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Review Article

Dietary Supplements of Vitamins E, C, and β-Carotene to Reduce Oxidative Stress in Horses: An Overview

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ABSTRACT

Oxidative stress is the excess generation of free radicals and/or a decrease in the response of the antioxidant system. It is known to cause damage to the equine health by unbalancing the stable molecules. The dietary supplementation of vitamins E, C, and β-carotene cause beneficial effect on horses' health. These supplements could transform free radicals into the stable radicals, thereby showing importance in the prevention of diseases associated with oxidative stress. Adding vitamins E, C, and β-carotene to the horses' diets in stressful conditions could decrease the production of free radicals that cause inflammation and tissue damage, the typical characteristics that have been associated with oxidative stress. This review spotlights the available evidence of the benefits of dietary supplements of vitamins E, C, and β-carotene towards the reduction of oxidative stress in horses.

1. Introduction

Reactive oxygen species (ROS) are free radicals which cause death to the cells by reacting with biomolecules [1]. These ROS show harmful effect on biological systems due to the antioxidant deficiency [1]. The imbalance caused by the oxidative stress can generate serious diseases that worsen the tissue injuries in horses and other mammals. However, this damage can also be attributed to a deficiency of protective substances, such as antioxidants, which have a close relationship with the nutritional contribution and its nutritional demands of the animals [2].

Antioxidants (that are not enzymatic in nature) bind to free radicals and transform them into the less aggressive radicals [3]. After donating an electron, vitamin C is transformed into the ascorbic radical, which is relatively stable and shows low reactivity. When it loses a second electron, a more stable reduction is obtained [4]. Vitamin E (an antioxidant) also protects from the attack of free radicals, thereby giving protection to tissue lipids. β-carotene also shows beneficial impact by suppressing superoxide O_2 and acting in antioxidant defense [5].

The possibility of preventing imbalances by adding dietary supplements of vitamins E, C, and β-carotene in diets of the horses under stress conditions can prevent the production of free radicals that promote the typical damage associated with the oxidative stress [5]. Thus, the present review discusses how antioxidant supplements of vitamins E, C, and β-carotene in horses' diets could benefit them against oxidative stress.

2. Reactive Oxygen Species

The ROS are formed when a free electron is added to an O_2 molecule primarily in the mitochondria that is responsible for cellular respiration. Different biochemical reactions generate a wide variety of molecules like oxygen ions, free radicals, superoxide anion, nitric oxygen, and hydrogen peroxides [6]. All of them are highly reactive small molecules with unpaired electrons derived from cellular metabolism and other exogenous sources. ROS cause harmful damage to the organisms by unbalancing the sta-

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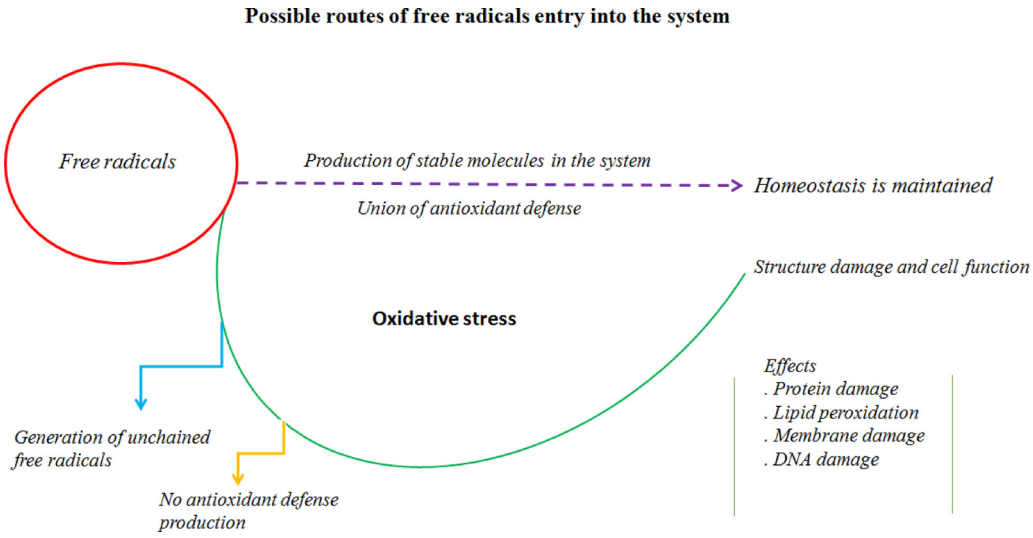

