

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of vitamin E as a feed additive for all animal species^{1,2}

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)^{3,4}

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from European Commission, the European Food Safety Authority was asked to deliver a scientific opinion on the safety and efficacy of vitamin E as a feed additive for all animal species.

Vitamin E is a well-established micro-nutrient for all animal species. Vitamin E-based additives are globally used in animal nutrition, and have been for decades, to prevent vitamin E deficiency.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) examined data from three active substances (all-rac- α -tocopheryl acetate, RRR- α -tocopheryl acetate and RRR- α -tocopherol) currently authorised as vitamin E. The biopotency of these forms is different: one International Unit (IU) of vitamin E is defined as 1 mg all-rac- α -tocopheryl acetate, as 0.74 mg of RRR- α -tocopheryl acetate and as 0.67 mg of RRR- α -tocopherol.

The additives described containing those forms of vitamin E do not present major stability or homogeneity issues. However, sensitivity to light and moisture, as well as to oxygen and heat for RRR-preparations, must be appropriately managed in order to maintain the amount of vitamin E nominally available to farm animals.

Vitamin E at the current use levels is safe for all animal species. Information on hypervitaminosis E is not sufficiently consistent to derive a maximum content for vitamin E in feedingstuffs, based on safety for target species.

A conservative consumer exposure assessment indicates that the UL (300 mg α -tocopherol equivalents/day) is not exceeded even assuming high background intake and levels in animal feeds far higher than practical use. The FEEDAP Panel concludes that the use of vitamin E at practical use levels is safe for the consumer.

¹ On request from European Commission, Question No EFSA-Q-2009-00816, adopted on 25 May 2010.

² This scientific opinion has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

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No concern for user safety is expected from the use of the active substances vitamin E in feed additives. However, to draw conclusions on the final formulated additives, specific studies would be required.

Vitamin E occurs in nature and its use in animal nutrition will not result in a substantial increase in concentration in the environment. Therefore, no concern for the environment is expected.

All-*rac*- α -tocopheryl acetate, RRR- α -tocopherol and RRR- α -tocopheryl acetate are efficacious in all animal species in satisfying the requirements for vitamin E.

The FEEDAP Panel makes specific recommendations concerning (i) labelling of vitamin E in IU, (ii) restricting the use of oily RRR- α -tocopherol to premixture manufacturers, (iii) the introduction of a recommended maximum of 200 IU vitamin E/kg complete feedingstuffs, (iv) the specification of the additives.

KEY WORDS

Nutritional additives, vitamins, RRR- α -tocopherol, all-*rac*- α -tocopheryl acetate, RRR- α -tocopheryl acetate, safety, efficacy

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BACKGROUND

Regulation (EC) No 1831/2003⁵ establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the VITAC-EEIG-Vitamins Authorisation Consortium⁶ for authorisation of the product vitamin E, to be used as a feed additive for all animal species and categories (category: Nutritional additives; functional group: vitamins, provitamins and chemically well defined substances having a similar effect) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.⁷ According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 30 October 2009.

Vitamin E has been authorised without a time limit under Council Directive 70/524/EEC⁸ for its use in all species as a nutritional additive.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the efficacy and safety for the target animal(s), consumer, user and the environment of the product vitamin E when used under the conditions described in Table 1.

⁵ OJ L 268, 18.10.2003, p. 29.

⁶ VITAC-EEIG-Vitamins Authorisation Consortium, avenue Louise, 120-Box 13, 1050 Brussels, Belgium (Adisseo France SAS; ADM Speciality Ingredients BV; BASF AG; DSM Food Speciality BV; Europe-Asia Import Export GmbH; Lohmann Animal Health GmbH; Sunvit GmbH; Vitae Caps SA).

⁷ EFSA Dossier reference: FAD-2008-0047.

⁸ OJ L 271, 30.09.2006, p. 12.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive		Vitamin E				
Registration number/EC No/No (if appropriate)						
Category(ies) of additive		Nutritional additives				
Functional group(s) of additive		Vitamins, provitamins and chemically well defined substances having a similar effect				
Description						
Composition, description		Chemical formula	Purity criteria (if appropriate)		Method of analysis (if appropriate)	
All rac alpha-tocopheryl acetate		C31H52O3	Min. 93 %		Gas chromatography (PhEur 2.2.28)	
RRR alpha-tocopheryl acetate		C31H52O3	Min. 40 %		Gas chromatography (PhEur 2.2.28)	
RRR alpha-tocopherol		C29H50O2	Min. 67%		Gas chromatography (PhEur 2.2.28)	
Trade name (if appropriate)						
Name of the holder of authorisation (if appropriate)						
Conditions of use						
Species or category of animal	Maximum Age	Minimum content		Maximum content		Withdrawal period (if appropriate)
		mg or Units of activity or CFU kg ⁻¹ complete feedingstuffs		of		
All animal species and categories	--	--		--		--
Other provisions and additional requirements for the labelling						
Specific conditions or restrictions for use (if appropriate)		Refer to (1) labelling proposal (Section 2: Annex 2.5.3) and (2) biopotency statements (Section 4: Annexes 4.0a and 4.0b)				
Specific conditions or restrictions for handling (if appropriate)		None				
Post market monitoring (if appropriate)		No specific requirements other than the traceability and complaint system implemented in compliance with the requirements of Regulation No 183/2005.				
Specific conditions for use in complementary feedingstuffs (if appropriate)		Applicable in premix, feed supplement or water. Direct incorporation of the additives necessitates specific equipment in the production site, in order to ensure proper and homogeneous mixing. It is recommended that the additives be formulated as a preparation before being incorporated in premixtures and in feeds. The additives shall be emulsified with authorised emulsifier and other carriers before being diluted in water. In water and / or milk replacers the additives should be formulated as a preparation before being dispersed.				

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
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ASSESSMENT

This opinion is based in part on data provided by a consortium of companies involved in the production/distribution of vitamin E. This data covers a fraction of existing additives containing vitamin E. The composition of the additives, critical for stability during feed processing and storage, is not subject of this application. The Panel has sought to use the data provided together with data from other sources to deliver an opinion and to produce recommendations for the authorisation which would secure the safety of future uses of vitamin E as feed additive.

1. Introduction

Vitamin E is a feed additive authorised without a time limit under Council Directive 70/524/EEC⁹ for its use in all species as a nutritional additive; no maximum total levels of vitamin E in feeds are established in the EU. The applicant (consortium) asks for the re-evaluation of the use of vitamin E in the form of three active substances (all-*rac*- α -tocopheryl acetate, RRR- α -tocopheryl acetate and RRR- α -tocopherol) as a nutritional additive for all animal species.

The dossier contains data from four sources of all-*rac*- α -tocopheryl acetate, two sources of RRR- α -tocopheryl acetate and one source of RRR- α -tocopherol. The biopotency of these forms is different, one International Unit (IU) of vitamin E is defined as 1 mg all-*rac*- α -tocopheryl acetate, 0.74 mg of RRR- α -tocopheryl acetate and 0.67 mg of RRR- α -tocopherol (US Pharmacopeia, 2010; Bieri and McKenna, 1981). Other tocopherols and tocotrienols, not subject of this application, also exert (mainly lower) vitamin E activity.

