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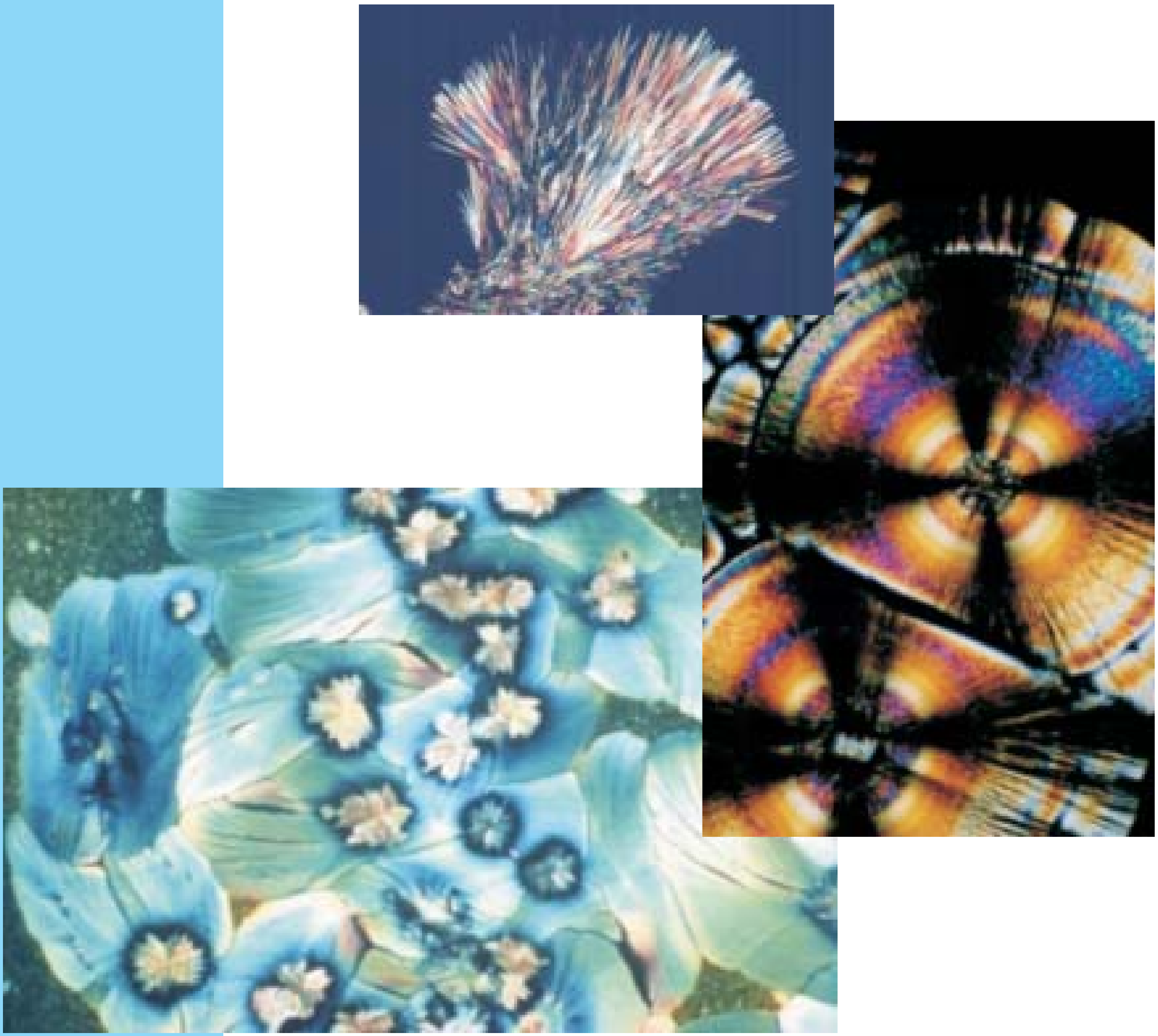
(Ed.)

Vitamins

in animal nutrition



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Economic Association AWT

The AWT is a German Economic Association formed to represent, safeguard and promote the professional, economical and technical interests of leading German manufacturers and processors of feed additives for animal nutrition on a national and international level.

Missions and objectives

- To safeguard members‘ interests and represent them towards public authorities, government representatives, legislative organs, professional organisations and other national institutes
- To represent German interests in feed additives on an international level
- To provide members with information and advice in all professional matters, especially on current projects in legislation
- To inform the public on the benefits, safety and quality of feed additives in animal nutrition

Vitamins in Animal Nutrition

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1.1. What are vitamins?

Vitamins are organic substances that are indispensable to the normal metabolic processes of animal organisms. They are essential to maintain health and performance, and have to be supplied with the feed. Vitamins can also be ingested as pro-vitamins, which are converted into the corresponding vitamins by the animal organism. In general, the animal organism itself is not able to synthesise vitamins.

1.2. How do vitamins work?

A deficiency or complete lack of one or more vitamins may lead to multiple malfunctions of the metabolism resulting in depressed performance, growth retardation, fertility problems or diseases. Furthermore, an increased supply of certain vitamins has positive effects e.g. on immunity or hoof quality.

There are two main groups of vitamins: fat-soluble and water-soluble. The two groups also indicate two different types of activity. While the fat-soluble vitamins have specific functions in the development and maintenance of tissue structures, the water-soluble vitamins participate in catalytic functions or act as control mechanisms in the metabolism, e.g. as co-enzymes. For these physiological effects only very small quantities are needed.

Every single vitamin fulfils specific tasks that cannot be accomplished in the same way by any other vitamin.

1.3. Vitamin research

More than 80 years ago, the function of vitamins was revealed in feeding experiments. Rats and mice that had been fed with vitamin-free diets of carbohydrates, protein, fat and minerals died within a very short time. When small quantities of milk were added to the diet, the lifespan of the animals was prolonged. The conclusion of this experiment was that milk contained essential active substances hitherto unknown.

It soon became evident that there were at least two substances involved: a fat-soluble factor A and a water-soluble factor B. When trying to isolate factor B, scientists discovered in 1912 a substance containing nitrogen which was chemically an amine, and which was in therefore named »vitamin« (vita = life). This name was soon used for a whole group of essential organic compounds, although it was later discovered that they were not always nitrogen-containing substances with an amine character.

Table 1: Key dates in the history of vitamins

Vitamin or pro-vitamin	Discovery	Elucidation of the structure	First synthesis
β-Carotene	1831 in palm oil	1930	1950
Niacin	1867	1873	1894
Vitamin B_1	1897 in rice bran	1936	1936
Vitamin A	1909 in fish liver oil	1930	1947
Vitamin C	1912 in lemon juice	1933	1933
Vitamin D_3	1918 in fish liver oil	1936	1959
Vitamin B_2	1920 in egg white	1935	1935
Vitamin E	1922 in wheatgerm oil	1938	1938
Vitamin B_{12}	1926 in liver	1955	1972
Vitamin K	1929 in alfalfa	1939	1939
Pantothenic acid	1931 in liver	1940	1940
Biotin	1931 in liver	1942	1943
Vitamin B_6	1934 in rice bran	1938	1939
Folic acid	1941 in liver	1946	1946

With more and more elaborate animal experiments, scientists were soon able to sub-divide the fat-soluble factor A and the water-soluble factor B into an increasing number of different substances, which were named in alphabetical order. Since then, vitamins have been divided into two groups: fat-soluble (A, D, E, K) and water-soluble (B, C). Medical doctors, veterinarians and biologists attempted to discover in animal experiments as many of these vitamins as possible, while chemists worked on resolving their structure, the first step towards chemical synthesis.

Table 1 is taken from W. Friedrich´s “Handbuch der Vitamine” (Manual of vitamins, 1987). It lists the dates of the first evidence, the discovery of the structure and of their first synthesis.

1.4. Use and processing

The following explanations deal mainly with the importance of vitamins and with recommendations for the vitamin supply of livestock and pets. The most important commercial products are also described and information on their application, stability and analysis is given.

2. Vitamins and their biological functions

Vitamins are complex organic compounds. They are essential for the metabolism, since they maintain normal physiological functions such as growth and development, life functions, health and reproduction. Vitamin deficiency or insufficient absorption will produce deficiency symptoms resulting in specific diseases and reduced performance. Most domestic animals are not capable of synthesising vitamins at all or cannot produce sufficient quantities for their own requirement. Above all, this applies to vitamins A, D, E and K, partly to vitamin C and to the vitamins of the B group (B_1, B_2, B_6, B_{12}, biotin, folic acid, niacin, pantothenic acid) and to choline.

Vitamins are divided into two groups: fat-soluble and water-soluble.

2.1. Fat-soluble vitamins

The vitamins A, D, E, K and ß-carotene (precursor of vitamin A) belong to the fat-soluble vitamins. The main functions of these vitamins are listed in Table 2. The hydrophobic character of these vitamins is a result of the long side-chain within the molecule. The fat-soluble vitamins consist of only carbon, oxygen and hydrogen, and are relatively sensitive to external influences such as oxidation, heat, ultraviolet light, metal ions and specific enzymes.

In the body, the fat-soluble vitamins are found in relationship with fats and are absorbed together with them. The mechanisms of absorption are similar. The body is able to store considerable quantities of fat-soluble vitamins depending on species and age. The sites of storage are inner organs such as the kidneys and liver, the muscles, the brain and fat tissue. Excretion normally only occurs after transformation during metabolism.

Vitamin	**Main function**
Vitamin A	Protection of the epithelium
ß-Carotene	Precursor of vitamin A
Vitamin D	Regulation of the calcium and phosphorus metabolism
Vitamin E	Antioxidant
Vitamin K	Blood coagulation

Table 2: Main functions of the fat-soluble vitamins

2.1.1. Vitamin A

Natural sources and bioavailability

Vitamin A (retinol) is found only in feeds of animal origin, e.g. liver, fish oil and high-fat fishmeal. The vitamin A content of milk and eggs is low. Feeds of plant origin (grass, carrots) only contain ß-carotene, a precursor that can be converted into vitamin A. The ratio of conversion of ß-carotene into vitamin A differs according to species, as shown in Table 3, and it also depends on the quantities consumed. If the animal consumes sufficient quantities for its requirement, 80 to 90% of vitamin A is absorbed in the small intestine. With higher consumption, this percentage will not decrease noticeably.

Physiological role

- Formation, protection and regeneration of skin and mucous membranes (epithelium protection)
- Promotion of fertility by improving ovulation and implantation of the ovum, embryonic and foetal development and hormone activation for pregnancy
- Control of growth and differentiation processes of the cellular metabolism by influencing the transcription of more than 300 genes (genetic expression)
- Increased resistance to infectious diseases

Deficiency symptoms

- Cornification of skin and mucous membranes and subsequent risk of infection
- Retarded maturation of the ova and embryo mortality
- Disturbed embryonic development
- Increased risk of infections

Additional effects

- Immune reaction: increased antibody production and phagocytosis

Table 3: Conversion ratio of ß -carotene into vitamin A depending on animal species

Species	Vitamin A per mg β-Carotene	Conversion ratio
Dairy cows	370 IU	8–10 : 1
Fattening cattle	440 IU	7–8 : 1
Horses	420 IU	6–10 : 1
Sheep	480 IU	6–8 : 1
Pigs	510 IU	6–7 : 1
Poultry	1667 IU	2 : 1

2.1.2. ß-Carotene

Natural sources and bioavailability

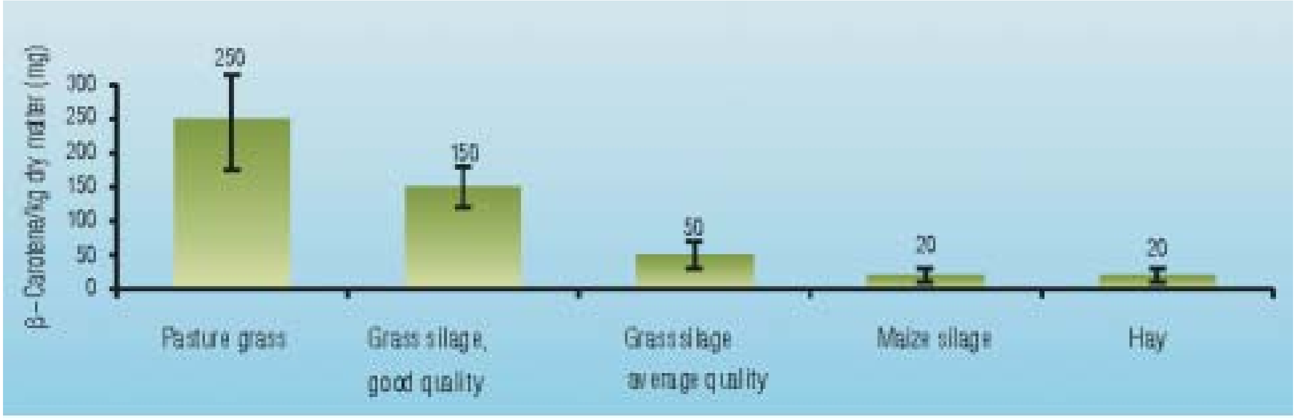
ß-Carotene only occurs in plants. Plants rich in ß-carotene are alfalfa, grass and grass silage and carrots. The ß-carotene contents of cereals and milling by-products are low.

Depending on vegetation period, time of harvest, type of preservation (hay, silage), drying temperature and duration of storage, the natural ß-carotene content of the feed will vary considerably (Figure 1). Absorption and storage will differ with animal species; in yellow-fat species (cattle, horses) it is high, in white-fat species (pigs, buffalos, sheep, goats, dogs, cats, rodents) it is low or nil.

