

## THE IMPORTANCE OF VITAMIN E IN ZOO MAMMALS

*Het belang van vitamine E voor zoo-zoogdieren*

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### ABSTRACT

The chemical structure, metabolism, biological function and general requirements for vitamin E are highlighted. The clinical symptoms, including the diagnosis and treatment of vitamin E deficiency, are briefly discussed for different zoo mammals. The physiological levels of vitamin E are presently available for most zoo animals, and supplementation of vitamin E in the diet has become common practice in zoo and wildlife medicine. Due to this knowledge and adapted supplementation, clinical cases of vitamin E deficiency are becoming increasingly rare.

### SAMENVATTING

In dit artikel wordt een overzicht gegeven van het belang van vitamine E bij zoo-zoogdieren. Naast de chemische structuur, het metabolisme, de biologische functie van en de behoeften aan deze stof worden de klinische symptomen met de diagnose en de behandeling kort belicht bij verschillende diersoorten. Heden zijn de fysiologische waarden van vitamine E voor de meeste zoedieren voldoende gekend waarbij vitamine E toegevoegd wordt aan het dieet in de meeste zoo's. Hierdoor worden klinische gevallen van vitamine E-tekort steeds zeldzamer.

### INTRODUCTION

Vitamin E is an essential nutrient for all animals, including zoo mammals. It is a natural antioxidant that, together with selenium, prevents the oxidation of unsaturated lipids. A whole set of symptoms can be observed when a deficiency of this vitamin occurs, a deficiency often leading to death in domesticated and non-domesticated animals. The most important symptoms are myopathy and cardiomyopathy. Vitamin E deficiency is most commonly known as "white muscle" or "yellow fat" disease (Dierenfeld, 1996).

Nowadays domestic animals fed adequate diets rarely show vitamin E-related disorders. These disorders are still important in the less well known species, however. The present paper is intended to provide an overview of the importance of vitamin E in zoo animals.

### CHEMICAL STRUCTURE, METABOLISM AND BIOLOGICAL FUNCTION

Vitamin E is a fat soluble vitamin which is heat resistant but easily oxidized. Eight different forms of vitamin E can be found in nature: 4 tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ) and 4 tocotrienols ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ). The differences between the tocopherols and the tocotrienols are due to an unsaturated side chain in the tocotrienols (Figure 1); the difference between  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  forms finds its origin in the incorporation of methyl groups at different places on the ring. Both the tocopherols and the tocotrienols are antioxidants;  $\alpha$ -tocopherol is considered to be the most powerful antioxidant of this group (McDowell, 1989). However, Wagner *et al.* (2001) showed that  $\gamma$ - and  $\delta$ -tocotrienols also have strong antioxidative properties.

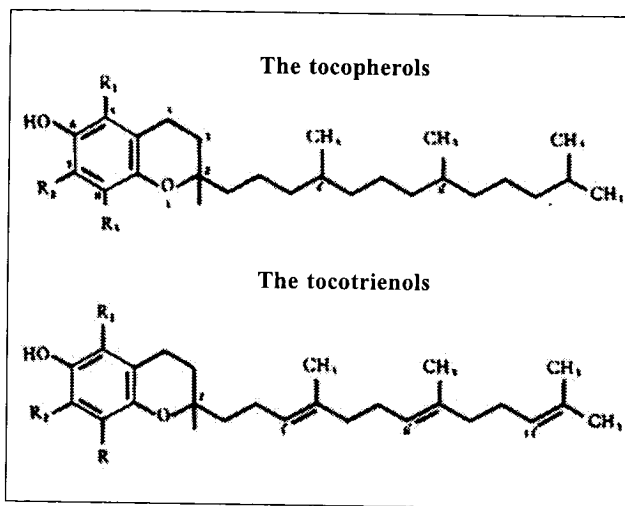


Figure 1. Biochemical structure of the tocopherols (from Putnam and Comben, 1987).

The stereo configuration of  $\alpha$ -tocopherol in forages is RRR- or d- $\alpha$ -tocopherol. Vitamin E supplements in commercial foods often contain a racemic mixture of all eight stereoisomers, the so-called dl  $\alpha$ -tocopherol. It has been proven that there is a synergism between these isomers, such that the racemic mixture is more potent than the natural  $\alpha$ -tocopherol. The synthetic dl- $\alpha$ -tocopheryl acetate is accepted as the International Standard (1 mg = 1 IU) (Putnam and Comben, 1987).

