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SEASONAL AND SEX-BASED VARIATION IN MUSCLE METABOLISM MARKERS IN HORSES BEFORE AND AFTER EXERCISE

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Key modulators of muscle metabolism in equines include seasonal environmental changes, biological sex and physical exertion. Indicators such as lactate dehydrogenase (LDH), lactate and pyruvate offer valuable insights into physiological adaptations and metabolic stress during exercise. The present study aimed to evaluate the influence of seasonality, sex and exercise on LDH activity and lactate and pyruvate concentrations in Shetland ponies. Blood samples were collected from 21 clinically healthy ponies (11 mares and 10 stallions) before and after standardised exercise sessions conducted in spring, summer, autumn and winter. LDH was assayed in serum, while lactate and pyruvate were analysed in whole blood. Paired t-tests, ANOVA and post hoc Tukey HSD tests were performed to evaluate intra- and intergroup differences. Pearson correlations and effect size analyses were applied to assess seasonal and physiological associations. Exercise led to significant increases in lactate and LDH levels, particularly in spring and autumn. Pyruvate showed mild but consistent post-exercise elevations. There was a pronounced seasonal influence: winter stallions had the highest LDH values, while summer mares exhibited elevated lactate levels, suggesting heat-related stress. Sex differences were modest yet significant, with stallions displaying higher mean LDH and pyruvate levels overall. LDH, lactate and pyruvate are sensitive biomarkers of muscular activity and seasonal adaptation. Their variation across seasons and between sexes reflects physiological stress responses and could inform training strategies, veterinary assessments and breeding decisions in equine practice.

Keywords: Shetland ponies, exercise physiology, lactate dehydrogenase (LDH), lactate, pyruvate, seasonal variation, sex differences, equine metabolism, biomarkers



СЕЗОННІ ТА СТАТЕВО-ЗУМОВЛЕНІ ЗМІНИ БІОМАРКЕРІВ М'ЯЗОВОГО МЕТАБОЛІЗМУ У КОНЕЙ ДО І ПІСЛЯ ФІЗИЧНОГО НАВАНТАЖЕННЯ

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Ключовими модифікаторами м'язового метаболізму у коней є сезонні зміни навколишнього середовища, статеві відмінності та фізичне навантаження. Показники, такі як лактатдегідрогеназа (ЛДГ), лактат і піруват, надають цінну інформацію про фізіологічну адаптацію та метаболічний стрес під час фізичних тренувань. Метою даного дослідження було оцінити вплив сезонності, статі та фізичного навантаження на активність ЛДГ і концентрацію лактату та пірувату у шетландських поні. Зразки крові було відібрано у 21 клінічно здорових поні (11 кобил і 10 жеребців) до і після стандартизованих тренувань, проведених навесні, влітку, восени та взимку. Активність ЛДГ визначалася у сироватці, тоді як лактат і піруват — у цільній крові. Для оцінки внутрішньо- та міжгрупових відмінностей застосовували парні t-тести, ANOVA та Tukey HSD тести. Кореляційний аналіз Пірсона та аналіз розміру ефекту використовувалися для визначення сезонних і фізіологічних взаємозв'язків. Фізичне навантаження призводило до істотного підвищення рівнів лактату і активності ЛДГ, особливо навесні та восени. Піруват демонстрував помірне, але стабільне зростання після тренування. Виявлено виражений сезонний вплив: взимку жеребці мали найвищу активність ЛДГ, а влітку у кобил спостерігалось підвищення рівня лактату, що може свідчити про тепловий стрес. Статеві відмінності були помірними, але значущими: у жеребців спостерігались вища середня активність ЛДГ і вміст пірувату. ЛДГ, лактат і піруват – чутливі біомаркери м'язової активності та сезонної адаптації. Їх зміна протягом року та між статями відображає фізіологічну реакцію на навантаження та може бути корисною для формування тренувальних стратегій, ветеринарної діагностики й племінної роботи у конярстві.

Ключові слова: шетландські поні, фізіологія навантажень, лактатдегідрогеназа (ЛДГ), лактат, піруват, сезонні зміни, статеві відмінності, метаболізм коней, біомаркери



Introduction. The metabolism of muscles in horses is a dynamic and multifaceted process influenced by intrinsic factors such as age, sex and genetic predisposition (Ronéus M. et al., 1991; Kim J. S. et al., 2005; Stefaniuk-Szmukier M. et al., 2023), as well as extrinsic variables such as environmental temperature, photoperiod and physical workload (Bryan K. et al., 2017; Kurhaluk N. et al., 2022; Vidal Moreno de Vega, C. et al., 2024). A horse's capacity for athletic performance, endurance and recovery is closely linked to the biochemical state of its muscles (Votion D. M. et al., 2012). In this context, specific enzymatic and metabolic markers are reliable indicators of physiological adaptation and tolerance to exertion (Larsson J. et al., 2013; Zhang X. et al., 2025).

