DOI 10.32900/2312-8402-2023-129-4-15
UDC 636.12: 577.112.4

PHOTOPERIOD-DEPENDENT ALTERATIONS IN OXIDATIVELY MODIFIED PROTEINS IN THE PLASMA OF SHETLAND PONY MARES AND STALLIONS INVOLVED IN RECREATIONAL HORSEBACK RIDING

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This study focuses on the photoperiod-induced variability in the levels of oxidatively modified proteins in the plasma of Shetland pony mares and stallions before and after exercise. We have analyzed the effect of photoperiods and exercise on the levels of aldehydic (AD) and ketonic (KD) derivatives of oxidatively modified proteins (OMP) in the blood of Shetland pony mares and stallions involved in recreational horseback riding in the central Pomeranian region (Pomeranian Voivodeship, northern part of Poland). Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) aged 6.5 ± 1.4 years old were used in this study. All horses participated in recreational horseback riding. Training started at 10:00 AM, lasted 1 hour, and consisted of a ride of cross country by walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min), and walking (10 min). Blood was drawn from the jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (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. The level of oxidatively modified proteins (OMP) was evaluated by the content of protein carbonyl derivatives in the reaction with 2,4-dinitro-phenylhydrazine (DNFH). There was a statistically significant reduction in the levels of aldehydic derivatives of OMP in the plasma of ponies during the winter photoperiods only after exercise in both sexes. A decrease in the levels of ketonic derivatives of OMP in the summer photoperiod was observed. These changes were observed independently of the sex and only after exercise. Levels of aldehydic and ketonic derivatives of OMP varied depending on the photoperiod and exercise session in our studies. These changes were dependent on the baseline levels of the enzymatic and non-enzymatic antioxidant defense systems in the ponies, which differed between the mares and the stallions (statistically significant differences in the winter period) both before and after exercise (winter).

Keywords: **oxidatively modified proteins, plasma, exercise, seasonal alterations, photoperiods, Shetland ponies, mares and stallions.**

The most important environmental factor is the duration of daylight hours, which has a strong impact on physiological processes [39]. According to modern concepts, melatonin in photoperiodic animals is a powerful regulator of the activity of the endocrine system and the seasonal rhythm of many processes [17]. Animals make use of changes in photoperiod to adapt their physiology to the forthcoming breeding season [15]. The pineal gland is a neurochemical transducer that converts nerve signals of light perception into endocrine signals [20]. Its main hormone, melatonin, is involved in the coordination of circadian rhythms in mammals and is responsible for controlling seasonal rhythms [40], determining seasonal differences in body weight, appetite, coat, metabolic rate, and other physiological characteristics [18, 19, 25, 37], including reproductive function [11, 24, 36]. Seasonal changes in night length (scotoperiod) induce parallel changes in the duration of melatonin secretion (which occurs exclusively at night) so that it is longer in winter and shorter in summer. These changes in the duration of nocturnal melatonin secretion, in turn, trigger seasonal changes in behavior [40]. At both physiological and pharmacological concentrations, melatonin attenuates and counteracts oxidative stress and regulates cellular metabolism [36]. The study of seasonal levels of oxidative stress and antioxidant defenses in these conditions is an important issue in animal adaptation to season-induced changes. High ambient temperature has been reported to increase oxidative stress by increasing lipid peroxidation and decreasing antioxidant defense in transition dairy cows [6]. Exposure of adult female Wistar rats to high ambient temperature and humidity of the hot season increases neuroendocrine stress, and oxidative stress and decreases antioxidant defense in them [7].

Recently, seasonal fluctuations in the antioxidant status determined by association with final and initial substrates of energy metabolism in the blood of horses have attracted increasing interest as a function of their sex and ability to exercise [28, 35]. Investigation of the photoperiod-induced (seasonal) features of the processes of lipid peroxidation and oxidative modification of proteins together with basic antioxidants, such as antioxidant protection enzymes, is particularly useful for the prevention and treatment of cardiovascular, cerebrovascular, and laminitis diseases in ponies. These animals are characterized by a broad spectrum of physiological and biochemical characteristics of the functioning of various metabolic systems, which has been convincingly shown in a number of studies [8-10].

