Effect of different types of exercise on salivary biochemical indices in the horse

Lucie Hostovská[1], Martin Hostovský[2], Katarina Nenadović[3], Eva Voslářová[1], Vladimír Večerek[1]

[1]University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Animal Protection and Welfare and Veterinary Public Health, Brno, Czech Republic
[2]University of Veterinary Sciences Brno, Faculty of Veterinary Medicine, Department of Physiology, Brno, Czech Republic
[3]University of Belgrade, Faculty of Veterinary Medicine, Department of Animal Hygiene, Belgrade, Serbia

Received June 5, 2024
Accepted July 11, 2024

Abstract

The aim of this study was to investigate the biochemical indices in the saliva of leisure and sport horses undergoing different levels of workload. The experiment was conducted on 40 horses (4–28 years): sport horses (n = 20), and leisure horses (n = 20). The saliva samples were collected non-invasively 15 min before riding (A), 1 min before riding (B), 1 min after riding (C) and 15 min after riding (D). Albumin (Alb), alkaline phosphatase (ALP), α-amylase (AMY), creatine kinase (CK), creatinine (Crea), glucose (Glc), lactate (Lac), triacylglycerols (TG), total protein (TP), urea and lipid peroxidation (TBARS) were measured in saliva. Riding caused a significant change in many biochemical indices (Alb, CK, Glc, Lac, TBARS and urea) compared to baseline values in leisure horses ($P < 0.05$) but only in a few indices (Glc, Crea and TBARS) in sport horses. On the other hand, when comparing groups of leisure and sport horses, higher concentrations of Alb, Glc, TG and urea were found in sport horses whereas raised concentrations of CK and Crea were found in leisure horses. The obtained results indicate the adaptation of trained horses to physical effort. Moreover, this study confirms other possibilities of using salivary biochemical properties for physical stress assessment. Other more detailed comparative studies of load response in horses may provide useful information to quantify the reference range of individual stress indices.

Equine, training, adaptation, physiological response, horse riding

Different types of exercise, their intensity and duration, are associated with different workloads and cause various physiological responses in the horse (*Equus ferus cabbalus*). Stress in horses is often connected with inappropriate conditions during training, transport or improper management (Ayala et al. 2012). Deficiencies in management and animal care are the most common findings detected during welfare inspections (Svestkova et al. 2024). Coping with stress in horses is associated with physiological as well as behavioural responses. Measuring biochemical indicators of stress can provide a better evaluation of the physical status of a horse (Sheriff et al. 2011). Equestrian sport has undergone significant changes in recent years that have not been reflected in different management and training practices yet. Sport horses but even the majority of leisure horses undergo training that challenges their physiology. However, the relationship between the degree of workload and its impact on an individual is still not clear (Strzelec et al. 2011). Elite equestrian sport has been often perceived as horse abuse by the public and animal protection organizations and charities (Dashper 2017). They claim that equine sport is a human invention and that the line between the use and abuse of horses is unclear (Campbell 2013). On the other hand, there was an initiative in 2007 by Julie Fiedler (Australia) who first presented the concept of "Social License to Operate" (SLO). The SLO is a complex of aspects that represent public acceptance of using horses with regard to welfare and safety (Douglas et al. 2022).

Address for correspondence:
MVDr. Martin Hostovský, Ph.D.
Department of Physiology
Faculty of Veterinary Medicine
University of Veterinary Sciences Brno
Palackého tř. 1946/1, 612 42 Brno, Czech Republic

Email: hostovskym@vfu.cz
http://actavet.vfu.cz/

For monitoring the adaptation of horses to training, it is necessary to evaluate the duration and intensity of the type of exercise, and to know the response of the organism on each level of training. Understanding physiological changes during different types of training helps provide appropriate training and recovery phases (Wing 2019) and avoid overtraining and detraining (Marlin and Nankervis 2013). Biochemical markers can serve as markers of physiological changes caused by different levels of exercise. Various physical, haematological, and biochemical markers have been studied in various equestrian disciplines, such as racing (Mukai et al. 2007), endurance (Santos et al. 2001; Teixeira-Neto et al. 2008), cross-country events (Munoz et al. 1999), or jumping (Hura et al. 2013). Some of the physiological processes, functions, and anatomical structures can adapt as a result of stress and exercise due to repeated activity, i.e. training or conditioning (Marlin and Nankervis 2013).

