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J.D. Pagan



BETA-CAROTENE: AN ESSENTIAL NUTRIENT FOR HORSES?

ED KANE

Stuart Products, Bedford, Texas

Introduction

Beta-carotene (β -carotene) is one of many carotenoids found in nature. In the human nutrition field, evidence suggests that higher blood levels of β -carotene as well as other carotenoids such as alpha-carotene, lycopene, lutein, zeaxanthin, and β -cryptoxanthin may be associated with lower risk of several chronic diseases. β -carotene, α -carotene, and β -cryptoxanthin have pro-vitamin A activity, while the other carotenoids have no pro-vitamin A activity (DRI, 2000).

Earlier publications on nutrient requirements of horses (NRC, 1961) suggested that horses had both a carotene and vitamin A requirement; however the latest edition (NRC, 1989) has published only a vitamin A requirement and not a carotene requirement for all horse classes. Thus, nutritionists typically do not consider the intake of carotenoids when formulating rations for horses.

Do all horse classes only need pre-formed vitamin A and not β -carotene, or is there also a need to supplement some classes of horses with β -carotene as well as vitamin A?

What Are Carotenoids?

Carotenoids occur in almost all plants and animals. Carotenoids are essential to green plants for photosynthesis, acting in light harvesting, and protecting against destructive photooxidation. Without carotenoids photosynthesis in an oxygen-containing atmosphere would be impossible. In addition to those in green plants, carotenoids are also compounds easily recognized as the orange-red colors of foods like oranges, tomatoes, and carrots. Some animals use carotenoids for coloration, especially birds (yellow and red feathers; e.g., flamingos), fish (e.g., goldfish and salmon), and a wide variety of invertebrate animals (shrimp, lobster, and other crustaceans), where binding with protein may modify their colors to blue, green, or purple.

Straub (1987) described 563 different carotenoids, not counting their various cis- and trans-isomers. A few of the main carotenoids and polyenes found in foodstuffs and feeds are α - and β -carotene, zeaxanthin, lutein, β -Apo-8'-carotenoids,

β -cryptoxanthin, astaxanthin, canthaxanthin, citranaxanthin, lycopene, neoxanthin, phytoene and phytofluene, and violaxanthin. The naturally occurring carotenoids are fat-soluble and are completely insoluble in water. They are often associated with lipids, to which they impart their color (e.g., milk fat, egg yolks, and animal fat). In fish and shrimp, however, carotenoids are typically protein-bound.

Of the major carotenoids, β -carotene has the highest pro-vitamin A activity, thus it has received most attention by scientists. More recently, protective effects against serious disorders such as cancer, heart disease, and degenerative eye disease have been recognized for β -carotene and the other carotenoids lutein, lycopene, and zeaxanthin. Roles other than pro-vitamin A activity have stimulated intensive research into various effects of carotenoids in humans and animals. Of the commercially available carotenoids, β -carotene is the least expensive.

β -carotene Absorption, Transport, and Tissue Retention

Like other fat-soluble nutrients, β -carotene must be emulsified prior to metabolism and/or absorption via the action of bile acids. β -carotene can either be converted in the mucosa to retinal by the action of a specific enzyme—15,15'-dioxygenase—and subsequently reduced to retinol or absorbed intact and incorporated in the chylomicrons. The β -carotene is then transported via the lymph into the blood. In either case, β -carotene or vitamin A must then be incorporated into chylomicrons and passed into the lymphatics. There are no data in horses, but in humans 60-75% of the β -carotene is absorbed as vitamin A, while 15% is absorbed intact (Goodman et al., 1966). β -carotene is transported in the blood exclusively via lipoproteins, predominantly by low-density ones (LDL). β -carotene is excreted in the urine and bile.

