DOI 10.32900/2312-8402-2024-131-253-261
UDC 636.13: 612.111: 577.112.4

EXERCISE-INDUCED CHANGES IN SOME BLOOD BIOCHEMICAL INDICES IN HORSES INVOLVED IN RECREATIONAL HORSEBACK RIDING

Halina Tkaczenko, Doctor of Biological Sciences
https://orcid.org/0000-0003-3951-9005
Natalia Kurhaluk, Doctor of Biological Sciences
https://orcid.org/0000-0002-4669-1092
Institute of Biology, Pomeranian University in Słupsk, Poland
Ievgenii Aksonov, Candidate of Agricultural Sciences
https://orcid.org/0000-0002-6292-78198
Iryna Tkachova, Doctor of Agrarian Sciences
https://orcid.org/0000-0002-4235-7257
Livestock farming institute of NAAS of Ukraine, Kharkiv

The aim of this study was to evaluate the changes in some biochemical indices [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities, lactate concentration] of horses involved in recreational riding before and after exercise. Fourteen healthy adult horses from the Central Pomeranian region of Poland, aged 8.7 ± 1.1 years, including 7 Hucul ponies, 2 thoroughbreds, 1 Anglo-Arabian and 4 horses of unknown breed, were used in this study. All horses were involved in recreational riding. Blood samples were taken from the animals' jugular veins in the morning, 90 minutes after feeding, while the horses were in the stable (between 8.30 am and 10 am) and immediately after the exercise test (between 11 am and 2 pm). The training started at 10:00 and lasted 1 hour, consisting of a cross-country ride consisting of walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min) and walking (10 min). Blood alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase activities and lactate levels were analysed in horses before and after exercise. The results of the current study showed a statistically non-significant increase in ALT, AST and LDH activity. There was also a statistically significant increase in lactate concentration post-exercise compared to pre-exercise. A non-significant change in serum LDH activity was observed in horses during exercise. This may indicate a normal course of aerobic-anaerobic glycolysis in recreational horses before and after exercise. The concentration of lactic acid in the blood of horses increases after exercise with small changes in LDH activity. The significant post-exercise increase in lactic acid concentration that we recorded demonstrates the dynamics of aerobic-anaerobic reactions and emphasises the influence of anaerobic glycolysis in the overall energy supply for muscle activity.

Key words: *alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), lactate, horses, exercise, recreational riding*

ВИКЛИКАНІ ФІЗИЧНИМ НАВАНТАЖЕННЯМ ЗМІНИ ДЕЯКИХ БІОХІМІЧНИХ ПОКАЗНИКІВ КРОВІ У КОНЕЙ, ЯКІ БЕРУТЬ УЧАСТЬ У РЕКРЕАЦІЙНІЙ ВЕРХОВІЙ ЇЗДІ

Галина Ткаченко, д. б. н., https://orcid.org/0000-0003-3951-9005
Наталія Кургалюк, д. б. н., https://orcid.org/0000-0002-4669-1092
Інститут біології, Поморський університет у Слупську, Польща
Євгеній Аксьонов, к. с.-г. н., https://orcid.org/0000-0002-6292-78198
Ірина Ткачова, д. с.-г. н., https://orcid.org/0000-0002-4235-7257
Інститут тваринництва НААН України, Харків

