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Comparison of Oxidative Stress and Antioxidant Markers between Race and Endurance Horses

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ABSTRACT

The purpose of this study was to evaluate oxidative stress and antioxidant markers in sport horses, comparing pre-exercise and post-exercise between two types of sport. Twenty horses (ten race horses and ten endurance horses) were used in this study. The exercise intensity varied based on sport-specific formats. Blood samples were collected at pre-exercise, immediately, and three hours after exercise for oxidative stress and antioxidant markers analysis. The results showed that malondialdehyde (MDA) and protein carbonyl levels were significantly increased just after exercise, followed by a significant decrease of MDA 3hours after exercise in both types of sport horses. In contrast, protein carbonyl level was not significantly decreased 3hours after exercise. The superoxide dismutase (SOD) levels were significantly increased just after exercise in both types of sport horses, whereas catalase levels were significantly increased just after exercise only in race horses, indicating their adaptive responses to oxidative stress. Moreover, plasma nitrate/nitrite (NOx) levels showed no significant differences between periods of exercise and types of sports, although NOx levels tended to decrease just after exercise in both types of sports. These findings suggest that exercise patterns influence the level of oxidative stress in sport horses, as race horses exhibit higher oxidative stress response than endurance horses, supporting the notion that exercise-induced oxidative stress depends on exercise type, intensity, and duration. Further studies are needed to clarify the accumulation of ROS in different muscle fiber types to provide more understanding about the oxidative and antioxidative response during exercise in sport horses.

Key words: Race Horse, Oxidative stress, Antioxidant, Endurance, Nitric oxide,

INTRODUCTION

The exercise-induced generation of reactive oxygen species (ROS) is a well-known phenomenon in both humans and animals (Dillard et al. 1978; Powers et al. 2020; Wang et al. 2021). The most important source of ROS during exercise is mitochondria (Vargas-Mendoza et al. 2021). In addition, activated phagocytes and increased activity of several oxidase enzymes also contribute to an increased ROS formation (Mills et al. 1996; Moffarts et al. 2004; Bouviere et al. 2021). During exercise, the increase of oxygen flux into active skeletal muscles leads to enhanced ROS production and oxidative stress, resulting in an imbalance between free radicals and antioxidants (Kinnunen et al. 2005; Powers et al. 2020; Zohier et al. 2023). Moreover, a higher maximal oxygen uptake (VO_{2max}) is associated with an increase in superoxide anion ($O_2^{\bullet-}$), one of the most important free radicals (Fazio et al. 2016; Ankur et al. 2018). ROS produced from contracting muscles during exercise potentially causes damage to tissues by oxidation of cellular components, such as DNA, proteins, and membrane lipids (Moffarts et al. 2004; Powers et al. 2020). This oxidative stress contributes to accelerated muscle fiber damage and muscle fatigue, leading to exercise intolerance and decreased physical performance (Sen et al. 2000; Wang et al. 2021).

Exercise-induced oxidative stress causes muscle damage and an increase in lipid peroxidation level in horses as well (Kent et al. 2023). Muscle fibers of equine athletes are classified into slow-twitch (Type I) or fast-twitch (Type II) fibers, which feature different structural, metabolic, and functional properties (Rivero et al. 2014; Vasileiadou et al. 2023). The athletic abilities of horses are, in part, associated with variations of their muscle fiber composition

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(Hodgson et al. 2013). In race horses, their athletic performance heavily relies on the utilization of fast-twitch muscle fibers, whereas in endurance horses, their endurance ability relies on the utilization of slow-twitch muscle fibers (Hodgson et al. 2013). In the previous studies, the differences in redox signaling and oxidative stress were examined between supplement-treated and non-treated groups, young and old groups, or trained and untrained animal groups (Fazio et al. 2016; Smarsh et al. 2017; Bis-Wencel et al. 2020; Molinari et al. 2020; Stucchi et al. 2025). However, the investigation of the differences between the horses enrolled in the different types of sport, which represent the usage of different types of muscles (slow and fast twitch), is rare (Krumrych 2010; Zohier et al. 2023). In fact, horses participating in different types of athletic practices are trained to develop physical status, including muscles suitable for their specific purpose (Zohier et al. 2023). In fact, testing for oxidative stress and antioxidant status in a single type of sport horse may not fully address the question of how oxidative stress changes vary across different sports (Liu et al. 2000; Moffarts et al. 2004; Andriichuk et al. 2014; Shono et al. 2020). Therefore, the aim of this study was to compare the oxidative stress and antioxidant status in plasma between race and endurance horses.

