

# Influence of Source and Quantity of Supplemental Vitamin E on Equine Serum and Cerebrospinal Fluid $\alpha$ -Tocopherol and Its Implication for Neurologic Diseases

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Practitioners should consider supplementing horses with neurologic disorders with micellized natural vitamin E at a level of 5–10 IU per lb body weight (10–20 IU per kg). A 1,000-lb. horse should receive 5,000–10,000 IU daily. Authors' addresses: 2414 1st Avenue #506, Seattle, Washington 98121 (Kane); 112 Bedford Road, Bedford, Texas 76022 (Stuart) and University of California, 1 Shields Avenue, Davis, California 95616 (Pusterla); e-mail: ekaneed@aol.com. © 2010 AAEP.

## 1. Introduction

Vitamin E ( $\alpha$ -tocopherol) is a powerful antioxidant, shown to protect cell membranes and tissues from oxidant free-radical damage and prevent propagation of lipid peroxidation, which may cause severe damage to cell membranes and intracellular components.<sup>1–3</sup> Free-radical production and damage may also become excessive in neurologic tissue after neurologic injury or disease, causing further neuronal injury.<sup>4–6</sup> It is also known that vitamin E enhances humoral and cell-mediated immunity.<sup>7,8</sup> Therefore, vitamin E could have additional beneficial effects in animals with neurologic disease.

The difference between natural and synthetic vitamin E is their chemical structures. Natural vitamin E (d- $\alpha$ -tocopherol) contains one isomer, RRR- $\alpha$ -tocopherol. Synthetic vitamin E (d,l- $\alpha$ -tocopherol) contains equal molar amounts of eight

stereoisomers, of which only one is identical to the natural RRR-isomer. The body preferentially transports and incorporates the natural isomer, thereby making the bioavailability of natural vitamin E greater than an equal quantity of synthetic vitamin E.<sup>9</sup>

Green forages are the primary source of naturally occurring vitamin E, with cereal grains and stored forages containing minimal amounts. Level of vitamin E supplementation is especially important when considering vitamin E needs of confined horses that have limited access to pasture and consume stored forages.

Vitamin E deficiency has been shown to be linked to equine degenerative myeloencephalopathy (EDM) and equine motor neuron disease (EMND). Other equine neurologic diseases include equine protozoal myeloencephalitis (EPM), equine herpesvirus-1 myeloencephalopathy, cervical vertebral myelopa-

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## NOTES

thy, West Nile virus encephalitis, and traumatic injury to the central nervous system (CNS).

## 2. EDM

EDM is a diffuse degenerative disease of the spinal cord and brain stem, with signs of slowly progressive ataxia and weakness.<sup>10</sup> Although first observed in the Northeast, several breeds of horses with ataxia and neuroaxonal dystrophy were subsequently noted, including Appaloosas, Arabians, Quarter Horses, Thoroughbreds, Standardbreds, Paso Finos, Morgan horses, Paint horses, Norwegian Fjord horses, Welsh ponies, and mules.<sup>11–15</sup> The disease was also seen in Grant zebras (*E. burchelli*) and Mongolian wild horses (*E. przewalskii*) in captivity.<sup>16,17</sup>

Most EDM-affected horses exhibit ataxia during their first year, with onset of clinical signs before 6 mo of age.<sup>10,12</sup> The clinical signs of EDM were initially described as being upper motor neuron signs with general proprioceptive deficits as well as a distinct tendency for generalized hyporeflexia and hypometria. Signs of EDM include clumsiness, inability to do complicated movements (i.e., tight turns), malpositioning of limbs at rest and during movement, and obvious ataxia or incoordination.<sup>18</sup>

EDM, commonly seen in young horses, was shown to have a familial component, but it is also linked to low serum vitamin E levels and/or vitamin E deficiency.<sup>15,18,19</sup> Several factors suggested vitamin E deficiency. Pathologic findings of neuroaxonal dystrophy and lipofuscin-like pigment accumulation are common features in experimentally induced vitamin E deficiency in several species and EDM-affected horses. Low to deficient serum  $\alpha$ -tocopherol values have been reported in several studies of EDM. Supplementary administration of vitamin E to foals from sires known to produce EDM decreased the incidence of the disease, and adult horses with EDM treated with 6,000 IU/day of  $\alpha$ -tocopheryl acetate for  $\geq 1$  yr showed neurologic improvement.