The biological effects of vitamin E are predominantly seen in the prevention of resorption of fetuses, testicular degeneration, muscle dystrophy, anaemia and encephalomalacia, the classical signs of vitamin E deficiency in animals. The influence of vitamin E on the immune system has also become an important issue (Politis et al., 1995; Politis et al., 1996). Vitamin E acts as an antioxidant but the specific molecular basis of its action is still unclear.

Vitamin E can be added to food (Regulation (EC) No 1925/2006¹⁰), the active substances all-*rac*- α -tocopheryl acetate; RRR- α -tocopheryl acetate and RRR- α -tocopherol are listed in Annex II of the Regulation as formulations. Vitamin E (the substances listed above) may also be used in food supplements (Directive 2002/46/EC¹¹). Vitamin E, either orally or parenterally administered, is also used in veterinary medicine (Regulation (EC) No 997/1999¹²).

In this opinion, the word 'additive' is used for the final (stabilised) formulation containing the vitamin E active substance, ready to be marketed. The word 'product' refers to the raw product, either synthetic or extracted, the source of the active substance, which forms the basis of the additive.

2. Characterisation

At present, numerous additives containing vitamin E active substances are commercially available in Europe. They may differ in the source of vitamin E (plant extracts, esterified or not, chemical synthesis), physical properties (oily, powder (adsorbate, spray dried)) and vitamin E concentration.

2.1. Characterisation of the active substance¹³

Vitamin E is the active principle of the additives under application. The active substances are all-*rac*- α -tocopherol, product of chemical synthesis, consisting of eight stereoisomers (RRR, RRS, RSS, RSR,

⁹ OJ L 271, 30.09.2006, p. 12.

¹⁰ OJ L 404, 30.12.2006, p. 26.

¹¹ OJ L 183, 12.7.2002, p. 51.

¹² OJ L 122, 12.5.1999, p. 24.

¹³ This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

SRR, SSR, SRS and SSS) in equal quantities, and the single isomer RRR- α -tocopherol, usually obtained from natural sources. Both tocopherols (all-rac and RRR) can be esterified commonly with acetate.

The vitamin E activity of the additives refers only to the α -tocopherols under application, it does not consider additional potential activity resulting from the occurrence of other tocopherols and tocotrienols.

2.1.1. All-rac- α -tocopheryl acetate

The analysis of five products (five batches each) from four sources and two synthetic routes (fully described in the dossier) shows high content uniformity and negligibly small differences between the products (mean values of all-rac- α -tocopheryl acetate: product 1: 95.0 %; product 2: 94.5 %; product 3: 93.7 %; product 4: 93.4 %; product 5: 93.7 %).

The main impurities resulting from the synthesis consist of tocopherol-related products (impurity A: all-rac-trans-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-yl acetate; impurity B: all-rac-cis-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-yl acetate; impurity C: all-rac- α -tocopherol; impurity D: 4-methoxy-2,3,6-trimethyl-5-[(all-RS,E)-3,7,11,15-tetramethylhexadec-2-enyl] phenylacetate; impurity E: (all-RS,all-E)-2,6,10,14,19,23,27,31-octamethyldotriaconta-12,14,18-triene). On average from five products, they amount to approximately 2 %.

The products specifications for other impurities can be summarised as max. 0.1 % for sulphated ashes, max. 20 mg heavy metals (expresses as lead)/kg and max. 2.0 % free (organic and inorganic) acids. Residual solvents are regularly monitored and the VICH thresholds not exceeded. The available data indicate no safety concern.

2.1.2. RRR- α -tocopherol

RRR- α -tocopherol is produced from oilseeds. It is used as such (oily form) or as free flowing powder by dilution with an (inorganic) carrier.

Two RRR- α -tocopherol products are characterised by different contents of RRR- α -tocopherol. The analysis of five batches of each product was provided, showing a range of 69.7 to 71.5 % RRR- α -tocopherol for one product and 94.0 to 95.5 % for the other. The product with the lower tocopherol content contained between 27.3 and 29.0 % extractable vegetable oil components, the product with the higher tocopherol content between 3.0 and 4.7 %. Impurities (other tocopherols (0.5 to 0.9 %), sterols (0.1 to 0.4 %) and fatty acids (0.2 to 0.6 %)) are low. The tocopherol-related impurities that were identified, but not quantified, are RRR- δ -tocopherol, RRR- β -tocopherol and other RRR-tocopherols. In all ten batches benzo(a)pyrene was below 2 μ g/kg, heavy metals (expressed as lead) below 10 mg/kg.

2.1.3. RRR- α -tocopheryl acetate

RRR- α -tocopheryl acetate is derived from oilseeds followed by esterification with acetate.

Each of the two producers described two products with different purities. The products of one source are characterised by at least 50 % RRR- α -tocopheryl acetate (range of active substance 51 to 52.4 % in five batches) and by at least 70 % (range of active substance 70 to 74 % in five batches). The main impurities were fatty acids and esters followed by other extractable vegetable oil components, sterols and sterol-acetates, other tocopherols and tocopherol-acetates. The products of the second source showed typical concentrations of the active substance of 74 and 96 %. The impurities, not given in detail, are quantitatively similar to those described for RRR- α -tocopherol.

2.2. Characterisation of the additive

Vitamin E sources are sensitive to light (and moisture), the all-rac-preparations to alkalis, the RRR-preparation to oxygen and heat. The applicant noted that the RRR-preparations contain stabilised forms of tocopherol or tocopheryl acetate. No information is given on the process by which stabilisation is reached.

The content of active substances in vitamin E-containing additives depends on the producer, the physical form (liquid or dry) and galenic preparation of the additive therefore varying considerably. Dry products are mostly in the range of 25 to 55 % active substance, other organic matrix between 46 and 70 % and inorganic matrix (mostly SiO₂) between 3 and 50 %. Oily formulations may contain up to 94 % active substance.

Three batches of an additive containing RRR- α -tocopherol (55 %) were analysed for heavy metals and arsenic, PAHs, organic solvents, chlorinated hydrocarbons and pesticides, other pesticides, aflatoxins (B₁, B₂, G₁, G₂) and ochratoxin A. With the exception of copper (0.06 mg/kg), cadmium (0.006 mg/kg), lead (0.2 mg/kg) and arsenic (0.006 mg/kg in one batch) all values were below the quantification limit. Dioxins were determined in one batch (0.217 ng WHO-PCDD/F-PCB-TEQ/kg). No safety concern could be identified for those impurities.

Three samples of an additive containing RRR- α -tocopheryl acetate (40 %) and representing the production of three different years were analysed for heavy metals and arsenic, PAHs, organic solvents, chlorinated hydrocarbons and pesticides, other pesticides, aflatoxins (B₁, B₂, G₁, G₂) and ochratoxin A. With the exception of copper (about 2 mg/kg), all values were below the quantification limit for heavy metals and arsenic and the below the detection limit for other substances. The data did not indicate any safety concern. Control methods including dioxins are in place.

The applicant provided some selected data on the particle size of different products, some of them being characterised by only one value, others by mean values based on five to ten batches. According to this list, the dry all-rac powders show percentages between 0.2 to 25 % of critical fraction below 50 μ m. This fraction is below 1 % for six products and above 1 % for the other eight. For products/batches based on RRR-tocopherol and its ester (seven products), the fraction below 50 μ m ranges from 1.2 to 34 %. For products of which the fraction below 50 μ m exceeds 1 %, inhalatory toxicity studies would normally be required.

