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FACTORS AFFECTING SERUM
VITAMIN E CONCENTRATIONS IN
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FACTORS AFFECTING SERUM VITAMIN E CONCENTRATIONS IN YEARLING AND ADULT MARES

By

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ABSTRACT

FACTORS AFFECTING SERUM VITAMIN E CONCENTRATIONS IN YEARLING AND ADULT MARES

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Stacey Angel Moore-Doumit

Three adult and three yearling mares were fed a controlled amount of a pelleted, moderately low-vitamin E diet (31 μg α-tocopherol [α-T]/g) and oat straw and were given ad libitum access to water for 248 d. Serum was collected initially and at 1- or 2-wk intervals for assay of α-T by HPLC. Serum α-T concentrations varied among horses and samples. Mean d 0 values for adults and yearlings were 5.6 and 4.4 μg/ml, declining by 12 and 53%, respectively, by d 245. From d 175 to d 245, one adult and one yearling were exercised at a fast walk and trot for up to 12 min/d in an attempt to increase vitamin E depletion. No effect of exercise on serum α-T concentration was observed. On d 248, two adults and one yearling were offered an alfalfa-grass hay ad libitum. One adult and two yearlings were turned into an alfalfa-grass pasture. These treatments were continued for 30 d, with serum collected on d 0, 1, 3, 10, 17, 23, and 30. Mean d 0 serum α-T concentrations were 3.4 and 2.8 μg/ml, increasing to d 30 by 8 and 46% for mares on hay and pasture, respectively. Serum α-T concentration appeared to be a relatively insensitive measure of vitamin E status in adult horses. However, the yearlings exhibited levels that varied more directly with dietary vitamin E.

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Introduction

Feed additives and nutritional supplements are marketed in great varieties for horses. They are promoted to cure or aid in the prevention of numerous ailments and conditions. One nutrient whose need by the horse has not been well-researched, but is frequently used as a supplement and is available in many different forms, is the fat-soluble vitamin E.

Vitamin E supplementation of performance horses is a particularly widespread practice. Such supplementation has been recommended to alleviate muscle soreness and tying-up, and vitamin E deficiency has been implicated as a possible cause of equine degenerative myeloencephalopathy. However, the effectiveness of vitamin E supplements has not been clearly established. Serum α-tocopherol assays have been used as a means of assessing vitamin E stores in the horse, although the relationship of serum α-tocopherol concentration to vitamin E levels in liver, muscle, and adipose tissue has not been established with certainty. In fact, serum vitamin E levels in horses that are "normal" have not been well defined. The hypothesis to be tested in this study was that serum α-tocopherol assays are a useful means of assessing

vitamin E status of the horse. Status is defined as the amount of vitamin E available from stores or diet in relation to need.

Though vitamin E is often plentiful in dry forages, such as hay, and fresh forage in pastures, many producers feel a vitamin E supplement should still be added to the diet. The following studies were designed to determine the effects of age and physical activity on vitamin E depletion in serum α-tocopherol levels in horses and to compare serum vitamin E levels after repletion by dry or fresh forages. Increased knowledge in this area of equine nutrition may influence how or whether we add vitamin E to the diet.

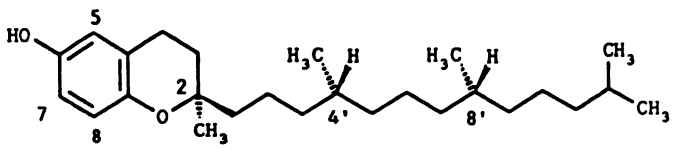
In the first phase of these studies, serum α-tocopherol concentrations were determined throughout a 248-d period of vitamin E intake below the presumed requirement. This was done to evaluate serum α-tocopherol as a measure of the vitamin E status of yearling and adult mares over a prolonged period of depletion. One adult and one yearling were exercised during a portion of the study in an attempt to increase vitamin E requirements and to produce a more rapid decline in vitamin E status as detected by changes in serum α-tocopherol concentration. At 248 d, a 30-d study was initiated to assess the effectiveness of fresh and dry forages in replenishing vitamin E stores and in elevating serum α-tocopherol concentrations. It is hoped that the results of these studies will improve our understanding of the

effectiveness and limitations of serum α-tocopherol concentration as a measure of vitamin E status of horses.

Review of Literature

History Of Vitamin E

Many aspects of fat-soluble vitamin E metabolism have been and continue to be studied in the human and animal sciences. In 1922, a fat-soluble vitamin was found to prevent fetal resorption in the rat (Evans and Bishop, 1922). Named vitamin "E," following the discovery of vitamin D (Evans, 1925), it was found that there are eight naturally occurring forms, including α- , β- , γ- , and δ-tocopherol and α- , β- , γ- , and δ-tocotrienol, with α-tocopherol being the most biologically active. The tocopherols are methyl derivatives of tocol, while the other four forms, identified some 25 yr later, are methyl derivatives of tocotrienol. In Greek, the term "tocopherol" translates "to bring forth offspring." Vitamin E, in the α-tocopherol form, was isolated from wheat germ oil by Evans et al. in 1936. In 1938, Fernholz established the structure of α-tocopherol.



Structure of tocopherol (Machlin, 1991)

The natural stereoisomer is called *RRR*-α-tocopherol, or *d*-α-tocopherol, while the α-tocopherol prepared from synthetic phytol is called all-*rac*-α-tocopherol, or *dl*-α-tocopherol. Tocopherols must be in the free phenolic form to function as

antioxidants, whereas the esters (acetate forms such as α-tocopheryl acetate) are inactive until they are hydrolyzed.

Both α-tocopherol and α-tocopheryl acetate are clear, odorless, viscous oils, which are used as dietary supplements, with α-tocopheryl acetate being the most common. The natural ("*d*" or "*RRR*") and synthetic ("*dl*" or "all-*rac*") forms are both commonly marketed as dietary supplements. Vitamin E occurs naturally in plants, primarily as a mixture of free alcohols in green leaves and seeds. Tocopherols are widely distributed in an unesterified form and in the absence of oxygen are relatively stable. Plants which contain greater amounts of α-tocopherol, compared to tocotrienols, are better suited to provide animals with dietary vitamin E of high biological activity. Tocopherols occur at their highest concentration in cereal grain oils, alfalfa meal, and green, leafy vegetables. Wheat germ oil may contain as much as 2 mg tocopherol/g. Materials containing α-tocopherol should always be protected from oxidation, as oxygen will cause a decrease in vitamin E activity. In addition, oxidation of α-tocopherol is accelerated by exposure to light and the presence of unsaturated fatty acids. Vitamin E inhibits deterioration of fats and oils, which would otherwise cause undesirable odors and flavors (Ullrey, 1981).

Not all forms of vitamin E have the same biopotency. To compare various forms, Table 1 shows various biopotencies with the international unit (IU) equivalent to 1 mg all-*rac*-α-

tocopheryl acetate (*dl*-α-tocopheryl acetate).

Table 1. Various forms of α-tocopherol and their biopotencies

Form of Vitamin E	IU/mg
all-*rac*-α-tocopheryl acetate	1.00
all-*rac*-α-tocopherol	1.10
RRR-α-tocopheryl acetate	1.36
RRR-α-tocopherol	1.49

Machlin, 1991

Functions Of Vitamin E

Vitamin E functions as an antioxidant, inhibiting auto-oxidation of fats in contact with molecular oxygen, and protecting cellular membranes from free radicals (Dam, 1957). Other antioxidants include ascorbic acid, β-carotene, and the enzymes superoxide dismutase, catalase, glutathione peroxidase, and phospholipid glutathione peroxidase, all of which aid in maintaining cell integrity. The primary function of a lipid antioxidant such as vitamin E is to donate hydrogen to a lipid peroxyl radical and break a chain reaction. Free radicals are generated during peroxidation of unsaturated lipids. The primary effect of an antioxidant is to prevent free radicals (R·) from creating hydrogen peroxides (R00H) (see reaction below), which damage cells.

$$R\cdot + O_2 \text{ -------> } ROO\cdot$$

$$RH + ROO\cdot \text{ -------> } ROOH + R\cdot$$

Types of cell damage that can occur include hemolysis of red blood cells, membrane damage to lysosomes which can magnify

cellular damage, and damage to mitochondria and microsomes (Tappel, 1962).

Erythrocyte stability has been found to be influenced by tocopherol content as well (Stowe, 1968). Vitamin E acts with a number of small molecules (e.g., glutathione peroxidase, superoxide dismutase, and catalase) for defense against oxygen radical damage. With this assistance, the body is able to sustain its normal functions and to marshall a defense against disease and environmental toxins.

Selenium as a part of certain enzymes also acts as a lipid antioxidant and interacts metabolically with vitamin E. Studies have led to an understanding that vitamin E and selenium-containing glutathione peroxidase function as part of an antioxidant defense system (NRC, 1989). The importance of selenium as a nutrient was shown by Schwarz and Foltz (1957). These workers demonstrated that hepatic necrosis in vitamin E-deficient chicks and rats could be prevented by supplements of dietary selenium.

Vitamin E Deficiency

Vitamin E deficiency may affect different animal species in different ways. Dam and Granados (1945) found peroxides in adipose tissue of chicks and rats fed low vitamin E diets. These animals exhibited a variety of vitamin E deficiency signs, such as exudation, diffuse hemorrhage, and brown discoloration of adipose tissue. Vitamin E deficiency signs in humans include debilitating neuropathy with severe fat

malabsorption. Mason (1925) found male rats with vitamin E deficiency had degeneration of testes. In swine, it has been shown to cause defective development of the embryo and steatitis. Another condition caused by a deficiency of vitamin E is nutritional muscular dystrophy (Dam et al. 1952), which has been found to occur in many livestock species and small animals. Machlin et al. (1977) determined that vitamin E deficiency caused chronic necrotizing myopathy in rats. Willman and co-workers (1945) found that a lack of vitamin E in lamb diets may cause stiff-lamb disease. Post-mortem examinations revealed muscular lesions, appearing as whitish areas to the naked eye, in most of the skeletal muscles of lambs determined to have stiff-lamb disease. The number of stiff lambs decreased with the substitution of wheat bran for some of the oats and barley in the lamb diets. The disease was almost entirely prevented with the addition of wheat germ meal and was later found to be prevented by feeding an oil solution of mixed tocopherols (Willman et al., 1945). Exudative diathesis (Dam and Glavind, 1938), found to be provoked by cod liver oil and lard, encephalomalacia (Pappenheimer and Goettsch, 1931), erythrocyte hemolysis (Rose and György, 1949), and anemias, are other conditions found to be caused by vitamin E deficiency in chickens.

