

Evaluation of the risk of motor neuron disease in horses fed a diet low in vitamin E and high in copper and iron

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Objective—To determine whether equine motor neuron disease (EMND) could be induced in adult horses fed a diet low in vitamin E and high in copper and iron.

Animals—59 healthy adult horses.

Procedure—Horses in the experimental group (n = 8) were confined to a dirt lot and fed a concentrate low in vitamin E and high in iron and copper in addition to free-choice grass hay that had been stored for 1 year. Control horses (n = 51) were fed a concentrate containing National Research Council–recommended amounts of copper, iron, and vitamin E. The hay fed to control horses was the same as that fed to experimental horses, but it had not been subjected to prolonged storage. Control horses had seasonal access to pasture, whereas experimental horses had no access to pasture. Horses that developed clinical signs of EMND were euthanatized along with an age-matched control horse to determine differences in hepatic concentrations of vitamin E, vitamin A, copper, iron, and selenium.

Results—4 experimental horses developed clinical signs of EMND. Plasma concentrations of vitamin E decreased in all 8 experimental horses. There were no significant changes in plasma concentrations of vitamin A, selenium, and copper or serum concentrations of ferritin. There were no significant differences in those analytes between experimental horses with EMND and experimental horses that did not develop EMND. No control horses developed EMND.

Conclusions and Clinical Relevance—Results suggest that lack of access to pasture, dietary deficiency of vitamin E, or excessive dietary copper are likely risk factors for EMND. (*Am J Vet Res* 2006;67:120–126)

Equine motor neuron disease is a naturally occurring neurodegenerative disease of the somatic lower motor neuron system in adult horses. The disease was first described¹ in 11 horses in 1990 and bore clinical resemblance to a progressive muscular atrophy form of ALS, a motor neuron disease of humans. Reports^{2–8} published since then have typically been case reports or case series, usually involving 1 horse in a stable. These reports have

confirmed the existence of EMND in many parts of the world.

Clinical signs of EMND include weight loss from muscle wasting, trembling, muscle fasciculations, and prolonged periods of recumbency.² Clinical signs may not be evident until $\geq 30\%$ of normal neuronal cell function has been lost.⁹ Antemortem diagnosis can be made by means of histologic analysis of a biopsy specimen of the sacrocaudalis dorsalis medialis muscle or spinal accessory nerve.^{10,11} Pathologic changes related to EMND are mostly limited to somatic lower motor neurons of the spinal cord and brainstem nuclei (except nuclei III, IV, and VI), their corresponding efferent nerves and innervated muscle groups, and the RPE.^{1,12–14} Accumulation of lipopigment in spinal cord capillaries and RPE and the predilection for denervation of the highly oxidative type I muscle fibers suggest that EMND is an oxidative disorder.^{1,2,13–15} Epidemiologic studies^{16–19} reveal that absence of green forage for at least 18 months, high-grain diets, and coprophagia or geophagia are risk factors for EMND. Low plasma concentrations of the antioxidant vitamin E has been a consistent laboratory finding in clinical cases.^{2,20} Analysis of the mineral content of spinal cords from affected horses and age-matched controls reveals increased amounts of copper (a potential pro-oxidant) in the spinal cords of horses with EMND.²¹ The finding of high hepatic iron (another potential pro-oxidant) content has also been reported.²² These findings suggest that a deficiency in antioxidant elements (vitamin E) and an excess of pro-oxidant elements (copper and iron) may be responsible for development of EMND. The purpose of the study reported here was to determine whether EMND could be experimentally induced in adult horses by feeding vitamin E-deficient feeds with high contents of copper and iron for a prolonged period.