MATERIALS AND METHODS

Animals and experimental design horse

Ethical approval for this study was obtained from the Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU-84/64), based on the Ethics of Animal Experimentation of the National Research Council of Thailand. A total of twenty horses, namely 10 each of race and endurance horses, were selected from a standard farm and a sports club in Thailand. The gender includes stallion, gelding, and mare. The average age was 7 to14 years old, and the average weight was 350 to 475kg. For health status, horses underwent the physical examination, complete blood count, aspartate aminotransferase (AST) test, and creatinine phosphokinase (CK) test. The inclusion criteria for participation are: the horse had a normal health status, participated in a competition at least once, and had not been treated with an antioxidant supplement for at least 6months. In addition, the horses must complete an exercise program or competition without injury.

Type of sports

In each study group, there were differences in exercise-specific formats for that particular sport. The exercise intensity levels of each group of horses were assessed using the guidelines reported previously (Rivero 2007; Hinchcliff et al. 2008; Hodgson et al. 2013). Exercise intensity and the various exercise formats represent the full utilization of physical fitness specific to that particular sport by the level of VO_{2max} at 80-100% and blood lactate concentration of 4mmol/L, which is the same intensity of VO_{2max} at 80-100% (Rivero 2007). The exercise protocol was defined as follows: a racehorse (high-intensity acute exercise) ran two laps on the racing track at an approximate speed of 60km/h, covering 2.4–3.2km in total, with a duration of 4–5 minutes. An endurance horse (moderate-intensity chronic exercise) had participated in a 40km competition on the track, maintaining an approximate speed of 20 km/hour, totaling 3 hours.

Blood sample collection

10mL of blood samples were collected from the external jugular vein of each animal, and put into potassium-EDTA for oxidative stress and antioxidant markers. The blood samples were collected at three time periods: pre-exercise, immediately, and 3 hours post-exercise. Subsequently, plasma samples were separated by refrigerated centrifugation. The samples were stored at -20°C until analyzed.

Blood profile and biochemical assessments

Complete blood count was done using a hematology analyzer (ABX micros ESV 60, HORIBA Instruments Incorporation, California, USA). AST level was determined using a blood chemistry analyzer (ABX Pentra 400, HORIBA Instruments Incorporation, California, USA.) and CPK level was measured using a blood chemistry analyzer (Olympus AU 400, Olympus, Tokyo, Japan). All these hematological parameters were measured to assess the normal health status of horses before inclusion in this study (data not shown).

Oxidative stress markers

Malondialdehyde (MDA)

The concentration of plasma MDA was determined using thiobarbituric acid as described previously (Luangaram et al. 2007; Nakmareong et al. 2011) and the results were presented as µM.

Protein carbonyl content

Protein carbonyl concentration was measured using a previously described method (Nakmareong et al. 2012). In brief, plasma samples were incubated with 15mM DNPH. The protein was precipitated with TCA. The pellet was washed with ethyl acetate and ethanol to remove free DNPH and lipid contaminants, and then dissolved in 6M guanidine. The carbonyl content was measured with an ELISA reader at 360nm. The quantity obtained is expressed as nmol/mg of protein.

Antioxidant markers

Superoxide Dismutase (SOD)

SOD was measured spectrophotometrically using the superoxide dismutase activity assay kit (Merck KGaA, Darmstadt, Germany) according to the manufacturer's protocol. The SOD activity is expressed as a percentage inhibition.