Although widely used, biochemical markers in blood samples are of limited use in current research as they can be affected by the invasive sampling itself. Therefore, non-invasive sampling has been favoured. Apart from its easy and painless collection, saliva can be sampled in field conditions and in cases where repeated sampling is required. It is well known that stress and excessive workload have a significant impact on blood properties and their changes are also reflected in saliva. In mammals, the main role of saliva is to lubricate the oral cavity, protect oral tissues and initiate enzymatic digestion (Tabak 2004). Current studies have indicated the possibility of using the saliva fluid as a matrix for stress assessment. Increasing interest in saliva analysis corresponds with higher demand for better welfare and the growing knowledge of baseline activity of biochemical markers and stress response in saliva (Thoma et al. 2012). To this date, salivary analytes have been studied in different animal species such as pigs (Cook et al. 2013; Rubio et al. 2019), sheep (Cook and Jacobson 1995; Contreras-Aguilar et al. 2019a, Nemeckova et al. 2022), goats (Popelkova et al. 2022) as well as horses (Contreras-Aguilar et al. 2019b).

Welfare concerns for horses, namely, the negative effects of improper care, excessive workload but also detraining require further research. However, current knowledge on relevant biochemical indices and their changes during optimal or improper training is limited to understanding some effects of adaptations for training, such as an increase in muscle enzyme activity, namely, alkaline phosphatase (ALP), creatine kinase (CK), an increase of glucose concentration (Glc) and triacylglycerols (TG) and a decrease of post-exercise lactate (Lac) concentration (Marlin and Nankervis 2013). Furthermore, muscle oxidative damage by the thiobarbituric acid reactive substances (TBARS) can be detected. Some studies focused on training assessed only lactate or uric acid concentrations and, especially in horses, also salivary alpha-amylase (AMY) (Contreras-Aguilar et al. 2021), but multifactorial studies are necessary for better understanding and clarification.

The aim of this study was to evaluate the changes in salivary biochemical markers - albumin (Alb), ALP, AMY, CK, creatinine (Crea), Glc, Lac, TG, total protein (TP) and urea in horses undergoing different workloads, low and high. We expected differences in the organism's physiological response in race and leisure horses; a subtask was to find out how these indices are measurable in saliva. High intensity exercise was represented by show jumping competition horses. Show jumping is supposed to be a physically demanding activity that requires intensive training (St George et al. 2019). The high workload of these horses is shown in the predominance of anaerobic metabolism (Gomes et al. 2020). Low workload was represented by leisure horses that are mostly ridden at irregular intervals with no precise training plan. Their metabolism is commonly fully supplied by oxygen during the light walk; however, it may vary (Gomes et al. 2020).

Materials and Methods

Ethical statement

All procedures in this study were done in compliance with the national legislation of the Czech Republic (Act No. 246/1992 Coll., on the Protection of Animals Against Cruelty, as amended) and with the EU legislation (Directive 2010/63/EU revising Directive 86/609/EEC on the Protection of Animals Used for Scientific Purposes, as amended). Ethics Committee on Animal Welfare of the University of Veterinary Sciences Brno was informed of all procedures and issued the consent to carry out the research (Reference Number: PP-IGA 205/2020/FVHE). Animal husbandry and care were under the professional management of private horse owners and trained personnel.