Young foals showing signs of incoordination were supplemented with 6,000 IU vitamin E per day. They appeared normal by 2 yr of age.<sup>20</sup> Horses with EDM responded to massive doses of the vitamin, a factor to assist practitioners in diagnosing the disease. It is highly recommended that horses at risk to the disease be supplemented with the vitamin. The incidence of EDM on one farm was reduced from 40% to 0% through supplementation of 1,000–2,000 IU/day of d,l  $\alpha$ -tocopheryl acetate.<sup>15,21</sup> Affected horses fed 6,000 IU of d,l  $\alpha$ -tocopheryl acetate per day mixed with 60 ml of corn oil and fed with sweet feed or grain showed improvement of the neurologic scores.

In children with neurologic deficits caused by malabsorption of vitamin E that were supplemented with massive doses of vitamin E before 5 yr of age returned to normalcy.<sup>22</sup> Similarly, in young horses, the earlier the gait deficits in predisposed or

sporadic cases of EDM are diagnosed, the better will be the response to vitamin E supplementation.

The reasons for the low  $\alpha$ -tocopherol values in horses have been hypothesized to be possibly because of several factors: inadequate vitamin E in the feed, reduced absorption of vitamin E because of lack of bile acids or intestinal malfunction, or failure to make chylomicrons in the enterocytes necessary to carry the vitamin E through the lymphatic system. Additional factors include the absence of the specific hepatic protein (tocopherol transfer protein [TPP]) postulated to transfer the vitamin E from the chylomicrons to the low-density lipoprotein (LDL) and high-density lipoprotein (HDL), an inadequate number of cellular vitamin E receptors, and increased use and/or excretion of the vitamin.

Adequate dietary and/or supplemental vitamin E has been shown to be a factor in treatment and prevention of EDM.<sup>11,18,20</sup> Vitamin E is valued for its prime role as a scavenger of free radicals and therefore, its antioxidant protection of neuronal lipid membranes. The disease is often confirmed, not only by the ataxia seen in young horses but also by low plasma or serum  $\alpha$ -tocopherol status ( $< 3 \mu\text{g/ml}$ ), although these low  $\alpha$ -tocopherol values are also seen in normal horses without neurologic disease. A known history of EDM within the horse's bloodline can help confirm the diagnosis.

Supplementing stallions with 1,500 IU vitamin E per day decreases the incidence of EDM in their foals from 40% to 10%.<sup>15</sup> A vitamin E-responsive degenerative myeloencephalopathy in Standardbred and Paso Fino horses from 3 to 30 mo of age has been described. Symmetric ataxia and paresis, along with laryngeal adductor, cervicofacial, and local cervical and cutaneous trunci hyporeflexia, were characteristic. No clinical signs were observed before 3 mo of age. The onset of gait abnormality is usually abrupt. Clinical signs have been seen to remain static or progress for weeks or months. Severely affected animals often fall while running. In one study, the mean ( $\pm$  SD) serum  $\alpha$ -tocopherol concentration of 13 ataxic weanlings was  $0.62 \pm 0.13$  (range = 0.47–0.84)  $\mu\text{g/ml}$ . A number of non-ataxic weanlings had similar values, although vitamin E supplementation markedly reduced the incidence of the syndrome on affected farms. Based on genetic studies, this disorder seems to have a familial disposition.