In horses, winter-associated hypometabolism has been interpreted as the capacity for seasonal adaptation to environmental conditions with the aim to reduce energy expenditure [8, 33]. Domesticated horses seem to have maintained the capacity for seasonal adaptation to environmental conditions through seasonal fluctuations in their metabolic rate [8]. In addition to day length, factors such as environmental temperature further modulate seasonal changes in different organ systems. The environmental temperature may also play an important adjuvant zeitgeber for the timing of the first ovulation of estrus in the mare [14]. During winter, free-living herbivores are often exposed to a reduced energy supply at the same time that energy needs for thermoregulation increase. Several wild herbivores as well as robust horse breeds reduce their metabolism during times of the low ambient temperature and food shortage [9]. Ponies acclimatize to different climatic conditions by changing their metabolic rate, behavior, and some physiological parameters. When exposed to energy challenges, ponies, like wild herbivores, exhibited hypometabolism and nocturnal hypothermia [10].

The oxidative modification of proteins plays a key role in the molecular mechanisms of oxidative stress and is the trigger mechanism for the oxidative destruction of other molecules (proteins, lipids, DNA) in the cell [16]. Since the oxidative modification of proteins is selective and specific and its products are markers of early oxidative stress, further studies of this process will contribute to improving the diagnosis and treatment of a number of pathological conditions in horses. The products of oxidative modification of proteins themselves, as well as lipid peroxidation products, have a cytotoxic effect. It was previously shown that during the cytotoxic action of oxidatively modified proteins (OMP), peroxide reactions are induced, leading to the modification of a number of the most important biomolecules in membranes and nuclear chromatin, primarily proteins and DNA [12]. This study focuses on the photoperiod-induced variability in the levels of oxidatively modified proteins in the plasma of Shetland pony mares and stallions before and after exercise. We have analyzed the effect of photoperiods and exercise on the levels of aldehydic (AD) and ketonic (KD) derivatives of oxidatively modified proteins (OMP) in the blood of Shetland pony mares and stallions involved in recreational horseback riding in the central Pomeranian region (Pomeranian Voivodeship, northern part of Poland).

Materials and methods. Horses. The experiments were conducted in compliance with the Guidelines of the European Union Council and the current laws. Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) aged 6.5 ± 1.4 years old from the central Pomeranian region in Poland (Strzelinko, N54º30'48.0" E16º57'44.9") were used in this study. All horses participated in recreational horseback riding. The horses were housed in individual boxes, with feeding (hay and oat) provided twice a day at 08.00 and 18.00 h and water available ad libitum. All horses were thoroughly examined clinically and screened for hematological, biochemical, and vital parameters, which were within reference ranges. The females were non-pregnant.

Exercise. Training started at 10:00 AM, lasted 1 hour, and consisted of a ride of cross country by 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 drawn from the jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (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 tubes with K_3-EDTA and 3.8% sodium citrate and kept on the ice until centrifugation at 3,000 rpm for 10 minutes. The plasma was removed. The erythrocyte suspensions (one volume) were washed with five volumes of PBS (pH 7.35) three times and centrifuged at 3,000 rpm for 5 minutes.

Assay of carbonyl derivatives of protein oxidation. The level of oxidatively modified proteins (OMP) was evaluated by the content of protein carbonyl derivatives in the reaction with 2,4-dinitro-phenylhydrazine (DNFH) using the method described by Reznick and Packer (1994) and Levine and co-workers (1994). Proteins were precipitated with cold 20% trichloroacetic acid (TCA). After adding 1 mL of 2.5 mM 2,4-DNFH in 2.5 N HCl, the samples were incubated for 1 hour at room temperature. A 2.5 N solution of HCl was added to the control samples. The samples were centrifuged at 3,000 rpm for 15 min, and residual amounts of 2,4-DNFH were removed by washing the precipitate with an ethanol: ethyl acetate solution (1:1). 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 $M^{-1} \cdot cm^{-1}$ [32].