Fig. 1. Comparison of free radicals entry into a system with and without antioxidant defense.

ble molecules, causing oxidative stress due to the low amounts of antioxidants and high amount of ROS [2].

Free radicals can be generated in different forms such as during metabolic reactions, when cells transform food into ATP, during intense exercise, by the exposure to the external agents such as pollution and ionizing radiation, and by the addition of an electron from a stable molecule [1]. Free radicals are a group of small number of molecules. Compared to non-radicals, they interact with the stable molecules by oxidation-reduction reactions to reach stability through the donation of free electrons. However, after electron's donation, the stable molecules are transformed into a free radical [5]. Although free radicals can cause negative effects on organisms, they have important physiological functions, including their participation in phagocytosis as well as collagen synthesis, in the activation of enzymes in the cell membrane, and in the synthesis of catecholamines [2].

3. Oxidative Stress

Oxidative stress is triggered by ROS (Fig. 1). It is also defined as the imbalance caused by the excess production of ROS and/or by decrease in the antioxidants. When the amount of ROS in cells exceeds the number of stable molecules, there is an increasing oxidative activity inside the cell. Under oxidative stress, free radicals are formed, affecting macromolecules and damaging their structure and function, including the proteins and lipids of DNA and RNA [7].

4. Pathological Changes Associated With Oxidative Stress

The presence of ROS and oxidative stress is caused by the damages that can be chronic, degenerative, or age-derived [6]. Aging is a cumulative process where cell death has increased, generating biochemical and physiological changes. Chronic changes that can produce free radicals have been found to suppress the generation of antioxidant defense, reduce the proteolytic activity, and promote the accumulation of oxidized proteins [7].

Cancer is a process in which abnormal or harmful cells multiply uncontrollably due to the induction of mutations in the DNA of a somatic cell, with a tumor stimulation of the mutated cells and subsequently generating the possibility of malignant tumor development [8]. When free radicals cause damage to endothelial cells, it releases protease, and thus causes the rapid degradation of the membrane. Angiogenesis is one of the processes of tumor cell development and diffusion [9].

Sclerosis is considered a multifactorial pathology which has the participation of many genetic, environmental, and immunological factors. This pathology begins with the formation of the arteriosclerotic plaque and the uptake of low-density lipoproteins. The released products from the breakdown of oxidized low-density lipoproteins have an atherogenic potential before being taken up by the macrophages [9].

There are other factors that can cause the lead of sclerosis. Hypertension, hypercholesterolemia, and smoking induce an imbalance between pro-oxidation and anti-oxidation [10]. Sclerosis is one of the most significant diseases in horses because the thoracic limbs and joints support more than 70% of their body weight [9].

5. Cellular Antioxidant Defense Systems

There is a physiological mechanism of antioxidant defenses to avoid excess oxidation at the cellular level to control the damage caused by ROS. The antioxidant mechanism can inhibit the generation of free radicals or cancel their reactivity by different antioxidant systems that act against the harmful effect of radicals through the action of enzyme molecules and chemical sequesters. The primarily defenses against oxidative damage are enzymatic antioxidants such as superoxide dismutase which remove O_2 and hydrogen peroxide. However, non-enzymatic antioxidants also act against free radicals (Fig. 2). The defense action of these antioxidants is to sacrifice their molecular integrity to avoid molecular alterations [10].

