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LIPID PEROXIDATION AND THE TOTAL ANTIOXIDANT CAPACITY IN THE PLASMA OF SHETLAND PONY MARES AND STALLIONS INVOLVED IN RECREATIONAL HORSEBACK RIDING: ROLE OF PHOTOPERIOD AND EXERCISE

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This study focuses on the photoperiod-induced variability in the levels of biomarkers of lipid peroxidation (2-thiobarbituric acid reactive substances, TBARS) and the total antioxidant capacity (TAC) in the blood of Shetland pony mares and stallions before and after exercise. We have analyzed the effect of photoperiods and exercise on the levels of TBARS and TAC 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 lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) for determining the malonic dialdehyde (MDA) concentration. The TAC level in samples was estimated by measuring the TBARS level after Tween 80 oxidation. In the stallions and mares before exercise (at rest) in the summer photoperiod, we obtained minimum TBARS values, while maximum levels were determined in the winter photoperiod. In our study, the TAC level illustrated the baseline levels of enzymatic and non-enzymatic antioxidant defense systems, which were different in the mares than in the stallions (statistically significant in the winter photoperiod) both before and after exercise (winter photoperiod). The results of the current study demonstrated that changes in the lipid peroxidation and the total antioxidant capacity in the blood of Shetland ponies are photoperiod-, sex-, and exercise-dependent. The maximum statistically significant values for the effect of the photoperiod-exercise combination defined as the F values were observed. The multivariate analysis of the influence of the three parameters: photoperiods, sex, and exercise on the lipid peroxidation and the total antioxidant *capacity in the blood of the ponies indicated the leading role mainly of the photoperiod factor, and next the exercise and sex factors.*

Key words: 2-thiobarbituric acid reactive substances, total antioxidant capacity, plasma, exercise, seasonal alterations, photoperiods, Shetland ponies, mares and stallions.

ПЕРЕКИСНЕ ОКИСНЕННЯ ЛІПІДІВ І ЗАГАЛЬНА АНТИОКСИДАНТНА АКТИВНІСТЬ У ПЛАЗМІ КОБИЛ І ЖЕРЕБЦІВ ШЕТЛАНДСЬКИХ ПОНІ, ЯКІ БЕРУТЬ УЧАСТЬ У РЕКРЕАЦІЙНІЙ ВЕРХОВІЙ ЇЗДІ: РОЛЬ ФОТОПЕРІОДУ ТА ФІЗИЧНОГО ТРЕНІНГУ

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У цьому дослідженні ми зосередилися на вивченні індукованих фотоперіодом змінах рівнів біомаркерів перекисного окиснення ліпідів (реактивні речовини, які взаємодіють з 2-тіобарбітуровою кислотою, TBARS) і загальної антиоксидантної активності (TAC) у плазмі кобил і жеребців шетландських поні до і після тренування. Ми проаналізували вплив фотоперіоду і фізичних вправ на рівні TBARS і TAC у крові кобил і жеребців шетландських поні, які беруть участь у рекреаційній верховій їзді 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 перекисного окиснення ліпідів визначали кількісно за допомогою ТБК-активних речовин (TBARS), зокрема малонового діальдегіду (MDA). Рівень TAC у зразках оцінювали шляхом вимірювання рівня TBARS після окиснення Tween 80. Як показали результати наших досліджень, у жеребців і кобил перед навантаженням (у стані спокою) в літній фотоперіод ми отримали мінімальні значення TBARS, а максимальні – у зимовий фотоперіод. У нашому дослідженні рівень TAC ілюстрував базові рівні ферментативних і неферментативних систем антиоксидантного захисту, які відрізнялися у кобил, ніж у жеребців (статистично значущі в зимовий фотоперіод) як до, так і після фізичного навантаження. Результати поточного дослідження показали, що зміни перекисного окиснення ліпідів і загальної антиоксидантної активності в крові шетландських поні залежать від фотоперіоду, статі та фізичних вправ. Спостерігалися максимальні статистично значущі *значення ефекту комбінації фотоперіод-вправи, визначені як значення F. Багатофакторний аналіз впливу трьох параметрів: фотоперіодів, статі та фізичного навантаження на перекисне окиснення ліпідів і загальну антиоксидантну активність у крові поні вказав на провідну роль переважно фактора фотоперіоду, в порівнянні до таких факторів, як фізичне навантаження та стать.*

Ключові слова: реактивні речовини, які взаємодіють з 2-тіобарбітуровою кислотою (TBARS), загальна антиоксидантна активність (TAC), плазма, фізичні навантаження, сезонні зміни, фотоперіод, Шетландські поні, кобили та жеребці.

The adaptation of a living organism, forming in the process of natural selection, as well as in the background of the natural change of the seasons, with a regularly repeated change in climate, and food conditions, does not manifest itself in the same way as an experimental adaptation to cold (Ebi, K. L., & Rocklöv, J., 2014). Seasonal adaptation occurs in the background of preliminary, advanced preparation of animals (storage of feed, fattening before the crucial season, migration, change of coat, etc.) to difficult conditions (cold, decrease of feed, etc.) with gradual adaptation to them, which cannot be the case with experimental adaptation to cold, since it is almost impossible to create real environmental conditions (Gaughan J. B. et al., 2018; Young B. A., 1981). Thermoregulation in horses primarily depends on heat loss through evaporation, in particular through sweating. Under thermoneutral conditions, these mechanisms are sufficient to enable horses to perform the high-intensity exercise for extended periods of time. Under conditions of thermal stress, especially in high-humidity environments, the efficiency of evaporative heat loss mechanisms is reduced, which can lead to the development of hyperthermia in horses (Guthrie A. J. & Lund R. J. 1998).