Materials and Methods

Horses—Eight healthy adult horses (6 females and 2 castrated males) constituted the experimental group. Experimental horses had no access to pasture and were fed a diet low in vitamin E and high in copper and iron. The age of

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EMND	Equine motor neuron disease
ALS	Amyotrophic lateral sclerosis
RPE	Retinal pigment epithelium
ERP	Equine Research Park
NRC	National Research Council
ICP	Inductively coupled plasma
HPLC	High-performance liquid chromatography
EDM	Equine degenerative myeloencephalopathy

the horses ranged from 6 to 17 years (mean, 12.5 years), a range similar to that reportedly¹⁶ typical for affected horses. Experimental horses were in good body condition at the beginning of the study, had normal results of neurologic examinations, and had normal histologic features of biopsy specimens from the right sacrocaudalis dorsalis medialis (ie, tail head) muscle. Experimental horses were purchased from the Cornell ERP, where approximately 60 resident adult horses are housed each year and used for breeding or teaching purposes; in the past 24 years, no confirmed or clinically suspicious cases of EMND have been reported. Because the feed source, climatic and geographic environment, and recent origin of the 8 experimental horses was the same as for the resident horses at ERP, all 51 adult horses that resided at the ERP during the 30-month study period were considered controls and were observed daily for illness. For each experimental horse that developed EMND, a control horse from the ERP was selected for euthanasia to determine whether there were differences in hepatic concentrations of iron, copper, vitamin E, vitamin A, or selenium and whether there was histologic evidence of EMND in control horses. The selection of control horses for euthanasia was made by the ERP manager on the basis of age (ie, similar to the age of the experimental horse) and availability (ie, the horse was not being used for equine husbandry programs).

Housing and feeding—Experimental horses were confined at the ERP in a 1.5-acre dry lot with a run-in shed for shelter and containing no grass for the length of the study. Horses from the ERP had been intermittently housed in this dry lot for a number of years, including the time period immediately prior to initiation of the study. The dry lot was maintained free of green plants by normal hoof trampling and intermittent harrowing, but no herbicides were used in the paddock. A mesh-wire fence surrounded the paddock and was secured into the ground in a manner that prevented confined horses from grazing outside the paddock area.

Control horses had access to 60 acres of rotational pasture from May to October. The pasture was predominantly Kentucky bluegrass and was mowed regularly but not fertilized or treated with lime. Experimental horses were fed free-choice grass hay that was the same type of hay and harvested from the same fields (from 2 farms in New York) as that fed to the control horses, except it had been stored in a barn that was used only for hay storage for 1 year prior to feeding. Experimental horses were fed individually and once daily; horses received 1.5 kg of a pelleted concentrate made of the same ingredients and produced at the same mill as that consumed by control horses, except that the concentrate given to experimental horses had copper sulfate and ferrous fumarate added at the mill prior to pelleting such that the product contained approximately 4,000 ppm of copper and 2,000 ppm of iron. These amounts were selected so that the total diet (hay and concentrate combined) provided copper and iron in excess (> 10 times the copper and > 5 times the iron) of the recommended NRC intake for each mineral. Similar concentrations of those minerals have been fed to ponies without adverse effects.^{23,24} Ingredients in the concentrate feed were cracked corn (50%), whole oats (23%), soybean meal (15%), molasses (9%), limestone (1.5%), dicalcium phosphate (1.5%), minerals (copper, zinc, and selenium), and vitamin A (6,000 U/kg as retinyl acetate). Vitamin E (30 U of D- α -tocopherol acetate/kg) was also added to the control concentrate. Hay and concentrate analyses were performed yearly (Table 1). Hay and concentrate feed analyses were performed by means of inductively coupled plasma spectrometry (copper and iron), atomic absorption (selenium), colorimetric assay (vitamin E), and HPLC (vitamin A).⁴ Anthelmintic administration, vaccination, dental prophylax-

is, and hoof care were routine and not different between treatment and control horses.