Platt and Whitwell (1971) observed widespread necrosis of adipose tissue with associated steatitis in eight pony and donkey foals. Although tissue vitamin E levels of the foals

were not determined, they speculated that the condition they observed may have been an equine form of yellow fat disease.

Vitamin E Deficiency In Equines

Wilson and co-workers (1976) found what they thought to be nutritional myodegeneration in foals and concluded that it could be due to vitamin E deficiency. Five case studies involving horses found to have lesions that were indicative of dystrophic myodegeneration were studied by Owen and co-workers (1977). Dystrophic myodegeneration was recognized in foals from birth to 7 mo of age. Initial signs included stiffness in gait and in carriage of the head and neck. The foals then became recumbent and death followed. There have been Scandinavian cases of adult dystrophic myodegeneration determined as well. Of the five cases studied, two included measurements of serum vitamin E concentrations. Serum α-tocopherol values of the two foals were 9.0 and 10.8 μg/ml. These levels were considered normal, though serum selenium was measured and found to be low in comparison to normal horses.

Liu et al. (1983) determined that six Mongolian (*Equus przewalskii*) horses were diseased with degenerative myelopathy and concluded it was due to vitamin E deficiency. Clinical signs included uncoordinated movement of the hindlimbs and an abnormally wide-based gait and stance. Some of the horses had mild ataxia. Plasma α-tocopherol values were determined in five of the six affected horses. Plasma values ranged from <.3 to .8 μg/ml. Seven clinically normal horses from the same

herd ranged from <.3 to 3.0 μg/ml serum α-tocopherol. Equine degenerative myeloencephalopathy has been shown by Mayhew et al. (1987) to be a vitamin E-responsive disorder. They also concluded that low serum vitamin E levels in horses may be caused by feeding processed (i.e., heated, pelleted) grains, no fresh forage in the diet, or poor quality hay.

A possible link was observed between neuroaxonal dystrophy and vitamin E deficiency in Haflinger horses by Baumgärtner et al. (1990). Two Haflinger mares, 1- and 2-yr old full siblings, were revealed to have neuroaxonal dystrophy after histologic examination. These mares had reduced serum vitamin E values of .22 and .23 μg α-tocopherol/ml, respectively, compared to five clinically normal Haflingers which served as control horses with a mean value (±SD) of 1.40±.21 μg α-tocopherol/ml. There were no significant changes in mean serum lipid concentrations of 3.10±.92 mg/ml and 3.39±.06 mg/ml of the dystrophic and control horses, respectively. Mean serum selenium values were 122 ng/ml and 106 ng/ml in the dystrophic mares. The authors concluded that this condition may have a familial hereditary basis, because of the full-siblings that were studied.

More recently, Blythe et al. (1991) conducted a 4-yr study on two generations of a family of Appaloosa horses (n=11) with a historically high rate of clinical ataxia and pathologic signs of equine degenerative myeloencephalopathy. Serum vitamin E concentrations were determined to be

consistently low prior to death in affected horses that died during the study. Serum α-tocopherol values ranged from .4 to 2.6 μg/ml, with the authors using ≤1.5 μg/ml as the margin between deficiency and adequacy, based on a definition by Mayhew et al. (1987). They speculated but did not confirm that the neurologic dysfunction of horses affected with equine degenerative myeloencephalopathy may have been due to a vitamin E deficiency.

Recently, it has been proposed that equine motor neuron disease (EMND) may be linked to low vitamin E levels. This was reported by Divers and co-workers at Cornell University as told to Hovdey (1993). EMND is a condition very similar to amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's Disease. In horses, it causes inexplicable weight loss, shortened gait, lethargy, quivering limbs, and failure of the respiratory muscles. It usually affects older horses, 10 to 15 yr of age. These workers proposed that the primary cause of EMND is a deficiency of antioxidants in the body. They noted that extremely low levels of antioxidants were found in some horses with EMND, although no data were shown. Another finding was that most EMND horses were kept in stables in cities, with no access to pasture or fresh forages.

Metabolism

Vitamin E circulates in the blood and lymph bound to lipoproteins and is most generally distributed according to fat composition of each fraction. Most is transported in the

low density lipoproteins (LDL), and there is a high correlation between total lipid or cholesterol in serum and α-tocopherol level. Red blood cells transport vitamin E, with a vitamin E concentration 20% of that in plasma, with all vitamin E found in the membrane. Red blood cell and platelet vitamin E concentrations usually reflect changes in plasma levels. Tissues vary considerably in their vitamin E levels with no consistent relationship to lipid concentrations. Highest tissue vitamin E concentrations are found in adipose tissue, adrenal, pituitary, testis, and platelets (Machlin, 1991).

For maximum absorption of vitamin E, bile and pancreatic juices are necessary. Apparently absorbed as a lipid-bile micelle with free fatty acids, monoglycerides and other fat-soluble vitamins penetrate the epithelial cells through the plasma membrane of the absorptive cells in the brush border membrane (Machlin, 1991). Maximum absorption takes place in the medial portion of the small intestine, whereas none takes place in the large intestine. Efficiency of absorption of fat-soluble vitamins is enhanced by simultaneous digestion and absorption of certain dietary lipids. Following absorption, initial transport occurs primarily through the lymphatic system where vitamin E is transported as part of a lipoprotein complex (Gallo-Torres, 1980).

Analysis of Vitamin E

Several procedures are used to determine the

concentration of α-tocopherol in plants and animals. The Emmerie-Engel reaction, spectrofluorometry, and chromatography (thin layer, gas, liquid, HPLC) are some examples. Bioassay procedures include fetal resorption assay, muscular degeneration tests, and an erythrocyte hemolysis test (Machlin, 1991). High pressure liquid chromatography (HPLC) is one of the most common methods available and was the method used in this study.

Requirements and Supplementation

Different livestock species require and absorb different amounts of vitamin E. The form of vitamin E, whether it be natural or synthetic, fat- or water-soluble, also determines how much vitamin E is absorbed and needs to be added to the diet. Much research has been done in an attempt to determine vitamin E requirements of domestic animals.

Vitamin E requirements are influenced by body weight, dietary concentrations of selenium, type of diet available, and amount of exercise incurred.

In Equines. The National Research Council (1989) has recommended 50 IU/kg as the vitamin E requirement (concentration in diet DM) for a horse at maintenance. Pregnant and lactating mares, growing horses, and working horses may require 80 IU/kg and maximum tolerance levels were estimated to be 1,000 IU/kg.

Stowe (1968) determined that foals considered deficient in vitamin E required 27 μg of intramuscular or 233 μg of oral

α-tocopherol/kg of BW/d to maintain erythrocyte stability. From these data, 1 mg of α-tocopherol administered intramuscularly appears to be equal to 8.6 mg of orally administered α-tocopherol.

Lindholm and Asheim (1973) found serum α-tocopherol levels in 10 mares varied widely between individuals (2 to 7 μg/ml). There was a tendency for differences between individuals to be maintained over a 13-mo period. Seasonal variation was seen, with elevated values appearing from July to September when fresh forages were grazed, with α-tocopherol concentrations of 4.49 to 5.25 μg/ml. Reduced serum α-tocopherol was observed from March to May when dry forages were fed, with α-tocopherol concentrations of 3.73 to 3.99 μg/ml. Lindholm and Asheim (1973) also studied 22 trotting horses for 7 mo, with 11 being supplemented with 2,500 IU of α-tocopherol/d. All were in training and were regularly raced. Monthly serum samples were taken. The concentration of α-tocopherol in control horses showed consistent levels (mean monthly values of 3.49 to 5.61 μg/ml) but with differences between individual horses. Horses supplemented with vitamin E had greater variation between individuals than unsupplemented horses, ranging from 2 to 11 μg/ml. The average serum α-tocopherol concentration of the supplemented horses was higher (5.52 μg/ml) than that of the control group (4.17 μg/ml). No relationship was found between the concentration of vitamin E in serum and the incidence of

myositis ("tying-up") in race horses.

Maylin et al. (1980) found seasonal variations of vitamin E in plasma concentrations of Standardbred and Thoroughbred horses. These variations may have resulted from variations in vitamin E concentrations in winter pasture versus spring and summer pasture and the effects of processing and storage of hays and grains. Plasma α-tocopherol was measured and ranged from 1.67 to 9.5 μg/ml. Wide variations between horses also was observed. An additional study was performed in which Thoroughbred horses were supplemented orally with 200 IU vitamin E/d (a specific form of vitamin E was not identified). After 11 wk, plasma vitamin E levels ranged from 3.8 to 4.8 μg/ml, with no significant effect of supplementation.

Roneus et al. (1986) fed a low vitamin E diet (119 mg of total tocopherols/d) to 12 adult Standardbred horses, then repleted with vitamin E to determine vitamin E requirements of the horse. A 2½ mo depletion period was conducted in all treatment groups except the control, which remained on the low vitamin E diet. Repletion was at vitamin E levels of 200, 600, 1,800, and 5,400 mg/d for 2 mo, followed by an additional depletion period. Serum total lipid content was measured and remained unchanged during the entire 8-mo period. Of the tissues measured, adipose tissue had the highest concentration of vitamin E followed by the liver and skeletal muscle. Conclusions regarding recommended daily vitamin E supplementation were 600 to 1,800 mg of all-*rac*-α-tocopheryl

acetate, corresponding to a vitamin E diet providing 1.5 to 4.4 mg/kg of BW.

Standardbred and Finnish trotters (n=142) were bled during January and July, and serum α-tocopherol levels were measured (Mäenpää et al., 1987). These authors observed an increase in serum α-tocopherol levels during summer months and a decrease and possible deficiency in winter months. Values also varied among stables, possibly reflecting differences in the quality of feeds and feeding practices. In the spring and summer, fresh hay and grass were available, whereas only stored hay and oats were available in the winter months. Mäenpää et al. (1988) also found seasonal differences in α-tocopherol levels in mares and foals. Concentrations of serum α-tocopherol were lowest from February to May. During this period, concentrations for mares ranged from 1.5 to 1.75 μg/ml and foals 1 to 1.5 μg/ml. Mares and foals had their highest serum α-tocopherol concentrations from June through August (2.5 to 2.75 μg/ml and 1 to 2.25 μg/ml, respectively). Levels were much lower in foals their first 4 mo of life than in mares. In countries such as Finland, horses are fed dried, processed feeds and stabled during the winter months. They are kept on pasture or fed fresh forages in stables during summer months. The authors concluded that hay and stored oats are not sufficient to ensure adequate circulating levels of vitamin E in pregnant mares and weanlings in the winter and that a deficiency may develop within a few months.

