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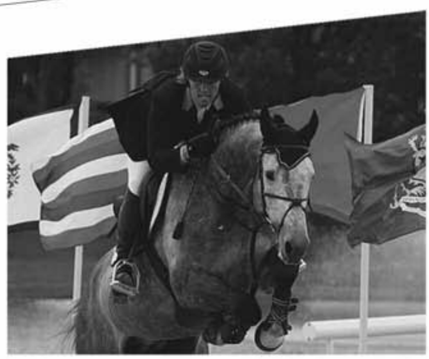

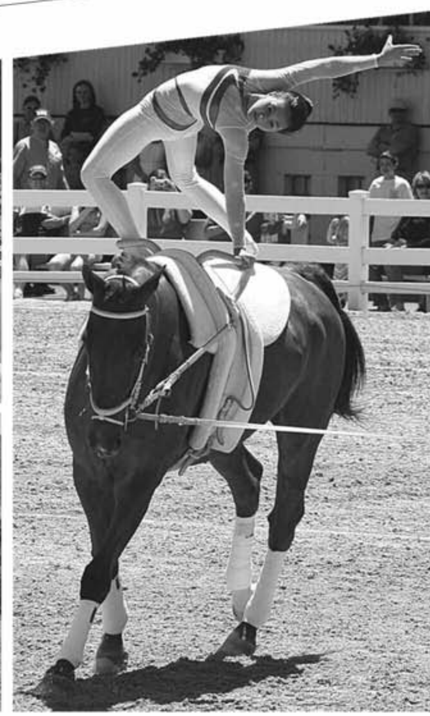

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Antioxidant Research and Its Application to Feeding Horses

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INTRODUCTION

Ever tried looking up "oxidative stress" on the Internet? Google brings up just under four million results; when adding "horse" to this search you get about 124,000. Try "antioxidant" and you get over eight million results on Google. Any way you look at it, these are hot topics. A lengthy review has recently been published detailing oxidative stress and antioxidant supplementation in horses and points out the many conflicting results (Kirschvink et al., 2008). In the following article I will try to highlight studies from my laboratory along with other pertinent studies of antioxidant supplementation in exercising horses.

Oxidative Stress Background

The welfare of competing sport horses has attracted public attention following deaths at the Olympics and other championships. Welfare may be assessed partially by objective indicators of stress (heart rate and various blood metabolites). Evidence of oxidative stress in horses has been described in reports dealing with intense (Chiaradia et al., 1998; White et al., 2001) and endurance exercise (Marlin et al., 2002; Williams et al., 2004a).

Oxidation provides energy for maintenance of cellular integrity and functions. Most of the consumed oxygen forms carbon dioxide and water; however, 1 to 2% of the oxygen is not completely reduced and forms reactive oxygen species (ROS). When antioxidant systems are insufficient, oxidative processes may damage DNA and lipids, and contribute to degenerative changes such as aging and cancer. Lipids are protected directly by alpha-tocopherol (TOC) in the membranes and by other antioxidants, including ascorbic acid (ASC) in the cytisol or external spaces around cells.

Antioxidants are interrelated and may prevent oxidant damage in several ways: scavenging of ROS; decreasing the conversion of less reactive ROS to more reactive ROS; facilitating repair of damage caused by ROS; and providing an environment favorable for activity of other antioxidants (Clarkson and Thompson, 2000). Lipid peroxidation occurs in tissues with a high concentration of polyunsaturated fatty acids such as cell and organelle membranes, lipoproteins, adipose tissue, and brain.

Antioxidant Supplementation Trials

Vitamin E is the most commonly supplemented antioxidant in horses. One study found that a single bout of submaximal exercise does not affect plasma TOC concentration, but horses conditioned for several weeks may require higher levels of vitamin E supplementation than recommended (Siciliano et al., 1996).