Yang and Tume (1993) suggested that differences in the selective absorption process in the small intestine are responsible for the various concentrations of carotenoids observed in different species of animals. It is generally thought that carotenoids move into the enterocytes by passive diffusion (Furr and Clark, 1997). If this is the case, species differences in intestinal pH, gut motility, liposome and micelle formation, as well as the variation in the type and amount of dietary fat consumed, may influence carotene absorption. In addition, little is known concerning how carotenoids are incorporated into lipoprotein fractions and enter the circulation (Furr and Clark, 1997). Species variations in lipoprotein handling are also likely to be large contributors to differences in the accumulation of carotenoids (Slifka et al., 1999).

Horses and certain breeds of cattle have the ability to absorb β -carotene intact as well as be converted to vitamin A (Parrish et al., 1947; Van der Noot et al., 1964; Bondi and Sklan, 1984). This conversion appears not to be very efficient in horses, and differences in utilization of carotenes from various forages may occur (NRC, 1989).

The utilization of β -carotene is dependent on the animal species and on the carotene and vitamin A supply status. In ruminants, with a vitamin A supply approximately

covering the requirement, a conversion rate of β -carotene to vitamin A of 6:1 (1.8 μg of β -carotene provides 0.3 μg of vitamin A alcohol = 1 IU of vitamin A) can be assumed. For horses the conversion is similar, 1mg β -carotene provides 333 IU vitamin A activity (Fonnesbeck and Symons, 1967).

Measuring tissue concentration, horse-related species (*Perissodactyla*) had carotenoids ranging from not detectable in the black rhinoceros to 13 $\mu\text{g}/\text{dl}$ in the Grant's zebra (Slifka et al., 1999). The serum concentration of β -carotene in the Grant's zebra was similar to the 14.6 $\mu\text{g}/\text{dl}$ reported in horses by Baker et al. (1986), but considerably lower than the 52.46 $\mu\text{g}/\text{dl}$ value reported for horses by Van der Noot et al. (1964).

Functions and Actions

For the horse, the main recognized function of β -carotene is as a precursor to vitamin A. Through other actions, it may provide benefits other than a source of vitamin A, especially to those horses not consuming adequate β -carotene from green, lush pasture. β -carotene, like vitamin E, can serve as an antioxidant and enhance the immune system. It has been shown to enable immune cells to act more efficiently by increasing lymphocyte response to mitogens, and to assist helper T cells and natural killer cells (Santos et al., 1996; Hughes et al., 1997; Kramer and Burri, 1997). Disease resistance has also been observed in animals with high circulating β -carotene levels (DRI, 2000). Whether the action is due to the β -carotene-moiety or its conversion to vitamin A is still being debated.

Benefit to Reproduction and Lactation

In the horse and other species, β -carotene supplementation has been shown to enhance reproduction (Van der Holst, 1984; Brief and Chew, 1985; Michal et al., 1994; Chew et al., 1994). Fertility, especially of females, can be improved through the consumption of adequate β -carotene. Reduced β -carotene intake occurs mainly when horses do not have access to feedstuffs containing high levels of β -carotene, such as lush, green grass. Confinement feeding and/or feeding β -carotene-low diets has been shown to reduce circulating levels of β -carotene in animals. β -carotene supplementation was found to have a positive effect on fertility in cattle. Its deficiency resulted in higher incidence of silent estrus, decreased conception rates, increased embryonic death, early abortion, and poor-quality colostrum (Simpson and Chichester, 1981).

Pres et al. (1993) found that sows fed supplemental β -carotene had more piglets per litter, and that synthetic β -carotene in addition to vitamin A supplementation increased the number of viable embryos and corpus lutei in sows slaughtered on the 28th day of gestation. Moreover β -carotene subsequently improved the fertility

of sows in the next reproductive cycle. Coffey and Britt (1993) reported that sows injected with various levels of β -carotene had decreased embryonic mortality and higher plasma β -carotene levels for up to 13 days post-injection. By day 18, there were no differences in plasma levels.

Iwanska and Strusinska (1997) suggested that β -carotene specifically, not as a precursor of vitamin A, was an important factor in bovine reproduction. They found that the number of inseminations per cow was reduced and the conception rate was significantly higher in cows supplemented with 300 mg of synthetic β -carotene with or without vitamins A, D₃ and E.

