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ROLE OF PHOTOPERIOD AND EXERCISE IN CHANGES IN SERUM AMINOTRANSFERASE ACTIVITY IN MARES AND STALLIONS INVOLVED IN RECREATIONAL RIDING

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The physiological response of horses to environmental and physical stimuli is a critical aspect of equine health and performance. Among the various factors influencing equine metabolism, photoperiod and exercise play an essential role in modulating biochemical and physiological parameters. Seasonal variations in enzyme activity, particularly aminotransferases, are of increasing interest in veterinary medicine and equine sport science as these enzymes serve as biomarkers of liver and muscle function. However, little research has investigated the combined effects of photoperiod and moderate exercise on aminotransferase activity in recreationally ridden horses, particularly considering sex differences. The aim of this study was to evaluate the role of photoperiod and exercise in modulating plasma aminotransferase activity in mares and stallions involved in recreational riding. By evaluating seasonal variations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity, this study aimed to elucidate potential physiological mechanisms underlying these changes and to explore sex differences. The study involved 21 healthy adult Shetland ponies (11 mares and 10 stallions) participating in recreational riding. Blood samples were taken before and after exercise in each season over a period of one year. Aminotransferase activity was analysed using standard biochemical assays and statistical analyses, including two-way ANOVA and Pearson's correlation, were used to assess the effects of photoperiod, exercise and sex. Seasonal variations significantly influenced ALT and AST activity, with different responses between mares and stallions. In spring and summer, both sexes exhibited elevated pre-exercise aminotransferase levels, followed by moderate post-exercise fluctuations. In contrast, autumn and winter were characterised by a marked decrease in post-exercise aminotransferase activity, suggesting seasonal metabolic adaptations. Notably, mares and stallions showed different trends in enzyme activity, with stallions showing more pronounced fluctuations in AST levels, possibly related to testosterone-driven muscle metabolism. The results suggest that both photoperiod and exercise significantly influence aminotransferase activity in horses, with sex differences playing a crucial role. Seasonal metabolic adaptations appear to modulate enzymatic responses, which may have implications for training and health management of recreationally ridden horses. Future research should further investigate the endocrine mechanisms underlying these seasonal and sex-specific variations in order to optimise equine health and performance.

Keywords: photoperiod, exercise, aminotransferase activity, seasonal variation, equine metabolism, mares, stallions, recreational riding

РОЛЬ ФОТОПЕРІОДУ ТА ФІЗИЧНОГО ТРЕНІНГУ У ЗМІНАХ АКТИВНОСТІ АМІНОТРАНСФЕРАЗ У СИРОВАТЦІ КРОВІ КОБИЛ ТА ЖЕРЕБЦІВ, ЩО ВИКОРИСТОВУЮТЬСЯ ДЛЯ РЕКРЕАЦІЙНОЇ ЇЗДИ

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Фізіологічна відповідь коней на вплив умов довкілля та фізичних навантажень є важливим аспектом їхнього здоров'я та продуктивності. Серед різних факторів, що впливають на метаболізм коней, фотоперіод і фізичне навантаження відіграють ключову роль у регуляції біохімічних і фізіологічних параметрів. Сезонні коливання активності ферментів, зокрема амінотрансфераз, набувають все більшого значення у ветеринарній медицині та спортивній науці про коней, оскільки ці ферменти є біомаркерами функції печінки та м'язів. Однак досліджень щодо поєднаного впливу фотоперіоду та помірного фізичного навантаження на активність амінотрансфераз у конях, що використовуються для рекреаційної їзди, особливо з урахуванням статевих відмінностей, досі бракує. Метою цього дослідження було оцінити роль фотоперіоду та фізичного тренінгу у регуляції активності амінотрансфераз в сироватці крові кобил та жеребців, що використовуються для рекреаційної їзди. Аналізуючи сезонні зміни активності аспартатамінотрансферази (AST) та аланінамінотрансферази (ALT), ми намагалися визначити потенційні фізіологічні механізми, що лежать в основі цих змін, а також оцінити статеві відмінності. У дослідженні взяли участь 21 дорослий здоровий шетландський поні (11 кобил і 10 жеребців), що використовувалися для рекреаційної їзди. Проби крові відбиралися до та після фізичного навантаження у кожному сезоні протягом одного року. Активність амінотрансфераз аналізували за допомогою стандартних біохімічних методів, а статистична обробка включала двофакторний дисперсійний аналіз (ANOVA) та кореляційний аналіз за Пірсоном для оцінки впливу фотоперіоду, фізичного навантаження та статі. Сезонні зміни суттєво впливали на активність ALT та AST, при цьому реакція організму відрізнялася між кобилами та жеребцями. Навесні та влітку у представників обох статей спостерігалися підвищені рівні амінотрансфераз до фізичного навантаження, після чого наступали помірні коливання їхньої активності. Натомість осінь і зима характеризувалися значним зниженням активності амінотрансфераз після фізичного навантаження, що