To reach a tentative diagnosis of EDM, a neurologic and cerebrospinal (CSF) exam is recommended. Measuring various parameters within the CSF are suggested, and analysis of CSF  $\alpha$ -tocopherol may be of value.<sup>23</sup>

## 3. EMND

EMND is a naturally occurring neurodegenerative disease, an oxidative disorder of the somatic motor neuron system in adult horses.<sup>24–26</sup> Clinical signs include weight loss from muscle wasting, trembling, muscle fasciculations, and prolonged periods of re-

cumbency.<sup>27</sup> Pathologic changes are primarily limited to the somatic lower motor neurons of the spinal cord and brainstem nuclei, their corresponding efferent nerves and innervated muscle groups, and the retinal pigment epithelium.<sup>28-30</sup>

Vitamin E deficiency is a primary factor, because studies show that horses without access to adequate vitamin E (green forage or pasture) for 18 mo or more are at high risk for the disease.<sup>31,32</sup> Low serum  $\alpha$ -tocopherol is a consistent laboratory finding in horses with EMND. Excessive amounts of copper (Cu) in the spinal cord and hepatic iron (Fe) are seen in horses with EMND.<sup>25</sup>

Four of eight horses fed low vitamin E diets (hay stored for >1 yr, 19.8 IU/kg; grain, 25.7 IU/kg) with excess Cu (>4,000 ppm) and Fe (>2,000 ppm) exhibited signs of EMND at 21, 27, 28, and 28 mo, with mean plasma  $\alpha$ -tocopherol concentrations of 0.25  $\mu$ g/ml. The other four horses, although they did not exhibit signs of EMND within 30 mo, did show decreased plasma  $\alpha$ -tocopherol levels (mean = 0.39  $\mu$ g/ml). Histological changes in the spinal cord, peripheral nerves, and muscles were characteristic of EMND in all four affected horses. The other four horses, although they had no clinical signs of EMND, did show some nerve lesions histologically. Mean hepatic  $\alpha$ -tocopherol concentration was significantly low (2.57  $\mu$ g/g, dry weight) in EMND-affected horses compared with control horses (21.1  $\mu$ g/g). Mean hepatic Cu concentrations (503 ppm) of horses with EMND were approximately 50 times that of control horses. It was concluded that vitamin E deficiency had a major causative role in EMND. Vitamin E was not measured in the spinal-cord tissue of the horses, but in humans,  $\alpha$ -tocopherol concentrations in CSF are highly correlated with serum concentrations.<sup>33</sup>

In relation to the etiology of EMND, synthetic vitamin E ester (d,l- $\alpha$ -tocopheryl acetate) commonly added to concentrates has both lower bioavailability and lower potency than natural vitamin E (d- $\alpha$ -tocopherol).<sup>25,34</sup>

#### 4. Supplementation of Vitamin E and Neurologic Diseases

Two studies were conducted at the University of California at Davis to measure effects of vitamin E supplementation on serum and CSF  $\alpha$ -tocopherol levels.<sup>23,35</sup> All procedures were approved by the Institutional Animal Care and Use Committee of the University of California.

The first study was conducted to determine if there were differences in serum and CSF  $\alpha$ -tocopherol levels between two groups of horses supplemented with two levels of vitamin E as water-soluble natural micellized d- $\alpha$ -tocopherol.<sup>23</sup>

Five horses per treatment group were supplemented for 10 days with either 1,000 IU or 10,000 IU vitamin E. From days 10 to 20, horses received no supplementation. Serum samples were taken pre-

during, and post-supplementation, and CSF samples were taken on days 0 and 10.

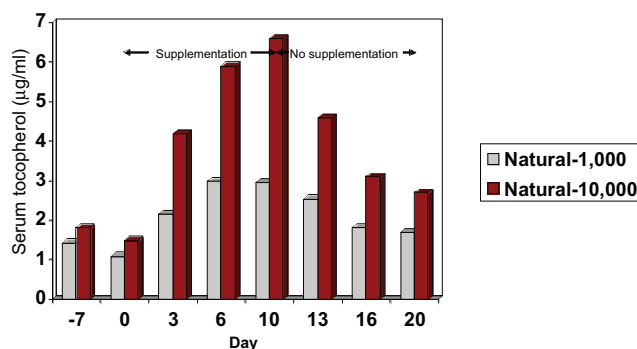
CSF samples were collected from the atlanto-occipital space, which presumably provides accurate values of  $\alpha$ -tocopherol concentrations around the brain and cervical portion of the spinal cord, and this is where the most damage to the CNS is detected in horses.

