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ASSESSMENT OF THE LEVELS OF OXIDATIVELY MODIFIED PROTEINS IN THE BLOOD PLASMA OF MARES AND STALLIONS OF PONIES INVOLVED IN RECREATIONAL HORSEBACK RIDING

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Introduction. Recreational horse riding has become an increasingly popular leisure activity and sport worldwide, attracting participants of all ages and skill levels [Dąbek et al., 2015]. While the physical and psychological benefits of riding are well recognised, the potential impact of this activity on the health and well-being of participating equestrians remains a topic of interest and

concern [Janczarek and Wilk, 2017]. In particular, oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defence mechanisms, has been implicated in several equine health conditions, including musculoskeletal injuries, metabolic disorders and immune dysfunction [Wong et al., 2012].

Oxidatively modified proteins (OMPs) serve as biomarkers of oxidative stress and reflect the extent of protein damage induced by ROS-mediated reactions. Assessment of OMP levels in biological samples, such as blood, plasma, and erythrocytes provides valuable insight into the systemic oxidative status and overall health of individuals [Dalle-Donne et al., 2005]. In the context of recreational equestrianism, where riders are exposed to physical exertion, environmental stressors and dietary factors, monitoring OMP levels may provide a means of assessing the physiological response to equestrian activities and identifying potential risk factors for oxidative damage [Balogh et al., 2001]. Despite the growing interest in the relationship between oxidative stress and equine health, little research has focused specifically on assessing OMP levels in horses participating in recreational equestrian activities [Williams, 2016]. Furthermore, the influence of sex on oxidative stress biomarkers in horses remains poorly understood, with potential differences in antioxidant capacity and susceptibility to oxidative damage between mares and stallions or geldings [Andriichuk and Tkachenko, 2017; Kurhaluk et al., 2022].

Therefore, the present study aims to investigate the levels of oxidatively modified proteins in the plasma of mares and pony stallions involved in recreational riding. By quantifying OMP levels and examining their association with riding intensity, duration and other relevant factors, we aim to elucidate the impact of recreational riding on the oxidative status of equine athletes. In addition, we aim to investigate potential sex differences in oxidative stress biomarkers and gain insight into the physiological responses of male and female horses to equestrian activities. This study focuses on the seasonal variability in the levels of oxidatively modified proteins in the plasma of Shetland pony mares and stallions before and after exercise. We analysed the effect of season and exercise on the levels of aldehydic (AD) and ketonic (KD) derivatives of oxidatively modified proteins (OMP) in the blood plasma of Shetland pony mares and stallions involved in recreational riding in the Central Pomeranian region (Pomeranian Voivodeship, northern part of Poland).

Materials and methods. ***Horses.*** The experiments were conducted in accordance with the guidelines of the Council of the European Union and current legislation. Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) aged 6.5 ± 1.4 years from the Central Pomeranian region of Poland (Strzelinko, N54º30'48.0" E16º57'44.9") were used in this study. All horses participated in recreational riding. Horses were housed in individual stalls with feed (hay and oats) provided twice daily at 08:00 and 18:00 and water available *ad libitum*. All horses underwent a thorough clinical examination and haematological, biochemical and vital parameters were within reference ranges. Females were not pregnant.

Exercise. The training started at 10:00 am, lasted 1 hour and consisted of a cross-country ride consisting of walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min) and walking (10 min).

Blood samples. Blood was collected from the animals' jugular veins in the morning, 90 minutes after feeding, while the horses were in the stable (between 8.30 and 10 am) and immediately after the exercise test (between 11 am and 12 am). Blood samples were taken once per season for one year: summer and winter. Blood was stored in VACUETTE™ K_3EDTA tubes and kept on ice until centrifuged at 3,000 rpm for 10 minutes. The plasma was removed. The erythrocyte suspensions (one volume) were washed three times with five volumes of PBS (pH 7.35) and centrifuged at 3,000 rpm for 5 minutes.

Assay of carbonyl derivatives of protein oxidation. The level of oxidatively modified proteins (OMP) was assessed by the content of protein carbonyl derivatives in the reaction with 2,4-dinitrophenylhydrazine (DNFH) according to the method described by Reznick and Packer (1994) with some modifications. Aldehydic (AD) and ketonic (KD) derivatives of OMP were determined in the samples. Optical density was measured at a wavelength of 370 and 430 nm with the molar extinction coefficient of 22,000 $M^{-1} \cdot cm^{-1}$.

Statistical analysis. The results were expressed as mean ± S.D. Significant differences between means were measured using a multiple range test at least $p < 0.05$. Data not normally distributed were log transformed. Statistical tests with 95% confidence intervals ($\alpha = 0.05$) were used to determine the significance of differences between the parameters studied. The data were tested for homogeneity of variance using Levene's test and normality using the Kolmogorov-Smirnov test. Basic statistical analysis (significance of regression slopes, analysis of variance for significance) was performed using the STATISTICA 13.3 package (TIBCO Software Inc., USA).

