Short- and long-term effect of oral administration of micellized natural vitamin E (D-α-tocopherol) on oxidative status in race horses under intense training

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ABSTRACT: This study tested the effect of micellized vitamin E (D-α-tocopherol; 1,400 IU/d) administered 12 and 1 h orally before training for 1 d (ST-VitE) or 8 d (LT-VitE) compared with an unsupplemented control (CONTROL) on plasma α-tocopherol, thiobarbithuric acid-reactive substances (TBARS), total glutathione (GSHt), and trolox equivalent antioxidant capacity (TEAC) in 10 race horses. Different sampling times [immediately before training (BEF) and after intense training (END) or 8 h after recovery (+8h)] were investigated. Plasma α-tocopherol concentration was greater in the ST-VitE group than the CONTROL group at +8h (P < 0.05). Natural vitamin E supplementation increased plasma α-tocopherol (P < 0.001) in the LT-VitE group by approximately 1.6-fold at BEF, END, and +8h. In all groups, TBARS tended to be slightly greater (P = 0.087) immediately after training when compared with values BEF or +8h and the lowest TBARS values were observed at +8h in LT-VitE. Vitamin E supplementation did not affect the GSHt concentrations at BEF, END, or +8h. The TEAC values were modified by the vitamin E administration (P = 0.010). The greatest TEAC was found in the LT-VitE group at all sampling times and similar concentrations were reached in the ST-VitE group at +8h. The CONTROL group was not able to maintain TEAC after training (P < 0.001), indicating consumption of antioxidants (mainly vitamin E) and consequently oxidative stress because of the antioxidant system being overwhelmed by a reduced antioxidant supply. In conclusion, micellized natural vitamin E at 1,400 IU/d for 8 d efficiently increased plasma α-tocopherol concentration of race horses undergoing intense training conditions and maintained the general oxidative status.

Key words: natural α-tocopherol, oxidative status, race horses, thiobarbithuric acid-reactive substances, total glutathione, trolox equivalent antioxidant capacity

INTRODUCTION

Prolonged aerobic exercise is 1 of the causes that promotes increased oxidant production and modifies cellular redox balance leading to molecular damage (Soares et al., 2011; Powers et al., 2011). Consequently, the use of antioxidants is a common practice to prevent exercise-induced oxidative lesions in human and animal athletes, despite lack of information about the optimum dose for inducing beneficial effects.

Vitamin E has been one of the most intensely studied compounds because it is an effective chain-breaking antioxidant present in cell membranes and, therefore, plays an important role in capturing free radicals and other reactive substances (Halliwell, 1994). Hence, the NRC (2007) recommended that horses in moderate to intense work should receive supplements of vitamin E greater than 80 IU/kg DM and, in instances of continuous exercise, at least 300 IU/kg DM are needed (Siciliano et al., 1997). Recent studies show that vitamin E supplementation more than NRC (2007) guidelines (3,000 IU DL-α-tocopheryl acetate/d) can influence certain measures of oxidative stress in intensely exercising horses (Duberstein et al., 2009).

On the other hand, it has recently been demonstrated that the natural form of vitamin E (D-α-tocopherol) is...
accumulated more efficiently than the synthetic form (Hoppe and Krennrich, 2000). In addition, natural micellized vitamin E has been found to be superior at elevating plasma α-tocopherol when compared with either the synthetic or natural vitamin E (Pagan et al., 2005). Moreover, antioxidant defenses are affected not only by dietary supplementation but also by training status, which decreases peroxidative phenomena after exercise (Avellini et al., 1999; Krumrych, 2010).

It was hypothesized that oral micellized natural vitamin E (1,400 IU/d) given for a short or long term might improve the plasma α-tocopherol concentration and the oxidative status in race horses under intense training conditions. The objective of the present research was to study the effect of direct oral administration (1,400 IU/d) of micellized natural vitamin E (D-α-tocopherol) for a 1 or 7 d period on the oxidative status measured as α-tocopherol concentration, total glutathione (GSHt), trolox equivalent antioxidant capacity (TEAC), and thiobarbituric acid reactive substance (TBARS) before and after intense training in plasma of race horses.