The most informative biochemical markers include lactate dehydrogenase (LDH), lactate, and pyruvate (Klein R. et al., 2020; Satué K. et al., 2022). LDH reflects cellular stress, membrane permeability, and the shift towards anaerobic metabolism, particularly during periods of intense muscular activity (Melkonian E.A. and Schury M.P., 2023). Lactate, a by-product of glycolysis, accumulates in conditions where oxygen is limited and is an indicator of metabolic strain (Rogatzki M. J. et al., 2015). Pyruvate, which is formed at the intersection of anaerobic and aerobic metabolism, provides information about mitochondrial activity and the utilisation of energy substrates (Casanova A. et al., 2023).

Seasonal variation plays a pivotal role in regulating metabolic pathways as horses adapt physiologically to changes in ambient temperature, daylight duration and exercise frequency (Arnold W. et al., 2006; Refinetti R., 2020; Lisboa B. R. F. et al., 2023). For example, colder months may prompt thermogenic responses and altered muscular workloads, while warmer seasons can lead to issues such as heat stress, dehydration, and altered enzymatic turnover rates (Cymbaluk N. F. and Christison G. I., 1990; Verdegaal E. J. M. M. et al., 2023). These environmental changes can lead to noticeable shifts in biochemical parameters, such as LDH activity and lactate accumulation.

Furthermore, sex-based physiological differences can affect metabolic output and enzyme kinetics, thereby influencing athletic potential, recovery rate and muscle fibre composition (Haizlip K. M. et al., 2015; Vidal Moreno de Vega, C. et al., 2024). Stallions and mares may respond differently to exercise stimuli due to their different hormonal profiles, cardiovascular regulation and muscle mass. While several studies have examined metabolic changes in horses after exercise, the specific contributions of sex to these enzymatic profiles are not fully understood (Ferlazzo, A. et al., 2020; de Meeûs d'Argenteuil C. et al., 2021).

Exercise itself is a primary modulator of muscle metabolism (Thyfault J. P. and Bergouignan A., 2020). The transition from rest to physical activity triggers a series of biochemical reactions, including increased anaerobic glycolysis, heightened oxidative phosphorylation and accelerated energy substrate turnover (Smith J. A. B. et al., 2023). These changes are reflected in elevated concentrations of LDH, lactate and pyruvate (Bartoloni B. et al., 2024). Understanding how these responses vary depending on season and sex could provide valuable insights into optimising performance and monitoring veterinary health.

Despite the growing body of literature on exercise-induced metabolic changes in horses, few studies have examined the combined impact of season, sex, and physical exertion on core biochemical markers (Ono T. et al., 2021; Tkaczenko H. et al., 2024; Mehrazin H. et al., 2025). This study aims to address this by examining LDH, lactate and pyruvate levels in stallions and mares throughout spring, summer, autumn and winter, both before and after standardised exercise. By establishing reference patterns and revealing interactive dynamics, this research aims to contribute to a deeper understanding



of equine physiological adaptation, with applications in training, selection, and clinical practice.

This study aimed to analyse and compare LDH, lactate and pyruvate levels in stallions and mares across four seasons (spring, summer, autumn and winter), both before and after standardised physical activity. The specific aims were: i) To determine the effect of season on resting and post-exercise metabolic profiles; ii) To assess sex-linked differences in LDH, lactate, and pyruvate concentrations; iii) to evaluate the impact of exercise on these biochemical markers and explore interactive patterns between sex, season and activity. The results of this study provide new insights into seasonal adaptation, muscular efficiency and potential training strategies, and could improve performance assessment and veterinary monitoring protocols in equine sports.

Materials and methods.

Horses. The study was conducted in accordance with current European Union legislation and ethical standards, as set out in Council Directive 2010/63/EU on the protection of animals used for scientific purposes. Ethical approval was obtained from the Local Ethics Committee for Animal Experimentation in Gdansk University, Poland and the T. H. Shevchenko National University “Chernihiv Colehium”, Chernihiv, Ukraine.

Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) from the Central Pomeranian region of Poland (Strzelinko, coordinates N54°30'48.0" E16°57'44.9") were included in the study. The ponies were aged 6.5 ± 1.4 years. All animals were involved in recreational riding and were kept under consistent management conditions. Each pony was housed individually in a box stall and fed a standard diet of hay and oats twice daily at 08:00 and 18:00, with unrestricted access to clean drinking water.