Weng et al. (2000) found that β -carotene was taken up by canine blood and luteal and endometrial tissues in a dose-dependent manner, as had been shown in sows (Chew et al., 1994), and cows (O'Fallon and Chew, 1994). They suggested that β -carotene may be beneficial in optimizing the functional integrity of these tissues and, as an antioxidant, protecting ovarian and endometrial tissues from oxidative stress. As the endometrium undergoes dramatic changes to ensure successful implantation and survival of the conceptus, β -carotene may act to decrease embryonic mortality. They stated that "uptake of β -carotene by the uterine endometrium could protect the highly active uterine environment against oxidative damage, thereby ensuring a more optimal uterine environment for embryo development."

For horses, Van der Holst (1984) reported that β -carotene supplementation produced stronger heats, improved conception rates, and tended to reduce embryonic mortality. Ferraro and Cote (1984) suggested that feeding 100 mg β -carotene/day induced earlier and stronger heats, improved conception rates, and aided in maintenance of pregnancy. Ralston et al. (1985) found that 17-19 mg β -carotene /kg DM in grass hay was adequate for semen production and libido in stallions.

Schweigert and Gottwald (1999) found that β -carotene concentrations in colostrum were positively correlated with corresponding plasma levels in mares during the period 12 weeks around parturition. They suggested the possible reasons for the increase in plasma β -carotene around parturition may be an improved absorption of carotene and/or reduced conversion to vitamin A, as well as mobilization from tissue storages or a reduced uptake in tissues other than the mammary gland. Increased β -carotene in the colostrum would be favorable to enhanced immune status in the newborn foal. Mares readily pass significant amounts of β -carotene to their offspring via the colostrum.

Intake and Supplementation

What is the daily vitamin A intake of horses grazing green pasture? The answer is zero! Unlike carnivores, grazing horses do not consume vitamin A per se. They consume β -carotene, which is converted to vitamin A (retinol) in the intestine. *Nutrient Requirement for Horses* (NRC, 1961) noted a specific requirement for carotene and that those horses on grain and forage diets consume approximately 360 mg natural

β -carotene per day. It stated that a horse at maintenance required 1.5 mg/100 lb body weight; thus a 1200-lb mare would require 18 mg β -carotene daily. A lactating mare required 7 mg β -carotene per 100 lb body weight; thus a 1200-lb lactating mare would have a daily requirement of 84 mg β -carotene.

Horses on fresh green pasture typically consume β -carotene at much higher levels than horses consuming stored roughages. β -carotene is also found in yellow corn at much lower levels. It is rapidly oxidized by light and heat, and therefore cured and storage forages readily lose β -carotene content over time. After two years of storage, β -carotene content of hay declined to less than 10% of its original concentration (Waite and Sastry, 1949). If hay is rained on during curing and drying time is extended, further losses will occur. β -carotene content of fresh growing grass and alfalfa is 400-600 mg/kg DM, whereas content in alfalfa and timothy hay (US 1) is 40 and 20 mg/kg DM, respectively (NRC, 1989).

β -carotene intake also varies seasonally from a high level during early spring and summer, and in some climates during the fall, to a decline during winter that lasts until new plant growth begins. Garton et al. (1964) noted that plasma β -carotene concentration of mares was 7.9 μ g/dl during the winter on hay and 114.1 μ g/dl on early spring pasture, with a subsequent decrease to 15.6 μ g/dl during the following winter. They reported a mean concentration of summer and fall pastures of 204 mg/kg DM. Fannesbeck and Symons (1967) found that Standardbreds consuming hay (bromegrass, canarygrass, fescue, red clover, and alfalfa) supplying 198 mg β -carotene/day did not maintain initial plasma vitamin A concentrations, which declined from 14.6 to 8.5 μ g retinol/dl over a 24-week feeding period. Plasma β -carotene was very low and ranged from 1.9 to 8.5 μ g/dl during the same time period. Ahlswede and Konermann (1980) reported that horses on pasture had plasma carotene concentrations 8 to 13 times higher than horses kept in stables, and that β -carotene supplementation of stabled mares tended to improve ovarian activity. Other researchers (Mäenpää et al., 1988) found seasonal variation in vitamin A status, an indicator of seasonal β -carotene intake. In a survey of pastured horses, Barton (1997) found that serum β -carotene levels ranged from a low of 3.3 μ g/dl to a high value of 293 μ g/dl with an average of 55 μ g/dl. In stabled horses, the range was 1.8 to 16 with an average of 8.4 μ g/dl.

