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THE ROLE OF VITAMINS IN GROWTH OF HORSES

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Equine nutritionists have long concerned themselves with both the absolute amounts and the balance of minerals in the diets for young, growing horses. The role of vitamins in growth is often ignored. A primary reason for the lack of interest may be that all but vitamins A and E are produced in the body, so it is assumed that even young growing horses are producing everything they need. Vitamins C and D as well as the B vitamin niacin are produced by the horse; the rest of the B vitamins and vitamin K are produced by microbes in the horse's cecum and large intestine (Lewis, 1995). Many of these vitamins have key roles in the regulation of calcium and phosphorus and in proper skeletal development. The following paper will discuss several vitamins with known influences on skeletal growth and indirect relationships with healthy growth. In addition, this paper will evaluate the potential need for vitamin supplementation in growing horse diets.

Vitamins Required in the Diet

The two important vitamins which the horse cannot produce itself are the fat-soluble vitamins, A and E. Because they are fat-soluble, they require at least a small amount of fat in the diet to be absorbed properly. The amount of fat typically found in green grass is usually enough to aid in the absorption of these nutrients, under normal conditions.

VITAMIN A

Vitamin A is best known for its role in vision but also has functions in reproduction, gene expression, differentiation of epithelial cells, embryogenesis and growth. Vitamin A is found in abundant quantities in fresh green forages in the form of carotenes, which are converted to vitamin A by enzymes in the intestinal mucosal cell of the horse. The importance of fresh green forage in maintaining good health in the horse was well understood even before the early days of vitamin A research (Howell et al., 1941). Once forage is cut, there is rapid oxidation of carotenes (up to 85% within the first 24 hours and then about 7% per month during storage) which results in hay being practically devoid of carotenes after extended storage (McDowell, 1989). Horses on hay-only diets demonstrated depletion of vitamin A liver stores over a relatively short period of time (Fonnesbeck and Symons, 1967; Greiwe-Crandell et al., 1995). The inability of hay to supply adequate vitamin A to the horse is the rationale behind the inclusion of vitamin A in commercial horse feed mixes and supplements. Manufacturers cannot know whether a client is going to give a horse access to pasture or not.

Vitamin A has a distinct role in growth of the horse. Both deficiency and toxicity of vitamin A adversely affected growth, body weight, rate of gain and heart girth within young growing ponies (Donoghue et al., 1981). This retardation of growth may have reflected impaired cell proliferation and differentiation. Bone remodeling is modulated by vitamin A in the growing animal. Vitamin A's role in bone remodeling is in the proper functioning of osteoclasts, the bone cells responsible for resorption of bone. Without sufficient vitamin A, excessive deposition of periosteal bone occurs. The appearance of bones in vitamin A deficiency is actually shorter and thicker than normal (Fell and Mellanby, 1950). This is in part caused by the dysfunction of osteoclasts, but also by a reduction in the degradation of glycosaminoglycans and the synthesis of proteoglycans also caused by deficiency (Dingle et al., 1972). In addition, effects of overall bone changes may result in mechanical pressure on certain nerves, such as the optic or auditory nerves, which can result in blindness and/or deafness.

It is possible that some of the systemic effects of vitamin A on growth, as well as the poor growth usually associated with vitamin A deficiency, are related to its effects on growth hormone secretion. Vitamin A takes different functional forms once it is working in the body, one of which is retinoic acid. Retinoic acid has been found to affect growth hormone regulation (Sporn et al., 1994). Retinoic acid can synergize with either thyroid hormone or glucocorticoids to enhance the transcriptional activity of the growth hormone gene, and subsequently of growth hormone secretion from cells (Bedo et al., 1989). Retinoic acid is also essential for embroyonic development. Retinoic acid has been implicated in the expression of hox genes, which determine the sequential development of various parts of a developing fetus and in the morphogenesis of the vertebrate limb (DeLuca, 1991; Thaller and Eichle, 1987).

Intakes estimated to prevent signs of severe vitamin A deficiency (9.7 to 10.6 IU/kg body weight/day) in horses by Stowe (1968) are suggested to be far too low for maximal growth in young horses by Donoghue and coworkers (1981), who recommended 63 IU/kg body weight per day. This is above what the NRC is currently recommending, which is 45 IU/kg body weight per day. For horses grazing sufficient quantities of green pastures, their vitamin A requirement can be met entirely by the carotenes in the forage (Greiwe-Crandell et al., 1997). In northern states and countries, vitamin A supplementation is particularly important because of the short growing season of grasses. Weanling foals supplemented with 40,000 IU of vitamin A per day (~160 IU/kg body weight per day) along with hay and oats had improved serum levels during winter and spring, but supplementation had no effect during the summer when the horses were on pasture (Mäenpää et al., 1988). Toxicity levels for vitamin A are estimated to be around 1000 IU/kg body weight per day (NRC, 1989). Carotenes have not been found to be toxic at any level in the horse because it appears that the enzyme which converts carotenes to vitamin A is found in limited amounts in the body and therefore excessive conversion is not possible.