Animals

The subjects of the study were 40 horses (*Equus ferus cabbalus*, 20 sport horses and 20 leisure horses of various breeds but of a similar type and constitution, mostly Czech Warmblood). Horses ranged in age from 4 to 28 years, with the average of 12 years. Horses showed no clinical signs of disease or discomfort and were clinically healthy. They were kept individually in indoor horse boxes (4 × 3.5 m) with access to adjacent paddocks for at least 6 h per day. Horses were fed a commercial diet based on oats and barley twice a day. They had access to water and hay *ad libitum*. Racehorses were checked by a veterinarian on arrival at the race, recreational horses were inspected by a trained person before riding in the same way as racehorses. All horses included in the study were found to be healthy and fit to ride.

Sample collection and biochemical analysis of saliva

Saliva samples were collected non-invasively with a cotton swab from the specialized collecting device Salivette® (Sarstedt AG & Co., Nümbrecht, Germany). The cotton swab held by Pean forceps (Medin a.s., Nové Město na Moravě, Czech Republic) was put into the horse's mouth in the interdental space right in front of premolars for 1 min. Saliva samples were obtained from horses during their typical workload, i.e. during show jumping competition for the group of sport horses and during walks into the countryside in all three gaits for the group of leisure horses. The first set of samples were obtained 15 min before riding (sample A = basal level) when the horses were stabled and relaxed. Samples B were obtained immediately (1 min) before riding, samples C immediately (1 min) after riding and samples D were obtained 15 min after riding when the horses had calmed down and relaxed. After collection, the samples were centrifuged (9,391 × *g*, 4 °C, 10 min) and stored at −80°C until analysis.

Analysis of Alb, ALP, AMY, CK, Crea, Glc, Lac, TG, TP and urea by commercial kits BioVendor (BioVendor Laboratorní medicína, Brno, Czech Republic) was performed by the biochemical analyser Thermo Scientific INDIKO (Thermo Fisher Scientific Inc., Santa Clara, USA). Determination of oxidative stress was performed via measuring of lipid peroxidation (TBARS) in blood (Ohkawa et al. 1979) modified to saliva and measured spectrophotometrically by Varioskan™ Flash Multimode Reader (Thermo Fisher Scientific). All indices were measured in duplicate.

Statistical analysis

Statistical analysis was performed using SAS System for Windows 9.4 (SAS). Each of the quantitative parameters was expressed as a mean ± standard error of the mean (SEM). Values were compared using ordinary one-way ANOVA method and Tukey-HSD was used for multiple comparison with means. Values of $P < 0.05$ were considered significant.

Results

Results of analysis of salivary biochemical indices in both leisure and sport horses 15 min before riding (A - basal level), 1 min before riding (B), 1 min after riding (C) and 15 min after riding (D) are shown in Table 1.

In leisure horses, significant ($P < 0.05$) changes were found in concentrations of Alb, CK, Glc, Lac, TBARS and urea. An increase was found in Alb, CK, Glc, Lac and TBARS after riding compared to basal values. In contrast, a decline in urea was detected 1 min before riding as well as 1 min after riding compared to basal values, whereas 15 min after riding urea concentrations did not differ ($P > 0.05$) from basal values.

In sport horses, significant ($P < 0.05$) changes were found in concentrations of Glc, Crea and TBARS. Glc increased from basal values during riding and kept increasing even after riding. A significant increase was found also in Crea 15 min after riding compared to concentrations detected 1 min before riding, and in TBARS when comparing basal values with values 1 min after riding.

Table 1. Effect of exercise on salivary biochemical indices in leisure and sport horse groups (mean ± SEM).