Statistical analysis. The results were expressed as mean ± S.D. Significant differences between the means were measured using a multiple range test at min. $p < 0.05$. Data with no normal distribution were log-transformed. Statistical tests with 95% confidence intervals ($\alpha = 0.05$) were applied to determine the significance of differences between the parameters studied [34]. The data were tested for homogeneity of variance using Levene's test, and normality was checked with the Kolmogorov-Smirnov test. The data of MANOVA analysis were also confirmed by the results of the sum-of-squares test (total SS model) *vs*. residual SS regarding the values of the multiple correlation analysis (R), the coefficient of determination (R^2), and its corrected form reduced by random errors (R^2 adjusted) in the data analysis. The basic statistical analysis (significance of regression slopes, analysis of variance for significance) was done using the

STATISTICA 13.3 package (TIBCO Software Inc.). We used the SS test to describe the share of all analyzed biomarkers of oxidative stress and biochemical parameters in the assessment of the antioxidant defenses (the F test and its significance) [34].

Results and discussion. We investigated the content of aldehydic (AD) and ketonic derivatives (KD) of OMP in the plasma of the Shetland ponies influenced by the three factors (photoperiods, sex, exercise). Levels of aldehydic and ketonic derivatives of oxidatively modified proteins in the plasma of Shetland pony mares and stallions before and after exercise in summer and winter are presented in Fig. 1.