VITAMIN E

Vitamin E may not be directly linked to growth, but its roles in immune response, nerve and muscle function, and its powerful antioxidant properties make it vital to the health of the young growing horse. Together with selenium, vitamin E acts to maintain normal muscle function, aid in the prevention of muscular disease, and provide antioxidant protection to body tissue, particularly cell membranes, enzymes and other intracellular substances, from oxidation induced damage (McDowell, 1989). A deficiency of vitamin E may cause a variety of different symptoms and pathological changes**,** which may include nutritional muscular dystrophy (weak and poorly oxygenated muscles) and poor immunity to diseases (e.g. recurrent cold and cough) (Moore and Kohn, 1991; Bendich, 1993)**.** Growth rates were found to decrease during periods of illness in Thoroughbred weanling foals (Greiwe-Crandell, unpublished research). Both vitamin E and selenium may help leukocytes and macrophages survive the toxic products that are produced during phagocytosis of invading bacteria (McDowell, 1989). The importance of vitamin E in the diet of the young growing horse deserves more attention in research.

Vitamin E, like vitamin A, is found in abundant quantities in fresh green forage, and horses consuming adequate quantity of green forage have not been found to have vitamin E deficiency. However, vitamin E rapidly disappears during harvesting for hay, with 30 to 85% being lost initially and further loss occurring during storage. The amount of vitamin E in hay is quite variable, depending on the type of forage and the harvesting procedures. Because of the large number of horses that have access only to hay, manufacturers routinely add vitamin E to commercial feed mixes. Vegetable oils are relatively high in vitamin E but are generally not fed in sufficient quantities to make a big impact on the supply of this vitamin in the diet. The increasing popularity of high fat feeds may have an impact on the fortification of vitamin E in feeds since these feeds require extra vitamin E to prevent oxidation of fat in the feed. Horses are not very efficient in storing vitamin E, although body stores may contain sufficient vitamin E to cover four months of inadequate intake in a well-repleted adult. Foals born to mares depleted in vitamin E may have little or no reserves, which would make them more susceptible to infectious diseases (McDowell, 1989). The NRC has estimated adequate intakes of vitamin E for young growing foals at 80 IU/kg of diet. Mäenpää and coworkers (1988) were able to improve serum vitamin E levels in weanling horses through the long, grassless winters in Finland with 400 IU/day of vitamin E. No signs of vitamin E toxicity in the horse have been reported (NRC, 1989). However, extrapolating from other animals, the NRC has set an upper safe limit of 20 IU/kg of body weight per day.

Vitamins Produced in the Body

Vitamins C, D and all of the B vitamins are produced in the body of the horse by either enzymatic conversion or microbial synthesis of substrates normally found in the diet. Since the production of these vitamins is hard to quantitate, there are still questions on precisely what external or internal factors influence production of these vitamins and whether synthesis is adequate to meet the demands of a growing horse.

VITAMIN C

The well-publicized role of vitamin C as a powerful antioxidant overshadows the many other functions it has in the body. In the forms of ascorbate (ascorbic acid) and dehydroascorbic acid, vitamin C acts as a cofactor or co-substrate for eight different enzymes in the body (Ziegler and Filer, 1996). Perhaps the most important role of vitamin C for the growing horse is its function in collagen synthesis and repair. Collagen is the tough, fibrous, intercellular material (protein) that is the principal component of skin and connective tissue, the organic substance of bones and teeth, and ground substance between cells. Three enzymes require ascorbate for proline or lysine hydroxylation in collagen synthesis. Without lysine there is poor formation of cross-links in collagen fibers. Without hydroxyproline (from proline) non-fibrous collagen is formed instead of fibrous collagen, which results in unstable extracellular matrix. Without proper collagen synthesis, proper growth is not possible.

Vitamin C is also important in energy production and hormone and amino acid synthesis. Two enzymes require ascorbate for biosyntheses of carnitine, which is used by mitochondria for transmembrane electron transfer in ATP synthesis. Two more enzymes require ascorbate for corticosteroid hormone biosynthesis. Vitamin C is also needed for production of the amino acid tyrosine.