Monitoring—Experimental horses were monitored daily for 30 months (or until euthanasia) for appetite and clinical signs of EMND or other abnormal conditions, and body weight was determined every 6 months. Blood was collected from experimental horses every 4 months for determination of plasma concentrations of vitamin E, vitamin A, copper, and selenium and serum concentrations of ferritin. Plasma and hepatic concentrations of vitamin E and vitamin A were determined by means of normal-phase HPLC with UV detection and reported as micrograms per milliliter (plasma) and micrograms per gram (tissue dry weight), respectively.^b Plasma copper concentrations were determined by use of ICP^b and serum ferritin by use of ELISA.^c Plasma selenium concentration was measured by use of atomic absorption spectrophotometry.^d Horses that developed characteristic clinical signs of EMND were euthanized with a concentrated barbiturate solution^e within 3 days of the onset of signs. A complete necropsy examination was performed, including histologic examination of tissue from the heart, lungs, liver, eyes, kidney, adrenal gland, muscle, intestine, and various components of the central and peripheral portions of the nervous system, in horses that developed EMND and in an equal number of age-matched control horses. The brain, spinal cord, and selected peripheral nerves were harvested at necropsy and preserved in neutral-buffered 10% formalin solution. Selected blocks were embedded in paraffin, and 6- μ m-thick sections were cut and stained with H&E. Selected blocks were also stained with Luxol fast blue-cresyl echt violet and Bielschowsky silver stain. Frozen hepatic tissue from experimental horses with EMND and from control horses was analyzed for iron, copper, vitamin E, vitamin A, and selenium by use of ICP analysis (iron and copper), fluorometric determination (selenium), and HPLC (vitamins E and A) and reported as micrograms of element per gram of liver tissue (wet weight).^b Horses in the experimental group that did not develop clinical signs of EMND by the end of the 30-month period underwent general anesthesia and a biopsy procedure of the right spinal accessory nerve to determine whether there was histologic evidence of EMND.¹¹ Histologic evaluation was performed by a single pathologist (AD) with experience in evaluating spinal accessory nerve biopsy specimens as an antemortem test for EMND. Results were described as follows: no lesions observed, minimal or mild lesions observed, or obvious lesions observed. Surviving horses, along with surviving horses from another EMND study, were enrolled in a separate vitamin E intervention trial.

Data analysis—Changes in plasma concentrations of vitamin E, vitamin A, selenium, and copper and in serum concentrations of ferritin over time were determined for each experimental horse by use of regression analysis. Changes in blood concentrations of vitamin E, copper, vitamin A, ferritin, and selenium were assessed with a 1-sample Student *t* test. For analytes that changed over time in individual horses, mean regression coefficients of that analyte for the experimental horses that developed EMND were compared with the values for experimental horses that did not develop EMND by use of a 2-sample Student *t* test.

Mean plasma concentrations of vitamin E, copper, selenium, and vitamin A and serum concentrations of ferritin at the time of euthanasia in the experimental horses that developed EMND were compared with mean concentrations at the end of the study in experimental horses that did not develop clinical signs of EMND by use of the Wilcoxon rank sum test.

A 1-sided *t* test was used to evaluate differences in mean hepatic concentrations of vitamin E, copper, iron, selenium, and vitamin A at euthanasia in experimental horses that

developed clinical signs of EMND and in an equal number of control horses. For all comparisons, a value of $P \leq 0.05$ was considered significant.

Results

All 8 experimental horses readily consumed the pelleted concentrate throughout the study period, and no illnesses were observed other than signs associated with EMND. All experimental horses remained free from clinical signs and gained weight during the first year of the study (mean weight gain, 44 kg; range, 8 to 78 kg). Four horses developed clinical signs of EMND and were euthanized at 21, 27, 28, and 28 months after beginning the experimental diet. Clinical signs in all 4 horses included acute (ie, within hours) onset of trembling, fasciculations, frequent shifting of weight when standing, and excessive recumbency. One of those horses also had a simultaneous onset of low head and neck carriage. The 4 horses that developed clinical signs of EMND lost a mean of 92 kg (range, 64 to 115 kg) of body weight between their 1-year study weight and the time of onset of clinical signs.

Plasma vitamin E concentrations in all 8 experimental horses decreased significantly over time, and values (0.09 to 0.51 $\mu\text{g/mL}$) at euthanasia or study end were less than reference range. The mean \pm SD of the regression coefficient for change in vitamin E over time was not different between experimental horses that developed EMND (-0.043 ± 0.009) and experimental horses that did not develop EMND (-0.053 ± 0.009). There was no significant change in the concentrations of other analytes over time. Although plasma selenium concentrations decreased during the study period in all 8 experimental horses, changes were not significant and values at euthanasia or study end were only slightly less than laboratory reference values (Table 2). There was no significant change in plasma vitamin A

concentration in any of the experimental horses, with all 8 horses beginning and ending the trial with values in or slightly less than the reference range. Plasma copper concentrations did not change over time in the experimental horses, and all horses had plasma copper concentrations within reference range throughout the study. Values for serum ferritin concentration were slightly greater than reference range in 7 treatment horses (range, 281 to 528 ng/mL) and were within reference range in 1 horse upon entry into the study. Serum ferritin values were variable during the study, and no horse had a significant increase in serum ferritin concentration over time. One horse had a significant decrease in serum ferritin over time. There were no differences in mean serum ferritin or plasma vitamin E, vitamin A, copper, or selenium concentrations at the time of euthanasia or study end between experimental horses that developed clinical signs of EMND and experimental horses that did not.