Griewe-Crandell et al. (1997) fed vitamin A- and β -carotene-depleted mares either 72,000 IU vitamin A palmitate or 216 mg β -carotene /d utilizing a 10% water-dispersible beadlet equivalent to 72,000 IU vitamin A. They found that the beadlet form of β -carotene was poorly absorbed and did not improve vitamin A status of depleted mares. β -carotene concentrations in serum were found to be undetectable. Watson et al. (1996) also found poor availability from a water-dispersible beadlet when fed to Thoroughbreds and ponies. They found approximately 40% of the supplemental beta-carotene in the feces. Kienzle et al. (2002) fed approximately 225 mg β -carotene from either grass meal (natural-source) or a water-dispersible beadlet for 4 weeks. Plasma β -carotene levels increased from approximately 2.5 μ g/dl to 30 μ g/dl when oil was added to the diet. When oil was not added, the magnitude of response was not as high (Figure 1).

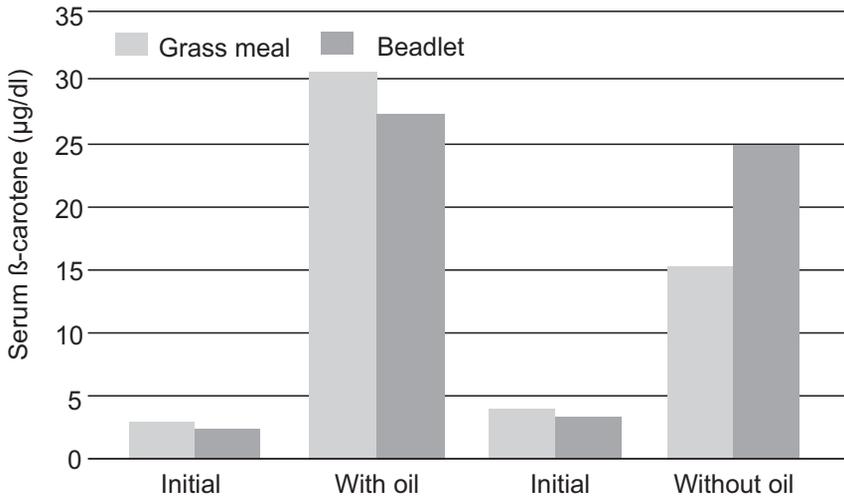


Figure 1. Effects of natural or synthetic β -carotene sources on β -carotene status in ponies (Kienzle et al., 2002).

These studies demonstrate the variation in the bioavailability of β -carotene products supplemented to horses. Some appeared to improve β -carotene status, while others did not. More research is needed to determine what commercial sources are effective in enhancing β -carotene status of horses. A product containing β -carotene does not necessarily mean that the form and source is biologically available to the horse.

Recommended Intakes

It should be kept in mind that β -carotene has pro-vitamin A activity to meet a horse's vitamin A needs; however, if beta-carotene is needed for other metabolic purposes, vitamin A cannot meet those needs. The 1989 NRC's vitamin A requirement ranges from 30 to 60 IU/kg BW. Guilbert et al. (1940) suggested that the minimal requirement of β -carotene for horses could be defined as that level to prevent nyctalopia (night blindness) to be 20-30 μ g/kg BW; that would be equivalent to 6.6-10 IU vitamin A/kg BW. This level supported normal growth and freedom from signs of deficiency. The minimal carotene requirement for significant tissue storage and reproduction was raised to 125 μ g/kg BW, but that level was based on research with other species besides horses.