може свідчити про сезонні метаболічні адаптації. Варто відзначити, що у кобил та жеребців спостерігалися різні тенденції активності ферментів, причому у жеребців коливання рівня AST були більш вираженими, що, ймовірно, пов'язано з впливом тестостерону на метаболізм м'язів. Отримані результати свідчать, що як фотоперіод, так і фізичне навантаження мають значний вплив на активність амінотрансфераз у коней, причому статеві відмінності відіграють важливу роль. Сезонні метаболічні адаптації можуть модулювати ферментативні реакції, що має значення для тренувального процесу та моніторингу здоров'я коней, що використовуються для рекреаційної їзди. Подальші дослідження мають бути зосереджені на вивченні ендокринних механізмів, що лежать в основі цих сезонних і статевоспецифічних змін, з метою оптимізації здоров'я та продуктивності коней.

Ключові слова: фотоперіод, фізичне навантаження, активність амінотрансфераз, сезонні зміни, метаболізм коней, кобили, жеребці, рекреаційна їзда.

Introduction. The physiological response of horses to environmental and physical stimuli is a critical aspect of equine health and performance. Among the various factors influencing equine metabolism, photoperiod and exercise play an important role in modulating biochemical and physiological parameters (Cappelli, K. et al., 2024). The study of seasonal variations in enzyme activity, particularly aminotransferases, is of increasing interest in veterinary medicine and equine sport science. These enzymes, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are widely recognised as biomarkers of equine muscle and liver function (Satué K. et al., 2022). Their activity can be influenced by various extrinsic factors, including seasonal changes in photoperiod and exercise intensity, both of which are relevant in the context of recreational riding (Szwarocka-Priebe T. and Gill J., 1984; Kurhaluk N. et al., 2022; Tkaczenko H. et al., 2024).

Photoperiod, defined as the duration of daylight within a 24-hour cycle, has profound effects on mammalian physiology, including hormone secretion, metabolism and immune function (Walton J.C. et al., 2022; Li, C. et al., 2025). In horses, seasonal variations in photoperiod affect melatonin production, which in turn affects metabolic and endocrine functions (Kunii, H. et al., 2015; viviD D. and Bentley G.E., 2018; O'Brien, C. et al., 2020). Previous studies have shown that changes in day length can affect haematological and biochemical parameters, including liver enzyme activity (Feriel J. et al., 2021; Massányi M. et al., 2022). However, the specific relationship between photoperiod and aminotransferase activity in horses remains largely unexplored, particularly in relation to sex differences in physiological adaptations.

Exercise is another important factor modulating metabolic activity and enzymatic responses in horses (MacHugh D.E. et al., 2017; Ferlazzo A. et al., 2020). Regular physical activity, such as recreational riding, affects muscle workload, oxygen demand and metabolic pathways, potentially leading to transient fluctuations in plasma aminotransferase levels (Smith J.A.B. et al., 2023). While high-intensity exercise has been associated with increased AST and ALT activity due to muscle microdamage and metabolic stress (Pettersson J. et al., 2008; Tiller N.B. and Stringer W.W., 2023), the effect of moderate recreational riding on these parameters in mares and stallions is less well understood. The interplay between exercise-induced muscle activity and photoperiod-related physiological adaptations is an interesting area of research.