Different defense systems work together for the effective protection of ROS. Preventive antioxidants have a fundamental role in eliminating indicators that are of oxidative stress. In this preventive mechanism, various proteins act because it has the ability to form bonds with metals and sequesters before generating damage. Although other metals that have a central iron core, including transferrin, ferritin, and myoglobin, the antioxidants prevent the formation of harmful ROS [6].

Dietary components are the main sources of antioxidants and microelements for the synthesis of antioxidant enzymes. Antioxidants perform important functions such as protecting cells from damage by oxidative stress [2]. All these aforementioned antioxidant systems must work together to achieve homeostatic balance. Alternative oxidases use some metals (Cu, Zn, Se, Mn, and Fe) and acids (folic, α-tocopherol, and β-carotene) as cofactors or components to sequester ROS [11].

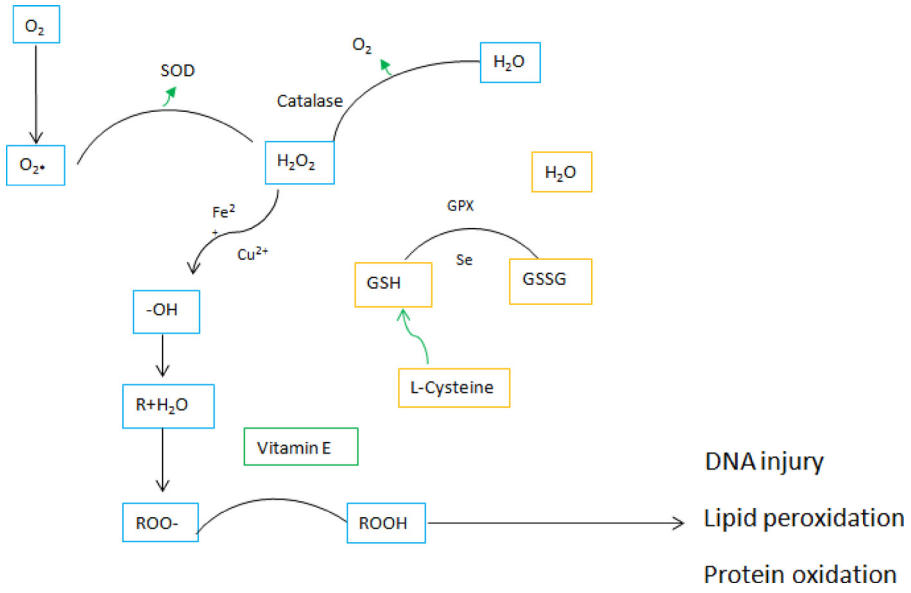

Fig. 2. Outline of the mechanisms of action of enzymatic and non-enzymatic antioxidants. SOD: Superoxide dismutase; GSH: Reduced glutathione; GSSG: Oxidized glutathione. O_2: Oxygen; $\cdot OH$: Hydroxyl radical; O_2^*: Superoxide radical; H_2O_2: Hydrogen peroxide; H_2O: Water.

Table 1
Non-enzymatic antioxidants, mechanism of action, and their physiological functions.

Antioxidant	Origin/Place of the Action	Action	Physiological function	References
Vitamin E	Exogenous/intracellular and extracellular	Neutralizes singlet oxygen. Captures hydroxyl free radicals. O_2 capture. Neutralizes peroxides.	To maintain antioxidants, present in the cell membrane.	[12]
Vitamin C	Exogenous/intracellular and extracellular	Neutralizes singlet oxygen. Captures hydroxyl free radicals. O_2 capture. Regenerates the oxidized form of vitamin E.	Free radical scavenging effect and recycle vitamin E.	[13]
Carotenoids	Exogenous	Neutralizes singlet oxygen.	Lipid antioxidant.	[14]

6. Non-Enzymatic Antioxidants (Vitamin C, E, and β-Carotene)

Non-enzymatic antioxidants are a group of hydrophobic molecules that capture free radicals. These are primarily found in the cytosol, mitochondrial matrix, nuclear, and extracellular fluids [5]. Non-enzymatic antioxidants generally consist of vitamins and molecules (Table 1). Vitamin C and E are part of the main non-enzymatic antioxidants.