The influence of environmental factors is especially susceptible to animals kept in the open when grazing (Schieltz J. M. & Rubenstein D. I. 2016). Meanwhile, knowledge of the biological characteristics of individual breeds of farm animals makes it possible to establish the critical moments of their life, depending on the specific conditions of the external environment. The habitat of farm animals throughout the year is not the same. It is highly susceptible to changes in the seasons of the year (especially when they are kept on pasture all year round) and thus affects the biological needs of animals for feed (energy) at the conditions of keeping. By targeted satisfaction of the physiological needs of the body in certain periods of the season, it is possible to reduce the negative impact of environmental factors and achieve high productive qualities in animals, as well as improve health (Wróbel B. et al.,. 2023)

In our previous study, we also revealed that the seasonal variations in the antioxidant defences, as well as substrates of energy metabolism in the blood of mares and stallions, depending on exercise capacity, could be an important aspect in the ability of endogenous adaptive mechanisms of animals to react in advance to environmental changes associated with seasons (Kurhaluk N. et al., 2022). The influence of seasons and sex on body size and hematological and biochemistry parameters of Noma horses, a native Japanese breed was evaluated by Ono and co-workers (2021). Body size was larger in winter than in summer. Laboratory testing variables, including erythrocytic parameters and urea nitrogen, total cholesterol, and creatinine kinase levels, were higher in winter, while the eosinophil count was higher in summer. These seasonal differences may be related to the increased energy consumption of horses due to heat stress. The higher eosinophil counts may have been related to dermatitis observed in the summer. Stallions tended to have smaller bodies compared with mares (Ono T. et al., 2021).

In the mechanisms of adaptation of the body to free radical lipid peroxidation and the development of oxidative stress at the molecular level, a special place is given. Unlike conventional molecules, molecules and atoms of free radicals (FRs) have one or more electrons with unpaired spin in their outer energy orbital and, therefore, are highly reactive (Jakubczyk K. et al., 2020). FR production in a healthy organism is carried out in small amounts during physiological processes (phagocytosis, oxidation of arachidonic acid, prostaglandin synthesis, mitochondrial respiration, and peroxisomal oxidation) (Di Meo S. et al., 2016). Many enzymatic and non-enzymatic redox processes are known, the products of which are various types of FR, the largest number is formed from oxygen (Pham-Huy, L. A. et al., 2008). Molecular oxygen in the basic state is a biradical that has two unpaired electrons in atomic orbitals. Less than 5% of molecular oxygen undergoes a one-electron reduction in mitochondria and microsomes of cells, especially phagocytes. These reactions result in superoxide anion radical ($\cdot O_2^{-}$), singlet form of oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and hydroperoxyl radical (HO_2^{-}) (Ignatowicz, E., & Rybczyńska, M. 1994). The general name of these products is reactive oxygen species (ROS), which have the ability to react with endogenous substrates that form cell structures, and primarily with phospholipid biomembranes (Brieger K. et al., 2012). The consequence of such reactions is the destruction of cellular components, including proteins (with inactivation of enzymes, especially those containing sulfhydryl groups), membrane lipids (with the formation of lipid peroxides from polyunsaturated fatty acids, PUFAs), and nucleic acids (with the formation of breakdowns and mutations) (Ward J. P. T., 2017). Thus, ROS are highly reactive radical oxygen compounds formed as a result of incomplete oxygen reduction.

The entry of aggressive FR into reactions with PUFAs of phospholipids with the formation of hydroperoxides occurs regardless of the cause that caused the increase in ROS levels. The cascade of lipid peroxidation reactions leads to the appearance and accumulation of a wide range of toxic metabolites, the tension of antioxidant defense mechanisms, and the development of oxidative stress (Ayala A. et al., 2014). At a certain intensity, lipid peroxidation (LPO) is a normal physiological process involved in the self-renewal and restructuring of membrane structures, regulation of ion transport, and changes in the activity of membrane-binding enzymes (Spiteller, G., 2003). LPO processes play the role of a necessary link in the biosynthesis of prostaglandins, steroid hormones, and leukotrienes, as well as in the processes of phagocytosis (Ricciotti E., & FitzGerald G. A., 2011). Excessive LPO activation plays an important and sometimes key role in the development of many pathological conditions, which makes it justified to consider this process as a universal typical link in the pathogenesis of various pathological conditions. The action of external prooxidants and the activation of endogenous mechanisms of ROS generation lead to the tension of antioxidant defense mechanisms and the development of oxidative stress, an important pathogenetic factor in many pathological processes and conditions that can manifest itself at the cellular, tissue, and organism levels. LPO and its products, acting as a "primary mediator" of stress represent one of the earliest regulatory mechanisms and are a non-specific pathogenetic link in the development of various diseases (Ayala A. et al., 2014)..

This study focuses on the photoperiod-induced variability in the levels of biomarkers of lipid peroxidation (2-thiobarbituric acid reactive substances, TBARS) and the total antioxidant capacity (TAC) in the blood of Shetland pony mares and stallions before and after exercise. We have analyzed the effect of photoperiods and exercise on the levels of TBARS and TAC 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).

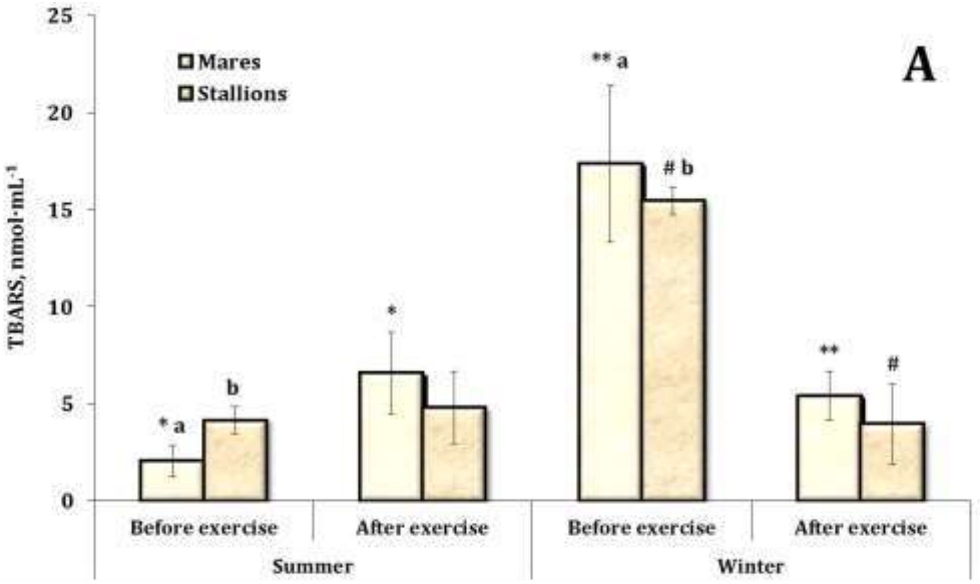
Blood samples. Blood was drawn from the jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM), and immediately after the exercise test (between 11 AM and 12 AM). Blood samples were taken once per season for one year: summer 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.