Indicator	Sampling A	Sampling B	Sampling C	Sampling D
		Leisure horses (n = 20)		
Alb (g/l)	$0.59 \pm 0.08^{a,x,y}$	$0.62 \pm 0.10^{a,x}$	$1.04 \pm 0.15^{b,x,y}$	$0.78 \pm 0.10^{a,b,x,y}$
ALP (µkat/l)	$0.32 \pm 0.05^{a,x,y}$	$0.30 \pm 0.03^{a,x,y}$	$0.45 \pm 0.07^{a,x,y}$	$0.32 \pm 0.05^{a,x,y}$
AMY (µkat/l)	$0.25 \pm 0.09^{a,x,y}$	$0.32 \pm 0.10^{a,x,y}$	$0.46 \pm 0.15^{a,x,y}$	$0.37 \pm 0.09^{a,x,y}$
CK (µkat/l)	$0.06 \pm 0.02^{a,x,y}$	$0.09 \pm 0.01^{a,x,y}$	$0.21 \pm 0.05^{a,x,y}$	$2.28 \pm 0.66^{b,x}$
Crea (µmol/l)	$70.51 \pm 6.88^{a,x,y}$	$62.50 \pm 3.46^{a,x,y}$	$72.98 \pm 5.79^{a,x}$	$63.93 \pm 3.93^{a,x,y}$
Glc (mmol/l)	$0.85 \pm 0.15^{a,x,y}$	$1.17 \pm 0.25^{a,b,x,y}$	$1.85 \pm 0.47^{a,b,x}$	$2.25 \pm 0.19^{b,x}$
Lac (mmol/l)	$1.21 \pm 0.20^{a,x,y}$	$1.47 \pm 0.18^{a,b,x,y}$	$2.70 \pm 0.45^{b,x,y}$	$2.50 \pm 0.38^{b,x,y}$
TBARS (µmol/ml)	$4.18 \pm 0.39^{a,x,y}$	$6.71 \pm 0.52^{b,x,y}$	$8.73 \pm 1.08^{b,x,y}$	$6.33 \pm 0.42^{a,b,x,y}$
TG (mmol/l)	$0.23 \pm 0.07^{a,x}$	$0.11 \pm 0.03^{a,x,y}$	$0.20 \pm 0.08^{a,x,y}$	$0.40 \pm 0.12^{a,x,y}$
TP (g/l)	$3.29 \pm 0.44^{a,x,y}$	$3.24 \pm 0.30^{a,x,y}$	$4.67 \pm 0.70^{a,x,y}$	$3.05 \pm 0.43^{a,x,y}$
Urea (mmol/l)	$2.95 \pm 0.29^{a,x,y}$	$0.90 \pm 0.25^{b,x}$	$0.43 \pm 0.21^{b,x}$	$3.27 \pm 0.23^{a,x,y}$
		Sport horses (n = 20)		
Alb (g/l)	$1.09 \pm 0.11^{a,x,y}$	$1.18 \pm 0.15^{a,y}$	$1.22 \pm 0.13^{a,x,y}$	$0.97 \pm 0.13^{a,x,y}$
ALP (µkat/l)	$0.30 \pm 0.05^{a,x,y}$	$0.25 \pm 0.05^{a,x,y}$	$0.28 \pm 0.06^{a,x,y}$	$0.28 \pm 0.06^{a,x,y}$
AMY (µkat/l)	$0.14 \pm 0.07^{a,x,y}$	$0.16 \pm 0.07^{a,x,y}$	$0.14 \pm 0.06^{a,x,y}$	$0.16 \pm 0.06^{a,x,y}$
CK (µkat/l)	$0.27 \pm 0.05^{a,x,y}$	$0.31 \pm 0.07^{a,x,y}$	$0.31 \pm 0.07^{a,x,y}$	$0.21 \pm 0.04^{a,y}$
Crea (µmol/l)	$47.71 \pm 4.04^{a,b,x,y}$	$45.76 \pm 4.06^{a,x,y}$	$47.09 \pm 5.10^{a,y}$	$71.17 \pm 10.37^{b,x,y}$
Glc (mmol/l)	$0.50 \pm 0.17^{a,x,y}$	$0.86 \pm 0.39^{a,x,y}$	$4.45 \pm 0.76^{b,y}$	$5.62 \pm 1.13^{b,y}$
Lac (mmol/l)	$0.90 \pm 0.18^{a,x,y}$	$1.74 \pm 0.42^{a,x,y}$	$1.77 \pm 0.49^{a,x,y,}$	$2.09 \pm 0.51^{a,x,y}$
TBARS (µmol/ml)	$5.47 \pm 0.60^{a,x,y}$	$7.23 \pm 0.68^{a,b,x,y}$	$9.20 \pm 1.44^{b,x,y}$	$7.86 \pm 0.91^{a,b,x,y}$
TG (mmol/l)	$0.95 \pm 0.29^{a,y}$	$0.60 \pm 0.11^{a,x,y}$	$0.65 \pm 0.16^{a,x,y}$	$0.62 \pm 0.15^{a,x,y}$
TP (g/l)	$2.81 \pm 0.36^{a,x,y}$	$3.41 \pm 0.55^{a,x,y}$	$3.10 \pm 0.42^{a,x,y}$	$3.42 \pm 0.29^{a,x,y}$
Urea (mmol/l)	$2.50 \pm 0.35^{a,x,y}$	$2.37 \pm 0.26^{a,y}$	$2.54 \pm 0.31^{a,y}$	$2.79 \pm 0.30^{a,x,y}$