Vitamin C or ascorbic acid is an organic compound called lactone; a sugar-acid derived from gluonic acid synthesized from the glucose molecule. It is the most abundant water-soluble antioxidant in the blood. It is present in various tissues and organs like spleen, lungs, testicles, brain, lymph nodes, small intestine, pancreas, kidney, leukocytes, and salivary glands [15].

The two important biological forms of the vitamin C are the reduced (ascorbic acid) and the oxidized (dehydroascorbic acid) form. The reduced form is transported into the cells by glucose transporters and some specific ones such as sodium dependent vitamin C transporters. It is oxidized to dehydroascorbic acid and then reduced back to ascorbic acid by the action of glutathione. Vitamin C (as an antioxidant) has the function of neutralizing the free radicals and helps in the synthesis of carnitine and collagen type I and II. Most mammals synthesize vitamin C endogenously in the liver from glucose and galactose (Table 1). The reduction of the acid is important for the stability of mitochondria that needs antioxidant mechanisms to act against oxidative phosphorylation because oxidative damage is greater in mitochondrial DNA than that of nuclear. Vitamin C has a protective effect on oxidative stress [16]. Vitamin E as an antioxidant has a great ability to trap free radicals. Vitamin E shows a greater reactivity too. Vitamin E prevents the tissue oxidation by protecting tissue lipids damage because of the rapid reactions of the peroxyl radicals on radial peroxyls [17].

The effects of vitamin E on the immune system are important due to the protection it generates. Vitamin E contains certain susceptibility to oxidative damage led by ROS such as hydroxyl radicals, alkoxyl, peroxyl, singlet oxygen, and perhaps several metals bound to oxygen in microsomes [17]. Vitamin E deficiency shows decreased cell-mediated immunity and interleukin production, destabilization of the cell membranes of the immune system, decreased delayed hypersensitivity, and decreased immunoglobulin. Like vitamin C, vitamin E has also been studied as an antioxidant. However, vitamin E is the most active form of α-tocopherol succinate that induces directly or indirectly in apoptosis in tumor cells [17].

β-carotene is one of the most common carotenoids in food which is composed of double-linked systems (polygenic chain) [18]. Plasma is the transport of carotenoids, which is why the possibility of using the antioxidants of α- or β- carotene as biomarkers is considered as the purpose of knowing the intake of vegetables or fruits. β carotene has the greatest correlation with diet which makes it of great importance in nutrition in horses and their health. It has not yet been possible to clearly specify the mechanism that carotene uses to generate an effect in the diet [18].

Table 2
Dosage of dietary supplements and their effect on horses.

