs the symptoms are usually enlarged joints, broken bones, stiffness of the joints and occasionally. paralysis. The growth rate is generally adversely affected. The term 'rickets' is confined to young growing animals; in older animals vitamin D deficiency causes osteomalacia, in which there is reabsorption of bone already laid down. Osteomalacia due to vitamin D deficiency is not common in farm animals, although a similar condition can occur in pregnant and lactating animals, which require increased amounts of calcium and phosphorus. Rickets and osteomalacia are not specific diseases necessarily caused by vitamin D deficiency; they can also be caused by lack of calcium or phosphorus or an imbalance between these two elements. In poultry, a deficiency of vitamin D causes the bones and beak to become soft and rubbery; growth is usually retarded and the legs become weak. Egg production is reduced and eggshell quality deteriorates. Most foods of pigs and poultry, with the possible exception of fishmeal, contain little or no vitamin D, and the vitamin is generally supplied to these animals, if reared indoors, in the form of fish-liver oils or synthetic preparations.

Vitamin E

Chemical nature

Vitamin E is a group that includes a number of closely related active compounds. Eight naturally occurring forms of the vitamin are known, and these can be divided into two groups according to whether the side chain of the molecule, as shown below, is saturated or unsaturated. The four saturated vitamins are designated alpha, beta, gama and delta-tocopherol. the alpha form is the most biologically active and most widely distributed. The unsaturated forms of the vitamin have been designated Alpha,beta, gama and delta tocotrienols. Of these only the alpha form appears to have any significant vitamin E activity, and then only about 13 per cent of its saturated counterpart.

Sources

Vitamin E, unlike vitamin A, is not stored in the animal body in large amounts for any length of time and consequently a regular dietary source is important. Fortunately, the vitamin is widely distributed in foods. Green fodders are good sources of alpha tocopherol, young grass being a better source than mature herbage. The leaves contain 20–30 times as much vitamin E as the stems. Losses during haymaking can be as high as 90 per cent, but losses during ensilage or artificial drying are low. Cereal grains are also good sources of the vitamin, but the tocopherol composition varies with species .Wheat and barley grain resemble grass in containing mainly alpha tocopherol, but maize contains, in addition to alpha tocopherol, appreciable quantities of gama tocopherol. During the storage of moist grain in silos, the vitamin E activity can decline markedly. Reduction in the concentration of the vitamin from 9 to 1 mg/kg DM has been reported in moist barley stored for 12 weeks. Animal products are relatively poor sources of the vitamin, although the amount present is related to the level of vitamin E in the diet.

Metabolism

Vitamin E functions in the animal mainly as a biological antioxidant; in association with the selenium-containing enzyme glutathione peroxidase and other vitamins and trace-element-containing enzymes, it protects cells against oxidative damage caused by free radicals. Free radicals are formed during cellular metabolism and, as they are capable of damaging cell membranes, enzymes and cell nuclear material, they must be converted into less reactive substances if the animal is to survive. This protection is particularly important in preventing oxidation of polyunsaturated fatty acids, which function as primary constituents of subcellular membranes and precursors of prostaglandins. Oxidation of unsaturated fatty. Vitamin E also plays an important role in the development and function of the immune system. In recognition of this the National Research Council requirements for dairy cows have been increased to reduce the incidence of mastitis. In studies with several species, supplementation of diets with the vitamin provided some protection against infection with pathogenic organisms. Recent research has indicated that vitamin E is also involved in the regulation of cell signalling and gene expression. Like vitamin A, it was thought that the transfer of vitamin E across the placenta was limited, with the neonate relying on colostrum to meet its requirements. More recent evidence in sheep indicates that placental transfer does occur, with increased muscle and brain concentrations in lambs born from ewes fed higher levels. Nonetheless, colostrum is a very important source of vitamin E for the new born.

Free radicals and antioxidants

Antioxidants are required to protect the animal's cells from damage due to the presence of free radicals. These are highly reactive molecules containing one or more unpaired electrons and can exist independently (e.g. superoxide, $O^{*}2-$, and hydroxyl, OH^{*}). Their high reactivity is a result of their trying to lose or gain an electron to achieve stability.Within cells hydrogen peroxide (H_2O_2) can easily break down, especially in the presence of transition ions (e.g. Fe^{2+}), to produce the hydroxyl radical, which is the most reactive and damaging of the free radicals:

$$H_2O_2 + Fe^{2+} \rightarrow OH^{*} + OH^{-} + Fe^{3+}$$

Free radicals are generated during normal cellular metabolism owing to leakage from the electron transport chain in mitochondria and leakage from peroxidation of polyunsaturated fatty acids in the pathway of conversion of arachidonic acid to prostaglandins and related compounds. Also O_2^{*-} plays an essential role in the extracellular killing of microorganisms by activated phagocytes, and activation of this system can lead to further leakage. All classes of biological molecules are vulnerable to free radical damage, but especially lipids, proteins and DNA. Cell membranes are an important target because of the enzyme systems contained within them. Lipids are the most susceptible; oxidative destruction of polyunsaturated fatty acids can be extremely damaging, since it proceeds as a self-perpetuating chain reaction. The more active cells, such as muscle cells, are at greatest risk of damage because they depend on the utilisation of lipids as energy sources. To maintain cell integrity the animal's cells require protection mechanisms and these are provided by the antioxidant system, which involves a group of vitamins and enzymes containing trace elements working in series.The initial line of defence is by the enzymes superoxide dismutase (containing copper), glutathione peroxidases (containing selenium) and catalase. Superoxide dismutase eliminates superoxide radicals formed in the cell and prevents the reaction of the radical with biological membranes or their participation in the production of more powerful radicals. Glutathione peroxidase detoxifies lipid hydroperoxides that are formed in the membrane during lipid peroxidation. Catalase can also break down hydrogen peroxide. If large amounts of radicals are produced the enzyme systems will be insufficient to prevent damage and the second antioxidant system is brought into action.Antioxidants break the chain reaction by scavenging peroxyl radicals and thus interfere with the propagation steps in the lipid peroxidation process.Vitamin E is the main antioxidant but the carotenoids, vitamin A and vitamin C are also involved. In mammalian cells vitamin E is located in the mitochondria and endothelial reticulum. It donates a hydrogen atom to the free radical to form a stable molecule, thereby breaking the chain. The amount of vitamin E in the cell membranes is low and it must be regenerated so that there is sufficient to act against other radicals.The regeneration is carried out by reaction with vitamin C and the ascorbate radical in turn is reduced by NADH-dependent enzymes . It has been reported that vitamin C also acts as an antioxidant in extracellular fluid, where it operates as a scavenger preventing the initiation of lipid peroxidation. It contributes up to one-quarter of the total antioxidant activity in plasma.

Deficiency symptoms

The most frequent and, from a diagnostic point of view, the most important manifestation of vitamin E deficiency in farm animals is muscle degeneration (myopathy). Nutritional myopathy, also known as muscular dystrophy, frequently occurs in cattle, particularly calves, when they are turned out on to spring pasture. It is associated with low vitamin E and selenium intakes during the in-wintering period and possibly the relatively high concentration of polyunsaturated fatty acids in the young grass lipids. The requirement for the vitamin increases with increasing concentrations of polyunsaturated fatty acids in the diet. The myopathy primarily affects the skeletal muscles and the affected animals have weak leg muscles, a condition manifested by difficulty in standing and, after standing, a trembling and staggering gait. Eventually, the animals are unable to rise, and weakness of the neck muscles prevents them from raising the head. A popular descriptive name for this condition is white muscle disease’, owing to the presence of pale patches or white streaks in the muscles.The heart muscle may also be affected and death may result. Serum creatine phosphokinase and glutamic oxaloacetic transaminase levels are elevated in animals deficient in vitamin E.

Vitamin K

Vitamin K was discovered in 1935 to be an essential factor in the prevention of haemorrhagic symptoms in chicks.The discovery was made by a group of Danish scientists, who gave the name ‘koagulation factor’ to the vitamin, which became shortened to the K factor and eventually to vitamin K. Vitamins K are relatively stable at ordinary temperatures but are rapidly destroyed on exposure to sunlight.

Metabolism

Vitamin K is necessary for the synthesis of prothrombin in the liver. In the blood-clotting process, prothrombin is the inactive precursor of thrombin, an enzyme that converts the protein fibrinogen in blood plasma into fibrin, the insoluble fibrous protein that holds blood clots together. Prothrombin normally must bind to calcium ions before it can be activated. If the supply of vitamin K is inadequate, then the prothrombin molecule is deficient in _-carboxyglutamic acid, a specific amino acid responsible for calcium binding. Proteins containing _-carboxyglutamic acid, dependent on vitamin K for their formation, are also present in bone, kidney and other tissues.

Deficiency symptoms

Symptoms of vitamin K deficiency have not been reported in ruminants, horses and pigs under normal conditions, and it is generally considered that bacterial synthesis in the digestive tract supplies sufficient vitamin for the animal’s needs. A number of microorganisms are known to synthesise vitamin K, including *Escherichia coli*. Medicines that affect the bacteria in the gut may depress the production of vitamin K.A disease of cattle called ‘sweet clover disease’ is associated with vitamin K. Sweet clover (*Melilotus albus*) naturally contains compounds called coumarins which, when the crop is preserved as hay or silage, may be converted by a variety of fungi, such as the *Aspergillus* species, to dicoumarol.This compound lowers the prothrombin content of the blood and thereby impairs the blood-clotting process.The disease can be overcome by administering vitamin K to the animals. For this reason dicoumarol is sometimes referred to as an ‘anti-vitamin’ The symptoms of vitamin K deficiency in chicks are anaemia and a delayed clotting time of the blood; birds are easily injured and may bleed to death. It is doubtful whether, in birds, microbially synthesised vitamin K is available by direct absorption from the digestive tract, because the site of its formation is too distal to permit absorption of adequate amounts except by ingestion of faecal material (coprophagy).