**MATERIAL AND METHODS**

Procedures performed in this study were in accordance with the approved protocols by the Committee of Animal Care of the University Complutense of Madrid.

**Animals and Experimental Design**

Ten race-trained horses (6 males and 4 females; English Thoroughbred) ranging from 2 to 5 yr old (Hipódromo de la Zarzuela, Madrid, Spain) were used. The horses were trained daily with similar intensity for 1.5 to 2 h (3,700 m walking, 1,000 m trotting, and 2,700 m cantering). All animals were stabled in straw boxes and after intense training in plasma of race horses. No oral supplements (mineral–vitamin complex providing vitamin C, vitamin E, selenium, etc.) were administered from 4 d before and during the experiment. After this 4-d washout period, blood samples from horses were taken \( n = 10 \) to check the baseline status, and these were considered as control. Oral natural vitamin E was administered 7 d after checking the baseline for 1 d and then blood samples were collected again. Horses received the oral supplement of natural vitamin E for the next 7 d and blood samples were taken. The experiment design involved 3 feeding regimens in the same race horses over a time period of 2 wk. Each horse received all 3 treatments. Because plasma kinetics and storage characteristics of the form of vitamin were unknown in race horses and to ensure that natural vitamin E would be available in plasma, it was orally administered (1,400 IU/d) in 2 doses of 700 IU, 1 dose between 18 and 19 h (before horses received feed) and the other dose between 6 and 7 h (1 to 2 h before training). Experimental groups were as follows: 1) horses with no oral supplementation of vitamin E before or during the training protocol (CONTROL), 2) horses received 1,400 IU of natural vitamin E in a micellized alcohol form (D-α-tocopherol; H2E EQUUS 70%; Prebia Feed Extracts, S.L., Talavera de la Reina, Toledo, Spain) administered in 2 doses of 700 IU (70 IU/mL) provided 12 and 1 h before training for 1 d (ST-VitE), and 3) horses received 2 doses of 700 IU (1,400 IU total) of natural vitamin E in a micellized alcohol form provided 12 (700 IU) and 1 h (700 IU) before training for 1 wk (LT-VitE). Therefore, each horse served as its own control.

**Sample Collection**

Blood samples (5 mL) were taken from a jugular vein of horses immediately before training (BEF), immediately after training (END), and 8 h after training (+8h) by venipuncture into vacuum EDTA (BD vautainer, 10.8 mg) tubes. All blood samples were immediately placed on ice after collection. The plasma was then separated by centrifugation at 600 × g for 10 min at 4°C and the supernatant was kept in a freezer at –20°C until analysis. Analyses were performed within the next 2 wk.

**Laboratory Analysis**

**Tocopherol Quantification in Plasma.** The α-tocopherol concentration in plasma from horses was quantified as described by Rey et al. (2006) by direct extraction. Plasma samples were mixed with 0.054 M dibasic sodium phosphate buffer adjusted to pH 7.0 with HCl and absolute ethanol. After mixing, tocopherol was extracted with hexane by centrifugation (600 × g for 10 min at 4°C); the upper layer was evaporated to