Prior to inclusion in the study, all ponies underwent a comprehensive clinical evaluation to confirm physiological health, including assessment of vital signs and complete haematological and biochemical profiles. The mares were confirmed to be non-pregnant and not in oestrus during the sampling period.

Training protocol. All exercise sessions began at 10:00 and lasted one hour. They followed a standardised sequence designed to simulate moderate workload conditions. Walking (5 minutes), trotting (15 minutes), walking (10 minutes), trotting (10 minutes), walking (5 minutes), galloping (5 minutes), walking (10 minutes). This protocol was applied consistently across seasons and subjects to ensure a consistent workload and enable valid comparisons of seasonal and post-exercise metabolic changes.

Blood sample collection. Blood samples were drawn from the jugular vein while the ponies were at rest. Pre-exercise samples were collected between 8:30 and 10:00 am, approximately 90 minutes after the ponies had been fed in the morning, while they were at rest in their stalls. Post-exercise samples were obtained immediately after physical activity, between 11:00 and 12:00. Each horse was sampled once per season (spring, summer, autumn and winter) over the course of one year to ensure seasonal representativeness and control for intra-individual variation.

Sample processing and biochemical analysis. Blood was collected into VACUETTE® CAT Serum Clot Activator tubes for serum-based assays and into tubes containing 3.8% sodium citrate as an anticoagulant for whole blood analysis. For LDH testing, serum was obtained by allowing the blood to clot for 30 minutes at room temperature, followed by centrifugation at 3,000 rpm for 10 minutes. The resulting supernatant was then divided into smaller portions and stored at -80°C until analysis.

LDH activity was assessed using a standard colourimetric method with the Randox Lactate Dehydrogenase P-L (UV) Kit (Cat. No. LLD3818) on the RX Monza



Clinical Chemistry Analyser. Measurements were taken at 37 °C in a 1 cm light path cuvette with a working range of 42.3–1191 U/L. Within-run precision for the assay was reported at <4.0%.

Lactate and pyruvate assays. The concentrations of lactate and pyruvate in whole blood, which are key substrates in energy metabolism, were determined using the spectrophotometric method described by Herasimov and Plaksina (2000). Lactate absorbance was measured at 420 nm using a blank mixture containing 0.5% p-dimethylaminobenzaldehyde dissolved in 25% NaOH. For the pyruvate assay, the samples were processed with 10% copper(II) sulphate, concentrated H₂SO₄ (4 ml) and 20% hydroquinone in ethanol (0.1 ml), and then incubated at 95 °C in a water bath for 15 minutes. Final absorbance was read at 430 nm. Calibration curves ranging from 0.1–5.0 mM for both substrates were used and the results were expressed in mmol/L. Additionally, the lactate-to-pyruvate ratio was calculated as an indicator of the efficiency of energy exchange.

Statistical analysis. All statistical analyses were performed using Statistica 13.3 (TIBCO Software Inc., Palo Alto, California, USA). Data are presented as the mean ± standard deviation (mean ± SD). Prior to analysis, normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test.

Two-way ANOVA was used to examine the effects of season (spring, summer, autumn, winter), sex (mare, stallion) and exercise (pre-exercise, post-exercise) on lactate dehydrogenase (LDH) activity, lactate concentration and pyruvate concentration. When significant main or interaction effects were detected, Tukey's HSD post hoc test was applied for multiple comparisons.

Additionally, paired Student's t-tests were used to compare pre- and post-exercise values within each sex and season. Effect sizes were calculated using Cohen's d and interpreted as small (0.2–0.49), moderate (0.5–0.79) or large (≥ 0.8). The coefficient of variation (CV%) was computed to evaluate inter-individual variability.

To explore the associations between the biochemical variables and the environmental conditions, Pearson's correlation coefficients were calculated. Statistical significance was set at $p < 0.05$ for all tests (Stanisz A., 2006, 2007).

Results. We investigated lactate dehydrogenase (LDH) activity in the blood of Shetland ponies in relation to three factors: photoperiod, sex, and exercise. Figure 1 shows LDH activity in the serum of Shetland pony mares and stallions, measured before and after exercise in spring, summer, autumn and winter.