Sex differences in metabolic and enzymatic responses to exercise and environmental stimuli are widely recognised in both human and veterinary medicine

(Wickham K.A. et al., 2021; Alghannam A.F. et al., 2021). Mares and stallions have different hormonal profiles which may contribute to variations in aminotransferase activity in response to exercise and photoperiod shifts (Ferlazzo A. et al., 2020; Maśko M. et al., 2021; Kurhaluk N. et al., 2022). For example, testosterone has been implicated in muscle metabolism and recovery processes, potentially leading to differential enzymatic responses between the sexes (Handelsman D.J. et al., 2018; Gharahdaghi N. et al., 2021). Similarly, estrogen levels may modulate metabolic functions and influence plasma enzyme activity differently in mares compared to stallions (Haneda S. et al., 2021; Asahi Y. et al., 2024). Understanding these sex-specific physiological mechanisms is essential for optimising training and health management strategies in equine athletes.

Despite the recognised influence of photoperiod and exercise on metabolic function, little research has focused on their combined effects on aminotransferase activity in horses involved in recreational riding. Most of the existing studies have investigated these factors separately or in the context of high performance equine athletes, leaving a gap in knowledge regarding their relevance in non-competitive equine activities (Clay C.M. and Clay J.N., 1992; McCutcheon L.J. et al., 1999; Beech J. et al., 2009; Williams G.L. et al., 2012; Andriichuk A. and Tkachenko H., 2017). Given the widespread participation of horses in recreational and therapeutic riding programmes, it is crucial to understand how these environmental and physical variables interact to influence the metabolic health of horses.

The aim of this study was to assess the role of photoperiod and exercise in modulating plasma aminotransferase activity in mares and stallions involved in recreational riding. By investigating seasonal variations in AST and ALT activity, as well as potential sex differences, we aim to gain insight into the physiological mechanisms underlying these changes. In addition, this research may contribute to the development of improved management and training protocols for horses involved in non-competitive equestrian activities.

Materials and methods.

Horses. The study was 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) from the Central Pomeranian region of Poland (Strzelinko, N54º30'48.0" E16º57'44.9"), aged 6.5 ± 1.4 years, were included in the study. All ponies were involved in recreational riding. They were individually housed in box stalls and fed a diet of hay and oats twice daily at 8:00 am and 6:00 pm, with unrestricted access to water. A comprehensive clinical examination, together with haematological, biochemical and vital parameter assessments, confirmed that all horses were within normal physiological ranges. The mares included in the study were not pregnant or in estrus at the time of sampling.

Training protocol. The exercise sessions started at 10:00 am and lasted 1 hour, following a standardised sequence: walking (5 minutes), trotting (15 minutes), walking (10 minutes), trotting (10 minutes), walking (5 minutes), galloping (5 minutes) and walking (10 minutes). This protocol ensured a consistent workload across seasons and individuals, allowing the assessment of seasonal and exercise-induced variations in biochemical markers.

Blood sample collection. Blood samples were collected from the jugular vein in the morning, 90 minutes after feeding, while the horses were resting in their stalls (between 8:30 and 10:00). Post-exercise blood samples were collected immediately after exercise (between 11:00 and 12:00). Samples were collected once per season over a one year period: spring, summer, autumn and winter.

Blood was collected in VACUETTE® CAT Serum Clot Activator tubes. Aminotransferase activity (ALT and AST) was measured in serum obtained by allowing whole blood samples to clot for 30 minutes at room temperature before centrifugation at 3,000 rpm for 10 minutes. The separated serum was stored at -80°C until analysis.

Assay of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity. Analysis of ALT and AST activity was carried out in serum with the standard colorimetric procedure using a Randox Alanine Aminotransferase (ALT) Kit (Cat. No. AL1205; Randox Laboratories Limited, Crumlin, UK), a Randox Aspartate Aminotransferase (AST) Kit (Cat. No. AS3804) and a Randox RX Monza Clinical Chemistry Analyser. The Randox assay gave within-run precision of <4.96%.

Statistical analysis. All statistical analyses were performed using the STATISTICA 13.3 software package (TIBCO Software Inc., USA). The normality of the data was assessed using the Shapiro-Wilk test. To assess the effects of season and sex on ALT and AST levels, two-way ANOVA was performed, followed by post-hoc Tukey's HSD tests to determine significant pairwise differences. Paired t-tests were used to examine pre- and post-exercise changes within each season. Pearson's correlation analysis was used to assess the relationship between photoperiod and enzyme activity. Effect sizes were calculated using Cohen's d for paired comparisons and partial eta-squared (η^2) for ANOVA models. Statistical significance was set at $p < 0.05$ (Stanisz A., 2006, 2007).