The 2-Thiobarbituric acid reactive substances (TBARS) assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration (Kamyshnikov V. S. (2004). This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as the extinction coefficient.

Measurement of total antioxidant capacity (TAC). The TAC level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova L. P. et al., 1998). The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

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 (Stanisz A. 2006; Zar J. H., 1999). 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 (the F test and its significance) (Stanisz A. 2006; Zar J. H., 1999).

Results. We investigated the levels of TBARS and TAC in the blood of the Shetland ponies influenced by the three factors (photoperiods, sex, and exercise). Levels of TBARS and TAC in the blood of Shetland pony mares and stallions before and after exercise in summer and winter are presented in Fig. 1.



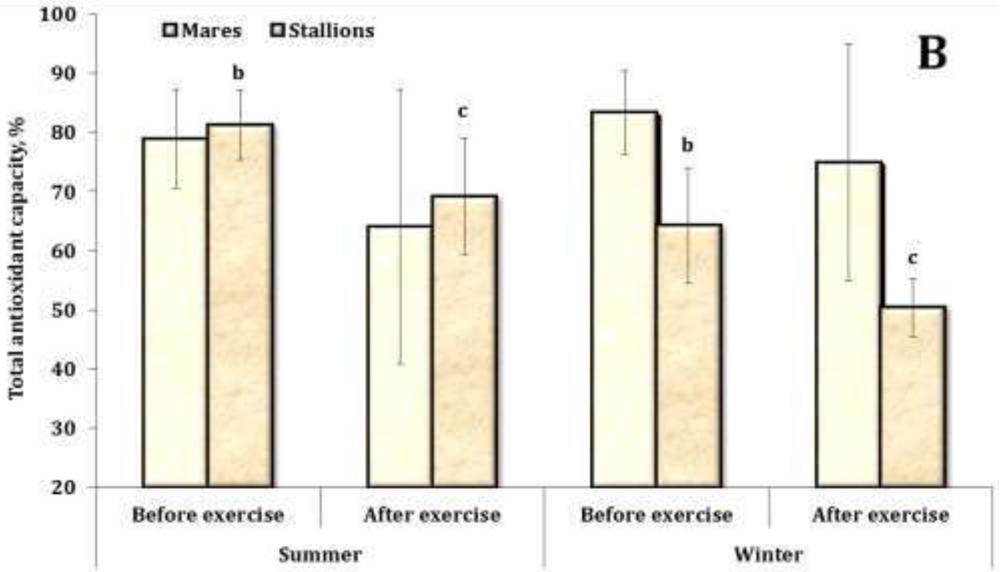


Fig. 1. Levels of 2-thiobarbituric acid reactive substances (TBARS, nmol·mL^{-1}) (A) and total antioxidant capacity (TAC, %) (B) in the blood of Shetland pony mares (n = 11) and stallions (n = 10) before and after exercise in summer and winter.

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 in summer;*

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

 # – between values obtained before and after exercise in stallions in winter;

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

b – between values obtained in stallions before exercise in winter and summer;

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

Results of our study revealed that levels of TBARS in the plasma of Shetland pony mares in summer were statistically significantly increased after exercise to (6.58 ± 2.09 nmol·mL^{-1}) compared to the state before exercise (2.05 ± 0.81 nmol·mL^{-1}). On the other hand, in stallions, a statistically non-significant increase in levels of TBARS in the plasma after exercise (4.81 ± 1.85 nmol·mL^{-1}) compared to the state before exercise (4.15 ± 0.70 nmol·mL^{-1}) was observed. The percentage of increase was 221% ($p < 0.05$) for mares and 15.91% ($p > 0.05$) for stallions, respectively. Contrarily, in the winter, levels of TBARS in the plasma of both mares and stallions of Shetland ponies were statistically significantly decreased after exercise to (5.41 ± 1.28 nmol·mL^{-1}) and (3.96 ± 2.04 nmol·mL^{-1}) compared to the state before exercise (17.37 ± 4.05 nmol·mL^{-1}) and (15.46 ± 0.71 nmol·mL^{-1}), respectively. The percentage of decrease was 68.86% ($p < 0.05$) for mares and 74.39% ($p < 0.05$) for stallions (Fig. 1A).

After exercise, levels of TBARS in the plasma of both mares and stallions of Shetland ponies in summer were similar to the values obtained in the winter. Before exercise, levels of TBARS in the plasma of both mares and stallions of Shetland ponies in winter were higher [(17.37 ± 4.05 nmol·mL^{-1}) and (15.46 ± 0.71 nmol·mL^{-1})] compared to the values obtained in the summer, i.e. (2.05 ± 0.81 nmol·mL^{-1}) for mares and (4.15 ± 0.70 nmol·mL^{-1}) for stallions. The percentage of decrease was 88.2% ($p < 0.05$) for mares and 73.16% ($p < 0.05$) for stallions (Fig. 1A).