Physiological role

- Precursor (pro-vitamin) of vitamin A
- By specific means of metabolic transport (cattle: 80% high-density lipoproteins) ß-carotene is carried into specific organs (e.g. corpus luteum, follicle, udder) were it is converted into vitamin A (enzyme: carotenase)
- Stimulation of progesterone synthesis, necessary for the formation of the mucous membranes of the uterus
- Probable influence independent from vitamin A by antioxidative effect on cell-degrading lipid radicals, resulting in increased hormonal activity (FSH, LH) and improved immunity (multiplication of lymphocytes)

Figure 1: ß-Carotene content per kg dry matter in some forages



Deficiency symptoms

- Fertility problems, e.g. prolonged oestrus and silent oestrus
- Retarded follicle maturation and ovulation
- Cyst growth in follicle and corpus luteum
- Embryo losses and early abortion
- Increased somatic cell counts in milk, mastitis
- Increased susceptibility of young animals to infectious diseases

Additional effects

- Increased resistance of young animals owing to the high content in the colostrum (unspecific immunity)
- Synergistic antioxidant effect with other carotenoids (zeaxanthin, lutein, lycopene etc.)

2.1.3. Vitamin D_3

Natural sources and bioavailability

Vitamin D is found in very few products, e.g. as vitamin D_3 (cholecalciferol) in whole milk and liver oils, and as vitamin D_2 (ergocalciferol) in sun-dried green forage.

Vitamin D_2 is formed under the influence of UV radiation from ergosterol in plants when they are dried. Vitamin D_3 is formed in the epidermis from 7-dehydrocholesterol by UV radiation (exceptions: dogs, cats).

The production of vitamin D_3 is limited when animals are confined to the stable for long periods.

Owing to the limited availability in nature, natural sources of vitamin D are not important for covering requirements. Furthermore, animals are only able to utilise vitamin D precursors of plant origin to a limited degree.

Physiological role

Vitamin D_3 has no direct metabolic activity. In the liver, it is converted into 25-hydroxyvitamin D_3, which is then converted into 1,25-, 24,25- and 1,24,25-hydroxyvitamin D_3 in the kidneys. 1,25-Hydroxyvitamin D_3 is the form with the largest biological effect. In the organism, vitamin D fulfils the following tasks:

- It regulates calcium and phosphate metabolism and promotes calcium and phosphate absorption in the intestine
- It controls the excretion of calcium and phosphate by the kidneys and the storage of calcium and phosphate in the skeleton
- It mobilises calcium and phosphorus from the skeleton
- It promotes germ cell production
- It increases the performance of the immune system, and inhibits auto-immunisation
- It controls the transcription of more than 50 genes

It is economically and scientifically doubtful whether the direct oral administration of D_3 metabolites, e.g. in order to improve eggshell quality or to prevent milk fever, has any benefit.

Deficiency symptoms

- Disorders of calcium and phosphate metabolism
- Inhibited mineralisation of bone during growth (rickets)
- Extraction of mineral substances from the bones
- Deformed bones and joints (softening of the bones)
- Growth disorders
- Spontaneous bone fractures
- Poor eggshell stability

2.1.4. Vitamin E

Natural sources and bioavailability

Vitamin E is a generic term for various compounds based on tocopherol or tocotrienol. It is found in plants and animals. However, it is not the total tocopherol content that is important, but the content of the biologically active d-α-tocopherol.

Grass, clover, alfalfa, green meal and uncrushed oilseeds are rich in vitamin E. Extracted oilseed meals are poor in vitamin E.

Humidity and long storage have an adverse effect on vitamin E stability and content. Conserved green forages and cereals are the types of feed mostly affected.

Cereals and middlings mainly contain β-, γ-, and δ-tocopherols (70–90%) with a biological activity significantly lower than that of α-tocopherol.

Biological efficiency of various vitamin E compounds:

α-Tocopherol	100%
β-Tocopherol	15–40%
γ-Tocopherol	1–20%
δ-Tocopherol	1%
α-Tocotrienol	15–30%
β-Tocotrienol	1–5%
γ-Tocotrienol	1%
δ-Tocotrienol	1%

Physiological Role

- Reduces the production of lipid peroxyl radicals from highly unsaturated fatty acids
- Antitoxic effect in cell metabolism
- Reduces the incidence of liver necrosis and muscular degeneration
- Antioxidant effect, i.e. phospholipids in the cell membrane and other substances sensitive to oxidation, e.g. vitamin A, carotenoids and their intermediates, are stabilised. There is a close relationship in the functions of vitamin E and selenium in protecting the cell membrane from oxidation. While vitamin E acts within the cell membrane, the effect of selenium is based on peroxide degradation by glutathione

peroxidase in the soluble constituents of the cell. To achieve a sufficient production of selenium containing glutathione peroxidase, a selenium content of 0.2-0.3 mg per kg dry matter in the feed is necessary

- Controls metabolism of the hormones via the anterior lobe of the hypophysis
- Maintains membrane stability, especially of the cardiac and skeletal muscles
- Controls the development and function of the gonads
- Stimulates antibody production (improved resistance to diseases), phagocytosis and the bactericide effects of phagocytes
- Preparation for pregnancy and protection against abortion

Deficiency symptoms

- Damage to cardiac and skeletal muscles (dystrophy, myopathy)
- Sudden death through damage to the heart muscle (mulberry heart disease)
- Fertility disorders
- Changes in the vascular and nervous system (encephalomalacia, oedema in the cerebellum by increased plasma secretion, causing abnormal posture of the head and uncoordinated movements)
- Liver lesions and changes in fat deposits (yellow-fat disease in mink, brown coloration of bacon)
- Locomotory disorders and muscle incurvation (banana disease) in pigs
- White muscle disease due to dystrophic alteration in calves and lambs
- Reduced hatchability and exsudative diathesis (increased plasma secretion of the blood) in poultry

Additional effects

- Stabilisation of fat (protection against oxidation) in animal products (meat, milk, eggs)

2.1.5. Vitamin K

Natural sources and bioavailability

Vitamin K is a generic term for vitamin K_1 (phylloquinone), K_2 (menaquinone) and K_3 (menadione).

Green plants are rich in vitamin K_1, whereas cereals, beets, meat and fishmeals are poor. Vitamin K_2 is produced by bacteria in the rumen and in the large intestine.

Vitamin K_3 (menadione) is an industrial form, which is offered in various water-soluble menadione compounds for animal nutrition:

- Menadione sodium bisulphite (MSB)
- Menadione dimethylpyrimidinol bisulphite (MPB)
- Menadione nicotinamide bisulphite (MNB)

The fat-soluble forms K_1 and K_2 can only be absorbed when pancreas lipase and bile acid are secreted. This is not necessary for the water-soluble vitamin K_3 forms. All three forms serve as a basis for the production of menaquinone-4, which is highly active in the metabolism.

Physiological role

- Synthesis of blood coagulation factors II (pre-thrombin), VII, IX and X
- Production of the calcium transport protein osteocalcin for bone mineralisation
- Participation in carboxylation of other proteins

Deficiency symptoms

- Haemorrhages in various tissues and organs
- Blood coagulation disorders
- Growth disorders

Antagonists

- Dicoumarol
- Coumarin derivatives
- Sulphonamides
- Mycotoxins

2.2. Water-soluble vitamins

The water-soluble vitamins of the B group, i.e. B_1, B_2, B_6, B_{12}, biotin, folic acid, niacin and pantothenic acid, act as co-enzymes, and are hence very important for the metabolism (Table 4). Each co-enzyme is specialised on specific metabolic reactions. An insufficient supply of the B vitamins will reduce the activity of the corresponding enzyme and result in metabolic disorders.

Vitamin C and choline are also water-soluble; however, there is currently no evidence for any co-enzyme function.

An insufficient supply of B vitamins leads to disorders of the skin, mucous membranes and hair, an impaired immune system and reduced performance.

Table 4: The most important co-enzymes of the water-soluble vitamins and their main functions

Vitamin	**Main co-enzymes**	**Main functions**
Vitamin B_1	Thiamine pyrophosphate	Carbohydrate metabolism
Vitamin B_2	FAD, FMN (hydrogen transfer)	Energy metabolism
Vitamin B_6	Pyridoxal phosphate	Amino acid metabolism
Vitamin B_{12}	Cyanocobalamin (transfer of methyl groups)	Protein turnover
Biotin	Pyruvate-acetyl-CoA -carboxylase	Fatty acid metabolism and energy metabolism
Folic acid	Tetrahydrofolic acid	Amino- and nucleic acid metabolism
Niacin	NAD, NADP (hydrogen transfer)	Energy metabolism
Pantothenic acid	Co-enzyme A	Fat metabolism and energy conversion
Vitamin C	–	Redox reactions
Choline	–	Fat metabolism, transmission of neural impulses

The B vitamins can be produced by microbes in the stomach and intestine. In ruminants, auto-synthesis occurs when the rumen system is functioning normally. In pigs, bacterial synthesis of the B vitamins takes place in the large intestine, where they are absorbed only to a limited degree.

Animals are not able to store major quantities of the water-soluble vitamins, so that a continuous supply has to be assured.

2.2.1. Vitamin B_1

Natural sources and bioavailability

Vitamin B_1 (thiamine) occurs in all feeds in various concentrations. Cereals and middlings, oilseed meals, dairy products and brewer´s yeast are rich in vitamin B_1, whereas tapioca, dried sugar beet pulp, meat meal, fishmeal and coconut meal are poor.

The vitamin B_1 in feedstuffs is well utilised by animals. However, there are antagonists that can limit utilisation considerably.

Physiological effects

In its phosphorylated form (thiamine pyrophosphate), vitamin B_1 is a co-enzyme of various decarboxylases (pyruvate dehydrogenase, α-ketoglutarate dehydrogenase) and of transketolase, and therefore has the following functions:

- It is indispensable to degradation processes in carbohydrate metabolism
- It is important for the function of neural and cardiac tissue
- It is necessary for the peristalsis of the stomach and intestine

In the form of thiamine triphosphate, it is a possible activating substance for the stimulation of peripheral nerves.

Deficiency symptoms

In deficiency, a great number of serious disorders can occur, mainly in the nervous system and in cardiac and vascular tissue:

- Polyneuritis, irritability, spasms, paralysis and cerebrocortical necrosis in calves, cattle and sheep
- Reduced pulse (bradycardia), heart failure, heart damage
- Reduced feed consumption, insufficient energy utilisation, growth depression, weakness

Antagonists

- Thiaminases in the rumen, produced by rumen microbes when feed rich in starch but poor in fibre is consumed
- Thiaminases in fresh fish (mink feed)
- Feed contaminated with bacteria or fungi
- Amprolium (coccidiostat), especially when administered at high levels
- Phenol derivatives and heavy metals, e.g. arsenic and mercury

2.2.2. Vitamin B_2

Natural sources and bioavailability

Vitamin B_2 (riboflavin) is contained in feed of plant and animal origin. Feedstuffs of animal origin, especially dairy products such as skim milk and whey powders and brewer´s yeast, have a high vitamin B_2 content. Feedstuffs of plant origin, e.g. cereals and tapioca, have a low vitamin B_2 content.

The vitamin B_2 contained in the feed is only partly bioavailable. Experiments with pigs showed a precaecal digestibility of approximately 60% with maize and wheat bran.

Physiological role

Riboflavin, which is almost always bound to proteins (flavoproteins), is a component of the co-enzymes FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide), and is of importance for the following:

- Hydrogen transfer within the respiratory chain for energy metabolism
- Oxidation and reduction processes for producing and breaking down fatty acids and amino acids

Deficiency symptoms

- Inflammatory skin disorders (atrophy, hyperkeratosis, hyperplasia)
- Neurological disorders
- Retarded growth, poor feed conversion efficiency and diarrhoea
- »Curled Toe Paralysis« in chicks
- In poultry, reduced hatchability and higher losses during rearing
- Smaller litters in sows, especially gilts

2.2.3. Vitamin B_6

Natural sources and bioavailability

Vitamin B_6 (pyridoxine) is found in feed of plant and animal origin. Feedstuffs of plant origin such as cereals, milling by-products, extracted oilseed meals and brewer´s yeast are rich in vitamin B_6. Feedstuffs of animal origin and tapioca are poor in vitamin B_6.

The vitamin B_6 contained in feed of plant origin is only partially bioavailable: 65% in soybean meal, and approx. 50% in maize.

Physiological role

Vitamin B_6 as a component of the co-enzyme pyridoxal-5´-phosphate plays a central part in:

- transamination, decarboxylation and racemising processes during the metabolism of amino acids.

The breaking down of tryptophan (e.g. in niacin synthesis) requires the enzyme kynureninase, which is linked to vitamin B_6.

- Carbohydrate metabolism by participating in phosphorylation

Deficiency symptoms

- Retarded growth, reduced feed consumption and protein retention
- Skin inflammation, damage to liver and heart, disorders of blood parameters
- Malfunction of the peripheral and central nervous systems (uncontrolled movements, excitedness, spasms)
- Reduced hatchability in poultry

Antagonists

- Inhibiting factor in linseed

2.2.4. Vitamin B_{12}

Natural sources and bioavailability

Vitamin B_{12} (cobalamin) only occurs in feed of animal origin. Fishmeal, fish solubles and skim milk powder are rich in vitamin B_{12}.

Microbes will produce sufficient quantities of this vitamin in the rumen if feed with a sufficient cobalt content (> 0.1 mg/kg dry matter) is consumed.

The vitamin B_{12} present in feed is normally well utilised.

Physiological role

- Production of blood cells and growth
- Production of the co-enzyme 5-desoxyadenylcobalamin, which is necessary for the utilisation of propionic acid and thus for the production of glucose and lactose in ruminants
- Production of the co-enzyme methylcobalamin, which is necessary for methylation reactions and hence e.g. for the metabolism of methionine

Deficiency symptoms

- Reduced synthesis of DNA and protein, growth disorders, lower feed conversion, anaemia, rough coat and inflammation of the skin
- Poor plumage, reduced hatchability and increased embryo mortality in poultry
- In ruminants, weight loss in regions with a low cobalt content in plants

Antagonists

- Tannic acid reduces the absorption of vitamin B_{12}

2.2.5. Biotin

Natural sources and bioavailability

Biotin is present in many feeds of animal and plant origin. Biotin-rich products are brewer´s yeast and extracted oilseed meals. Poor sources of biotin are cereals and tapioca.