Catalase

The catalase activity in plasma was measured by Goth's colorimetric method as described previously by Goth (1991). The absorbance of the supernatant was measured at 405nm, and the data were presented as kU/L.

Nitrate/nitrite (NOx)

The level of plasma NOx, the end products of NO metabolism, was measured using an enzymatic conversion method as described previously (Nakmareong et al. 2011). Briefly, samples were deproteinized by ultrafiltration using centrifugal concentrators (Nanosep^{TM}, Pal Filtron, USA), then the supernatant was treated with converting enzymes and reacted with a Griess solution for 15 minutes. After incubation, the absorbance of samples was measured at 540nm with a microplate spectrophotometer (Epoch^{TM} 2 Microplate Spectrophotometer, BioTek). The data were given as µM.

Statistical Analysis

The data were analyzed using the normality test in SPSS for Windows version 26. All data are expressed as the mean ± standard error of the mean (SEM). Statistical difference among groups was done using Two-way repeated measures analysis of variance (ANOVA). The level of significance was determined at $P<0.05$.

RESULTS

Kinetic changes of oxidative stress and antioxidant markers before and after exercise

MDA levels were examined before-, just after-, and 3 hours after-exercise in both race and endurance horses. As shown in Fig. 1, in both types of sport horses, MDA levels were significantly increased just after exercise. The degree of MDA increase just after exercise was much more dominant in race horses than in endurance horses. In both types of horses, MDA levels returned to almost normal levels 3 hours after exercise. The protein carbonyl levels were significantly increased at just, and 3 hours after exercise when compared to those before exercise in both types of sport horses ($P<0.05$). This result is shown in Fig. 2. In race horses, the protein carbonyl levels did not significantly differ between just after and 3 hours after exercise. In contrast, in endurance horses, the highest level of protein carbonyl was observed 3 hours after exercise ($P<0.05$; Fig. 2).

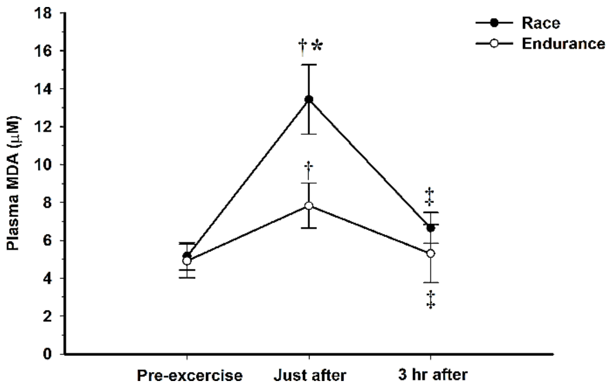

Fig. 1: Plasma MDA levels compared between the period of exercise and types of sports. $^{\dagger}P<0.05$ compared to pre-exercise, $^{\ddagger}P<0.05$ compared to post-exercise, $^{*}P<0.05$ compare to endurance at the same time.

In race horses, catalase levels increased significantly ($P<0.05$; Fig. 3) just after exercise, but returned to the normal level 3 hours after exercise. In the endurance horse, catalase levels did not change significantly just or 3 hours after exercise ($P>0.05$; Fig. 3). In race horses, SOD levels were significantly increased just and 3 hours after exercise compared with that of before-exercise ($P<0.05$; Fig. 4), although SOD levels just and 3 hours after exercise were not statistically different from each other ($P>0.05$; Fig. 4). In endurance horses, SOD level was significantly increased just after exercise, but returned to the pre-exercise level at 3 hours after exercise ($P<0.05$; Fig. 4).

Kinetic changes of plasma NOx levels before and after exercise in both types of horses are shown in Fig. 5. As can be seen in the graph, in part due to relatively large variation, any significant difference of plasma NOx levels was not observed during the period of times in both types of sport horses ($P>0.05$), although it tended to decrease just after exercise in both types of horses, especially in endurance horses ($P>0.05$; Fig. 5).