Significant correlation was found between serum and CSF  $\alpha$ -tocopherol on day 0 but not on day 10 (Figs. 1 and 2). The lack of correlation between serum and CSF  $\alpha$ -tocopherol on day 10 may have been attributed to a longer supplementation time needed to achieve steady state. Although median and maximum CSF  $\alpha$ -tocopherol levels were higher when 10,000 IU was fed compared with 1,000 IU, differences were not significant.

In the study, serum  $\alpha$ -tocopherol concentration increased rapidly in a non-linear manner when 10,000 IU of supplemental d- $\alpha$ -tocopherol was administered daily compared with 1,000 IU. Serum

#### Influence of Level of Natural Vitamin E on Serum $\alpha$ -tocopherol levels in Horses

Higgins, et al. 2008. Am J Vet Res. 69:785-790



Each value represents the mean of 5 horses. Vitamin E was supplemented from day 0-10

Fig. 1. Influence of level of natural vitamin E on serum  $\alpha$ -tocopherol levels in horses.

#### Influence of Level of Natural Vitamin E on CSF $\alpha$ -tocopherol levels in Horses

Higgins, et al. 2008. Am J Vet Res. 69:785-790

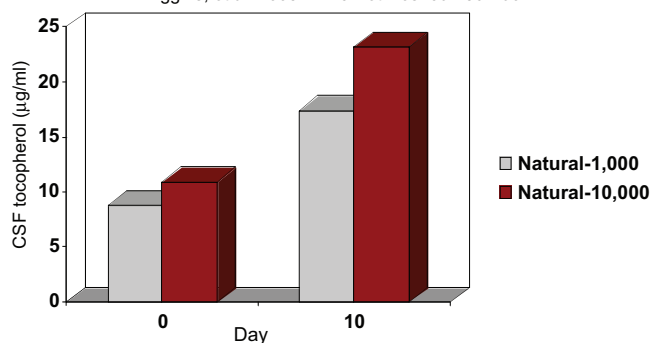


Fig. 2. Influence of level of natural vitamin E on CSF  $\alpha$ -tocopherol levels in horses.

$\alpha$ -tocopherol concentrations in both groups increased significantly throughout the period of vitamin E administration. From baseline values at day 0, they reached a plateau by day 10 (mean = 2.96  $\mu\text{g/ml}$  for 1,000 IU; mean = 6.6  $\mu\text{g/ml}$  for 10,000 IU). Median and maximum CSF  $\alpha$ -tocopherol concentrations were higher in horses treated with 10,000 IU d- $\alpha$ -tocopherol compared with those receiving 1,000 IU, suggesting that high doses of supplemental vitamin E are likely to have greater antioxidant benefit. Data from both groups between day 0 and 10 showed a 1.3- to 3.4-fold increase in CSF  $\alpha$ -tocopherol in 9 of 10 horses.

These results showed that daily administration of 10,000 IU vitamin E, as micellized d- $\alpha$ -tocopherol, was able to cross the blood-brain barrier in healthy horses. This allowed for vitamin E to reach the target site, the brain and spinal cord, and be potentially beneficial for horses with neurologic disease.

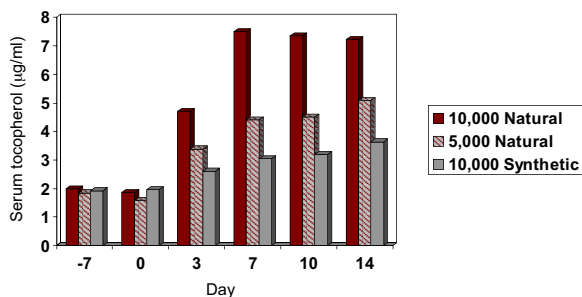
The second study was conducted to determine if there were differences in plasma and CSF  $\alpha$ -tocopherol levels when horses were supplemented with natural versus synthetic vitamin E.<sup>35</sup> Five horses per treatment group were supplemented for 14 days with 5,000 IU water-soluble natural micellized d- $\alpha$ -tocopherol, 10,000 IU water-soluble natural micellized d- $\alpha$ -tocopherol, or 10,000 IU synthetic (d,l  $\alpha$ -tocopheryl acetate). Serum samples were taken 1 wk before supplementation and on days 0, 3, 7, 10, and 14, and CSF samples were taken on days 0 and 14.