2.3. Stability and homogeneity

Shelf life of the additive

Different formulations of all-rac- α -tocopheryl acetate (spray dried, adsorbate) were studied for 24, 36 and 48 months (25 °C, 60 % RH). All the data support stability up to the end of the study. A comparable shelf life (48 months) was shown for RRR- α -tocopheryl acetate. The stability of RRR- α -tocopherol (in oily and powder form) was demonstrated for 24 months.

Stability in premixtures and feed

Stability in premixtures, during feed processing and in feed was studied for additives containing all-rac- α -tocopheryl acetate, RRR- α -tocopherol or RRR- α -tocopheryl acetate.

No main differences between the active substances were detected. Recovery in premixtures for different target species, containing different levels of choline-chloride and trace elements, after six months of storage at about 25 °C, was not lower than 80 % (up to 100 %). The product was stable to pelleting at 75 °C, but 40 % of the active substance was lost during extrusion. Recovery in feed after three months at about 25 °C was in the range of 80 to 100 %.

Stability in water was studied for two batches of a spray-dried additive (three sub-samples each) containing all-rac- α -tocopheryl acetate. Recovery after 24 hours was 96 % at 25 °C. No data for RRR-

α -tocopheryl acetate were provided. However, no reasons for a different stability to all-rac- α -tocopheryl acetate were identified. RRR- α -tocopherol (three batches) lost 12.5 % during a 24 hour-storage at 21 °C. Microbial contamination was not observed.

Homogeneity

The applicant provided a statistical method to calculate homogeneity in feed (setting the maximum coefficient of variation (CV %) at 10 %) for two preparations (five and three batches) of all-rac- α -tocopheryl acetate (50 %). The calculations resulted in a CV % of around 3 % for the concentration of all-rac- α -tocopheryl acetate in feed. Since the additives based on the three active substances show approximately the same particle size distribution, the FEEDAP Panel applies the result of this calculation also to dry vitamin E additives containing RRR- α -tocopheryl acetate and RRR- α -tocopherol. The homogeneity of oily additives was not demonstrated.

In a study with two batches of an additive containing 50 % all-rac- α -tocopheryl acetate mixed with water (25 °C), the additive showed a homogeneous distribution in water (CV %: 2.9 and 1.9 %, respectively).

2.4. Conditions of use

The applicant proposes that vitamin E additives (oily, adsorbate or spray dried) should be incorporated in feed directly or via premixtures without minimum or maximum content. Spray dried formulations (also containing emulsifier) could be used in water or feed which is administered on liquid basis (milk replacer).

The FEEDAP Panel expresses its reservation concerning the incorporation of an oily formulation directly in feed. The small quantities of vitamin E (5 to 50 IU/kg feed, equal to 5.3 to 53 mg oil) could only be homogeneously distributed in feed if added via a premixture.

Considering the different biopotencies of the different active substances, the FEEDAP Panel recommends that the vitamin E content should be labelled in IU (see Recommendations).

2.5. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of vitamin E in animal feed. The Executive Summary of the CRL report can be found in Appendix A.

3. Safety

No distinction is made in this section between the different active substances. The safety is addressed for the overall form of Vitamin E as α -tocopherol, considering different biopotencies when relevant.

3.1. Safety for the target species

According to the Regulation (EC) No 429/2008, tolerance studies are not required for vitamin E.

At present, the information on hypervitaminosis E in animals is limited. Former studies in rats and chickens (Yang and Desay, 1977; Alam and Alam, 1981; March et al., 1973) and more recent in laying hens (Sunder and Flachowsky, 2001) indicate that 1000 IU vitamin E/kg complete feed is tolerated. This concentration is 20 to 200 times higher than the supplementation level commonly used in practice (5 to 50 IU/kg feed). Therefore, the FEEDAP Panel concludes that vitamin E at the current use levels is safe for all animal species.

However, scientific evidence indicate that high doses (generally well above the 1000 IU) of vitamin E may reverse the principal beneficial effects of vitamin E, e.g. they can exert pro-oxidative properties, reducing glutathione peroxidase (Thomas and Stocker, 2000), reduce retinol and xanthophyll utilisation, also leading to less coloured egg yolks (Sünder and Flachowsky, 2001) and affect reproduction in poultry (Sunder et al., 1999; Engelmann et al., 2001; Danikowski et al., 2002). Some studies indicate

that reversal of favourable immunomodulation may be the most sensitive effect (Yasunaga et al., 1982), being observed at dietary concentrations in the 200 IU range in poultry (Leshchinsky and Klasing, 2001). The available data are not sufficiently consistent to derive a maximum content for vitamin E in feedingstuffs, based on safety for the target species.

3.1.1. Conclusions on safety for the target species

Vitamin E at the current use levels is safe for all animal species. Information on hypervitaminosis E in animals is limited. The available data are not sufficiently consistent to derive a maximum content for vitamin E in feedingstuffs, based on safety for the target species. However, the FEEDAP Panel considers that available data is indicative of a need to set an upper limit that should not be exceeded in feeding practice.

3.2. Safety for the consumer

3.2.1. Absorption, distribution, metabolism and excretion of tocopherols

A detailed description of absorption, distribution, metabolism and excretion of tocopherols based on former EFSA opinions (EFSA, 2006; EFSA, 2008) and a bibliographic review submitted by the applicant is given in Appendix B.

The tocopherols and their esters (and tocotrienols homologues) are intestinally absorbed in a similar manner. The absorption rate varies, depending on the intake, between 20 % and 80 %. Absorption of vitamin E requires the presence of dietary fat and further depends on pancreatic function and biliary secretion to form micelles for the transfer across intestinal membranes. Absorption is controlled by a passive process, however a possible receptor has been recently identified. The chylomicrons containing vitamin E are secreted into the lymphatic system where vitamin E is transported to about 90 % as α -tocopherol. Already at absorption level there is a preference for RRR-tocopherols compared to other stereoisomers, particularly in dairy cows.

The different lipoproteins act as the main systemic transport system of tocopherol in the blood stream. After hepatic uptake, vitamin E is re-secreted mainly in the form of α -tocopherol. RRR- α -tocopherol and the 2R forms of the all-rac- α -tocopherol are the predominant forms secreted by the liver.

The mechanisms involved in the transfer of α -tocopherol from feed via plasma lipoproteins to milk in ruminants are not known, but only a low percent is secreted into milk.

Tocopherol is excreted as a water soluble conjugated compound resulting from different oxidation steps.

3.2.2. Toxicological studies

The toxicological profile of Vitamin E has already been described in detail in previous EFSA opinions (EFSA, 2006; EFSA, 2008). In summary, Vitamin E exerts a very low acute toxicity in several laboratory animals (> 2000 mg/kg bw), and in subchronic studies in rats the NOAEL was established at 125 mg RRR- α -tocopheryl acetate/kg bw due to hepatotoxic effects in the higher dose.

In a chronic rat study with doses of 500, 1000 or 2000 mg all-rac- α -tocopheryl acetate/kg bw, the animals showed at all dose levels spontaneous haemorrhages in the gut, urinary tract, meninges, orbit and at sites of minor injury correctable by administration of Vitamin K₃. No evidence of carcinogenicity was observed and a NOAEL could not be established (WHO, 1986).