Levels of the total antioxidant capacity in the plasma of both mares and stallions of Shetland ponies in summer were decreased after exercise to (64.06 ± 23.26 %) and (69.22 ± 9.8 %) compared to the state before exercise (78.89 ± 8.35 %) and (81.26 ± 5.82 %), respectively. The percentage of decrease was 18.8% ($p > 0.05$) for mares and 14.82% ($p > 0.05$) for stallions, respectively. Similarly, in the winter, TAC levels in the plasma of both mares and stallions of Shetland ponies were statistically non-significantly decreased after exercise to (74.94 ± 20.03 %) and (50.40 ± 4.87 %) compared to the state before exercise (83.37 ± 7.06 %) and (64.27 ± 9.82 %), respectively. The percentage of decrease was 4% ($p > 0.05$) for stallions. The percentage of decrease was 10.12% ($p > 0.05$) for mares and 21.58% ($p > 0.05$) for stallions, respectively (Fig. 1B).

Before exercise, TAC levels in the plasma of stallions of Shetland ponies in summer were higher (81.26 ± 5.82 %) compared to the values obtained in the winter (64.27 ± 9.82 %). The percentage of decrease was 20.91% ($p < 0.05$). TAC levels in the plasma of mares of Shetland ponies in summer were at the same levels obtained in the winter. After exercise, TAC levels in the plasma of stallions of Shetland ponies in winter were less (50.40 ± 4.87 %) compared to the values obtained in the summer, i.e. (69.22 ± 9.8 %). The percentage of decrease was 27.18% ($p < 0.05$) (Fig. 1B).

Discussion. The results of our study revealed that in the stallions and mares before exercise (at rest) in the summer photoperiod, we obtained minimum TBARS values, while maximum levels were determined in the winter photoperiod. In our study, the TAC level illustrated the baseline levels of enzymatic and non-enzymatic antioxidant defense systems, which were different in the mares than in the stallions (statistically significant in the winter photoperiod) both before and after exercise (winter photoperiod). The next step of our study was to determine statistically significant differences between the values of oxidative stress biomarkers of the maintenance of homeostatic mechanisms of antioxidant defenses in the blood in Shetland ponies depending on the photoperiod, sex, and exercise. TBARS levels in the blood of these horses were shown to differ statistically significantly with a maximum coefficient of variability of $F_{15.152}$ = 38.47 ($p = 0.000$). These data were also confirmed by the results of the sum-of-squares test (total SS model) *vs*. residual SS regarding the values of the correlation coefficient

($R = 0.89$), the coefficient of determination ($R^2 = 0.79$), and its adjusted form $R^2_{ad} = 0.77$ at the value of $F = 38.47$ ($p = 0.000$). The plasma of the stallions and mares before exercise (at rest) exhibited minimum TBARS levels in the summer photoperiods and maximum values of the parameter in the winter photoperiods. The ANOVA post-hoc Tukey test (HSD) revealed statistically significant differences in the TBARS levels between the groups in the different photoperiods, depending on the sex and exercise. Before exercise (at rest) for a group of pony stallions, these dependencies were significant for summer-winter photoperiod ($p < 0.05$).

The total antioxidant capacity (TAC) was statistically significantly correlated with the sex, photoperiods, and exercise of the Shetland ponies in our study. The changes in this parameter are probably related to the baseline levels of enzymatic and non-enzymatic antioxidant defense systems in ponies, which are different in mares compared to stallions (statistically significant in the winter period) both before and after exercise (winter period). The TAC data differed statistically significantly ($F_{15.152} = 10.71$; $p = 0.000$) in the total SS model vs. residual SS regarding the values of the correlation coefficient ($R = 0.71$), the coefficient of determination ($R^2 = 0.50$), and its adjusted form $R^2_{ad} = 0.45$ at the value of $F = 10.06$ ($p = 0.000$).

Various studies on the gradual adaptation of melatonin rhythms to the photoperiod in humans and rodents have been reported in the literature. Murphy and co-workers (2011) revealed that melatonin rhythms in horses may be dependent on light to a greater extent. The authors believe that melatonin is not a suitable marker of the circadian phase in this species, because melatonin biosynthesis in the pineal glands of some ungulates is largely determined by the ambient light cycle, and the circadian oscillator located in the mammalian hypothalamus suprachiasmatic nucleus (SCN) has a minor role in this process (Murphy B. A. et al., 2011). The importance of the hypothalamus SCN, i.e. the site of the main mammalian pacemaker (Murphy, B. A., et al., 2007), has been previously shown in a number of studies. The SCN controls the circadian rhythms of various physiological and behavioral phenomena such as rest and activity cycles, hormone secretion, and body temperature (Nagy, P., et al., 2011). The role of the circadian system is to provide endogenous mechanisms to optimize the survival and adaptation processes. This is important for the formation of anticipatory pathways of adaptive activity of physiological functions. Murphy and co-workers (2007) suggested that the rapid resynchronization of the melatonin rhythm and the central circadian pacing of the horse may exhibit a particularly strong response to restructuring (Murphy, B. A., et al., 2007).

An important role in the physiological effects of melatonin is assigned to its antioxidant properties, as convincingly demonstrated in recent studies (Kurhaluk N., 2021; Kurhaluk N. & Tkachenko H. 2020; Tordjman S. et al., 2017). Possibly, the seasonal dominants of melatonin release help to explain the statistically significant effects of the decrease in TBARS levels in the plasma of the Shetland ponies (Fig. 1). These data are also confirmed by the results of the TBARS levels in the sum-of-squares test (total SS model) vs. residual SS regarding the values of the correlation coefficient ($R = 0.89$), the coefficient of determination ($R^2 = 0.79$), and its adjusted form $R^2_{ad} = 0.77$ at the value of $F = 38.47$ ($p = 0.000$). This may suggest higher statistical correlations between these processes in our study. Thus, before exercise (at rest), the plasma of the stallions and mares exhibited minimum TBARS levels in the summer photoperiod and maximum values in the winter photoperiod (Fig. 1).