Sampling A – 15 min before riding; Sampling B – immediately (1 min) before riding; Sampling C – immediately (1 min) after riding; Sampling D – 15 min after riding.
Alb – albumin; ALP – alkaline phosphatase; AMY – α-amylase; Glc – glucose; CK – creatine kinase; Crea – creatinine; Lac – lactate; TBARS – thiobarbituric acid reactive substance; TG – triglycerides; TP – total protein.

[a,b] Different superscripts in the same row indicate a significant difference ($P < 0.05$) in the given biochemical indicator between the sampling times (A, B, C, D) within the same horse group (sport or leisure).
[x,y] Different superscripts in the same column indicate a significant difference ($P < 0.05$) in individual biochemical indicator within the same sampling time between leisure and sport horses.

Comparing groups of leisure and sport horses, significant ($P < 0.05$) differences in the monitored salivary biochemical indices between the two groups were found in TG 15 min before riding, in Alb and urea 1 min before riding, in Crea, Glc and urea 1 min after riding and in CK and Glc 15 min after riding. Higher concentrations of Alb, Glc, TG and urea were found in sport horses whereas higher concentrations of CK and Crea were found in leisure horses.

Discussion

Physical exercise is a part of physiological processes and functions that, to a given degree, can help an organism to cope with higher environmental demands and changes (Marlin and Nankervis 2013; Fazio et al. 2014). The organism acts in this way because it seeks to minimize the disruption of homeostasis influenced by exercise and increasing demands. Consequently, optimal training in horses can be very beneficial (Marlin and Nankervis 2013; Witkowska-Pilaszewicz et al. 2021).

Physical activity obviously entails higher metabolic demands (Masko et al. 2021). Increased activity tends to be associated with the release of Glc into the blood and the body (Pösö and Hyyppä 1999). Our results show that Glc metabolism (Glc and Lac) was dependent on the type of horse training and duration of exercise. The increasing trend of Glc concentration was more evident in the leisure horse group. This finding suggests that the horses were not sufficiently prepared for the workload. However, it is countered by the fact that comparing the absolute Glc values, Glc concentrations in the saliva were higher in the sport horses' calming down phase.

During prolonged activity, Glc tends to decrease (Marlin and Nankervis 2013) but with short and intense activity, both decreases and increases can occur (Pösö and Hyyppä 1999). The decrease or increase depends on the intensity, training, and nutritional status. The concentration of Lac as a product of anaerobic glycolysis is closely related to Glc concentrations (Gomes et al. 2020). However, Lac is strongly related to the anaerobic capacity of the horse, and well-trained horses in regular training have a higher capacity for gluconeogenesis (Pösö and Hyyppä 1999; Gomes et al. 2020). The indisputable connection of Lac is associated with metabolic changes in the muscles (Pösö and Hyyppä 1999). Our study showed that values of Lac before exercise (samples B) increased and were higher in leisure horses.