Метою даного дослідження була оцінка змін деяких біохімічних показників [активність аланінамінотрансферази (АЛТ), аспартатамінотрансферази (АСТ) і лактатдегідрогенази (ЛДГ), концентрація лактату] в крові коней, які беруть участь у рекреаційній верховій їзді до та після фізичних навантажень. У цьому дослідженні було використано 14 здорових дорослих коней із Центрально-Поморського регіону Польщі віком 8,7 ± 1,1 років, включаючи 7 поні, 2 чистокровних коней, 1 англо-арабського та 4 коней невідомої породи. Всі коні беруть участь у рекреаційній верховій їзді. Зразки крові відбирали з яремних вен тварин вранці, через 90 хвилин після годування, під час перебування коней у стайні (між 8.30 ранку та 10 ранку) та відразу після тесту з навантаженням (між 11 ранку та 14 годинами дня). Тренування розпочиналося о 10:00 і тривало 1 годину, а також складалося з кросової їзди, яка включала ходьбу (5 хв), рись (15 хв), ходьбу (10 хв), рись (10 хв), ходьбу (5 хв), галоп (5 хв) і ходьбу (10 хв). Активність біохімічних показників аналізували в крові коней, відібраної до та після тренування. Результати цього дослідження показали статистично незначуще підвищення активності АЛТ, АСТ і ЛДГ. Також спостерігали статистично істотне збільшення концентрації лактату після тренування порівняно з періодом до тренування. Під час фізичного навантаження у коней спостерігалася незначна зміна активності ЛДГ у сироватці крові. Це може вказувати на нормальний перебіг аеробно-анаеробного гліколізу у рекреаційних коней до та після фізичних вправ. Концентрація молочної кислоти в крові коней підвищувалася після фізичного навантаження з невеликими змінами активності ЛДГ. Зафіксоване нами значне підвищення концентрації молочної кислоти після фізичного навантаження демонструє динаміку аеробно-анаеробних реакцій і підкреслює вплив анаеробного гліколізу на загальне енергозабезпечення м'язової діяльності.

Ключові слова: *аланінамінотрансфераза (АЛТ), аспартатамінотрансфераза (АСТ), лактатдегідрогеназа (ЛДГ), лактат, коні, фізичні навантаження, рекреаційна верхова їзда*

Improving methods of training horses and advancing equestrian sport is impossible without improving our knowledge of the physiology and biochemistry of training. Research in this direction is necessary to find ways of targeting the animal's body in order to increase its performance and maintain its health under extreme stress. In recent decades, the relationship between various physiological and biochemical indicators of horses and their performance has been well studied (Hinchcliff K.W. & Geor R.J., 2008). On the basis of a large amount of data, the optimal values of these indicators associated with high performance in horses have been determined, training systems have been improved and various means of increasing the performance of horses have been proposed (Bergero D. et al., 2005).

It is known that during the long-term adaptation of the horse's body to physical exercise of varying intensity, specific changes occur in the metabolic reactions aimed at reducing the release of energy as heat and at the same time accumulating it in the form of macroergic compounds (ATP) (Anderson M. G., 1975; Muñoz A. et al., 2022). Changes in energy metabolism during exercise in horses cause an increase in the concentration of energy sources such as creatinine phosphate and glycogen in skeletal muscle, an increase in the activity of glycolytic and Krebs cycle enzymes, beta-oxidation of fatty acids, etc. (Marlin D. & Nankervis K., 2002). Metabolites responsible for energy supply to skeletal muscle and enzymes reflecting the tendency of biochemical changes during exercise can be used as indicators of the level of physical condition in sport horses (Harris P. A. et al., 1998; McGowan C. M. et al., 2002; Padalino B. et al., 2007; Mack S. J. et al., 2014). Such markers include lactate and pyruvate, lactate dehydrogenase activity, alanine and aspartate aminotransferases (Harris P. A. et al., 1998; Kedzierski W. et al., 2009). Changes in the activity of the above enzymes during exercise in trained horses reflect specific changes in metabolic reactions (Davies R. & Pethick D. W., 1983; McGowan C. M. et al., 2002; Padalino B. et al., 2007).

In our previous study (Andriichuk A. & Tkachenko H., 2017), we evaluated the influence of exercise of average intensity on haematological and biochemical values, as well as on the acid resistance of erythrocytes in mares and stallions of the Holsteiner breed. A total of seventeen Holstein horses (seven mares and ten stallions aged 6 years) were used in this study. Blood samples were analysed for haematocrit (HCT), haemoglobin concentration (HGB), red blood cells (RBC), white blood cells (WBC), platelets (PLT), leukogram, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red cell distribution width (RDW) and platelet distribution width (PDW). Serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) and biomarkers of oxidative stress were analysed. Stallions showed a significant increase in leukocyte and granulocyte counts, as well as erythrocyte, haemoglobin and haematocrit levels after the exercise test. The pre-exercise mean corpuscular haemoglobin concentration was higher in stallions. At the same time, mares showed a significant decrease in platelet volume after the exercise test. Exercise induced a significant increase in aspartate aminotransferase activity in stallions. Exercised mares and stallions showed a decrease in lipid peroxidation after exercise. Exercise also caused an increase in oxidatively modified protein of erythrocytes in stallions, indicating exercise-induced oxidative stress. The resistance of erythrocytes in 0.1 m HCl was similar between females and males. No statistically significant differences were observed in the percentage of haemolysed erythrocytes before and after exercise (Andriichuk A. & Tkachenko H., 2017).