Dill et al. (1989) found serum vitamin E concentrations varied between three different age groups of horses (<12 mo old, 12 to 18 mo old, and ≥18 mo old) whether or not they had equine degenerative myeloencephalopathy. These authors concluded that horses affected with degenerative myeloencephalopathy did not have serum vitamin E values significantly different from those of control horses. Mean serum α-tocopherol levels (μg/ml) were as follows: foals <12 mo old - affected, 3.1, control, 2.5; 12 to 18 mo old - affected, 3.8, control, 3.5; ≥18 mo old - affected, 4, control, 3.95.

Craig et al. (1989) monitored serum α-tocopherol levels in 12 horses at 3-hr intervals for 72 hr. Their results showed that a single serum sample assay is an unsatisfactory indicator of vitamin E status in the horse. Results from their study also determined that the majority of within-animal variance for vitamin E, total lipid, and cholesterol, was not attributable to laboratory errors but to variation within each animal. Craig et al. (1989) also determined that mean serum α-tocopherol concentration in younger horses (2.58 μg/ml) was significantly less than in older horses (3.59 μg/ml).

More recently, Saastamoinen and Juusela (1993) measured serum vitamin E concentrations in 40 adult horses fed three levels of supplementation for 1 yr. Blood samples were taken on the first d of every third mo during the experimental period. These horses were orally supplemented with 1, 3, or

5 mg of a water-miscible form of vitamin E/kg of BW/d and were exercised 1 to 2 h/d (a specific form of vitamin E was not identified in the text). Twelve of the horses were harness racing horses, and their exercise consisted of a training program that included trotting on a race track or graveled roads. The remaining 28 horses were riding horses (dressage and jumping) that were exercised in different gaits, primarily in an indoor riding arena. Supplementation was initiated at the beginning of the winter months (September) when feeding was entirely indoors, or the middle of the winter months (January). These horses received a basal diet providing .3 to .5 mg α-tocopherol/kg BW (159 to 254 mg) and included the following: timothy hay, 7 kg/d; oats, 1 to 6.5 kg/d; wheat bran or sugar beet pulp, 200 to 300 g/d. Vitamin E (α-tocopherol) content of basal feeds was timothy hay (n=4) 19.5 to 30 mg/kg DM, oats (n=4) 10 to 20 mg/kg DM, and grass (n=1) 24 to 55 mg/kg DM.

Seasonal variation was observed, with serum vitamin E concentration increasing during the grazing period and decreasing during the indoor feeding period. Mean serum α-tocopherol (±SE) was 1.73±.27 μg/ml in June and increased to 2.21±.59 μg/ml by September. Mean serum α-tocopherol levels decreased during the indoor feeding period (no access to pasture) to a low of 1.89±.19 μg/ml in the control horses. Supplementing with 1 mg/kg BW did not increase serum vitamin E concentration during the indoor feeding period. These

authors concluded that minimum daily vitamin E intakes of >1.5 mg/kg of BW/d are necessary to sustain serum vitamin E levels. If high serum vitamin E levels are advantageous, a daily supplement of 3 to 5 mg/kg BW would be recommended.

In Swine. The vitamin E form *RRR*-α-tocopherol may be more effectively absorbed and retained by weanling swine than all-*rac*-α-tocopheryl acetate (Chung et al., 1992). Their data suggested a biological activity of 2.44 IU/mg of *RRR*-α-tocopherol relative to all-*rac*-α-tocopheryl acetate for weanling swine when comparing serum, liver, lung, heart, and longissimus muscle tissue levels. These data imply that actively growing tissues in young swine incorporate α-tocopherol into cellular components, and the amount retained depends on the quantity of vitamin E ingested and source provided.

In Cattle. Schingoethe et al. (1978) compared long term diets of stored feeds to fresh feeds in dairy cows. Total tocopherol concentrations of feeds were measured several times during the experiment and mean values were as follows: stored alfalfa-brome hay, 14.5 mg/kg DM; alfalfa-brome haylage, 15.6 mg/kg DM; alfalfa-brome pastures, 147 mg/kg DM; and concentrate mixtures, 16.3 mg/kg DM. The authors determined that year-long feeding of stored feeds had no adverse effects on milk production, health, or reproductive efficiency. However, vitamin E in milk was decreased, and consequently the milk was more susceptible to the development of oxidized

flavors.

Roquet et al. (1992) assessed the effect of different chemical forms and routes of administration of vitamin E in cattle by measuring plasma and red blood cell α-tocopherol levels. Supplementation was equivalent to 228 mg of α-tocopherol/d from one of the following forms: *RRR*-α-tocopheryl acetate, all-*rac*-α-tocopheryl acetate, *RRR*-α-tocopheryl polyethylene glycol 1,000 succinate, and a blend of *RRR*-α-tocopheryl acetate and *RRR*-α-tocopheryl polyethylene glycol 1,000 succinate. These forms were compared to each other as well as to a control (no vitamin E supplementation). All of the supplements produced an increase in plasma and red blood cell tocopherol concentration, with the highest increase from the *RRR*-α-tocopheryl acetate and the blend of *RRR*-α-tocopheryl acetate and *RRR*-α-tocopheryl polyethylene glycol 1,000 succinate. In an additional experiment, cattle received a single dose of 810 mg of *RRR*-α-tocopheryl acetate placed in the rumen or the duodenum via cannulas. Plasma α-tocopherol levels were higher in cattle that were ruminally dosed rather than duodenally dosed.

Vitamin E supplements (all-*rac*-α-tocopheryl acetate) of 300 IU/d for 9 mo, 1,140 IU/d for 67 d, or 1,200 IU/d for 38 d, were fed with a high concentrate diet to feedlot cattle (Arnold et al., 1992). While these supplements had no effect on performance or carcass characteristics, they were effective in extending the color stability of beef.

In Sheep. Cattle and sheep were supplemented with a single dose of either all-*rac*-α-tocopherol or all-*rac*-α-tocopheryl acetate, at 50 mg/kg BW or 100 mg/kg BW, respectively. Hidiroglou et al. (1989) found an increase in serum α-tocopherol levels over a 300-h period in sheep and a 500-h period in cattle, as well as a higher biological potency for all-*rac*-α-tocopherol than for all-*rac*-α-tocopheryl acetate.

Njeru et al. (1992) found an increase in serum α-tocopherol levels over a 360-h period in lambs given a single intramuscular injection of all-*rac*-α-tocopherol at 0, 125, 250, 500, or 1,000 IU. Serum α-tocopherol concentrations rapidly increased to a maximum concentration during the first 8 to 12 h, with a 0-h mean serum α-tocopherol concentration of .69 μg/ml and peak values of 6.68, 9.62, 21.66, and 50.75 μg/ml for treatments 2 through 5, respectively. Peak concentrations were followed by rapid declines to pretreatment values.

Ochoa et al. (1992) measured serum and tissue α-tocopherol concentrations in 35 crossbred wethers orally supplemented with 1,000 IU/d for 56 d with one of six different forms of vitamin E or a control (no vitamin E). The six different forms were 1) emulsifiable all-*rac*-α-tocopheryl acetate - dry, 2) non-emulsifiable all-*rac*-α-tocopheryl acetate - dry, 3) emulsifiable all-*rac*-α-tocopheryl acetate - liquid 4) emulsifiable all-*rac*-α-tocopherol - liquid, 5)

micellized all-*rac*-α-tocopheryl acetate - liquid, and 6) micellized all-*rac*-α-tocopherol - liquid. No differences were observed in tissue α-tocopherol concentrations among supplemented groups. Comparing all supplemental treatments, the liver (~6 to 7.5 mg/100 g) and pancreas (~2.5 to 5 mg/100 g) exhibited the highest α-tocopherol concentrations and the kidney and gluteus medius the lowest (~.1 to 1 mg/100 g). All supplemental forms increased serum α-tocopherol concentrations to relatively the same level and all were concluded to be suitable forms of supplemental vitamin E.

Five supplemental forms of vitamin E and combinations of some of the forms were administered to lambs to determine their bioavailability (Hidiroglou et al. 1992). The treatments (forms) included the following: 1) control (no supplemental vitamin E, 2) all-*rac*-α-tocopheryl acetate, 3) *RRR*-α-tocopheryl acetate, 4) *RRR*-α-tocopheryl succinate, 5) *RRR*-α-tocopheryl polyethylene glycol 1,000 succinate, 6) all-*rac*-α-tocopheryl nicotinate, 7) all-*rac*-α-tocopheryl nicotinate and *RRR*-α-tocopheryl polyethylene glycol 1,000 succinate, and 8) *RRR*-α-tocopheryl acetate and *RRR*-α-tocopheryl polyethylene glycol 1,000 succinate. After 60 d of supplementation, treatment 8 produced the highest serum α-tocopherol levels, with the next highest being treatment 3. The range of mean serum α-tocopherol concentrations after 60 d was .39 μg/ml in control animals to 3.52 μg/ml in treatment 8 animals.

In Humans/Rats. Changes in plasma and red blood cell α-tocopherol levels after administration of all-*rac*-α-tocopheryl acetate and *RRR*-α-tocopherol were compared in vitamin E-deficient rats both orally and intravenously by Ogihara and co-workers (1985). After intravenous administration of all-*rac*-α-tocopheryl acetate (4 mg/ml α-tocopherol equivalents at a dose of 10 mg/kg BW) at 6 h, plasma α-tocopherol levels were higher than those elevated by the *RRR*-α-tocopherol supplementation (4 mg/ml). Red blood cell α-tocopherol levels were lower after the infusion of all-*rac*-α-tocopheryl acetate than after the *RRR*-α-tocopherol infusion. After *RRR*-α-tocopherol supplement administration, both plasma and red blood cell α-tocopherol levels were higher than after all-*rac*-α-tocopheryl acetate administration. Baker et al. (1980) compared all-*rac*-α-tocopherol and all-*rac*-α-tocopheryl acetate in humans. Forty-eight humans were orally dosed with 400, 800, or 1,600 mg all-*rac*-α-tocopherol or all-*rac*-α-tocopheryl acetate. α-Tocopherol concentrations were higher in the plasma of all-*rac*-α-tocopherol-supplemented patients than in the plasma of patients supplemented with all-*rac*-α-tocopheryl acetate.

Materials and Methods

Feeding and Handling Methods

Three adult (no. 1-3) and three yearling (no. 4-6) Arabian mares were fed a pelleted diet (Table 2), moderately low in vitamin E (27.6 IU/kg), and oat straw (15.6 IU/kg) for 248 d.

