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Vitamins in Animal Nutrition

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Authors:

René BLUM
Geoff BROWN
An BUYENS
Yueming DERSJANT-LI
Elena MICELI
Chantal NUYTS
Manfred PEISKER
Lisa SAIBI
Tracey SAINSBURY
Eleanor TREDWAY
Gerritjan VAN DER VEN

ISBN 978-2-9601289-2-5

Table of Contents

Disclaimer

This booklet is intended to provide the best-of-our-knowledge basic information to anyone interested to get a better understanding about Vitamins in Animal Nutrition. However, FEFANA does not take any responsibility for whatever use of the information provided herewith, by either the general public or any actor in the food and feed chain.

For more detailed information on specific use please refer to the technical documentation and safety data sheets as provided by supplier.

Preamble

FEFANA is the European Association of Specialty Feed Ingredients and their Mixtures. With over 100 member companies from 28 European countries, it represents business operators active throughout the feed chain, such as the specialty feed ingredient producers, premixtures manufacturers, users, importers and distributors.

Established in 1963, FEFANA has loyally represented and served the interests of its industry ever since 1963, and it is recognized as a representative partner to national and international authorities and fellow organizations in the feed and food chain.

Specialty Feed Ingredients and their Mixtures are essential ingredients in animal feed, ensuring balancing the nutritional quality of the feed and hence contributing to animal health and welfare. Quality and safety being two main cornerstones, we also focus on innovation and sustainability, which we believe to be present and future key features of our business.

Having a broad range of members, representing the majority of business leaders as well as many Small and Medium Enterprises, our organization is capable of making use of the exceptional knowledge of its members, combining product expertise, practical and commercial experience with science.

This booklet is the fruit of the work of the FEFANA Working Group on Vitamins and gives an overview of the state-of-the-art knowledge on the use and application of vitamins in animal nutrition.

Prior to focusing on the detail of each individual vitamin and its use as a feed ingredient, we will provide basic information on the physical and chemical characteristics of each vitamin. This information will be given for the active substance, as well as for the available commercial products.

Relevant information on the natural occurrence in feedstuffs, on in-feed recommendations, as well as on safety, production methods and on activity levels will be dealt with. Attention will also be paid to the commercially available products, and to the most essential quality criteria.

Some basics on the regulatory background and indications on the classification, labelling and packaging are given in the booklet to help the reader understanding the high standards requested and applied to these products which are fundamental in today's farming practice. We hope you find this booklet useful to better appreciate the landscape our industry operates in.

An Buyens
Chairperson of FEFANA Working Group Vitamins

Marco Bruni
FEFANA President

Introduction

A BIT OF HISTORY

Earlier in the 20th century, 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 unknown before.

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 therefore named “vitamin” (from the Latin “*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. See **Table 1** for some historical information of vitamins.

Table 1 - *Dates of discovery, elucidation of the structure, and of their first synthesis*

Vitamin, provitamin or vitamin-like substance	Discovery	Elucidation of the structure	First synthesis
Betaine	1869 in beet juice		1912
Biotin	1931 in liver	1942	1943
L-carnitine	1905 in meat extracts	1927	1970
β-carotene	1831 in palm oil	1930	1950
Choline chloride	1849 in pig bile	1864	1866
Folic acid	1941 in liver	1946	1946
Inositol	1849		1915
Niacin	1867	1873	1894
D-pantothenic acid	1931 in liver	1940	1940
Taurine	1827 in ox bile		
Vitamin A	1909 in fish liver oil	1930	1947
Vitamin B_1	1897 in rice bran	1936	1936
Vitamin B_2	1920 in egg white	1935	1935
Vitamin B_6	1934 in rice bran	1938	1939
Vitamin B_{12}	1926 in liver	1955	1972
Vitamin C	1912 in lemon juice	1933	1933
Vitamin D	1918 in fish liver oil	1936	1959
Vitamin E	1922 in wheat germ oil	1938	1938
Vitamin K_3	1929 in alfalfa		1939

WHAT ARE VITAMINS?

Vitamins are organic substances that are indispensable to the normal metabolic processes of animals. Vitamins are required in very small amounts and are classified as micronutrients. They are essential to maintain health and performance, and have to be supplied as part of the overall diet. Vitamins can also be ingested as pro-vitamins, which are converted into the corresponding vitamins by the animal organism. In most cases, the animal organism itself is not able to synthesise vitamins. A deficiency or complete lack of one or more vitamins may lead to

multiple metabolic malfunctions, possibly resulting in depressed performance, growth retardation, fertility problems or diseases and possibly death. Furthermore, an increased supply of certain vitamins can have positive effects on health.
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.

CLASSIFICATION OF VITAMINS

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. Vitamins have than been divided into two groups: fat-soluble (A, D, E, and K) and water-soluble (B, C).

The two groups also have different functions. 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 small quantities are needed.

Table 2 provides for an overview of vitamins' main classification and functions used in animal nutrition as listed in this booklet.

Fat-soluble vitamins

The vitamins A, D, E, and K belong to the fat-soluble vitamins. 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. This is a reason why well balance diets in fat soluble vitamins, especially vitamin A and D is needed. 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 liver and kidneys, the brain, fat and muscles tissue. Excretion normally only occurs after metabolic transformation.

Vitamin A, D, E and K are commonly supplemented via premixtures in animal feed, as in general the intake from basal ingredients is not sufficient for meeting the requirement of animals.

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, D-pantothenic acid, and vitamin C, act as co-enzymes. Each co-enzyme has a specific metabolic function. Insufficient supply of the B vitamins will reduce the activity of the corresponding enzyme and result in metabolic disorders. In general, B-vitamins can be synthesized by microorganisms inhabiting the intestinal tract. In ruminants, abundant synthesis occurs in the fore stomachs of healthy animals. In pigs, bacterial synthesis of the B vitamins mainly takes place in the large intestine; however, they are absorbed only to a limited degree.

Vitamin-like substances and provitamins

We can also identify vitamin-like substances, like betaine, inositol or L-carnitine. These are substances that exhibit biological activity but are dietary essential for only certain species. Vitamin-like substances include a number of compounds that resemble vitamins in their activity, can be synthesized in the animal body, but not necessarily in adequate amounts to satisfy the animal requirements.
There exist also substances that can be converted into a vitamin within the body. These are provitamins, which have as such little or

no vitamin activity, but in the body they can be converted to an active form by normal metabolic processes. For example, β-carotene is provitamin of vitamin A.

Table 2 - *Vitamins, their classification and main functions as currently used in animal nutrition*

VITAMIN	MAIN FUNCTION
Fat-soluble vitamins	
Vitamin A	Proliferation of new tissues
Vitamin D	Regulation of the calcium and phosphorus metabolism
Vitamin E	Antioxidant and immune system modulation
Vitamin K	Blood coagulation
Water-soluble vitamins	
Biotin	Fatty acid metabolism and energy metabolism
Folic acid	Amino and nucleic acid metabolism
Niacin	Energy metabolism
D-pantothenic acid	Fat metabolism and energy conversion
Vitamin B_1	Carbohydrate metabolism
Vitamin B_2	Energy metabolism
Vitamin B_6	Amino acid metabolism
Vitamin B_{12}	Protein turnover and blood synthesis
Vitamin C	Antioxidant and immune system modulation
Vitamin-like substances	
Betaine	Protects against cellular osmotic stress
L-carnitine	Fat and energy metabolism
Choline chloride	Fat metabolism, transmission of neural impulses
Inositol	Synthesis of phospholipids
Taurine	Osmoregulation and membrane stability
Provitamins	
β-carotene	Precursor of vitamin A

Monographs

The monographs hereafter summarise basic information on vitamins used in animal nutrition.

Each vitamin monograph provides for information on the active substance per se, as well as for the available commercial products. Monographs are structured as follows:

For the Active Substance

- Introduction
- Chemical formula
- Classification
- Molecular weight
- Alternative names (of common use, and/or IUPAC name)
- CAS number
- Key natural sources
- Biological functions
- Benefits for the animal
- Signs of deficiency
- Tolerance to high intake
- Analytical methods (EURL if applicable, or the one currently proposed)
- Antagonists (where known)

For Commercial Products

- Common commercial forms
- Common physical parameters
- Stability under ambient conditions:
  - In feed and premixtures
  - In manufactured forms (Note: please refer to the manufacturer's datasheet for more précised information)

Furthermore, at the end of the book, you will find additional tables providing for information on recommended supplementation levels.

BETAINE

FOR ACTIVE SUBSTANCE

Introduction	Betaine is the trimethyl derivate of the amino acid glycine and is widely used as feed additive in livestock animal species such as poultry, pigs, ruminant, and aquaculture species.
Chemical formula	$C_5H_{11}NO_2$
Classification	Water-soluble vitamin-like substance
Molecular weight	117,15 g/mol
Alternative names	Trimethylglycine (TMG) Glycine betaine IUPAC: 2-trimethylammonioethanoate (betaine anhydrous) 2-(trimethylazaniumyl)acetate hydrochloride (betaine hydrochloride)
CAS No.	107-43-7 (betaine anhydrous) 590-46-5 (betaine hydrochloride) 590-47-6 (betaine monohydrate)
Key natural sources	Betaine is widespread among both flowering plants and algae. A betaine-rich product source is sugar beets. Betaine is also an endogenous compound in animal species.
Biological functions	The betaine molecule has two important functions as a methyl donor via transmethylation and as an osmolyte, the effects of which may modify carcass properties. Osmoregulation: Betaine serves as an organic osmolyte, which protects cells against osmotic stress, drought, high salinity and high temperature. Methyl donor function: Free methyl groups are generated for several metabolic reactions, such as synthesis of creatine, carnitine and others. Methyl group metabolism is amongst others important in preventing fatty liver syndrome. Betaine has sparing effect on dietary methionine and choline due to methyl donation property.
Benefits to the animal	Betaine is supplied to improve performance and carcass quality (lower carcass fat and higher lean carcass percentage).
Signs of deficiency	Usually none if choline, methionine and other amino acids are sufficiently supplied in the diet.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.

FOR ACTIVE SUBSTANCE

Analytical methods	EURL evaluation report 2011, CRL/100190, CRL/100036, CRL/100165: In feed additive: Titration or HPLC-RI for the determination of betaine hydrochloride; ref. USP 31. HPLC-RI for the determination of betaine anhydrous. In premixtures, feed and water: HPLC-RI for the determination of betaine anhydrous and betaine hydrochloride.

FOR COMMERCIAL PRODUCTS

Common commercial forms	Plant extracted betaine anhydrous: • Dry form: 91-97% betaine content • Liquid form: 38 and 47% betaine content: Chemically synthesized betaine sources: • Betaine hydrochloride 93% and 98% • Betaine monohydrate 91% • Betaine anhydrate 96%
Common physical parameters	From white over pale yellow to brown crystalline powder or a brown liquid.
Stability under ambient conditions	Stability in manufactured form: up to 2 years (liquid product); up to 3 years (crystalline product).

BIOTIN

FOR ACTIVE SUBSTANCE	
Introduction	Biotin is classified as a vitamin from the B-complex, therefore it is water soluble. It will be found in plants in a free chemical form, in organisms bound to lysine residues which are connected to proteins. Biotin has three asymmetric centres. From 8 stereoisomers only (3aS,4S,6aR), D-(+)-biotin has full biological activity as vitamin.
Chemical formula	$C_{10}H_{16}N_2O_3S$
Classification	Water-soluble
Molecular weight	244,31 g/mol
Alternative names	Vitamin B_7 Coenzyme R Vitamin H IUPAC: 5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl] pentanoic acid
CAS No.	58-85-5
Key natural sources	Biotin is present in many feed of animal and plant origin. Biotin-rich products are brewer's yeast and extracted oilseed meals, egg yolk, soy beans, oat flakes.
Biological functions	Biotin is required as a co-enzyme for the production of a number of carboxylases. These biotin-dependent enzymes play an important role in several metabolic processes: fatty acid synthesis; gluconeogenesis; propionic acid metabolism; decomposition of leucine; synthesis of DNA and RNA (via purine synthesis).
Benefits to the animal	Improved immune response. Stabilization of tissue and fat in animal products. Preparation for pregnancy and maintaining integrity and optimal function of the reproductive organs.
Signs of deficiency	Various symptoms occur according to the severity and duration of the deficiency: Common: retarded growth and fertility disorders. Poultry: poor plumage, inflammatory lesions of beak, legs and toes, fatty liver and kidney syndrome (FLKS). Pigs: hair loss, inflammation of the hooves and hoof-sole lesions. Cattle, sheep and horses: brittle horns and grooves and cracks in hooves.
Tolerance to high intake	No evidence on adverse effects on animals by high levels of oral intake.