Vitamin E absorption is closely related to fat absorption. The absorption is facilitated by the lipase enzymes of the bile and pancreas. A vitamin E deficiency might occur when there is an impaired pancreatic or liver function and/or when the release of bile (e.g. obstruction) is abnormally delayed or stopped. Vitamin E is absorbed as an alcohol whereby esters are firstly hydrolyzed before absorption. Afterwards vitamin E incorporates in chylomicrons and is transported to the liver (McDowell, 1989).

Vitamin E is delivered to tissues in different ways (Dierenfeld, 1999). First of all, the chylomicrons with vitamin E are broken down into remnants and are incorporated in the liver in very low density lipoproteins (VLDLs). This mechanism is mediated by the so-called tocopherol binding protein. A genetic deficiency in the gene decoding for this protein may cause inheritable vitamin E deficiency. It has been suggested, but not proven, that this phenomenon occurs in the black rhinoceros. Another mechanism is the direct transfer of vitamin E into the different tissues, including muscles and adipose tissue, using lipoprotein lipase activity.

Vitamin E can be stored in all body tissues, with the highest concentration being found in the liver. Small

amounts of vitamin E will persist for a longer time in the body, but the major stores will be exhausted rapidly due to poly-unsaturated fatty acids (PUFAs) in the tissues. The excretion of Vitamin E takes place mainly by bile in a free form (McDowell, 1989).

The main function of vitamin E derives from its capability of acting as an intercellular and intracellular antioxidant, preventing the oxidation of unsaturated lipids in the cell wall and the cell organelles. In cases of deficiency, lipid hydroperoxides can be produced, which will destroy the structural integrity of the cell and result in cellular tissue damage. Vitamin E is considered a "first-line defence" against oxidation, but even with a sufficient amount of vitamin E, some noxious peroxides can be produced by the body (Putnam and Comben, 1987; McDowell, 1989).

Selenium, on the other hand, is a "second line defence". It is an integral part of the enzyme glutathione peroxidase. Even when there is a sufficient amount of vitamin E present, some hydroperoxides are formed. Glutathione peroxidase converts these peroxides into alcohols (Putnam and Comben, 1987). Selenium and vitamin E have a sparing effect on each other and, up to a critical point, they are mutually replaceable. However, in the absence of selenium, cells will contain excessive amounts of peroxides, which attack unsaturated lipids even when adequate amounts of vitamin E are present, and thus deficiency symptoms may occur (Putnam and Comben, 1987).

Vitamin E also plays a role in the blood clotting system by inhibiting platelet integration. Both vitamin E and selenium protect the leukocytes and macrophages during fagocytosis and provide protection against the toxicity of heavy metals (cadmium, mercury, arsenic, selenium and lead) (McDowell, 1989).

### Requirements and natural sources

It is not easy to give daily requirements for vitamin E for all species of animals because of the existence of large individual – as well as intra- and interspecies – variations. Generally it has been accepted that the diets need to contain 15 to 100 mg/kg dry matter, with an average of 30 mg/kg dry matter (Fowler, 1986). However, other factors have a major influence on the availability of the vitamin E. If large amounts of PUFAs or other fat soluble vitamins (A, D, K) are also present in the food, then the vitamin E requirements are higher (0.5 to 3 mg  $\alpha$ -tocopherol/g dietary PUFA). What is more, the demands in distressed or sick animals are also greater than in normal animals. If reasonable amounts of antioxidants or selenium are incorpo-

rated into the food, then the requirements are considered to be lower (McDowell, 1989; Dierenfeld, 1999).

The determination of the kind of tocopherol is often not possible when diets for zoo animals are prepared. Some of the tocopherols are less active (particularly  $\gamma$ -tocopherol) and can be found in some diets in greater amounts (2 to 4 times more) than in other diets. For these reasons, the vitamin E values are easily overestimated when total tocopherol contents are determined. Overall, the sum of different tocopherols gives a better and more realistic idea of the actual amount of vitamin E in a diet. The amount of  $\alpha$ -tocopherol provides the best indication of the vitamin E content of a diet. The total biologically active vitamin E can be calculated using one of the following equations: total vitamin E = amount of  $\alpha$ -tocopherol + 0.5  $\beta$ -tocopherol + 0.1  $\gamma$ -tocopherol + 0.3  $\delta$ -tocopherol, or: total vitamin E = 1.2  $\alpha$ -tocopherol (McDowell, 1989).