Type of antioxidant	Dose	Effect	References
Vitamin C	20 g/d	Increased antibody response to vaccines in older horses, especially those with pituitary dysfunction or Cushing's syndrome	[24]
	30 mg/kg of body weight/d	Horses with recurrent airway obstruction	[24]
	10–30 mg/kg of body weight/d	Promote recovery and airway obstructions.	[25]
	25 mg or a measure for horses in general	Periods of stress, hot weather conditions or an additional antioxidant requirement.	[26]
	Adult horses 25 g/d	Present in immune processes, development and healing. For growing and training horses.	[7]
Vitamin E	50 IU vitamin E/kg dry food materials	Suitable for most stages of the life cycle and moderate activity.	[27]
	10 IU/kg body weight per day	Prevents muscle weakness	[17]
	5,000 IU/d of soluble vitamin E and then decreasing the regimen to transition to 5,000 IU/d of oral acetate	Restore vitamin E levels (prolonged increase in CSF concentrations 8 wk after starting supplementation.)	[24]
	1,500 IU of vitamin E/d	In mares, the incidence of EDM in foals decreased from 40% to 10%.	[26]
	Green and leafy grasses (45–400 IU/kg MS) (alfalfa particularly)	Maintain stable vitamin E levels.	[26]
	27 μg of parenterally (intramuscular) α-tocopherol or 233 μg of oral α-tocopherol/kg of body weight/d	In foals with vitamin E deficiency to maintain the stability of erythrocytes.	[26]
	Daily oral supplement from 600–1,800 IU of synthetic vitamin E acetate, equivalent to 1.5–4.4. IU/kg body weight.	Standard-breed adult horses fed a diet low in vitamin E.	[26]
	50 IU/kg of MS or 0.75–1.0 IU/kg of body weight	Growing horses, pregnant and lactating mares, and performance horses.	[26]
	NRC VA range from 375 IU/d (horse 500 kg) to 1200 IU/d (working horse and lactating mare)	Maintenance of a 500 kg horse, 500 kg lactating mare, and a 500 kg working horse.	[26]
	1 IU/kg PV	Maintenance in nutrition.	[10]
	2 IU/kg PV	Growth, reproduction, lactation, and gestation	[28]
	2, 2.25, and 2.5 IU/kg PV	For light, moderate, and intense exercise respectively.	[28]
	Up to 6,000 IU/d may be recommended	For certain neurological or muscular diseases.	[24]
	1.5 mg/100 lb body weight/d	Horse in maintenance	[24]
β-carotene	18 mg of β-carotene daily	1,200-pound mare.	[24]
	7 mg of β-carotene/100 pounds of body weight	Lactating mare.	[24]
	Supplementation with β-carotene 50 mg/d or more	Indicated for breeding mares and stallions to maintain optimal performance and health.	[29]

Carotenoids have the function of inactivating some molecules that are in an excited state [18]. β-carotene inhibits the effect of ROS and the process of singlet oxygen produced by the lipid peroxidation in liposomes. Although carotenoids as antioxidants prevent damage by ROS, they do not act alone. There are other factors or molecules that act as antioxidants, although the combinations or the way in which these mechanisms act are not known yet [18].

7. Horses and Metabolism

Horses have been one of the most important animals for humanity [19]. Although there is only one species of domestic horse, there are about 400 different breeds which perform different tasks related to humans. Thus, the health of these animals is of great importance [20]. Oxidative stress causes a large number of diseases that affect horses including respiratory difficulties (asthma, pulmonary fibrosis, and bronchitis) [11]. The horse is a domesticated perissodactyl mammal of the Equidae family, which can be classified as non-ruminant herbivores. Horses can partially or totally supply their nutritional demand by consuming only grass [21]. The entry of food into the digestive system begins through the mouth, where the speed of intake is determined by the structure of the food. The horse can ingest around 2.5 mg of dry matter per kg of metabolic weight for each chew. During the chewing process, the food is crushed into particles of a maximum of 2 mm diameter and 1–4 mm in length which facilitates the maintenance of normal intestinal transit, because the digestive system is characterized by a small stomach and a very developed intestine. After food passes through the stomach, it quickly passes into the small intestine where most of the nutrients in the food, including soluble carbohydrates and proteins are digested and absorbed. The small intestine and stomach make up only 40% of the digestive tract so by increasing the rate of passage between the two, the absorption of nutrients decreases [22].

The large intestine of the horse is the largest and the most complex of domestic animals. It can also serve as a reservoir of water and electrolytes that are volatile to maintain performance during exercise. The large intestine is composed of the cecum and colon. These make up approximately 60% of the horse's digestive tract [22].

8. Antioxidant Therapies

Antioxidant therapies present an opportunity to prevent functional deterioration caused by oxidative stress. Although the benefits of antioxidants are known, there are also several contradictory or inconclusive studies on their applications. The efficacy of antioxidant therapies depends on several aspects for their effectiveness such as the type of disease, circumstances, and that the conditions are conducive to these treatments being successful [23]. In this case, the antioxidant therapies that interest us are those in which dietary supplements of vitamin E, C, and β-carotene were used (Table 2).