<table>
<thead>
<tr>
<th>Item</th>
<th>Oat</th>
<th>Hay</th>
<th>Concentrate</th>
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<tr>
<td>Ash, %</td>
<td>2.8</td>
<td>12.3</td>
<td>6.5</td>
</tr>
<tr>
<td>CP, %</td>
<td>8.5</td>
<td>20.6</td>
<td>12.0</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>4.8</td>
<td>2.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>12.5</td>
<td>22.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.08</td>
<td>1.55</td>
<td>0.9</td>
</tr>
<tr>
<td>P, %</td>
<td>0.33</td>
<td>0.24</td>
<td>0.5</td>
</tr>
<tr>
<td>Cu, mg/kg</td>
<td>5.0</td>
<td>–</td>
<td>55.0</td>
</tr>
<tr>
<td>Se, mg/kg</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
<td>Zn, mg/kg</td>
<td>25.0</td>
<td>–</td>
<td>220.0</td>
</tr>
<tr>
<td>Vitamin E, IU/kg</td>
<td>12.0</td>
<td>30.0</td>
<td>300.0</td>
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</table>
dryness and dissolved in ethanol before analysis. Tocopherols were analyzed by reverse phase HPLC (HP 1100, equipped with a diode array detector; Agilent Technologies, Waldbronn, Germany) as described elsewhere (Rey and López-Bote, 2001). Identification and quantification were performed by means of a standard curve ($R^2 = 0.99$) built using the pure compound (Sigma, Alcobendas, Madrid). All samples were analyzed in duplicate. Results were expressed as microgram of $\alpha$-tocopherol per milliliter of plasma.

**Oxidative Status of Plasma Samples.** The malondialdehyde (MDA) concentration as measure of TBARS was quantified in plasma according to a modification of the spectrophotometric method of Buege and Aust (1978). Perchloric acid was added to plasma samples to precipitate proteins. The protein-free supernatant was collected and mixed in a proportion 1:2 with thiobarbituric acid (TBA) reagent (0.026 $M$ TBA and 0.92 $M$ trichloroacetic acid in 250 mL of water with 62.5 mL 1 $M$ HCl). Then samples were centrifugated at 600 $\times$ g for 10 min at 4°C and absorbance was read at 532 nm using a spectrophotometer (UV-Vis, ScanGo; ThermoFisher Scientific, Alcobendas, Spain). The TBARS concentrations were calculated using $1.56 \times 10^5 M^{-1} \times cm^{-1}$ as the molar absorption coefficient. Results were expressed as micromoles of MDA per milliliter plasma.

The TEAC concentration was measured using the procedure described by Re et al. (1999). The 2,2-azinobis-(3-ethylbenzothiazoline 6-sulphonate; ABTS) radical cation (ABTS$^+$) was produced by reacting 25 mL of 14 mM aqueous ABTS with an equal volume of 4.9 mM potassium persulfate (final concentration: 7 mM ABTS in 2.45 mM potassium persulfate). The mixture was incubated in the dark for 16 h to allow the complete radicalization of the ABTS. The ABTS$^+$ radical solution was diluted with PBS (pH 7.4) to reach an absorbance of 0.70 ± 0.03 at 734 nm and was equilibrated at 30°C (ScanGo; ThermoFisher Scientific, Alcobendas, Spain). For the samples, 0.010 mL of plasma was mixed with 0.3 mL of diluted ABTS$^+$ solution and the absorbance at 734 nm was recorded after 6 min of incubation at 30°C. All determinations were performed by duplicate. Additionally, trolox standards (concentration ranging from 0 to 1 mM) and appropriate blanks were run. The percentage of inhibition of the ABTS$^+$ radical was calculated as

\[
\text{Inhibition} = \frac{[A_{0-B1} - A_6]}{A_{0-B1}} \times 100,
\]

where $A_{0-B1}$ is absorbance at 0 minutes - blank absorbance, and $A_6$ is absorbance at 6 minutes. Results were expressed as millimoles of trolox equivalents per liter of plasma.

Total glutathione was quantified spectrophotometrically at 405 nm in deproteinized plasma using the corresponding diagnostic colorimetric kit (Arbor Assays, Ann Arbor, MI). The concentrations obtained were expressed as micromoles of glutathione.

**Statistical Analysis**

The experimental unit for analysis of all data was the individual horse. Data were analyzed using a completely randomized design using the GLM procedure (SAS Inst. Inc., Cary, NC). A repeated measurement test was used to assess the effects of time and treatment on plasma status and interaction. A comparative analysis between means was conducted using the Tukey test. Data are presented as the mean and SEM, and differences between means were considered statistically significant at $P < 0.05$.