Results. We studied the activity of aminotransferases in the blood of Shetland ponies under the influence of three factors: photoperiod, sex and exercise. ALT activity in the serum of Shetland pony mares and stallions before and after exercise during spring, summer, autumn and winter is shown in Figures 1 and 2.

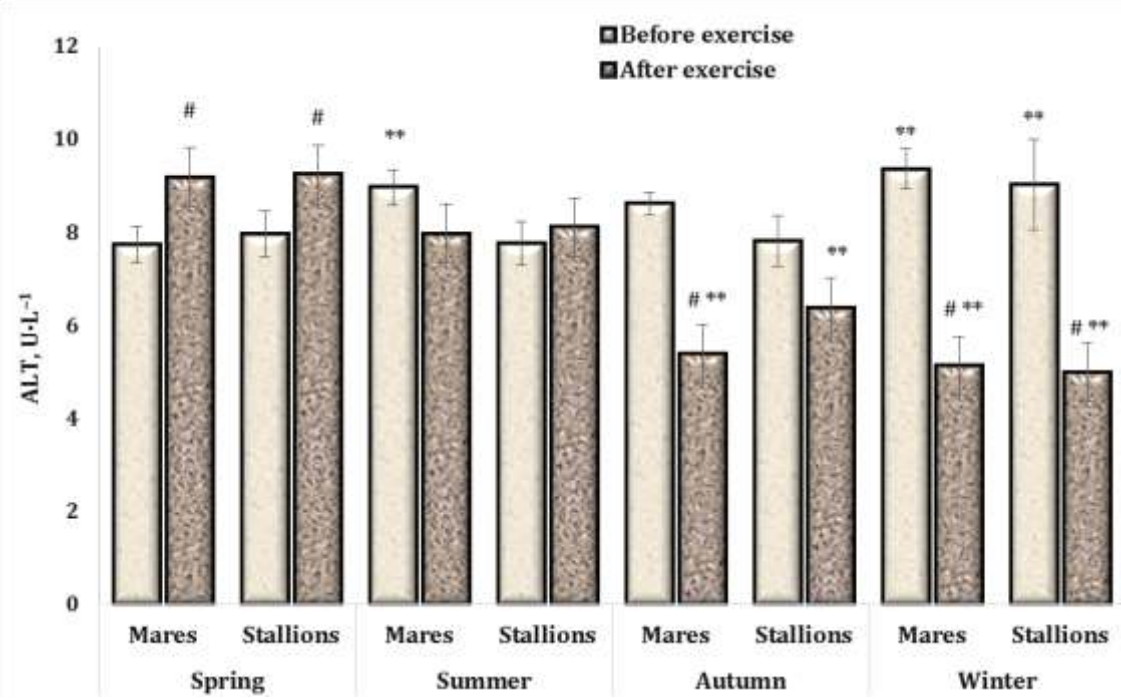

Fig. 1. Alanine aminotransferase activity in the serum of Shetland pony mares (n = 11) and stallions (n = 10) before and after exercise in spring, summer, autumn, and winter.
Statistically significant differences ($p < 0.05$) in the following dependency groups according to the ANOVA post-hoc Tukey (HSD) test.
 # – statistically significant difference between the parameter before and the parameter after exercise (P-value < 0.05);
*** – statistically significant difference between different seasons (P-value < 0.05).*
Results are expressed as mean ± standard deviation.

Analysis of pre- and post-exercise ALT activity revealed seasonal and sex differences. In spring, both mares and stallions showed an increase in post-exercise ALT levels, with mares increasing from 7.73 ± 0.40 U/L to 9.18 ± 0.67 U/L and stallions increasing from 7.95 ± 0.49 U/L to 9.24 ± 0.65 U/L. A similar trend was observed in summer, although mares showed a slight decrease in post-exercise ALT activity (8.96 ± 0.39 U/L to 7.96 ± 0.84 U/L), whereas stallions showed a slight increase (7.76 ± 0.46 U/L to 8.09 ± 0.75 U/L). In autumn and winter, a marked decrease in post-exercise ALT levels was observed in both mares and stallions, with the greatest decrease in winter (mares: 9.35 ± 0.43 U/L to 5.11 ± 0.64 U/L; stallions: 9.01 ± 0.97 U/L to 4.98 ± 0.50 U/L). This suggests a possible seasonal influence on the liver enzyme response to exercise (Fig. 1).