Schrammel and co-workers (2016) analyzed the effects of artificially long days on rectal temperature, heart rate, heart rate variability, hematology, coat changes, semen parameters, and plasma testosterone concentrations in Shetland stallions stabled overnight and assigned to a control group (CON, n = 9) kept under natural photoperiod, and a treatment group exposed to a long-day light program from 15 December to 20 March (AL, n = 9). During the 8-month study, rectal temperature, heart rate, and heart rate variability at no time differed between groups. Plasma total protein, hematocrit, leukocyte, and lymphocyte counts first increased and then decreased during the study period but did not differ between groups. Length of the guard hair decreased over time and this decrease occurred earlier in AL than in CON stallions. Hair regrowth was faster in CON than in AL stallions. Total sperm count increased from January to April (AL) and May (CON) but did not differ between groups. Sperm motility and percentage of membrane-intact spermatozoa showed no clear seasonal changes and semen parameters did not differ between groups. Thus, Shetland stallions showed seasonal variations in hair coat and total sperm count but only changes in hair coat but not semen parameters were advanced by a long-day light program (Schrammel N. et al., 2016). In another study, these researchers analyzed the effect of artificially long days on the quality of cooled-stored and cryopreserved semen in Shetland stallions. The motility of cooled-stored semen was reduced in January and increased in stallions kept under a long daylight programme for at least 30 days cold-stored sperm (Deichsel K. et al., 2016). Semen quality was impaired in midsummer when low sperm motility and viability were combined with elevated DNA fragmentation and Ca^{2+} level of sperm (Wach-Gygax L. et al., 2017). The results of Janett and co-workers (2003) demonstrated that in climatic conditions of Switzerland, clear seasonal differences occur in semen quality of fresh and frozen-thawed semen and that cryopreservation of stallion semen should preferably be performed in autumn (Janett F. 2003a). Also, individual and seasonal differences occurred in the semen quality of Franches-Montagnes stallions. Ejaculates collected in autumn (September, October, and November) demonstrated good quality, especially regarding sperm morphology, and were more suitable for cryopreservation because of better motility in frozen-thawed semen collected during autumn than in winter (Janett F. 2003 b).

A growing body of evidence has suggested the beneficial role of regular physical exercise. The results of Mills and co-workers (1994) indicate that lipid peroxidation and the excretion and clearance of hydroxyproline increase when unfit thoroughbreds are strenuously exercised on a treadmill (Mills P. C. et al., 1994). Several studies using exogenous supplementation of vitamin E, vitamin C, and alpha-lipoic acid have shown positive results in decreasing the effects of exercise (endurance and intense exercise)-induced oxidative stress and increasing the antioxidant status based on the markers and antioxidants measured (Kinnunen S. et al., 2009; Marañón G. et al., 2008; McMeniman N. P. et al., 1992; Ono K. et al., 1990; Williams C. A. et al., 2016, 2004), whereas other studies using superoxide dismutase showed little effects on the exercise horses (Lamprecht E. D. & Williams C. A. 2012). In old horses (22 ± 2 years), lipid peroxidation levels and blood antioxidant concentrations were similar to those found in younger but mature (12 ± 2 years) horses both at rest and during exercise (Williams C. A. et al., 2004, 2008). Other studies found that yearlings (18 ± 2.4 months) that are novel to forced exercise had less lipid peroxidation and greater antioxidant status than mature mares (13 ± 2.1 years) prior to exercise training (Smarsh D. & Williams C. 2014). Exercise training reduced oxidative stress markers and improved antioxidant status in mares, whereas few effects were seen in yearlings (White S. H. & Warren L. K. 2017). This indicates that youth provided more defense against oxidative stress due to exercise than the exercise training program (Smarsh D. & Williams C. 2016).

In our previous study, we determined the level of oxidative stress biomarkers in sports horses involved in eventing before and after training (Andriichuk A. et al., 2013). All horses were in regular systematic training and had the same diet. A significant increase in the 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 (Andriichuk A. et al., 2013).

We also 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 (Andriichuk A. et al., 2013). The exercise test induced a significant increase in erythrocyte values, hemoglobin concentration, and hematocrit in horses of both breeds. Regular training induces 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 (Andriichuk A. et al., 2016).

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 (Andriichuk A. et al., 2014). 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 was observed, which may indicate a protective response of superoxide dismutase and catalase against exercise-induced oxidative stress (Andriichuk A. et al., 2014). 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 (Andriichuk A. et al., 2017).

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 were also assessed (Kurhaluk N. et al., 2023; Pażontka-Lipiński P. et al., 2017; Tkachenko H. et al., 2016; Witaszek M. et al., 2017). 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 oxidatively modified proteins (OMP) increased after exercise by 88% ($p < 0.05$) and 77% ($p < 0.05$), respectively (Tkachenko H. et al., 2016) 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 aldehydic 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 (Kurhaluk N. et al., 2023, Witaszek M. et al., 2017). 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 (Kurhaluk N. et al., 2023, Witaszek M. et al., 2017). 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 circulation as a result of the increased splenic function (Andriichuk A. & Tkachenko H., 2015; Tkachenko H. et al., 2016)

Only with knowledge of the biological needs of the body, it is possible to establish scientifically based feeding norms depending on the season of the year. Knowledge of seasonal adaptive changes in morphological structures, as well as biochemical and physiological reactions of animals makes it possible, using the biological characteristics of individual breeds and offspring, to significantly increase their training capacity and health. Seasonal changes in the thickness of the skin and the structure of the hairline of horses were studied by Shkyratova and co-workers (2019) as signs of adaptation to environmental factors. The minimum skin thickness in winter was found in mares on the back and shoulder blade (4.3 and 4.4 mm), and the maximum – on the side and thigh (4.5 and 4.6 mm). Compared with the summer period, the increase on the side was 0.8 mm, and on the back, shoulder blade, and thigh – 0.4 mm ($p \leq 0.001$). In spring, skin thickening was also noted in the same topographic areas within 0.1-0.3 mm compared to autumn. Quantitative indicators of hair cover varied depending on the season of the year. In winter, the hairline contained more down (23.10%), but fewer guard hairs (68.24%). In summer, there was a lower content of down (4.33%), but more guard hairs – 94.01%. There are sharp seasonal changes in the length of the hair. The longest hairs was found in winter and spring (4.96 and 4.26 cm), and the shortest was in summer and autumn (0.94 and 1.90 cm, respectively) (Shkuratova G. M. et al., 2019).