Monogastric animals are not always able to assimilate a sufficient percentage of biotin from plant feed (0–10% in wheat, 20–30% in barley). Higher levels of utilisation are achieved with maize and soybean meal.

Physiological role

Biotin is required as a co-enzyme for the production of a number of enzymatic systems (carboxylases). These biotin-dependent enzymes play an important role in the several metabolic processes:

- Fatty acid synthesis (acetyl-CoA carboxylase)
- Gluconeogenesis (pyruvate carboxylase)
- Propionic acid metabolism (propionyl-CoA carboxylase)
- Decomposition of leucine (methyl crotonyl-CoA-carboxylase)
- Synthesis of DNA and RNA (via purine synthesis)

Deficiency symptoms

Various symptoms occur according to the severity and duration of the deficiency:

- Retarded growth and fertility disorders
- Skin disorders
- Poor plumage, inflammatory lesions of beak, legs and toes, fatty liver and kidney syndrome (FLKS) in poultry
- Hair loss, inflammation of the hooves and hoof-sole lesions in pigs
- Brittle horns and grooves and cracks in hooves in cattle, sheep and horses

Antagonists

- Avidin in raw egg white

2.2.6. Folic acid

Natural sources and bioavailability

Folic acid (pteroylglutamic acid) is a generic term for various compounds, also known collectively as folates. The biologically active form of folic acid is tetrahydrofolic acid.

Folates are found in feeds of both plant and animal origin.
Folate-rich feedstuffs are lucerne green meal and brewer´s yeast.
Folate-poor feedstuffs are tapioca and cereals.

In feed, folates are found as monoglutamates and as polyglutamates. Polyglutamates have a very low bioavailability, so that natural folic acid can only partly be utilised by monogastric animals. Only 20–60% of the folates in cereals is utilised by poultry and pigs.

Physiological role

Folic acid in the form of tetrahydrofolic acid is biologically active as a co-enzyme, with the following metabolic functions:

- Transfer of specific C1 units (methyl and formyl groups), which are important for cell growth, cell division and cell differentiation in the metabolism of proteins and of DNA and RNA
- Together with vitamin B_{12}, it converts homocysteine into methionine

Deficiency symptoms

- Macrocytic anaemia
- Damage to the skin and mucous membranes
- In poultry, disorders of growth, bad plumage and depigmentation, perosis, increased embryo mortality, reduced hatchability and laying performance
- Hair loss and fertility disorders in pigs
- Fertility disorders in cattle

Antagonists

- Sulphonamides and aflatoxins in feed and in drugs to inhibit intestinal microflora

Additional effects

- Increased antibody production

2.2.7. Niacin (nicotinic acid/nicotinamide)

Natural sources and bioavailability

Niacin is found as nicotinic acid in varying concentrations in almost all feeds of plant origin. Brewer´s yeast, bran, green forage and plant protein feeds are rich in niacin. Maize, rye and dairy products are poor in niacin.

Nicotinamide is frequent in animal cells. Minor quantities are produced by microbial synthesis in the intestine and by transformation of the amino acid tryptophan.

From a physiological point of view, nicotinic acid and nicotinamide can be considered as equivalent sources of niacin.

Pigs, poultry and ruminants possess a limited capability to utilise niacin derived from wheat and middlings.

Physiological role

- Constituent of NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) which act as hydrogen-transferring co-enzymes and participate in vital metabolic reactions (carbohydrates, fats and amino acids)
- Key functions in energy metabolism

Deficiency symptoms

- Functional disorders of the nervous system
- Skin disorders (pellagra)
- Increased peristalsis of the gastrointestinal tract
- Retarded growth
- Inflammation and ulcers of the mucous membranes
- Disorders of feather development and reduced laying activity and brood capability in poultry
- Black tongue disease in dogs

Additional effects

- A daily extra supplement of 6–12 g niacin can increase performance and lower the risk of ketosis in high-performance dairy cows. Lower supplements in proportion to body weight can be given to smaller ruminants.

2.2.8. Pantothenic acid

Natural sources and bioavailability

Pantothenic acid is found in almost all types of feed. Dairy products, fish solubles, brewer´s yeast, middlings, green meals and oilseed meals are rich in pantothenic acid.
Beans, dried beet pulp and meat meal are poor in pantothenic acid.

Pantothenic acid in feed stuffs can be well utilised.

Physiological role

- As a constituent of co-enzyme A in synthesis and degradation processes in the metabolism of proteins, carbohydrates and fats
- Production of acetylcholine for the function of neural cells
- Function of skin and mucous membranes
- Pigmentation of hair

Deficiency symptoms

- Alterations of the skin and mucous membranes
- Loss of pigmentation
- Rough coat
- Loss of hair and feathers
- Decreased synthesis of steroid hormones
- Poor appetite and diarrhoea due to functional disorders in the gastrointestinal tract
- In poultry, scabby crusts on the toes and beak, secretions around the eye, low hatchability, increased embryonic mortality and poor plumage
- In pigs, brown exsudate around the eyes and a jerky gait as a result of a functional disorders of the nervous system

2.2.9. Vitamin C

Natural sources and bioavailability

Vitamin C (ascorbic acid) is not found in many feedstuffs, and degrades rapidly during storage and processing. Feedstuffs rich in vitamin C are green forage and potatoes.

The vitamin C present in stuffs can be utilised very well.

Primates, guinea pigs and some species of fish (trout, salmon etc.) are not capable of synthesising vitamin C, since they lack the enzyme L-gluconolactone oxidase. Other mammals and fish produce vitamin C in the liver, birds in the kidneys.

Physiological role

- Removal of radicals and lipid peroxyl compounds in the cell metabolism in co-operation with other antioxidative vitamins such as vitamin E and ß–carotene
- Collagen synthesis in bones, cartilage, muscles, skin and eggshell
- Regulation of calcium metabolism by activating vitamin D_3 metabolites
- Function of macrophages, granulocytes and lymphocytes in the immune system
- Inhibition of stress reactions caused by reduced hormone production (cortisol)
- Improved fertility-linked properties such as sperm quality, follicle maturation and progesterone synthesis
- Improved resorption of iron
- Reduction of the toxic effects of heavy metals such as lead, cadmium and nickel

Deficiency symptoms

- Susceptibilty to infections and parasites
- Retarded growth
- Bone diseases
- Delayed healing of wounds, umbilical bleeding in piglets
- Reduced eggshell stability
- Increased susceptibility to stress factors such as heat, transport, housing changes
- Reduced immune reaction in general and after vaccination
- Decreased fertility in both males and females

Additional effects

- Increased antibody production
- Better resistance of younger animals through increased content in the colostrum (unspecific immunity)

2.2.10. Choline

Natural sources and bioavailability

Choline is present in all feeds. Feeds rich in choline are protein-based feeds of animal origin, yeasts and some extracted oilseed meals. Tapioca and corn have a poor choline content.

Choline from soybean meal is bioavailable to 60–70%. The bioavailability of choline from cereals is lower, and in the case of rapeseed meal falls to only 25%.

With a sufficient supply of methionine, serine, folic acid and vitamin B_{12}, choline can be produced in the liver. Young animals and broilers are not capable of producing sufficient choline quantities for their own requirements.

Physiological role

- Production of phospholipids (e.g. lecithin) and lipoproteins
- Transport and metabolism of fats
- Production of electrical signals in nerve cells (involved in the production of acetyl choline)
- In a phospholipid form, choline is a constituent in most cell types
- Methyl group donor in metabolism (other methyl group donors in metabolism are e.g. methionine and betaine)

Deficiency symptoms

- Functional disorders in fat metabolism and fatty liver
- Functional disorders in joints and bones (perosis of poultry, splayed legs in piglets, adult pigs sitting in a dog-like posture)
- Retarded growth, mainly of young animals
- Increased mortality in chicks

Additional effects

- Higher choline supplements may improve growth and feed conversion of high-fat rations, especially in broiler production.

2.3. Other vitamin-like substances

2.3.1. p-Amino-benzoic acid (PABA)

para-Amino-benzoic acid is a constituent of the folic acid molecule, and can be produced by green plants and various micro-organisms. For some micro-organisms, PABA is a growth promoter, but it also seems to fulfil certain tasks in the metabolism of higher organisms. In chickens receiving only marginal amounts of folic acid, PABA administration gives positive effects (growth and plumage).

PABA is found in a great number of feeds, so that animals should be able to cover their requirements from natural sources. Fish in particular have a high requirement for PABA. In trout this is around 100–200 mg per kg feed.

2.3.2. Betaine

Betaine acts as a methyl group donor in metabolism, and together with choline and methionine is a lipotropic factor (protection against fatty liver). However, it is not capable of replacing the other specific functions of choline and methionine. It is uncertain whether it participates in osmosis control under specific conditions.

2.3.3. Inositol

The physiological effects of this six-valent alcohol are largely unknown. It has a lipotropic effect in preventing fatty liver. Livestock are capable of producing sufficient quantities of inositol, and use it for the synthesis of phospholipids and lipoproteins.

To cure fatty liver syndrome in laying hens, supplements of 1000 mg per kg feed are added. For salmonids, 350–500 mg per kg feed is recommended.

2.3.4. Essential fatty acids (EFAs)

Essential fatty acids (EFAs) include the omega-3 fatty acids eicosapentaenoic acid (EPA 20:5), docosahexaenoic acid (DHA 22:6) and α-linolenic acid (18:3), and the omega-6 fatty acids linoleic acid (18:2), γ-linolenic acid (18:3) and arachidonic acid (20:4). Mammals are not capable of de novo synthesis of these molecules.

These fatty acids play an important role as constituents of membrane lipids and as prostaglandin precursors.

In modern fish farming, EFAs are vital. Nowadays, linoleic acid is a routine ingredient in commercial mixed feed formulations for laying hens.

Fatty acid deficiencies are manifested as disorders of the skin, water metabolism and reproduction. The correct ratio of omega-3 and omega-6 fatty acids in feed is important. Normally, the latter are present in excess quantities.

There is a metabolic correlation between unsaturated fatty acids and vitamin E. The availability of unsaturated fatty acids in the feed must be considered when the amount of supplemental vitamin E is determined.

2.3.5. Carnitine

L-Carnitine occurs in mammal muscles but also in yeast, wheat germs, fish and milk. The muscles contain approx. 85% of the total stores of L-carnitine, the blood plasma less than 1%. L-carnitine is mainly synthesised in the liver.

L-carnitine has a variety of functions, the most important one being its role as a carrier in fat metabolism to transport active fatty acids into the mitochondria for energy metabolism, and as a storage site for activated acetyl radicals. This function is of great importance when the muscles work extremely hard, in ketotic situations and during periods of hunger, and it represents the major part of the requirement.

Requirements for L-carnitine are increased during reproduction, in young animals, at high growth rates and when the liver metabolism is under stress.

2.3.6. Taurine

Taurine is to be found in all stuffs of animal origin, but never in stuffs of plant origin. In contrast to livestock, cats have a very restricted capability of synthesising this substance from cysteine.

In the organism, taurine is mainly linked to cholic acid (taurocholic acid). In the gall bladder, taurocholic acid is present as bile salt and takes part in fat emulgation by promoting fat degradation. Taurine also probably acts as an inhibiting neurotransmitter, plays an important role in the development of the central nervous system and influences the transport processes of 2-valent metal ions.

In cats, taurine deficiency will result in a degeneration of the photoreceptors in the eye and possible blindness. It is also involved in the development of cardiomyopathies as another deficiency symptom. Therefore, cats should receive 400 to 500 mg taurine per kg feed.

3. Vitamin supply

3.1. Basic considerations

Nowadays, demands for healthy and ecological animal nutrition are higher than ever before. One of the most important factors in modern animal nutrition is an optimal vitamin supply. During the past few decades, there have been fundamental developments in our knowledge of the vitamin requirements of livestock. Whereas in the fifties, the prime purpose of adding vitamins to feedstuffs was to protect animals from deficiency, nowadays animal health, ecology and economy are the most important aspects. The main objective of an optimised vitamin supply is to ensure health under practical conditions of animal husbandry.

3.1.1. Factors influencing vitamin supply

The vitamin supply is the amount of vitamins given to the animal in its feed, according to individual requirements. It is dependent on several factors:

- Animal
  - Species (e.g. cattle, pig, poultry, horse, fish, pets)
  - Age (e.g. chicken, dogs of old age)
  - Use (e.g. reproduction, production, hobby)
  - Performance (e.g. meat, milk, eggs, wool, leather, endurance, long life)
  - Progress in breeding
  - Health (e.g. in general, antioxidation, improved immunity)
  - Stress (e.g. animal groups, transport)
  - Animal welfare (e.g. protection against vitamin deficiencies, well being)
- Environment
  - Housing conditions
  - Hygiene (e.g. contamination with germs, mycotoxins)
  - Climate and weather conditions.
- Product quality
  - Improved stability toward oxidation (meat, milk, eggs) and improved processing quality (e.g. wool, leather)
- Feed
  - Natural variations in nutrients owing to growth periods, harvest, drying and storage
  - Biological availability (only 50% α-tocopherol in vitamin E from cereals, biotin availability for poultry and pigs only 10% in wheat)

- Vitamin antagonists (coumarin, thiaminases, avidin)
- Storage conditions and time
- Feed composition (content of energy, proteins, fat, minerals, trace elements, acids)
- Economic advantage
- Cost/benefit ratio

3.1.2. Vitamin requirements as a basis for optimum supply

The optimum vitamin supply is based on the animals´ requirements. In general, we distinguish between the minimum requirement, the optimum requirement and the additional specific requirement (improved immunity, meat quality etc.). Because of the many influencing factors and the fact that sufficient data are not available, a factorial approach of vitamin requirement is not possible in the same way as for energy or protein requirements. The influence of vitamins on specific metabolic activities is difficult to assess, often not precisely defined and sometimes not even known.