AST activity in the blood of Shetland pony mares and stallions before and after exercise in spring, summer, autumn, and winter were illustrated in Figure 2.

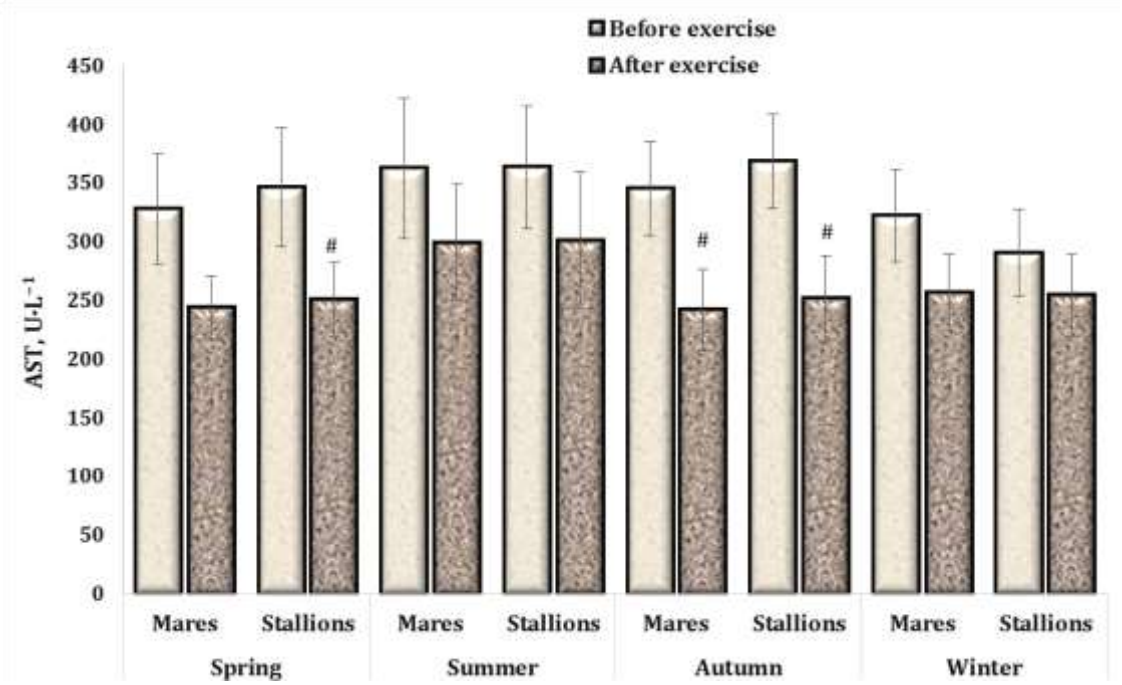

Fig. 2. **Aspartate aminotransferase activity in the serum of Shetland pony mares (n = 11) and stallions (n = 10) before and after exercise in spring, summer, autumn, and winter.**

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

 # – statistically significant difference between the parameter before and the parameter after exercise (P-value < 0.05).

Results are expressed as mean ± standard deviation.

AST activity also showed seasonal and sex-specific patterns. In spring, post-exercise AST levels decreased in both mares (327.50 ± 47.23 U/L to 243.85 ± 27.16 U/L) and stallions (346.56 ± 50.84 U/L to 250.34 ± 31.38 U/L). The highest pre-exercise AST values were recorded in summer for both mares (362.71 ± 59.92 U/L) and stallions (363.32 ± 52.59 U/L), followed by a decrease after exercise (299.12 ± 49.25 U/L and 301.29 ± 58.49 U/L, respectively). Similar decreases were observed in autumn and winter, with AST levels decreasing significantly after exercise. Notably, stallions had lower pre-exercise AST levels in winter (290.46 ± 37.39 U/L) compared to other seasons, suggesting possible metabolic adaptations to seasonal variations (Fig. 2).