In general, the supply of β -carotene appears to be adequate when horses are grazing lush pasture. Due to higher cost compared to vitamin A, β -carotene is not typically supplemented in horse diets. From review of the literature, breeding horses and foals in confinement or on poor winter pasture and fed stored forages could benefit from β -carotene supplementation.

From various horse studies, β -carotene has been fed at levels of 100 mg/day (Ferraro and Cote, 1984); 400 mg/day (Van der Holst, 1984); and 500 mg/day (Ahlsvede and Konermann, 1980; Eitzer and Rapp, 1985). While the NRC (1989) suggests that β -carotene supplementation is not beneficial to mares on pasture or fed forages containing high levels, it does suggest that the daily dietary requirement for β -carotene to supply vitamin A activity is 72-144 μ g/kg BW.

Horses grazing lush pasture appear not to require additional β -carotene supplementation, no matter what their life stage or physical condition. However, young foals, yearlings, and breeding animals not on pasture should be supplemented with 1000 mg β -carotene per horse daily. Working performance horses and breeding stallions should be supplemented daily with 500 mg β -carotene (Table 1). Since excess β -carotene supplementation has not caused any reported toxicities in horses, these recommended supplemental levels are well within safe levels (NRC, 1989).

Table 1. Supplemental β -carotene recommendations for horses.

<i>Class</i>	<i>Forage source</i>	<i>Supplemental β-carotene (mg/day)</i>
Foals and yearlings	Stored roughages	1,000
	Lush grass	Not needed
Working horses	Stored roughages	500
	Lush grass	Not needed
Pregnant and Lactating mares	Stored roughages	1,000
	Lush grass	500-1,000
Stallions	Stored roughages	500
	Lush grass	Not needed

Summary

β -carotene is a naturally occurring plant pigment readily obtained by horses grazing lush pasture. A 500-kg horse on pasture consuming 1.5-2% of its body weight as forage would consume approximately 3000 mg natural β -carotene daily. Due to β -carotene losses during processing and storage, a confined horse would consume approximately 300 mg. Though its primary functions are as the major pro-vitamin A source and as an antioxidant, β -carotene has been shown to enhance immunity and be beneficial to reproduction. It is found in abundance in the mare's colostrum and corpus luteum. There is also evidence it is beneficial for stallion fertility. Because all commercial sources of supplemental β -carotene are not equally bioavailable, it is important to supplement a horse with the most biologically absorbed source of β -carotene.

β -carotene is naturally absorbed with the aid of bile acids and micelle formation, so therefore micellized sources of β -carotene allow for more efficient absorption and uptake. β -carotene should be supplemented to breeding animals, especially gestating and lactating mares that foal during early spring after being wintered on stored roughages low in natural β -carotene.