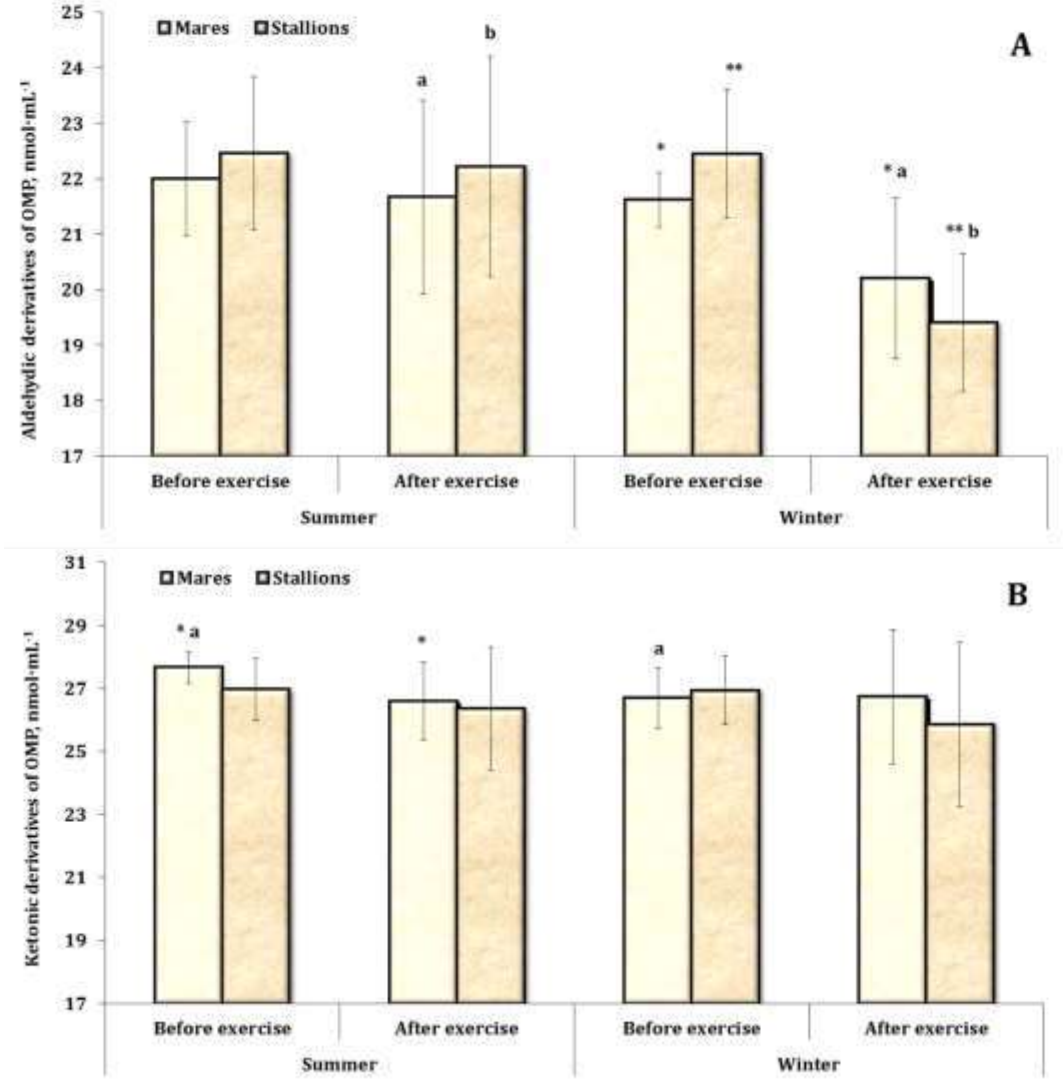

Fig. 1. Levels of aldehydic (A) and ketonic derivatives of oxidatively modified proteins (B) in the plasma ($nmol \cdot mL^{-1}$) of Shetland pony mares (n = 11) and stallions (n = 10) before and after exercise in summer and winter.

Statistically significant differences (p < 0.05) in the following dependency groups according to the ANOVA post-hoc Tukey (HSD) test:

** – between values obtained before and after exercise in mares;*

*** – between values obtained before and after exercise in stallions;*

a – between values obtained in mares after exercise in winter and summer;

b – between values obtained in stallions after exercise in winter and summer.

Results of our study revealed that levels of aldehydic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of Shetland pony in summer were statistically non-significant decreased after exercise to (21.67 ± 1.75 $nmol \cdot mL^{-1}$)

and (22.22 ± 1.99 nmol·mL^{-1}) compared to the state before exercise (22.0 ± 1.03 nmol·mL^{-1}) and (22.46 ± 1.38 nmol·mL^{-1}), respectively. Similarly, in the winter, levels of aldehydic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of Shetland pony were statistically significantly decreased after exercise to (20.20 ± 1.45 nmol·mL^{-1}) and (19.41 ± 1.24 nmol·mL^{-1}) compared to the state before exercise (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 (Fig. 1A).

Before exercise, levels of aldehydic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of Shetland pony in summer were similar to the values obtained in the winter. After exercise, levels of aldehydic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of Shetland pony in winter were less [(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 (Fig. 1A).

Similarly, levels of ketonic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of Shetland pony in summer were decreased after exercise to (26.6 ± 1.24 nmol·mL^{-1}) and (26.36 ± 1.96 nmol·mL^{-1}) compared to the state before exercise (27.67 ± 0.50 nmol·mL^{-1}) and (26.97 ± 0.99 nmol·mL^{-1}), respectively. Similarly, in the winter, levels of ketonic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of 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}) compared to the state before exercise (26.7 ± 0.95 nmol·mL^{-1}) and (26.94 ± 1.08 nmol·mL^{-1}), respectively. The percentage of decrease was 4% ($p > 0.05$) for stallions (Fig. 1B).

Before exercise, levels of ketonic derivatives of oxidatively modified proteins in the plasma of both mares and stallions of Shetland pony in summer were similar to the values obtained in the winter. After exercise, levels of ketonic derivatives of oxidatively modified proteins in the plasma of stallions of Shetland pony in winter were less (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% ($p > 0.05$) (Fig. 1B).