**RESULTS**

**Plasma Tocopherol Concentration**

Plasma $\alpha$-tocopherol concentrations from horses supplemented with natural vitamin E over the sampling times (BEF, END, and +8h) are presented in Fig. 1. Oral administration of 1,400 IU of D-$\alpha$-tocopherol for 1 d (ST-VitE) did not modify the $\alpha$-tocopherol concentration in plasma during the first hours before and after training when compared with the CONTROL group. However, plasma $\alpha$-tocopherol concentration was greater in the ST-VitE than in the CONTROL group at 8 h after training. Horses LT-VitE showed increased plasma $\alpha$-tocopherol ($P < 0.001$) by approximately 1.6-fold immediately before and after training and 8 h later when compared with the CONTROL. Moreover, plasma $\alpha$-tocopherol concentrations of horses had different response to oral vitamin E supplementation (treatment $\times$ time interaction $P = 0.004$). The $\alpha$-tocopherol concentration increased in the ST-VitE group 8 h after training whereas a small decrease at 8 h after training was observed in other treatments.

**Oxidative Status in Horse Plasma**

As an indication of the oxidative stress because of intense exercise, MDA concentrations are presented in Fig. 2. Oral natural vitamin E supplementation slightly affected the MDA concentration at 8 h after training, but no effect was observed at BEF or END. The LT-VitE treated horses had lower MDA values than the CONTROL group at 8 h after training. Supplementation of vitamin E for 1 d resulted in slightly greater MDA concentration at +8h than in
the LT-VitE group and the greatest MDA immediately after training (END) were found in the CONTROL group. The MDA tended to be greater immediately after exercise (END) in all groups when compared either with those values before training or 8 h later ($P=0.087$). There was no treatment × time interaction.

The GSHt concentration, as an indication of the antioxidant power, in horse plasma is presented in Table 2. In the present study, oral natural vitamin E supplementation at 1,400 IU did not affect the total GSHt concentrations immediately before or after exercise and 8 h later.

Trolox equivalent antioxidant power is presented in Fig. 3, and the effects of treatment ($P=0.010$), time ($P=0.031$), and treatment × time ($P<0.001$) were found. As in the α-tocopherol concentration, plasma TEAC was greatest in the LT-VitE group at all 3 sampling times. Moreover, the ST-VitE group that received vitamin E supplementation for 1 d also reached TEAC values similar to those of the LT-VitE group 8 h after training. On the other hand, immediately before and after training, the antioxidant capacity of the ST-VitE group was not different from the CONTROL group. Also, TEAC changed differently because of the treatment and sampling times (interaction, $P<0.001$) where ST-VitE and LT-VitE groups showed increased TEAC with the sampling time, but its value in the CONTROL group decreased and reached the least concentration 8 h after training.

**DISCUSSION**

Because natural vitamin E (D-α-tocopherol) was expected to be more efficiently accumulated than the synthetic form, in the present study, a dose of 1,400 IU/d was used. This dose is within the range of minimum daily vitamin E requirement for adult horses recommended in the NRC (2007) guidelines (80 IU/kg DM, equivalent to 1.6, 1.8, and 2 IU/kg BW for light, moderate, and other levels of work) and was approximately one half of the dose recommended by the NRC (2007) to maintain blood vitamin E concentration in horses undergoing exercise (300 IU/kg DM, equivalent to 6 IU/kg BW).

Supplementation with 1,400 IU natural vitamin E/d for 1 or 8 d increased plasma α-tocopherol concentration of race horses measured 8 h after training when compared with the CONTROL group. This increase in plasma α-tocopherol concentrations was approximately 40% and was not affected by exercise in the LT-VitE group. The α-tocopherol concentration found in plasma samples of the present study was greater than that reported by other authors who also supplemented horses with natural vitamin E (Higgins et al., 2008). Hence, those authors found 2.98 and 5.9 μg/mL and 63 and 74% increase when horses were given 1,000 and 10,000 IU for 6 d, respectively. However, Higgins et al. (2008) started the study with a much lower (1.0 and 1.5 μg/mL) initial vitamin E concentration than the present study (6.35 μg/mL) and their subjects were not
being exercised. Other authors (Fiorellino et al., 2009) reported that vitamin E in horses is more efficiently accumulated when the micellized alcohol or liquid form was administered and found values between 4.85 to 6.55 μg/mL after 7 d of supplementation of 4,000 IU, which are closer to the concentrations found in the present study. Similar findings were reported by Pagan et al. (2005) who found that natural micellized vitamin E was superior in increasing plasma α-tocopherol compared with either the synthetic or natural vitamin E after short-term administration.