The results of reproductive toxicity studies in rats indicated that vitamin E (administered as the watersoluble RRR- α -tocopherol (polyethylene glycol succinate)) did not have adverse effects on the reproductive function at doses up to 2 % of the diet (Krasavage and Terhaar, 1977). In a mice study, RRR- α -tocopherol was not teratogenic (Hook et al., 1974). However, in a rat developmental study, cell signaling and synaptic plasticity in developing hippocampus of offspring of highly supplemented

mothers (1000 mg/kg bw) was influenced by vitamin E, inducing neurobehavioral (cognitive) impairment in the adult offspring (Betti et al., 2010).

No studies designed to investigate the potential genotoxicity of vitamin E *per se* were identified. However, when studying the modulating effect of vitamin E on the mutagenicity/clastogenicity of genotoxic compounds, no indications of any genotoxicity in vitamin E controls were identified (Ansari et al., 2006; Rosignoli et al., 2008; Umemura et al., 2009).

3.2.3. Assessment of consumer safety

3.2.3.1. Tolerable upper intake level (UL)

The Scientific Committee on Food (2003) set a tolerable upper intake level (UL) for Vitamin E expressed as RRR- α -tocopherol equivalents. The UL was established at 300 mg/day (equivalent to 450 IU vitamin E), derived from a NOAEL of 540 mg/day, based on impaired blood clotting in human studies, applying an uncertainty factor of two and rounding to 300 mg/day. Since no specific concerns for children and adolescents were identified, the UL for younger age groups is derived by scaling the adult UL on the basis of body surface area (SCF, 2003). The SCF did not consider the UL to apply to subjects with vitamin K deficiency.

Miller et al. (2005) performed a meta-analysis of the dose-response relationship between vitamin E supplementation and total mortality by using data from randomised, controlled trials (a total of 135967 patients participating in 19 clinical trials, nine of which tested vitamin E alone and ten vitamin E combined with other vitamins or minerals). The dosages of vitamin E ranged from 16.5 to 2000 IU/d (median, 400 IU/d). Nine of 11 trials testing high-dosage vitamin E (> 400 IU/d) showed increased risk for all-cause mortality in comparisons of vitamin E vs. control. A dose-response analysis showed a statistically significant relationship between vitamin E dosage and all-cause mortality, with increased risk in dosages greater than 150 IU/d. However, high-dosage (> 400 IU/d) trials were often small and were performed in patients with chronic diseases (most often coronary heart disease). The extent of these findings to healthy adults is uncertain. A precise estimation of the threshold at which risk increases is therefore difficult. The authors concluded that high-dosage (> 400 IU/d) vitamin E supplements may increase all-cause mortality and should be avoided.

3.2.3.2. Consumer exposure

The applicant provided data on vitamin E content in edible tissues (muscle, liver, kidney and fat) from mammals and birds, in fish and in eggs and milk (all data from literature). The data which are used for the calculation of consumer exposure are given in Appendix C.

Values obtained with diets showing vitamin E concentrations higher than 1000 mg/kg, which are already far above practical use levels, were not considered. For the worst case calculation (Table 2) the FEEDAP Panel (i) considered vitamin E concentration in tissue mostly analysed as α -tocopherol as RRR- α -tocopherol, (ii) selected the following maximum vitamin E concentrations in tissues/products (mg RRR- α -tocopherol/kg fresh matter): liver (hen)126; skin/fat (hen) 72 (half of the value for abdominal fat); fish (turbot (*Scophthalmus maximus*)) 256; whole egg 377; milk 1.3. In the consumer exposure calculation, kidney was not considered because of its negligible contribution and meat was not considered because the worst case value was much lower than fish (51 mg/kg fresh matter, leg muscle, hens).

Table 2: Theoretical consumer intake of vitamin E based on the consumption model from Regulation (EC) No 429/2008

Amount consumed	α -tocopherol equivalents (mg/kg tissue or product)	α -tocopherol equivalents (mg/person day)
300 g fish*	256	77
100 g liver (from hens)	126	13
50 g skin/fat (from hens)	72	4
100 g egg	377	38
1.5 L milk	1.3	2
Total		134

* This data are from eviscerated juvenile whole fish, edible flesh from farmed fish show considerably lower α -tocopherol concentrations.

The theoretical exposure from products from animals treated with vitamin E, mostly at levels far higher than practical use, amounts to 45 % of the UL. Even adding the 95 percentile of human total exposure resulting from a nutrition survey in Ireland (38.3 mg/day, which includes food of plant and animal origin (SCF, 2003)), the total exposure would be 57 % of the UL.

Any possible reduction of the UL is likely to be accommodated by the large margin of safety when considering more realistic consumption data and practical use levels in animal nutrition.

These theoretical considerations demonstrate that there is no safety concern for the consumer resulting from the intake of food from all animal species fed with high vitamin E levels. Under this aspect, the FEEDAP Panel does not see a need to propose a maximum content for vitamin E in feed.

3.2.3.3. Proposal for maximum residue limits

The FEEDAP Panel does not see a need for proposing MRLs.

3.2.4. Conclusions on safety for the consumer

A conservative exposure assessment indicates that the UL is not exceeded, even assuming high background intake and levels in animal feeds far higher than practical use. Therefore, the FEEDAP Panel concludes that no safety concern for the consumer is identified and there is no need to propose a maximum content for vitamin E in feed under this aspect.

3.3. Safety for the user

The user is exposed to the final form in which the additive is placed on the market. No studies with additives concerning user safety have been provided.

3.3.1. Effects on skin and eyes

The applicant provided studies on the dermal and ocular irritation of the product all-rac- α -tocopheryl acetate in rabbits.¹⁴ No irritating effects were observed.

3.3.2. Effects on the respiratory system

As mentioned under point 2.2 (particle size), the vitamin E additives described would have required an examination of inhalatory toxicity. However, considering the very low systemic toxicity of oral vitamin E and lack of irritancy, studies do not appear necessary. A risk from inhalatory exposure is considered unlikely.

¹⁴ Technical dossier, section III, Ref. 3.3.01 and 3.3.02.

3.3.3. Sensitisation

No sensitisation studies have been provided. The occurrence of allergic skin reactions after the use of vitamin E-containing cosmetics have been reported (Ramirez Santos et al., 1996; Bazzano et al., 1996). No comparable findings are published concerning the use of vitamin E for other purposes.

3.3.4. Conclusions on safety for the user

No concern for user safety is expected from the use of the active substances vitamin E in feed additives. To draw conclusions on the final formulated additives, specific studies would be required.

3.4. Safety for the environment

Vitamin E occurs in nature and its use in animal nutrition will not result in a substantial increase in concentration in the environment. Therefore, no further assessment of the impact on the environment is required.

4. Efficacy

Vitamin E has been globally used in animal nutrition since decades to prevent vitamin E deficiency. Data on requirement, allowances and recommendations for feed supplementation are easily accessible as standard literature for animal nutrition experts. Also the interactions of vitamin E requirement with the dietary supply of other vitamins, selenium and polyunsaturated fatty acids (PUFAs) are well-known and routinely considered in feed formulation.

In addition to its classical use to prevent deficiency, vitamin E may be used for particular purposes, e.g. enrichment of foods and products of animal origin where it exerts antioxidant properties (protection against off flavour by oxidation during storage, longer maintenance of (beef) meat colour), or improvement of immune response. For those particular purposes, dietary vitamin E levels considerably exceeding nutritional requirements are necessary and used.