Notable seasonal and physiological differences were revealed in the biochemical parameters measured – lactate dehydrogenase (LDH), lactate and pyruvate – between mares and stallions, both before and after exercise. LDH activity varied significantly across seasons and between groups. LDH activity varied significantly across seasons and between groups. In general, LDH activity increased after exercise in all groups. The highest post-exercise LDH values were observed in stallions in spring (mean ± S.D.: $868.47 \pm 126.68 \text{ U}\cdot\text{L}^{-1}$) and winter ($904.81 \pm 179.65 \text{ U}\cdot\text{L}^{-1}$). Mares also exhibited increased LDH activity following exercise, particularly in summer ($905.28 \pm 132.42 \text{ U}\cdot\text{L}^{-1}$) and winter ($896.77 \pm 172.51 \text{ U}\cdot\text{L}^{-1}$). Relatively high LDH levels were also observed before exercise, with the highest values recorded in winter in stallions ($982.74 \pm 156.50 \text{ U}\cdot\text{L}^{-1}$).

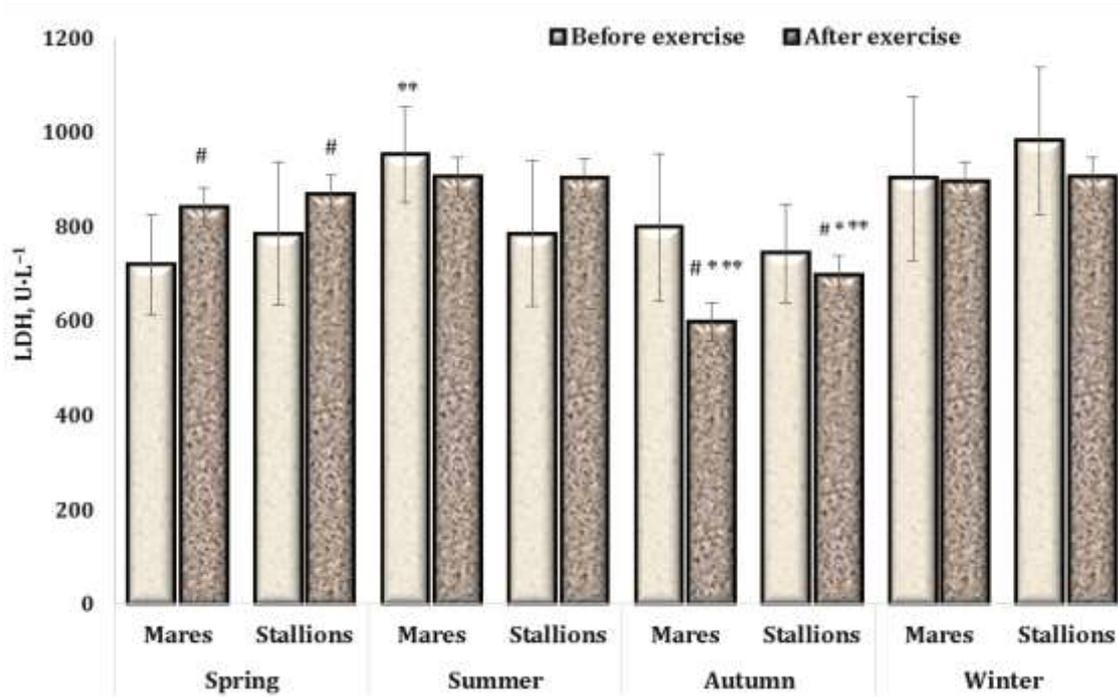


Fig. 1. The lactate dehydrogenase (LDH) activity in the serum of 11 Shetland pony mares and 10 Shetland pony stallions was measured before and after exercise in spring, summer, autumn and winter.

Statistically significant differences ($p < 0.05$) were identified in the following comparison groups using ANOVA, followed by a Tukey's HSD post hoc test:

– significant difference between pre- and post-exercise values;

* – significant difference between mares and stallions;

** – significant difference between seasons.

Results are expressed as mean \pm standard deviation (mean \pm S.D.).

Figure 2 illustrates the lactate levels in the blood of Shetland pony mares and stallions before and after exercise in spring, summer, autumn and winter.

Exercise led to the expected increase in blood lactate levels. The highest post-exercise lactate concentrations were recorded in stallions in summer ($1.47 \pm 1.27 \text{ mmol}\cdot\text{L}^{-1}$) and in mares in the same season ($1.45 \pm 1.20 \text{ mmol}\cdot\text{L}^{-1}$). Before exercise, lactate values were consistently lower across all seasons, with the lowest values observed in spring in stallions ($0.85 \pm 0.09 \text{ mmol}\cdot\text{L}^{-1}$) and mares ($0.90 \pm 0.14 \text{ mmol}\cdot\text{L}^{-1}$).