**Table 1.  $\alpha$ -tocopherol values in different oils and grains frequently used in zoo mammals (adapted from McDowell 1989).**

Nutrient component	Content (ppm)
Corn oil	112
Cottonseed oil	389
Palm oil	256
Soybean oil	101
Wheat germ oil	1330
Alfalfa meal, 17%, dehydrated	73
Alfalfa hay	53
Barley	36
Rice, bran	61
Oat	20

The richest sources of vitamin E are the vegetable oils, cereal products containing these oils, eggs, liver, vegetables and green plants (Table 1) (McDowell, 1989). The vitamin E values for different whole-prey species used as feed for zoo animals are presented in Table 2 (Dierenfeld, 1996).

The plasma  $\alpha$ -tocopherol values of many zoo species have now been established and can be used as a guideline in determining vitamin E deficiency (Brush and Anderson, 1986).

**Table 2. Active vitamin E values (IU/kg dry feed) in whole prey used as feed for zoo mammals (adapted from Dierenfeld, 1996).**

Type of prey	IU/kg dry matter
<i>Fish</i>	
Herring	74.4
Mackerel	141.3
Capelin	116.3
Smelt	164.1
Spearing	166.7
Rainbow trout	236.1
<i>Other vertebrates</i>	
Mice	46.2
Rats	190.0
Prairie dogs	53.8
Rabbits	18.1
Chicks	258.7
Chickens	30.4
Quails	44.7

## VITAMIN E DEFICIENCY SYNDROME IN DIFFERENT SPECIES

### Piscivores

Although fish, the natural food of marine zoo mammals, contains large amounts of vitamin E, deficiency (electrolyte imbalances, lowered plasma tocopherol levels and molt irregularities) has been reported in seals and Californian sea lions (Engelhardt and Geraci, 1978; Worthy, 1990). The reasons for this are multiple. Fish contains high values of PUFAs, which are highly prone to rancidity. Furthermore, fish are sometimes eviscerated, which results in an important source of vitamin E being lost. Vitamin E in fish can be very quickly oxidized and inactivated, even when frozen at very low temperatures. It is advisable to provide a supplementation of 100 IU vitamin E per kg fish (Engelhardt and Geraci, 1978; Robbins, 1993).

### Carnivores

Vitamin E deficiency in carnivores has been described in African lions, jaguars, tigers (Wallach, 1970), foxes and minks (McDowell, 1989). The vitamin E is mainly stored in fat, viscera and muscles. When the animals are fed only lean and chunk meat, deficiency may occur (Robbins, 1993). Vitamin E deficiency also occurs when non-ruminant meat (horse or pig meat), oily fish meal or cod liver oil, all of which are rich in PUFAs, are incorporated in diets for carnivores (Wallach, 1970; Bush *et al.*, 1987). Most zoos supplement meat with 50 to 150 IU vitamin E per kg dry matter, either by direct injection into the meat or by simple mixing with the meat (Bush, 1987).

### Herbivores

Tocopherol levels in grass and plants increase during early growth but decrease dramatically as seed becomes more mature, making hay a poor source of vitamin E. Leaching during the haying process and excessive storage times also reduce the amount of active tocopherols. Furthermore, in ruminants there is a possibility of vitamin E destruction in the rumen.

Virtually all wild ruminants and camelids are prone to vitamin E deficiency. Sudden deaths associated with cardiomyopathy have been reported in nyalas at the Bronx zoo, (Liu *et al.*, 1982) and has also been seen in old world (Finlayson *et al.*, 1971) and new world camels (Fowler, 1998). Old world camels are apparently more susceptible to vitamin E deficiency than new world camels.