FOR ACTIVE SUBSTANCE	
Analytical methods	EURL evaluation report 2011, CRL/100012: In feed additive: Potentiometric titration assay and optical rotation identification: PhEur 6th ed. method 01/2008:1073. In preparations, premixtures and feed: RP-HPLC-MS/MS.
Antagonists	Avidin in raw egg white.

FOR COMMERCIAL PRODUCTS	
Common commercial forms	Biotin pure substance Biotin 1% Biotin 2% Biotin 10%
Common physical parameters	White or almost white powder. Adsorbate powders. Spray dried coated powders for water dispersible products.
Stability under ambient conditions	Stability in feed and premixtures: 6 months minimum. Stability in manufactured form: up to 2 years.

L-CARNITINE

FOR ACTIVE SUBSTANCE

Introduction	L-carnitine is a vitamin-like compound and a natural component of all animal cells. It was discovered in 1905 as a component of muscle tissue (hence the name: Latin carnis = flesh or meat). Indications of the biological function were provided by vitamin requirements studies with the discovery of a growth factor that proved to be essential for the metamorphosis of the mealworm (Tenebrio molitor).
Chemical formula	$C_7H_{15}NO_3$
Classification	Water-soluble vitamin-like substance
Molecular weight	161,20 g/mol
Alternative names	Vitamin BT IUPAC: γ-trimethylamino-β-hydroxybutyrate, 1-Propanaminium, 3-carboxy-2-hydroxy-N,N,N-trimethyl-, hydroxide, inner salt, (R)
CAS No.	541-15-1
Key natural sources	L-carnitine can be formed in the body of humans and animals, with the amino acids lysine (in protein peptide linkage) and methionine as precursors. L-carnitine occurs in feedstuffs in varying amounts. Animal based feedstuffs are rich in L-carnitine, whereas plant-based feed contain little. High amounts of L-carnitine can be found in meat and fish meals (80 to 150 mg/kg), extracted soya meals or cereals contain less than 15 mg/kg. The D-form does not occur in nature; however, it can be synthesized chemically.
Biological functions	L-carnitine plays an important role in the energy metabolism. L-carnitine is an essential cofactor in the transport of long-chain fatty acids into the mitochondria producing large amounts of high energy ATP molecules. L-carnitine is also associated with many other aspects of fat and carbohydrate metabolism. The D-form has inhibiting effects.
Benefits to the animal	Ensures energy provision in conditions of sustained exertion (pregnancy and lactation). Improves growth performance in animals in grower-finisher periods. Supports endurance performance for racing and sport animals. Supports general metabolic health and well-being.

FOR ACTIVE SUBSTANCE

Signs of deficiency	Common: accumulation of fat in muscle (myolipidosis), pronounced muscle weakness, rapid fatigue including cardiac muscle and muscular pain. Poultry: reduced feed efficiency; decrease egg production and hatchability; higher risk for fatty liver syndrome. Swine: decrease of reproductive performance; reduced feed efficiency. Ruminants: higher risk for ketosis; impaired fertility.. Pets: increased fat storage; reproduction disorder. Fish: reduction in feed efficiency; increase of disease stress situations.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.
Analytical methods	EURL evaluation report 2011, CRL/100150: In feed additive: Titration, PhEur 6th ed., monograph 1339. In premixtures: Ion chromatography with electrical conductivity detection (IC-ECD) or spectrophotometric method after enzymatic reaction with carnitine-acetyl-transferase. In feed: RP-HPLC using a fluorometric detector or spectrophotometric method after enzymatic reaction with carnitine- acetyl-transferase. In water: Potentiometric titration with hydrochloric acid.

FOR COMMERCIAL PRODUCTS

Common commercial forms	L-carnitine is hygroscopic and thus formulations of L-carnitine with carriers or salt with L-tartaric acid are used and approved products. Pure crystalline L-carnitine mainly for liquid applications. Formulated L-carnitine with carriers and anticaking substances, salt of L-carnitine as L-carnitine L-tartrate and rumen protected forms for use in ruminants.
Common physical parameters	L-carnitine products are white to off-white crystalline powders.
Stability under ambient conditions	Stability in feed: 3 months minimum. Stability in premixtures: 6 months minimum.

ß-CAROTENE

FOR ACTIVE SUBSTANCE

Introduction	It was around 1929 when, in England, Moore showed that the plant pigment carotene was converted to the colourless form of vitamin A in liver tissues. In 1930, Karrer and his colleagues in Switzerland determined the structure of both carotene and vitamin A.
Chemical formula	$C_{40}H_{56}$
Classification	Fat-soluble
Molecular weight	536,9 g/mol
Alternative names	All-trans- β-carotene β,β-carotene Provitamin A IUPAC: (all-E)-3,7,12,16-Tetramethyl-1,18-bis(2,6,6-trimethylcyclohex-1-enyl)octadeca-1,3,5,7,9,11,13,15,17-nonaene
CAS No.	7235-40-7
Key natural sources	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. 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.
Biological functions	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) where 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). ß-carotene also plays a role as such in the fertility of dairy cows, through their presence in corpus luteum.
Benefits to the animal	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.)

FOR ACTIVE SUBSTANCE

Signs of deficiency	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.
Tolerance to high intake	LD50 in mice 30% > 20000mg/kg bw
Analytical methods	EURL evaluation report 2010, CRL/090016: In feed additive: UV-VIS spectrophotometry: JECFA Volume 4, 2006, total in crystalline material and ref. 2.6.02: PhEur 3rd ed., monograph 1069. In preparation: Protease digestion/ spectrophotometry. In premixtures and feed: HPLC.

FOR COMMERCIAL PRODUCTS

Common commercial forms	Beadlets, water dispersible. Red-orange free flowing capsule.
Common physical parameters	Brown-red or brownish-red, crystalline powder 10% formulation.
Stability under ambient conditions	Stability in feed and premixtures: 6 months minimum. Stability in manufactured form: up to 2 years.

CHOLINE CHLORIDE

FOR ACTIVE SUBSTANCE

Introduction	Choline was first isolated from pig bile by Strecker in 1849. Choline's role in nutrition was not known until the 1930's, following the discovery of insulin by Banting and Best (1922). Choline chloride is the salt of Choline hydroxide, a quaternary saturated amino alcohol, and HCl. Choline chloride in its anhydrous form is very hygroscopic, hence the product is typically commercialized either as a solution in water or as a powder. There are different Choline salts used for this purpose, but Choline chloride is the most commonly used form.
Chemical formula	$C_5H_{14}NO{\cdot}Cl$
Classification	Water-soluble vitamin-like substance
Molecular weight	139,63 g/mol
Alternative names	IUPAC: ethanaminim, 3-hydroxy-N,N,N-trimethyl-, chloride
CAS No.	67-48-1
Key natural sources	Dried brewer's yeasts, liver, meat (especially pork), egg yolk, legumes including soybean and peanut and in the germ of cereals.
Biological functions	Choline serves a series of critical functions in the body. a. Improves fat transport and metabolism in the liver b. Serves as a source of methyl donors for methionine regeneration from homocysteine; c. Builds and maintains cell wall structure; d. supports nervous system function; e. serves as a source of osmolytes that regulate cell volume.
Benefits to the animal	Improves animal growth through fat metabolism. After a two-step enzymatic oxidation, choline serves as an important donor of methyl groups, important for regenerating methionine. Choline is essential for the body, in case there is choline deficit the body will synthesize de novo choline from glycine or serine.
Signs of deficiency	Common: fatty liver; reduced growth. Poultry: perosis. Swine: splayed stance (piglets). Ruminants: reduced milk production, milk fat level and protein yield; ketosis; carcass characteristics. Young ruminants have a dietary need for choline. Fish: haemorrhagic kidney and intestine. Pets: see common signs.

FOR ACTIVE SUBSTANCE

Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of intake.
Analytical methods	EURL evaluation report 2010, CRL 100009: In feed additives, premixtures, feed and water: Ion Chromatography with Conductivity Detection (IC-CD).
Antagonists	Betaine

FOR COMMERCIAL PRODUCTS

Common commercial forms	Choline chloride 75% solution in water. Choline chloride on a carrier (50 -70 wt. %). Choline chloride crystals (>95%). Rumen protected Choline chloride.
Common physical parameters	Depending on the form, powder or liquid.
Stability under ambient conditions	Stability in feed and premixtures: 6 months minimum. Stability in manufactured form: up to 24 months.

FOLIC ACID

FOR ACTIVE SUBSTANCE

Introduction	Folates occur naturally in several sources of human and animal food. Folates and folic acid derive their names from the Latin word folium (which means «leaf»). Leafy vegetables are a principal source as well as fresh vegetables, mushrooms and yeast, grasses and citrus fruit. Among foods of animal origin, liver and eggs, and especially egg yolk, are considered a good source. Vitamin B9 (folic acid and folate) is essential to numerous functions in the body.
Chemical formula	$C_{19}H_{19}N_7O_6$
Classification	Water-soluble
Molecular weight	441,4 g/mol
Alternative names	Vitamin B_9 IUPAC: (2S)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid
CAS No.	59-30-3
Key natural sources	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 feed of both plant and animal origin. Folate-rich feedstuffs are lucerne green meal and brewer´s yeast. Folate-poor feedstuffs are tapioca and cereals.
Biological functions	Folic acid is a member of the vitamin B family that stimulates the hematopoietic system. Folic acid is used in the treatment and prevention of folate deficiencies and megaloblastic anemia. 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 B12, it converts homocysteine into methionine.
Benefits to the animal	Increased antibody production

FOR ACTIVE SUBSTANCE

Signs of deficiency	Common: macrocytic anaemia; damage to the skin and mucous membranes. Poultry: disorders of growth; bad plumage and depigmentation; Perosis: increased embryo mortality; reduced hatchability and laying performance. Pigs: hair loss and fertility disorders. Cattle: fertility disorders.
Analytical methods	EURL evaluation report 2011, CRL 100007: In feed additive: Liquid Chromatography coupled to UV detection LC-UV, PhEur 6th ed. 01/2008: 0067. In premixtures: RP-HPLC-UV. In feed and water: Total folates (including added folic acid: microbiological assay based on CEN-ring trial validated method EN 14131.
Antagonists	Sulphonamides and aflatoxins in feed and in drugs to inhibit intestinal microflora.

FOR COMMERCIAL PRODUCTS

Common commercial forms	Folic acid min 96% on anhydrous basis. Folic acid 80% spray-dried on a carrier.
Common physical parameters	Yellowish to orange, crystalline powder.
Stability under ambient conditions	Stability in manufactured form: 24 months minimum.

INOSITOL

FOR ACTIVE SUBSTANCE	
Introduction	Inositol is considered as a vitamin-like substance that was identified 100 years ago in the urine of diabetics. It exists in nine possible stereoisomers, of which the most prominent form with biologic activity is myo-inositol. It is occurring in plants as phytic acid and in animals as myo-inositol. Inositol in the human body is virtually present in all tissues.
Chemical formula	$C_6H_{12}O_6$
Classification	Water-soluble vitamin-like substance
Molecular weight	180,2 g/mol
Alternative names	Myo-inositol Meso-inositol IUPAC: cis-1,2,3,5-trans-4,6-cyclohexanehexol
CAS No.	87-89-8
Key natural sources	Inositol occurs in food and feedstuffs in three forms: free myo-inositol, phytic acid and phosphatidylinositol. In plants, the hexaphosphate of inositol, phytic acid or its salts are found. The predominant form occurring in plant materials is phytic acid. Livestock are capable of producing sufficient quantities of inositol. However, most animals lack the enzymes to utilize phytic acid as a source of myo-inositol. Fish meals usually contain 700 to 800 mg myo-inositol per kg dry weight.
Biological functions	Inositol and some of its mono and polyphosphates function as the basis for a number of signalling and secondary messenger molecules. They are involved in a number of biological processes, including Insulin signal transduction, intracellular calcium (Ca2+) concentration control and cell membrane potential maintenance.
Benefits to the animal	Proper inositol supplementation will optimize performance, cure fatty liver syndrome in laying hens.
Signs of deficiency	Poultry: reduction of fatty liver syndrome; reduced feed efficiency. Fish: impaired growth; reduced feed efficiency.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.

FOR ACTIVE SUBSTANCE	
Analytical methods	EURL evaluation report 2012, CRL/100007: In feed additive: Identification by liquid chromatography/IR absorption spectrophotometry, PhEur 01/2008: 1805. Quantification by microbiological activity analysis. In premixtures and feed: Quantification by microbiological activity analysis.

FOR COMMERCIAL PRODUCTS	
Common commercial forms	Min 97.0% inositol
Common physical parameters	White crystalline powder.
Stability under ambient conditions	Stability in feed: 3 months minimum. Stability in premixtures: 6 months minimum.