Schmidt and co-workers (2017) hypothesized that the housing of stallions in a thermoneutral temperature zone during autumn and winter not only influences metabolism and hair shedding but also improves the characteristics of raw and processed semen. These researchers revealed in a study that environmental temperature during autumn and winter had clear results on body temperature as well as hair coat characteristics in Shetland stallions. Simultaneously determined effects on semen characteristics were minimal indicating that reproductive function in the horse is more dependent on day length i.e. the geophysical year than on other environmental factors (Schmidt K. et al., 2017).

Conclusions. We have analyzed the effect of photoperiods and exercise on the levels of TBARS and TAC 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). We obtained the maximum statistically significant values for the effect of the photoperiod-exercise combination defined as the F values. The TBARS levels were indicated as biomarkers of lipid peroxidation in plasma with a maximum value of the adjusted form of the coefficient of determination. In the stallions and mares before exercise (at rest) in the summer photoperiod, we obtained minimum TBARS values, while maximum levels were determined in the winter photoperiod. In our study, the TAC level illustrated the baseline levels of enzymatic and non-enzymatic antioxidant defense systems, which were different in the mares than in the stallions (statistically significant in the winter photoperiod) both before and after exercise (winter photoperiod).

The results of the current study demonstrated that changes in the lipid peroxidation and the total antioxidant capacity in the blood of Shetland ponies are photoperiod-, sex-, and exercise-dependent. The multivariate analysis of the influence of the three parameters: photoperiods, sex, and exercise on the lipid peroxidation and the total antioxidant capacity in the blood of the ponies indicated the leading role mainly of the photoperiod factor, and next the exercise and sex factors.

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References

Andriichuk, A., & Tkachenko, H. (2017). Effect of gender and exercise on haematological and biochemical parameters in Holsteiner horses. *Journal of animal physiology and animal nutrition*, 101(5), e404–e413. https://doi.org/10.1111/jpn.12620.

Andriichuk, A., & Tkachenko, H. (2015). Seasonal variations of hematological indices in equines involved in recreational horse riding. *Journal of Ecology and Protection of the Coastline (Baltic Coastal Zone)*, 19, 11-22.

Andriichuk, A., Tkachenko, H., & Kurhaluk, N. (2014). Gender Differences of Oxidative Stress Biomarkers and Erythrocyte Damage in Well-Trained Horses During Exercise. *Journal of Equine Veterinary Science*, 34(8), 978-985. https://doi.org/10.1016/j.jevs.2014.05.005.

Andriichuk, A., Tkachenko, H., Kurhaluk, N., & Tkachova, I. (2013). Markery stresu oksydacyjnego i parametry biochemiczne we krwi koni biorących udział we Wszechstronnym Konkursie Konia Wierzchowego w dynamice treningu. *Słupskie Prace Biologiczne*, 10, 5-25.

Andriichuk, A., Tkachenko, H., & Tkachova, I. (2016). Oxidative Stress Biomarkers and Erythrocytes Hemolysis in Well-Trained Equine Athletes Before and After Exercise. *Journal of Equine Veterinary Science*, 36, 32-43. https://doi.org/10.1016/j.jevs.2015.09.011.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*, 2014, 360438. https://doi.org/10.1155/2014/360438

Brieger, K., Schiavone, S., Miller, F. J., Jr, & Krause, K. H. (2012). Reactive oxygen species: from health to disease. *Swiss medical weekly*, 142, w13659. https://doi.org/10.4414/smw.2012.13659

Deichsel, K., Schrammel, N., Aurich, J., & Aurich, C. (2016). Effects of a long-day light programme on the motility and membrane integrity of cooled-stored and cyropreserved semen in Shetland pony stallions. *Animal reproduction science*, 167, 68–73. https://doi.org/10.1016/j.anireprosci.2016.02.008

Di Meo, S., Reed, T. T., Venditti, P., & Victor, V. M. (2016). Role of ROS and RNS Sources in Physiological and Pathological Conditions. *Oxidative medicine and cellular longevity*, 2016, 1245049. https://doi.org/10.1155/2016/1245049

Ebi, K. L., & Rocklöv, J. (2014). Climate change and health modeling: horses for courses. *Global health action*, 7, 24154. https://doi.org/10.3402/gha.v7.24154

Galaktionova, L. P., Molchanov, A. V., El'chaninova, S. A., & Varshavskiĭ, B. I.a (1998). Sostoianie perekisnogo okisleniia u bol'nykh s iazvennoĭ bolezn'iu zheludka i dvenadtsatiperstnoĭ kishki [Lipid peroxidation in patients with gastric and duodenal peptic ulcers]. *Klinicheskaia laboratornaia diagnostika*, (6), 10–14.

Gaughan, J. B., Sejian, V., Mader, T. L., & Dunshea, F. R. (2018). Adaptation strategies: ruminants. *Animal frontiers: the review magazine of animal agriculture*, 9(1), 47–53. https://doi.org/10.1093/af/vfy029

Guthrie, A. J., & Lund, R. J. (1998). Thermoregulation. Base mechanisms and hyperthermia. The Veterinary clinics of North America. *Equine practice*, 14(1), 45–59. https://doi.org/10.1016/s0749-0739(17)30211-0

Ignatowicz, E., & Rybczyńska, M. (1994). Some biochemical and pharmacological aspects of free radical-mediated tissue damage. *Polish journal of pharmacology*, 46(3), 103–114.