Table 2. Formulated pellet for vitamin E depletion diet

Item	% in Diet
Corn cob byproduct[a]	34.00
Wheat middlings	28.00
Soybean meal (48% CP)	21.50
Alfalfa meal, dehy. (17% CP)	5.00
Cane molasses	5.00
Corn grain	3.70
Soybean oil	1.00
Calcium carbonate	0.60
Sodium chloride	0.50
Vitamin-trace mineral premix[b]	0.60
Mold inhibitor	0.10
TOTAL	100.00

[a]Bracts and pith.
[b]Vitamin-trace mineral premix containing: 11,880 ppm Fe, 100 ppm I, 198 ppm Cu, 748 ppm Mn, 14,960 ppm Zn, 661 ppm riboflavin, 3,965 ppb vitamin B_{12}, 3,524 ppm niacin, 25,374 ppm choline, 2,646 ppm pantothenic acid, 660,793 IU/kg vitamin A, and 132,158 IU/kg vitamin D.

Each horse was fed approximately 1.5% of its BW/d, split evenly between two feedings, with half provided by pellets and half by straw. These amounts were adjusted as needed to maintain each horse in a good body condition. Water was provided ad libitum. Housing through d 248 consisted of

individual box stalls with daily turnout, between feedings, as a group into a dirt lot. Blood serum was collected by jugular venipuncture initially and at 1- or 2-wk intervals throughout the 248-d period. Serum was harvested, flushed with N_2, and samples were frozen and stored at -20°C for later analysis. From d 175 to d 245, one adult (no. 1) and one yearling (no. 4) were exercised daily on a treadmill at a fast walk and trot for up to 12 min in an attempt to increase the rate of vitamin E depletion. On d 248, horses 1, 2, and 6 were turned into a dirt lot and fed a dry forage diet, ad libitum, consisting of alfalfa-grass hay (16.2 IU α-tocopherol/kg). Horses 3, 4, and 5 were turned into an alfalfa-grass pasture. These treatments were maintained for 30 d, with blood serum collected on d 0, 1, 3, 10, 17, 23, and 30.

Analysis of Serum α-Tocopherol

Analytical Equipment. Concentrations of α-tocopherol were determined by high pressure liquid chromatography (HPLC) according to a procedure by Whetter and Ku (1982), a modification of the method used by Bieri and co-workers (1979). For most of the samples, the HPLC equipment (Waters Chromatography Div., Millipore Corporation, Milford, MA) consisted of a model M-45 solvent delivery system, a model U6K injector, and a model 440 absorbance detector with a 280 nm filter. The column was a 4.6 x 150 mm Nova-pak C-18 (Waters Chromatography Div.). A guard column with a Guard-PAK precolumn insert was attached to the primary column. Later in

the project, an HPLC (Waters Chromatography Div.) was used that included automated controls with a refrigerated 712 WISP, automatic injector, a dual 510 pump, the same column as above (both at ambient temperature), and the same type of absorbance detector.

The mobil phase was HPLC-grade methanol:water, 95:5, degassed through a Millipore filtration unit with a .45-μm filter for all analyses. Samples were injected with a 100-μl syringe (Hamilton Co., Reno, NV). For spectrophotometric determinations of α-tocopherol, a Beckman DU 2400 and a Beckman DU 7400 (Fullerton, CA) spectrophotometer were used. All solvents used, which included methanol, ethanol, hexane, and water, were HPLC-grade. Saturated ascorbic acid, 34 g to 100 ml distilled, deionized H_2O, as used by Whetter and Ku (1982), butylated hydroxy toluene (BHT) used at .05% in hexane, and nitrogen (N_2) were also used.

Working Standards. The standards used were *RRR*-α-tocopherol and *RRR*-α-tocopheryl acetate (Eastman Kodak Co., Rochester, NY). The α-tocopherol was in a viscous oil form. To prepare a working *RRR*-α-tocopherol standard, it was warmed to room temperature and dissolved in ethanol to produce a concentration of approximately 2 mg/ml, layered with N_2, and stored at -20°C. Before use, this was diluted 1:19 with ethanol. The absorbance of the solution was measured on a spectrophotometer at 292 nm. This reading was divided by .00758 to determine the exact concentration of α-tocopherol in

the working standards based on an extinction coefficient of α-tocopherol in ethanol of 75.8, as reported by the National Institute of Standards and Technology.

Internal Standard. To prepare a working *RRR*-α-tocopheryl acetate standard, it was also warmed to room temperature and dissolved in ethanol to produce a concentration of approximately 2 mg/ml, then diluted .5:9.5 ethanol, creating a concentration of 100 μg/ml, and layered with N_2, and stored at -20°C.

Analysis of Samples. Initially, 2 ml of ethanol were pipeted into 15 x 150 mm pyrex tubes, .3 ml of saturated ascorbic acid was added and .1 ml of the tocopheryl acetate working solution added as an internal standard. For tubes used as standards, calculated predetermined amounts of the α-tocopherol working standard were added to make three different standards. All other tubes had 1 ml of designated serum samples added. All samples and standards were prepared in duplicate, layered with N_2, and tightly capped. Tubes were vortexed using a "Big Vortexer" model VB-3 (Glas-col Apparatus Co., Terre Haute, IN), which held 20 tubes. It was pulsed six times, at top speed for each set of 20 tubes. Next, 3 ml of BHT-spiked hexane were added to all tubes, they were layered with N_2, capped, and pulse-vortexed for an additional minute. Tubes were then centrifuged at 1435 x *g* at 5°C for 10 min. Using a disposable pasteur pipet, as much as possible of each hexane (top) layer was transferred to 25 ml flasks. An

additional 3 ml of BHT-hexane was added to each tube and the top layer was transferred to flasks again. The tubes were then emptied and washed. The flasks were layered with N_2, capped, and placed in a vacuum oven at room temperature. The oven trap was packed with dry ice and acetone. Samples were evaporated to dryness for approximately 1 h. Flasks were removed and the oil remaining in each flask was layered with N_2. One ml of methanol was added to each flask, the flasks were layered with N_2, and capped. (The newer system required that only .5 ml methanol be added to the 25 ml flasks when they were removed from the vacuum oven.) The extract in each flask was then transferred to a microfilterfuge tube with a .45-μm Nylon-66 membrane filter (Rainin Instrument Co., Woburn, MA) and centrifuged at 1435 x *g* at 5°C for 3 min. Filters were discarded and samples were layered with N_2 and capped.

One hundred μl of a 1:9 dilution of the working *RRR*-α-tocopheryl acetate standard:methanol was injected initially, periodically between samples, and lastly. One hundred μl of each standard and sample were injected as well. Flow rate was 2.5 ml/min, and the detector was set at .02 attenuation. The recorder was set at .25 cm/min. The newer HPLC required that the 1:9 dilution of tocopheryl acetate and methanol be injected at 200 μl and only 50 μl injections of samples were necessary. Flow rate was 2 ml/min, the sensitivity was .05 OD, and chart speed was .4 cm/min.

Calculations. For the majority of the samples, peak heights were measured for both α-tocopherol and α-tocopheryl acetate. The newer system automatically calculated areas-under-the-curve. The average peak height (or area) of tocopheryl acetate (internal standard) was divided by the extracted tocopheryl acetate peak of each standard or sample trace and multiplied by the α-tocopherol peak height. Linear regression from Lotus 1,2,3, version 2.2 was used to determine concentrations of the samples, entering the adjusted peak heights of the standards and their concentrations, calculated from the spectrophotometer reading divided by .00758 and then multiplied by the amount of α-tocopherol standard that was added in μl.

Analysis of α-Tocopherol in Fresh Forages

Analytical Equipment. Concentrations of α-tocopherol were determined by HPLC according to a modified procedure by Burton et al. (1985). For all samples, the HPLC equipment (Waters Chromatography Division) consisted of a model M-45 solvent delivery system, a model U6K injector, and a model 440 absorbance detector with a 280 nm filter. The column was a 4.6 x 150 mm Nova-pak C-18 (Waters Chromatography Div.). A guard column with a Guard-PAK precolumn insert was attached to the primary column.

The mobil phase was HPLC-grade methanol:water, 94:6, degassed through a Millipore filtration unit with a .45-μm filter for all analyses. Samples were injected with a 100-μl

syringe (Hamilton Co.). For spectrophotometric determinations of α-tocopherol, a Beckman DU 7400 spectrophotometer was used. All solvents used, which included methanol, ethanol, hexane, and water, were HPLC-grade. L-ascorbic acid, lauryl sulfate, sodium salt (Sigma Chemical Co., St. Louis, MO), and nitrogen were used. Butylated hydroxy toluene (BHT) was used at 0.05% to spike hexane. The lauryl sulfate was used to make .1 M sodium dodecyl sulfate (SDS), which consisted of 7.21 g of lauryl sulfate brought to 250 ml with distilled H_2O. A tissue homogenizer was used to mix samples during the procedure.

Working and Internal Standards. The standard compounds used were *RRR*-α-tocopherol and *RRR*-α-tocopheryl acetate (Eastman Kodak Co.). The working standards were prepared as noted above in the serum α-tocopherol analysis procedure.

Analysis of Samples. Initially, .5 g of plant tissue were weighed and placed into 35-ml short glass test tubes. Five ml of distilled water were pipeted into the tubes, and .5 ml of the tocopheryl acetate working solution was added as an internal standard to all tubes. For tubes used as standards, predetermined amounts of the α-tocopherol working standard were added to make three different standards. All samples and standards were prepared in duplicate and layered with N_2. Approximately 20 mg of sodium ascorbate powder was added to each tube, and the contents of each tube were homogenized for ~1 min. Between each homogenization, the mixing probe was cleaned with forceps and rinsed with distilled H_2O, ethanol,

and acetone. Next, 2 ml of SDS were added to all tubes followed by 5 ml of ethanol. Six ml of BHT-spiked hexane were added to all tubes. Tubes were layered with N_2, capped, and vortexed for 1 min. and then centrifuged at 1435 x *g* at 5°C for 10 min. Using a disposable pasteur pipet, as much as possible of each hexane (top) layer was transferred to 25 ml flasks. The tubes were then emptied and washed. The flasks were layered with N_2, capped, and placed in a vacuum oven at room temperature. The oven trap was packed with dry ice and acetone. Samples were evaporated to dryness for approximately 1 h. Flasks were removed and the oil remaining in each flask was layered with N_2. One ml of methanol was added to each flask, they were layered with N_2, and capped. The extract in each flask was then transferred to a microfilterfuge tube with a .45 μm Nylon-66 membrane filter (Rainin Instrument Co.) and centrifuged at 1435 x *g* at 5°C for 3 min. Filters were discarded and samples were layered with N_2 and capped.