Minimum requirement: This safely protects the animal from deficiency symptoms under optimum conditions of housing and hygiene. The minimum requirement is normally established in scientific feeding experiments with specific diets under laboratory conditions.
Optimum requirement: This not only covers minimum requirements but will guarantee full performance potential, good health and resistance to disease.
Additional effects: Results from recent research show that apart from their main functions, many vitamins produce additional metabolic effects with a positive influence on animal health and fertility and on the quality of the animal products (Table 5).
Optimum supply: This is the vitamin quantity actually supplied in feed to the animals, depending on their optimum requirement. If an increased specific effect is to be achieved beyond the optimum requirement, an additional vitamin supply may be beneficial (see Figure 2).

3.1.3. Recommendations

Scientific laboratories, authorities, associations and companies offer varying recommendations for vitamin supply, which they base on different approaches for calculating vitamin requirements.

Table 5: Vitamins and their effects

Vitamin	Main effect	Additional effect
A	Protection of the epithelium	Fertility, cell metabolism, immunity
β-Carotene	Vitamin A precursor	Health, fertility
D	Metabolism of calcium and phosphorus	Immunity
E	Antioxidant	Health, immunity, quality of meat , milk, eggs
K	Blood coagulation	Protein carboxylation
B_1	Carbohydrate metabolism	Transmission of stimuli, nervous system
B_2	Energy metabolism	
B_6	Protein metabolism	Immunity
B_{12}	Blood production and protein metabolism	
Biotin	Carbohydrate and fat metabolism	Quality of skin, hair, horn
Folic acid	Carbohydrate and nucleic acid metabolism	Fertility
Niacin	Energy metabolism	Metabolic activity, ketosis protection
Pantothenic acid	Energy metabolism	
C	Antioxidant	Stress reduction, health, immunity
Choline	Fat metabolism, methyl group donor	Transmission of stimuli, nervous system

Many official recommendations (e.g. NRC, ARC, DLG) cover only the minimum requirement, which is not sufficient in normal practice. Housing conditions, hygiene, nutritional influences and general stress may considerably increase the animal´s requirements. Companies (e.g. breeding associations, producers of feeds and feed additives) therefore normally base their recommendations on the optimum requirement. Figure 3 gives an example of the optimum vitamin E-supply for finishing pigs with the additional objective of improving meat quality (oxidation stability) and offering a high-fat diet.

Figure 2 : Vitamin supply = optimum requirement (+ additional effects)

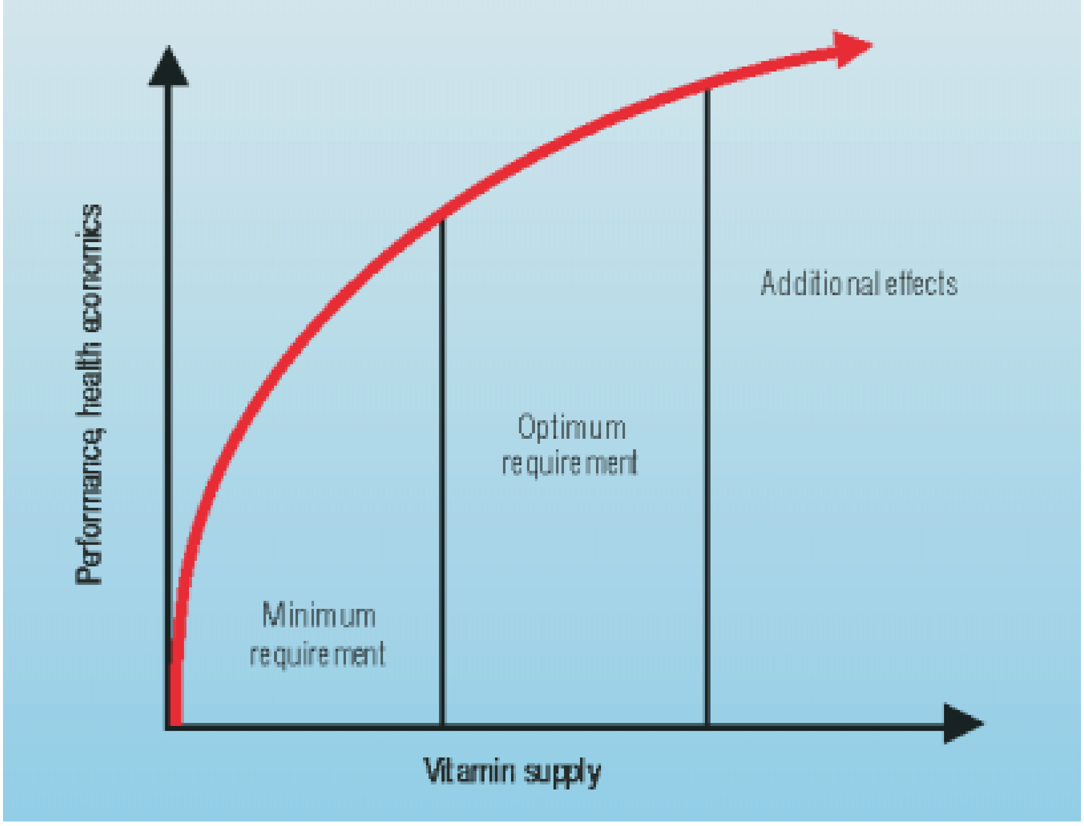
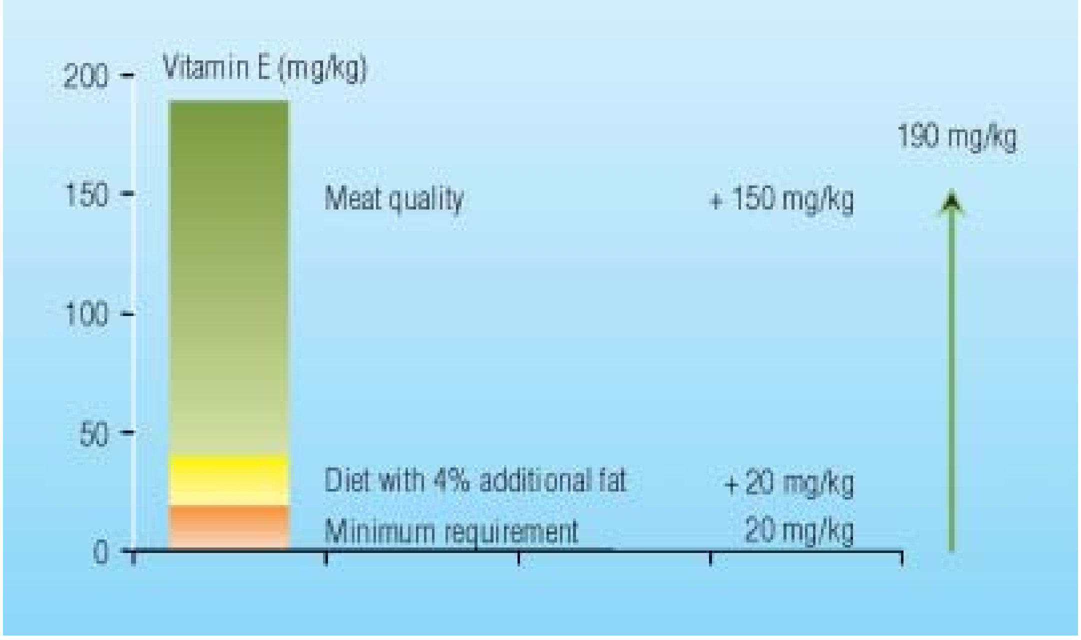


Figure 3 shows that a diet for finishing pigs with 4% additional fat should contain 190 mg vitamin E per kg feed, if improved meat quality is desired.
The vitamin content is also influenced by feed composition and production processes. The natural vitamin content may vary considerably within individual feedstuffs. For ruminants consuming forage in large amounts, the natural vit-

Figure 3: Example of an optimum vitamin E supply of a diet containing 4% additional fat for finishing pigs to improve meat quality



amin contents can be considered, e.g. of ß-carotene and vitamin E in grass and grass silage. In general, the natural vitamin content in mixed diets for poultry and pigs varies widely and can hardly be considered in calculations of a regular vitamin supply to the animals. Some feedstuffs even contain anti-nutritive or antagonistic factors, which limit or neutralise the effect of specific vitamins. Interactions between the individual feed ingredients must also be considered. If feed with a higher fat content or a higher content of polyunsaturated fatty acids (PUFAs) is consumed, the vitamin E requirements will rise. The manufacturing processes of mixed feed also influence the vitamin content. Chapter 4 will explain the impact of feed manufacturing technology (pelleting, expansion, extrusion) in detail.

3.1.4. Benefits and cost of vitamins

During recent years, vitamin supplements in feed have also been considered from an economic viewpoint. There are two models: one referring to the optimum requirement, the other to additional effects.

3.1.4.1. Optimum requirement

When calculating the economic benefit on the basis of the optimum requirement, the total costs of vitamin supplementation are set against the benefits gained from higher performance (milk, eggs, meat). Economic and practical experience have shown that a higher vitamin supply results in an increased economic benefit when performance and stress levels rise.

This is confirmed by a major study carried out in the USA (Coelho and Cousins, 1997) with finishing pigs. Five groups of 424 finishing pigs were given one of five different levels of vitamin supplement (Table 6). The lowest level in supplementation, corresponding to the American NRC recommendation (vitamin supplementation taking native contents in consideration) was fed to group A. The other groups B-E contained increasing amounts of vitamins according to information from feed manu-

Table 6: Vitamin supplementation regimes (amount per kg feed)

	Dietary groups				
Vitamin	**A**	**B**	**C**	**D**	**E**
Vitamin A (IU)	418	3 300	5 500	8 470	10 560
Vitamin D (IU)	176	550	1 100	1 760	2 200
Vitamin E (mg)	1.3	11.0	21.3	38.0	47.4
Vitamin K_3 (menadione) (mg)	0.6	0.8	1.9	4.3	5.4
Vitamin B_1 (mg)	-	-	0.6	1.6	1.9
Vitamin B_2 (mg)	-	2.6	4.2	6.1	7.7
Vitamin B_6 (mg)	-	-	1.0	2.3	2.9
Vitamin B_{12} (µg)	5	13	21	29	36
Biotin (µg)	-	-	70	190	240
Folic acid (mg)	-	-	0.3	1.2	1.5
Niacin (mg)	-	17.5	26.1	38.5	48.1
D-Pantothenic acid (mg)	2.0	11.9	16.3	22.3	27.8

Coelho and Cousins, 1997

facturers based on practical experience. All five groups were exposed to low, average and high stress levels (Table 7).

Economic evaluation was based on fattening results (daily growth, feed utilisation) and sales prices depending on

Table 7: Experimental stress factors

Stress factor	**Low**	**Average**	**High**
Density (m^2/pig)	2.75	2.05	1.65
Pigs per pen	3	4	5
E. coli[1] challenge (organisms per pig)	0	500 000	1 000 000
Salmonella[1] challenge (organisms per pig)	0	100 000	200 000
Mycotoxin[2] challenge (ppm)	0	50	100
Nutrient content of feed	Low[4]	Average[5]	High[6]

[1] E. coli and salmonella field strains from local farm, oral administration on 7th day of experiment
[2] Mycotoxins, fusarium strains B_1, B_2 and B_3
[4] 13.4 MJ (3197 kcal) ME, 12.4% protein
[5] 14.3 MJ (3417 kcal) ME, 13.0% protein
[6] 15.2 MJ (3638 kcal) ME, 13.8% protein

Coelho and Cousins, 1997

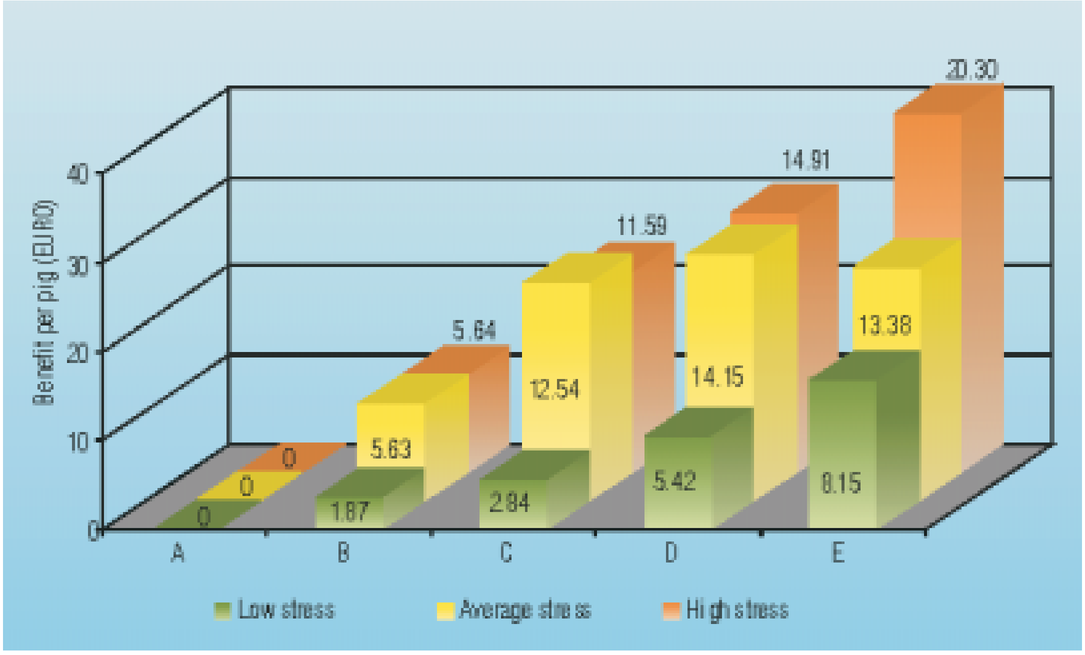


Figure 4: Economic benefit of vitamin supplementation in pig fattening experiments at various stress levels

carcass quality (Figure 4).