The antioxidant action of vitamin C is that of an electron donor (or reducing agent) for intra- and extracellular chemical reactions (Ziegler and Filer, 1996). The ability of vitamin C to work both inside and outside the cell is due to its water-soluble nature and because it is distributed throughout the body in the body water. Because oxidants (normally reduced by ascorbate) may affect DNA transcription or could damage DNA, protein or membrane structures, ascorbate may have a central role in cellular oxidant defense. Uncontrolled oxidation in the body could affect growth and development. Also ascorbate can transfer electrons to tocopherol radicals (reduced vitamin E) in lipid particles or membranes. Essentially, vitamin C can help recycle vitamin E.

Vitamin C deficiency is not normally observed in horses because they can synthesize ascorbic acid from glucose in the liver. Animals that cannot synthesize vitamin C (such as humans and primates) are lacking in the enzyme L-gluconolactone (Lewis, 1995). Supplemental vitamin C for the horse has not been found to affect blood levels unless it is given in very large amounts (Stillions et al., 1971). The absorption of vitamin C in horses appears to occur in the ileum

by the process of passive diffusion (Lewis, 1995). Production of vitamin C in the liver can be limited, and in some circumstances, the supply may not be adequate to meet the requirement of the horse. Supplementation of vitamin C may be beneficial during dietary deficiencies of energy (in particular glucose and glucose substrates), protein, vitamin E, selenium, and iron; rapid growth; stressful situations like performance for competition; transportation; a change of environment; disease (bacterial and viral infections); and parasitic infection (McDowell, 1989).

Since supplemental vitamin C is poorly absorbed in the horse, research has been done to find the amounts that will show increases in blood levels and which forms are more effective. Daily supplementation had an effect on plasma levels while single feeding did not (Snow et al., 1987). Daily supplementation of 20 g of vitamin C did make a difference in plasma ascorbate in racehorses in training (Snow and Frigg, 1987b). Feeding more than 4.5 g of ascorbic acid did not increase plasma concentrations any more than 20 g (Snow et al., 1987). Ascorbyl palmitate appears to be better absorbed in the horse than ascorbic acid or ascorbyl stearate (Snow and Frigg, 1987a). No toxic levels of vitamin C intake have been observed in the horse (NRC, 1989).

VITAMIN D

Vitamin D is known as the sunshine vitamin since it is made on the skin from 7-dehydrocholesterol by a reaction catalyzed by ultraviolet (UV) light. Studies conducted near the turn of the 19th century documented severe bone-deforming disease in children with limited exposure to sunlight, compared to healthy children with normal exposure to the sun. This established a link between the sun, the sunshine vitamin (vitamin D) and skeletal health.

The function of vitamin D is maintenance of calcium homeostasis in the blood (McDowell, 1989). Circulating calcium is used for normal mineralization of bone as well as for a host of other body functions. Parathyroid hormone (PTH) and calcitonin function with vitamin D to control blood calcium and phosphorus concentrations. When blood calcium is low, the parathyroid is stimulated to release PTH. PTH travels to the kidney and stimulates 1-hydroxylation of 25-OH vitamin D to form the active vitamin (1,25 OH vitamin D). Active vitamin D then stimulates intestinal calcium uptake, stimulates bone mineral release and stimulates resorption of calcium by the kidney, all in an effort to restore blood calcium levels (Linder, 1991). Calcitonin regulates high serum calcium by depressing gut absorption, halting bone demineralization and slowing reabsorption in the kidney.

Since vitamin D is readily synthesized and absorbed from the skin, is it necessary to supplement vitamin D in the diet? El Shorafa and coworkers (1979) determined young Shetland ponies kept outdoors in Florida do not need vitamin D supplementation. However, ponies deprived of sunlight with no vitamin D supplementation lost their appetite and had difficulty standing. Oral supplementation with vitamin D (approximately 400 IU/kg diet) prevented the signs of vitamin

deficiency (El Shorafa et al., 1979). In modern horse production systems young show horses are often kept out of the sunlight to prevent dulling of the hair coat. For horses not exposed to sunlight or artificial light with an emission spectrum of 280-315 nm, the National Research Council (NRC, 1989) has established a requirement for dietary vitamin D. Growing horses require 800 IU of vitamin D per kilogram of diet dry matter according to the NRC (1989). If an 800 lb yearling eats 2% of its body weight per day in dry feed, this would equate to a vitamin D requirement of 5800 IU/day. Eating a diet consisting of 50% fresh, sun-cured alfalfa hay (1800 IU/kg) would easily satisfy the requirement established by the NRC, 1989. In addition, feeding a grain fortified with vitamin D at a concentration of 1100 IU/kg would supply the yearling with a daily vitamin D intake (hay + grain) of 10,500 IU/day. The actual vitamin D intake would likely be less than calculated since vitamin D is lost at a rate of 7.5% per month with hay storage (Lewis, 1995). The NRC (1989) establishes the maximum tolerance level 2200 IU/kg of diet dry matter, a value of 16,000 IU/day in the above example.