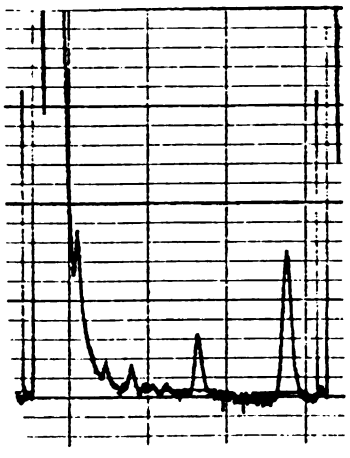
One hundred μl of a 1:9 dilution of the working *d*-α-tocopheryl acetate standard:methanol was injected initially, periodically between samples, and lastly. One hundred μl of each standard and sample were injected as well. Flow rate was 2.5 ml/min, and the detector was set at .05 attenuation. The recorder was set at .25 cm/min.

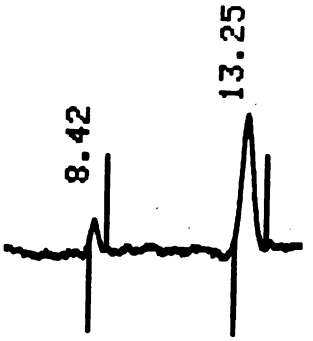
Calculations. Peak heights were measured for both α-tocopherol and α-tocopheryl acetate. The average peak height of tocopheryl acetate (internal standard) was divided by the

extracted tocopheryl acetate peak of each standard or sample trace and multiplied by the α-tocopherol peak height. Linear regression from Lotus 1,2,3, version 2.2 was used to determine concentrations of the samples, entering the adjusted peak heights of the standards and their concentrations, calculated from the spectrophotometer reading divided by .00758 and then multiplied by the amount of α-tocopherol standard that was added in μl.

Printouts from the manual-inject HPLC (left) and the WISP, automatic-inject HPLC (right) are shown below, with α-tocopherol being the smaller peak on the left and *RRR*-α-tocopheryl acetate on the right.

Repeated measures analysis of variance was used to analyze serum α-tocopherol mean values of the mares and yearlings.





Results

The provision of a moderately low vitamin E pellet and oat straw for 248 d was an attempt to deplete serum α-tocopherol concentrations in all six horses. The pelleted feed and oat straw contained 27.6 IU/kg and 15.6 IU/kg of vitamin E, respectively (Hazelton Labs, Madison, WI). However, no substantial average decrease in serum levels was observed. Table 3 shows samples taken from all six horses over a 279-d period. Serum α-tocopherol concentrations varied between individual horses and from sample to sample. In Figure 1, mean adult serum α-tocopherol concentrations as well as their individual concentrations are shown, exhibiting the variation observed over time as well as at each sampling period. Figure 2 demonstrates the same effect with mean and individual yearling levels. However, in this study, from d 0 to d 248 a difference ($P<.05$) between mean adult and yearling serum α-tocopherol levels was evident (Figure 3). There was also a trend toward an age by period interaction. Mean initial values for adults and yearlings were 5.6 and 4.4 μg/ml, declining by 12 and 53%, respectively, by d 245 (Figure 3).

During this period, adult 1 and yearling 4 were exercised (d 175 to d 245), and a slight decrease over time in serum α-tocopherol concentrations was observed. Adult 1 decreased from 4.98 to 4.20 μg/ml and yearling 4 decreased from 2.82 to 1.86 μg/ml (Figure 4).

From d 249 to d 278 (30 d), when the mares were in two groups, one on pasture (Figure 5) and the other fed hay (Figure 6), the mean d 0 serum α-tocopherol concentrations were 3.4 and 2.8 μg/ml, increasing to d 30 by 8 and 46% for mares offered hay and pasture, respectively. In addition, yearlings on pasture showed a marked increase in serum α-tocopherol concentrations compared to the adult mare (Figure 5). Yearlings 4 and 5 increased from 1.63 to 3.23 μg/ml and 2.15 to 4.47 μg/ml, respectively. The serum α-tocopherol concentration of yearling 6 (fed hay) did not change (Figure 6).

Five fresh forage samples were analyzed from a nearby alfalfa field (Table 4) to determine the relative vitamin E intake of the three horses on pasture. Comparing the vitamin E levels in the hay (16.2 IU/kg, Hazelton Labs, Madison, WI) and fresh forage diets (381 IU/kg or 256 μg α-tocopherol/g DM) with the serum α-tocopherol concentrations from each of the two groups indicates that the serum α-tocopherol levels of the yearlings were more reflective of dietary vitamin E supplies than those of the mares.

Table 3. Serum α-tocopherol concentrations (μg/ml) of six horses over a 279-d pd

Horse	Days												
	0	7	14	21	28	35	42	49	56	63	70	77	84
1	5.42	3.84	5.16	3.89	3.49	3.19	4.14	4.16	3.08	3.52	4.60	4.19	3.71
2	6.98	5.39	5.84	5.82	3.37	3.17	5.96	5.64	4.32	4.65	5.65	4.28	4.41
3	4.06	4.06	4.40	3.89	4.48	3.66	4.52	4.43	3.65	4.73	4.57	4.35	5.52
4	2.31	2.33	2.90	2.89	2.02	1.67	2.72	1.94	2.18	2.40	2.57	2.36	2.92
5	6.79	4.12	5.47	4.14	3.63	2.97	4.01	3.65	3.18	3.32	4.21	3.42	3.53
6	4.17	2.97	4.90	3.52	2.29	2.04	2.32	1.78	2.49	3.00	2.72	2.49	4.06
	91	98	119	126	133	140	147	154	161	168	175	182	189
1	3.27	4.76	4.38	4.19	3.43	3.67	4.37	4.60	4.53	2.71	4.31	4.70	4.63
2	4.60	5.50	5.24	5.61	5.62	5.75	6.57	5.85	6.12	4.38	5.67	5.64	5.96
3	3.50	4.30	3.99	4.62	4.12	4.07	4.70	4.89	4.82	3.02	5.13	3.77	4.49
4	2.35	2.51	2.39	2.21	2.49	2.11	2.57	2.57	2.90	1.60	2.67	2.61	2.20
5	3.43	4.71	4.06	3.87	3.99	4.02	4.32	4.52	4.43	2.68	3.57	2.89	3.57
6	2.66	3.65	2.60	3.35	2.86	2.43	3.13	3.46	3.94	1.68	2.87	2.69	2.86
	203	210	224	231	238	245	249	250	252	259	266	273	279
1	4.98	4.09	4.60	4.32	3.98	4.20	3.57	3.15	4.21	3.97	3.13	4.47	3.72
2	7.50	4.03	5.27	5.49	4.89	5.77	4.55	5.20	6.56	4.53	6.15	5.20	5.21
3	6.23	3.38	3.57	4.91	3.47	4.87	4.73	5.24	5.08	5.10	4.75	5.74	4.73
4	2.82	1.56	2.62	2.07	1.56	1.86	1.63	2.24	2.47	1.93	2.83	3.31	3.23
5	4.08	3.05	2.88	2.90	2.76	2.47	2.15	3.35	3.60	3.86	5.09	5.06	4.47
6	3.16	2.22	2.21	2.68	2.12	1.92	2.16	1.89	2.93	2.30	2.64	2.35	2.31

Table 4. α-Tocopherol in five fresh alfalfa samples[a]

			α-Tocopherol		
Sampling Date	% NDF	% DM	Leaf:Stem	μg/g DM	IU/kg DM
6/09/92	47.6	33.3	.57	206	307
6/29/92	39.8	41.9	.88	199	297
7/28/92[b]	40.9	32.7	.98	296	441
8/19/92	39.6	29.3	1.14	354	527
9/11/92	42.4	40.3	.96	223	332

[a]Samples were cut at the 1-2 bud stage and wilted in the field for silage. Samples were collected at time of chopping.
[b]Represents regrowth from 7/11/92, a cutting that was lost due to rain.

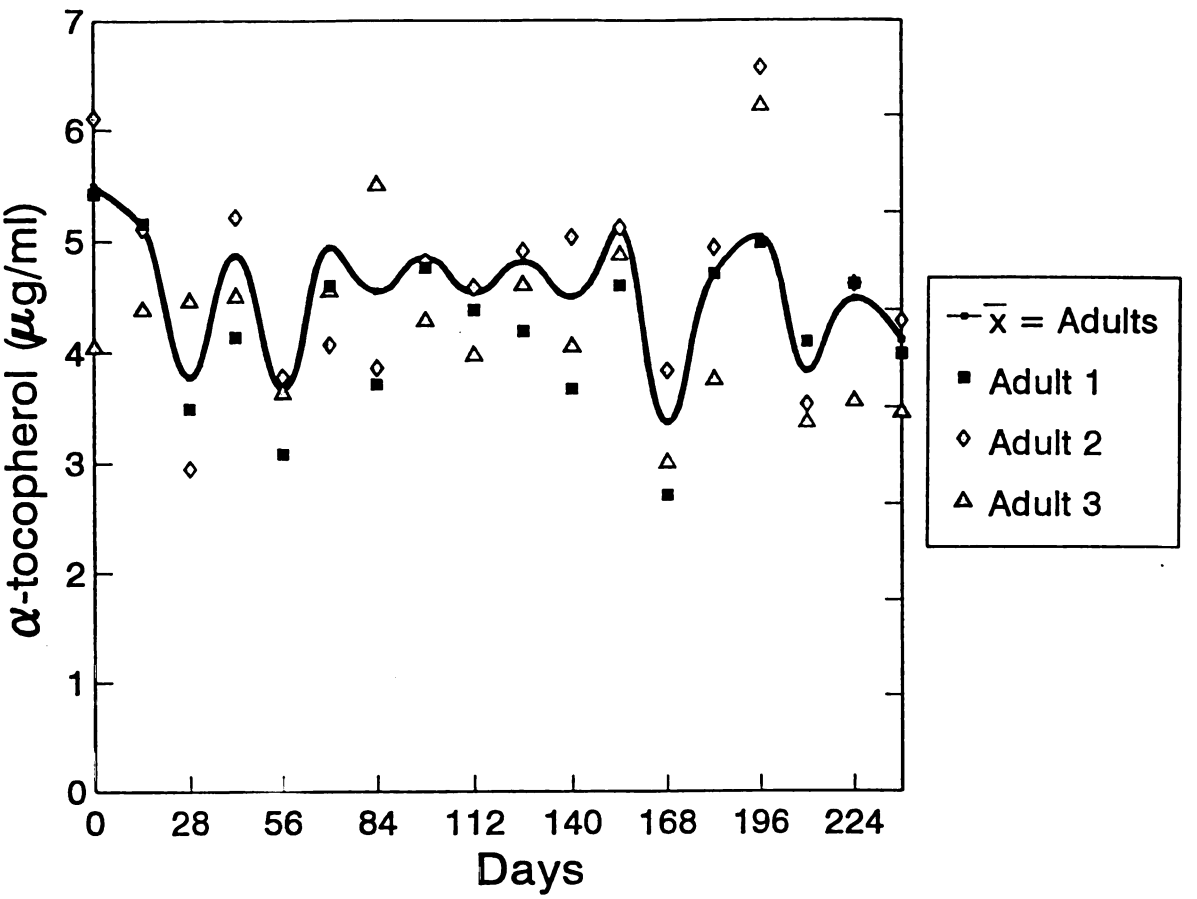


Figure 1. Mean and individual serum α-tocopherol concentrations of adults, d 0 to d 248.