This experiment shows the economic advantage of a higher vitamin supply when stress levels rise. Similar results have been obtained with broilers and turkeys.

3.1.4.2. Additional effects

To evaluate the economic benefit of additional effects, the effect of an individual vitamin or vitamin complex (e.g. the antioxidant effect of vitamin E, C and ß-carotene) on a specific aspect of performance is examined. This requires precise and reproducible data to be monitored from as many animals as possible. Some traditional applications are e.g. ß-carotene and fertility in cattle, biotin and hoof health in cattle, vitamin E and mastitis in dairy cows or vitamin E and improved resistance to disease. Practical experience from ß-carotene and fertility in cattle demonstrates that the benefits outweigh the costs by far when feed low in ß-carotene is supplemented with ß-carotene. The economic benefits lie in fewer inseminations, shorter calving intervals, longer active life, lower veterinary costs and healthier calves.

3.2. Native vitamin contents of forages and commercial feedstuffs

The native contents of vitamins in feedstuffs vary considerably. Next to climate, species, site of growth and use of fertiliser, the main influences are storage and treatment, especially in fresh feeds. The figures given in Table 8 for the vitamin content of various feedstuffs are therefore only to be seen as a guideline. Furthermore, biotin, niacin and choline are only partially bioavailable. The vitamins A, D_3 and C are practically absent from feedstuffs and are hence not listed. For practical reasons, only ß-carotene and vitamin E are listed with average values and variations for forages. The values for commercial feedstuffs refer to the air-dry substance, those for forages to 100% dry matter.

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Table 8:
Average vitamin content of various feedstuffs

Commercial feed-stuffs (amount per kg air-dry substance)	ß-Carotene mg	Vit. E mg	Vit. K mg	Vit. B_1 mg	Vit. B_2 mg	Vit. B_6 mg	Vit. B_{12} µg	Biotin µg	Folic acid mg	Niacin mg	Panto-thenic acid mg	Choline mg
Barley	< 5	8		10	2	4		150	0.5	55	8	1050
Beans		5		6	2	3		90		25	3	2310
Beet pulp, dried	< 1			< 1	< 1	1				20	1	520
Brewer´s yeast, dried		2		100	38	45		1050	15.5	445	110	3800
Feed yeast, dried	< 1			7	49	27		1170	17.4	495	80	3070
Grass meal	100	75	< 1	4	14	9		220	3.5	59	18	1020
Oats	< 1	9		6	2	2		210	0.4	15	12	1060
Lucerne green meal	120	100	16	4	15	7		320	4.2	40	29	1440
Maize	< 5	9	< 1	4	1	5		70	0.3	20	6	500
Maize gluten feed	8	14		2	2	14		270	0.3	75	16	1870
Molasses, beets	< 1	4	< 1	2		7		50	0.2	50	5	990
Peas	< 1	5		7	2	2		200	0.4	30	24	1550
Rapeseed meal	< 1	15	2	3	4	10		940	2.4	150	10	6850
Rye	< 1	15		4	2	3		60	0.6	15	9	450
Sorghum	< 1	8	< 1	4	1	4		260	0.2	40	11	700

Table 8 (continued):
Average vitamin content of various feedstuffs

Commercial feed-stuffs (amount per kg air-dry substance)	ß-Carotene mg	Vit. E mg	Vit. K mg	Vit. B_1 mg	Vit. B_2 mg	Vit. B_6 mg	Vit. B_{12} µg	Biotin µg	Folic acid mg	Niacin mg	Panto-thenic acid mg	Choline mg
Soybean meal	< 1	3		4	3	5		330	2.5	30	15	2800
Sunflower meal	< 1	11		3	4	13		1040	1.5	205	29	3310
Tapioca	< 1			< 1	< 1	1		30	0.2	10	6	
Triticale	< 1	9			< 1							
Wheat	< 1	12	< 1	5	2	4		100	0.4	55	11	840
Wheat bran	< 5	17	< 1	8	4	10		280	2.0	210	29	1200
Fish meal		10	2.4	< 1	8	8	280	300	0.8	75	13	4010
Meat meal		1		< 1	4	2	100	50	4	0.9	150	2050
Skim milk powder		5		4	20	4	40	10	34	0.5	290	1440
Whey powder		0		4	29	3	20	10	54	0.8	380	1910

Table 8 (continued):
Average vitamin content of various feedstuffs

Forages (amount per kg dry matter)		ß-Carotene mg	Vit. E mg	Vit. K mg	Vit. B_1 mg	Vit. B_2 mg	Vit. B_6 mg	Vit. B_{12} µg	Biotin µg	Folic acid mg	Niacin mg	Panto-thenic acid mg	Choline mg
Grass	Average	200	200										
	Range	100-400	100-400										
Grass silage	Average	100	60										
	Range	20-250	10-200										
Grass hay	Average	20	30										
	Range	0-100	5-80										
Alfalfa	Average	250	150										
	Range	100-500	50-300										
Alfalfa hay	Average	50	20										
	Range	10-150	5-60										
Maize silage	Average	20	15										
	Range	0-50	5-40										

3.3. AWT recommendations for vitamin supply

The AWT recommendations in Table 9 are based on optimum supply. Additional supplementations for specific effects are marked and explained in the footnotes. If the animals are mainly fed a compound feed (e.g. poultry, pigs), recommendations are indicated per kg of feed. If a basal feed is supplemented with a concentrate (e.g. ruminants, horses), recommendations are given per animal and day or per 100 kg body weight and day.

Recommendations are based on following factors: vitamin E supplements are indicated as vitamin E acetate, vitamin B_1 as vitamin B_1 mononitrate or vitamin B_1 hydrochloride, and vitamin B_6 as hydrochloride. For further information, see Chapter 6.

3.4. Vitamin interactions

Since vitamins have multiple functions in metabolism, they interact with many other nutrients, including other vitamins, minerals and trace elements, amino acids and proteins, fats and fatty acids, certain plant substances with antagonistic effects and some orally administered drugs to cure diseases (e.g. sulphonamides).

Especially the interactions between the fat-soluble vitamins A and E have been the subject of scientific investigation. It was found that they can influence each other in the following ways:

- In digestion in connection with other fat components in the feed
- In the absorption of fat-soluble substances in the intestine
- Through competition in intermediary metabolism during plasma transport, intracellular uptake and intracellular transport

Tests have shown that only extremely high quantities of vitamin A or E have an adverse effect on utilisation. A very high vitamin A level in the feed reduces the vitamin E content of plasma and liver. When vitamin A and E are given according to AWT recommendations, no interactions will occur.

Table 9:
AWT recommendations for vitamin supplementation (amounts per kg compound feed)

Poultry

	A	D_3	E	K_3 (Mena-dione)	B_1	B_2	B_6	B_{12}	Biotin	Folic acid	Niacin	D-Panto-thenic acid	C	Cho-line
	IU	IU	mg	mg	mg	mg	mg	µg	µg	mg	mg	mg	mg	mg
Chicks starter	12 000–15 000	2 500–3 000	40–60 150–200 1)	2–4	2–3	8–10	4–6	20–40	100–150	1–2	40–60	10–15	100–200 2)	400–700
Pullets	8 000–10 000	1 500–2 500	20–30	1–3	2–3	4–6	3–5	10–20	80–120	1	30–50	8–10	100–150 2)	250–400
Laying hens	8 000–12 000	2 000–3 000	20–30	2–3	2–3	5–8	3–5	15–25	50– 80	1	25–40	8–10	100–150 2)	300–500
Breeding hens	12 000–15 000	2 500–3 500	40–60	2–3	2–3	8–10	4–6	20–30	100–200	1–2	40–60	10–15	150–200 2)	400–600
Broilers	8 000–12 000	2 500–4 000	30–50 150–200 3)	2–3	2–3	5–7	3–5	15–25	100–150	1	30–50	10–12	100–150 2)	300–600
Turkey starter	10 000–14 000	3 500–5 000	40–60 150–200 1)	2–3	3–4	10–14	5–7	30–40	250–300	2–3	70–100	15–20	100–200 2)	800–1 200
Turkey grower	8 000–12 000	3 000–4 000	30–50	2–3	2–3	6–8	3–5	20–30	200–250	2	60–80	10–15	100–150 2)	600–800

Table 9:
AWT recommendations for vitamin supplementation (amounts per kg compound feed)
Poultry

	A	**D_3**	**E**	**K_3 (Mena-dione)**	**B_1**	**B_2**	**B_6**	**B_{12}**	**Biotin**	**Folic acid**	**Niacin**	**D-Panto-thenic acid**	**C**	**Cho-line**
	IU	**IU**	**mg**	**mg**	**mg**	**mg**	**mg**	**µg**	**µg**	**mg**	**mg**	**mg**	**mg**	**mg**
Turkey finisher	8 000–12 000	2 500–3 500	30–40 150–200 3)	2–3	2–3	4–6	3–5	15–25	150–200	1	40–60	8–12	100–150 2)	500–700
Turkey breeder	12 000–15 000	3 000–4 000	40–60	2–3	2–3	8–10	4–6	25–35	300–400	2–3	60–80	10–15	100–200 2)	500–700
Ducks/geese	10 000–13 500	2 500–3 500	40–60	2–4	2–3	6–8	4–6	25–35	100–150	1	40–60	8–12	100–150 2)	400–600

1) For improved immunity
2) Under stress and/or for improved reproductive performance in breeding hens
3) For improved meat quality

Table 9:
AWT recommendations for vitamin supplementation (amounts per kg compound feed)
Pigs

	A	D_3	E	K_3 (Menadione)	B_1	B_2	B_6	B_{12}	Biotin	Folic acid	Niacin	D-Pantothenic acid	C	Choline	ß-Carotene
	IU	IU	mg	mg	mg	mg	mg	µg	µg	mg	mg	mg	mg	mg	mg
Piglet pre-starter	15 000–20 000	1 800–2 000	80–120 200–250[1]	2–4	3–4	6–8	5–7	40–60	250–350	2	40–50	15–20	150–200	400–600	
Piglet starter	12 000–20 000	1 500–2 000	70–100	2–3	2–3	5–7	4–6	30–50	150–250	2	30–40	12–16	100–150[3]	300–500	
Grower	10 000–12 000	1 500–2 000	60–80	2–3	2	4–6	3–5	20–30	100–150	1	20–30	10–14	50–100[3]	250–400	
Finisher	8 000–10 000	1 000–1 500	40–60 150–200[2]	2	1	3–5	2–4	15–25	50–80	1	15–25	8–12	50–100[3]	200–350	
Breeding animals	12 000–20 000	1 500–2 000	60–80	2	2	5–7	4–6	20–30	200–300	2–3	30–40	12–16	150–200[3]	300–500	300[4]

[1] For improved immunity
[2] For improved meat quality
[3] Under stress and/or for improved reproductive performance
[4] For improved fertility from weaning and successful new mating (mg/animal and day)

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Table 9:

AWT recommendations for vitamin supplementation (amounts per head or per 100 kg body weight and day)

Ruminants and horses

	A	D_3	E	K_3 (Mena-dione)	B_1	B_2	B_6	B_{12}	Bio-tin	Folic acid	Nia-cin	D-Pan-tothenic acid	C	Choline	ß-Carotene
	IU	IU	mg	mg	mg	mg	mg	µg	µg	mg	mg	mg	mg	mg	mg
Calves, milk replacer[1]	20 000-25 000	2 500-3 000	80-120 150-200[2]	2	8-10	4-6	3-4	20-30	100-200	1	30-40	10-20	150-250	200-300	100[3]
Heifers	30 000-50 000	3 000-5 000	100-150		10-20										
Fattening cattle	40 000-70 000	4 000-7 000	200-300 1 000-1 500[4]		30-50						1000-2000				
Dairy cows	80 000-120 000	8 000-12 000	200-400 1 000-1 500[5]								4000-6000				200-300
Sheep/goats[8]	4 000-8 000	400-800	50-80												

Table 9 (continued):

AWT recommendations for vitamin supplementation (amounts per head or per 100 kg body weight and day)

Ruminants and horses

	A	D_3	E	K_3 (Menadione)	B_1	B_2	B_6	B_{12}	Biotin	Folic acid	Niacin	D-Pantothenic acid	C	Choline	ß-Carotene
	IU	IU	mg	mg	mg	mg	mg	µg	µg	mg	mg	mg	mg	mg	mg
Foals [9]	10 000-12 000	1 000-1 200	100-120	3	8-10	8-12	6	60-80	200-300	6	10-20	8-10	150-250	200	
Leisure horses [9]	6 000-8 000	600-800	60-80	2	6-8	6-8	4	50-70	200[10]	4	10-15	6-8	150-250	100	
Race and breeding horses [9]	12 000-15 000	1 200-1 500	200-300	3	8-12	8-12	6	60-80	200-300[10]	8	15-25	10-12	300-400	200-300	400-500[11]

[1] Values per kg feed
[2] For improved immunity
[3] During the first 14 days following colostral milk phase
[4] For improved meat quality 100 days before slaughtering
[5] For improved udder health during dry periods and during the first 10 weeks of lactation
[6] Two weeks before calving until period of maximum lactation
[7] Two weeks before calving until successful insemination, low ß-carotene basal feed
[8] Per head and day
[9] Per 100 kg body weight and day
[10] For improved hoof quality 15 000–20 000 µg/animal/day for at least 6 months
[11] Values per animal and day, 4 weeks before until 10 weeks after birth

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Table 9:

AWT recommendations for vitamin supplementation (amounts per kg compound feed)

Pets and fish

	A	D_3	E	K_3 (Menadione)	B_1	B_2	B_6	B_{12}	Biotin	Folic acid	Niacin	D-Pantothenic acid	Choline	C	ß-Carotene
	IU	IU	mg	mg	mg	mg	mg	µg	µg	mg	mg	mg	mg	mg	mg
Dogs	8 000-12 000	800-1 200	80-120	1	2-4	4-6	3-5	30-40	150-250	1	20-25	8-10	1 000-1 200	80-120	
Cats	15 000-25 000	1 000-1 800	100-150	1	6-10	5-8	3-5	30-40	150-250	1	45-60	10-14	1 200-1 400	80-120	
Rabbits	8 000-12 000	800-1 200	30-50	1	1-2	3-6	2-3	10-20	50-100	1	40-60	10-14	600-800		20
Minks	8 000-12 000	800-1 200	80-120	1	6-10	6-10	6-10	30-40	300-400	1	20-40	10-14	600-800	80-120	
Trout and salmon	7 000-9 000	1 500-2 000	180-250	6-8	10-15	20-30	10-15	30-40	800-1 000	5-7	140-160	40-50	800-1 000	150-250	
Carp	8 000-10 000	1 500-2 000	150-200	4-6	8-12	10-20	8-12	20-30	600-800	3-5	60-80	30-40	600-800	150-250	

3.5. The safety of vitamins

Only the continuous availability of vitamins to the metabolism guarantees that many vital functions of the animal organism can be maintained. Vitamins have shown their efficacy as feed supplements over many decades. From a chemical point of view, vitamins are organic substances with a rather simple molecular structure, with the exception of vitamin B_{12}. Because to their low molecular weight, their potential as allergens can be taken as minimal. In general, vitamins are considered to be substances with a high degree of safety during manufacture and application.