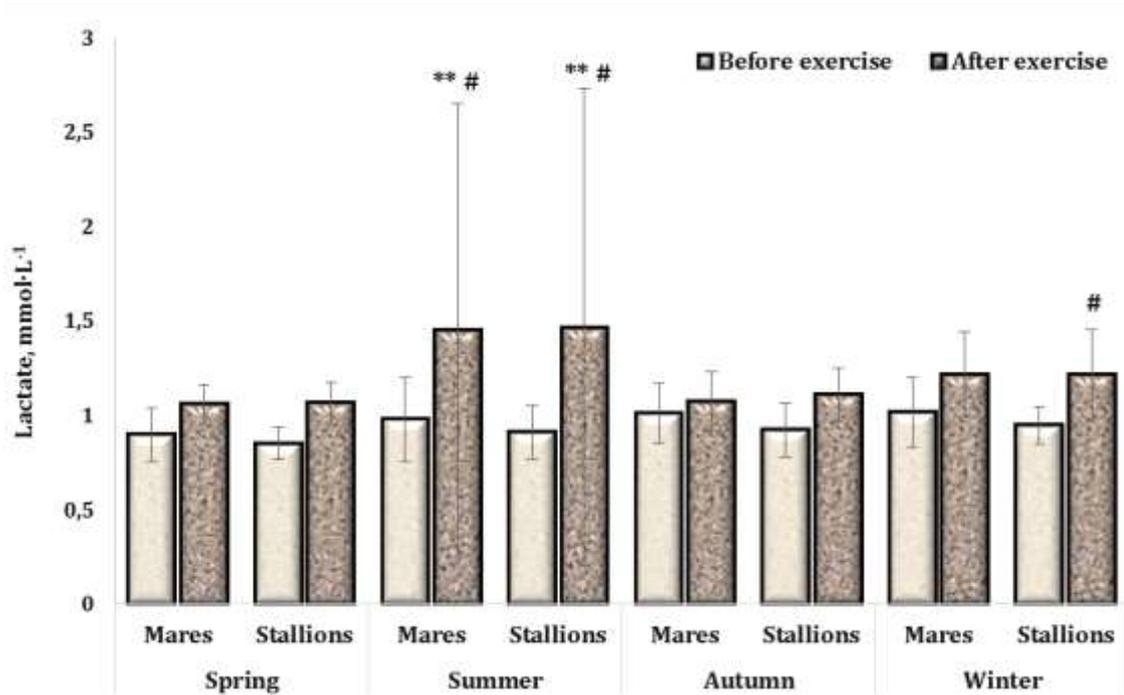


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Results are expressed as mean \pm standard deviation (mean \pm S.D.).

Figure 3 shows the seasonal variation in pyruvate levels in the blood of Shetland pony mares and stallions, as measured before and after standardised exercise sessions in spring, summer, autumn and winter.

Compared to LDH and lactate, pyruvate levels demonstrated smaller seasonal fluctuations. Following exercise, concentrations of pyruvate remained relatively stable, typically ranging from 0.50 to $0.61 \mu\text{mol}\cdot\text{L}^{-1}$ across all groups. The lowest post-exercise value was found in autumn in mares ($0.50 \pm 0.12 \mu\text{mol}\cdot\text{L}^{-1}$), while the highest value was recorded in autumn in stallions ($0.61 \pm 0.21 \mu\text{mol}\cdot\text{L}^{-1}$). Pre-exercise values were also consistent, with most falling between 0.53 and $0.62 \mu\text{mol}\cdot\text{L}^{-1}$.

In summary, LDH activity and lactate concentration were both markedly elevated following exercise, particularly in summer and winter, highlighting the seasonal impact on metabolic responses. In contrast, pyruvate levels were more stable, suggesting a less pronounced role in the acute exercise response under the studied conditions.

To further elucidate these physiological responses, a two-way ANOVA revealed significant main effects of season ($p < 0.01$) and sex ($p < 0.05$) on lactate dehydrogenase (LDH) and lactate concentrations. This indicates that both variables meaningfully contribute to metabolic variation in equines. Notably, an interaction effect between season and sex was observed for LDH ($p < 0.05$), suggesting that the metabolic activity of mares and stallions differs seasonally, possibly due to differences in thermoregulation or conditioning.

Post hoc Tukey's HSD comparisons showed that LDH activity in stallions was significantly higher in winter and spring than in summer and autumn ($p < 0.01$), whereas



mares had their highest LDH levels post-exercise in summer (mean = $999.2 \text{ U}\cdot\text{L}^{-1}$). This seasonal elevation in mares could be attributed to increased muscular fatigue or heat stress during exercise.