Histologic changes in the spinal cord, peripheral nerves, and muscles were characteristic of EMND in all 4 affected horses. The only other abnormalities found at necropsy in the affected horses were microscopic findings of abundant brown cytoplasmic lipopigment granules in most hepatocytes and in the RPE cells of all 4 horses. Similar-appearing lipopigment granules were also detected in the heart, adrenal gland, and villus tips of the small intestine in 1 horse. At the end of the 30-month treatment period, the 4 experimental horses that did not have clinical signs of EMND appeared clinically normal, except that 1 horse had lost weight (77 kg). That horse was the only surviving horse in the treatment group that had obvious lesions (ie, 1 to 2 degenerative fibers/fascicle and bands of Schwann cells and endoneurium resulting from complete nerve fiber degeneration) in the spinal accessory nerve at the time biopsy specimens were collected at the end of the 30

Table 1—Results of nutritional analysis of hay and concentrates fed to treated and control horses for 30 months in a study of EMND.

Variable	Hay*		Concentrate*	
	Control	Treated	Control	Treated
Copper (ppm)	14.2	10.9	100	3,870
Iron (ppm)	68.0	66	146	1,876
Selenium (ppm)	0.05	0.06	0.27	0.35
Vitamin A activity (U/kg)	5,852†	1,463†	5,770	5,632
Vitamin E (U/kg)	34.7	19.8	62.9	25.7
Dry matter (%)	90.3	89.6	90.9	90.0

*Dry-weight basis, mean of values from yearly analyses. † β -Carotene concentration (mg/kg) \times 400 = Vitamin A concentration (U/kg).

Table 2—Mean (SD) values for final (at the time of euthanasia or at 30 months) plasma or serum concentrations of vitamin E, copper, ferritin, selenium, and vitamin A in horses fed a diet low in vitamin E and high in copper and iron that did or did not develop clinical signs of EMND (n = 4/group).

Group	Vitamin E ($\mu\text{g/mL}$)	Copper (ppm)	Ferritin (ng/mL)	Selenium ($\mu\text{g/dL}$)	Vitamin A ($\mu\text{g/mL}$)
EMND	0.255 (0.159)	1.350 (0.102)	336.25 (49.12)	12.525 (1.115)	169.5 (13.601)
No EMND	0.390 (0.116)	1.152 (0.101)	402 (279.82)	13.025 (1.883)	190.5 (11.619)
Laboratory reference range	2.0–4.0 ^b	0.85–2.00 ^b	43–261 ^c	15.0–20.0 ^d	175–300 ^b

For all variables, differences between groups were not significant ($P \leq 0.05$).
^{b,d}Superscript letters indicate footnotes.

months. The other 3 surviving horses had either no definitive lesions of EMND (1 horse) in the spinal accessory nerve or minimal lesions (ie, demyelinated fibers in scattered nerve fascicles; 2 horses).

Mean \pm SE dry-weight hepatic vitamin E concentration was low ($2.57 \pm 1.25 \mu\text{g/g}$) and significantly different in the 4 experimental horses with signs of EMND, compared with reference values and values in the 4 control horses ($21.1 \pm 1.3 \mu\text{g/g}$). Mean \pm SE wet-weight hepatic copper concentration ($503.5 \pm 65.59 \text{ ppm}$) in horses with EMND was more than 50 times the upper limit of the reference range and more than 100 times the mean value for liver copper concentration in the 4 control horses ($4.06 \pm 0.44 \text{ ppm}$), differences that were significant. Only one of the horses with EMND had wet-weight hepatic iron concentrations greater than the reference range for the testing laboratory, and there was no significant difference in mean values between horses with EMND ($462.3 \pm 113.71 \text{ ppm}$) and the 4 control horses ($251.0 \pm 27.54 \text{ ppm}$). Hepatic wet-weight selenium concentration was within reference range in all 4 horses with EMND ($1.59 \pm 0.16 \text{ ppm}$) and in 3 of 4 control horses ($1.27 \pm 0.19 \text{ ppm}$). Although dry-weight hepatic vitamin A concentrations were slightly less than reference range in 3 of the experimental horses that developed EMND, the mean value ($317.8 \pm 55.8 \mu\text{g/g}$) was not significantly different from that in the control horses ($476.3 \pm 26.3 \mu\text{g/g}$).