European legislation has set up maximum levels in feed for vitamins A and D. The limited vitamin A content of compound feed for fattening animals is based on the fact that animals have a high physiological storage capacity (>90% of the supply) for vitamin A in the liver. The vitamin A content of the animal liver is therefore dependent on the vitamin A supply through the feed. The consumption of liver can therefore lead to excess intake levels of vitamin A in humans.

The restrictions on vitamin D are due to its capability to mobilise and deposit calcium in the blood vessels. All other vitamins can be consumed by animals in quantities that may exceed the AWT recommendations by far without any adverse effects. The short-term administration of higher vitamin supplements is easily tolerated.

4.1. Vitamin production

There are three main production processes for vitamins:

- Chemical synthesis
- Fermentation
- Isolation from substances of plant or animal origin

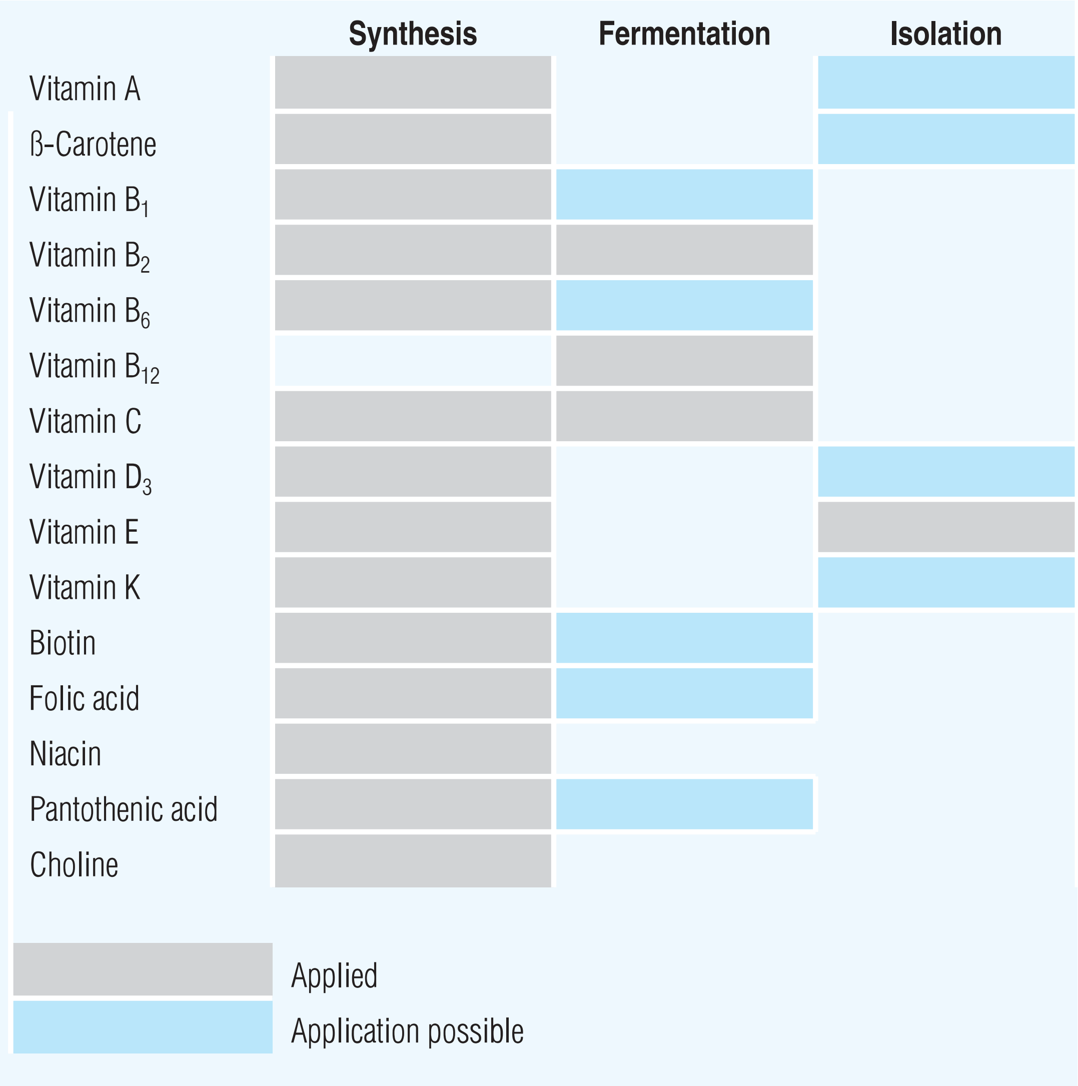
The vitamins used in animal nutrition are almost exclusively produced by the first two methods. Because of their cost, vitamins extracted from plants or animal products are mainly used in human nutrition.

Even though chemical synthesis is nowadays the major source of vitamin production, fermentation is expected to become more and more important. Although some vitamins, such as vitamin B_2, can be produced by either process, fermentation is normally preferred, whereas 10 years ago, chemical synthesis was the only way of obtaining vitamin B_2. Vitamin B_{12} has a very complicated structure and is therefore almost exclusively obtained by fermentation.

In the fermentation process, suitable micro-organisms capable of producing the desired vitamin are identified and selected. The vitamins are then separated from the fermentation broth and purified. Genetic engineering allows the productivity of the micro-organisms to be increased. At the same time, it reduces the burden on the environment and results in a better use of resources. Figure 5 lists the various production methods.

Chemical synthesis is normally based on raw materials such as crude oil or gas. These materials are split into small units, which are subsequently recombined in multistep processes to form the desired vitamin. The synthesis of vitamin A takes more than 15 process steps.

Whether vitamins are obtained by fermentation or chemical synthesis, they are identical to those occurring in nature, and therefore produce the same biological effects. Synthetic vitamins are sometimes superior to natural ones, since some, e.g. biotin, niacin and choline are only available to a limited extent because of the nature of their chemical bonds.



	Synthesis	Fermentation	Isolation
Vitamin A	▨		🟦
ß-Carotene	▨		🟦
Vitamin B_1	▨	🟦	
Vitamin B_2	▨	▨	
Vitamin B_6	▨	🟦	
Vitamin B_{12}		▨	
Vitamin C	▨	▨	
Vitamin D_3	▨		🟦
Vitamin E	▨		▨
Vitamin K	▨		🟦
Biotin	▨	🟦	
Folic acid	▨	🟦	
Niacin	▨		
Pantothenic acid	▨	🟦	
Choline	▨		

▨ Applied

🟦 Application possible

Figure 5: Technical processes of vitamin production

Chemical synthesis is, however, only a part of the whole production process. Before vitamins are actually used, they normally need to be formulated, in order to guarantee their beneficial properties and to rule out possible problems. A most important aspect is the stability of vitamins during the various application processes and in the final premixes and compound feeds. The vitamins A, D_3 and E are obtained by synthesis in the form of oils and must be converted into powders before they can be applied in the feed industry. Normally, modern spray-drying processes are used, accompanied by methods to improve stability. Sometimes this is also done afterwards, e.g. in the case of vitamin A products by crosslinking.

In vitamin formulation, manufacturers need to fulfil various requirements. The most important are listed in Table 10.

4.2. Commercial forms and quality criteria

The choice of the most appropriate vitamin product form for an individual application depends in each individual case on product price, planned use, processing, and the estimated shelf life of the mixed feed. Nearly all vitamins are sold in various commercial forms, which can differ considerably in their application properties. The most important distinctive properties and criteria under practical aspects are:

- Solubility or dispersability of the product in water in various fields of application (milk substitutes, administration in drinking water)
- Stability during special processing (expansion, extrusion)
- Product behaviour in view of specific technical conditions during processing (particle size, hygroscopic or electrostatic behaviour etc.)

The classification of vitamins as fat-soluble and water-soluble only refers to pure vitamins, not the solubility of commercial vitamin products.

Formulations of fat-soluble vitamins are not necessarily fat-soluble, since they may contain formulation aids. Likewise, formulations of water-soluble vitamins are not always water-soluble, since they may contain insoluble matter such as carriers.

Table 10: Prerequisites of vitamin products for the feed industry

Criteria	Requirements
Concentration	At least as indicated
Stability	No or minor losses during processing and storage
Bioavailability	A high biological availability is necessary
Flowability	No lumps, free flowing
Appearance	Typical colour and particle size
Dust	Low or minor dusting
Particle size	Narrow distribution
Mixability	Good behaviour during mixing, no demixing tendency

Vitamins of the B group are often supplied as pure substances, i.e. their vitamin content is a minimum of 95%. Commercial vitamin B_2 is normally produced by fermentation and offered as a product with a vitamin B_2 content of 80%. Biotin and vitamin B_{12} are normally sold at concentrations of 1 or 2%, owing to the small amounts necessary to fulfil requirements.

The fat-soluble vitamins A, D_3 and E are normally offered as formulations, since they are obtained as oils at the end of the manufacturing process and must be converted into powders for practical use in mixed feed. For special applications, e.g. in veterinary products, they are also used in their oily form.

Various formulation processes are available and are necessary e.g. to convert liquids into powders, to adjust the desired concentration and to improve product stability by the use of additives or through the process itself.

Of major importance are granulation and spraying techniques, next to chemical modifications of the active substance (e.g. vitamin E-acetate instead of vitamin E-alcohol) and adjustment of the appropriate particle size.

There are two general spraying techniques: spray-drying and spray-formulation. Spray-drying produces fine powders with particle amounts exceeding one million per gram, whereas spray-formulation processes produce coarser particles, so-called beadlets.

Spray-formulation is a very important process in vitamin A production, since it allows the manufacture of products with particle diameters of 0.1–0.5 mm, which corresponds to 100 000–200 000 particles per gram.

An important step in vitamin A formulation consists in a subsequent crosslinking (hardening) of the matrix to stabilise the product and facilitate its application in premixes and mineral feeds, and during processing such as pelleting and extrusion. This method essentially consists of a Maillard reaction to combine or crosslink the proteins and sugar molecules, thus making the product form insoluble in water.

Processes which cover the active substance with an additive or embed and thus protect it from outside influence are known as coating processes. The final products are referred to as »coated«.

When water-soluble vitamins are offered as pure substances, the concentrations given to the dry matter, which means that the indicated values must be converted into those of the original substance. However, in general no major changes in concentration will result, since most of the vitamins have a very low water content, with the exception of folic acid. Since this vitamin contains about 8% chemically bound water, the converted value is lower than the original.

For B-group vitamins with a declared active substance of 80% or below, this figure refers to the original substance.

4.2.1. Commercial fat-soluble vitamins

4.2.1.1. Vitamin A

Vitamin A is produced in three types of formulation, with differing solubility in water:

1. Beadlets, which are insoluble in water as a result of a crosslink process. These products are very stable in all mixes and applications.
2. Coated products, which are not crosslinked and are water-dispersible. They are less stable.
3. Spray-dried fine powders, which are readily dispersible in water and are specially formulated for liquid mixes.

The active substance of these products is vitamin A acetate. The standard vitamin A content is normally 500 000 IU/g, apart from the USA where it is 650 000 IU/g.

Next from pure vitamin A products, combinations of vitamin A and vitamin D_3 are offered. In Europe, these usually have a vitamin A content of 500 000 IU/g and a vitamin D_3 content of 100 000 IU/g. There are two different product qualities on the market:

1. Products based on a mixture of oily vitamin A and oily vitamin D_3. And which therefore contain the same quantity of vitamin A and vitamin D_3 in each particle. These products are crosslinked, which offers stability advantages even for vitamin D_3.

2. Products based on a mixture of vitamin A particles and vitamin D_3 particles. Normally, these mixtures contain non-crosslinked vitamin A and vitamin D_3 formulations, since there are no crosslinked vitamin D_3 forms.

Vitamin A is very sensitive to oxidation, and the manufacturer therefore requires considerable experience in formulation technology. Sometimes only highly stabilised products are able to fulfil the necessary stability requirements in mixes (mixed feed, premixes, mineral feed).

4.2.1.2. ß-Carotene

Commercial products have a minimum ß-carotene content of 10% (100 000 mg/kg) and are offered as beadlets. They are not crosslinked and are dispersible in water.

4.2.1.3. Vitamin D_3

Vitamin D_3 formulations normally have a vitamin D_3 content of 500 000 IU/g; others are of minor importance. All products are water-dispersible or offered as combinations with vitamin A as mentioned above.