Later, we also determined the photoperiod-induced variations and the effects of exercise on biomarkers of oxidative stress [2-thiobarbituric acid reactive substances (TBARS), aldehydic (AD) and ketonic (KD) derivatives of oxidatively modified proteins (OMP), total antioxidant capacity (TAC) and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] and biomarkers of metabolic changes [glucose, urea and uric acid and activity of lactate dehydrogenase (LDH)] in the blood of Shetland pony mares and stallions involved in recreational riding. Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) aged 6.5 ± 1.4 years from the Central Pomeranian region of Poland were used in this study. Blood samples were collected once per season for one year: spring, summer, autumn and winter. A MANOVA analysis showed that photoperiod had a leading role in the changes of these biomarkers, while exercise and sex of the ponies had a lesser effect. The lipid peroxidation biomarkers, for example, the plasma TBARS level, showed the maximum adjusted coefficient of determination $R^2_{ad} = 0.77$. Prior to exercise (at rest), the plasma of stallions and mares showed minimum TBARS levels in the spring and summer photoperiods and maximum levels in the autumn and winter. A statistically significant reduction in the levels of both aldehydic and ketonic derivatives of OMP in the blood of ponies was observed during the autumn and winter periods; in addition, the level of ketonic derivatives of OMP decreased after exercise in the spring. TAC was statistically significant in the spring and winter photoperiods both before and after exercise. SOD activity did not show a pronounced photoperiod-induced pattern, but was sex- and exercise-dependent. CAT activity varied and was statistically significant only in the plasma of mares after exercise in the spring, summer and winter photoperiods. The minimum GPx activity in the blood of mares before exercise (at rest) was observed in autumn, while the maximum was observed in winter and summer. Photoperiod and exercise-induced changes in markers of oxidative stress and antioxidant defences may contribute to the adaptation of animals to exercise, depending on sex. The seasonal variations in antioxidant defences and substrates of energy metabolism in the blood of mares and stallions as a function of exercise capacity demonstrated in our study may be an important aspect of the ability of endogenous adaptive mechanisms in animals to respond in advance to seasonal environmental changes (Kurhaluk N., 2022).

It is important to understand the biochemical changes produced by different types of exercise because they reflect changes in the function of different systems and the type of energy used (Räsänen L. A. et al., 1995; Nostell K. et al., 2006). For this reason, the correct interpretation of metabolic changes after endurance exercise in horses is necessary, as it can help the veterinarian, trainer, or owner to select appropriate training and post-exercise recovery. Therefore, the aim of this study was to evaluate the changes in some biochemical indices of horses involved in recreational riding before and after exercise.

Materials and methods.

Horses. Fourteen healthy adult horses from the Central Pomeranian region of Poland (Strzelinko village, N54º30'48.0" E16º57'44.9"), aged 8.7 ± 1.1 years, including 7 Hucul ponies, 2 thoroughbreds, 1 Anglo-Arabians and 4 horses of unknown breed, were used in this study. All horses were involved in recreational riding. Horses were housed in individual stalls with feed (hay and oats) provided twice daily, at 08:00 and 18:00, and water available *ad libitum*. All horses underwent a thorough clinical examination and haematological, biochemical and vital parameters were within reference ranges. Females were not pregnant. Our current scientific project was undertaken in the frame of the cooperation program between the Institute of Biology (Pomeranian University in Słupsk, Poland) and Institute of Animal Science of the National Academy of Agrarian Science of Ukraine, Kharkiv, Ukraine. This work was supported by The International Visegrad Fund, and the authors are cordially grateful for this.

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

Blood samples. Blood samples were taken from the animals' jugular veins in the morning, 90 minutes after feeding, while the horses were in the stables (between 8.30 am and 10 am) and immediately after the exercise test (between 11 am and 2 pm). Blood was collected in tubes containing K_3-EDTA and kept on ice until centrifuged at 3,000 rpm for 15 minutes. The plasma was removed. The erythrocyte suspension (one volume) was washed three times with five volumes of saline and centrifuged at 3,000 rpm for 15 minutes. Plasma aliquots were frozen and stored at -25°C until assayed.