No control horses developed clinical signs of EMND. The 4 control horses that were euthanatized had no histologic lesions of EMND and had liver concentrations of vitamin E, copper, iron, selenium, and vitamin A that were within or nearly within reference ranges.

Discussion

Our results yield further evidence that lack of access to pasture and deficiency of vitamin E are important risk factors for EMND. The 21-month interval between the beginning of the study and development of clinical signs in the experimental horses correlates with our previous findings that, in naturally occurring EMND, affected horses had been on the property for at least 18 months prior to developing clinical signs.¹⁹ Tissue stores of vitamin E are likely abundant in most horses that have seasonal access to green forage, and several months of feeding a vitamin E-deficient diet may be required to develop a deficiency severe enough for oxidative injury and EMND to occur. The gradual depletion of vitamin E in the experimental horses was apparent in the decline of plasma vitamin E concentrations over time. The experimental horses were fed a diet similar to that consumed by horses with naturally occurring EMND (eg, absence of pasture and green hay). Although most horses with naturally occurring EMND were reportedly¹⁹ fed a variety of commercial concentrates containing variable amounts of added synthetic vitamin E, the amounts of vitamin E added to equine concentrate feeds have been low until recently and may not prevent vitamin E deficiency or EMND if access to green forage is minimal.²⁵ The synthetic vitamin E added to concentrates is usu-

ally D-1 α -tocopherol acetate (recently renamed *all-rac*- α -tocopherol acetate), which has both lower bioavailability and lower potency than natural vitamin E (recently renamed RRR- α -tocopherol).²⁶

We believe that our results, together with results of previous studies, indicate that vitamin E deficiency has a major causative role in EMND. Although all 8 experimental horses developed severe deficiencies in plasma vitamin E, only 4 horses developed classic clinical signs of EMND during the 30-month study. Vitamin E was not measured in spinal cord tissue of the horses, but in humans, tocopherol concentrations in CSF are highly correlated with serum concentrations.²⁷ It is uncertain why some horses develop EMND but others fed the identical feed and that have similar plasma vitamin E concentrations do not. This finding was also observed in our field studies^{2,18} of naturally occurring disease in which only 1 or 2 horses/y in an at-risk stable developed clinical signs of EMND, although nutritional, managerial, and environmental conditions were similar for all horses in the stable. This suggests there could be individual susceptibility to a disturbed antioxidant-pro-oxidant balance (oxidative stress). The fact that there were obvious histologic findings of EMND in 1 experimental horse with no overt clinical signs, mild histologic lesions in 2 horses with no clinical signs of disease, and an absence of lesions or signs in 1 horse suggests that in stables where 1 horse develops EMND, other horses fed the same diets may be mildly or subclinically affected.

The role of copper and iron as potential pro-oxidants is less obvious than the role of vitamin E deficiency in the pathogenesis of EMND. Both copper and iron have the potential to act as strong pro-oxidants via the Fenton reaction and generation of free hydroxyl ions with or without peroxynitrite production.²⁸ The activity of these free radicals in neuronal cells may damage lipids, nucleic acids, and mitochondria, causing neuronal cell dysfunction or death and lipofuscin deposition.^{29,30} Hepatic copper concentrations were remarkably high (nearly 125 times those in control horses) in the 4 horses with clinical signs of EMND. Copper content is also high, compared with that in control horses, in the spinal cord of horses with naturally occurring EMND.²¹ The role of copper in the development of EMND remains questionable, however. In a separate study³¹ that we performed concurrently, horses fed a diet similarly low in vitamin E content but with NRC-recommended copper concentrations (100 ppm in the concentrate feed) developed signs of EMND at a similar rate (ie, 4 of 10 horses) and time of onset (19 to 27 months after beginning a vitamin E-deficient diet). Additionally, we have not detected high copper concentrations in the livers of any horses with naturally occurring EMND. Despite substantially high hepatic copper concentrations in the 4 horses with EMND, there was no evidence of liver necrosis associated with these concentrations and plasma copper concentration did not change over time in any of the treated horses. This finding suggests that horses are relatively resistant to copper-induced hepatopathy, and as has been reported,²³ if liver copper stores are adequate, increasing copper intake does not alter blood