NIACIN

FOR ACTIVE SUBSTANCE

Introduction	Niacin (nicotinic acid) was first synthesized in 1867 by oxidative degradation of nicotine. It had been chemically identified long before its importance as an essential nutrient was recognized. In the early 1900'ies it was discovered that pellagra, a disease with symptoms affecting the skin, gastrointestinal tract, and central nervous system, was caused by niacin deficiency. In 1935 niacin was shown to be a moiety of the coenzymes NAD and NADP, indispensable for many biochemical reactions.
Chemical formula	$C_6H_5NO_2$ nicotinic acid $C_6H_6N_2O$ nicotinamide
Classification	Water-soluble
Molecular weight	123,11 g/mol nicotinic acid 122,13 g/mol nicotinamide
Alternative names	Vitamin B_3 Vitamin PP Pyridine 3-carboxylic acid Pyridine 3-carboxamide
CAS No.	59-67-6 nicotinic acid 98-92-0 nicotinamide
Key natural sources	Nicotinic acid is the form present in varying concentrations in almost all feed of plant origin. Meat and meat products contain Nicotinamide in varying concentrations. Brewer´s yeast, bran, green forage and plant protein feed are rich in niacin. Not all of the natural niacin is fully bioavailable for humans and animals. Niacin in cereals and in oil seeds is present in a bound complex. Minor quantities are produced by microbial synthesis in the intestine and by transformation of the amino acid tryptophan.
Biological functions	Niacin is one of the B vitamins with coenzyme function. In its active forms, the pyridine coenzymes NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate),are all involved in the pathways of energy metabolism, like glycolysis, citric acid cycle, oxidations of fatty acids, gluconeogenesis and de novo fatty acid synthesis. From a physiological point of view, nicotinic acid and nicotinamide can be considered as equivalent sources of niacin.
Benefits to the animal	Proper niacin supplementation will: maximize weight gain; optimize feed efficiency; ensure proper utilization of other nutrients; and support general health and well-being.

FOR ACTIVE SUBSTANCE

Signs of deficiency	Common: functional disorders of the nervous system; skin disorders (pellagra); increased peristalsis of the gastrointestinal tract; reduction in growth; inflammation and ulcers of the mucous membranes. Poultry: reduced feed efficiency; decrease egg production and hatchability; disorders in feather development. Swine: reduced feed efficiency; decrease of reproductive performance. Ruminants: reduction of performance parameters; decrease of reproductive performance; increase of risk of ketosis. Pets: reproduction disorder; nervous disorders; black tongue disease in dogs. Fish: reduction in feed efficiency; increase of disease stress situations.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.
Analytical methods	EURL evaluation report 2011, CRL/100139, CRL/100173, CRL/100240, CRL/100170, CRL/100369: In feed additive: Titration: for nicotinic acid, PhEur 6th ed. monograph 0459; for nicotinamide, PhEur 6th ed. monograph 0047. In premixtures, feed and water: Ion-pair RP-HPLC-UV or VDLUFA (Method Bd. III, 13.9.1) using RP-HPLC-UV.

FOR COMMERCIAL PRODUCTS

Common commercial forms	Nicotinic acid and nicotinamide used as pure substances for all applications.
Common physical parameters	Nicotinic acid and nicotinamide are white to off-white powders and granules.
Stability under ambient conditions	Stability in feed: 3 months minimum. Stability in premixtures: 6 months minimum.

D-PANTOTHENIC ACID

FOR ACTIVE SUBSTANCE

Introduction

D-pantothenic acid was discovered in 1931 as a growth factor for microorganism. It is found in two enzymes, coenzyme A (CoA) and acyl carrier protein (ACP), which are involved in many reactions in carbohydrate, fat and protein metabolism. Animals require D-pantothenic acid to synthesize coenzyme-A (CoA). D-pantothenic acid is hygroscopic and not very stable. Therefore, Calcium D-pantothenate and D-panthenol are the forms of the vitamin used in practice.

Chemical formula

$C_9H_{17}NO_5$	D-pantothenic acid
$Ca[C_9H_{16}NO_5]_2$	Calcium D-pantothenate
$C_9H_{19}NO_4$	D-panthenol

Classification

Water-soluble

Molecular weight

219,23 g/mol	D-pantothenic acid
476,53 g/mol	Calcium D-pantothenate
205,25 g/mol	D-panthenol

Alternative names

Vitamin B_5

IUPAC:

- N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-β-alanine (D-pantothenic acid)
- Calcium bis[3-[[(2R)-2,4-dihydroxy-3,3-dimethylbutanoyl] amino] propanoate] (Calcium D-pantothenate)
- 2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutanamide (D-panthenol)

CAS No.

79-83-4	D-pantothenic acid
137-08-6	Calcium D-pantothenate
81-13-0	D-panthenol

Key natural sources

D-pantothenic acid is widely distributed in feedstuffs of animal and plant origin. High quantities are found in various organ meats like liver and in egg yolk. Milk and other dairy products, alfalfa hay, yeast, rice and wheta bran are good sources of D-pantothenic acid. Corn and soybean meal diets are low in D-pantothenic acid.

Biological functions

D-pantothenic acid is found in two enzymes, coenzyme A (CoA) and acyl carrier protein (ACP), which is involved in many reactions in carbohydrate, fat and protein metabolism.
CoA is necessary for acetylation in the intermediate metabolism. It is essential in the citric acid cycle and the basis of numerous syntheses which the body is able to perform in the presence of adequate pantothenic acid, namely the biosynthesis of long-chain fatty acids, phospholipids, cholesterol and steroid hormones.
Only the optical active D-form has vitamin activity.

FOR ACTIVE SUBSTANCE

Benefits to the animal

More efficient use of nutrients.
Improved performance.

Signs of deficiency

Common: functional disorders of the nervous system; lesions of the skin; reduction in growth; gastrointestinal disturbances; inhibition of antibody formation.
Poultry: reduced feed efficiency; poorer hatchability; reduced laying performance; rough and uneven feathering; increased embryonic mortality.
Swine: reduced feed efficiency; reduced protein formation; lesions of the skin.
Pets: convulsions; fatty degeneration of the liver.
Fish: swelling of the gills.

Tolerance to high intake

No evidence of adverse effects on animals caused by high levels of oral intake.

Analytical methods

EURL evaluation report 2011, EURL/090043, EURL/100057:
In feed additive:
Titration: for Calcium-D-pantothenate, PhEur monograph 0470; for D-panthenol PhEur monograph 0761.
In premixtures and feed:
RP-HPLC-MS for Calcium D-pantothenate.
In water:
RP HLPC-UV for D-panthenol.

FOR COMMERCIAL PRODUCTS

Common commercial forms

D-pantothenic acid is viscous, pale yellow oil. The oil is hygroscopic and easily destroyed by acids, bases and heat.
Two types of D-pantothenic acid are commercially available:
Calcium D-pantothenate (assay: min 98%), and D-panthenol (assay: min 98%).

Common physical parameters

Calcium d-pantothenate is a white to off-white powder.
D-panthenol is a viscous transparent liquid.

Stability under ambient conditions

Stability in feed: 3 months minimum.
Stability in premixtures: 6 months minimum.

TAURINE

FOR ACTIVE SUBSTANCE

Introduction	Taurine is a β-amino acid, an essential dietary nutrient for cats. Taurine is not an amino acid in the usual biochemical meaning of the term, it is not incorporated into proteins. Taurine is one of the most abundant free amino acids in mammals, being particularly high in brain, heart and skeletal muscle. Taurine is not found in proteins and is made in the liver of most species from cysteine. The synthesis of taurine appears to be severely limited for strict carnivores, but not in most herbivores or omnivores.
Chemical formula	$C_2H_7NO_3S$
Classification	Water-soluble vitamin-like substance
Molecular weight	125,15 g/mol
Alternative names	β-aminoethylsulfonic acid, tauric acid IUPAC name 2-amino-ethanesulfonic acid
CAS No.	107-35-7
Key natural sources	Animal tissues, in particular brain, heart and skeletal muscle.
Biological functions	Taurine is involved in foetal development, growth, reproduction, neuromodulation, sight, hearing, heart function, osmoregulation, fat emulsification, neutrophil function, immune response, anti-oxidation, bile acid and xenobiotic conjugation and act as an anticonvulsant.
Benefits to the animal	Cats cannot synthesize taurine. Taurine prevents that a cat's retina slowly degenerates, causing eye problems and eventually irreversible blindness.
Signs of deficiency	Taurine deficiency in cats results in feline central retinal degradation (FCRD) and blindness; dilated cardiomyopathy (DCM) and heart failure; inadequate immune response; poor neonatal growth; reduced auditory brain stem evoked potentials resulting in deafness; poor reproduction resulting in a low number of foetuses, resorptions, abortions, decreased birth weight, and low survival rate of the kittens; congenital defects including hydrocephalus and anencephaly.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.

FOR ACTIVE SUBSTANCE

Analytical methods	EURL evaluation report 2012, CRL/100050: In feed additive: Ion-exchange chromatography with post column ninhydrin derivatisation, Ph. Eur. 6.6 – 2.2.56 – Method 1. In premixtures and feed: • Ion-exchange chromatography with post column ninhydrin derivatisation and photometric detection. • RP-HPLC coupled to fluorescence detector (AOAC 999.12). In water: Liquid Chromatography coupled to UV or fluorescence detector (AOAC 997.05).

FOR COMMERCIAL PRODUCTS

Common commercial forms	Taurine 99%
Common physical parameters	White, odourless, crystalline powder.
Stability under ambient conditions	Stability in manufactured form: up to 3 years.

VITAMIN A

FOR ACTIVE SUBSTANCE

Introduction	Retinyl esters providing vitamin A supplementation in feed can be hydrolysed in the intestine and retinol is released to be then absorbed. The retinol form functions as storage form of the vitamin, and can be converted to and from aldehyde form, retinal, which is acting insuring good vision. The associated acid (retinoic acid), a metabolite that can be irreversibly synthesized from vitamin A, has only partial vitamin A activity, and does not function in the retina for the visual cycle. Nearly 90% of total vitamin A body store are in liver.	
Chemical formula	$C_{22}H_{32}O_2$ $C_{36}H_{60}O_2$ $C_{23}H_{34}O_2$ $C_{20}H_{30}O$	(acetate) (palmitate) (propionate) (retinol)
Classification	Fat-soluble	
Molecular weight	328,49 g/mol 342,51 g/mol 524,86 g/mol 286,45 g/mol	(acetate) (propionate) (palmitate) (retinol)
Alternative names	Retinyl esters Retinol	
CAS No.	127-47-9 7069-42-3 79-81-2 68-26-8	(acetate) (propionate) (palmitate) (retinol)
Key natural sources	Vitamin A in its active form exists only in animal tissues of mammals and marine fishes (liver, fish oil, high-fat fishmeal). However, a vitamin A precursor is found in plant tissues (grass, carrots) in the form of carotenoid pigments (particularly β-carotene).	
Biological functions	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 on processes of the cellular metabolism by influencing the transcription of genes. Increase of resistance to infectious diseases. Normal bone development. Rhodopsin synthesis (mechanism of vision).	
Benefits to the animal	Improved: growth, bone development, reproduction performance, health and tissue / membrane integrity, vision.	

FOR ACTIVE SUBSTANCE

Signs of deficiency	Poultry: reduction in growth; decrease resistance to disease; eye lesions; muscular incoordination; decrease egg production and hatchability; alteration in bone growth. Swine: decrease of reproductive performance; nervous trouble (mobility disorder); vision deficiency. Ruminants: keratinisation of the epithelial tissue; decrease of reproductive efficiency; blindness (or night-blindness); failure in bone development; increase susceptibility to infection. Pets: lack of growth; epithelial tissue mucous glands are impaired; night blindness; bone thickness; reproduction disorder. Fish: impaired growth; degeneration of retina; depigmentation.
Tolerance to high intake	Poultry: presumed upper safe levels are 4 to 10 times the nutritional requirements (from 15,000 IU/kg for broiler and turkey to 40,000 IU/kg for duck). Swine: presumed upper safe levels for swine go from 20,000 IU/kg (growing) to 40,000 IU/kg (breeding). Cattle and sheep: upper safe levels are 30 times the nutritional requirements (from 45,000 IU/kg for sheep to 66,000 IU/kg for cattle). Fish: 25,000 to 33,330 IU/kg upon species.
Analytical methods	EURL evaluation report 2011, CRL/100058: In feed additive: Thin layer chromatography and UV detection (TLC-UV), PhEur 6th ed. monograph 0217. In premixtures and feed: RP-HPLC with UV or fluorescence detection.
Antagonists	None.

FOR COMMERCIAL PRODUCTS

Common commercial forms	Liquid oily formulation or micro emulsion. Solid dry powder or granules.
Common physical parameters	Orange, fine powder.
Stability under ambient conditions	Stability in manufactured form: up to 1 year.