The active substances which are used in vitamin E-containing additives are efficacious in all animal species. The different biopotency of the different isomers is taken into account by calculating (and expressing) the vitamin E supplementation in terms of IU.

5. Post-market monitoring

No risks associated with the use of the product are foreseen. It is considered that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation¹⁵ and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The additives described containing vitamin E do not present major stability or homogeneity issues. However, sensitivity to light and moisture, as well as to oxygen and heat for other vitamin E-containing additives, should be appropriately managed in order to maintain the amount of vitamin E nominally available to farm animals.

Vitamin E at the current use levels is safe for all animal species. Information on hypervitaminosis E in animals is limited. The available data are not sufficiently consistent to derive a maximum content for vitamin E in feedingstuffs, based on safety for the target species; however, they indicate that a level higher than 200 IU/kg feed is not desirable in feeding practice.

A conservative exposure assessment indicates that the UL (300 mg/day) is not exceeded, even assuming high background intake and levels in animal feeds far higher than practical use. Therefore,

¹⁵ OJ L 35, 8.2.2005, p. 1.

the FEEDAP Panel concludes that no safety concern for the consumer has been identified and there is no need to propose a maximum content for vitamin E in feed under this aspect.

No concern for user safety is expected from the use of the active substances vitamin E in feed additives. To draw conclusions on the final formulated additives, specific studies would be required.

Vitamin E occurs in nature and its use in animal nutrition will not result in a substantial increase in concentration in the environment. Therefore, no concern for the environment is expected.

All-*rac*- α -tocopheryl acetate, RRR- α -tocopherol and RRR- α -tocopheryl acetate are efficacious in all animal species in satisfying the requirements for vitamin E.

RECOMMENDATIONS

Considering the different biopotencies of the active substances, the FEEDAP Panel recommends that the vitamin E content should be labelled in IU. For conversion, the following factors are proposed: 1 mg RRR- α -tocopherol is equivalent to 1.49 IU, 1 mg RRR- α -tocopheryl acetate to 1.36 IU and 1 mg all-*rac*- α -tocopheryl acetate to 1.00 IU.

Since oily additives may contain up to 94 % RRR- α -tocopherol, their use should be restricted to premixture manufacturers to guaranty homogeneous distribution in final feed.

Considering that (i) high doses of vitamin E may exert inverse responses to the normally described beneficial effects and (ii) the present data are not sufficiently consistent to derive a mandatory maximum content for vitamin E, the FEEDAP Panel recommends to introduce in the register entry for vitamin E, under 'other provisions': '200 IU vitamin E/kg complete feedingstuffs for all species should normally not be exceeded'.

The following provisions should be made, under 'description of the additive':

- All-*rac*- α -tocopheryl acetate, stabilised, from chemical synthesis;
- RRR- α -tocopheryl acetate, stabilised, extracted from oilseeds;
- RRR- α -tocopherol, stabilised, extracted from oilseeds.

GENERAL REMARK

The FEEDAP Panel notes that a potential authorisation of vitamin E should consider the prerequisite under which the opinion on vitamin E has been adopted, i.e. the formulated products contain, beside feed materials, only processing aids (e.g. antioxidants, emulsifiers, anti-caking agents) used under the same conditions as they are approved as feed additives.

DOCUMENTATION PROVIDED TO EFSA

1. Vitamin E as feed additive for all animal species. September 2008. Submitted by VITAC-EEIG-Vitamins Authorisation Consortium
2. Vitamin E as feed additive for all animal species. Supplementary information. March 2010. Submitted by VITAC-EEIG-Vitamins Authorisation Consortium.
3. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for vitamin E.
4. Comments from Member States received through the ScienceNet.

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APPENDICES

APPENDIX A

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for vitamin E

In the current application authorisation is sought for *vitamin E* under the category nutritional additives, functional group '3a' vitamins, pro-vitamins and chemically well-defined substances having similar effect, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for use of vitamin E for all animal species and categories.

Vitamin E consists of a group of active substances. Three active substances are applied for in this dossier: (1) *all-rac-alpha-Tocopheryl acetate*, (2) *RRR-alpha-Tocopheryl acetate* and (3) *RRR-alpha-Tocopherol*. *RRR-alpha-Tocopherol acetate* and *RRR-alpha-Tocopherol* are derived from vegetable oil with the minimum purity of 40 % and 67 %, respectively. *All-rac-alpha-Tocopheryl acetate* is produced by chemical synthesis with the minimum purity of 93 %. Preparations of *vitamin E* consisting of these forms are variable depending on the producers, and are usually marketed in the form of adsorbate (powder) and spray-dried powder. No final concentrations of *feed additive* in *feedingstuffs* are proposed by the applicant.

For the determination of the three active substances mentioned above in the *feed additive*, the applicant proposed several European Pharmacopoeia (EP) methods, based on gas chromatography with flame ionisation detector (GC-FID): EP-0439 for *all-rac-alpha-Tocopheryl acetate* (oil), EP-0691 for *all-rac-alpha-Tocopheryl acetate* (powder form), and EP-1256 for *RRR-alpha-Tocopherol* (oil).

The CRL recommends for the determination of *vitamin E* (oil form) in *feed additives* the following GC-FID methods recommended by the European Pharmacopoeia: EP-0439 for *all-rac-alpha-Tocopheryl acetate*; EP-1257 for *RRR-alpha-Tocopheryl acetate* and EP-1256 for *RRR-alpha-Tocopherol*. For the determination of *vitamin E* (powder form) in *feed additives* the CRL recommends the following EP methods: EP-0691 for *all-rac-alpha-Tocopheryl acetate*; EP-1801 for *RRR-alpha-Tocopheryl acetate* and for *RRR-alpha-Tocopherol*.

For the determination of all three active substances in *premixtures* and in *feedingstuffs* the CRL recommends the Community method (Commission Regulation (EC) No 152/2009) at the validated concentration range (from 60 to 17000 mg *vitamin E*/kg), as proposed by the applicant.

In addition, the applicant reported that this method is also applicable to the determination of the target analyte in water (extension of scope). However, experimental data showing the validity of this approach have not been provided, thus the CRL cannot comment on the suitability of the Community method to the analysis of the matrix "water".

Further testing or validation is not considered necessary.

APPENDIX B

Metabolism and deposition of vitamin E in animal tissues and products

B.1.1 Metabolism

The applicant provided an overview of the metabolism and deposition of Vitamin E (based on EFSA opinions (EFSA, 2006; EFSA, 2008) and a bibliographic review).