The changes in levels of ketonic derivatives of oxidatively modified proteins were independent of the sex and were observed only after exercise. It was noted that the aldehydic derivatives of OMP in the plasma of the Shetland ponies were distributed statistically significantly $F_{15.152} = 11.91$ ($p = 0.000$). There were statistically significant differences in levels of aldehydic derivatives of OMP as a function of sex and physical activity, as described below, at different photoperiods. In the mare group after exercise, these relationships were statistically significant ($p = 0.000$) in the spring-autumn and summer-autumn photoperiods. After exercise in the stallions, there were statistically significant differences in the level of aldehydic derivatives of OMP ($p < 0.05$) between spring and summer, spring and winter, summer and autumn, and summer and winter photoperiods. The data on the aldehydic derivatives of OMP were also confirmed by the results of the statistical ANOVA sum-of-squares test (total SS model) *vs.* residual SS regarding the values of the correlation coefficient ($R = 0.74$), the coefficient of determination (R^2=0.54), and its adjusted form $R^2_{ad} = 0.49$ at the value of F=11.91 ($p = 0.000$).

Another analyzed parameter was ketonic derivatives of OMP in plasma, which was shown to be statistically significant with $F_{15.152} = 5.07$ ($p = 0.000$). There were statistically significant sex differences in levels of the ketonic derivatives of OMP only during the post-exercise periods. In the group of mares after exercise, these differences appeared to be statistically significant in both the spring-autumn and autumn-winter seasons. After exercising, these differences in the levels of ketonic derivatives of OMP in the stallion group were statistically significant ($p = 0.000$) between the spring-autumn and summer-autumn photoperiods. These data were also confirmed by the results of the statistical ANOVA analysis of the total SS model *vs*. residual SS regarding the values of the correlation coefficient ($R = 0.58$), the coefficient of determination ($R^2 = 0.33$), and its adjusted form $R^2_{ad} = 0.28$ at the value of $F = 5.07$ ($p = 0.000$).

A growing body of evidence has suggested the beneficial role of regular physical exercise. Exercise mode, intensity, and duration, as well as the subject population tested, all can impact the extent of oxidation [13]. Regular moderate training appears beneficial for oxidative stress and health. Conversely, acute exercise leads to increased oxidative stress, although this same stimulus is necessary to allow an up-regulation in endogenous antioxidant defenses (hormesis) [27]. This beneficial effect is associated with the fact that exercise-induced reactive oxygen species (ROS) production is necessary for oxidative stress-related adaptations [29, 31]. Endurance exercise-related angiogenesis, up to a significant degree, is regulated by ROS-mediated activation of hypoxia-inducible factor 1α [31]. Some of these mechanisms may be related to the lower production of oxidants, up-regulation of antioxidant capacity, or even higher DNA repair activity. This specific signaling may be crucial for adaptive responses induced by physical exercise. In this sense, acute exercise increases the production of ROS in the muscle tissue, which can act as the initiation of two important redox-sensitive signaling pathways including nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) [13, 31]. Based on our results, it is possible to suggest that the reduced OMP level in the plasma of the pony mares and stallions involved in recreational horseback riding in winter could be attributed to exercise-induced adaptation for minimizing the transient oxidative stress caused by exercise (Fig. 1).

Exercise can induce the activity of the proteasome complex, which is involved in the degradation of oxidatively modified proteins [29]. Increased activity of proteasomes could be an important determinant of the rate of protein turnover and the remodeling of skeletal muscle after injury [30]. An increased rate of protein turnover during exercise decreases the accumulation of oxidative damage, thereby exerting a beneficial effect on the physiological function of proteins. The proteasome complex plays a critical role in this process [29]. Thus, the significant decrease in the aldehydic and ketonic derivatives of OMP in the plasma of ponies after the exercise is the result of exercise-induced adaptation, especially in the winter period (Fig. 1).