Biochemical analysis. The Randox kit method (RANSOD, Cat. N AL7930 and Cat. N AS3804 Randox Laboratories Limited, UK) was used for the determination of alanine aminotransferase (ALT, E.C. 2.6.1.2) and aspartate aminotransferase (AST, E.C. 2.6.1.1) activity. Lactate dehydrogenase (LDH, E.C. 1.1.1.27) was determined by the colourimetric method of Sevela M. and Tovarek J. (1959).

Lactate concentration was measured using the method described by Herasimov I. and Plaksina O. (2000). One mL of the sample was added to 6 mL of distilled water and 1 mL of 10% metaphosphoric acid. The mixture was centrifuged at 800 g for 5 min to separate the supernatant. To the supernatant, 1 mL of 25% cupric sulphate and 500 mg calcium hydroxide were added and mixed for 30 min. The mixture was centrifuged at 1,000 g for 10 min. For the lactate concentration assay, the resulting supernatant was resuspended in 3 mL of p-dimethylaminobenzaldehyde (0.5% dissolved in dimethylsulfoxide) and 1 mL of 25% NaOH. The mixture was incubated at 37ºC for 45 minutes and then centrifuged at 1,000 g for 10 minutes. Absorbance was measured at 420 nm. A mixture of 0.5% p-dimethylaminobenzaldehyde and 25% NaOH was used as a blank. The calibration curve of lactate (0.1-50 μM) was used and the results were expressed as nmol lactate per L (Herasimov I. & Plaksina O., 2000).

Statistical analysis. Results are expressed as mean ± S.E.M. All variables were tested for normal distribution using the Shapiro-Wilk test ($p>0.05$). To find significant differences (significance level, $p < 0.05$) between the pre- and post-exercise states, the Wilcoxon signed-rank test was applied to the data (Zar J. H., 1999). All statistical analyses were performed using STATISTICA 13.3 software (TIBCO Software Inc., USA).

Results. Measuring the levels of liver biomarkers (AST, ALT) and an indicator of muscle damage (LDH) after different training programmes can help to better understand the acute and chronic effects of resistance training (Guy P. S. & Snow D. H., 1977; Krzywanek H. et al., 1996). The results of blood biochemical indices in horses are shown in Table 1.

Table 1

Mean values of blood biochemical parameters in horses before and after exercise

Parameters	Before exercise	After exercise
Alanine aminotransferase activity, $IU \cdot L^{-1}$	8.11 ± 0.48	8.45 ± 0.51
Aspartate aminotransferase activity, $IU \cdot L^{-1}$	362.35 ± 17.94	378.56 ± 21.45
Lactate dehydrogenase activity, $IU \cdot L^{-1}$	379.25 ± 20.06	412.18 ± 25.74
Lactate concentration, $nmol \cdot L^{-1}$	1.39 ± 0.11	1.93 ± 0.10*

**– change was statistically significant (p < 0.05) before and after exercise.*

In our study, non-significant changes in ALT, AST and LDH activities were observed in the blood of horses involved in recreational riding (Table 1). There was a statistically non-significant increase in ALT, AST and LDH activity of 4.2% ($p > 0.05$), 4.48% ($p > 0.05$) and 8.69% ($p > 0.05$) before and after exercise, respectively. In addition, lactate concentration was statistically significantly increased after exercise (by 38.85%, $p < 0.05$) compared to the pre-exercise state (Table 1).

Discussion. This study aimed to evaluate the changes in some biochemical indices (ALT, AST and LDH activities, lactate concentration) of horses involved in recreational riding before and after exercise. The results of the current study revealed a statistically non-significant increase in ALT, AST and LDH activity. In addition, lactate concentration was statistically significantly increased after exercise (by 38.85%, $p < 0.05$) compared to the pre-exercise state (Table 1).