Most vitamin D_3 formulations are fine powders. They are sometimes also offered in beadlet form with a slightly coarser structure. Dispersibility in water is slightly reduced, but these types generally offer improved stability.

4.2.1.4. Vitamin E

With only a few exceptions, vitamin E products have a vitamin E content of 50%. There are two different types:

- Vitamin E adsorbates
- Spray-dried vitamin E formulations

Vitamin E adsorbates consist of oily vitamin E acetate (dl-α-tocopheryl acetate), which is bound to precipitated silica. This is a mineral with numerous small cavities into which the oily vitamin E penetrates and where it is adsorbed. This "rather simple" method is possible since vitamin E acetate is a very stable substance (in contrast to vitamin E alcohol).

Spray-dried vitamin E is water-dispersible. Under specific conditions, e.g. with high MgO contents in premixes and mineral feeds, these formulations show an improved stability.

4.2.1.5. Vitamin K_3

Vitamin K_3 is offered in three different product forms:

1. Menadione sodium bisulphite (MSB), with a minimum content of 50% menadione
2. Menadione pyrimidinol bisulphite (MPB), with a content of approx. 23% or 43% menadione
3. Menadione nicotinamide bisulphite (MNB), with a content of 23% or 43% menadione and 16% or 31% nicotinamide

Stability is the most important distinctive feature of these three types. Normally, the highest vitamin losses occur with MSB, which is the only commercial K_3 vitamin that is completely water-soluble. MPB is more stable in most mixes, but the highest stability of all is obtained with MNB, which demonstrates its superiority mainly in premixes and mineral feed.

In extrusion and expansion processes, these three products hardly show any stability differences which is quite in contrast to other vitamin types (e.g. vitamin A, vitamin C).

In practical use, it is not always quite clear whether the reference is to vitamin K_3 as the active substance or to one of the commercial types. Concentrations should always be indicated as active substance. The general opinion is that with regard to the menadione content, the biological effect of the various vitamin K_3 products is identical.

4.2.2. Commercial water-soluble vitamins

4.2.2.1. Vitamin B_1

Vitamin B_1 is offered in two commercial forms: vitamin B_1 mononitrate and vitamin B_1 hydrochloride. Both forms have a minimum vitamin B_1 content of 98%.

Vitamin B_1 hydrochloride has a higher solubility in water than vitamin B_1 mononitrate. If this is not an issue for the user, vitamin B_1 mononitrate should be used in mixtures, since it is more stable.

4.2.2.2. Vitamin B_2

Nowadays, mainly vitamin B_2 from fermentation processes with a minimum vitamin B_2 content of 80% is used. The remaining 20% consist of carrier or residues from the fermentation process.

Vitamin B_2 from chemical synthesis is also available. Its vitamin B_2 content of 96% is sometimes diluted to a concentration of 80%.

Because of their specific formulation, products obtained from fermentation are easier to process than those obtained from chemical syntheses. For example, the former are antistatic, which definitely prevents sticking to the processing machinery

4.2.2.3. Vitamin B_6

The commercial product is a pure product with a content of at least 98% vitamin B_6 hydrochloride.

4.2.2.4. Vitamin B_{12}

Vitamin B_{12} is normally offered as a 0.1% or 1% blend with calcium carbonate as a carrier. The active substance of vitamin B_{12} is cyanocobalamin, but several manufacturers offer vitamin B_{12} products with various other vitamin B_{12} active compounds in various concentrations.

2.2.2.5. Biotin

Biotin is normally sold as a 2% product. Most products are spray-dried, are readily soluble in water and have a very high particle number, which guarantees homogeneous mixing in mixed feeds even at low supplementation levels. Next to spray-dried products, biotin blends with a carrier are also offered. Since the biotin particles in these products are coarser, the active substance is not always homogeneously mixed in the feed.

4.2.2.6. Folic acid

Folic acid is offered as a pure substance with 95% or 80% active substance. 80% products have better processing properties, e.g. they show better flowability and reduced dusting.

4.4.2.7. Niacin (nicotinic acid/nicotinamide)

The minimum vitamin concentration in commercial products is 99%. The solubility of nicotinamide in water is higher than that of nicotinic acid.

4.4.2.8. Pantothenic acid

Producers offer calcium D-pantothenate with 98% active matter, and calcium DL-pantothenate with approx. 45% active matter (calcium D-pantothenate). Since animals can only assimilate the D-form, only the D-part can be considered when the animal supply is calculated. Another reason for preferring calcium D-pantothenate to DL-products is because it is easier to process and has a better biological effect.

4.2.2.9. Vitamin C

Nowadays, three types of vitamin C are in practical use:

1. Crystalline vitamin C (pure ascorbic acid), with a vitamin C content of at least 99%
2. Coated (formulated) vitamin C, with a vitamin C content between 50 and 97.5%
3. Vitamin C phosphate, with a vitamin C content of 25–45%

In heat-treated mixed feeds, premixes or mineral feeds, pure vitamin C is rather unstable. Depending on the processing methods and shelf life, coated products or even vitamin C phosphate should be preferred. Up to a few years ago, vitamin C sulphate was also recommended as a heat-stabilised product; however, owing to its poor biological effect, this substance is no longer used.

Coated products are normally more stable in premixes than crystalline vitamin C, although the individual products show significant differences depending on their stabilising matrix. This also applies to stability in pelleted feed.

In extruded and expanded feed, the use of vitamin C phosphate is recommended in order to reduce losses to a minimum even after prolonged storage.

4.2.2.10. Choline

Choline is available as aqueous solutions of 75% choline chloride or as adsorbates on an organic or inorganic carrier with a content of 50%, 60% or more.

Liquid choline chloride (75%) is designed to be used in mixed feed and should be restricted to this application when used in dry mixes.

Choline powders are mainly used in premixes and mineral feeds; however, they can also be used in mixed feeds. The choice of the carrier — organic (corncob or dry chips) or inorganic (silica) — is mainly determined by the processing conditions. In milk replacers, choline adsorbed onto silica is generally preferred, since it is finer and whiter.

4.3. Stability in feed

For various reasons (improved digestibility and hygiene, better feed consumption), mixed feed manufacturers frequently use production methods which have a negative influence on the stability of additives. For technical reasons, additive premixes are often used. They contain highly concentrated active substances that may react with one another.

One of the main ambitions of vitamin producers is to find appropriate processes that will ensure good stability of vitamins during all conditions of processing and storage. The most important methods are:

- Synthesis of stable compounds, e.g. α-tocopheryl acetate instead of unstable α-tocopherols
- Coating by spraying processes, as in vitamin A and vitamin D_3 dry powders

- The use of antioxidants and other stabilising agents, e.g. ethoxyquin (EQ) and butyl hydroxytoluene (BHT) in vitamin formulations sensitive to oxygen
- Matrix crosslinking, to make the product insoluble in water and thus more stable

During processing, vitamin stability is mainly influenced by the following factors:

1. Processing

- Mixing
- Conditioning
- Pelleting
- Expansion
- Extrusion

2. Mixture composition

- Vitamin concentrate
- Mixed feed
- Mineral feed
- Premixes (vitamins and trace elements).

3. Storage conditions and duration
4. Quality of vitamin formulation

Vitamin stability can also be influenced by chemical and physical factors, e.g.

- Humidity
- Temperature
- Pressure
- Acidity and alkalinity (pH)
- Oxidising and reducing agents (e.g. trace elements)

Because of the many »stress factors« that can affect vitamins, it is only possible to indicate average values of vitamin losses in specific production processes or for different mixture compositions. Above all, this applies to premixes with their great variety of constituents and compositions. In production processes, conditioning time and temperatures during pelleting, expansion and extrusion may vary considerably.

In general, losses will increase with temperature and time.

The water content of premixes and feeds has an adverse influence on vitamin stability. In the European Union, the maximum permitted water content of mixed feeds is 14%; however, only a considerably higher level is expected to have an impact on vitamin stability. In premixes and mineral feeds, however, even small quantities of water can result in measurable vitamin losses. The addition of molasses and other aqueous ingredients for dust prevention should be reduced to a minimum.

Some trace-element compounds contain large amounts of crystallisation water. For example, iron sulphate heptahydrate contains 45% crystallisation water, whereas iron sulphate monohydrate has a lower content of 10% and is therefore preferable for mixtures. In premixes, 3–4% free water should not be exceeded.

The loosely bound crystallisation water is sometimes released during storage. Prolonged and continuous heat or the action of hygroscopic constituents such as choline chloride or betaine will increase the content of free water in a mixture, resulting in an increased reactive potential and higher vitamin degradation.

Shelf life and storage temperatures are further influencing factors. The longer products are stored and the higher the storage temperature, the higher the final losses will be.

4.3.1. Individual vitamins

When stored at low temperatures, individual pure vitamins are stable for at least one year. Manufacturers give a stability guarantee, which varies and depends on the vitamin type.

4.3.2. Vitamin premixes

If a carrier with a low water content (max. 8%) is used, there are no or very small vitamin losses during the normal six months of the stability guarantee. For stability reasons, vitamin premixes should not contain any choline chloride.

4.3.3. Premixes and mineral feeds

Premixes normally consist of vitamins and trace elements. They may also contain other feed additives, minerals and amino acids. Their percentage in feed is between 0.2 and 1%, sometimes even higher.

Mineral feeds are used as supplements which consist mainly of individual mineral compounds. They are added to the final feed in quantities of 2–5%. They normally contain all major minerals, trace elements, vitamins and sometimes other additives necessary for a balanced diet. Cattle are given mineral supplements as high as 200 g per day to the forage and concentrates.

Vitamin losses are mainly attributed to shelf life and mixture composition. In general, premixes added to feed at lower levels will show higher vitamin losses, since they have a higher concentration of trace elements (catalytic effect).

The details on vitamin stability in mineral feed given in Table 11 also apply to 0.5% to 1% premixes if they do not contain any choline chloride.

All vitamins are stable within a certain pH range. The optimum pH of most vitamins is around the neutral point (pH 6–8), although the vitamins B_1 and B_6 prefer acidic conditions (pH 3–5). This may also explain why B_1 and B_6 are somewhat more prone to degradation in mixtures with a higher pH. In high alkaline mixtures, major losses of vitamin E must be expected. This mainly affects mineral feeds with a high content of manganese oxide, since this substance has a pH value of approx. 11. For mixtures containing more than 8–10% MgO, it is therefore recommended to use the more stable, sprayed (coated) form of vitamin E instead of vitamin E adsorbate.

Choline chloride is a hygroscopic substance with an adverse effect on the stability of some vitamins, e.g. K_3, B_1, B_6 , C and folic acid, especially when it is added at higher levels (> 5%). The shelf life of such premixes should not exceed approx. 4 weeks.

4.3.4. Mixed feed

After two months storage, only minor losses of below 5% of most vitamins are registered in mixed feed, with the exception of vitamin K_3 (MSB) with 20% degradation and vitamin C with 40% degradation.

Table 11: Vitamin stability in mineral feeds (averages)

	Vitamin retention after storage (%)	
	2 months	**4 months**
Vitamin A (crosslinked)	95	90
Vitamin A (not crosslinked)	60	35
Vitamin D_3	95	90
Vitamin E*	90	85
Vitamin K_3 (MSB)	40	30
Vitamin K_3 (MNB**)	80	70
Vitamin B_1***	80	70
Vitamin B_2	85	80
Vitamin B_6	80	75
Vitamin B_{12}	85	80
Biotin	90	85
Folic acid	80	75
Niacin	95	95
Calcium D-pantothenate	95	95
Vitamin C (coated****)	60	30
Vitamin C phosphate	95	95

* As vitamin E-adsorbate
** Vitamin K_3 (MPB) has a slightly lower stability
*** As vitamin B_1 monohydrate; vitamin B_1 hydrochloride is slightly less stable
**** Commercial products show different stabilities

In pelleted, expanded and extruded feeds, vitamin losses depend on temperature and duration of processing and may be much higher. Retention rates in feed can be found in Tables 12 to 14.

Table 12: Vitamin stability in pelleted mixed feed (averages)

	Vitamin retention after 2 months storage (%)
Vitamin A (crosslinked)	90
Vitamin A (not crosslinked)	65
Vitamin D_3	90
Vitamin E	95
Vitamin K_3 (MSB)	50
Vitamin K_3 (MNB)	75
Vitamin B_1	90
Vitamin B_2	95
Vitamin B_6	85
Vitamin B_{12}	80
Biotin	95
Folic acid	80
Niacin	95
Calcium D-pantothenate	95
Vitamin C (crystalline)	30
Vitamin C phosphate	95

Table 13: Vitamin stability after expansion (averages)

	Vitamin retention after 2 months storage (%)
Vitamin A (crosslinked)	85
Vitamin A (not crosslinked)	30
Vitamin D_3	90
Vitamin E	90
Vitamin K_3	20
Vitamin B_1	90
Vitamin B_2	95
Vitamin B_6	95
Vitamin B_{12}	90
Biotin	95
Folic acid	80
Niacin	95
Calcium D-pantothenate	90
Vitamin C (crystalline)	15
Vitamin C phosphate	95

Table 14: Vitamin stability after extrusion (averages)

	Vitamin retention (%)		
	After extrusion	**After 1 month**	**After 3 months**
Vitamin A (crosslinked)	95	90	80
Vitamin A (not crosslinked)	85	50	25
Vitamin D_3	95	90	85
Vitamin E	95	90	80
Vitamin K_3	25	20	20
Vitamin B_1	95	90	85
Vitamin B_2	95	90	90
Vitamin B_6	95	90	85
Vitamin B_{12}	80	80	80
Biotin	100	95	95
Folic acid	95	90	85
Niacin	100	95	95
Calcium D-pantothenate	100	95	95
Vitamin C (crystalline)	10	5	0
Vitamin C phosphate	95	90	90

4.4. Product forms and stabilising methods

Some vitamins are offered in different product forms, which may vary considerably in stability depending on the type of mixture and treatment process. During extrusion, losses of vitamin C (pure substance) may reach 90–100%, whereas vitamin C phosphate does not deteriorate at all. This shows the importance of selecting the appropriate product form. Which stabilisation technology to choose is another important factor. There are for example significant differences in vitamin A stability. Some products are crosslinked after the spray-formulation to render them insoluble in water. In premixes, mineral feed and mixed feeds, these products have a considerably higher stability than products that are not crosslinked. Tables 11 to 14 give details on the varying stability of crosslinked and not-crosslinked products.