In our study, levels of oxidatively modified proteins after exercise were decreased compared to the state before exercise, especially in the winter season. Other trends were obtained in the study of researchers from the Department of Experimental Medicine and Public Health, University of Camerino, Camerino, Macerata, Italy. Martarelli and co-workers (2011) determined the combined effects of cold and exercise on oxidative stress during submaximal exercise. Sixteen amateur male cyclists pedaled at a constant speed corresponding to 85% of maximal HR as determined in normal conditions. Eight athletes pedaled indoors at 23 °C while 8 athletes pedaled outdoors at a temperature of 4-6 °C. The levels of reactive oxygen metabolites and plasma levels of antioxidants after exercise were evaluated. Results of these researchers revealed that performing a physical task in cold conditions increased free radical production, as demonstrated by the augmented levels of reactive oxygen metabolites and the concomitant decrease of plasma levels of antioxidants in outdoor cyclists as compared to indoor cyclists. The overall ANOVA and the post hoc comparisons revealed a significant exercise and temperature effect. The mean level of reactive oxygen metabolites in athletes who exercised indoors was significantly lower than that of the outdoor athletes. Moreover, the outdoor group presented plasma levels of antioxidants significantly lower than those of the indoor group. Thus, cyclists, football and rugby players, and runners are all affected by the elevation in oxygen radicals induced by cold and should take appropriate precautions, such as specific antioxidant integration [22]. Oxidative stress and antioxidant changes during a 24-hour mountain bike endurance exercise in master athletes were studied by Martarelli and Pompei (2009). Exposure to intense and prolonged exercise induced a marked increase in the reactive oxygen metabolite levels in master athletes, only partially counterbalanced by antioxidants in blood plasma [21].

On the other hand, Muñoz Marín and co-workers (2018) evaluated the oxidative stress, lipid peroxidation indexes, and antioxidant vitamins in long and middle-distance athletes during a sports season (12 months). Twenty-three long and middle-distance male athletes participated in this study. Basal malonic dialdehyde (MDA) as a biomarker of lipid peroxidation on plasma and antioxidant vitamins in plasma and erythrocytes were measured at four moments along the season (0, 3, 6, and 9 months). Fatty acid concentrations in erythrocytes were obtained to determine lipid peroxidation indexes. In plasma, vitamin C suffered significant decreases at 3 and 6 months compared with the beginning ($P < 0.01$), and an increase at 9 months, compared with 3 months. On the other hand, vitamin A level was significantly lower at 9 months compared with the other periods ($P < 0.01$ compared with 0 and 6 months; $P < 0.05$ compared with 3 months). In erythrocytes, significant decreases were observed in vitamin E during the season at 6 months and an increase from 6 to 9 months ($P < 0.05$). Vitamin A suffers a significant decrease in both for competitive periods, at 3 and 9 months, compared with the beginning of the season. The most of changes in lipid peroxidation indexes were produced in the first 3 months. Thus, physical training improves the antioxidant systems in order to reduce lipid peroxidation in trained athletes during the season [23].

In our previous study, we determined the level of oxidative stress biomarkers in sports horses involved in eventing before and after training [4]. All horses were in regular systematic training and had the same diet. Significant increases in the 2-thiobarbituric acid reactive substrates (TBARS) level in the blood of horses after exercise was observed. There were no significant differences in erythrocyte TBARS levels between the resting period and after exercises. Significant decreases by 8% ($p < 0.05$) in the aldehydic derivatives of protein oxidation in the plasma after training were noted. Exercise can induce the activity of the proteasome complex, which is significantly involved in the degradation of oxidatively modified proteins. The preventive effect of regular exercise leads to adaptation to prolonged exercises, which is accompanied by an increase of oxidative stress-induced adaptation and changes in redox homeostasis, increased antioxidant defenses, lower oxidative damage, and increased resistance to oxidative stress. Regularly performed exercise might induce an adaptive enhancement in skeletal muscle and erythrocytes of the defense mechanisms that protect them against oxidative stress [4].