Regular exercise induces adaptive processes that lead to changes in haematological and biochemical indices. The magnitude of these changes depends on several factors: type of exercise, intensity of exercise (intensity, duration and frequency) and individual variation. Physiological increases in ALT and AST have been shown to occur in the absence of tissue destruction (Giannini E. G. et al., 2005; McGill M. R., 2016). Aspartate aminotransferase is a cytoplasmic and mitochondrial enzyme that catalyses the deamination of aspartate to oxaloacetate, which can enter the Krebs cycle (Rennie M. J. & Tipton K. D., 2000). Increases in plasma aminotransferase activity may be due to hepatocyte injury, muscle injury or *in vitro* haemolysis (Kumar V. et al., 2009). ALT is a pyridoxal-dependent enzyme that catalyses the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate (Sakagishi Y., 1995). The magnitude of changes in AST, ALT and LDH activities depends on the type of exercise (Pettersson J. et al., 2008).

In veterinary medicine, lactate dehydrogenase and aminotransferase activity are often used to assess the functional status of the musculoskeletal system and to determine the appropriate level of exercise for exercise horses (Harris P. A. et al., 1998; Padalino B. et al., 2007; Kedzierski W. et al., 2009). A significant increase in aspartate activity caused by strenuous exercise in sport horses may indicate the development of overtraining syndrome and muscle damage (Padalino B. et al., 2007). However, in the process of adaptation to regular exercise, aminotransferase activity may change from lower to higher values in training dynamics. In particular, the study by Fazio F. and co-workers (2011) observed a significant increase in AST activity in the blood of purebred horses on the sixtieth day of intense exercise (Fazio F. et al., 2011). However, at the end of the training macro-cycle, on day eighty, AST activity decreased significantly, accompanied by an increase in physical condition and adaptation of the horses to regular exercise (Fazio F. et al., 2011).

Bashiri J. and co-workers (2010) found that two months of resistance training resulted in a non-significant increase in serum ALT and AST levels in non-athletic students (Bashiri J. et al., 2010). Therefore, non-significant changes in LDH and AST activity suggest some form of adaptation, and if exercise stress is followed by proper recovery, it will prevent further damage to the liver and muscles (Bashiri J. et al., 2010). LDH catalyses a redox reaction, the reversible conversion between pyruvate and L-lactate. The formation of L-lactate consumes reduced nicotinamide adenine dinucleotide ($NADH_2$) and produces oxidised nicotinamide adenine dinucleotide (NAD^+), whereas the oxidation of L-lactate to pyruvate produces $NADH_2$. LDH converts pyruvate, the end product of glycolysis, to lactate when oxygen is absent or scarce, and performs the reverse reaction during the Cori cycle in the liver (Andrews F. M. et al., 1995). In our study, a non-significant change in serum LDH activity was observed in horses during exercise (Table 1). This may indicate a normal course of aerobic-anaerobic glycolysis in horses involved in recreational riding before and after exercise.

Lactate (lactic acid) is a product of anaerobic glucose metabolism. It is formed from pyruvate in an enzymatic reaction with LDH (Berg J.M. et al., 2002). In the presence of sufficient oxygen, pyruvate enters the Krebs cycle and undergoes metabolic changes in the mitochondria to produce the end product – carbon dioxide and water. During vigorous exercise with hypoxia in skeletal muscle, the enzymatic reaction with LDH proceeds mainly towards the formation of lactate from pyruvate. Lactate, which accumulates in skeletal muscle during active exercise, enters the bloodstream and is taken up by hepatocytes. In the liver, lactic acid is oxidised to pyruvate in an opposite enzymatic reaction involving LDH (Cori cycle). Part of the pyruvate is used to replace glucose in gluconeogenesis and the rest is oxidised in the Krebs cycle with subsequent

ATP synthesis. Glucose produced in gluconeogenesis is transported by the blood to the muscles to replenish glycogen stores. Therefore, by converting pyruvate to lactate for subsequent use in gluconeogenesis in the liver (Cori cycle), skeletal muscle not only disposes of the "extra" lactic acid produced in extremely large amounts during vigorous exercise, but also ensures a high $NAD^+/NADH^+$ ratio necessary for the active action of glycolysis (Berg J.M. et al., 2002). Our research shows that the concentration of lactic acid in the blood of horses increases after exercise with small changes in LDH activity (Table 1). The significant post-exercise increase in lactic acid concentration that we recorded demonstrates the dynamics of aerobic-anaerobic reactions and emphasises the influence of anaerobic glycolysis in the overall energy supply for muscle activity.