In dairy cows, Pumfrey et al. (1993) compared the natural and synthetic vitamin E and the alcohol and acetate forms as well as micellized and nonmicellized formulations and reported greater plasma tocopherol concentrations with an oral administration of the natural micellized alcohol form. In turkeys (Soto-Solanova, 1995) and piglets (Amazan et al., 2012), authors found a similar superiority of the micellized natural vitamin E. Moreover, Texeira et al. (2009) have reported that athletes showed increased plasma values of α-tocopherol without additional supplementation, which could also explain in part the greater initial plasma vitamin E concentrations observed in the present study. On the other hand, Williams and Carlucci (2006), using 5,000 IU of the synthetic form (DL-α-tocopheryl acetate) in intensely exercised horses over a 4-wk period, found the values from 3.6 to 4.5 μg/mL, a 20% increase. The alcohol form of the vitamin has been shown to be more bioavailable than acetate supplements (Lauridsen et al., 2001) and the natural form of vitamin E (RRR-D-α-tocopherol) is accumulated more efficiently than the synthetic form (Hoppe and Krennrich, 2000), which is composed of a mixture of 8 stereoisomers. This is due to the greater affinity of the α-tocopherol transfer protein for the natural RRR-stereoisomer (Hosomi et al., 1997). In addition, micelles, which transport vitamin E, can be solubilized in their inner core at a small size, probably maximizing its absorption (Jones and Leroux, 1999). Consequently, oral natural vitamin E supplementation (given as micellized alcohol liquid) results in greater plasma α-tocopherol concentrations than nonmicellized/synthesized vitamin E supplements and the concentrations may also depend on the exercise status of the animals.

Plasma α-tocopherol concentrations in race horses supplemented with natural micellized vitamin E were similar before and after an intense training session. However, the response with time was different depending on the treatment. Hence, horses in the ST-VitE group, which showed similar plasma α-tocopherol concentrations to the CONTROL group immediately before and after training, had increased α-tocopherol concentrations at +8h whereas plasma α-tocopherol concentration dropped at +8h in the CONTROL group. Siciliano et al. (1997) and Marlin et al. (2002) found that exercise did not affect plasma vitamin E status after a single bout of medium to high intensity training; however, concentrations decreased in horses conditioned for several weeks (Siciliano et al., 1997). On the other hand, Pagan et al. (1998) reported that exercise may affect digestibility and the rate of passage of diet because exercised horses consume more water, which travels faster through the equine digestive tract. Differences observed in ST-VitE group of the present study may in part be explained because, at sampling (1 to 2 and 12 h after oral administration), the supplemented nutrient may still not be totally absorbed because the total absorption of some nutrients in horse has been estimated to take approximately between 24 and 36 h after intake (Pagan et al., 1998; Hembroff,

Table 2. The effect of natural vitamin E supplementation and sampling time on total glutathione concentration (μM) in plasma of 10 horses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time1</th>
<th>3BEF</th>
<th>END</th>
<th>+8h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td></td>
<td>4.37</td>
<td>3.70</td>
<td>3.76</td>
</tr>
<tr>
<td>ST-VitE</td>
<td></td>
<td>3.28</td>
<td>3.80</td>
<td>4.17</td>
</tr>
<tr>
<td>LT-VitE</td>
<td></td>
<td>3.86</td>
<td>3.89</td>
<td>4.64</td>
</tr>
</tbody>
</table>

1SEM = 0.13 and 0.06 μM for treatment and time, respectively; P-values = 0.50, 0.28, and 0.16 for treatment, time, and their interaction, respectively.