Intestinal absorption of tocopherols varies between 20–80 % depending on intake and, with lower figures at higher dietary supply (EFSA, 2006). Nearly all the Vitamin E absorbed across the intestinal mucosa is free tocopherol. All forms of vitamin E, including all tocopherols and tocotrienol homologues are absorbed in a similar manner by a passive process (EFSA, 2008). Inhibition of the scavenger receptor class B type I (SR-BI) blocks up to 80 % of α -tocopherol up-take, and suggest a role of SR-BI in intestinal α -tocopherol transport (Reboul et al., 2006). The intestinal absorption of vitamin E requires the intake and digestion of dietary fat, the latter being enhanced by the production of bile acids from the liver (Traber et al., 1990a). Vitamin E absorption is limited if there is an inadequate fat intake (Traber, 2007); in addition, intestinal absorption of lipids and fat-soluble vitamins depends on pancreatic function and biliary secretion to form micelles with the hydrolysed fat for the transfer across intestinal membranes. Moreover, chylomicron secretion is also required for transportation from the intestine to the circulation. Chylomicrons containing vitamin E are assembled and secreted into the lymph (Kayden and Traber, 1993). About 90% of the free α -tocopherol is transported via the lymphatic system into the bloodstream, where it is distributed into lipoproteins for passage into the liver. The main systemic transport system of tocopherol is the Low-Density Lipoprotein (LDL)-fraction (55–65 %) followed by the High Density Lipoprotein (HDL) (24–27 %) and Very Low Density Lipoprotein (VLDL) (8–18 %). There is a preferential incorporation of RRR α -tocopherol into VLDL (Traber et al., 1992). Under fasting condition, LDL and HDL transport most of the plasma α -tocopherol into the liver and may also be an important source of plasma vitamin E for hepatic uptake (Rigotti, 2007).

After hepatic uptake, the α -tocopherol form of vitamin E is resecreted into the circulation. The α -tocopherol transport protein (α -TTP), a 32kDa cytosolic lipid-binding protein found in a number of tissues but mainly the liver (Murphy et al., 1981), plays an important role for the secretion and incorporation of vitamin E in the form of α -tocopherol into VLDL in the liver. It is also involved in the subsequent transport of vitamin E to the various tissues, such as muscle, adipose tissue and brain (Traber and Arai, 1999; Blatt et al., 2001; Clarke et al., 2008). The only forms of the vitamin secreted by the liver are the natural RRR- α -tocopherol and the four 2R forms of the all rac α -tocopherol. Small amount of the other tocopherols and tocotrienol homologues are also secreted (Machlin, 1991).

Recent studies indicates that vitamin E is excreted in the form of a conjugated hydrosoluble compound called carboxyethylhydroxychromans (CEHCs) in urine (Brigelius-Flohè and Traber, 1999) or bile (Kiyose et al., 2001). The CEHCs are degradation products of two oxidation steps, an initial omega-oxidation by cytochrome P450s (CYPs) is followed by a beta-oxidation.

CHECs are sulfated or glucuronidated (Schultz et al., 1995; Brigelius-Flohe and Traber, 1999; Stahl et al., 1999; Swanson et al., 1999; Pope et al., 2002). Tocopherols appear to be preferentially metabolized by different CYPs and to different extent (Lodge JK, 2005) α -tocopherols primarily metabolized by CYP3A4 (Birringer et al., 2001) but only a small fraction of the dose (< 1 %) is found as alpha-CHEC (Leonard et al., 2005) whereas γ -tocopherols are primarily metabolized by CYP4F2 (Sontag and Parker, 2002) and further extensively converted into gamma-CHEC (Galli et al., 2003).

Kinetics of different vitamin E sources

Kinetics human studies (Acuff et al., 1994; Traber et al., 1992) with deuterated RRR and all-rac- α -tocopheryl acetate simultaneously administered orally indicated a bioavailability ratios of RRR: all-rac close to 2:1 (Food and Nutrition Board, 2000), differing from the accepted biopotency ratio 1.36:1,

but equivalent kinetics could be shown in a non competitive uptake approach (Lodge, 2005; Proteggente et al., 2005) when RRR and all-rac- α -tocopherol were administered separately.

The apparent half-life of RRR- α -tocopherol in plasma is 48 h (Traber, 1994) up to 60 h (Bruno et al., 2005), whereas that of SRR- α -tocopherols is only 15h (Trabert, 1994). The 2S- α -tocopherols disappear relatively fast, within 48 hours nearly 90 % of the 2S forms were removed from the plasma, while 50 % of the 2R forms remain (Traber, 2007). However the data on factors influencing vitamin E bioavailability are still limited and many studies, including clinical trials, are performed without the indication of bioavailability.

The relative biopotencies of each individual α -tocopherol stereoisomer, ranging from 100 % for RRR to 21 % for SSR, determined by the fetal rat absorption model (Weiser and Vecchi, 1982), are related to the binding to the hepatic α -TTP, which has higher affinity to the RRR than to 2R forms of α -tocopherol (Traber and Kayden, 1989; Trabert et al., 1990b).

The relative biopotencies of the different stereoisomers of α -tocopherol in the fetal rat absorption assay were RRR 100 %, RRS 90 %, RSS 73 %, RSR 57 %, SSS 60 %, SRS 37 %, SRR 31 % and SSR 21 %. The configuration of the carbon 2 on the chromanol ring is the most important asymmetric carbon with respect to determining the biological activity of α -tocopherol, and has also an important role in the biodiscrimination of the different stereoisomers (Weiser et al., 1996). 2R α -tocopherols are preferentially found in the tissues of humans (Traber et al., 1990b), rats (Weiser et al., 1996) and pigs (Lauridsen et al., 2002), as well as in eggs (Piiironen et al., 1991). It is analytically difficult to quantify the different stereoisomers in animal fluids and tissues. Therefore, only few data is available on transfer and deposition of different stereoisomers of α -tocopherol from feed to plasma, milk and tissues in various farm animals consuming different sources of vitamin E.

B.1.2. Deposition

Ruminants

Conflicting results on the degradation of α -tocopherol in the rumen are reported in the literature. According with Alderson et al. (1971) 50 % of α -tocopheryl acetate could be degraded in the rumen, when a high amount of concentrate was fed, but Weiss et al. (1995) could not confirm this high ruminal losses. The tocopherol acts as an antioxidant in feed and intestine, which may cause losses prior its absorption (Halliwell et al., 2000).

The mechanism involved in the translocation of α -tocopherol from feed to milk is poorly understood. The process involved in the final transportation from plasma lipoproteins into milk fat is not known, but only a few percent is secreted into milk (Weiss and Wyatt, 2003).

In both studies, in cows administered all-rac- α -tocopheryl acetate the RRR form was the predominant stereoisomer in plasma and milk (84 to 88 % of all α -tocopherol stereoisomers), the SS forms were absent. Meglia et al. (2006) found highest concentrations after administration of RRR- α -tocopheryl acetate compared to all-rac- α -tocopheryl acetate and RRR- α -tocopherol.

Feeding 2500 IU vitamin E/day as RRR- or all-rac- α -tocopheryl acetate to dairy cows, starting 14 days before anticipated calving continuing until 14 days post-parturition resulted in significantly higher α -tocopherol concentrations in milk (colostrum, transition milk and milk at the end of the study) of the group given the RRR stereoisomer. The concentration of RRR in plasma and milk of the RRR fed cows represented 87–94 % of all isomers. For the all-rac group the RRR isomer amounted to 60 to 65 % (Weiss et al., 2009).

The results of Jensen et al. (2005) concerning the plasma levels of tocopherol stereoisomers following exposure in cows are compared in figure B1 with those in rats fed 1 mg all-rac- α -tocopheryl acetate

for ten days. Cows apparently discriminate in favour of the RRR isomer to a much higher degree than rats.