In our previous study, we also have investigated the effect of an exercise test of moderate intensity on oxidative stress biomarkers, antioxidant enzyme activity, and osmotic resistance of erythrocytes in well-trained equine athletes [5]. The exercise test induced a significant increase in erythrocyte values, hemoglobin concentration, and hematocrit in horses of both breeds. Regular training induced activation of the antioxidant enzymes and thereby can reduce oxidative stress in athletic horses. The exercise test in horses of both breeds attenuated oxidative stress and was accompanied by a significant decrease in lipid peroxidation and oxidatively modified proteins in erythrocytes after exercise. Our data suggest that oxidative stress and enzymatic antioxidant defense biomarkers can be used for the monitoring of fitness levels, health benefits, and performance of equine athletes [5].

The effects of gender differences on the blood oxidative stress biomarkers, antioxidant defenses, and resistance of erythrocytes to hemolytic agents of trained horses before and after exercise were evaluated in other our study [3]. The study was carried out on nine mares and 14 stallions of Ukrainian Warmblood well-trained horses, involved in jumping, eventing, and dressage. Trained stallions showed a decrease in lipid peroxidation and higher glutathione reductase activity, whereas mares presented a higher superoxide dismutase activity after exercise. The resistance of erythrocytes was similar in females and males. No statistically significant differences were observed in the percentage of hemolyzed erythrocytes between after and before exercise. A correlation between the oxidative stress biomarkers and antioxidant defenses in the stallions after exercises were observed, which may indicate a protective response of superoxide dismutase and catalase against exercise-induced oxidative stress [3]. Stallions showed a significant increase in leucocytes and granulocytes amount, as well as erythrocytes, hemoglobin, and hematocrit levels after the exercise test. Pre-exercise level of mean corpuscular hemoglobin concentration was higher in stallions. Trained mares and stallions showed a decrease in lipid peroxidation after exercise. Exercise also caused an increase in the oxidatively modified protein of erythrocytes in stallions indicated by exercise-induced oxidative stress. The resistance of erythrocytes in 0.1N HCl was similar between females and males. No statistically significant differences in the percentage of haemolysed erythrocytes before and after exercise were observed [1].

Our prior studies have assessed the seasonal-induced variations of exercise impact on hematological indices and oxidative stress biomarkers in plasma and erythrocytes in horses involved in recreational horseback riding [38]. The results of our study showed statistically significant alterations of oxidative stress biomarkers in erythrocytes after the exercise test in autumn. The results observed in our study suggest that ROS production may contribute to exercise-induced damage to the erythrocyte membrane. There were no statistically significant alterations in the derivatives of protein destruction level in the erythrocytes of trained horses involved in recreational horseback riding before and after exercise. Only in the autumn season, aldehydic and ketonic derivatives of OMP increased after exercise by 88% ($p < 0.05$) and 77% ($p < 0.05$), respectively [26]. There were no statistically significant alterations in the aldehydic derivatives of protein destruction level in the plasma of trained horses involved in recreational horseback rides before and after exercise in the spring and summer seasons. In the autumn season, aldehydic and ketonic derivatives of OMP were increased after exercise by 7.2% and 12.8% ($p < 0,05$), respectively. On the contrary, aldehydic and ketonic derivatives of oxidatively modified proteins were decreased after a training session in winter. Increasing the level of oxidative-modified proteins suggests the activation of oxidative stress during a training session in spring and autumn as adaptive changes in the horse's body due to changing ambient temperatures. Significant reductions in aldehyde derivatives of oxidatively modified proteins in the plasma of horses after training in winter are the result of adaptation induced by constant ambient temperature and exercise. This is an important seasonal adaptive reaction to stabilize ambient temperature [41]. The increase in plasma lipid peroxidation level in horses after exercise could be attributed to oxidative damage in various organs mainly in muscle tissue, owing to free radicals being produced, as a consequence of endurance exercise. Based on our results, it is concluded that endurance exercises lead to specific metabolic changes accompanied by a redistribution of energy supply for improving resistance to exercises and the athletic performance of horses [41]. A significant increase in erythrocytes amount after exercise tests both in the summer and autumn seasons was observed. Moreover, increased erythrocytes count by 18% ($p < 0.05$) in the autumn compared to the value in the spring season after the exercise test was noted. Hemoglobin level was increased after exercise in the summer season (by 15%, $p < 0.05$). Our results also revealed the increased hematocrit level after exercise tests in the summer, autumn, and winter seasons. We assume that it was due to the release of erythrocytes into the circulation as a result of the increased splenic function [2, 38].