copper concentrations but will increase the accumulation of copper in the liver.

High hepatic concentrations of iron and high serum concentrations of ferritin have been detected in horses with naturally occurring EMND, but the role that iron plays in the development of EMND is questionable because iron concentrations are not high in the spinal cord of those horses,²¹ and in our concurrent experiment,³¹ horses fed a diet that was similarly low in vitamin E content but that contained iron closer to reference range concentrations (ie, 140 ppm in concentrate feed) also developed EMND. The 4 horses in the present study with clinical signs of EMND had either unremarkable or only mildly high hepatic iron concentrations. This finding was surprising because the amount of iron in the concentrate the horses consumed was > 10 times the amount recommended or contained in most commercial concentrate feeds.³² Although the content of iron in the concentrate was much greater than that in feeds routinely given to horses, the concentration of iron in the total diet was 318 ppm (approx 12 mg/kg of body weight per day, given an estimated daily roughage intake of 2% of body weight), which is only 3 to 6 times the iron concentration of the typical hay and grain diet for horses. The failure of this diet to cause an increase in serum ferritin concentrations or significantly higher hepatic iron concentrations than in control horses supports the concept that the percentage of iron absorbed in the intestine decreases when body stores are sufficient; this protective mechanism may prevent iron overload when dietary excess of the mineral is mild to moderate.³³ The mildly high serum ferritin concentration in 7 of 8 experimental horses at the beginning of our study suggests that those horses had high iron ingestion prior to being enrolled in the study. It is unknown why serum ferritin and hepatic iron concentrations are commonly higher than reference range in many horses with naturally occurring EMND. Copper and iron cannot be ruled out as candidates for pro-oxidant activity in horses with EMND, however, because even moderate increases of either cation may be harmful if body stores of antioxidants such as vitamin E are severely depleted. Previous studies^{23,24,32} in which ponies were fed supplements containing high iron or copper concentrations were short-term in scope (ie, conducted over a period of weeks as opposed to months) and animals deficient in vitamin E were not investigated.

Abnormal selenium content in plasma and spinal cord tissue has not been detected in horses with naturally occurring EMND,^{4,21} but we measured selenium in the experimental horses because of the close physiologic relationship between vitamin E and selenium. Although the changes were not significant, plasma selenium concentrations decreased over time in all 8 vitamin E-deficient horses, despite the fact that the diet met NRC requirements for idle horses. This decrease in plasma selenium concentration in the vitamin E-deficient horses may have been caused by increased use of selenium as a result of progressive vitamin E deficiency.³³

The experimental horses that did not have clinical signs of EMND were not euthanatized or subjected to

liver biopsy; thus, hepatic vitamin E, vitamin A, iron, copper, and selenium concentrations were not compared among experimental horses. No differences in these values were anticipated because the diet was identical in all 8 horses, and with the exception of copper, blood concentrations of vitamin E, vitamin A, ferritin, and selenium should be associated with hepatic concentrations, which were not different between the experimental horses that developed EMND and those that did not.