Conclusions. The aim of this study was to evaluate the changes in some biochemical indices (ALT, AST and LDH activities, lactate concentration) of horses involved in recreational riding before and after exercise. The results of the current study showed a statistically non-significant increase in ALT, AST and LDH activity. There was also a statistically significant increase in lactate concentration after exercise compared to before exercise. A non-significant change in serum LDH activity was observed in horses during exercise. This may indicate a normal course of aerobic-anaerobic glycolysis in recreational horses before and after exercise. The concentration of lactic acid in the blood of horses increases after exercise with small changes in LDH activity. The significant post-exercise increase in lactic acid concentration that we recorded demonstrates the dynamics of aerobic-anaerobic reactions and emphasises the influence of anaerobic glycolysis in the overall energy supply for muscle activity.

Acknowledgements. *Our current scientific project was carried out within the framework of the cooperation programme between the Institute of Biology (Pomeranian University in Słupsk, Poland) and Livestock farming institute of NAAS of Ukraine, Kharkiv. This work was supported by The International Visegrad Fund, and the authors are cordially grateful for this.*

References

Anderson M. G. (1975). The influence of exercise on serum enzyme levels in the horse. *Equine Veterinary Journal*, 7(3), 160-165. https://doi.org/10.1111/j.2042-3306.1975.tb03258.x.

Andrews, F. M., Geiser, D. R., White, S. L., Williamson, L. H., Maykuth, P. L., & Green, E. M. (1995). Haematological and biochemical changes in horses competing in a 3 Star horse trial and 3-day-event. *Equine Veterinary Journal. Supplement*, (20), 57-63. https://doi.org/10.1111/j.2042-3306.1995.tb05009.x.

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), 404-413. https://doi.org/10.1111/jpn.12620.

Bashiri, J., Gaini, A. A., & Nickbaht, H. (2010). Concurrent effect of monohydrate creative supplementation and resistance training on the activity of serum liver enzymes in male non-athletes. *Iranian Journal of Endocrinology and Metabolism*, 12, 42-47.

Berg J. M., Tymoczko J. L., Stryer L. (2002). *Biochemistry,* 5th edition. W H Freeman, New York.

Bergero, D., Assenza, A., & Caola, G. (2005). Contribution to our knowledge of the physiology and metabolism of endurance horses. *Livestock Production Science*, 92, 167-176.

Davies, R., & Pethick, D. W. (1983). Blood lactate as a measure of work intensity in standardbred horses in training. *Australian Veterinary Journal*, 60(12), 380–381. https://doi.org/10.1111/j.1751-0813.1983.tb02855.x.

Fazio, F., Assenza, A., Tosto, F., Cassela, S., Piccione, G., & Caola, G. (2011). Training and haematochemical profile in Thoroughbreds and Standardbreds: A longitudinal study. *Livestock Journal*, 141, 221-226. https://doi.org/10.1016/j.livsci.2011.06.005.

Giannini, E. G., Testa, R., & Savarino, V. (2005). Liver enzyme alteration: a guide for clinicians. *CMAJ: Canadian Medical Association Journal - Journal De l'Association Medicale Canadienne*, 172(3), 367–379. https://doi.org/10.1503/cmaj.1040752.

Guy, P. S., & Snow, D. H. (1977). The effect of training and detraining on muscle composition in the horse. *The Journal of Physiology*, 269(1), 33–51. https://doi.org/10.1113/jphysiol.1977.sp011891.

Harris, P. A., Marlin, D. J., & Gray, J. (1998). Plasma aspartate aminotransferase and creatine kinase activities in thoroughbred racehorses in relation to age, sex, exercise and training. *Veterinary Journal* (London, England: 1997), 155(3), 295–304. https://doi.org/10.1016/s1090-0233(05)80026-7.

Herasimov, I., & Plaksina, O. (2000). Non-enzymatic assessment of lactate and pyruvate concentrations in blood samples. *Laboratorna Diagnostyka*, 2, 46–48 (In Ukrainian).

Hinchcliff, K.W., & Geor, R.J. (2008). The horse as an athlete: a physiological overview. In: *Equine Exercise Physiology. The Science of Exercise in the Athletic Horse*. K.W. Hinchcliff, R.J. Geor, A.J. Kaneps (eds.). Saunders Company, Edinburgh, p. 2-11.