Both TP and Alb concentrations are very often associated with cardiovascular and metabolic adaptations due to increased activity (Munoz et al. 1999). However, our study showed an increase in Alb concentrations only in leisure horses during riding, and they decreased immediately after the calm-down phase. On the contrary, sport horses showed higher Alb concentrations immediately before exercise compared to leisure horses, but no changes were found in response to their exercise time. In our study, leisurely used horses showed significantly lower concentrations of urea after activity but a sharp increase in urea concentration was observed in the calm-down phase. While urea concentrations in sport horses did not significantly change during riding, they were significantly higher shortly before and shortly after riding compared to that of leisure horses at the same sampling times. Blood urea has shown a demonstrable upward trend among groups of horses during exposure to workload stress (Massányi et al. 2022) and also during transportation (Tateo et al. 2012). Therefore, salivary urea concentrations may reflect blood nitrogen metabolism and urea excretion, just as blood urea is affected by many other factors.

Important markers of muscle metabolism include CK (Lefebvre et al. 1996) and Crea (Stepanova et al. 2023). An increase in saliva has been observed also in diseased horses (Contreras-Aguilar et al. 2019a). In our study, significantly different concentrations of Crea were found in sport horses before and after riding compared to their calming down phase. However, Crea was significantly higher immediately after exercise in leisure horses compared to sport horses sampled in the same phase. While CK changed its activity after exercise in leisure horses, CK in the saliva of sport horses remained unchanged during the monitored period. In some cases, inappropriate or intense training leads to accumulation of muscle damage (Kim et al. 2005; McGowan and Whitworth 2008). This is related to the previously mentioned lack of preparedness of horses for this load and its relation to lactate concentrations.

Although baseline TG concentrations in the saliva of sport horses were higher compared to leisure horses monitored in our study, no differences were found between sampling times in any group. In this case, TG concentrations may be influenced by the nutritional status and feed ration of sport horses (Marlin and Nankervis 2013).

Assessment of lipoperoxidation and its products (TBARS) in the blood has become an integral part of many current studies focused on stress associated with transport or prolonged activity (Siqueira et al. 2014; Bottegaro et al. 2018). In our study, we found a regular increase of TBARS in saliva over time in both trained (sport) and leisure groups

of horses. Ultimately, the type and duration of exercise significantly influence oxidative stress and response to lipoperoxidation (Smarsh and Williams 2017; Bottegaro et al. 2018).

In conclusion, the leisure group of horses showed more significant changes in the monitored salivary biochemical properties in response to physical exercise that might have resulted from training or detraining. In sport horses, higher concentrations of the monitored indices were often detected than in irregularly trained leisure horses. These findings document that the salivary biochemical indices obtained non-invasively in this study can be used in research focused on workload and stress in horses. Nevertheless, only horses habituated to such exercise should be sampled, and knowledge of the basal saliva values is necessary in order to interpret data accurately and avoid bias.

Acknowledgement

This study was supported by the Internal Grant Agency of the University of Veterinary Sciences Brno, Czech Republic (project nr. IGA VETUNI 205/2020/FVHE).