Elephants and black rhinoceroses are extremely susceptible to vitamin E deficiency. The main symptom in elephants is myopathy. In the black rhinoceros, vitamin E deficiency has been suspected but never proven in cases of hemolytic anemia and encephalomalacia (Papas *et al.*, 1991). The browse in the wild is much richer in tocopherols than the hay fed in zoos. This might explain the higher vitamin E levels in animals not kept in zoo situations. Research has also shown that supplementing the diet with the commercially available dl- $\alpha$ -tocopheryl acetate does not cause a significant increase in circulating blood  $\alpha$ -tocopherol. However, the water-soluble d- $\alpha$ -tocopheryl polyethylene glycol 1,000 succinate (TPGS) causes a marked increase in circulating blood  $\alpha$ -tocopherol, thus making it the vitamin E additive of choice for these animal species (Papap *et al.*, 1991).

Wild perissodactyls can also develop vitamin E deficiency. Liu *et al.* (1983) reported the occurrence of degenerative myeloencephalopathy in six Przewalski horses with clinical signs of ataxia, while spinal ataxia has been observed in zebras (Wallach, 1970).

Swine and especially young pot belly pigs are very sensitive to vitamin E and/or selenium deficiency. Deficiency is most often associated with sudden death. Icterus, difficult locomotion, weakness, peripheral cyanosis and a weak pulse may or may not be seen before death (McDowell, 1989).

Wallabies, too, are extremely susceptible to vitamin E deficiency because selenium does not appear to have a sparing effect in these animals. Symptoms occur mainly when animals are kept in small pens (Fowler, 1986).

Nowadays it is recommended to provide herbivores on a hay diet with a supplement of 15 to 80 IU vitamin E/kg hay and 120 to 300 IU/kg pellets (McDowell, 1989).

### Monkeys

Vitamin E deficiency has been described in rhesus monkeys (anemia, McDowell, 1989), gelada baboons (cardiomyopathy, Liu *et al.*, 1984) and owl monkeys (anaemia, Garner, 2002). The occurrence is rare, as in humans, because captive primates receive a wide variety of foodstuffs. Deficiency occurs mainly due to insufficient intake of food containing vitamin E because of the "over choice" in snacks with low nutritional value included in the diet (Liu *et al.*, 1984).

## DIAGNOSIS AND TREATMENT

The clinical signs of vitamin E deficiency depend mainly on the organs affected. The animals are often weak and have difficulties standing, though muscle

stiffness can sometimes also be observed. Muscle deterioration and heart arrhythmias may also be present. In horses and zebras, myoglobinuria is often a clear clinical sign, while anemia is frequently the main symptom in monkeys (McDowell, 1989). Acute death was reported to occur in all species.

Additional specific tests can be performed to confirm the diagnosis. These include electrocardiogram and blood sampling, which are carried out mainly on anaesthetized wild animals. The levels of different enzymes (SGOT, ASPAT, LDH, CPK and MDH) are usually elevated. Vitamin E plasma levels lower than 0.5 µg/ml are considered to be abnormal, indicating vitamin E deficiency (McDowell, 1989).

Abnormal pale streaks in muscle and heart can be observed during the post mortem examination. This entity is often referred as "white muscle" disease. Steatitis may also be obvious, though mainly in carnivores (Brush and Anderson, 1986; McDowell, 1989). Focal myositis and myocarditis are microscopic indications of a general muscular degeneration, while hepatic necrosis and myelopathy may also be present (Liu, 1983, 1984; McDowell, 1989).

The therapy includes the parenteral administration (IM) of the free *dl* α-tocopherol at a dose of approximately 500 mg pro animal, possibly with a second injection after 3 days. The supplementation of vitamin E in the diet is certainly justified (Fowler, 1998). However, the treatment of animals with clinical symptoms of muscle problems induced by vitamin E deficiency only prevents further muscular necrosis but does not restore the lesions inside the muscles. Complete repair of the affected muscle can take from one week up to a number of months.

## TOXICITY OF VITAMIN E

Since vitamin E has such a wide safety margin, toxicity has been reported only in pelicans that were given relatively high doses in their diet (from 550 to 10,560 IU vitamin E per kg dry matter). The symptoms included severe hemorrhaging and prolonged clotting times. It was possible to partially reverse the clinical symptoms of the suspected toxicity using vitamin K (Robbins, 1993).

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