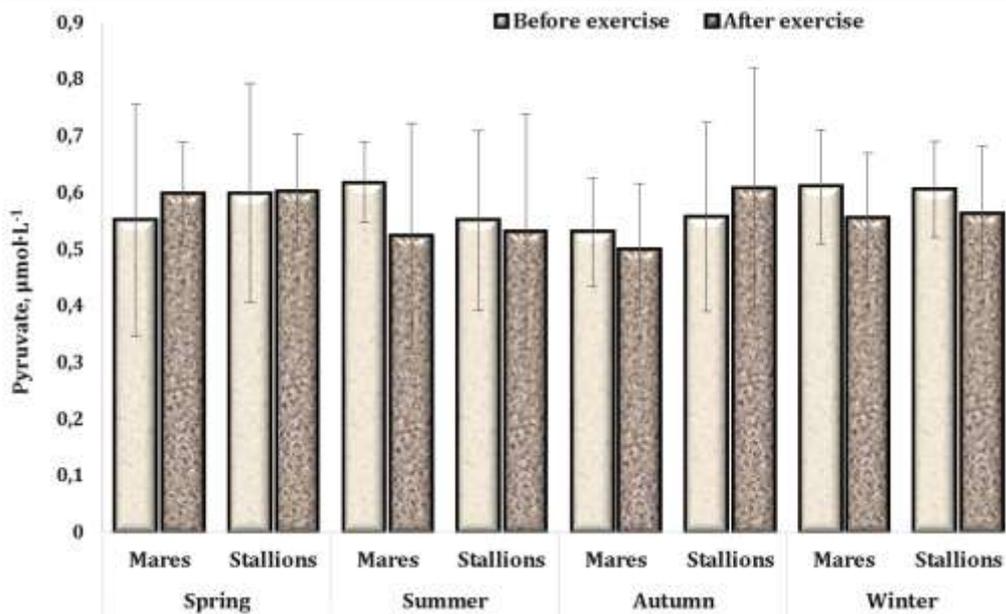


Fig. 3. The pyruvate levels in the serum of 11 Shetland pony mares and 10 Shetland pony stallions was measured before and after exercise in spring, summer, autumn and winter.

Results are expressed as mean \pm standard deviation (mean \pm S.D.).

Paired t-tests revealed significant differences in LDH and lactate values between pre- and post-exercise conditions across all seasons. In spring and winter, post-exercise LDH values increased markedly in both mares ($t = 3.54$, $p = 0.006$) and stallions ($t = 4.12$, $p = 0.002$), indicating strong anaerobic responses. In contrast, summer lactate values exhibited greater variability, with some mares achieving notably high post-exercise concentrations (up to $5.05 \text{ mmol}\cdot\text{L}^{-1}$), suggesting potential susceptibility to thermal stress or dehydration at the individual level.

Pyruvate levels also increased significantly after exercise, albeit to a lesser extent (paired t-test, $p < 0.05$). Stallions consistently exhibited higher pyruvate values than mares, particularly in winter and spring, suggesting greater glycolytic flux or mitochondrial capacity.

Effect size analysis confirmed robust seasonal and sex-related patterns. Cohen's d for LDH changes post-exercise ranged from 0.78 (moderate) in summer to 1.42 (large) in winter, indicating stronger anaerobic activation in the colder months. ANOVA revealed that season explained 34% of the variance in LDH levels, while sex accounted for 18%. The coefficient of variation (CV%) was highest for lactate in summer (21.3%), which is consistent with the observed differences in individual exercise tolerance.

Pearson's correlation analysis revealed a significant positive correlation between ambient temperature and post-exercise lactate levels ($r = 0.68$, $p = 0.007$), supporting the hypothesis that thermal stress exacerbates lactate accumulation. Furthermore, a moderate positive correlation was observed between pyruvate levels and LDH post-exercise ($r = 0.61$, $p = 0.014$), suggesting a coordinated metabolic response.

Discussion. This study investigated changes in key metabolic markers (LDH, lactate and pyruvate) in horses over the course of a year, and in relation to sex and



exercise. Statistical analysis revealed several significant trends and interactions that provide insight into equine muscular physiology and its responsiveness to intrinsic and extrinsic factors.