VITAMIN B_1

FOR ACTIVE SUBSTANCE

Introduction	Chemically, Vitamin B1 consists of pyrimidine and thiazole moieties linked by a methylene bridge. Isolated in pure form as the chloride it is very hygroscopic, hence the mononitrate salt is usually used. Thiamine hydrochloride is also commonly available.	
Chemical formula	$C_{12}H_{17}N_4OS{\cdot}NO_3$ $C_{12}H_{17}ClN_4OS{\cdot}HCl$	Thiamine mononitrate Thiamine hydrochloride
Classification	Water-soluble	
Molecular weight	327,36 g/mol 337,28g/mol	Thiamine mononitrate Thiamine hydrochloride
Alternative names	• Thiamin • Thiamine • Thiamine mononitrate • Thiamine nitrate	
CAS No.	532-43-4 67-03-8	Thiamine mononitrate Thiamine hydrochloride
Key natural sources	Dried brewer's yeasts and yeast products, animal by- products, legumes including soybean and groundnut and cereal germs.	
Biological functions	Main functions are connected to the role of thiamine as a coenzyme in the form of thiamine pyrophosphate, involved in about 25 enzymatic reactions. Carbohydrate metabolism, especially in the breakdown of glucose to energy. Activation of the key enzyme involved in the production of compounds like ATP, GTP, NADPH and nucleic acids DNA and RNA. Synthesis of acetylcholine, essential in the transmission of nervous impulses.	
Benefits to the animal	More efficient use of carbohydrates for energy. Improved performance especially in genetic lines with high meat yield.	
Signs of deficiency	Common: anorexia and poor growth; peripheral and central neuropathies; cardiac failure; muscle weakness; gastrointestinal malfunction. Poultry: polyneuritis with neck twisting; embryo mortality. Swine: vomiting. Ruminants: weakness; poor leg coordination, inability to rise and stand (Polioencephalomalacia, PEM). Pets: weight loss; neurological disorders; death from heart failure. Fish: ataxia	
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.	

FOR ACTIVE SUBSTANCE

Analytical methods	EURL evaluation report 2014, CRL/100032, CRL/100035, CRL/100184: In feed additive: Identification by HPLC-UV, USP 32. In premixtures: Quantification by HPLC-UV (VDLUFA Bd. III, 13.9.1) and HPLC-FL. In feed: Quantification by HPLC-FL. In water: quantification by HPLC with post-column derivatisation and FL.
Antagonists	Thiaminase enzymes in raw fish, some plants, bacteria and moulds.

FOR COMMERCIAL PRODUCTS

Common commercial forms	Thiamine mononitrate 98% Thiamine hydrochloride 98 %
Common physical parameters	Fine granular, white to pale yellow powder.
Stability under ambient conditions	Stability in manufactured form: up to 3 years.

VITAMIN B_2

FOR ACTIVE SUBSTANCE	
Introduction	Vitamin B2 naturally occurs as the free dinucleotide riboflavin and the 2 phosphorylated coenzymes - flavin mononucleotide (FMN) / riboflavin monophosphate and flavin adenine dinucleotide (FAD) / riboflavin adenosine diphosphate.
Chemical formula	$C_{17}H_{20}O_6N_4$
Classification	Water-soluble
Molecular weight	376,37 g/mol
Alternative names	Riboflavin Lactoflavin
CAS No.	83-88-5
Key natural sources	Yeasts and yeast products, animal by-products.
Biological functions	Flavin coenzymes FMN and FAD are essential for energy production via the respiratory chain. Involved in many metabolic reactions of carbohydrates, fats and proteins. Involved in synthesis of steroids, red blood cells and glycogen. Helps to maintain integrity of mucous membranes. Also important for antioxidant status within cell systems.
Benefits to the animal	Efficient use of nutrients, especially carbohydrates, amino acids and fatty acids. Growth and reproduction. Antioxidant functions.
Signs of deficiency	Common: reduced feed intake; growth depression or failure to grow; lesions of tissues and mucus membranes; impaired absorption of iron, zinc and calcium. Poultry: peripheral neuropathy; “curled-toe paralysis”, chickens walking on their hocks; decreased egg production; increased embryonic mortality; reduced hatchability. Swine: rough hair; dermatitis and alopecia; scours; ulcerative colitis; decreased immune response (in severe deficiency). Ruminants: deficiency usually demonstrated in young ruminants; redness of the mouth mucosa; lesions in the corner of the mouth; loss of hair. Pets: anorexia; weight loss; dermatitis and alopecia; ocular lesions. Fish: anorexia and poor growth; snout erosion and spin deformities; increased mortality.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.

FOR ACTIVE SUBSTANCE	
Analytical methods	EURL evaluation report 2013, CRL/100130: In feed additive: Spectrophotometry PhEur monograph 0292. In premixtures: HPLC-UV (VDLUFA method book, Vol.III, 13.9.1). In feed: HPLC (EN14152:2003).
Antagonists	Some mycotoxins

FOR COMMERCIAL PRODUCTS	
Common commercial forms	Spray dried powder containing 80% riboflavin.
Common physical parameters	Orange-brown to yellow-brown powder.
Stability under ambient conditions	Stability in manufactured form: up to 3 years.

VITAMIN B_6

FOR ACTIVE SUBSTANCE	
Introduction	Isolated in 1938 by Gyorgy and Lepkovsky. The term B6 was first used some years before by Gyorgy to distinguish this factor, isolated from rice polishing, from other hypothetical growth factors. Vitamin B6 is unique among the water-soluble vitamins with respect to the multiple functions it serves, its metabolism and its chemistry.
Chemical formula	$C_8H_{11}NO_3 \cdot HCl$
Classification	Water-soluble
Molecular weight	169,18 g/mol
Alternative names	Pyridoxine Pyridoxal Pyridoxamine
CAS No.	58-56-0
Key natural sources	Plant (cereals, milling by-products, extracted oilseed meals, brewer's yeast, etc.) and animal origin.
Biological functions	Involved in amino acids, fats and carbohydrate metabolism. Essential for RNA and DNA synthesis. Involved in the synthesis of niacin from tryptophan.
Benefits to the animal	Essential for energy production. Immune system integrity.
Signs of deficiency	Retarded growth; reduced feed consumption and protein retention; skin inflammation and dermatitis; rough and deficient plumage; damage to liver and heart; disorders of blood parameters (anaemia); malfunction of the peripheral and central nervous systems; reduced hatchability in poultry.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.
Analytical methods	EURL evaluation report 2011, EURL/0100182: In feed additive: Titration, PhEur. 6th ed. Monograph 0245. In premixtures: RP-HPLC-UV (VDLUFA Bd. III, 13.9.1). In feed and water: RP-HPLC-FL (EN14164:2008).

FOR COMMERCIAL PRODUCTS	
Common commercial forms	Pyridoxine hydrochloride 99%
Common physical parameters	White or almost white crystals or crystalline powder.
Stability under ambient conditions	Stability in manufactured form: up to 3 years.

VITAMIN B_{12}

FOR ACTIVE SUBSTANCE

Introduction	Vitamin B_{12} is the less abundant vitamin of all the vitamins found in nature. The name vitamin B_{12} is generic term for a specific group of cobalt-containing corrinoids with a biological activity for animals and human. This group of corrinoids is also known as cobalamins. The main forms of vitamin B_{12} are hydroxocobalamin, adenosylcobalamin and methylcobalamin, the last two being the active coenzyme forms. Cyanocobalamin is a form of vitamin B_{12} that is widely used in the industry due to its availability and stability. Vitamin B_{12} is absorbed by an active mechanism. Most of the vitamin is stored in the liver.
Chemical formula	$C_{63}H_{88}0_{14}N_{14}PCo$
Molecular weight	1355,37 g/mol
Alternative names	Cyanocobalamin α-(5,6-Dimethylbenzimidazolyl) cyanocobamide
CAS No.	68-19-9
Key natural sources	Animal products via microbial synthesis, Plant products are practically devoid of vitamin B_{12}.
Biological functions	• 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 e.g. for the metabolism of methionine; • Co-enzymes in the metabolism of nucleic acids and proteins, and also in the metabolism of fats and carbohydrates.
Benefits to the animal	Improved growth Improved reproduction performance
Signs of deficiency	Common: reduced synthesis of DNA and protein, growth disorders, lower feed conversion, anaemia, rough coat and inflammation of the skin. Poultry: poor plumage, reduced hatchability and increased embryo mortality. Ruminants: weight loss and reduced milk yield in regions with a low cobalt content in plants.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.

FOR ACTIVE SUBSTANCE

Analytical methods	EURL evaluation report 2013, CRL/100070, CRL/100076, CRL/100318: In feed additive: Thin layer chromatography (TLC) and spectrophotometry (UV/VIS), PhEur monograph 0547. Alternative methods: In premixtures and feed: Microbiological activity analysis. HPLC with UV detection.

FOR COMMERCIAL PRODUCTS

Common commercial forms	Market standard is concentrated 1%, but 0.1% preparations also available.
Common physical parameters	Pale pink to pink fluid powder.
Stability under ambient conditions	Stability in manufactured form: up to 2 years.

VITAMIN C

FOR ACTIVE SUBSTANCE

Introduction	Vitamin C occurs in two forms, namely L-ascorbic acid (reduced form) and dehydro-L-ascorbic acid (oxidized form). Although in nature the vitamin C is primarily present as ascorbic acid, both forms are biologically active. The L-isomer of ascorbic acid is biologically active; the D-isomer is not.
Chemical formula	$C_6H_8O_6$
Classification	Water-soluble
Molecular weight	176,13 g/mol
Alternative names	L-ascorbic acid
CAS No.	50-81-7
Key natural sources	Green forages, citrus products. Low in most dried feedstuffs.
Biological functions	• Antioxidant at cellular level; • Stimulation of phagocytic activity; • Biosynthesis of collagen; • Conversion of vitamin D to its active form; • Absorption of minerals; • Control of glucocorticoid synthesis; • Involved in wound healing.
Benefits to the animal	• Stimulation of immune response; • Involved in calcification of bones and teeth; • Adaptation to stress; • Maintenance of electrolytic balance.
Signs of deficiency	In stress conditions (e.g. high or cold temperatures) vitamin C deficiency enhances the negative impact of such conditions on productivity. Reduced immune response is also observed in vitamin C deficiency.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intakes.

FOR ACTIVE SUBSTANCE

Analytical methods	EURL evaluation report 2012, CRL/100181, CRL/100066: In feed additive: For ascorbic acid, sodium and calcium ascorbates: titrimetric or HPLC techniques according to the relevant PhEur monographs. For sodium and calcium ascorbyl phosphates: RP-HPLC. In premixtures and feed: For ascorbic acid, sodium and calcium ascorbates: titrimetric according to the relevant PhEur monographs. For sodium and calcium ascorbyl phosphates: HPLC-UV. In water: For L-ascorbic acid: titrimetric (AOAC 967.21) or HPLC-UV (EN14130:2003).

FOR COMMERCIAL PRODUCTS

Common commercial forms	L-ascorbic acid is the most important of the several compounds that have vitamin C activity. Ascorbic acid is commercially available as: • 100% crystalline L-ascorbic acid; • 97.5% L-ascorbic acid - ethyl cellulose-coated; • 35% phosphorylated Na/Ca salt of L-ascorbic acid (C6H9O9P MW. 256.11g/mol); • 50% phosphorylated Na salt of L-ascorbic acid ($C_6H_6O_9Na_3P{\cdot}H_2O$ MW. 358.08 g/mol).
Common physical parameters	Ascorbic acid: white to slightly yellow crystalline powder. Ethyl-cellulose coated: white to slightly yellowish powder. Phosphorylated products: beige, spray dried powder
Stability under ambient conditions	Stability in manufactured form: up to 2 years.

VITAMIN D_3

FOR ACTIVE SUBSTANCE

Introduction	Vitamin D designates a group of closely related compounds that possess antirachitic activity. It may be supplied through the diet or by irradiation (sunlight) of the body. The two most prominent forms of vitamin D are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Ergocalciferol is derived from a common plant steroid, ergosterol, whereas cholecalciferol is produced from the precursor 7-dehydrocholesterol, and derived exclusively from animal products. Vitamin D_3 is hydroxylated in the liver into 25-hydroxy-vitamin D (25-OHD_3), the circulatory and storage form of the vitamin. The 25-OHD_3 is transported to the kidney where it can be converted into the active hormonal form 1,25 dihydroxy-vitamin D 1,25-$(OH)_2D_3$ or calcitriol. The main products used in animal nutrition are vitamin D_3 and 25-OHD_3.
Chemical formula	$C_{27}H_{44}O$
Classification	Fat-soluble
Molecular weight	384,65 g/mol.
Alternative names	Cholecalciferol Calciol
CAS No.	67-97-0
Key natural sources	Some fish meals. Supplementation required for all species as feedstuffs supply limited amounts.
Biological functions	• Calcium and phosphorus absorption from the small intestine; • Mobilisation of calcium from bones; • Synthesis of calcium binding properties; • Regulation of calcification of egg shell and bones; • Regulation of immune cells.
Benefits to the animal	• Improved bone quality; • Improved growth; • Improved egg yield and quality (shell); • Improved meat quality (beef); • Improved health and welfare.
Signs of deficiency	All species: rickets and defects in mineralization, determining soft bones; reduction in growth. Poultry: depressed growth and feed conversion; egg production drop; decreased hatchability. Swine: stiffness; lameness. Ruminants: stiffness; lameness; weakness and occasionally tetany. Fish: anorexia; tetany.