Significant correlation between serum and CSF  $\alpha$ -tocopherol was shown for supplemented horses on day 14 (Figs. 3 and 4). Supplementation with natural vitamin E showed greater bioavailability than an equal IU amounts of synthetic vitamin E. It is suggested that amounts of vitamin E greater than National Research Council (NRC) requirements are needed to treat neurologic diseases.

It was concluded that vitamin E supplemented as water-soluble natural micellized d- $\alpha$ -tocopherol was

### Influence of Source and Quantity of Vitamin E on Serum $\alpha$ -tocopherol levels in Horses

Pusterla et al. 2010. Vet Record. 166:366-368.

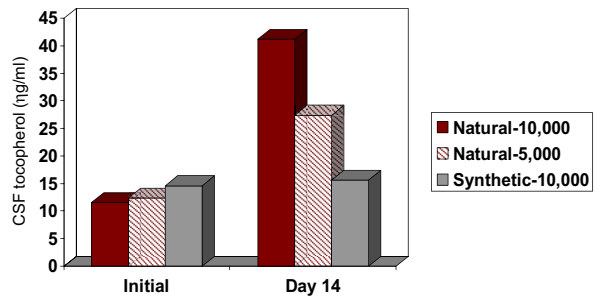


Each value represents the mean of 5 horses. Vitamin E was supplemented from day 0 -14

Fig. 3. Influence of source and quantity of vitamin E on serum  $\alpha$ -tocopherol levels in horses.

### Influence of Source and Quantity of Vitamin E on CSF $\alpha$ -tocopherol levels in Horses

Pusterla et al. 2010. Vet Record. 166:366-368.



Each value represents the mean of 5 horses. Vitamin E was supplemented from day 0 -14

Fig. 4. Influence of source and quantity of vitamin E on CSF  $\alpha$ -tocopherol levels in horses.

transferred across the blood-brain barrier more effectively than an equal IU quantity of synthetic vitamin E. Synthetic vitamin E was not effective in increasing CSF  $\alpha$ -tocopherol levels after 14 days of supplementation. It was similarly found in Parkinson's patients that oral supplementation of synthetic  $\alpha$ -tocopherol at levels up to 4,000 IU per day failed to increase ventricular CSF  $\alpha$ -tocopherol levels.<sup>36</sup> The horse study showed that feeding natural, micellized d- $\alpha$ -tocopherol showed greater bioavailability than synthetic (d,l- $\alpha$ -tocopheryl acetate) when fed on an equal (10,000 IU) basis, resulting in greater plasma and CSF  $\alpha$ -tocopherol levels.

### 5. Conclusions and Implications

Horses that present with neurologic disease are of different ages and on various diets. It has been postulated that horses with neurologic disease may have a higher vitamin E need compared with healthy horses. This may be because of an increased oxidant damage associated with the underlying neurologic disease.<sup>23</sup>

It may be advisable to sample serum and CSF of horses presented with various neurologic diseases and analyze for  $\alpha$ -tocopherol. In addition to the studies of CSF  $\alpha$ -tocopherol concentration in healthy horses, clinical studies to further evaluate the efficacy of daily oral administration of supplemental vitamin E should include investigation of  $\alpha$ -tocopherol concentrations in the CSF of horses with clinical signs of neurologic disease before and after vitamin E treatment. Such an investigation could evaluate the effects of similar treatment protocols in horses with inflammatory or degenerative neurologic diseases, including EDM, EMND, EPM, equine herpesvirus-1 myeloencephalopathy, cervical vertebral myelopathy, West Nile virus encephalitis, or traumatic injury to the CNS. Results of such research would help to further evaluate the clinical application of vitamin E treatment in affected horses.



It is recommended that because maintenance of sufficient cerebral  $\alpha$ -tocopherol concentrations is essential for neurologic function and of even greater importance when brain function is impaired by degenerative or inflammatory neurologic disorders, supplementation of a water-soluble natural micellized  $\alpha$ -tocopherol should be used instead of synthetic vitamin E when treating horses with neurologic disorders.

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