Results. The results of our study showed that the levels of aldehydic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of Shetland ponies in summer were statistically non-significantly decreased after exercise to (21.67 ± 1.75 nmol·mL^{-1}) and (22.22 ± 1.99 nmol·mL^{-1}) compared to the pre-exercise state (22.0 ± 1.03 nmol·mL^{-1}) and (22.46 ± 1.38 nmol·mL^{-1}), respectively. Similarly, in winter, the levels of aldehydic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of the Shetland pony were statistically significantly reduced after exercise to (20.20 ± 1.45 nmol·mL^{-1}) and (19.41 ± 1.24 nmol·mL^{-1}) respectively, compared to the pre-exercise state (21.62 ± 0.49 nmol·mL^{-1}) and (22.45 ± 1.17 nmol·mL^{-1}), respectively. The percentage of decrease was 6.57% ($p < 0.05$) for mares and 13.54% ($p < 0.05$) for stallions.

Before exercise, the levels of aldehydic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of the Shetland pony in summer were similar to those obtained in winter. After exercise, the levels of aldehydic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of the Shetland pony in winter were lower [(20.20 ± 1.45 nmol·mL^{-1}) and (19.41 ± 1.24 nmol·mL^{-1})] compared to the values obtained in the summer, i.e. (21.67 ± 1.75 nmol·mL^{-1}) for mares and (22.22 ± 1.99 nmol·mL^{-1}) for stallions. The percentage of decrease was 6.78% ($p < 0.05$) for mares and 12.65% ($p < 0.05$) for stallions.

Similarly, the levels of ketonic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of Shetland ponies in summer were reduced after exercise to (26.6 ± 1.24 nmol·mL^{-1}) and (26.36 ± 1.96 nmol·mL^{-1}), respectively, compared with the pre-exercise state (27.67 ± 0.50 nmol·mL^{-1}) and (26.97 ± 0.99 nmol·mL^{-1}). Similarly, in winter, the levels of ketone derivatives of oxidatively modified proteins in the plasma of both mares and stallions of the Shetland pony were statistically non-significantly decreased after exercise to (26.74 ± 2.13 nmol·mL^{-1}) and (25.86 ± 2.60 nmol·mL^{-1}), respectively, compared to the pre-exercise state (26.7 ± 0.95 nmol·mL^{-1}) and (26.94 ± 1.08 nmol·mL^{-1}), respectively. The percentage decrease in stallions was 4% ($p > 0.05$). Pre-exercise levels of ketonic derivatives of oxidatively modified proteins in the plasma of Shetland pony mares and stallions in summer were similar to those in winter. After exercise, the levels of ketone derivatives of oxidatively modified proteins in the plasma of Shetland pony stallions in winter were lower (25.86 ± 2.60 nmol·mL^{-1}) compared to the values obtained in the summer, i.e. (26.36 ± 1.96 nmol·mL^{-1}). The percentage of decrease was 1.9% ($p > 0.05$).

Thus, training activities are associated with changes in OMP levels, suggesting reduced oxidative stress in equine athletes exposed to physical exertion and environmental stressors. Importantly, our results suggest that season may influence the extent of oxidative damage, with higher levels of OMP observed in horses undergoing training activities during the summer. These findings underscore the importance of considering season as a factor contributing to oxidative stress in recreational riding horses and highlight the need for appropriate management strategies to mitigate the negative effects of prolonged or intense exercise on equine health.

In our previous study, we also investigated the effect of a moderate-intensity exercise test on oxidative stress biomarkers, antioxidant enzyme activity and erythrocyte osmotic resistance in well-trained equine athletes [Andriichuk et al., 2016]. Regular exercise induces the activation of antioxidant enzymes and may reduce oxidative stress in athletic horses. The exercise test attenuated oxidative stress in horses and was accompanied by a significant decrease in lipid peroxidation and oxidatively modified proteins in erythrocytes after exercise. Our data suggest that biomarkers of oxidative stress and enzymatic antioxidant defences can be used to monitor fitness levels, health benefits and performance in equine athletes [Andriichuk et al., 2016].

Conclusions. Our study revealed potential sex differences in OMP levels, with female horses (mares) exhibiting lower oxidative stress biomarkers compared to male horses (stallions). This sex dimorphism in oxidative stress response warrants further investigation to elucidate the underlying mechanisms and implications for equine health and performance. Overall, our findings highlight the importance of monitoring oxidative stress biomarkers in recreational riding horses as a means of assessing physiological responses to riding activities and identifying potential risk factors for oxidative damage. By understanding the oxidative status of equine athletes, veterinarians, trainers and riders can implement targeted interventions to support the health and well-being of horses, optimise performance and promote longevity in recreational riding horses.

Understanding the oxidative status of horses involved in recreational riding is essential for optimising management practices, promoting animal welfare and improving performance outcomes. By elucidating the relationship between riding activities and biomarkers of oxidative stress, this research will contribute to the development of evidence-based strategies to support the health and well-being of recreational equine athletes.

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