FOR ACTIVE SUBSTANCE

Tolerance to high intake	Poultry: as much as 100 times the requirement level may be tolerated. Swine: the upper safe dietary level for short-time exposure is 33,000 IU per kg. Cattle and sheep: the upper safe dietary level for short-term exposure is 25,000 IU per kg of diet. Fish: tolerance is high (several millions IU/kg diet).
Analytical methods	EURL evaluation report 2012, CRL/100180, CRL/100134, CRL/100231: In feed additive: HPLC-UV, PhEur 01/2008:0574, 0575, 0598. In premixtures and feed: HPLC-UV (VDLUFA 1997, method 13.8.1). In feed and water: RP-HPLC-UV (EN12821).

FOR COMMERCIAL PRODUCTS

Common commercial forms	Spray-dried vitamin D_3 preparations typically containing 500,000 iu/g. Combinations of vitamin A and D_3, usually in a 5:1 ratio.
Common physical parameters	Brownish, fine powder.
Stability under ambient conditions	Stability in manufactured form: up to 2 years.

25-HYDROXYVITAMIN D_3

FOR ACTIVE SUBSTANCE	
Introduction	25-hydroxyvitamin D_3 (25-OHD_3) is the first metabolite of vitamin D_3, produced in the liver by hydroxylation of calciferol. It is then transported to the kidney where it can be converted into the hormonal form 1,25-dihydroxy-vitamin D 1,25-$(OH)_2D_3$ or calcitriol. 25-OHD_3 is the main circulating form.
Chemical formula	$C_{27}H_{44}O_2 \cdot H_2O$
Classification	Fat-soluble
Molecular weight	418,7 g/mol.
Alternative names	Calcifediol Calcidiol
CAS No.	63283-36-3
Key natural sources	Active metabolite of vitamin D_3. Significant intakes only achieved via supplementation.
Biological functions	Calcium and phosphorus absorption from the small intestine. Mobilisation of calcium from bones. Synthesis of calcium binding properties. Regulation of calcification of egg shell and bones. Regulation of immune cells.
Benefits to the animal	Improved bone quality. Improved growth and meat yield. Improved egg yield and quality (shell). Improved health and welfare.
Signs of deficiency	All species: rickets and defects in mineralization, determining soft bones in all species; reduction in growth. Poultry: egg production drop; decreased hatchability. Swine: stiffness; lameness. Ruminants: stiffness; lameness; weakness and occasionally tetany. Fish: anorexia; tetany; increased liver and muscle liver content.
Tolerance to high intake	Poultry: 10 times the requirement level.
Analytical methods	In feed additive: HPLC-MS. In feed: RP-HPLC-UV.

FOR COMMERCIAL PRODUCTS	
Common commercial forms	25-hydroxyvitamin D_3 preparations
Common physical parameters	Fine, beige to brown, free-flowing spray-dried powder containing typically 1.25% of 25-hydroxyvitamin D_3.
Stability under ambient conditions	Stability in manufactured form: up to 1 year.

VITAMIN E

FOR ACTIVE SUBSTANCE

Introduction	Vitamin E is a generic term for various compounds based on tocopherol or tocotrienol. Alpha - tocopherol is recognized as vitamin E and is the active substance in vitamin E feed supplements. Alpha-tocopherol can be derived from natural sources or synthesized by chemical processes. Chemical synthesized α-tocopherol is a fully racemic mixture of stereoisomers, making it different from naturally occurring α-tocopherol. Plant extracted source of vitamin E (termed RRR-α-tocopherol) is derived from vegetable oils, consisting of 100% RRR-α-tocopherol stereoisomer. Chemically synthesized vitamin E (termed all-rac-α-tocopherol) is consisting of equal proportions of eight stereoisomers (12.5% each of RRR, RRS, RSR, RSS, SRR, SRS, SSR, SSS-α-tocopherol stereoisomer). These stereoisomers have different bio-activities compared to RRR-α-tocopherol stereoisomer.
Chemical formula	$C_{29}H_{50}O_2$
Classification	Fat-soluble
Molecular weight	430,7 g/mol tocopherol 472,8 g/mol tocopheryl acetate
Alternative names	IUPAC: Tocopherol;RRR-α-tocopherol (formerly d-α-tocopherol); all-rac-α-tocopherol (formerly dl-α-tocopherol)
CAS No.	59-02-9 (RRR-α- tocopherol) 58-95-7 (RRR α-tocopheryl-acetate) 7695-91-2 (all-rac α-tocopheryl-acetate)
Key natural sources	Wheat germ oil, grass, clover, alfalfa, uncrushed oil seeds, vegetable oils, liver, eggs.
Biological functions	Biological fat soluble antioxidant. Immune system stimulant. Regulation of DNA synthesis. Antitoxic effect (free radical quencher). Tissue protection. Development of reproductive organs. Regulation of gene expression.
Benefits to the animal	Improved immune response. Stabilization of tissue and fat in animal products. Preparation for pregnancy and maintaining integrity and optimal function of the reproductive organs. High amounts of vitamin E are commonly used in animal feed for improving immune function and meat quality.

FOR ACTIVE SUBSTANCE

Signs of deficiency	Common: muscular dystrophy; fertility disorders; reduced immune response. Ruminants: stiff lamb disease; retained placenta; increased mastitis; Pigs: mulberry heart disease; mastitis, metritis and agalactia (MMA) in sows; banana disease; yellow fat. Poultry: muscular dystrophy; encephalomalacia (crazy chick disease); yellow fat; reduced hatchability. Dogs: muscle degeneration; failure of gestation; weak, dead pups.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.
Analytical methods	EURL evaluation report 2010, CRL/080022: In feed additive: Gas chromatography, according to the relevant PhEur monographs. In premixtures and feed: HPLC.

FOR COMMERCIAL PRODUCTS

Common commercial forms	All-rac-α-tocopheryl acetate RRR-α-tocopheryl acetate RRR-α-tocopherol
Common physical parameters	Light yellow viscous oil (tocopherol; tocopheryl acetate) Powders cream-white (tocopheryl acetate) Adsorbate powders (tocopheryl acetate) Spray dried coated powders for water dispersible products (tocopheryl acetate)
Stability under ambient conditions	Stability in manufactured form: up to 2 years. Stability in feed and premixtures: 6 months minimum.

VITAMIN K_3

FOR ACTIVE SUBSTANCE	
Introduction	Vitamin K is known for its contribution to the blood clotting or coagulation process. Menadione (Vitamin K_3) is the form of vitamin K that is used in animal nutrition. Vitamin K^3 is offered in a number of forms: Menadione sodium bisulphite (MSB) Menadione nicotinamide bisulphite (MNB)
Chemical formula	$C_{11}H_{15}O_8SNa$ MSB $C_{17}H_{16}N_2O_6S$ MNB
Classification	Fat-soluble
Molecular weight	330,29 g/mol MSB 376,42 g/mol MNB
Alternative names	IUPAC: • 1,2,3,4-Tetrahydro-2-methyl-1,4-dioxo-2-naphthalenesulfonic acid, sodium salt, trihydrate; vitamin K3; K-Thrombin (MSB) • 1,2,3,4-Tetrahydro-2-methyl-1,4-dioxonapthalen-2-naphthalenesulfonic acid compound with 3-Pyridinecarboxamide, 2-Methoxysulfonyltetralin-1,4-dione with pyridine-3 carboxamide (MNB)
CAS No.	6147-37-1 MSB 73581-79-0 MNB
Key natural sources	Vitamin K_3 does not occur naturally. Green plants are rich in Vitamin K_1. Vitamin K_2 is produced by bacteria in the rumen.
Biological functions	Vitamin K contributes to blood clotting and coagulation. It also acts as a coenzyme in other metabolic processes related to bone mineralization, protein formation, and some specific to cell growth.
Benefits to the animal	Performance improvement. Supports general health and well-being. Avoid bleeding.
Signs of deficiency	Impairment of blood coagulation. Low prothrombin levels. Increased clotting time. Haemorrhages in various tissues and organs.
Tolerance to high intake	No evidence of adverse effects on animals caused by high levels of oral intake.

FOR ACTIVE SUBSTANCE	
Analytical methods	EURL evaluation report 2011, EURL/100156: In feed additive and water: Spectrophotometric method (VDLUFA Method Bd. III 13.7.1). In premixtures and feed: HPLC-UV.
Antagonists	Coumarin inhibits vitamin K.

FOR COMMERCIAL PRODUCTS	
Common commercial forms	MSB and MNB
Common physical parameters	White to brownish powders
Stability under ambient conditions	Stability in feed: 3 months minimum. Stability in premixtures: 6 months minimum.

Vitamin supplementation

Basic considerations

The needs 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. There have been fundamental developments in our knowledge of the vitamin requirements of livestock. In the past, the prime purpose of adding vitamins to feedstuffs was to protect animals from deficiency. Nowadays the focus has been broadened to include animal health and welfare, ecology and economy. Nevertheless the main objective of an optimised vitamin supply remains to ensure health under practical conditions of animal husbandry.

Factors influencing vitamin supplementation

The vitamin supply is the amount of vitamins given to the animal in its feed, according to individual requirements, influenced by several factors:

- Animal related: animal species, age, genotype, health;
- Environment related: hygiene, climate, and housing;
- Production related: performance, stress, welfare, end-product quality, economic advantage;
- Intended use related: reproduction, production, or hobby;
- Feed related:
  - → Natural variations in nutrients owing to growth periods, harvest, drying and storage;
  - → Biological availability (e.g. only 50% α-tocopherol in vitamin E from cereals; biotin availability from wheat only 10% for pigs and poultry);
  - → Vitamin antagonism (coumarin which inhibits vitamin K; thiaminases which inhibits vitamin B1; avidin which inhibits biotin);
  - → Vitamin stability during the feed production processes and storage.

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. It takes into account the different factors described above.
- *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.

For certain vitamins and/or certain animal species, beyond the requirement interval, also the maximum safe supplementation level should be taken into consideration: this is been referred to as "tolerance to high intake". For a limited group of vitamins and animal species adverse effects have been reported in literature when the requirement level is significantly exceeded (multiples), which allow setting maximum levels for those vitamins.

Recommendations

Scientific laboratories, authorities, associations and companies offer varying recommendations for vitamin supply, which they base on different approaches for calculating vitamin allowances.
Animals are not able to store major quantities of the water-soluble vitamins, hence continuous supply via feed or water is essential. On the contrary, fat soluble vitamins are stored in the body; the provision of high levels of vitamins at certain point of time might be an option, like in non-EU countries, where drenches could be used.
Many official recommendations (e.g. NRC or ARC) cover only the minimum requirement, which is not sufficient in normal practice. Housing conditions, hygiene, nutritional influences and general stress may considerably increase the animals' requirements. Companies (e.g. breeding companies, producers of feed and feed additives) therefore normally base their recommendations on the previously listed factors which are influencing the actual animal vitamin requirements.
FEFANA recommendations - provided in separate tables at the end of this booklet - are based on common practice. 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. When environmental conditions in the farm evolve, e.g. heat increase, presence of a disease, additional vitamins may be supplied through the drinking water supply.

Safety

Continuous availability of vitamins to the metabolism guarantees that many vital functions of the animal organism can be maintained. From a chemical point of view, vitamins are organic substances with a rather simple molecular structure, with the exception of vitamin B12. In general, vitamins are considered to be substances with a high degree of safety during manufacture and application.
Vitamins have shown their safety and efficacy as feed supplements over many decades. In an effort to further prove that, industry has provided the EU authorities with detailed registration dossiers.
European legislation can set maximum levels for some vitamins (e.g. vitamins A and D) for use in feed if required to ensure the appropriate level of intake of the animals or consumers of animal-derived foods such as meat, milk or eggs.

Production

There are three main production processes for vitamins:

- Synthesis
- Extraction
- Fermentation

Table 3 lists the various production methods for vitamins used in animal nutrition.

The vitamins used in animal nutrition are almost exclusively produced by synthesis.
Vitamins extracted from plants or animal products are commonly used in human nutrition; a few extracted vitamin products (such as Betaine and vitamin E) are also used in animal nutrition.
Even though chemical synthesis is nowadays the major source of vitamin production, fermentation is expected to become more and more important. In the case of Vitamin B_2 and B_{12} production by fermentation is already common practice today.
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 optimizes the productivity of the micro-organisms and at the same time, can reduce the burden on the environment as a result of a better use of resources.
Whether vitamins are obtained by fermentation, chemical synthesis or by extraction, they are identical to those occurring in nature, and therefore have the same biological effects.