4.5. Sampling and analysis

In order to determine the actual vitamin content of vitamin preparations, premixes, mineral and compound feeds, the following steps are necessary:

- Sampling of the lot to be analysed
- Preparation of sub-samples for analysis
- Determination of vitamin content of the prepared samples with the help of analytical instruments

Sampling procedures are described in the German Regulations for Feedstuff Sampling and Analysis (»Futtermittel-Probenahme- und Analysenverordnung)« and are legally binding. In the following sections only a few principles shall be explained, since the instructions are described in the appendix to the current feed legislation.

4.5.1. Sampling

It is not possible to analyse an entire lot or delivery. Samples must therefore be drawn. The correct procedure is indispensable. This includes:

- Sampling instruments as used for sampling cereals, in order to obtain representative samples
- Sampling of the entire lot, i.e. sampling from several places in the container, or sampling from several bags (the first and last bags of a lot should not be sampled)
- A sufficient number of samples. According to the official sampling instructions, individual samples must be drawn if the average vitamin content of a mixture is to be determined accurately and in a representative way (see Table below)

Individual samples		Minimum number of samples
Bags	Up to 4	1 per bag
	5-16	4
	>16	$\sqrt{\text{Number of bags, max. 20}}$
Bulk	Up to 2.5 t	7
	>2.5 t	$\sqrt{\text{tonnes x 20, max. 40}}$

The individual samples are subsequently pooled. For the official analyses, a sample divider is used to provide three final samples.

- The drawing of sufficient quantities. Samples in the order of 200–500 g, depending on the characteristics of the mixture, are required to determine vitamin concentration. To obtain an appropriate division of coarse samples, larger quantities are required. For the analysis of vitamin preparations, samples of 50 g are sufficient. An unequivocal identification is to be assured.

Mistakes or inaccuracies of the system are inevitable even when samples are carefully drawn from the premix. Appropriate and correct sampling minimises the extent of additional mistakes caused by the sampling procedure.

4.5.2. Sample preparation and analytical equipment

Modern analytical techniques such as high-pressure liquid chromatography (HPLC) and gas chromatography (GC) have improved the precision of vitamin analysis, and it has therefore significantly gained in importance. With this high-precision equipment, vitamins can be analysed down to the ppm level. Possible inaccuracies caused by these instruments are negligible.

Only laboratories familiar with all details of vitamin analysis should be charged with the analysis of vitamin samples. The procedures applied should be appropriate for the vitamin in question and calibrated to the expected concentrations in the mixtures.

4.5.3. Tolerance

German feed legislation defines levels of tolerance, in which the inherent technical range, (working precision) resulting in a certain inhomogeneity or in losses of activity due to processing techniques, are taken into consideration, along with the inaccuracies associated with sampling. This so-called technical latitude defines by how much the analysed values may differ from the declared values and can still be considered valid. The following tolerances are allowed according to § 19 of the German Feed Regulation:

1. max. 40% for up to 0.5 units
2. 0.2 units from 0.5 to 1.0 units
3. 20% for 1.0 to 50 units
4. 10 units for 50 to 100 units
5. 10% for 100 to 500 units
6. 50 units for 500 to 1000 units
7. 5% for > 1000 units

1 unit = 1 mg, 1 000 µg, 1 000 IU

4.5.4. Analytical latitudes

The determination of vitamin content is most precise in pure substances and vitamin preparations. With decreasing vitamin concentration and the disturbing influence of feed constituents, determination becomes more difficult and less precise, which is reflected in a larger analytical latitude.

The following analytical latitudes (comparability of different institutions or investigators) for the vitamins A, D_3, E and ß-carotene are based on the officially approved or provisional official methods of the VDLUFA, which have been established by ring tests. Analytical latitudes for other vitamins have not yet been determined.

Nowadays, higher vitamin concentrations as found in premixes and mineral feed are generally analysed by HPLC. A minimum analytical latitude of ±10% can be assumed for all vitamins not listed in Table 15. Because of their low content, the analysis of the water-soluble vitamins in compound feed is often carried out by microbiological assays. It is generally possible to apply these methods in premixes and in mineral feed. For microbiological assays, no analytical latitude has yet been defined. It should lie between 10 and 40%, depending on the concentration of the active substance, with the higher analytical latitude applying to compound feed with low vitamin concentrations.

Table 15: Analytical latitudes (comparability) for the determination of vitamins in feedstuffs and premixes

Vitamin	Range		Analytical latitude*
A	2 000 up to	4 000 IU/kg	± 1 000 IU/kg
	4 000 up to	100 000 IU/kg	± 25%
	100 000 up to	125 000 IU/kg	± 25 000 IU/kg
	125 000 up to	375 000 IU/kg	± 20%
	375 000 up to	600 000 IU/kg	± 75 000 IU/kg
	600 000 up to	800 000 IU/kg	± 12.5%
	800 000 up to	1 000 000 IU/kg	± 100 000 IU/kg
	> 1 000 000 IU/kg		± 10%
ß-Carotene			± 15%
D	1 000 up to	3 000 IU/kg	± 50%
	3 000 up to	6 000 IU/kg	± 1 500 IU/kg
	6 000 up to	40 000 IU/kg	± 25%
	40 000 up to	100 000 IU/kg	± 10 000 IU/kg
	> 100 000 IU/kg		± 10%
E		< 25 mg/kg	± 40%
	25 up to	50 mg/kg	± 10 mg/kg
	50 up to	150 mg/kg	± 20%
	150 up to	200 mg/kg	± 30 mg/kg
	200 up to	500 mg/kg	± 15%
	500 up to	750 mg/kg	± 75 mg/kg
	> 750 mg/kg		± 10%

* referring to the analysed content

Calculation example

In a mineral feed the declared vitamin A content is 800 000 IU/kg. The analysis reveals a content of 720 000 IU/kg. Is there reason for complaint? In this case the tolerance is 50 000 IU (see No. 6 under tolerances). This implies that at least 750 000 IU/kg must be found. An analytical latitude of ± 12.5% (= 90 000 IU) must be granted for the analysed value of 720 000 IU; therefore, the “true” value should lie in the area of 630 000 IU to 810 000 IU/kg.
The declaration is fulfilled, since the analysed value of 720 000 IU lies within the defined range.

4.6. Synonyms

4.6.1. Scientific designations

Most vitamins have a scientific name frequently used in the literature instead of the common designation. Designation is the same for names not appearing in the listing.

Vitamin A - Retinol
Vitamin D_3 - Cholecalciferol
Vitamin E - Tocopherols
Vitamin K_3 - Menadione
Vitamin B_1 - Thiamine
Vitamin B_2 - Riboflavin
Vitamin B_6 - Pyridoxine
Vitamin B_{12} - Cobalamin
Vitamin C - Ascorbic acid

4.6.2. Outdated vitamin designations

The designations of vitamins listed in this brochure are the ones commonly used nowadays. There is a multitude of designations that are only rarely used.

Since it is often not clear to which vitamin these designations refer to, they should not be used.
The following table was taken for the most part from the vitamin encyclopaedia by Bässler et al. (1997).

Table 16: Outdated vitamin nomenclature

Outdated nomenclature	**Active substance**
Anti-infectious vitamin	Vitamin A
Anti-xerophthalmic vitamin	Vitamin A
Anti-rachitic vitamin	Vitamin D
Anti-sterility vitamin	Vitamin E
Anti-haemorrhage vitamin	Vitamin K
Aneurine	Vitamin B_1
Anti-beriberi vitamin	Vitamin B_1
Anti-dermatitis factor	Vitamin B_6
Anti-pernicious factor	Vitamin B_{12}
Anti-anaemia vitamin	Vitamin B_{12}
Anti-seborrhoea vitamin	Biotin
Anti-scorbutic vitamin	Vitamin C

Table 16 (continued): Outdated vitamin nomenclature

Outdated nomenclature	**Active substance**
Epithelia protection vitamin	Vitamin A
Extrinsic factor	Vitamin B_{12}
Filtrate factor	Pantothenic acid
Fertility vitamin	Vitamin E
Coagulation vitamin	Vitamin K
Chick-anti-dermatitis factor	Pantothenic acid
Lactoflavin	Vitamin B_2
Lactobacillus casei factor	Folic acid
Pteroylglutamic acid	Folic acid
Vitamin A_2	Dehydroretinol
Vitamin B_3	Niacin or pantothenic acid
Vitamin B_4	Mixture of arginine, glycine and cystine
Vitamin B_5	Niacin or pantothenic acid
Vitamin B_9	Folic acid
Vitamin B_{13}	Orotic acid
Vitamin B_{14}	Nitrogen-containing mixture from human urine
Vitamin B_{15}	Pangamic acid
Vitamin B_C	Folic acid
Vitamin B_P	Anti-perosis factor for chicken, can be replaced by manganese and choline
Vitamin B_T	Carnitine
Vitamin B_W	Probably identical to biotin
Vitamin F	Essential fatty acids
Vitamin G	Vitamin B_2
Vitamin H	Biotin
Vitamin L	Vitamin L_1 and L_2: factors in yeast, essential for milk production
Vitamin M	Folic acid
Vitamin P (permeability vitamin)	Bioflavonoids

In the current EU legislation for animal nutrition, vitamins are considered as additives. However, in the appendix of the EU Directive for additives (Dir. 70/524/EC, chapter H), only the maximum contents of vitamin A and D in complete feed are indicated. A positive list which would correspond to the German »Futtermittelverordnung (FMV)« (Feed Regulation) and would list the admitted vitamins and their various forms does not yet exist on the European level. With reference to the sale, processing, labelling and use of vitamins, special regulations apply.

5.1. Sales

Under German law (March 2001) it is no longer necessary to prepare premixtures of vitamins (with the exception of vitamins A and D), which means that they may be added directly into the feed or can be sold directly to the livestock owner. The vitamins A and D can only be sold to:

- Approved manufacturers
- Approved traders of additives
- Registered manufacturers of mixed feed for pets
- Registered manufacturers of mixed feed with a special permission under § 31a,1

5.2. Processing

The vitamins A and D can only be added into mixed feed when premixed with carriers which must not be below 0.2% of the total mass of the mixed feed. However, the vitamins A and D may be used directly in mixed feed if it is intended for pets, and if the manufacturer is registered according to § 31.1. For all other mixed feeds, a special permission under § 31a is necessary.

Furthermore, it is possible — as an exception to the regulation — to reduce the percentage of the premix down to 0.05% of the total mass of the mixed feed, if the composition of the premix allows such a step, and if the manufacturer has a special permission according to § 31a,2.

5.3. Labelling

The labelling of vitamins is regulated in § 21 FMV. The label must not only indicate the name, but also provide information regarding the content of the active substance (for vitamin E this is to be expressed as α-tocopheryl acetate equivalents), and either the expiry date of the guaranteed vitamin content or the shelf life after the date of manufacture. Indication of the approval or registration number of the manufacturer is compulsory since 1 April 2001.

5.4. Use

There are maximum contents for the vitamins A and D in complete feed (depending on the total diet). In premixes and supplementary feed that only represent a part of the total diet these values must be considered on the basis of a complete feed with 88% dry matter.

6. Conversion factors

Table 17:
Active vitamin substance in various chemical forms

Vitamin products are available in many different forms and with different contents of active substance. Table 17 shows the international conversion factors of various vitamin compounds indicated as units of active substance.

Vitamin (active substance)	**Unit**	**Conversion factors of vitamin forms to active substance**	
A (retinol)	IU	0.3 µg vitamin A alcohol (retinol)	= 1 IU
		0.344 µg vitamin A acetate	= 1 IU
		0.359 µg vitamin A propionate	= 1 IU
		0.55 µg vitamin A palmitate	= 1 IU
D_3 (cholecalciferol)	IU	0.025 µg vitamin D_3	= 1 IU
E (tocopherol)	mg	1 mg dl-α-tocopheryl acetate	= 1 IU
		Bio-equivalence of various tocopherols:	
		1 mg d-α-tocopherol	= 1.49 IU
		1 mg dl-α-tocopherol	= 1.10 IU
		1 mg dl-α-tocopheryl acetate	= 1.00 IU
		1 mg dl-ß- tocopherol	= 0.33 IU
		1 mg dl-δ-tocopherol	= 0.25 IU
		1 mg dl-γ-tocopherol	= 0.01 IU
K_3 (menadione)	mg	1 mg menadione sodium bisulphite (MSB)	= 0.51 mg menadione
		1 mg menadione pyrimidinol bisulphite (MPB)	= 0.45 mg menadione
		1 mg menadione nicotinamide bisulphite (MNB)	= 0.46 mg menadione
B_1 (thiamine)	mg	1 mg thiamine mononitrate	= 0.92 mg thiamine
		1 mg thiamine hydrochloride	= 0.89 mg thiamine
B_6 (pyridoxine)	mg	1 mg pyridoxine hydrochloride	= 0.89 mg pyridoxine
Niacin	mg	1 mg nicotinic acid	= 1 mg niacin
		1 mg nicotinamide	= 1 mg niacin
D-pantothenic acid	mg	1 mg calcium D-pantothenate	= 0.92 mg pantothenic acid
		1 mg calcium DL-pantothenate	= 0.41–0.52 mg pantothenic acid
Choline	mg	1 mg choline chloride (basis choline ion)	0.75 mg choline
		1 mg choline chloride (basis choline hydroxy analogue)	0.87 mg choline

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