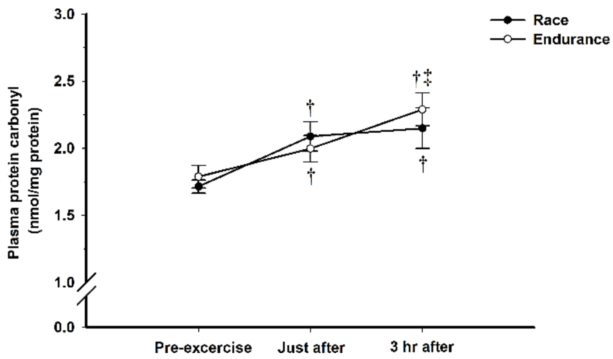

Fig. 2: Plasma protein carbonyl levels compared between the period of exercise and types of sports. $^{\dagger}P<0.05$ compared to pre-exercise, $^{\ddagger}P<0.05$ compared to post-exercise.

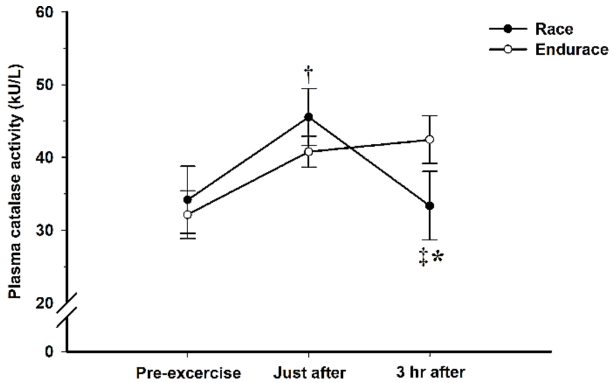

Fig. 3: Plasma catalase levels compared between the period of exercise and types of sports $^{\dagger}P<0.05$ compared to pre-exercise, $^{\ddagger}P<0.05$ compared to post-exercise, $^{*}P<0.05$ compared to endurance at the same time.

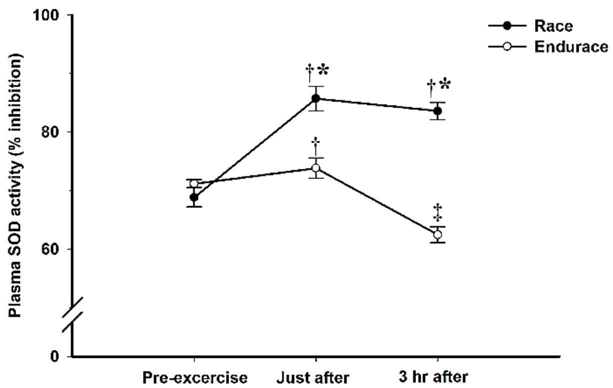

Fig. 4: Plasma SOD levels in plasma compared between the period of exercise and types of sports $^{\dagger}P<0.05$ compared to pre-exercise, $^{\ddagger}P<0.05$ compared to post-exercise, $^{*}P<0.05$ compared to endurance at the same time.

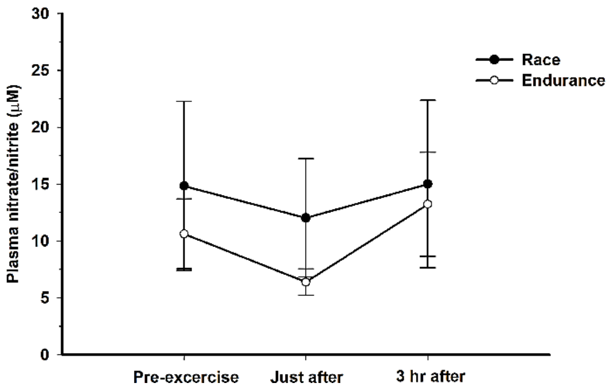

Fig. 5: Plasma nitrate/nitrite levels compared between the period of exercise and the type of sports.