Vitamin E, being one of the main fat-soluble antioxidants and the only vitamin that is not necessary in metabolic functions, is of great importance in the protection and proper functioning of the nervous and immune muscle systems, protecting cells from oxidation [27].

The symptoms of vitamin E deficiency are difficult to distinguish because they are found in glutathione peroxidase, like selenium (enzymatic antioxidant); but the most common symptom is muscle degeneration affecting skeletal and cardiac muscles, or tongue muscles. Horses that exhibit clinical signs of disease such as equine motor neuron, better known as vitamin E deficiency myopia, require vitamin E deficiency supplementation, mainly in horses that do not graze. Normal serum levels of vitamin E in equines are higher than 2.5 μg/ml. To be able to think about a vitamin E supplementation, critical factors must be taken into account to determine the need of the equine such as having the knowledge of the deficiency of the same, the formulation of the necessary dose (Table 2), and the term of the antioxidant treatment [27].

The type of supplementation may vary due to its forms of use. In the injectable form, vitamin E is normally presented with selenium and it is mostly used and prescribed for authorized veterinary use because it avoids the inhibition that has been observed in other oral compounds. It is also found in two synthetic forms of acetate (powder) which increases serum concentration over a period of approximately two months [27]. In the case of β-carotene in antioxidant therapy, horses convert it into vitamin A (retinol) [25]. Vitamin C in antioxidant therapy is a molecule that can effectively destroy nitrosamines through harmful processes of free radicals due to its ability to eliminate free radicals and is attributed to anti-carcinogenic effect by concentrating in the pulmonary alveoli and inhibiting tumor invasion [28].

9. Intestinal Microbiota

The intestinal microbiota has a rich and complex microbial community. Horse receives much of its dietary energy through microbial hydrolysis and fiber fermentation predominantly in the large intestine. When some type of disease appears, a reduction can be observed in the healthy microbial population and the amounts of anaerobic bacteria increase. The microbiota is unique and personal for each individual as if it were a fingerprint that identifies the individual because it has multiple missions to keep the equine healthy, avoid epithelial damage through protection mechanisms, providing the necessary resistance against pathogens, and generating greater absorption of nutrients [10].

The importance of the gut microbiota ranges from the possible identification of the core community of the microbiota to the prevention of diseases. The dysfunctional microbiota is called "Dysbiosis" and is associated with various diseases such as obesity, insulin resistance, colic, laminitis, allergies, and even behavioral diseases. The microbiota can be affected by the diet of the horse because the microbial community of the intestine is not as stable as human. Although there are different causes of alterations in the intestinal microbiota, any general illness can alter the intestinal flora, especially if they produce fever or dehydration, stressful situations (transport, shearing, eating, competition, and changes in livestock), and of course sudden changes in diet [22].

The horse gets most of its dietary energy through microbial hydrolysis and fiber fermentation, predominantly in the large intestine, while the microbiota ferments the fiber to produce volatile fatty acids which can be absorbed and used as energy sources [10]. Horses in particular are susceptible to gastrointestinal disturbances, mainly when they have sudden changes in their diet that can result in altered fermentation patterns or a metabolic disorder [10].

10. Conclusions

Horses face various stress situations throughout their lives, including intense or prolonged exercise, transport, dehydration, shearing, weeding, competition, livestock changes, and of course sudden changes in diet. Under normal conditions, horses can still produce oxidizing molecules, such as free radicals and ROS. These are highly toxic because it performs the oxidation of different molecules such as carbohydrates, lipids, proteins, and nucleic acids, affecting their function and structure. As a result of the oxidation of these molecules, oxidative damage is generated. This oxidative damage has been associated with the development of various diseases in horses. Horses are among the animals with more physical activity which increases susceptibility to the oxidative damage. Antioxidants such as vitamin C, E, and β-carotene have been reported to have beneficial effects by decreasing oxidative stress. The addition of antioxidants to the diet can help in the prevention of diseases, benefit in the health and maintenance of horses, and possibly provide therapeutic targets.

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