Kedzierski, W., Bergero, D., & Assenza, A. (2009). Trends of hematological and biochemical values in the blood of young race horses during standardized field exercise. *Acta Veterinaria (Beograd),* 59(5-6), 457-446. https://doi.org/10.2298/AVB0906457K.

Krzywanek, H., Mohr, E., Mill, J., & Scharpenack, M. (1996). Veränderungen von Serumenzymen, Lactat-, und Hämoglobinkonzentrationen im Blut junger Trabrennpferde durch Trainingsbelastung [Changes of serum enzymes, lactate and hemoglobin concentrations in the blood of young trotting horses due to training exertion]. *Zentralblatt fur Veterinarmedizin. Reihe A*, 43(6), 345–352.

Kumar, V., Atherton, P., Smith, K., & Rennie, M. J. (2009). Human muscle protein synthesis and breakdown during and after exercise. *Journal of Applied Physiology* (Bethesda, Md.: 1985), 106(6), 2026–2039. https://doi.org/10.1152/japplphysiol.91481.2008.

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.

Mack, S. J., Kirkby, K., Malalana, F., & McGowan, C. M. (2014). Elevations in serum muscle enzyme activities in racehorses due to unaccustomed exercise and training. *The Veterinary Record*, 174(6), 145. https://doi.org/10.1136/vr.101669.

Marlin, D., & Nankervis, K. (2002). *Equine Exercise Physiology*. Iowa State Press, Ames, Iowa, USA.

McGill M. R. (2016). The past and present of serum aminotransferases and the future of liver injury biomarkers. *EXCLI Journal*, 15, 817–828. https://doi.org/10.17179/excli2016-800.

McGowan, C. M., Golland, L. C., Evans, D. L., Hodgson, D. R., & Rose, R. J. (2002). Effects of prolonged training, overtraining and detraining on skeletal muscle metabolites and enzymes. *Equine Veterinary Journal. Supplement*, (34), 257–263. https://doi.org/10.1111/j.2042-3306.2002.tb05429.x.

Muñoz, A., Riber, C., Santisteban, R., Lucas, R. G., & Castejón, F. M. (2002). Effect of training duration and exercise on blood-borne substrates, plasma lactate and enzyme concentrations in Andalusian, Anglo-Arabian and Arabian breeds. *Equine Veterinary Journal. Supplement*, (34), 245–251. https://doi.org/10.1111/j.2042-3306.2002.tb05427.x.

Nostell, K., Funkquist, P., Nyman, G., Essén-Gustavsson, B., Connysson, M., Muhonen, S., & Jansson, A. (2006). The physiological responses to simulated race tests on a track and on a treadmill in standardbred trotters. *Equine Veterinary Journal. Supplement*, (36), 123–127. https://doi.org/10.1111/j.2042-3306.2006.tb05527.x.

Padalino, B., Rubino, G., Centoducati, P., & Petazzi, F. (2007). Training versus Overtraining: evaluation of protocols. *Journal of Equine Veterinary Science*, 27(1), 28-31. https://doi.org/10.1016/j.jevs.2006.11.013.

Pettersson, J., Hindorf, U., Persson, P., Bengtsson, T., Malmqvist, U., Werkström, V., & Ekelund, M. (2008). Muscular exercise can cause highly pathological liver function tests in healthy men. *British Journal of Clinical Pharmacology*, 65(2), 253–259. https://doi.org/10.1111/j.1365-2125.2007.03001.x.

Räsänen, L. A., Lampinen, K. J., & Pösö, A. R. (1995). Responses of blood and plasma lactate and plasma purine concentrations to maximal exercise and their relation to performance in standardbred trotters. *American Journal of Veterinary Research*, 56(12), 1651–1656.

Rennie, M. J., & Tipton, K. D. (2000). Protein and amino acid metabolism during and after exercise and the effects of nutrition. *Annual Review Of Nutrition*, 20, 457–483. https://doi.org/10.1146/annurev.nutr.20.1.457.

Sakagishi Y. (1995). Nihon rinsho. *Japanese Journal Of Clinical Medicine*, 53(5), 1146–1150.

Sevela, M., & Tovarek, J. (1959). Metoda stanovení laktikodehydrogenázy v télních tekutinách [Method for the estimation of lactic dehydrogenase]. *Casopis lekaru ceskych*, 98(27), 844–848.

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