References

- Ahlswede, L., and H. Konermann. 1980. Erfahrungen mit der Oralen und Parenteralen Applikation von Beta-Carotin beim Pferd. *Prakt. Tierarzt* 61:47.
- Baker, H., S.M. Schor, B.D. Murphy, B. DeAngelis, S. Feingold, and D. Frank. 1986. Blood vitamin and choline concentrations in healthy domestic cats, dogs, and horses. *Am. J. Vet. Res.* 47:1468.
- Barton, L. 1997. (Personal communication).
- Bondi, A., and P. Sklan. 1984. Vitamin A and carotene in animal nutrition. *Prog. Food Nutr. Sci.* 8: 165.
- Brief, S., and B.P. Chew. 1985. Effect of vitamin A and β -carotene on reproductive performance in gilts. *J. Anim. Sci.* 60:998.
- Chew, B.P., O. Szenci, T.S. Wong, V.L. Gilliam, P.P. Hoppe, and M.B. Coehlo. 1994. Uptake of β -carotene by plasma, follicular fluid, granulose cells, luteal cells, and endometrium in pigs after administering injectable β -carotene. *J. Anim. Sci.* 72 (Suppl 1):100.
- Chew, B.P., J.S. Park, T.S. Wong, H.W. Kim, B. C. Weng, K.M. Byrne, M.G. Hayek, and G.A. Rinehart. 2000a. Dietary β -carotene stimulates cell-mediated and humoral immune response in dogs. *J. Nutr.* 130:1910.
- Chew, B.P., J.S. Park, B.C. Weng, T.S. Wong, M.G. Hayek, and G.A. Rinehart. 2000b. Dietary β -carotene is taken up by blood plasma and leukocytes in dogs. *J. Nutr.* 130:1788.
- Chew, B.P., J.S. Park, B.C. Weng, T.S. Wong, M.G. Hayek, and G.A. Rinehart. 2000c. Dietary β -carotene absorption by blood plasma and leukocytes in domestic cats. *J. Nutr.* 130:2322.
- Coffey, M.T., and J.M. Britt. 1993. Enhancement of sow reproductive performance by β -carotene or vitamin A. *J. Anim. Sci.* 71:1198.
- DRI. 2000. Dietary reference intakes for vitamin C, vitamin E, selenium and the carotenoids. National Academy Press. Washington, DC.
- Eitzer, P., and H.J. Rapp. 1985. Zur Oralen Anwendung von Synthetischen β -Carotin bei Zuchstuten. *Prakt. Tierarzt* 66:123.
- Ferraro, J., and J.F. Cote. 1984. Broodmare management techniques improve conception rates. *Standardbred* 12:56.
- Fonnesbeck, P.V., and L.D. Symons. 1967. Utilization of carotene of hay by horses. *J. Anim. Sci.* 26:1030.

- Furr, H.C., and R.M. Clark. 1997. Intestinal absorption and tissue distribution of carotenoids. *J. Nutr. Biochem.* 8:364.
- Garton, C.L., G.W. Van der Noot, and P.V. Foncesbeck. 1964. Seasonal variation in carotene and vitamin A concentrations of the blood of broodmares in New Jersey. *J. Anim. Sci.* 23:1233 (Abstract).
- Goodman D.S., R. Blomstrand, B. Werner, H.S. Huang, and T. Shiritori. 1966. The intestinal absorption and metabolism of vitamin A and beta-carotene in man. *J. Clin. Invest* 45:1615.
- Greiwe-Crandell, K.M, D.S. Kronfeld, L.S. Gay, D. Sklan, W. Tiegs, and P.A. Harris. 1997. Vitamin A repletion in Thoroughbred mares with retinyl palmitate or β -carotene. *J. Anim. Sci.* 75:2684.
- Guilbert, H. R., C.E. Howell, and G.H. Hart. 1940. Minimum vitamin A and carotene requirements of mammalian species. *J. Nutr.* 19:91.
- Hughes, D.A., A.J. Wright, P.M. Finglas, A.C. Peerless, A.L. Bailey, S.B. Astley, A.C. Pinder, and S. Southon. 1997. The effect of beta-carotene supplementation on the immune function of blood monocytes from healthy male non-smokers. *J. Lab. Clin. Med.* 129:309.
- Iwanska S., and D. Strusinska. 1997. The effect of beta-carotene and vitamins A, D3 and E on some reproductive parameters in cows. *Acta Vet Hung.* 45:95.
- Kienzle, E., C. Kaden, P. Hoppe, and B. Opitz. 2002. Serum response of ponies to β -carotene fed by grass meal or a synthetic beadlet preparation with and without added dietary fat. *J. Nutr.* 132:1774S.
- Kim, H.W., B.P. Chew, T.S. Wong, J.S. Park, B.C. Weng, K.M. Byrne, M.G. Hayek, and G.A. Reinhart. 2000a. Modulation of humoral and cell-mediated immune response by dietary lutein in cats. *Vet. Immun. Immunopath.* 73:331.
- Kim, H.W., B.P. Chew, T.S. Wong, J.S. Park, B.C. Weng, K.M. Byrne, M.G. Hayek and G.A. Reinhart. 2000b. Dietary lutein stimulates immune response in the canine. *Vet. Immun. Immunopath.* 74:315.
- Kramer, T.R., and B.J. Burri. 1997. Modulated mitogenic proliferative responsiveness of lymphocytes in whole blood cultures after a low-carotene diet and mixed carotenoid supplementation in women. *Am. J. Clin. Nutr.* 65:871.
- Mäenpää, P.H., T. Koskinen, and E. Koskinen. 1988. Serum profiles of vitamins A, E and D in mares and foals during different seasons. *J. Anim. Sci.* 66:1418.
- Michal, J.J., L.R. Heirman, T.W. Wong, B.P.Chew, M. Frigg and L. Volker. 1994. Modulatory effects of dietary beta-carotene on blood and mammary leukocyte function in periparturient dairy cows. *J. Dairy Sci.* 77:1408.
- NRC. 1961. Nutrient Requirements of Horses. National Academy Press. Washington, DC.
- NRC. 1989. Nutrient Requirements of Horses (5th Ed.). National Academy Press. Washington, DC.
- O'Fallon, J.V., and B.P. Chew. 1984. The subcellular distribution of β -carotene in the bovine corpus luteum. *Proc. Soc. Exp. Biol. Med.* 177:406.