It has been found in various species that vitamin C potentiates the effects of vitamin E by reducing the tocopheroxyl radicals and restoring its activity (Chan, 1993). Under maintenance conditions horses have the ability to synthesize sufficient ascorbate, but the demand increases as stress on the body is increased. One study looking at the vitamin E and C interaction used 40 endurance horses competing in an 80-km race for the purpose of research (Williams et al., 2004b). Three weeks prior to the race, the horses were provided with vitamin E (5,000 IU/d alpha-tocopheryl acetate) or vitamin E plus vitamin C (same vitamin E dose, plus 7 g ascorbic acid/d). The 27% increase in RBC glutathione peroxidase (GPx) observed in the last two stages of this race in both treatment groups likely reflects a response to utilize reduced glutathione during the radical-scavenging process (reduced glutathione donates an electron to reduce a wide variety of hydroperoxides using GPx as a catalyst). It also reflects the consumption of prooxidants generated during exercise. In contrast to the RBC changes, novel findings were the changes in the WBC glutathione system. Fluctuations of WBC GPx during exercise and the sharp 41% increase during recovery may reflect replenishment of reduced glutathione. Compared to RBC, the higher concentration of WBC GPx and lower WBC total glutathione (GSH-T) may affect phagocyte oxidative burst and other immune functions during prolonged exercise.

Plasma ASC concentrations were lower in the horses supplemented with vitamin E alone than those receiving the vitamin E plus C at rest. This difference progressively diminished during the race as ASC increased in the vitamin E supplemented horses but remained unchanged in those also supplemented with C. This could be due to an increased mobilization of intracellular ASC stores when supplemented with only vitamin E, whereas when adding C they were able to maintain ASC levels using the exogenous source for its antioxidant capacity. These findings contrast with a previous study, in which a decrease in plasma ASC during a highly competitive and difficult 80-km race was found (Hargreaves et al., 2003).

A study of polo ponies used similar vitamin E and C supplemented groups (Hoffman et al., 2001). Throughout the polo season plasma TOC and ASC were higher in those also given vitamin C in hard-working ponies but not those in only light work. These observations may reconcile the endurance findings in which changes were observed in the highly competitive, midseason race (Hargreaves et al., 2003), but not in this lightly competitive, early-season race (Williams et al., 2004b). In a survey taken after the race, riders ranked the exertion level of the endurance ride easier than most of the rides later in the competition season. Also, ambient temperature was cooler in this race than in the summer when the majority of endurance competitions are held.

Vitamin E intake was calculated in competitive endurance horses via a pre-ride survey detailing intake two weeks prior to the 80-km endurance race (Williams et al., 2005). Pasture intake was estimated using 2.5% body weight eaten per day and subtracting amount of grain, hay, bran, and/or other supplements obtained from the surveys. Horses were estimated to consume 1150 to 4700 IU/d of vitamin E in their total diet during this time period. This level is 1.2- to 5-times higher than the recommended levels given by the NRC (2007), which at this intake averages 1000 IU/d. The horses with the lower vitamin E intake generally were the horses receiving mostly pasture and minimal grain to supplement their diets. A negative correlation was found between the vitamin E intake and both CK and AST, and a positive correlation was found with intake and plasma TOC adjusted for albumin at all sample times. Enzyme activity in plasma is used as an indicator of muscle leakage during exercise. As apparent in the correlations found in the present study, dietary intake of vitamin E is also a contributing factor in muscle enzyme plasma concentrations during exercise.

A negative correlation was found between finishing time and vitamin E intake for the 24 horses that finished the race (Williams et al., 2005). One hypothesis for this finding could be that the higher-placed horses were working at a greater intensity and/or being trained harder, thus having more sweet feed or supplements in the diet. Their higher level of conditioning may also have allowed these horses to work harder with lower muscle enzyme activities.

However, caution needs to be taken when supplementing with high levels of vitamin E. Other studies in my laboratory have investigated pharmaceutical levels of vitamin E on its impact of oxidative stress, muscle enzymes, and antioxidant status (Williams and Carlucci, 2006). Horses supplemented with vitamin E at nearly 10 times the NRC (2007) recommended level did not experience lower oxidative stress compared to control horses (Williams and Carlucci, 2006). Additionally, there was found to be lower plasma beta-carotene (BC) levels observed in this group compared to control or a moderately supplemented group, which may indicate that vitamin E has an inhibitory effect on BC metabolism. This study failed to show that supplementation above control levels is more beneficial to oxidative stress and antioxidant status in intensely exercising horses. However, this research has proven that supplementing with levels 10 times in excess may be detrimental to BC and should be avoided.