Table 3 - *Overview of current common vitamins' production processes*

	Synthesis	Fermentation	Extraction
Betaine	A		A
Biotin	A	P	
L-Carnitine	A	P	
ß-Carotene	A		P
Choline Chloride	A		
Folic Acid	A		
Inositol	A	P	
Niacin	A		
D-Pantothenic Acid	A		
Taurine	A		P
Vitamin A	A		
Vitamin B_1	A		
Vitamin B_2	P	A	
Vitamin B_6	A	P	P
Vitamin B_{12}		A	
Vitamin C	A		
Vitamin D_3	A		
Vitamin E	A		A
Vitamin K_3	A		

A = Common production process; P = Possible production process

Before vitamins are used, they often need to be formulated into stable preparations in order to guarantee the maintenance of their characteristics, their activity, or their safe handling and use. One important aspect is the stability of vitamins during the various production steps and in the final premixes and compound feed. The vitamins A, D_3 and E, for example, are obtained in the form of oils and are usually converted into powder before they can be applied in the feed industry. Normally, modern spray-drying processes are used, accompanied by methods to improve stability.

Commercial forms and quality criteria

The choice of the most appropriate vitamin product form for an individual application depends on the intended use, processing, and the estimated shelf life of the compound feed. Many vitamins are brought to the market in various commercial forms, which differ in their application properties. The most important distinctive properties and criteria are:

- Solubility or dispersibility 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, stability in premixes due to the presence of potential oxidants)

Be aware that the classification of vitamins as fat-soluble and water-soluble only refers to pure vitamins, and not to 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 materials such as carriers.

Conversion factors

A number of different molecules are commercialized with the same specific vitamin activity, and these different substances may be authorised as feed additives in their own right. It is then necessary to be able to relate the amount of the substances to the level of vitamin activity.

Table 4 provides for a summary for the main active substances and their conversion factors.

Table 4 - *Conversion factors*

Vitamin (active substance)	Unit	Conversion factor from 1 unit of active substance to amount of vitamin form
Betaine	1 mg	2.13 mg liquid betaine anhydrous (47%) 1.41 mg betaine HCl (93%) 1.27 mg betaine monohydrate (91%) 2.38 mg choline chloride 50%
L-Carnitine	1mg	1.49 mg L-carnitine L-tartrate
Choline Chloride	1 mg	1.34 mg choline chloride (basis choline hydroxy-analogue)
Niacin	1 mg	1 mg nicotinic acid 1 mg nicotinamide
D-Pantothenic Acid	1 mg	1.087 mg Calcium D- pantothenate
Vitamin A (retinol)	1 IU	0.300 µg retinol (vitamin A alcohol) 0.344 µg retinyl acetate 0.550 µg retinyl palmitate 0.359 µg retinyl propionate
Vitamin B_1 (thiamine)	1 mg	0.919 mg thiamine mononitrate 0.892 mg thiamine hydrochloride
Vitamin B_6 (pyridoxine)	1 mg	1.215 mg pyridoxine hydrochloride
Vitamin D_3 (cholecalciferol)	1 IU	0.025 µg cholecalciferol
Vitamin D_3 (25-OH-calciferol)	1 mg	1 mg of 25-OH-vitamin D3
Vitamin E (tocopherol)	1 IU	1.000 mg all-rac-α-tocopheryl acetate 0.735 mg RRR-α-tocopheryl acetate 0.671 mg RRR-α-tocopherol
Vitamin K_3 (menadione)	1 mg	1.96 mg menadione sodium bisulphite (MSB) 2.1 mg menadione nicotinamide bisulphite (MNB) 2.22 mg menadione dimethyl pyrimidinol bisulphite (MPB)

Regulatory Background

Globally, the CODEX ALIMENTARIUS working group on feed has defined vitamins as feed additives, linked to a submission of information related to the products to a pre-market assessment for the risk for the workers, the animals, the consumers of animal products and possibly for the environment. The submission leads to a registration process of vitamins in numerous countries around the globe. It has to be ensured that the vitamins to be used have followed the relevant registration process in the country where the vitamin product is marketed. Different assessments are done depending on the countries.

In Europe, commercially produced vitamin products added to animal feed are classified as feed additives and as such are closely regulated.

Regulation (EC) No 1831/2003* on additives for use in animal nutrition, provides the controlling legislation. Under the terms of this regulation, feed additives are classified into different categories and 'functional groups' and need to be authorised before being placed on the market.

Vitamin products are classified as follows (Annex I, Regulation (EC) No 1831/2003):

Additive Category:
3 - Nutritional additives
Functional Group:
(a) - Vitamins, pro-vitamins and chemically well-defined substances having similar effect

To gain authorisation, substances with vitamin activity are subject to a full assessment demanding detailed information relating to:

- the identity and chemical purity of the active substance(s)

- the forms in which the vitamins may be put on the market
- their safety for:
  - → people who are handling the products
  - → the target animals which will receive them
  - → consumers of foods derived from the animals if the vitamins are deposited in the tissues
  - → the environment
- their homogeneity and stability in different matrices such as premixtures, feed and water for drinking (for use in which specific authorisation is required)
- in case of new form of vitamins, their biological function

Regulation (EC) No 429/2008* and subsequent EFSA guidance documents provide detailed information relating to the content of dossiers submitted to support feed additive authorisation requests.

Dossiers are first submitted to the European Commission who then asks EFSA to undertake a risk assessment. In addition, detailed analytical methodology has to be provided, together with samples of the products. This is assessed separately by the European Union Reference Laboratory (EURL). Following publication of the EFSA opinion, the Commission, in their role as risk managers, then draft an authorising regulation for each additive, defining the required specification, target species and usage rates and indicating any specific conditions or labelling requirements.

In some cases maximum permitted levels in feed may be established in order to control any perceived risks.

Unless produced by a unique process, e.g. involving very specific strains of micro-organisms, vitamin authorisations are generic and not linked to particular manufacturers. Any producer able to meet the defined product specifications and who are compliant with other aspects of food and feed law may place products on the market.

Re-authorisation has been required to all feed additives already on the market prior to 2004, including vitamin products. Furthermore re-authorisation of all products is required every ten years in order to account for new knowledge gained during the interim period. As products become authorised, they are listed in the **Community Register of Feed Additives**, published and regularly updated by the European Commission. As the re-authorisation process proceeds, each vitamin will be assigned an identity number which reflects the additive category (3) and the functional group (a), followed by a numeric code.

Labelling of vitamins in feed is controlled by **Regulation (EC) No 767/2009*** on the placing on the market and use of feed.

It is mandatory to declare the added level of any feed additive for which a maximum level applies (in any species) in the 'additives' section of a feed label.

It is also permitted to make a voluntary declaration in the 'analytical constituents' part of the label. In this case, the amount of vitamin expected to be present throughout the shelf-life of the feed should be declared. Declarations are subject to technical tolerances as defined in Annex IV* of Regulation (EC) No 767/2009 and in addition, to analytical tolerances as may be reported by the laboratory concerned.

In order to incorporate vitamins into feed, manufacturers have to comply with specific feed hygiene requirements and to be registered or approved by their local competent authorities. This is governed by **Regulation (EC) No 183/2005* laying down requirements for feed hygiene**. This regulation defines the levels of control and the standards to which feed business operators should adhere.

* Documents: it refers to the relevant regulations and any subsequent amendments.

GHS/CLP

The GHS has been adopted at international level by the United Nations and provides a system to harmonise the criteria for classification of substances and mixtures relating to physical and health hazards during handling (e.g. worker safety), environmental hazards, as well as the rules of labelling and packaging for hazardous substances and mixtures. FEFANA has consolidated the classification for certain categories of substances based on the Globally Harmonised System (GHS) and in alignment with the EU legislation on Classification, Labelling and Packaging (CLP).

Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures (the so-called "CLP Regulation") implements the general principles of the GHS.

While Annex VI of the CLP Regulation lists the existing harmonised classification and labelling of substances according to the GHS, it does not cover every substance currently defined as a feed additive. Furthermore, the EU legislator mandates manufacturers and importers of the same substance to make every effort to agree on a single classification for that substance. In order to achieve this objective, FEFANA has been consolidating the classification for certain categories of substances, including vitamins. As far as vitamins are concerned, in Annex VI of the CLP Regulation, a harmonised classification appears only for the Vitamins D_2 and D_3, as well as for menadione and its salts (Vitamin K_3). Currently, all other vitamins are not covered by Annex VI. The FEFANA Working Group Vitamins has therefore classified vitamins, by common agreement, based on current classification (see Tables 6 and 7). For vitamins D_2, D_3 and K_3, the FEFANA classification reflects the one from Annex VI of the CLP Regulation; for all other vitamins, classification has been made based on currently available toxicity and ecotoxicity data. Please note: the table lists the classification from Annex VI for Vitamins D_2, D_3 and K_3.

For more information please see **Table 5** below and/or visit also the FEFANA website[1].

[1] http://www.fefana.org/clp-ghs/

Table 5 - *CLP/GHS for vitamins, provitamins and vitamin-like substances*

A. Vitamins and Provitamins

EEC NO	ADDITIVE	CHEMICAL FORMULA/ DESCRIPTION	HAZARD CLASSES PHYSICAL / HEALTH / ENVIRONMENTAL HAZARDS	LABEL / PICTOGRAMS	H PHRASES	REMARKS
E 672	**Vitamin A** **Retinol and its Esters**					**Classification refers to the active substance and not to formulations.** **Formulated products containing e.g. antioxidants may have a different classification**
		retinol acetate (as active substance)	Skin Irr. Cat 2 Repr. Cat 1B Aquatic Chronic Cat 4		H 315 Causes skin irritation H 360D H 413 May cause long lasting harmful effects to aquatic life.	
		retinol palmitate (as active substance)	Skin Irr. Cat 2 Repr. Cat 1B Aquatic Chronic Cat 4		H 315 Causes skin irritation H 360D H 413 May cause long lasting harmful effects to aquatic life.	

EEC NO	ADDITIVE	CHEMICAL FORMULA/ DESCRIPTION	HAZARD CLASSES PHYSICAL / HEALTH / ENVIRONMENTAL HAZARDS	LABEL / PICTOGRAMS	H PHRASES	REMARKS
E 671	**Vitamin D_3 Colecalciferol**	colecalciferol pre-colecalciferol	Acute toxicity oral 2 Acute toxicity dermal 3 Acute toxicity inhaled 2 STOT repeated 1	 	H300 Fatal if swallowed H311 Toxic in contact with skin H330 Fatal if inhaled H372 Causes damage to organs through prolonged or repeated exposure	**Classification according Annex VI (16.1.2012) of CLP Regulation 1278/2008** state all organs affected, if known state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard
	Calcifediol	25-hydroxycholecal-ciferol / 25-hydroxy-pre-cholecalciferol	Acute toxicity oral 3 Hazardous to aquatic environment - chronic toxicity 4 STOT repeated 1	 	H301 Toxic if swallowed H413 May cause long lasting harmful effects to aquatic life. H372 Causes damage to organs through prolonged or repeated exposure	state all organs affected, if known state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard
3a700	**Vitamin E**	all-rac-alpha tocopheryl acetate (all-rac-α TA) RRR-alpha tocopheryl acetate (RRR-α TA) RRR-alpha tocopherol (RRR-α T)	Not hazardous according CLP Not hazardous according CLP Not hazardous according CLP			

EEC NO	ADDITIVE	CHEMICAL FORMULA/ DESCRIPTION	HAZARD CLASSES PHYSICAL / HEALTH / ENVIRONMENTAL HAZARDS	LABEL / PICTOGRAMS	H PHRASES	REMARKS
	Vitamin K **Menadione and its Salts**					
		menadione sodium bisulphite (MSB)	Skin irritant 2 Eye irritant 2 Hazardous to aquatic environment – acute 1 Hazardous to aquatic environment – chronic 1		H315 Causes skin irritation H319 Causes serious eye irritation H 410 Very toxic to aquatic life with long lasting effects	**Classification according Annex VI (16.1.2012) of CLP Regulation 1278/2008**
		menadione nicotinamide bisulphite (MNB)	Skin irritant Cat. 2 Eye irritant Cat. 2 Hazardous to aquatic environment – acute 1 Hazardous to aquatic environment – chronic 1		H315 Causes skin irritation H319 Causes serious eye irritation H 410 Very toxic to aquatic life with long lasting effects	**Classification according Annex VI (16.1.2012) of CLP Regulation 1278/2008**
	Vitamin B_1 Thiamine and its Salts					
		thiamine hydrochloride	Not hazardous according CLP			
		thiamine mononitrate	Not hazardous according CLP			