Exercise-induced metabolic shifts. The consistent and significant elevation in LDH and lactate concentrations after exercise across all seasons and sexes confirms the metabolic stress response and anaerobic energy utilisation associated with physical exertion. LDH activity is a marker of muscle membrane permeability and anaerobic glycolysis, and an increase in LDH activity indicates muscle workload intensity (Smith J. A. B. et al., 2023). Post-exercise LDH levels were highest in winter and in stallions, suggesting a robust anaerobic response possibly linked to muscle conditioning or thermoregulatory adaptations in colder environments (Fig. 1). Lactate levels, particularly the high value observed in summer and in mares (approximately $5.05 \text{ mmol}\cdot\text{L}^{-1}$), may indicate acute muscular fatigue, subclinical myopathy or increased thermogenic strain in hot conditions (Wan J. J. et al., 2017; Siquier-Coll J. et al., 2024). Although rare, this outlier is consistent with literature describing heat-related increases in lactatemia in equines (Ebisuda Y. et al., 2023, 2024).

Seasonal variation and physiological adaptation. Animals in winter, especially stallions, demonstrated higher baseline LDH levels, suggesting a potential metabolic shift – such as an increased reliance on anaerobic processes due to lower ambient temperatures. While pyruvate levels were generally stable, they tended to increase post-exercise in both sexes, suggesting enhanced glycolytic flux and mitochondrial processing efficiency under stress (Gray L. R. et al., 2014). These observations are consistent with previous studies indicating that winter conditioning can alter mitochondrial density or shift fibre type composition towards more oxidative phenotypes (Yan Z. et al., 2011; Jacobs R. A. and Lundby C., 2013; Latham C. M. et al., 2021, 2022).

Sex-linked metabolic profiles. Stallions consistently exhibited higher levels of LDH and pyruvate than mares, both before and after exercise. This may be due to differences in muscle mass, hormonal influences (e.g. testosterone-driven anabolic activity) or behavioural factors, such as exercise intensity and reactivity (Kraemer W. J. and Ratamess N. A.; de Graaf-Roelfsema E. et al., 2007). Although lactate elevation was observed in both sexes, stallions exhibited more consistent lactate profiles, suggesting tighter regulation of glycolytic pathways. It is important to note that, while sex-related metabolic distinctions exist, their magnitude is relatively modest and there is substantial variation within groups.

The data confirm that LDH, lactate and pyruvate are sensitive biomarkers of equine physical stress and seasonal adaptation. Stallions appear to be more metabolically responsive, particularly in the colder months, whereas seasonal heat may exacerbate lactate accumulation in mares. These findings have implications for the management of performance horses, training schedules and even veterinary screening protocols during seasonal transitions (Coelho C. S. et al., 2022; Annan R. et al., 2023; Ramos G. V. et al., 2024).

The physiological implications of LDH and lactate dynamics. A significant increase in LDH and lactate levels after exercise indicates a shift towards anaerobic energy metabolism, which is characteristic of short-term, high-intensity muscular activity (Bartoloni B. et al., 2024). As a proxy for membrane permeability and tissue stress, LDH highlights the muscle workload imposed during trials (Callegari G. A. et al., 2017). The substantial increase in LDH levels observed in stallions during winter suggests greater anaerobic capacity or muscular recruitment in response to cold conditions, a phenomenon supported by thermogenic activation and altered cardiovascular dynamics during exercise in lower ambient temperatures (Hodgson D. R. et al., 1994; Geor R. J. and



McCutcheon L. J., 1998; Barreto, I. V. P. D. S. et al., 2023).

While lactate accumulation was expected, it presented with noteworthy variability, particularly in mares exposed to summer conditions. This heightened response may reflect thermoregulatory limitations or an electrolyte imbalance, given that heat stress has been shown to impair lactate clearance and amplify peripheral fatigue (Art T. et al., 1994; Lindinger M. I., 1999; Kang H. et al., 2023). The exceptionally high lactate values recorded in summer and mares warrant further investigation into hydration status, environmental factors and individual fitness profiles.

Pyruvate response and oxidative metabolic activity. Although changes in pyruvate concentration were modest compared to LDH and lactate, they remained statistically significant. This suggests nuanced shifts in glycolytic throughput and mitochondrial efficiency post-exercise (Glancy B. et al., 2021). Stallions consistently exhibited higher pyruvate levels, particularly in spring and winter, indicating greater oxidative potential or faster substrate conversion. This may be linked to fibre type distribution, as stallions have been reported to possess a higher proportion of type IIa fibres, which are capable of both anaerobic and aerobic function. This facilitates more efficient energy metabolism during intermediate-duration exertion (Curry J. W. et al., 2012; Mo M. et al., 2023).