Arabians trained to run on an equine treadmill were supplemented with vitamin E (E), lipoic acid (LA), or nothing (CON) before they underwent a simulated endurance exercise test of three exercise bouts totaling 55 km, with 20-minute vet checks separating each (Williams et al., 2004a). These results showed that apoptosis occurs in WBC during exercise, and it can be moderated by supplementation with vitamin E or LA. The E group had 50% lower and the LA group had 40% lower apoptosis compared to the CON group. The increase in antioxidant status in the E and LA groups aided the WBC in scavenging the ROS, thereby triggering the apoptosis in these cells.

Antioxidants are linked together in various ways, and this explains the increase in antioxidant status with supplementation of E and LA. In the present study LA increased the GSH-T concentrations in whole blood compared to CON (Williams et al., 2004a). The LA group also had increased levels of ASC and TOC in the plasma throughout the study. Both the E and LA groups had about 40% more GSH-T, 30% more TOC, and 15% more ASC than the CON group. This illustrates recycling and scavenging of antioxidant radicals using the exogenous sources of the vitamin E and LA.

Other Exercise Studies

Older horses are another group that might require antioxidant supplementation, especially if in combination with exercise. In my laboratory we have found that evidence of a disequilibrium oxidant balance during exercise and aging showed varying results (Williams et al., 2008). In old horses (22 ± 2 years), the amount of lipid peroxidation and blood antioxidant concentrations are similar to those found in mature but younger (12 ± 2 years) horses. Neither group had lipid peroxidation changes with either acute exercise or eight weeks of training, but there was a higher concentration of total glutathione in the pre- vs. post-training tests in both age groups. The observation that more total glutathione was needed during the pre-training graded exercise test for both old and younger groups of horses suggests that training helped the horses prime their systems for the intense post-training exercise tests. Our study also found that WBC apoptosis was significantly lower in the younger than in the older horses, signifying that age might have more of an impact on the immune system than on the oxidant/antioxidant system.

Elite three-day event horses competing internationally at a CCI** or CCI*** were found to have no differences between divisions for cortisol, TOC, retinol, BC, AST, and GPx (Williams and Burk, 2007). Total glutathione, however, was higher in the horses competing in the CCI** than horses in the CCI***. Total glutathione also peaked immediately after the cross-country phase, returning to baseline after 18 to 24 hours of recovery. Other measures including CK, AST, GPx, BC, retinol, cortisol, and lactate also peaked immediately after the cross-country phase and were typically lower before the competition started compared to 24 hours after the cross-country. Overall these results provided the first report of antioxidant status of horses competing in either a CCI** or CCI*** three-day event (Williams and Burk, 2007). Pre-event nutritional surveys were also undertaken to determine the intake level of antioxidants and other nutrients that would affect the level of stress during competition (Burk and Williams, 2008). These correlations have not yet been determined but they may explain, at least in part, the possible differences or lack of differences between divisions.

The effects of superoxide dismutase (SOD) on oxidative stress and inflammation in exercising horses systemically and locally (synovial fluid) were recently reported (Lamprecht, 2009). Previous studies in rats (Radak et al., 1995) and humans (Arent et al., 2009; 2010) have shown beneficial results; however, these horse studies failed to show similar results. Studies have tested a SOD derivative on exhaustively exercising rats and found that it provided effective protection against oxidative stress in the liver and kidney along with skeletal muscle (Radak et al., 1995). Recently, the effects of an oral SOD supplement on preseason collegiate soccer players (Arent et al., 2010) and football players (Arent et al., 2009) determined that performance improved by a greater magnitude in the soccer players supplemented with SOD, where the football players had greater improvements in peak power and lowered muscle breakdown, measured with CK, along with lower levels of 8-isoprostane.

Even though there have been many studies examining the levels of lipid peroxidation, antioxidant status, and other related metabolites or markers in the horse during exercise, we still have a long way to go before we fully understand the large variation in results both with and without antioxidant supplementation.

Implications

Overall these exercise studies have shown that oxidative stress was observed during endurance, intense, and treadmill exercise. The extent of the oxidative stress and muscle-enzyme leakage was dependent on the ambient temperature, conditioning level of the horse, and the intensity of work. Supplementing antioxidants like vitamin E, vitamin C, and lipoic acid is beneficial to horses by decreasing the oxidative stress and muscle-enzyme leakage, and increasing antioxidant status. Thus, we can provide better health and welfare to our equine athletes by supplementing with antioxidants before they are asked to perform under intense conditions. However, caution needs to be taken if supplementing above and beyond the recommended levels due to the possible interference with the absorption of other nutrients.

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