Comparison of oxidative stress and antioxidant markers between race and endurance horses

MDA levels between race and endurance horses were not significantly different at pre-exercise and 3hours post-exercise ($P>0.05$; Fig. 1), but MDA levels of the race horses was significantly higher than that of the endurance horses just after exercise ($P<0.05$; Fig. 1). In contrast, the protein carbonyl level was not significantly different between both type of horses at any time examined ($P>0.05$; Fig. 2).

For antioxidant markers, the catalase level of the endurance horses was significantly higher than that of the race horses at 3hours post-exercise ($P<0.05$; Fig. 3), but no statistical differences were observed between the type of horses at pre- and just after exercise ($P>0.05$; Fig. 3). The SOD levels at post-exercise just and 3hours post-exercise in race horses were significantly higher than those of the endurance horses ($P<0.05$; Fig. 4). No statistical difference was observed between race and endurance horses at pre-exercise ($P>0.05$; Fig. 4). Moreover, the plasma NOx levels were shown no significantly differences between type of sports ($P>0.05$; Fig. 5). However, just after exercise, the plasma nitrate/nitrite levels in endurance horses tended to be, although statistically not significant ($P>0.05$; Fig. 5), lower than that in race horses.

DISCUSSION

Our study demonstrated that the increase in oxidative stress and the upregulation of antioxidant enzymes are observed in both race and endurance horses after exercise. Plasma protein carbonyl, an oxidative stress marker, was not decreased 3 hours after exercise in both race and endurance horses. In contrast, plasma NOx was not significantly different between both types of sport horses during the study periods of exercise. A higher oxidative stress response was observed in race horses than in endurance horses.

An escalation of $O_2^{\bullet-}$ in horses can be derived from exercise, which is attributed to the increased blood circulation enabling the transportation of oxygen, an increase in the functioning of mitochondria, and the elevation of the activity of phagocytic cells (Fazio et al. 2016). Moreover, during physical activity and exercise, muscle spasm, accompanied by muscle fiber damage, initiates an inflammatory response to exercise and activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes, leading to an increase in the production of ROS (Miglio et al. 2024). The increase of $O_2^{\bullet-}$ causes lipid peroxidation, which could be determined by measuring the MDA level. In addition, $O_2^{\bullet-}$ also reacts with protein side chains within the aldehyde and ketone groups, leading to the formation of protein carbonyls. These carbonylated proteins are relatively stable and are often used as indicators for measuring oxidative stress levels (Dalle-Donne et al. 2003; Davies 2016). The results in this study revealed the increase of lipid peroxidation by exercise in both race and endurance horses, as indicated by the increase of plasma MDA and protein carbonyl levels. The MDA level was increased just after exercise compared to pre-exercise and returned to normal at 3 hours after exercise, corresponding with the results reported by Ankur et al. (2018) and Bottegaro et al. (2018). Same as MDA, the protein carbonyl level of both types of horses was increased just after exercise, corresponding with the study by Fogarty et al. (2011). However, different from MDA, the protein carbonyl level remained higher at 3 hours post-exercise in both types of horses, especially in endurance horses. These findings are consistent with the previous study in that protein carbonyl content increased just after exercise and increased further at 1 hour after exercise. A notable decline of protein carbonyl levels was found 24 hours after exercise (Mami et al. 2019). In addition, Liu et al. (2000) demonstrated that the accumulation of protein carbonyls tends to increase in slow-twitch muscle fibers during chronic exercise.

In this study, when the kinetic changes of the MDA levels before and after exercise of race horses and endurance horses were compared, the former was much higher than that of the latter. This result agrees with the previous work of Alessio et al. (1988) in that MDA level after high-intensity exercise was higher than that after moderate-intensity exercise. Furthermore, alongside engaging in high-intensity acute exercise, there is a possibility of triggering ischemic reperfusion injury. This serves as a stimulus to phagocytic cells to induce a substantial increase in the quantity of $O_2^{\bullet-}$ (McMichael 2004). Additionally, the studies by Liu et al. (2000) and Cunningham et al. (2005) revealed an increase in MDA levels in fast-twitch muscle fibers during exercise, indicating that the engagement in exercise activity that stimulates fast-twitch muscle fibers is a contributing factor to the accumulation of ROS in the body.