2CONTROL = horses unsupplemented with vitamin E; ST-VitE = horses supplemented with vitamin E 12 and 1 h before training; LT-VitE = horses supplemented for 8 d with vitamin E 12 and 1 h before training. n = 10.

3BEF = before training; END = immediately after training; +8h = 8 h after training. n = 30.

Figure 3. The effect of natural vitamin E supplementation (Control = horses un-supplemented with vitamin E, ST-VitE = horses supplemented with vitamin E 12 and 1 h before training and LT-VitE = horses supplemented for 8 d with vitamin E 12 and 1 h before training) and sampling times [before training (BEF), immediately after training (END), and 8 h after training (+8h)] on trolox equivalent antioxidant capacity (mM) in plasma of 10 horses. SEM = 0.131 and 0.056 mM for treatment (n = 10) and time (n = 30), respectively; P-values = 0.010, 0.031, and <0.001 for treatment, time, and their interaction, respectively. a,bMeans without common a common superscript differ, P < 0.05.
Absorption of vitamin E could depend not only on the form but also on the route of supplementation administration (Pumfrey et al., 1993). Hence, it has been reported maximum peak concentrations at d 1 after oral administration of the micellized alcohol form in horses (Fiorellino et al., 2009) and cows (Pumfrey et al., 1993).

However, in sheep, serum tocopherol concentration reached maximum concentration at d 3 after the oral administration of micellized DL-α-tocopherol (liquid; Ochoa et al., 1992). To our knowledge, there is not any more detailed study of the plasma vitamin E kinetics when the natural micellized alcohol form is used. On the other hand, the decrease in the plasma α-tocopherol concentration in the CONTROL group after training would indicate that vitamin E intake is required to maintain the oxidant/antioxidant balance of race horses even though fit horses have greater initial concentrations of plasma α-tocopherol.

The oxidative status of horses immediately before and after training and at 8 h later was also investigated. To our knowledge, there are no published data on the dose of natural micellized vitamin E necessary to reduce the oxidative stress in race horses. Therefore, natural vitamin E effects need to be explored in equine athletes under severe training conditions.

The MDA concentration was not affected by supplement administration before and immediately after exercise, but at 8 h after recovery, LT-VitE and ST-VitE horses showed lower oxidative stress than the CONTROL group. Zembron-Lacny et al. (2006) reported that a single dose of 1,000 mg α-tocopherol acetate administered 3 h before training enhanced oxidative defense in athletes. However, other authors (Duberstein et al., 2009) did not find any influence of supplemental vitamin E (3,000 IU DL-α-tocopheryl acetate) on plasma vitamin E or TBARS during a prolonged training program. This is, to our knowledge, the first study in which the effect of a natural source of vitamin E, administered as a micellized alcohol at different administration times, on oxidative stress was evaluated in race horses. In the present study, 1,400 IU of natural vitamin E supplementation during a period of 8 d was enough to reduce MDA concentrations at 8 h after a training session. Supplementation for 1 d produced intermediate MDA at +8h because it seems that an administration period of more than 12 h is necessary for orally administered vitamin E to ensure total absorption and reach maximum concentrations in plasma.

It is also noteworthy that exercise tended to increase TBARS concentrations because all groups reached the greatest values immediately after training. Strenuous physical activity is known to generate reactive oxygen species to a point that can exceed the antioxidant defense system and lead to oxidative stress. Texeira et al. (2009) reported that despite enhanced concentrations of plasma antioxidants, human athletes undergoing regular strenuous exercise exhibited more oxidative stress than sedentary groups. Likewise, Marlin et al. (2002) found plasma TBARS increase after exercise in horses competing in a 140-km endurance race. Similarly, TBARS concentrations were increased at the end of the race when horses were subjected to 60-, 90-, and 120-km endurance race (Al-Qudah and Majali-Al, 2006). In the present study, the LT-VitE group had the lowest MDA concentration immediately after training (1.7 vs. 2.1 μmol/mL) but differences were not statistically significant. The LT-VitE horses showed the least MDA increase (0.084 vs. 0.46 μmol/mL) after training whereas the CONTROL group had the least decline 8 h after recovery (0.18 vs. 0.38 μmol/mL). These results would indicate that an adequate concentration of antioxidants had been achieved in the supplemented groups 8 h after recovery.