EEC NO	ADDITIVE	CHEMICAL FORMULA/ DESCRIPTION	HAZARD CLASSES PHYSICAL / HEALTH / ENVIRONMENTAL HAZARDS	LABEL / PICTOGRAMS	H PHRASES	REMARKS
E 101 E 101 a	**Vitamin B_2 Riboflavin and its Esters**					
		riboflavin	Not hazardous according CLP			
		riboflavin-5'-phosphate	Not hazardous according CLP			
		ester monosodium salt (R-5'-P)				
3a831	**Vitamin B_6 Pyridoxine and its Salt**					
		pyridoxine hydrochloride	Not hazardous according CLP			
	Vitamin B_{12}					
		cyanocobalamin	Not hazardous according CLP			
		5'-desoxy-adenosyl-cobalamin (5'-d-AC)				
	Vitamin C L-Ascorbic Acid, its Esters and Salts					
E 300		L-ascorbic acid	Not hazardous according CLP			
E 301		sodium L-ascorbate	Not hazardous according CLP			
E 302		calcium L-ascorbate	Not hazardous according CLP			

EEC NO	ADDITIVE	CHEMICAL FORMULA/ DESCRIPTION	HAZARD CLASSES PHYSICAL / HEALTH / ENVIRONMENTAL HAZARDS	LABEL / PICTOGRAMS	H PHRASES	REMARKS
	Salts of Ascorbyl Phosphates					
		ascorbyl-monophosphate calcium (AMC)	Not hazardous according CLP			
		ascorbyl-monophosphate sodium (AMS)	Not hazardous according CLP			
3a841 3a842	**Pantothenic Acid**					
		calcium-D-pantothenate (Calpan)	Not hazardous according CLP			
		D-panthenol (D-Pan)	Not hazardous according CLP			
	Niacin					
3a314		nicotinic acid (NA)	Eye Irr. Cat. 2		H319 Causes serious eye irritation	
3a315		nicotinic acid amide (NAA)	Eye Irr. Cat. 2		H319 Causes serious eye irritation	
3a316	**Folic Acid**	folic acid	Not hazardous according CLP			
	Biotin	d- (+) -biotin	Not hazardous according CLP			
3a890	**Choline Chloride**	choline chloride (CCL)	Not hazardous according CLP			

B. Cofactors and other well-defined substances having a similar biological effect

EEC NO	ADDITIVE	CHEMICAL FORMULA/ DESCRIPTION	HAZARD CLASSES PHYSICAL / HEALTH / ENVIRONMENTAL HAZARDS	LABEL / PICTOGRAMS	H PHRASES	REMARKS
	Inositol					
		inositol	Not hazardous according CLP			
	L-Carnitine					
		L-carnitine	Not hazardous according CLP			
		L-carnitine-L-tartrate (CT)	Not hazardous according CLP			
	Betaine and its Salts					
		betaine hydrochloride	Eye Irr. Cat 2		H319 Causes serious eye irritation	
		betaine anhydrous			Not yet determined	
	Taurine					
		taurine	Not hazardous according CLP			
	Essential Fatty Acids:					
	Ω-3 and Ω-6 fatty acids		Not hazardous according CLP			

Tables of recommendations

- The unit used is mainly mg/Kg air-dry, unless differently indicated.
- For ruminants and horses the tables refers to animal (head) per day.
- Average weight for foals: 250 Kg.
- Average weight for horses: 550 Kg.

BETAINE

POULTRY	mg/kg
Broilers starter	200-2000
Broilers grower-finisher	200-2000
Broiler breeders	200-2000
Laying hens	200-2000
Turkey starter	200-2000
Turkey grower	200-2000
Turkey finisher	200-2000
Turkey breeder	200-2000
Ducks / geese	200-2000
Partridges / quails	0.2-2

SWINE	mg/kg
Piglets pre-starter	400-2000
Piglets starter	400-2000
Grower	300-1250
Finisher	300-1250
Gilts	300-1250
Sows	1200-3000
Boars	1000-2000
RUMINANTS AND HORSES	**g/head/d**
Dairy cows	20-50
Sheep and goats	Lactating goat and sheep: 4-6 Goat: 2-4 Lamb: 2kg/t

OTHER SPECIES	
Fish	0.5-2% Tilapia: 1500-2240 mg/kg
Crustaceans	0.5-2%

The recommended inclusion level is based on pure betaine levels. Final inclusion of the product should be recalculated for betaine concentration in the product.

BIOTIN

POULTRY	mg/kg
Broilers starter	0.2-0.4
Broilers grower-finisher	0.2-0.3
Broiler breeders	02.-0.4
Laying hens	0.1-0.15
Turkey starter	0.25-0.4
Turkey grower	0.25-0.3
Turkey finisher	0.2-0.25
Turkey breeder	0.4-0.6
Ducks / geese	0.1-0.15
Partridges / quails	0.15-0.25

SWINE	mg/kg
Piglets pre-starter	0.2-0.4
Piglets starter	0.2-0.4
Grower	0.15-0.3
Finisher	0.1-0.2
Gilts	0.3-0.5
Sows	0.5-0.8
Boars	0.5-0.8

RUMINANTS AND HORSES	mg/head/d
Dairy cows	20
Beef cattle	10-20
Calves	0.05-0.1
Heifers	10-20
Beef cows	20
Breeding bulls	20
Sheep and goats	5
Foals	2-3
Leisure horses	15-20
Race & breeding horses	15-20

OTHER SPECIES	mg/Kg
Dogs	0.25-0.8
Cats	0.25-0.8
Rabbit	0.1-0.2
Mink and foxes	0.3-0.6
Salmon and trout	0.8-1
Warm-water fish	0.5-1
Sea bream and sea bass	0.8-1
Shrimp	1-2
Eels	0.3-0.5

L-CARNITINE

POULTRY	mg/kg
Broilers starter	20-30
Broilers grower-finisher	20-30
Broiler breeders	25-35
Laying hens	25-40
Turkey starter	25-40
Turkey grower	25-40
Turkey finisher	25-40
Turkey breeder	30-45
Ducks / geese	20-30
Partridges / quails	30-40

SWINE	mg/Kg
Piglets pre-starter	40-60
Piglets starter	30-40
Grower	20-40
Finisher	20-40
Gilts	50-60
Sows	50-60
Boars	250

RUMINANTS AND HORSES [1]	g/head/d
Dairy cows	1-2
Beef cattle	100-150 [2]
Calves	200 [3]
Sheep and goats	200 [4]
Foals	2-5
Leisure horses	5
Race & breeding [5] horses	10-15

OTHER SPECIES	mg/Kg
Dogs [6]	300-500
Cats	200-500
Rabbit	40-50
Mink and foxes	40-50
Salmon and trout	500-1000
Warm-water fish	100-400
Sea bream and sea bass	100-400
Shrimp	500-1000
Eels	100-500
Pigeon [7]	25-50

[1] protected form is recommended to bypass ruminal degradation
[2] mg per 100 kg body weight daily
[3] mg per kg milk replacer powder
[4] mg per kg feed
[5] breeding horses 1-2 g, stallions 10-15 g/head/d
[6] racing dogs 500-1000 mg/kg
[7] permanent provision via drinking water: 10-15 mg per litre of water in layers and breeders

ß-CAROTENE

SWINE	mg/Kg
Sows	300

RUMINANTS AND HORSES [1]	mg/head/d
Dairy cows	300-1000
Calves	50-100
Heifers	300-500 [1] 500-1000 [2]
Beef cows	300-500
Race & breeding horses	400-800

OTHER SPECIES	mg/Kg
Dogs	30-50
Cats	30-50
Rabbit	10-20

[1] rearing
[2] 4-6 weeks before calving

CHOLINE[1]

POULTRY	mg/Kg
Broilers starter	400-700
Broilers grower-finisher	400-700
Broiler breeders	350-700
Laying hens	300-500
Turkey starter	1000-1200
Turkey grower	500-1000
Turkey finisher	400-600
Turkey breeder	1000-1200
Ducks / geese	300-500
Partridges / quails	400-600

SWINE	mg/Kg
Piglets pre-starter	500-800
Piglets starter	250-400
Grower	150-300
Finisher	100-200
Gilts	250-500
Sows	500-800
Boars	500-800

RUMINANTS AND HORSES	mg/head/d
Dairy cows	250-500
Beef cattle	250-500
Foals	300-400
Leisure horses	600-900
Race & breeding horses	1000-1400

OTHER SPECIES	mg/Kg
Dogs	1300-2700
Cats	1300-2700
Rabbit	600-800
Mink and foxes	500-1000
Salmon and trout	600-1000
Warm-water fish	600-1000
Sea bream and sea bass	600-1000
Shrimp	400-600

[1] dosages in the table expressed as Choline; to be multiplied by 1,34 to obtain Choline Chloride dose

FOLIC ACID

POULTRY	mg/Kg
Broilers starter	2-2.5
Broilers grower-finisher	2-2.5
Broiler breeders	1.5-2
Laying hens	1-1.5
Turkey starter	4-6
Turkey grower	2-3
Turkey finisher	2-2.5
Turkey breeder	2-3 (1) 4-6 (2)
Ducks / geese	1-2
Partridges / quails	2-4

SWINE	mg/Kg
Piglets pre-starter	1.5-3
Piglets starter	1.5-2.5
Grower	1-1.5
Finisher	0.5-1
Gilts	3.5-5.5
Sows	3.5-5.5
Boars	3.5-5.5

RUMINANTS AND HORSES	mg/head/d
Calves	0.2-0.3
Foals	15-20
Leisure horses	25-35
Race & breeding horses	45-65

OTHER SPECIES	mg/Kg
Dogs	0.6-2
Cats	0.6-2
Rabbit	0.2-0.5
Mink and foxes	0.6-1
Salmon and trout	6-10
Warm-water fish	4-7
Sea bream and sea bass	4-6
Shrimp	10-20
Eels	4-6

(1) growers
(2) starters and layers

INOSITOL

OTHER SPECIES	mg/Kg
Dogs	150-350
Cats	150-350
Salmon and trout	300-500
Warm-water fish	50-200
Sea bream and sea bass	50-200
Shrimp	500-1000

NIACIN

POULTRY	mg/Kg
Broilers Starter	60-80
Broilers Grower-finisher	50-80
Broiler breeders	50-60
Laying hens	50-60
Turkey starter	100-150
Turkey grower	80-100
Turkey finisher	60-80
Turkey breeder	100-150
Ducks / gees	60-80
Partridges / quails	60-80

SWINE	mg/Kg
Piglets pre-starter	60-80
Piglets starter	40-60
Grower	30-40
Finisher	20-40
Gilts	30-40
Sows	30-40
Boars	30-40

RUMINANTS AND HORSES	mg/head/d
Dairy cows	6-12[(1)]
Beef cattle	1[(2)]
Calves	30-40
Sheep and goats	10-20
Foals	40-60
Leisure horses	120-160
Race & breeding horses	140-180

OTHER SPECIES	mg/Kg
Dogs	50-170
Cats	50-170
Rabbit	40-60
Mink and foxes	50-100
Salmon and trout	180-200
Warm-water fish	180-200
Sea bream and sea bass	100-140
Shrimp	200-250

[(1)] g/head/d added per animal per day during 2 weeks prior and 100 days in lactation
[(2)] g/head/d added per animal per day feedlots

D-PANTOTHENIC ACID

POULTRY	mg/Kg
Broilers starter	15-20
Broilers grower-finisher	12-18
Broiler breeders	10-15
Laying hens	15-25
Turkey starter	30-35
Turkey grower	20-25
Turkey finisher	15-20
Turkey breeder	25-35
Ducks / geese	10-15
Partridges / quails	15-25

SWINE	mg/Kg
Piglets pre-starter	30-50
Piglets starter	25-45
Grower	25-45
Finisher	25-45
Gilts	15-30
Sows	30-35
Boars	20-30

RUMINANTS AND HORSES	mg/head/d
Calves	7-90

OTHER SPECIES	mg/Kg
Dogs	30-60
Cats	30-60
Rabbit	10-15
Mink and foxes	8-20
Salmon and trout	40-60
Warm-water fish	40-50
Sea bream and sea bass	50-100
Shrimp	100-180

TAURINE

OTHER SPECIES	mg/Kg
Dogs	2000 (1)
Cats	400-500
Mink and foxes	2000 (1)
Salmon and trout	2000 (1)

(1) maximum permitted level

VITAMIN A

POULTRY	IU/Kg
Broilers starter	11000-13000
Broilers grower-finisher	10000-12000
Broiler breeders	10000-14000
Laying hens	8000-12000
Turkey starter	11000-13000
Turkey grower	10000-12000
Turkey finisher	7000-9000
Turkey breeder	11000-15000
Ducks / geese	10000-15000
Partridges / quails	6000-6500