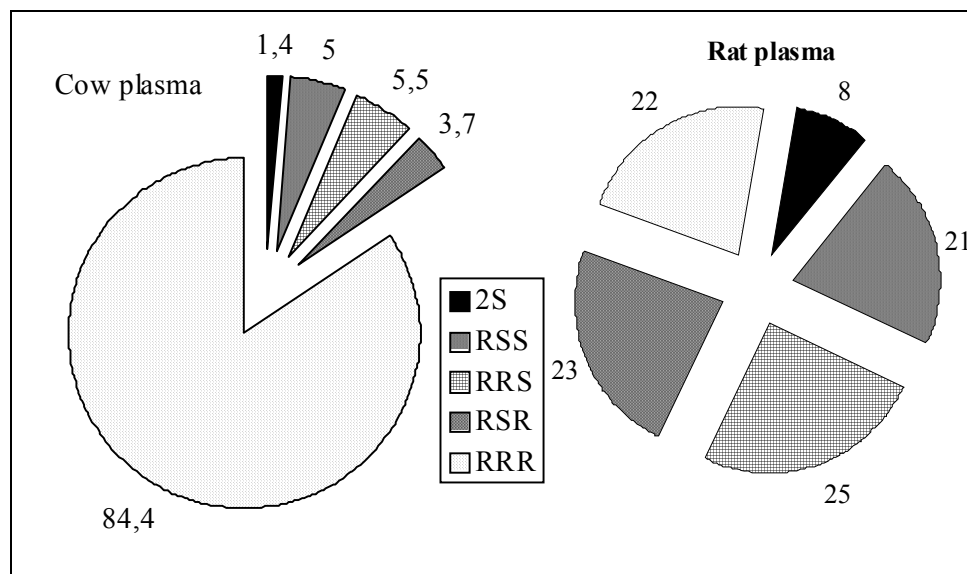


Figure B1: Relative distribution of α -tocopherol stereoisomers in plasma from cows fed 3000 mg all-rac- α -tocopheryl acetate daily for 16 days and rats fed 1 mg all-rac- α -tocopheryl acetate daily for 10 days (n = 5; means)

Calves fed milk replacer supplemented with 250 mg all-rac- α -tocopheryl acetate/kg DM (Jensen and Lauridsen, 2007) showed a similar distribution of α -tocopherol stereoisomers in plasma as rats in Figure B1.

Eicher et al. (1997) administered a single dose of 50 IU/kg bw of all-rac- α -tocopheryl acetate (powder) or RRR- α -tocopherol (liquid) by gastric tube to calves. Concentrations of α -tocopherol tended to be higher in plasma, liver and kidney for calves given RRR- α -tocopherol compared to all-rac- α -tocopheryl acetate.

Pigs

Some studies comparing tissue deposition with different vitamin E forms are available in piglets. A study to compare the bioavailability of RRR- α -tocopherol and all rac- α -tocopheryl-acetate, was conducted with two dietary vitamin E sources, RRR- α -tocopherol and all rac- α -tocopheryl-acetate, added to diets (16, 48 or 96 IU/kg feed each) for 35 days post weaning (Chung et al., 1992). At the higher supplementation level (96 IU) tissue and serum α -tocopherol concentrations were greater for RRR- α -tocopherol compared with all rac- α -tocopheryl-acetate. The highest tissue concentration was observed in the heart with both vitamin E forms.

In piglets, supplemented with vitamin E in drinking water (100–150 IU/L) at weaning, RRR- α -tocopheryl acetate showed a higher bioavailability than the acetate synthetic form of α -tocopherol based on α -tocopherol deposition in tissues (Wilburn et al., 2008). The highest deposition with both RRR and all-rac-forms was found in liver followed by heart and lung. The same authors found that both vitamin E forms were more effective in increasing plasma α -tocopherol when supplied to the pig's water at weaning than when added into the diet (Wilburn et al., 2008).

Several studies suggested that pancreatic esterase of weanling pigs may be a limiting factor for acetate forms for hydrolysis and absorption (Chung et al., 1992; Lauridsen et al., 2001; Jensen and Lauridsen, 2007).

Anderson et al. (1995) fed finishing pigs for 28 days with a single supplementary level (62 IU/kg) of all rac- α -tocopherol, all rac- α -tocopheryl acetate, RRR- α -tocopherol or RRR- α -tocopheryl acetate. Dietary supplementation with RRR- α -tocopheryl acetate resulted in the highest serum and tissue concentrations of α -tocopherol. The highest deposition was found in liver, in agreement with previous studies (Asghar et al., 1991; Jensen et al., 1988).

The contemporary administration of deuterated d3-RRR- α -tocopheryl acetate and d6 all-rac- α -tocopheryl acetate to farrowing sows (150 mg each daily for seven days before and seven days after giving birth) showed that in plasma, milk and in piglet tissues the concentration of d3-RRR: d6-all showed a ratio of 2:1. In suckling piglets highest concentration of deuterated vitamin E were found in liver, followed by lung, heart, kidney, muscles, intestine and brain. (Lauridsen et al., 2002; Jansen and Lauridsen, 2007).

In sows, feed supplementation with all rac- α -tocopheryl acetate before and during lactation increased α -tocopherol concentration in colostrum and in piglet tissues (Hidiroglou, 1993a; Hidiroglou, 1993b). Mahan et al. (2000) found higher α -tocopherol levels in milk as well as in serum and liver of 21-day-old nursing pigs when sows were fed with RRR- α -tocopheryl acetate compared with all-rac- α -tocopheryl acetate.

In another study (Jansen and Lauridsen, 2007) sows were fed feeds supplemented with 75 IU/kg feed of either all-rac- α -tocopheryl acetate or RRR- α -tocopheryl acetate one week before parturition and 28 days after parturition. The RRR form was the most predominant form of α -tocopherol in milk as well as in plasma, in sows and piglets, while the 2S-forms were present in very limited amounts, especially after feeding with the RRR- α -tocopheryl acetate (Jansen and Lauridsen, 2007). Thus sow metabolism leads to a preferential presence of RRR form in plasma and milk (Lauridsen et al., 2002a).

Poultry

Tissue and egg deposition was assessed in broilers and layers (Flachowsky et al., 2002) fed with basal diets supplemented with high to extremely high doses of α -tocopheryl acetate (0, 100, 1000, 10000 and 20000 mg/kg feed) for 30 (in broilers) or 70, 140, and 308 days (in layers). Alpha-tocopherol concentration were determined in muscle (breast, legs), liver, fat and eggs. At all dose levels tissue concentrations were liver>muscle>fat. Deposition in breast muscle was higher than in leg muscle at the lower supplementation levels (100 and 1000 mg/kg). Concentrations in egg yolk were much higher than in tissues.

In a recent study (Li et al., 2009), chickens were fed with feed supplemented with all rac- α -tocopheryl acetate (0, 10, 50, 100, 150, or 200 mg/kg feed); deposition of α -tocopherol was measured in breast and ilirotibialis muscles. At lower feed supplementation levels (10 and 50 mg/kg) deposition in ilirotibialis muscle was higher than in breast muscle; no such trend was observed at higher levels.

Differences in stereoisomer deposition in eggs were investigated by Piironen et al. (1991) in laying hens fed diets supplemented with all-rac- α -tocopherol; the enantiomeric RRR+SSS showed a greater egg transfer rate than others.