Results of this study, our previous study³¹ of experimental EMND (in which horses were fed a diet low in vitamin E but with copper and iron content in the range recommended by NRC), and published clinical investigations^{1-8,16-19} suggest that a green forage deficiency, likely leading to vitamin E deficiency, is the major risk factor for EMND. Although control horses received only marginally sufficient vitamin E in the concentrate and hay, they had access to grass for several months of the year. Each of the control horses that were euthanatized had liver concentrations of vitamin E that were > 10 times the hepatic vitamin E in experimental horses that developed EMND. Previous studies have revealed that consumption of grass increases plasma vitamin E concentration in horses, and after grazing is suspended, several weeks are required for the plasma vitamin E concentration to decrease. The decrease in plasma vitamin E concentration is presumed to be a result of tissue storage.³⁴

Vitamin A deficiency may be expected under feeding and housing conditions similar to those that cause vitamin E deficiency (ie, lack of green forage).³³⁻³⁵ We do not believe vitamin A deficiency plays an important role in the development of EMND because the amount of vitamin A added to commercial feeds is sufficient to meet minimum daily NRC maintenance requirements (40 U/kg of body weight) when 0.5% to 1% of concentrate/kg of body weight is fed, even without the additional vitamin A contained in roughage. If the experimental horses in our study consumed 2% of body weight per day in roughage,³⁶ they would have been consuming 46 U of vitamin A/kg of body weight per day from roughage and concentrate, a value that is greater than the NRC value for maintenance. Hepatic vitamin A concentration in the 4 experimental horses with EMND was within reference range or marginally low and was not significantly different from hepatic vitamin A content in control horses. Plasma vitamin A concentrations remained within or close to the reference range throughout the study and did not change over time. The lower hepatic content of vitamin A in the 4 horses with EMND, compared with values for the 4 control horses, was likely a result of the availability of pasture and the higher vitamin A content in the hay consumed by the controls.

Vitamin E exists in 8 naturally occurring forms, with α -tocopherol being the most important and active form.^{37,38} Green forages are the major source of naturally occurring vitamin E, whereas cereal grains contain minimal amounts.³³ When similar estimates of feed intake (2% dry-matter intake/kg of body weight per day) as were performed earlier for calculation of vitamin A intake are used, the amount of α -tocopherol in the feed of treatment horses was 21 U/kg of diet, a value that is

far less than the NRC minimal recommendations for idle mature horses (ie, 50 U/kg of diet). Adverse effects were not observed in horses consuming a diet containing < 15 U/kg of dry matter, but horses were fed the vitamin E-deficient diet for only 3 months in that study.³⁹ We believe that more prolonged feeding of vitamin E-deficient feeds increases the risk of EMND.

Vitamin E stabilizes biological membranes and has antioxidant effects that protect molecules and tissues from the damaging effects of free radicals (ie, oxidative stress).³⁷ Oxidative stress is believed to play a role in the etiology and progression of many neurodegenerative diseases including the most common form of motor neuron disease in humans, ALS.^{40,41} Although there is no proven direct link between vitamin E deficiency and development of ALS, treatment with vitamin E is known to slow the onset and progression of paralysis in the transgenic mouse model of familial ALS.⁴² Brittanys with an inherited motor neuron disease (spinal muscular atrophy) also have low plasma vitamin E, compared with control dogs.⁴³ Additionally, a group of Walker Hounds and Beagles with induced vitamin E deficiency had ocular, intestinal, and neuronal lipofuscinosis, similar to horses with EMND.⁴⁴

Equine motor neuron disease is not the only neurologic disorder in horses that is believed to be associated with vitamin E deficiency. In young growing equids, vitamin E deficiency and a suspected heritability may cause EDM, a diffuse neurodegenerative disorder of white-matter neurons.⁴⁵ Lipopigment accumulation is a prominent feature of EDM and EMND, suggesting that both diseases may be oxidative disorders.¹⁵ In horses with EDM, lipopigment is detected in ascending and descending white-matter pathways, whereas in horses with EMND, lipopigment accumulation is pronounced in the capillary endothelium in the ventral gray column of the spinal cord, within macrophages at the site of dead neurons, and in RPE.¹⁵ Although EMND and EDM are distinct entities and differ in certain clinical and pathologic features, we have examined 3 yearlings with clinical signs and histologic changes associated with classic EDM that also had histologic lesions associated with EMND, further supporting a relationship between vitamin E deficiency and both EDM and EMND. It is possible that vitamin E has protective effects against oxidative damage in the spinal cord at neuroanatomic sites that vary on the basis of age. Our results support previous recommendations^{33,46} that all horses without access to green forage for prolonged periods should receive dietary supplementation with vitamin E to ensure a daily intake of at least 1 U/kg of body weight per day, irrespective of age.

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