Jakubczyk, K., Dec, K., Kałduńska, J., Kawczuga, D., Kochman, J., & Janda, K. (2020). Reactive oxygen species – sources, functions, oxidative damage. *Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego*, 48(284), 124–127.

Janett, F., Thun, R., Bettschen, S., Burger, D., & Hassig, M. (2003b). Seasonal changes of semen quality and freezability in Franches-Montagnes stallions. *Animal reproduction science*, 77(3-4), 213–221. https://doi.org/10.1016/s0378-4320(03)00039-3

Janett, F., Thun, R., Niederer, K., Burger, D., & Hässig, M. (2003a). Seasonal changes in semen quality and freezability in the Warmblood stallion. *Theriogenology*, 60(3), 453–461. https://doi.org/10.1016/s0093-691x(03)00046-3

Kamyshnikov, V.S. (2004). *A reference book on the clinic and biochemical research and laboratory diagnostics*. MEDpress-inform, Moscow.

Kinnunen, S., Oksala, N., Hyyppä, S., Sen, C. K., Radak, Z., Laaksonen, D. E., Szabó, B., Jakus, J., & Atalay, M. (2009). alpha-Lipoic acid modulates thiol antioxidant defenses and attenuates exercise-induced oxidative stress in standardbred trotters. *Free radical research*, 43(8), 697–705. https://doi.org/10.1080/10715760903037673

Kurhaluk, N. (2021). Alcohol and melatonin. *Chronobiology international*, 38(6), 785–800. https://doi.org/10.1080/07420528.2021.1899198.

Kurhaluk, N., & Tkachenko, H. (2020). Melatonin and alcohol-related disorders. *Chronobiology international*, 37(6), 781–803. https://doi.org/10.1080/07420528.2020.1761372.

Kurhaluk, N., Lukash, O., & Tkachenko, H. (2022). Photoperiod-dependent changes in oxidative stress markers in the blood of Shetland pony mares and stallions involved in recreational horseback riding. *Chronobiology international*, 39(11), 1419–1434. https://doi.org/10.1080/07420528.2022.2115922.

Kurhaluk, N., Tkachenko, H., Tkachova, I., & Lukash, O. (2023). Photoperiod-dependent alterations in oxidatively modified proteins in the plasma of Shetland pony mares and stallions involved in recreational horseback riding. *Scientific and Technical Bulletin of the Institute of Animal Science of the National Academy of Agrarian Science of Ukraine*, 129, 4-15. https://10.32900/2312-8402-2023-129-4-15.

Lamprecht, E. D., & Williams, C. A. (2012). Biomarkers of antioxidant status, inflammation, and cartilage metabolism are affected by acute intense exercise but not superoxide dismutase supplementation in horses. *Oxidative medicine and cellular longevity*, 2012, 920932. https://doi.org/10.1155/2012/920932

Marañón, G., Muñoz-Escassi, B., Manley, W., García, C., Cayado, P., de la Muela, M. S., Olábarri, B., León, R., & Vara, E. (2008). The effect of methyl sulphonyl methane supplementation on biomarkers of oxidative stress in sport horses following jumping exercise. *Acta veterinaria Scandinavica*, 50(1), 45. https://doi.org/10.1186/1751-0147-50-45

McMeniman, N. P., & Hintz, H. F. (1992). Effect of vitamin E status on lipid peroxidation in exercised horses. *Equine veterinary journal*, 24(6), 482–484. https://doi.org/10.1111/j.2042-3306.1992.tb02882.x

Mills, P. C., Ng, J. C., Thornton, J., Seawright, A. A., & Auer, D. E. (1994). Exercise-induced connective tissue turnover and lipid peroxidation in horses. *The British veterinary journal*, 150(1), 53–63. https://doi.org/10.1016/S0007-1935(05)80096-X

Murphy, B. A., Elliott, J. A., Sessions, D. R., Vick, M. M., Kennedy, E. L., & Fitzgerald, B. P. (2007). Rapid phase adjustment of melatonin and core body temperature rhythms following a 6-h advance of the light/dark cycle in the horse. *Journal of circadian rhythms*, 5, 5. https://doi.org/10.1186/1740-3391-5-5

Murphy, B. A., Martin, A. M., Furney, P., & Elliott, J. A. (2011). Absence of a serum melatonin rhythm under acutely extended darkness in the horse. *Journal of circadian rhythms*, 9, 3. https://doi.org/10.1186/1740-3391-9-3

Nagy, P., Guillaume, D., & Daels, P. (2000). Seasonality in mares. *Animal reproduction science*, 60-61, 245–262. https://doi.org/10.1016/s0378-4320(00)00133-0

Ono, K., Inui, K., Hasegawa, T., Matsuki, N., Watanabe, H., Takagi, S., Hasegawa, A., & Tomoda, I. (1990). The changes of antioxidative enzyme activities in equine erythrocytes following exercise. Nihon juigaku zasshi. *The Japanese journal of veterinary science*, 52(4), 759–765. https://doi.org/10.1292/jvms1939.52.759

Ono, T., Inoue, Y., Hisaeda, K., Yamada, Y., Hata, A., Miyama, T. S., Shibano, K., Kitagawa, H., Ohzawa, E., & Iwata, E. (2021). Effect of seasons and sex on the physical, hematological, and blood biochemical parameters of Noma horses. *Journal of equine science*, 32(1), 21–25. https://doi.org/10.1294/jes.32.21

Pażontka-Lipiński, P., Witaszek, M., & Tkachenko, H. (2017). Seasonal alterations in exercise-induced resistance of erythrocytes in horses involved in recreational horseback riding. *Scientific and technical bulletin of Institute of Animal Husbandry, National Academy of Agrarian Sciences of Ukraine*, Kharkiv, 118, 22-29.