Conservative supplementation of vitamin D in the grain concentrate portion of the diet seems to be warranted due to the important role of vitamin D in bone growth and the variability young show horses may have in the opportunity for exposure to sunlight. In addition, losses in vitamin content of forage with prolonged storage make prediction of dietary vitamin D content difficult. Vitamin D should not be given in an effort to treat developmental orthopedic disease (DOD) by increasing calcium and phosphorus absorption and bone mineralization. DOD has not been shown to be caused by vitamin D deficiency and supplementation with vitamin D will not make up for diets that are not properly fortified with calcium and phosphorus. Oversupplementation of vitamin D to horses is toxic and results in extensive mineralization of cardiovascular and other soft tissues (Harrington and Page, 1983). Care should be taken to remain well below the maximum tolerance level (2200 IU/kg diet) established by the NRC, 1989.

VITAMIN K

Vitamin K was the last fat-soluble vitamin to be discovered (McDowell, 1989). For many years, vitamin K has been known for its blood-clotting function. Vitamin K is essential to activate several blood clotting factors including prothrombin (factor II) as well as factors VII, IX and X. These four blood-clotting proteins are synthesized in the liver in inactive precursor forms and are converted to biologically active proteins by the action of vitamin K (Suttie and Jackson, as cited by McDowell, 1989). The method of this activation is through carboxylation of specific glutamic acid residues associated with the inactive proteins (McDowell, 1989).

Recently, the carboxyglutamyl residues have been found in other proteins associated with a variety of tissues. Most notable is osteocalcin, a protein involved in bone metabolism. Osteocalcin is responsible for binding to

hydroxyapatite and facilitating bone mineralization. Undercarboxylated osteocalcin does not bind hydroxyapatite with the same affinity as carboxylated osteocalcin (Knapen et al., 1989). If vitamin K is in short supply, one would expect to find irregularities in blood clotting along with undercarboxylated osteocalcin. However, it is suspected that osteocalcin is more sensitive to low vitamin K activity than are the blood clotting proteins. Vermeer et al. (1996) concluded the liver is capable of efficiently extracting the required amount of vitamin K from the bloodstream, even at low circulating vitamin K concentrations. This is probably less so for extrahepatic tissues, notably bone. Therefore, it seems possible that bone tissue may be vitamin K deficient, while liver, and thus the blood clotting mechanism, is vitamin K adequate. Knapen et al. (1991) have speculated that osteocalcin is a far more sensitive marker for vitamin K status than are the blood coagulation factors.

If vitamin K has a positive effect on net bone formation, it might be expected that vitamin K antagonists (coumarin) have an opposite effect. Pastoreau et al. (1993) reported that lambs treated with vitamin K antagonists (warfarin) had strongly decreased bone formation indicated by a 30% lower bone mass in three months compared to controls. A deficiency in vitamin K would be expected also to have negative consequences for bone health. In humans, vitamin K intake is reported to decrease with age (Jie et al., 1995), and subjects with increased concentrations of undercarboxylated osteocalcin had a sixfold increased risk of hip fracture (Szulc et al., 1993). Supplementation of vitamin K increases the serum markers for bone formation, including osteocalcin and bone alkaline phosphatase, and may reduce urinary calcium and hydroxyproline excretion, a well-known marker for bone resorption (Knapen et al., 1989).

The NRC (1989) has not established requirements for vitamin K fortification of equine diets. Natural sources of vitamin K are phylloquinone (K-1), found in green leafy plants, and menaquinone (K-2) which is produced by bacteria in the digestive system. Both phylloquinone and menaquinone are converted to the active vitamin (hydroquinone) in the liver (Lewis, 1995). The NRC (1989) states that if the intake or intestinal synthesis of vitamin K are inadequate, horses will have an increased susceptibility to hemorrhage. With new functions of vitamin K being explored, the previous statement may no longer be true. Research being conducted at Colorado State University with exercising horses is studying the concentration of carboxylated and uncarboxylated osteocalcin as it relates to microfractures in bone (Siciliano, personal communication). Preliminary results of this research have encouraged the research group to take a closer look at vitamin K in equine diets. Another area that requires study is vitamin K status of young, growing horses. Foals undergo significant bone growth prior to having a completely functional hindgut and prior to eating a significant amount of green forage, the two principal sources of vitamin K. With current research interest, look for nutrient requirements for vitamin K in horse diets in the near future. At the present time, the NRC (1989) indicates that oral intake of phylloquinone (K1) and menadione (K3) appear to be essentially innocuous in horses (NRC, 1989).

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