- Olson, J.A. 1999. Carotenoids. In: Shils, M.E., J.A. Olson, M. Shike, and A.C. Ross (Eds.) *Modern Nutrition in Health and Disease* (9th Ed.). p. 525. Williams & Wilkens. Baltimore.
- Parrish, D.B., G.H. Wise, and J.S. Hughes. 1947. The state of vitamin A in colostrum and milk. *J. Biol. Chem.* 167:673.
- Pres, J., B. Fuchs, and A. Schleicher. 1993. The effect of carotene and vitamins A and E supplementation on reproduction of sows. *Arch. Vet. Pol.* 33:55.
- Ralston, S.L., S.A. Jackson, V.A. Rich, and E.L. Squires. 1985. Effect of vitamin A supplementation on the seminal characteristics and sexual behavior of stallions. In: *Proc. 9th Equine Nutr. Physiol. Soc. Symp.* p. 74. Michigan State University, East Lansing, Mich.
- Santos, M.S., S.N. Meydani, L. Leka, D. Wu, N. Fotouhi, M. Meydani, C.H. Hennekens, and J.M. Gaziano. 1996. Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. *Am. J. Clin. Nutr.* 64:772.
- Schweigert, F.J., and C. Gottwald. 1999. Effect of parturition on levels of vitamins A and E and of beta-carotene in plasma and milk of mares. *Equine Vet. J.* 31:319.
- Simpson, K.L., and C.O. Chichester. 1981. Metabolism and nutritional significance of carotenoids. *Ann. Rev. Nutr.* 1:351.
- Slifka, K.A., P.E. Bowen, M. Stacewicz-Sapuntsakis, and S.D. Crissey. 1999. A survey of serum and dietary carotenoids in captive wild animals. *J. Nutr.* 129:380.
- Stowe, H.D. 1968. Experimental equine avitaminosis A and E. In: *Proc. 1st Equine Nutr. Res. Soc.* p. 27. University of Kentucky. Lexington.
- Straub, O. 1987. *Key to Carotenoids*. Birkhauser Verlag, Basel, Boston.
- Van der Noot, G.W., P.V. Fonnesbeck, and C.L. Garton. 1964. Seasonal variation in carotene and vitamin A concentration of the blood of brood mares in New Jersey. *J. Anim. Sci.* 23:12.
- Van der Holst, M. 1984. Experiences with oral administration of beta-carotene to pony mares in early spring. p. 6 in *Proc. 35th Annu. Meet. Eur. Assoc. Anim. Prod.*
- Waite, R., and K.N.S. Sastry. 1949. The carotene content of dried grass. *J. Agr. Sci.* 39:174.
- Weng, B.C., B.P. Chew, T.S. Wong, J.S. Park, H.W. Kim, and A.J. Lepine. 2000. β -carotene uptake and changes in ovarian steroids and uterine proteins during the estrous cycle in the canine. *J. Anim. Sci.* 78:1284.