Conclusions. In conclusion, there was a statistically significant reduction in the levels of aldehydic derivatives of OMP in the plasma of ponies during the winter photoperiods only after exercise in both sexes. A decrease in the levels of ketonic derivatives of OMP in the summer photoperiod was observed. These changes were observed independently of the sex and only after exercise. Levels of aldehydic and ketonic derivatives of OMP varied depending on the photoperiod and exercise session in our studies. These changes were dependent on the baseline levels of the enzymatic and non-enzymatic antioxidant defense systems in the ponies, which differed between the mares and the stallions (statistically significant differences in the winter period) both before and after exercise (winter).

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ЗАЛЕЖНІСТЬ ВІД ФОТОПЕРІОДУ ЗМІНИ ВМІСТУ ОКИСНЮВАЛЬНО МОДИФІКОВАНИХ БІЛКІВ У ПЛАЗМІ КОБИЛ ТА ЖЕРЕБЦІВ ШЕТЛАНДСЬКИХ ПОНІ, ЯКІ БЕРУТЬ УЧАСТЬ У РЕКРЕАЦІЙНІЙ ВЕРХОВІЙ ЇЗДІ

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У цьому дослідженні зосередилися на вивченні індукованих фотоперіодом змінах окиснювально модифікованих білків у плазмі кобил і жеребців шетландських поні до і після тренування. Ми проаналізували вплив фотоперіоду і фізичних вправ на рівні альдегідних (АП) і кетонових (КП) похідних окиснювально модифікованих білків (ОМБ) у крові кобил і жеребців шетландських поні, які беруть участь у рекреаційній верховій їзди в центральній частині Поморського регіону (Поморське воєводство, північна частина Польщі). Двадцять один здоровий дорослий шетландський поні (11 кобил і 10 жеребців) віком 6,5 ± 1,4 років були використані в цьому дослідженні. Усі коні брали участь у рекреаційній верховій їзді. Тренування розпочиналося о 10:00, тривало 1 годину і складалося з кросу ходьбою (5 хв), риссю (15 хв), ходьбою (10 хв), риссю (10 хв), ходьбою (5 хв), галопом (5 хв) і ходьбою (10 хв). Кров брали з яремної вени тварин вранці, через 90 хвилин після годування, під час перебування коней у стайні (між 8:30 та 10 ранку) та відразу після тесту з фізичним навантаженням (між 11 ранку та 12 ранку). Забір крові проводили один раз за сезон протягом року: влітку та взимку. Рівень окиснювально модифікованих білків (ОМБ) оцінювали за вмістом карбонільних похідних білків у реакції з 2,4-динітрофенілгідразином (ДНФГ). Як показали результати наших досліджень, спостерігалося статистично істотне зниження рівнів альдегідних похідних ОМБ у плазмі поні обох статей протягом зимового фотоперіоду лише після фізичного навантаження. Подібне зниження рівнів кетонових похідних ОМБ показано нами також у літній фотоперіод. Ці зміни спостерігалися незалежно від статі і тільки після фізичних навантажень. Рівні альдегідних і кетонових похідних ОМБ змінювалися залежно від фотоперіоду та фізичних навантажень у наших дослідженнях. Ці зміни залежали від базових рівнів ферментативної та неферментативної систем антиоксидантного захисту у поні, які відрізнялися між кобилами та жеребцями (статистично значущі відмінності в зимовий період) як до, так і після фізичних навантажень (взимку).

Ключові слова: окиснювально модифіковані білки, плазма, фізичні навантаження, сезонні зміни, фотоперіод, Шетландські поні, кобили та жеребці.