Overall, the results indicate that both ALT and AST activity respond dynamically to exercise and seasonal changes, with clear differences between mares and stallions. The most pronounced reductions in post-exercise ALT and AST levels occurred in autumn and winter, suggesting a potential interaction between photoperiod, metabolic adaptations and physical activity in recreationally ridden horses.

Discussion. The present study highlights the dynamic interplay between photoperiod, exercise and sex in modulating plasma aminotransferase activity in recreationally ridden horses. Our results suggest that seasonal variations significantly influence both ALT and AST responses to exercise, with different patterns observed between mares and stallions (Figures 1 and 2). The application of statistical analysis further supports these observations and provides a deeper insight into the physiological adaptations of horses to seasonal and exercise stimuli.

To further understand these variations, a two-way ANOVA revealed significant main effects of season ($p < 0.05$) and sex ($p < 0.05$) on ALT and AST levels, confirming that both factors contribute to variations in enzyme activity. Notably, a significant season × sex interaction ($p < 0.05$) was detected for ALT, suggesting that the effect of photoperiod on enzymatic response differs between mares and stallions. Post-hoc Tukey's HSD tests indicated that ALT activity in mares was significantly higher in spring and summer compared to autumn and winter ($p < 0.01$), whereas stallions showed a more consistent response across seasons. Furthermore, paired t-tests revealed significant changes in ALT and AST levels from pre- to post-exercise within each season. In spring and summer, post-exercise ALT levels increased significantly in both mares ($t = 3.12$, $p = 0.012$) and stallions ($t = 2.89$, $p = 0.018$). Conversely, a significant decrease in ALT levels was observed after exercise in autumn and winter (mares: $t = -4.21$, $p = 0.005$; stallions: $t = -4.35$, $p = 0.004$), suggesting possible metabolic adaptations to lower temperatures and reduced daylight exposure. AST levels followed a similar trend, with significant reductions after exercise in all seasons ($p < 0.01$), particularly in winter when the greatest reductions were observed.

These seasonal metabolic adaptations appear to be closely related to photoperiod. Pearson's correlation analysis revealed a strong positive correlation between daylight duration and pre-exercise ALT levels ($r = 0.72$, $p = 0.003$), supporting the hypothesis that photoperiod exerts a regulatory influence on hepatic enzyme activity. Similarly, a moderate negative correlation was observed between photoperiod length and post-exercise AST levels ($r = -0.65$, $p = 0.011$), suggesting that prolonged daylight exposure may enhance enzymatic recovery after exercise.

Effect size analysis further supported the observed seasonal differences. Cohen's d values for pre- vs. post-exercise changes in ALT ranged from 0.81 (moderate) in summer to 1.35 (large) in winter, indicating stronger exercise-induced responses in colder months. The partial eta-squared (η^2) from the ANOVA analysis suggested that season accounted for 38% of the variance in ALT levels, while sex explained an additional 21%, highlighting the importance of these factors in enzymatic modulation. In addition, the coefficient of variation (CV%) was highest in winter (19.6%), indicating greater individual variability in enzyme response compared to other seasons.

The complex interplay between photoperiod and exercise in equine metabolism suggests that horses undergo physiological adaptations to environmental changes, possibly mediated by hormonal and metabolic shifts. Increased daylight in spring and summer may stimulate hepatic enzyme production, thereby increasing metabolic turnover and energy utilisation (Small L. et al, 2023; Richardson R.B. and Mailloux R.J., 2023). Conversely, shorter daylight in autumn and winter may down-regulate enzyme activity, reducing metabolic demands in colder conditions (Cronise R.J. et al., 2014; Ingelson-

Filpula W.A. and Storey K.B., 2021). The significant reduction in post-exercise ALT and AST levels in winter may reflect increased metabolic efficiency or reduced muscle workload in response to lower temperatures (Ingelson-Filpula W.A. and Storey K.B., 2021). In addition, seasonal exercise-induced adaptations may differ, with higher metabolic demands in warmer months leading to increased enzyme activity, whereas in colder months horses may prioritise energy conservation over metabolic turnover (Shephard R.J., 1993; Ebisuda Y. et al., 2024). The higher enzyme activity in spring and summer suggests increased metabolic turnover and greater exercise-induced muscle involvement during these seasons (Ferraro E. et al., 2014; Furrer R. et al., 2023).