On the other hand, daily training in race horses does not affect endogenous antioxidants, such as glutathione, involved in the redox system. Kirschvink et al. (2002) reported no changes in GSHT in trained healthy horses after an intermittent incremental exercise test. In humans, Camus et al. (1994) also found no changes in GSHT during exercise. However, Marlin et al. (2002) reported that total red cell hemolysate glutathione concentration was reduced by exercise and 16 h after recovery. That result was supported by Williams et al. (2008) who found greater concentration of GSHT before than after a training period. On the contrary, other authors (Williams and Carlucci, 2006) showed that exhaustive physical exercise caused an increase in red blood cell total glutathione that returned to baseline after 24 h, and Williams et al. (2004) found greater red blood cell GSHT in horses that finished the race at 80-km than horses that did not finish the race. Moreover, Krumrych (2010) reported the importance of enzymatic protection and the positive influence of training on antioxidative potential of blood in horses.

Vitamin E supplementation in the present study did not affect the plasma GSHT concentration. Similar to the current study, Duberstein et al. (2009) reported unchanged total glutathione concentrations during the training period by vitamin E supplementation. Meanwhile, Williams et al. (2004) found that vitamin E supplemented groups had about a 40% increase in GSHT.

This controversial finding on the effect of exercise and vitamin E on GSHT is probably due to complex regulation pathways between vitamin E concentrations and antioxidant enzymes. Hence, Chang et al. (2007) reported that vitamin E status and exercise training had an interactive effect on oxidative stress and glutathione peroxidize in skeletal muscle and, consequently, vitamin E deprivation augmented an exercise-induced increase in glutathione peroxidize activity. Consequently, results of the present study would indicate the predominant
importance of vitamin E in maintaining antioxidant balance in race horses under intense daily training.

Finally, it is interesting to note that the predominant effect of vitamin E on preserving the oxidative status was evidenced in the present study based on the TEAC values, which closely paralleled plasma $\alpha$-tocopherol concentrations. The 8 d of supplementation with natural vitamin E as a miscellaneous alcohol induced an increase in plasma TEAC in horses after training and at 8 h into recovery. Administration of vitamin E supplement for 1 d (12 h before training) only increased TEAC at 8 h after training. These results would confirm that the supplement administered for 8 d at the doses used in the present study was enough to improve the antioxidant/oxidant balance, thus controlling the radical oxygen production during a continuous period of exercise. However, the CONTROL group showed decreased TEAC as well as $\alpha$-tocopherol concentrations with time, indicating consumption of antioxidants (mainly vitamin E) and consequently oxidative stress because of the overwhelmed antioxidant system arising from a reduced antioxidant supply. Other authors (Stohrer et al., 2002) have reported a decrease in TEAC values in dogs after intense exercise that was ameliorated with vitamin E supplementation. However, this is the first study in which the micellized natural form of vitamin E has been evaluated in race horses.

In conclusion, oral supplementation of micellized natural vitamin E at 1,400 IU/d for 8 d increased plasma $\alpha$-tocopherol concentration in race horses immediately after intense training and at 8 h after recovery, thereby improving their antioxidant/oxidant status. Administration of natural vitamin E at the same dose for 1 d given approximately 18 to 20 h (to reach the maximum absorption) before training or competition would probably produce similar effects, but prolonged administration is needed to maintain the oxidative status in horses undergoing exercise. Although endogenous antioxidants, such as glutathione, are implicated in the maintenance of intracellular redox status and the control of cellular oxidative stress, the results of the present study indicate the predominant importance of vitamin E in maintaining antioxidant balance in race horses under intense daily training.

LITERATURE CITED