SWINE	IU/Kg
Piglets pre-starter	15000-25000
Piglets starter	10000-20000
Grower	8000-12000
Finisher	6000-8000
Gilts	8000-12000
Sows	10000-15000
Boars	10000-15000

RUMINANTS AND HORSES	IU/head/d
Dairy cows	75000-125000 (1) 80000-120000 (2)
Beef cattle	50000-100000
Calves	20000-25000
Heifers	40000-60000
Sheep and goats	6000-10000
Foals	10000-12000
Leisure horses	6000-8000
Race & breeding horses	12000-15000

OTHER SPECIES	IU/Kg
Dogs	10000-12000
Cats	20000-30000
Rabbit	10000-15000
Mink and foxes	10000-15000
Warm-water fish	8000-12000
Shrimp	10000-12000

(1) far off & close-up
(2) transition and lactation

VITAMIN B_1

POULTRY	mg/Kg
Broilers starter	3-4
Broilers grower-finisher	2-3
Broiler breeders	3-3.5
Laying hens	2.5-3
Turkey starter	4.5-5
Turkey grower	3-5
Turkey finisher	2-4
Turkey breeder	4-5
Ducks / geese	2-3
Partridges / quails	2-4

SWINE	mg/Kg
Piglets pre-starter	3.5-5.5
Piglets starter	3-5
Grower	2-3
Finisher	1-2
Gilts	1-2
Sows	2-2.5
Boars	1-2

RUMINANTS AND HORSES	mg/head/d
Beef cattle	60-250 [1]
Sheep and goats	100
Foals	20-25
Leisure horses	40-55
Race & breeding horses	70-110

OTHER SPECIES	mg/Kg
Dogs	4-8
Cats	5-10
Rabbit	1-2
Mink and foxes	20-50 [2]
Salmon and trout	10-20
Warm-water fish	10-20
Sea bream and sea bass	20-30
Shrimp	50-100

[1] upper level on high concentrate rations
[2] feeding raw fish add 50 mg/kg

VITAMIN B_2

POULTRY	mg/Kg
Broilers starter	8-10
Broilers grower-finisher	7-9 6-8
Broiler breeders	12-16
Laying hens	5-7
Turkey starter	15-20
Turkey grower	10-15
Turkey finisher	8-10
Turkey breeder	15-20
Ducks / geese	7-9
Partridges / quails	5-7

SWINE	mg/Kg
Piglets pre-starter	10-15
Piglets starter	10-15
Grower	7-10
Finisher	6-10
Gilts	6-10
Sows	6-10
Boars	6-10

RUMINANTS AND HORSES	mg/head/d
Foals	20-30
Leisure horses	30-40
Race & breeding horses	70-85

OTHER SPECIES	mg/Kg
Dogs	13-22
Cats	22-27
Rabbit	3-6
Mink and foxes	10-20
Salmon and trout	20-30
Warm-water fish	15-20
Sea bream and sea bass	20-30
Shrimp	40-80

VITAMIN B_6

POULTRY	mg/Kg
Broilers starter	4-6
Broilers grower-finisher	4-6
Broiler breeders	4-6
Laying hens	3.5-5
Turkey starter	6-7
Turkey grower	5-7
Turkey finisher	3-6
Turkey breeder	6-7

SWINE	mg/Kg
Piglets pre-starter	6-8
Piglets starter	6-8
Grower	2.5-4.5
Finisher	2-3.5
Gilts	3.5-5.5
Sows	3.5-5.5
Boars	3.5-5.5

RUMINANTS AND HORSES	mg/head/d
Calves	2.5-4.5
Foals	15-20
Leisure horses	25-35
Race & breeding horses	40-55

OTHER SPECIES	mg/Kg
Dogs	6-11
Cats	11-14
Rabbit	2-3
Mink and foxes	10-20
Salmon and trout	15-25
Warm-water fish	15-25
Sea bream and sea bass	20-25
Shrimp	50-120
Eels	10-15

VITAMIN B_{12}

POULTRY	µg/Kg
Broilers starter	20-40
Broilers grower-finisher	20-30
Broiler breeders	20-40
Laying hens	15-25
Turkey starter	40-50
Turkey grower	30-40
Turkey finisher	15-30
Turkey breeder	40-50
Ducks / geese	20-40
Partridges / quails	30-50

SWINE	µg/Kg
Piglets pre-starter	50-70
Piglets starter	40-60
Grower	30-50
Finisher	30-50
Gilts	30-50
Sows	30-50
Boars	30-50

RUMINANTS AND HORSES	µg/head/d
Foals	150-300
Leisure horses	350-650
Race & breeding horses	550-850

OTHER SPECIES	µg/Kg
Dogs	30-50
Cats	20-100
Rabbit	10-20
Mink and foxes	30-60
Salmon and trout	30-50
Warm-water fish	20-50
Sea bream and sea bass	100-200
Shrimp	20-50

VITAMIN C

POULTRY	mg/Kg
Broilers starter	100-200
Broilers grower-finisher	100-200
Broiler breeders	100-150
Laying hens	100-200
Turkey starter	100-200
Turkey grower	100-200
Turkey finisher	100-200
Turkey breeder	100-200
Ducks / geese	100-200
Partridges / quails	100-200

SWINE	mg/Kg
Piglets pre-starter	100-200
Piglets starter	100-200
Gilts	200-300
Sows	200-300
Boars	200-500

RUMINANTS AND HORSES	mg/head/d
Calves	250-500 (1)
Foals	500-750 (2)
Race & breeding horses	1000-2000

OTHER SPECIES	mg/Kg
Dogs	100-200
Cats	100-200
Rabbit	150-250
Mink and foxes	100-200
Salmon and trout	150-250
Warm-water fish	150-250
Sea bream and sea bass	150-250
Shrimp	250-500

(1) milk replacers
(2) 1st year

VITAMIN D_3

POULTRY	IU/Kg
Broilers starter	3000-5000
Broilers grower-finisher	3000-5000
Broiler breeders	3000-5000
Laying hens	3000-4000
Turkey starter	4000-5000
Turkey grower	3000-5000
Turkey finisher	3000-5000
Turkey breeder	4000-5000
Ducks / geese	3000-5000
Partridges / quails	3000-4000

SWINE	IU/Kg
Piglets pre-starter	1800-200
Piglets starter	1800-2000
Grower	1500-2000
Finisher	1000-1500
Gilts	1800-2000
Sows	1500-2000
Boars	1500-2000

RUMINANTS AND HORSES	IU/head/d
Dairy cows	25000-35000 (1) 30000-50000 (2)
Beef cattle	60-250
Calves	1400-1800
Heifers	2500-4000
Sheep and goats	600
Foals	4500-5500
Leisure horses	3500-4500
Race & breeding horses	6500-8500

OTHER SPECIES	IU/Kg
Dogs	780-1300
Cats	780-1300
Rabbit	800-1200
Mink and foxes	1500-2000
Salmon and trout	2000-2500
Warm-water fish	1500-2000
Sea bream and sea bass	1700-2200
Shrimp	2000-3500

(1) far off & close-up, and transition
(2) lactation

25-HYDROXYVITAMIN D_3

POULTRY	mg/Kg
Broilers starter	0.069
Broilers grower-finisher	0.069
Broiler breeders	0.069
Laying hens	0.069
Turkey starter	0.092
Turkey grower	0.092
Turkey finisher	0.092
Turkey breeder	0.092
Ducks / geese	0.069
Partridges / quails	0.069

SWINE	mg/Kg
Piglets pre-starter	0.05
Piglets starter	0.05
Grower	0.05
Finisher	0.05
Gilts	0.05
Sows	0.05
Boars	0.05

VITAMIN E

POULTRY	mg/Kg
Broilers starter	40-60 150-200 (1)
Broilers grower-finisher	20-30
Broiler breeders	30-50 150-200 (2)
Laying hens	20-30
Turkey starter	40-60 150-200 (1)
Turkey grower	30-50
Turkey finisher	30-40 150-200 (2)
Turkey breeder	40-60 80-120 (3)
Ducks / geese	40-60
Partridges / quails	40-60 80-120 (3)

SWINE	mg/Kg
Piglets pre-starter	80-150 200-250 (1)
Piglets starter	70-100
Grower	60-80
Finisher	40-60 150-200 (2)
Sows	60-80 100-150 (4)

RUMINANTS AND HORSES	mg/head/d
Dairy cows	200-400 1000-1500 (7)
Calves, milk replacer (5)	80-120 150-200 (1)
Heifers	100-150
Fattening cattle	200-300 1000-1500 (8)
Cows	2000-3000 (9) 1000-2000 (10)
Sheep and goats	50-80
Foals (6)	1000-1200
Leisure horses (6)	600-800
Race & breeding horses (6)	1200-1500

OTHER SPECIES	mg/Kg
Dogs	100-250
Cats	150-300
Rabbit	40-60
Fish	100-400

(1) for improved immunity
(2) for improved meat quality
(3) for improved immunity in newly hatched chicks
(4) lactating sow, in weaning piglets at 7 days before parturition till weaning
(5) Kg/feed
(6) per 100 Kg body weight per day
(7) for improved udder health during dry periods and during the first 10 weeks of lactation
(8) for improved meat quality 100 days before slaughtering
(9) 4 weeks pre-partum; for improved immunity during calving and in newborn calves
(10) 4 weeks post-partum; for improved immunity during calving and in newborn calves

VITAMIN K_3

POULTRY	mg/Kg
Broilers starter	3-4
Broilers grower-finisher	3-4
Broiler breeders	3-5
Laying hens	5-7
Turkey starter	4-5
Turkey grower	3-4
Turkey finisher	3-4
Turkey breeder	4-5
Ducks / geese	20-30

SWINE	mg/Kg
Piglets pre-starter	8-10
Piglets starter	5-6
Grower	2-4
Finisher	2-4
Gilts	1.5-3
Sows	4.5-5
Boars	4.5-5

OTHER SPECIES	mg/Kg
Dogs	1-2
Cats [1]	1-2
Rabbit	1-2
Mink and foxes	1-2
Salmon and trout [2]	8-12
Warm-water fish [2]	5-10
Sea bream and sea bass [2]	8-12
Shrimp [3]	40-60

[1] vitamin K_3 supplementation is particularly important in canned catfoods that contain >25% fish when fed for long periods
[2] amount to be increased by 30% for fry and broodstock
[3] at low stock density (<10p/m2) the lower levels are recommended

Acronyms

- AOAC: association of analytical communities
- ARC: Agricultural Research Council (UK), now Agricultural and Food Research Council
- CAS: Chemical Abstract Services
- CEN: European Committee for Standardization
- CLP: Classification, Labelling and Packaging
- DLG: German Society for Agriculture (Deutsche Landwirtschafts-Gesellschaft)
- EC: European Commission
- EFSA: European Food Safety Authority
- EN: European Standards test methods
- EURL [formally the CRL]: European Union Reference Laboratories
- FL: Fluorescence detection
- GHS: Globally Harmonised System
- HPLC: High-Performance Liquid Chromatography
- IUPAC: International Union of Pure and Applied Chemistry
- JECFA: Joint FAO-WHO Expert Committee Report on Food Additives
- MS: Mass Spectrometry
- NRC: (American) National Research Council
- PhEur: European Pharmacopoeia
- RI: Refractive Index
- RP: Reverse Phase
- UV: Ultraviolet
- UV/VIS: Ultraviolet–visible spectrophotometry
- VDLUFA: the Association of German Agricultural Analytic and Research Institutes, agricultural analysis and research institutions.

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Acknowledgements

This booklet is the result of efforts and commitment of the members of the FEFANA Working Group Vitamins. The FEFANA companies involved in the drafting of this booklet were the following: ADISSEO, ADM, BALCHEM, CHR. OLESEN, DSM, DUPONT, LONZA, ORFFA, and SUNVIT. Acknowledgements go in particular to René Blum, Geoff Brown, An Buyens, Yueming Derjant-Li, Elena Miceli, Chantal Nuyts, Manfred Peisker, Lisa Saibi, Eleanor Tredway, and Gerritjan van der Ven.

Furthermore a special acknowledgement goes to the authors of the AWT/FEFANA booklet “Vitamins in Animal Nutrition” published in 2002 by Agrimedia GmbH. This older publication was one of the main reference and source of information and inspiration upon which this new booklet was drafted. In particular we thank: Dr. N. Albers (BASF), Dr. G. Gotterbarm (Adisseo), Dr. W. Heimbeck (Degussa), Dr. Th. Keller (BASF), Dr. J. Seehawer (Roche Vitamins), and Dr. T.D. Tran (Vilomix).

www.fefana.org

ISBN 978-2-9601289-2-5

fefana

Avenue Louise, 130A Box 1 - 1050 Brussels - Belgium
Tel.: +32 (0)2 639.66.60 - Fax: +32 (0)2 640.41.11
E-mail: info@fefana.org