In contrast, in the present study, protein carbonyl levels of race horses and endurance horses were not significantly different before and after exercise. This finding is consistent with the study by Shi et al. (2007), where no significant difference in serum protein carbonyl levels was observed between anaerobic and aerobic exercise. A previous study by Fogarty et al. (2011) indicated that during exercise, the body tends to rely more on fat as an energy source than carbohydrates in a fasted state, resulting in an increase in lipid peroxidation more than protein oxidation. Moreover, the protein carbonyl levels during exercise may be influenced by many factors such as type of exercise, body temperature, nutritional status, and exercise-induced injuries (Delwing-de Lima et al. 2018).

Exercise-induced ROS generation leads to the activation of the antioxidant system in the body to counteract it. Catalase is one of the enzymes that can eliminate ROS from the body by converting H_2O_2 into oxygen and water (Terblanche 2000; Mami et al. 2019; Vasileiadou et al. 2023). Moreover, it is the major enzyme found in aerobic cells (Ankur et al. 2018). In race horses, the catalase levels just after exercise were significantly higher than those of the pre-exercise period, but rapidly decreased at 3 hours post-exercise. This is consistent with the previous study of Mami et al. (2019) in that catalase activity increased just after exercise and tended to decrease at 1 hour after exercise. In contrast to race horses, in the present study, the catalase levels of the endurance horse did not significantly alter before and after exercise. This aligns with the study of Parker et al. (2017), who showed the lower level of catalase in continuous moderate-intensity exercise than in high-intensity exercise. Moreover, the study by Ankur et al. (2018) showed no significant difference in catalase levels between pre- and post-exercise of horses that performed endurance training.

The SOD is classified as a primary enzyme used to eliminate $O_2^{\bullet -}$ (Yan et al. 2020). In the present study, in the race horses, SOD levels significantly increased just after exercise and remained at a high level until 3 hours post-exercise. This is in agreement with Hitomi et al. (2008), who reported that SOD was increased after exercise. Likewise, Mami et al. (2019) showed that SOD activity was increased 1 hour after exercise and returned to baseline 24 hours after exercise. The increase in SOD activity during exercise is due to the body's need to eliminate $O_2^{\bullet -}$; SOD can remain in the serum for a prolonged duration after exercise (Yan et al. 2020) and is associated with an increase in resistance to oxidative stress (Fielding et al. 1997; Mami et al. 2019). In this study, however, the increased SOD level of endurance horses returned to baseline level at 3 hours post-exercise. This is probably due to the increase in SOD activity just after exercise, which is sufficient to eliminate $O_2^{\bullet -}$ generated by exercise. No changes in SOD activity and antioxidant defense have been reported in the subjects that were trained and fit for the exercise test, indicating an adaptation of antioxidant systems to the specific condition of regular training (Niess et al. 1999; Lamprecht et al. 2012).

When catalase levels were compared between race horses and endurance horses, the latter exhibited higher catalase levels than the former 3 hours after exercise. This may be due to the adaptive response of catalase in peroxisomes and also stimulation of mitochondria by endurance training (Votion et al. 2012; D'Angelo et al. 2020). Terblanche (2000) reported that high-intensity exercise tended to increase H_2O_2 levels during exercise, and thus, in this study, catalase activity increased just after exercise to eliminate H_2O_2 into water. However, 3 hours after exercise, oxidative stress decreased as indicated by the reduction of plasma MDA. This may account for the decrease in catalase activity at 3 hours post-exercise in race horses in this study. In this study, SOD levels of race horses were higher than endurance horses both just and 3 hours after exercise. This result was corroborated by the study of Daud et al. (2006), who demonstrated that SOD activity was increased with the intensity of exercise.