Cortinas et al. (2004) fed broiler chickens at 100, 200 or 400 mg all rac- α -tocopheryl acetate/kg diet, in combination with two levels of dietary PUFA (15 or 61 mg/g). At the lower PUFA supplementation level the average distribution of the α -tocopherol stereoisomers was 20 % RRR, 21 % RRS, 17 % RSS, 15 % RSR, 28 % 2S α -tocopherol; at the higher PUFA level, the 2R stereoisomers predominated (69–100 %) on the 2S. Deposition patterns in liver and thigh muscle were consistently similar

Fish

In rainbow trout the uptake of all rac- α -tocopherol acetate is lower compared with RRR- α -tocopherol, based on the concentration of α -tocopherol in plasma, liver, kidney, spleen and heart, 4 h after oral administration of either form (Hung et al., 1982).

In juvenile marine fish, turbot (*Scophthalmus maximus L.*), halibut (*Hippoglossus Hippoglossus L.*), and sea bream (*Sparus aurata*), fed with diets supplemented with DL α -tocopheryl acetate (100-1000 mg/kg) a dose-related deposition in liver was observed. Interspecies differences were related to the background presence of vitamin E, as evaluated in the unsupplemented fish and deposition levels were higher in turbot, followed by halibut and sea bream. However, the relationship of feed concentrations with deposition of vitamin E in whole fish as well as with antioxidant enzymes and lipid peroxidation products in liver was less evident and markedly different among species.

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APPENDIX C
Deposition data used for calculation of consumer exposure

Sünder and Flachowsky (2001) fed a total of 45 laying hens of 20 weeks of age, divided into five groups, for 10 weeks diets all-rac- α -tocopheryl acetate concentrations of 0, 100, 1,000, 10,000 and 20,000 mg/kg, respectively. During the chick and growing periods, the same α -tocopheryl acetate levels were supplemented. The vitamin E content of the basal diet was calculated to be 15 mg/kg diet. Table C1 summarises the final α -tocopherol levels in different tissues.

Table C1: Vitamin E concentration [$\mu\text{g/g}$ fresh matter] of various tissues ($n = 9$, means \pm SD)

	all-rac-α-tocopheryl acetate supplementation (mg/kg diet)				
	0 ⁽¹⁾	100	1000	10000	20000
Liver	1.8 \pm 0.2 ^a	17 \pm 1 ^b	126 \pm 5 ^c	275 \pm 6 ^d	862 \pm 4 ^e
Abdominal fat	7 \pm 1 ^a	24 \pm 1 ^b	144 \pm 7 ^c	311 \pm 12 ^d	624 \pm 68 ^e
Breast muscle	3.3 \pm 0.2 ^a	8.0 \pm 0.3 ^b	35 \pm 4 ^c	65 \pm 2 ^d	81 \pm 6 ^e
Leg muscle	0.56 \pm 0.04 ^a	5.0 \pm 0.9 ^b	51 \pm 3 ^c	109 \pm 10 ^d	199 \pm 15 ^e
Egg yolk	78 \pm 7 ^a	251 \pm 7 ^b	1396 \pm 35 ^c	2502 \pm 128 ^d	3133 \pm 214 ^e

⁽¹⁾ Calculated background content of the basal diet: 15 mg vitamin E/kg

^{a-c} Different subscripts in the same row indicate significant differences between groups ($P < 0.05$).

Calculating the vitamin E concentration of eggs from the above yolk data, assuming a standardised egg with 27% yolk, 2.1, 6.8, 37.7, 67.6 and 84.6 mg vitamin E/100g egg resulted from the supplementation of 0, 100, 1000, 10000 and 20000 mg all-rac- α -tocopheryl acetate/kg diet, respectively.

Yang et al. (2009) fed groups of 5 pigs for 32 days diets supplemented with 22 mg vitamin E from all-rac- α -tocopheryl acetate/kg, and with 9, 11, 15 and 22 mg vitamin E/kg from RRR- α -tocopheryl acetate. The deposition of vitamin E in liver, kidney, muscle (loin) and adipose tissue is listed in Table C2.

Table C2: Effect of dietary vitamin E source and level on tissue α -tocopherol concentrations of finishing pig

Vitamin E (IU/kg feed)	22.00	9.13	11.33	14.96	22.00
Source of vitamin E	all-rac	RRR	RRR	RRR	RRR
Liver, α -tocopherol ($\mu\text{g/g}$)	2.63	2.86	2.75	3.43	4.59
Kidney α -tocopherol ($\mu\text{g/g}$)	1.76	1.72	1.91	2.27	2.68
Loin, α -tocopherol ($\mu\text{g/g}$)	1.12	1.37	1.83	1.56	1.75
Adipose, α -tocopherol ($\mu\text{g/g}$)	3.59	4.04	5.04	4.64	5.14

Tocher et al. (2002) fed juvenile marine fish of commercial importance in European aquaculture, namely turbot (*Scophthalmus maximus*), halibut (*Hippoglossus hippoglossus*) and gilthead sea bream (*Sparus aurata*) diets of identical unsaturation index supplemented with graded amounts of vitamin E. Table C3 reviews the vitamin E deposition.

Table C3: Effect of dietary vitamin E on tissue deposition (mg/kg tissue; mean \pm SD (n = 3)) whole fish of turbot (*Scophthalmus maximus*), halibut (*Hippoglossus hippoglossus*) and sea bream (*Sparus aurata*)

	Turbot			Halibut			Sea bream		
	E0	E100	E1000	E10	E100	E1000	E10	E100	E1000
Vitamin E									
Liver	160 \pm 15 ^b	706 \pm 766 ^b	6205 \pm 3423 ^a	71 \pm 45 ^b	25 \pm 22 ^b	1484 \pm 794 ^a	54 \pm 4 ^c	495 \pm 20 ^b	6661 \pm 631 ^a
Fish	62 \pm 6 ^b	135 \pm 69 ^b	256 \pm 98 ^a	2 \pm 1	7 \pm 6	56 \pm 97	n.a.	n.a.	n.a.

^{a,b,c} Mean values with different superscript letters within a row for each species are significantly different ($P < 0.05$).

Weiss et al. (2009) studied the concentrations of α -tocopherol stereoisomers in plasma and milk of periparturient dairy cows given orally 2,500 IU vitamin E/d as RRR- or all-rac- α -tocopheryl acetate, starting 14 days before anticipated calving continuing until 14 days post-parturition. Concentrations of α -tocopherol in colostrum, transition milk and milk were greatest for cows fed the RRR, intermediate for all-rac and lowest for cows fed no supplemental vitamin E. Concentrations of α -tocopherol in transition milk and milk were 1.24 and 1.43 times greater for cows fed the RRR compared with cows fed the all-rac (Table C4).

Table C4: Effect of type of supplemental vitamin E on α -tocopherol concentrations in colostrum and milk⁽¹⁾

Variable	Control	RRR	All-rac	SE
α -tocopherol, mg/L				
Colostrum ⁽¹⁾	6.79 ^a	13.91 ^c	10.45 ^b	1.05
Transition milk ⁽¹⁾	2.19 ^a	4.21 ^b	3.39 ^b	0.42
Milk ⁽¹⁾	0.41 ^a	1.29 ^c	0.90 ^b	0.088

^{a-c} Means in same row with different superscripts significantly differ ($P < 0.05$).

⁽¹⁾ Colostrum is the first milking following parturition; transition milk is a composite sample (by weight) of the second through sixth milking after parturition; and milk is a composite (a.m. and p.m.) sample of milk produced at 14 days post-parturition.

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