The positive correlation between pyruvate and LDH supports the idea of a tightly coordinated metabolic cascade triggered by physical stress, which could reflect enhanced NAD⁺ regeneration and sustained ATP turnover (Luengo A. et al., 2021).

Seasonal adaptation and individual variability. Seasonal differences in enzyme activity and metabolic response likely reflect adaptation to environmental stressors, such as temperature, humidity and photoperiod. Higher levels of LDH and lactate in colder months may indicate an increased metabolic demand for heat generation, whereas variability in summer could be due to fluid-electrolyte imbalances affecting muscular efficiency (Klein R. et al., 2020). The effect size and variability indices – particularly the coefficient of variation (CV%) for lactate in summer – highlight substantial divergence in individual responses. This emphasises the importance of personalised conditioning and monitoring strategies in equine sports, particularly during periods of environmental extremes (Geor R. J. et al., 1996; Marlin D. J. et al., 1996; Klous L. et al., 2020).

The observed increase in lactate and LDH levels after exercise, particularly in the colder seasons, suggests that horses may benefit from tailored warm-up strategies aimed at reducing anaerobic strain during the initial stages of exercise (Anderson M. G., 1976; Titotto A. C. et al., 2023). For stallions, whose LDH response was particularly pronounced in winter, incorporating longer aerobic warm-ups could promote more efficient oxygen utilisation and reduce cellular stress. Furthermore, the variability in lactate response among mares in summer highlights the importance of heat-adapted training schedules. Exercising during cooler hours, optimising hydration and gradually acclimatising to heat may reduce excessive lactate build-up and improve recovery rates (Lorenzo S. et al., 2010; De Maré L. et al., 2022).

Given the strong correlation between temperature and post-exercise lactate levels, seasonal biomarkers such as LDH and pyruvate could be valuable indicators in pre-competition veterinary examinations (Tennent-Brown B. S., 2011; Li G. et al., 2012; Satué K. et al., 2022). Horses exhibiting unusually high post-exercise values may require follow-up testing for subclinical muscular injury, metabolic syndrome or dehydration (Stefaniuk-Szmukier M. et al., 2023). Stallions with consistently high levels of pyruvate and LDH may also exhibit symptoms of overtraining (Dzięgielewska A. and Dunisławska A., 2022; Davie A. et al., 2023). Periodic blood screening across seasons could facilitate the early detection of exercise-induced stress, enabling more informed decisions regarding workload progression (Ferlazzo A. et al., 2020; Aragona F. et al., 2025).



Sex-related metabolic distinctions could indicate potential selection markers for performance horses (Andriichuk A. and Tkachenko H., 2017). Stallions with elevated oxidative and anaerobic responses may exhibit favourable traits for disciplines requiring high power output, while mares' thermoregulation and endurance in warmer seasons could be advantageous in events demanding sustained effort in hot conditions (Fonseca R. G. et al., 2013; Zhang X. et al., 2025). These insights could inform breeding decisions and encourage genetic studies on muscle fibre composition, enzymatic variability and heat tolerance. Such adjustments could promote peak performance while safeguarding metabolic health.

Conclusions. This study highlights the complex interplay between seasonal conditions, sex and physical activity in influencing metabolic responses in horses. The data clearly demonstrate that exercise significantly increases LDH, lactate and pyruvate levels in both sexes and at all times of year, confirming the reliance on anaerobic and glycolytic pathways during muscular exertion. There were pronounced seasonal effects, with winter stallions exhibiting the highest LDH levels post-exercise and summer mares showing substantial lactate concentration variability, including a notable outlier likely linked to heat stress. Sex differences were moderate but consistent: stallions had higher LDH and pyruvate levels overall, indicating greater muscle mass or glycolytic activity. Pyruvate levels increased modestly after exercise, indicating enhanced mitochondrial turnover and energy substrate utilisation.

Statistical analyses, including two-way ANOVA and post hoc tests, confirmed the main effects of season and sex, as well as a significant season \times sex interaction for LDH, reflecting nuanced patterns of physiological adaptation. Effect size calculations and correlation analyses further emphasised the influence of ambient temperature on lactate dynamics and the coordinated increase in LDH and pyruvate levels following exercise.

The practical implications of these findings include: 1) optimising seasonal training and recovery strategies, 2) making better-informed breeding and selection decisions based on metabolic resilience, and 3) developing enhanced veterinary screening tools that utilise LDH and lactate as biomarkers.

In summary, LDH, lactate and pyruvate are reliable indicators of equine metabolic activity that are sensitive to environmental context and individual traits. These markers can inform performance assessments, conditioning protocols and clinical evaluations, thereby supporting the safe and effective athletic development of horses.

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