References

Ayala I, Martos NF, Silvan G, Gutierrez-Panizo C, Clavel JG, Illera JC 2012: Cortisol, adrenocorticotropic hormone, serotonin, adrenaline and noradrenaline serum concentrations in relation to disease and stress in the horse. Res Vet Sci **93**: 103-107

Bottegaro NB, Gotic J, Suran J, Brozic D, Klobucar K, Bojanic K, Vrbanac Z 2018: Effect of prolonged submaximal exercise on serum oxidative stress biomarkers (d-ROMs, MDA, BAP) and oxidative stress index in endurance horses. BMC Vet Res **14**: 216

Campbell MLH 2013: When does use become abuse in equestrian sport? Equine Vet Educ **25**: 489-492

Contreras-Aguilar MD, Escribano D, Quiles A, Lopez-Arjona M, Ceron JJ, Martinez-Subiela S, Hevia ML, Tecles F 2019a: Evaluation of new biomarkers of stress in saliva of sheep. Animal **13**: 1278-1286

Contreras-Aguilar MD, Escribano D, Martinez-Subiela S, Martin-Cuervo M, Lamy E, Tecles F, Ceron JJ 2019b: Changes in saliva analytes in equine acute abdominal disease: a sialochemistry approach. BMC Vet Res **15**: 9

Contreras-Aguilar MD, Cerón JJ, Muñoz A, Ayala I 2021: Changes in saliva biomarkers during a standardized increasing intensity field exercise test in endurance horses. Animal **15**

Cook CJ, Jacobson LH 1995: Salivary cortisol as an indicator of stress in sheep (*Ovis ovis*). New Zeal Vet J **43**: 248

Cook NJ, Hayne SM, Rioja-Lang FC, Schaefer AL, Gonyou HW 2013: The collection of multiple saliva samples from pigs and the effect on adrenocortical activity. Can J Anim Sci **93**: 329-333

Dashper K 2017: Listening to horses: Developing attentive interspecies relationships through sport and leisure. Soc Anim **25**: 207-224

Douglas J, Owers R, Campbell MLH 2022: Social licence to operate: What can equestrian sports learn from other industries? Animals **12**: 1987

Fazio F, Casella S, Assenza A, Arfuso F, Tosto F, Piccione G 2014: Blood biochemical changes in show jumpers during a simulated show jumping test. Vet Arhiv **84**: 143-152

Gomes CLN, Alves AM, Ribeiro JD, Moraes FJ, Barreto RA, Fucuta RS, Ribeiro BM, Miranda LM 2020: Physiological and biochemical responses and hydration status in equines after two barrel racing courses. Pesqui Vet Brasil **40**: 992-1001

Hura V, Novotny F, Boldizár M, Rédl M, Noskovicová J, Hornák S, Petrovic V, Lazar G, Kovác G 2013: Biochemical responses to a non-standard exercise in horses trained for jumping. Acta Vet Brno **82**: 161-167

Kim JS, Hinchcliff KW, Yamaguchi M, Beard LA, Markert CD, Devor ST 2005: Exercise training increases oxidative capacity and attenuates exercise-induced ultrastructural damage in skeletal muscle of aged horses. J Appl Physiol **98**: 334-342

Lefebvre HP, Laroute V, Braun JP, Lassourd V, Toutain PL 1996: Non-invasive and quantitative evaluation of post-injection muscle damage by pharmacokinetic analysis of creatine kinase release. Vet Res **27**: 343-361

Marlin D, Nankervis KJ 2013: Equine exercise physiology. Wiley-Blackwell, Oxford, 304 p.

Masko M, Domino M, Jasinski T, Witkowska-Pilaszewicz O 2021: The physical activity-dependent hematological and biochemical changes in school horses in comparison to blood profiles in endurance and race horses. Animals **11**: 13

Massányi M, Halo M, Massányi P, Mlyneková E, Greń A, Formicki G, Halo M 2022: Changes in haematological and biochemical parameters in blood serum of horses during exposition to workload stress. Heliyon **8**: e12241

McGowan CM, Whitworth DJ 2008: Overtraining syndrome in horses. Comp Exerc Physiol **5**: 57-65

Mukai K, Takahashi T, Eto D, Ohmura H, Tsubone H, Hiraga A 2007: Heart rates and blood lactate response in Thorougbred horses during a race. J Equine Sci **18**: 58