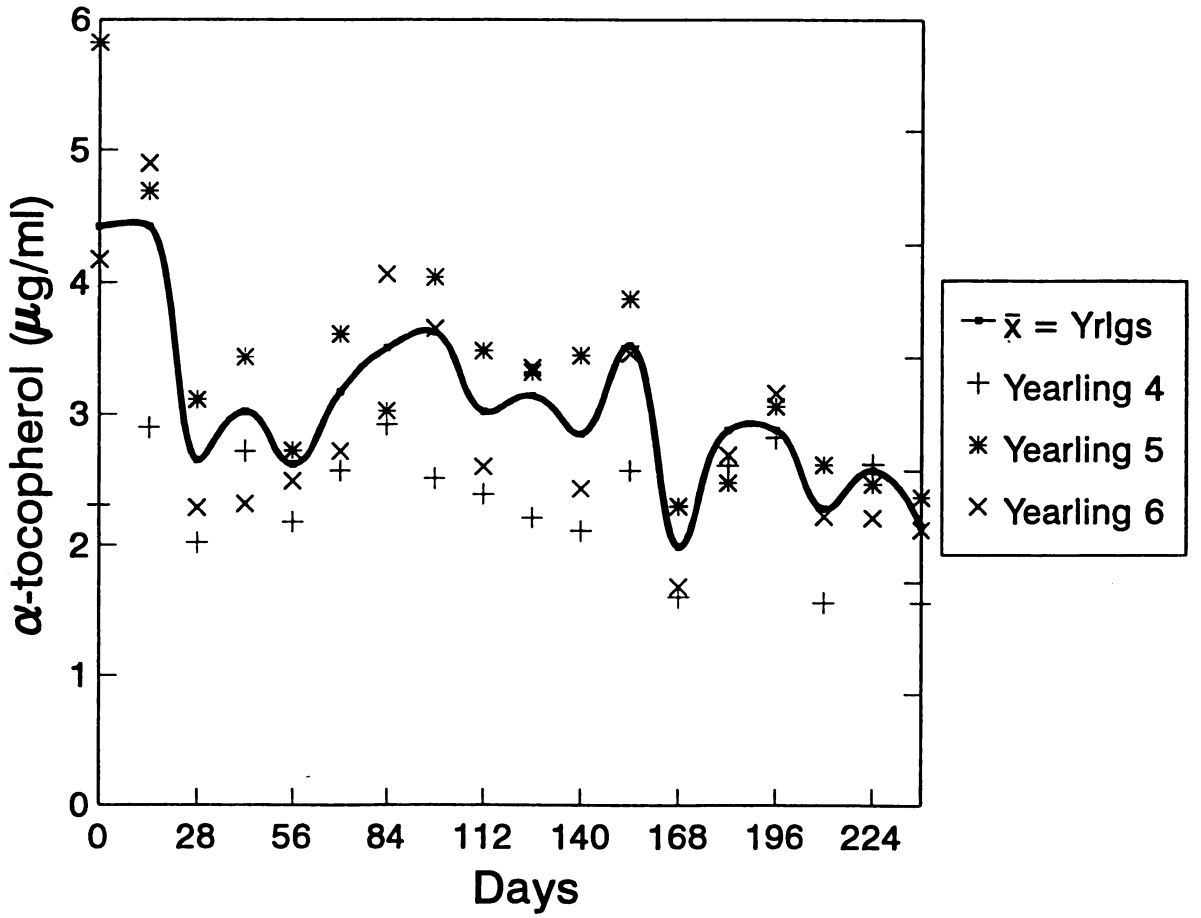


Figure 2. Mean and individual serum α-tocopherol concentrations of yearlings, d 0 to d 248.

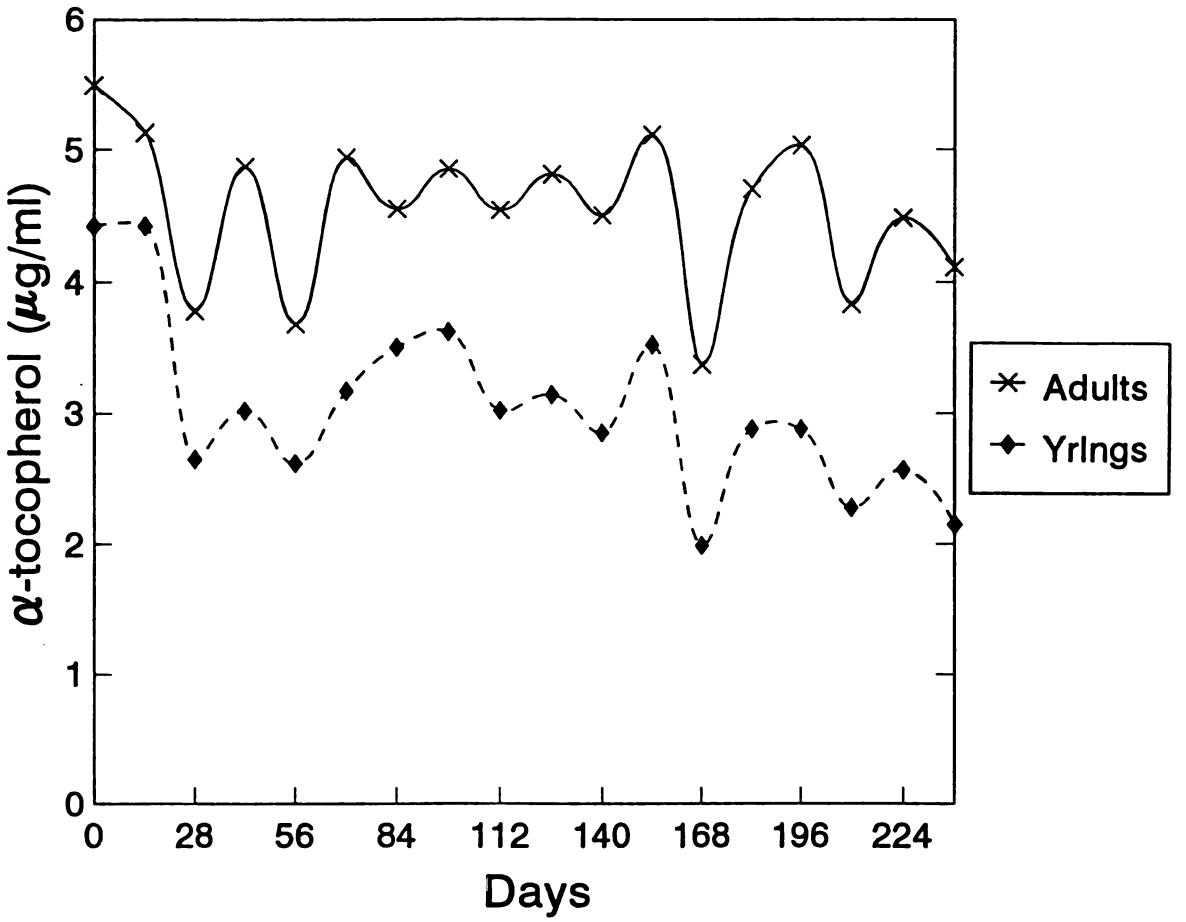


Figure 3. Mean serum α-tocopherol concentrations of adults and yearlings, d 0 to d 248.

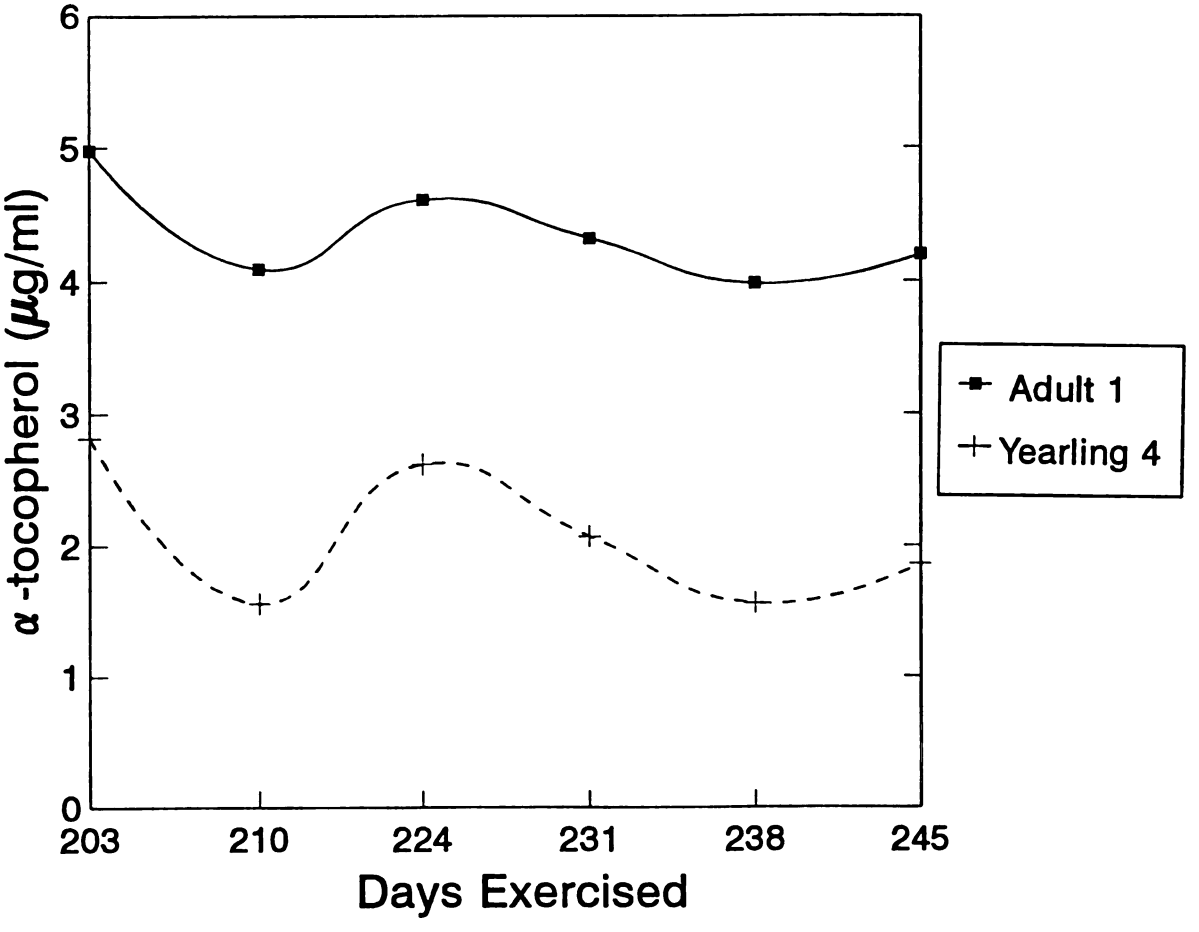


Figure 4. Serum α-tocopherol concentrations of horses 1 and 4 during a 30 d exercise period.