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS*, 4(2), 89–96.

Ricciotti, E., & FitzGerald, G. A. (2011). Prostaglandins and inflammation. *Arteriosclerosis, thrombosis, and vascular biology*, 31(5), 986–1000. https://doi.org/10.1161/ATVBAHA.110.207449

Schieltz, J.M., & Rubenstein, D.I. (2016). Evidence based review: positive versus negative effects of livestock grazing on wildlife. What do we really know? *Environmental Research Letters*, 11(11), 113003. https://doi.org/10.1088/1748-9326/11/11/113003.

Schmidt, K., Deichsel, K., de Oliveira, R. A., Aurich, J., Ille, N., & Aurich, C. (2017). Effects of environmental temperature and season on hair coat characteristics, physiologic and reproductive parameters in Shetland pony stallions. *Theriogenology*, 97, 170–178. https://doi.org/10.1016/j.theriogenology.2017.04.035.

Schrammel, N., Deichsel, K., Aurich, J., & Aurich, C. (2016). A long-day light program accelerates seasonal coat changes but is without effect on semen and metabolic parameters in Shetland pony stallions. *Theriogenology*, 85(5), 946–953. https://doi.org/10.1016/j.theriogenology.2015.11.003.

Shkuratova, G.M., Bazaron, B.Z., Khamiruev, T.N., & Dashinimaev, S.M. (2019). Adaptive changes of the hair coat of the horses of zabaykalsky aborigenous breed. *Siberian Herald of Agricultural Science*, 49(4), 101-105. (In Russian) https://doi.org/10.26898/0370-8799-2019-4-11.

Smarsh, D., & Williams, C. (2014). Effects of Age and Exercise Training on Oxidation of Skeletal Muscle and Blood in Mature and Yearling Horses. *Equine Veterinary Journal*, Volume 46: Abstracts from the 9th International Conference on Equine Exercise Physiology, 15-20 June, 2014 Chester, UK.

Smarsh, D., & Williams, C. (2016). Oxidative Stress and Antioxidant Status in Standardbreds: Effect of Age and Acute Exercise Before and After Training. *Journal of Equine Veterinary Science*, 47, 92-106. https://doi.org/10.1016/j.jevs.2016.07.019

Spiteller, G. (2003). Are lipid peroxidation processes induced by changes in the cell wall structure and how are these processes connected with diseases?. *Medical hypotheses*, 60(1), 69–83. https://doi.org/10.1016/s0306-9877(02)00333-x

Stanisz, A. 2006, 2007. *An affordable course of statistics using STATISTICA PL on examples from medicine*. Vol. 1-3. Basic Statistics. StatSoft Polska, Krakow, 2006, 2007. – 532 p., ISBN 83-88724-18-5.

Tkachenko, H., Pażontka-Lipiński, P., & Witaszek, P. (2016). Seasonal alterations in exercise-induced oxidative stress of horses involved in recreational horseback ride. In: *Globalisation and regional environmental protection. Technique, technology, ecology*. Eds Tadeusz Noch, Wioleta Mikołajczewska, Alicja Wesołowska. Gdańsk, Gdańsk High School Publ., P. 193–212.

Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., & Fougerou, C. (2017). Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Current neuropharmacology*, 15(3), 434–443. https://doi.org/10.2174/1570159X14666161228122115

Wach-Gygax, L., Burger, D., Malama, E., Bollwein, H., Fleisch, A., Jeannerat, E., Thomas, S., Schuler, G., & Janett, F. (2017). Seasonal changes of DNA fragmentation and quality of raw and cold-stored stallion spermatozoa. *Theriogenology*, 99, 98–104. https://doi.org/10.1016/j.theriogenology.2017.05.025

Ward, J. P. T. (2017). From Physiological Redox Signalling to Oxidant Stress. *Advances in experimental medicine and biology*, 967, 335–342. https://doi.org/10.1007/978-3-319-63245-2_21.

White, S. H., & Warren, L. K. (2017). Submaximal exercise training, more than dietary selenium supplementation, improves antioxidant status and ameliorates exercise-induced oxidative damage to skeletal muscle in young equine athletes. *Journal of animal science*, 95(2), 657–670. https://doi.org/10.2527/jas.2016.1130.

Williams, C. A. (2016). The effect of oxidative stress during exercise in the horse. *Journal of animal science*, 94(10), 4067–4075. https://doi.org/10.2527/jas.2015-9988.

Williams, C. A., Gordon, M. E., Betros, C. L., & McKeever, K. H. (2008). Apoptosis and antioxidant status are influenced by age and exercise training in horses. *Journal of animal science*, 86(3), 576–583. https://doi.org/10.2527/jas.2007-0585.

Williams, C. A., Kronfeldt, D. S., Hess, T. M., Saker, K. E., Waldron, J. N., Crandell, K. M., Hoffman, R. M., & Harris, P. A. (2004). Antioxidant supplementation and subsequent oxidative stress of horses during an 80-km endurance race. *Journal of animal science*, 82(2), 588–594. https://doi.org/10.2527/2004.822588x.

Witaszek, M., Pażontka-Lipiński, P., & Tkachenko, H. (2017). Sezonowe zmiany markerów stresu oksydacyjnego w osoczu krwi koni biorących udział w rekreacyjnych jazdach konnych w dynamice treningu. *Słupskie Prace Biologiczne*, 14, 185–208.

Wróbel, B., Zielewicz, W., & Staniak, M. (2023). Challenges of Pasture Feeding Systems – Opportunities and Constraints. *Agriculture*, 13(5), 974. https://doi.org/10.3390/agriculture13050974.

Young, B. A. (1981). Cold stress as it affects animal production. *Journal of animal science*, 52(1), 154–163. https://doi.org/10.2527/jas1981.521154x.

Zar, J.H. (1999). *Biostatistical Analysis*. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.