Munoz A, Riber C, Santisteban R, Rubio MD, Aguera EI, Castejon FM 1999: Cardiovascular and metabolic adaptations in horses competing in cross-country events. J Vet Med Sci **61**: 13-20
Nemecková M, Popelková T, Chloupek P 2022: Sampling methods for determination of cortisol in pig saliva and their use in the assessment of pig welfare. Acta Vet Brno **91**: 261-266
Ohkawa H, Ohishi N, Yagi K 1979: Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. Anal Biochem **95**: 351-358
Popelkova T, Nemeckova M, Tsponova Z, Pistekova V 2022: Factors influencing the welfare of dairy goats. Acta Vet Brno **91**: 333-337
Pösö AR, Hyyppä S 1999: Metabolic and hormonal changes after exercise in relation to muscle glycogen concentrations. Equine Vet J **31**: 332-336
Rubio CP, Mainau E, Ceron JJ, Contreras-Aguilar MD, Martinez-Subiela S, Navarro E, Tecles F, Manteca X, Escribano D 2019: Biomarkers of oxidative stress in saliva in pigs: analytical validation and changes in lactation. BMC Vet Res **15**: 7
Santos SA, Silva R, Azevedo JRM, Mello MAR, Soares AC, Sibuya SY, Anaruma CA 2001: Serum electrolyte and total protein alterations in Pantaneiro horse during long distance exercise. Arq Bras Med Vet Zoo **53**: 351-357
Sheriff MJ, Dantzer B, Delehanty B, Palme R, Boonstra R 2011: Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia **166**: 869-887
Siqueira RF, Weigel RA, Nunes GR, Mori CS, Fernandes WR 2014: Oxidative profiles of endurance horses racing different distances. Arq Bras Med Vet Zoo **66**: 455-461
Smarsh DN, Williams CA 2017: Oxidative stress and antioxidant status in standardbreds: effect of age and training in resting plasma and muscle. J Equine Vet Sci **48**: 93-102
St George L, Hobbs SJ, Sinclair J, Richards J, Roddam H 2019: Does equestrian knowledge and experience influence selection and training practices for showjumping horses? Comp Exerc Physiol **15**: 123-135
Stepanova LV, Kolesnik OV, Kolenchukova OA, Fedotova AS, Kolomeytsev AV, Makarov AV, Kratasyuk VA 2023: The prospects for using bioluminescent enzymatic analysis to reveal the exercise capacity of sports horses. Biology Bull **50**: 52-60
Strzelec K, Kankofer M, Pietrzak S 2011: Cortisol concentration in the saliva of horses subjected to different kinds of exercise. Acta Vet Brno **80**: 101-105
Svestkova M, Pistekova V, Takacova D, Vecerek V, Voslarova V 2024: Analysis of the major deficiencis detected during welfare inspections of farm animals in the Czech Republic. Acta Vet Brno **93**: 45-57
Tabak LA 2004: Dental, oral, and craniofacial research: the view from the NIDCR. J Dent Res **83**: 196-197
Tateo A, Padalino B, Boccaccio M, Maggiolino A, Centoducati P 2012: Transport stress in horses: Effects of two different distances. J Vet Behav **7**: 33-42
Teixeira-Neto AR, Ferraz GC, Moscardini ARC, Balsamao GM, Souza JCF, Queiroz-Neto A 2008: Alterations in muscular enzymes of horses competing long-distance endurance rides under tropical climate. Arq Bras Med Vet Zoo **60**: 543-549
Thoma MV, Kirschbaum C, Wolf JM, Rohleder N 2012: Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. Biol Psychol **91**: 342-348
Wing C 2019: A brief review of salivary biomarkers as stress indicators in sport and exercise. Strength Cond J **41**: 80-88
Witkowska-Pilaszewicz O, Grzedzicka J, Sen J, Czopowicz M, Zmigrodzka M, Winnicka A, Cywinska A, Carter C 2021: Stress response after race and endurance training sessions and competitions in Arabian horses. Prev Vet Med **188**: 5