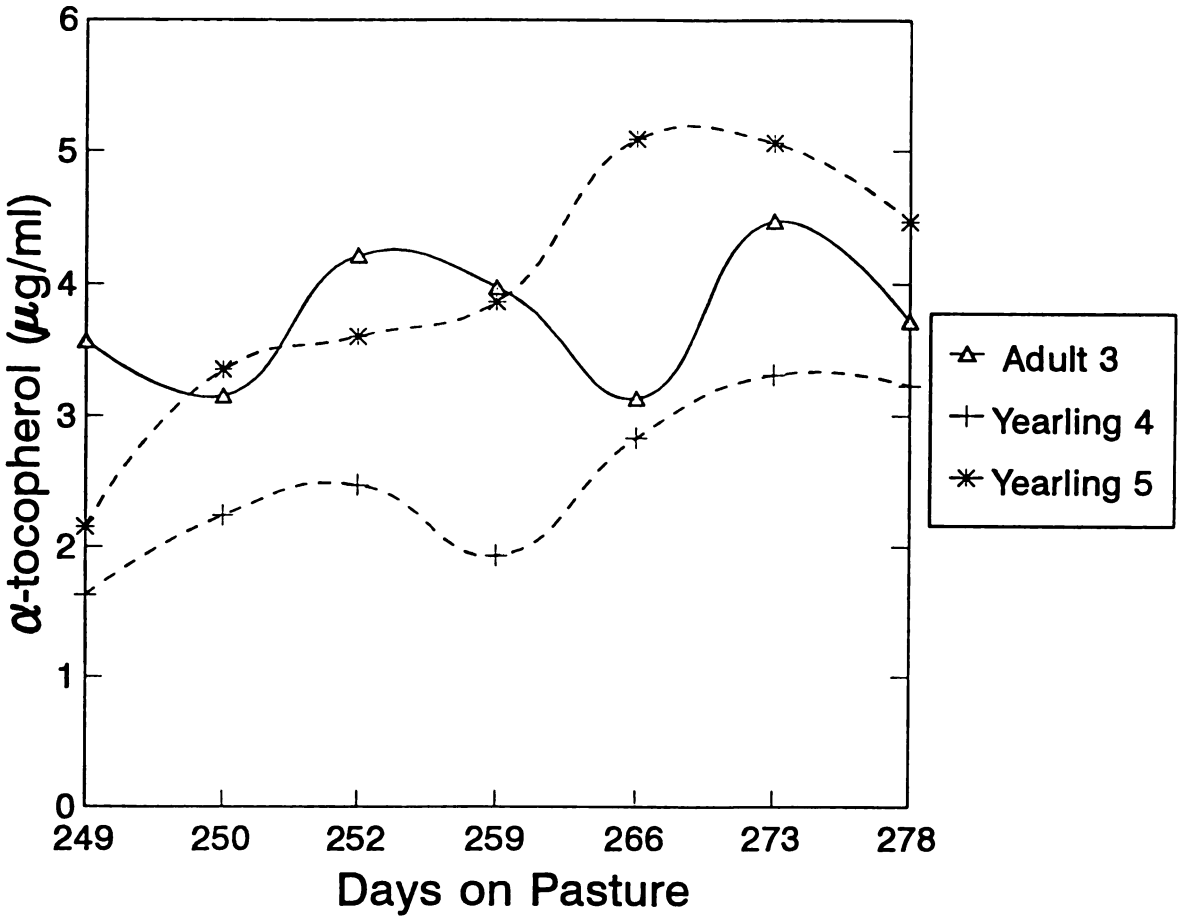


Figure 5. Serum α-tocopherol concentrations of horses 3, 4, and 5 on pasture, d 249 to d 278.

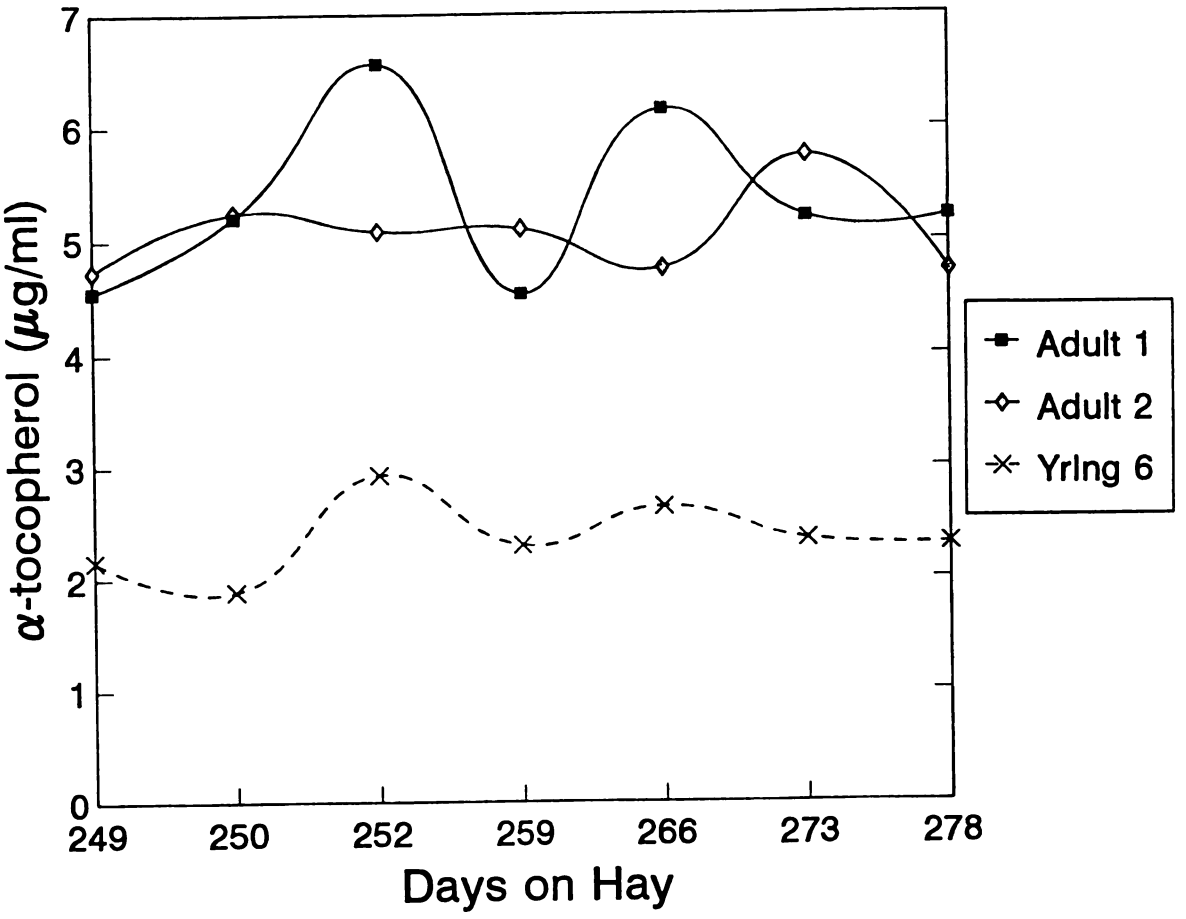


Figure 6. Serum α-tocopherol concentrations of horses 1, 2, and 6 fed hay, d 249 to d 278.

Discussion

Variation in Serum α-Tocopherol

Measuring serum α-tocopherol concentrations in horses has been thought to be a relatively non-invasive, rapid way to determine the vitamin E status of horses. However, many studies, including this one, have shown much variation between animals as well as within animals when measuring serum α-tocopherol. Lindholm and Asheim (1973) made monthly measurements of serum α-tocopherol on 12 unsupplemented mares over a 13-mo period. They stated that concentrations of serum α-tocopherol showed wide variations between individual horses (2 to 7 μg/ml), though individual tendencies were maintained. Ten of these mares were in foal, and the foals that were born had serum α-tocopherol concentrations similar to those of their mothers, with the same tendency toward individual variation. The authors did note that mares showing low concentrations could still have foals with relatively high values and vice versa. Lindholm and Asheim (1973) also investigated 22 trotting horses. Eleven were supplemented daily with 2,500 IU of α-tocopherol and 11 were used as controls. Determination of serum α-tocopherol concentrations over a 7-mo period (October through May) revealed wide variations between horses. The horses being supplemented showed more variation, but did have an average serum concentration higher than that of the control group.

Roneus and co-workers (1986) supplemented horses with

four different amounts of vitamin E and found that great variations occurred in serum α-tocopherol levels, especially in the two groups receiving the highest levels of vitamin E. Levels ranged from .4 to ~1.75 mg/g lipid. The horses in those groups were receiving 1,800 and 5,400 mg *dl*-α-tocopheryl acetate/d. All horses were fed a low vitamin E depletion diet (107.1 mequivalents of *dl*-α-tocopheryl acetate/d) for 45 d and then supplemented for 112 d. Mayhew et al. (1987) found that serum α-tocopherol concentrations reflected a deficient state in horses affected and unaffected with equine degenerative myeloencephalopathy on an unsupplemented vitamin E diet when compared to selected reference groups and to published values. The values ranged from .18 to 2.23 μg/ml, with 142 horses used in the study.

Serum α-tocopherol samples were collected from 142 race horses from 12 different stables throughout Finland and analyzed by Mäenpää et al. (1987). From 47 horses sampled in January and July, mean serum α-tocopherol ranged from 1.94 (January) to 2.64 (July) μg/ml. The range of mean serum α-tocopherol in horses from different stables was .95 to 3.06 μg/ml (n=93) during winter and 1.86 to 3.46 μg/ml (n=78) during summer.