NO is a mediator of various biological processes (Mueller et al. 2024). In muscle physiology, NO is involved in cell-cell interaction and metabolism, modulates muscle contraction, inhibits force output by altering excitation-contraction coupling (Reid 1998; Kumar et al. 2022) and acts as a relevant messenger in myogenesis for muscle repair after muscle injury (De Palma et al. 2012; Sibisi et al. 2022). Under physiological conditions, NO is produced at low levels by neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS). In the skeletal muscles, expression of nNOS increased by muscle activity, crush injury and ageing, whereas that of eNOS is increased by shear stress and chronic exercise (De Palma et al. 2012; Laird et al. 2025). The bioavailability of NO depends on the balance between the NO production and NO removal due to the interaction between NO and ROS (Dyakova et al. 2015). In this study, we found that there are no significant differences in the plasma NOx level among the timing periods and between the types of exercise. However, when compared between periods of exercise, there was a trend to decrease just after exercise in both race and endurance horses. These may correlate with the increase of plasma MDA and protein carbonyl just after exercise since NO reacts with $O_2^{-\bullet}$ to generate potent free radical peroxynitrite ($ONOO^-$), which induces oxidative damage. Therefore, reduction of NO bioavailability together with an increase in SOD and catalase activity is critical to counter oxidative stress just after exercise.

Several studies established that exercise-induced oxidative stress depends on the exercise type, intensity, and duration. Our results, therefore, fit in this idea that the intensity of exercise affects an adaptive response to oxidative stress; race horses are exposed to a higher degree of oxidative stress, leading to significant upregulation of antioxidants, whereas endurance horses show a lower accumulation of ROS and an antioxidant response during exercise. The exercise patterns of race horses induce a higher degree of oxidative stress and are prone to oxidative damage than the endurance exercise, if inappropriately trained. According to our findings at 3 hours post-exercise of both race and endurance horses, the rapid recovery of plasma MDA and antioxidant enzymes to baseline possibly indicates the horse's ability to adapt to oxidative stress when exposed to the exercise protocol, showing that they were trained carefully.

This study has some limitations that should be taken into consideration. Since the samples were collected from horses undergoing exercise resembling actual competition, it was not feasible to measure variables such as VO_{2max}. Nonetheless, in this study, exercise patterns and exercise intensity levels were referred to the standards that have been reported by Rivero and others (Rivero 2007; Hinchcliff et al. 2008; Hodgson et al. 2013). The referenced exercise intensity corresponds to the maximum physical fitness levels in each type of sport. In addition, it should be considered that circulating markers of oxidative stress and antioxidants may not reflect those within the muscle, which is the major site of ROS production during exercise.

Conclusion

The results of this study demonstrated that exercise-induced oxidative stress in both types of sport horses, with the highest degree of oxidative stress in race horses. However, both types of sport horses showed the induction of adaptive responses to oxidative stress, as evidenced by the increase in antioxidants levels during exercise. Since the exercise intensity and the different types of muscle fiber may generate varying levels of ROS and antioxidant response during exercise, additional studies are needed to clarify the accumulation of ROS in different muscle fiber types to obtain a better understanding of the oxidative and antioxidative response during exercise in sport horses.

DECLARATIONS

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: The data is available from the corresponding author(s) based upon a reasonable request.

Ethics Statement: The experimental protocols involving animals were approved by the Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU-84/64), based on the Ethics of Animal Experimentation of the National Research Council of Thailand.

Author's Contribution: Suwannachot P and Pinyanusorn P: Designed the study. Pinyanusorn P, Phutthachalee S, Senaphan K and Suwannachot P: Conducted the experiment, sample and data collection, and laboratory works. Pinyanusorn P, Phutthachalee S, Senaphan K and Suwannachot P: Analyzed the data. All authors: Wrote the original draft of the manuscript as well as read, reviewed and approved the final manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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