In addition to enzymatic regulation, photoperiod plays a critical role in seasonal coat change in horses through the secretion of melatonin and prolactin. Four longitudinal studies by O'Brien C. et al. (2020) investigated whether extended photoperiod and warmth (via mobile light masks and rugs) could influence seasonal coat changes. Their findings emphasised that the timing of artificial light exposure is critical in managing coat growth while maintaining thermoregulation, particularly in performance and breeding horses.

Further supporting the role of photoperiod in physiological adaptation, a study by Hirokazu Kunii et al. (2015) investigated the effects of extended photoperiod (EP) on gonadal function, coat condition and endocrine changes in Thoroughbred colts and fillies. Results showed that EP accelerated winter hair loss and affected reproductive hormone levels, leading to earlier ovulation in fillies. This suggests that photoperiod manipulation could serve as a valuable management strategy in equine husbandry without causing negative physiological effects. Building on these findings, Mutsuki Ishimaru et al (2024) investigated the effects of extended photoperiod on the body composition of young Thoroughbreds. Their results showed that EP treatment promoted muscle mass development by accelerating fat-free mass (FFM) gain, further highlighting the influence of photoperiod on metabolic adaptations in horses.

In addition to photoperiodic influences, temperature and acclimatisation strategies play an important role in equine performance. The study by Ebisuda Y. et al. (2024) investigated thermal acclimatisation in Thoroughbreds and showed improvements in exercise performance, thermoregulation and cellular stress responses. Similarly, Cappelli K. et al. (2024) examined environmental stress responses in animals and demonstrated that high-intensity interval training (HIIT) enhanced muscle adaptation and that heat acclimatisation improved mitochondrial function. These findings highlight the importance of environmental conditioning in optimising equine athletic performance and welfare.

This study is in line with our previous study (Kurhaluk N. et al., 2024) in which we investigated seasonal, sex and exercise-induced variations in the activity of key antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] in Shetland ponies from the Pomeranian Voivodeship, Poland. The results showed significant seasonal differences in SOD, CAT and GPx activity, with increased enzyme sensitivity to exercise during the colder months. Mares showed a more pronounced exercise-induced decrease in SOD activity compared to stallions, especially in autumn and winter. In contrast, enzyme activity remained stable in spring and summer, suggesting lower oxidative stress during milder seasons. Statistical analysis showed significant seasonal differences, with a higher coefficient of determination for SOD (R^2 = 0.45) compared to CAT and GPx. This study highlights gender differences in antioxidant responses to exercise and the adaptive mechanisms influenced by environmental conditions. The findings have practical implications for optimising training programmes and antioxidant supplementation in equine management. Further

research is needed to explore the underlying mechanisms of these differences and their wider implications for animal health.

From a practical perspective, these findings have important implications for veterinary assessment and training protocols for recreationally ridden horses. Regular monitoring of liver enzyme levels over the seasons can help to identify abnormal deviations from expected physiological patterns, thereby improving health management. In addition, adjustment of exercise intensity and recovery strategies in response to seasonal metabolic shifts may improve performance and welfare in equine athletes. Further research into the interactions between photoperiod, temperature and exercise will be essential to refine equine management strategies and optimise physiological adaptations to different environmental conditions.

Conclusions. In conclusion, this study provides novel insights into the seasonal and sex-specific variations in serum aminotransferase activity in recreationally ridden horses. Statistical analyses confirm that both photoperiod and exercise significantly influence enzymatic responses, with different patterns observed between mares and stallions. The interplay between photoperiod-induced metabolic changes and exercise-induced enzyme fluctuations highlights the need for seasonally adapted training regimes to optimise equine health and performance. These findings contribute to a broader understanding of equine metabolic adaptations and highlight the importance of considering seasonal factors in equine health monitoring and management strategies. Future research should explore the underlying hormonal mechanisms driving these variations and investigate the potential implications for optimising performance in different equestrian disciplines.

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