A study was conducted by Craig et al. (1989) to determine the relationship between serum vitamin E and total serum lipids during a 72-h period. Fluctuations in serum α-tocopherol concentration, cholesterol, and total lipids were

monitored in 12 horses (6 yearlings and 6 adults) at 3-h intervals. Serum α-tocopherol concentrations varied widely within horses, as did total serum lipids. The six yearlings had levels ranging from 1.2 to 4.1 μg/ml with a mean of 2.58 μg/ml. The six aged horses ranged from 2.09 to 5.84 μg/ml with a mean of 3.59 μg/ml. The authors determined that the variation observed was a within-animal variation. Variation between horses was attributed to differences in individual metabolism and was not unexpected. Also, there was a significant difference between the mean yearling and mean adult serum α-tocopherol concentrations (2.58 and 3.59 μg/ml, respectively). Similar results were observed in this study. The adult mares appeared to have sufficient vitamin E reserves to maintain normal serum α-tocopherol concentrations. Under these circumstances and considering the other studies discussed, determination of serum α-tocopherol concentration may be an insensitive measure of vitamin E status. In contrast to the adult mares in this study, serum α-tocopherol concentrations in the yearling mares varied more directly with dietary vitamin E supply, possibly because initial vitamin E reserves were limited.

Baumgärtner and co-workers (1990) studied two diseased, young Haflinger horses showing signs of neuroaxonal dystrophy and degenerative myelopathy. Both horses had reduced serum α-tocopherol levels (.22 and .23 μg/ml) and no alteration in serum total lipid concentrations or selenium values. The

authors could not conclude whether there was a link between vitamin E deficiency and the observed disease signs.

An examination of equine degenerative myeloencephalopathy from a clinical, viral, and genetic evaluation of a family of Appaloosas was conducted by Blythe and others (1991a). Two generations of Appaloosas (n=11), descendants of one mare, were studied over a 4-yr period. This family of horses was found to have a high incidence of clinical ataxia and pathologic features of equine degenerative myeloencephalopathy. Five of the horses had blood serum collected one to two times, and the initial samples were used for analysis of serum α-tocopherol. Values ranged from .4 to 3 μg/ml with a mean (±SD) of 1.43±.9 μg/ml. These horses were all considered to be clinically or pathologically affected by equine degenerative myeloencephalopathy. They were compared to a survey of 204 clinically normal, unrelated horses and with values in the literature, with a mean serum α-tocopherol value (±SD) of 2.67±1.38 μg/ml. Clinical signs of disease were observed in 7 of the 11 horses. Some of the signs of neurologic dysfunction included spastic forelimb and hindlimb gaits, occasional stumbling, frequent interference between limbs, and truncal ataxia. Karyotypes of a normal mare and an equine degenerative myeloencephalopathy-affected foal were compared and no differences were observed. The authors conducting this study felt it was evident that a wide range of serum vitamin E values could be found in normal and

neurologically deficient horses, with variations attributable to type of diet, season, and functional use and stated that serum vitamin E concentrations may not be the best indicator of vitamin E status in the horse.

Nine foals sired by a stallion with equine degenerative myeloencephalopathy were serially monitored as well as five age-matched foals raised in the same environment for the first year of life (Blythe et al., 1991b). Eight of the nine foals in the non-control group displayed neurologic deficits consistent with the above-mentioned disease on one or more occasions during the study period. The control foals showed normal gaits throughout the study. From 6 wk to 10 mo of age, the non-control foals had plasma α-tocopherol levels significantly lower than the control foals. Values ranged from 1.5 to 5.5 μg/ml and just above 2 to almost 6 μg/ml, respectively.

Saastamoinen and Juusela (1993) studied 40 horses in one of four groups: control, or an oral supplement of 1, 3, or 5 mg of a water-miscible form of vitamin E/kg of BW/d. It was concluded that the basal feed and the daily supplement of 1 mg vitamin E/kg of BW/d were not adequate to maintain or increase serum vitamin E concentrations during an indoor feeding period in exercising horses. A daily minimum intake of vitamin E in horses would seem to be greater than 1.5 mg of a water-miscible form/kg of BW/d.

Seasonal Serum α-Tocopherol Variation and its Relation to Forage α-Tocopherol

Thafvelin and Oksanen (1966) measured vitamin E in timothy hay (*Phleum pratense*) and determined α-tocopherol levels to be 108 μg/g in flowering stage and 52 μg/g in late development stage, exhibiting a decrease in levels over time. DM was between 90 and 92%.

Vitamin E levels were determined in dried forms of alfalfa by Bunnel et al. (1968). Sun-cured alfalfa (87% DM) and alfalfa hay contained 40.7 and 52.7 μg α-tocopherol/g, respectively. Wilson et al. (1976) found low vitamin E levels in feedstuff samples from an equine breeding stable where myodegeneration was diagnosed in a foal. Levels on a DM basis were as follows: hay, 8.42 μg/g; oats, 6.58 μg/g; and pasture grass, 23.15 μg/g (no form of vitamin E was specified). The authors felt that diagnosing the foal with equine nutritional myodegeneration was valid because of the low vitamin E feed and low serum selenium level in the foal's dam.

Hakkarainen and Pehrson (1987) collected eight samples each of Swedish grass hay and fresh grass in early summer and found fresh grass to contain a mean (±SD) of 128.9±58.5 μg α-tocopherol/g and grass hay to contain a mean (±SD) of 12.2±1.8 μg α-tocopherol/g. This agrees with observations in this study that fresh forages contain higher levels of vitamin E than dried forages.

The greater increase noted in this study in serum α-tocopherol levels on pasture compared to hay conforms with the

observations of Mäenpää et al. (1988) who found a pronounced difference in serum α-tocopherol levels in mares between winter and summer months. Dry forages were fed in winter and serum α-tocopherol levels were half of summer levels, when pasture was the primary forage.

Mutetikka and Mahan (1993) measured α-tocopherol levels in fresh forages during gestation and lactation periods of sows on two different pastures. They found that vitamin E levels declined from initial samples to those collected later in their study. The gestation pasture contained ≥90% orchardgrass and ryegrass, with the balance being alfalfa. All values were expressed on a DM basis. During initial gestation (4-25-91), samples contained 36.72 μg α-tocopherol/g, and at mid-gestation (6-06-91), 22.13 μg α-tocopherol/g. The lactation pasture contained ≥50% alfalfa with the balance being orchardgrass and ryegrass. Forage samples at initial lactation (8-18-91) contained 129.65 μg α-tocopherol/g. At mid-lactation (9-01-91) and weaning (9-14-91), samples contained 105.47 and 85.14 μg α-tocopherol/g, respectively. The pasture containing more alfalfa showed higher levels of α-tocopherol and also decreased over time.

This study agrees with other papers, confirming that vitamin E concentrations in fresh forage are appreciably greater than those in hay. In addition, yearlings on pasture showed a marked increase in serum α-tocopherol concentrations compared to the adult mare on pasture. Yearlings 4 and 5

increased from 1.63 to 3.23 μg/ml and 2.15 to 4.47 μg/ml, respectively. The adult mare on pasture showed much variation, but no significant changes over time, with serum concentrations ranging from 4.73 to 5.74 μg/ml. The serum α-tocopherol concentrations of the yearling and adult mares fed hay did not change, ranging from 3.13 to 4.60, 4.03 to 7.50, and 1.89 to 3.16 μg/ml for yearlings 1 and 2 and the adult mare 6, respectively.

Conclusions

There are many factors involved in determining nutritional needs, and the criteria used in assessing them greatly influence estimates of nutritional requirements. From this study, as well as others like it, it is apparent that horses from birth through approximately 3 yr, or initial maturation, are much more likely than older horses to reflect current vitamin E intakes in serum α-tocopherol concentrations. Aged horses, especially those having grazed pastures for significant periods of time, seem to store enough vitamin E to maintain serum concentrations over a prolonged period when vitamin E is not readily available in the diet. Dried forages, such as hays, have been shown to contain lower α-tocopherol levels than fresh forages, such as pasture. From this study, the yearlings on pasture had increased serum α-tocopherol levels from d0 compared to d30, where the yearling on pasture showed no serum α-tocopherol increase throughout its 30d on hay. The adult and yearling exercised in this study had serum α-tocopherol levels that did not change throughout their exercising period.

If serum α-tocopherol concentrations are to be used as a measure to determine vitamin E requirements, the amounts and periods of time over which vitamin E sources are consumed, as well as the type of exercise horses are performing, may affect serum vitamin E levels.

All nutritional requirements are presumed to differ in

relation to the productive state of horses. Horses at maintenance have minimal requirements, including those for vitamin E. This is understandable, since horses in this state are at rest, grazing, or being ridden or exercised minimally. Young, growing horses, lactating mares, breeding stallions, and horses performing at strenuous levels, presumably require higher levels of nutrients, including vitamin E. When these higher needs are combined with factors such as total confinement with no access to pasture and diets restricted to processed, dried feeds, an environment is created that may limit vitamin E intakes to levels below need, resulting in a decrease in vitamin E reserves in the body. Under these circumstances, incipient vitamin E deficiency may develop, although the quantitative requirements for vitamin E have not been well defined. However, vitamin E is still promoted as a "cure-all" supplement for many ailments. The horse industry should question purveyors of feed additives and supplements with respect to the validity of their claims and should insist upon evidence from controlled studies to support those claims.

Variations in serum α-tocopherol levels between individual horses, as well as from sample to sample in the same individual, suggest that measuring serum α-tocopherol may not be the most effective method of assessing vitamin E status of the horse. Though this is one of the least invasive methods of attempting to determine vitamin E status, it

appears not to be sufficiently diagnostic, and alternative methods should be investigated.

Implications

Prior to the endorsement of vitamin E supplementation, much more controlled research needs to be done. It is likely that some circumstances may require vitamin E in addition to that provided in the natural ingredients of the diet. Based on current evidence, if horses are provided with a combination of fresh and dry forages to complement an otherwise balanced diet, they receive most of the vitamin E they need to maintain a healthful state. Many factors should be considered when determining the vitamin E needs of horses. Type of diet, type of forage in the diet, exercise, quality and amount of pasture available, and the age of a horse may all influence vitamin E status in horses and lead to a potential need for supplemental vitamin E.

Although not possible in this study, more direct comparisons of the effects of age, type of forage, and exercise on concentrations of α-tocopherol in liver, muscle, and adipose tissue would have provided a clearer evaluation of serum α-tocopherol levels as indicators of vitamin E status.

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