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REVIEW ARTICLE

Usefulness of Creatine Kinase Activity Determination for Assessing the Effects of Physical Effort in Horses

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ABSTRACT

Creatine kinase (CK), one of the most characteristic muscle tissue enzymes, catalyzes transfer of phosphate from phosphocreatine to adenosine 5′-diphosphate (ADP). The CK activity during exercise depends largely on the duration and intensity of exercise. The physiological range of activity of this enzyme in the blood plasma of clinically healthy horses is in the range from 90 to 275 U/L. Common phenomenon, especially in endurance horses, is the occurrence of a much larger post-exercise CK activity. However, the increase in CK activity in the blood plasma is a frequent phenomenon in endurance horses and should not be interpreted as an undesirable symptom, unless it is accompanied by clinical signs of myopathy or is still persisting over a longer period of time to increase the activity of this enzyme in the blood. It is recommended to collect blood from the horse directly before and after (up to 5 min.) exercise and 15, 30, 60 minutes or 24 hours after the exercise. Due to the various analytical methods applied and various units in which the results are presented, it seems that it would be appropriate to present variability of post-exercise CK activity in percentages, which will facilitate the assessment of this phenomenon in different individuals, often subjected to different loads. In horses especially important for diagnostic purposes is a muscle isoform (M-CK), present only in striated muscle, which probably determines to a large extent, their potential aerobic. The paper discusses creatine kinase function and usefulness for determining the activity of this enzyme during the physical effort of horses.

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INTRODUCTION

Equine exercise physiology research has focused on assessing the physiological capacity and adaptability of horses to specific exercise loads (Malinowski *et al.,* 2002; Krumrych, 2010; Janicki *et al.,* 2013b, 2013c). Many observations have shown that creatine kinase (CK) activity in blood, is a good indicator of exercise-induced muscle cell damage (Harris *et al.,* 1990; Janicki *et al.,* 2013b). It is essential that horses are adequately prepared for performing often exhausting loads, because their values may approach the maximum physiological capacity of these animals, especially in the final stage of training and during competition. Post-exercise changes in the value of many blood parameters are generally very clear but they quickly subside (Zobba *et al.,* 2011). Overexertion usually makes these changes more persistent and may be indicative of overtraining (Padalino *et al.,* 2007). Therefore, the analysis of the blood CK activity may be useful for assessing the endurance adaptations of horses to exercise loads (Szarska, 2001).

Creatine kinase, discovered by Lohmann in 1934, is one of the most characteristic muscle tissue enzymes that catalyzes transfer of phosphate from phosphocreatine to adenosine 5′-diphosphate (Lohmann, 1934; McLeish and Kenyon, 2005; Kędzierski, 2011). A temporary small increase in serum CK activity is mainly associated with abnormal cell membrane permeability as a result of lipid peroxidation and muscle tissue hypoxia during physical effort (Nimmo and Snow, 1982). A particularly large role attributed to expression of the *CKM* candidate gene coding for a type of creatine kinase muscle isoenzyme, which occurs only in striated muscles and may influence the efficiency of sport horses (Gu *et al.,* 2010; Hill *et al.,* 2010; Schröder *et al.,* 2011). Type I muscle fibres are characterized by lower activity of muscle creatine kinase compared to type II fibres, which may be advantageous to sport horses whose muscles are composed primarily of type IIA and IIB fibres (Schröder *et al.,* 2011).

The present review is focused on the usefulness of determining creatine kinase activity for assessing the horse's adaptation to performing different exercise loads.

Role of creatine kinase in muscles: The main source of energy in the muscle cell is adenosino5`-triphosphate (ATP), the concentration of which is relatively low (2-5 mM), which allows muscles to contract for just several seconds. This being the case, additional metabolic processes have to take place in the activated muscle cell to maintain ATP homeostasis. It is determined by large reserves of phosphocreatine (20-35 mM), as a result of which ATP is regenerated in the cell after a reversible reaction catalyzed by creatine kinase. This enzyme transfers the phosphate group from phosphocreatine to adenosino5`-diphosphate (ADP) to generate cellular ATP. During ATP regeneration creatine kinase, by using ADP and $H^+$, prevents muscle acidification and an increase in ADP concentration, which could inhibit ATP-dependent processes. The increase in adenosino5`-monophosphate (AMP) during long and exhausting work of muscles stimulates AMP-activated protein kinase (AMPK), which inactivates enzymes through their phosphorylation and stimulates the oxidation of fatty acids as well as the transport and absorption of glucose in the muscles. A decrease in ATP levels may cause ADP to be converted to ATP and AMP via adenylate kinase, after which AMP is converted to inosino5`-monophosphate (IMP) by AMP deaminase with a concurrent release of ammonia (Grzyb and Skorkowski, 2008; Westerblad *et al.,* 2010; Janicki and Buzała, 2013a). In horses, the concentration of IMP and ammonia in the muscles and blood increases during intense physical effort. As a result of metabolic processes, IMP can be further converted by inosine, hypoxanthine and xanthine to uric acid. The presence of these metabolites in blood indicates that ATP use in muscles has exceeded the ATP turnover rate and the adenine nucleotides were completely lost (Schuback and Essen-Gustavsson, 1998; Castejon *et al.,* 2006).

The rate of CK release from the cells depends on its molecular weight and intracellular location, the type of attachment to different intracellular structures, the ratio of intracellular and extracellular concentration, as well as the extent and rate of muscle damage (Harris *et al.,* 1998b). A physiologically greater amount of muscle CK is associated with the myofibrillar M-line, and a certain part of this isoform is loosely connected with band 1, as well as sarcolemma and endoplasmic reticulum (Rojo *et al.,* 1991; Wallimann *et al.,* 1992; Hornemann *et al.,* 2003; Brancaccio *et al.,* 2007). Creatine kinase is necessary for efficient function of the calcium pump by ensuring calcium ion homeostasis in the activated muscle cells. The abnormal flow of Ca^{2+} ions through calcium channels in the cell, resulting from inhibition of the calcium pump in the endoplasmic reticulum, arrests Ca^{2+} ions in the sarcoplasm, which leads to a permanent muscle spasm (Grzyb and Skorkowski, 2008; Westerblad *et al.,* 2010; Janicki and Buzała, 2013a).

Effect of oxidative stress on the activity of creatine kinase: Although horses are endowed with many different adaptive mechanisms for performing exercise tasks, these animal vulnerable to the consequences of excessive exercise loads. During maximal exercise in horses more than 8-fold increase of the heart rate was demonstrated, while in a highly trained men this value is increased only less than 4-fold (Butler *et al.,* 1993; Art and Lekeux, 1995). In addition, there is also a more than twofold increase in the maximum oxygen consumption - $VO_{2\ max}$ (ml/min/kg) and to the ratio of lung volume to body weight than in humans. Other examples of physiological adaptation of horses to intense physical effort is the ability to empty the spleen from red blood cells as a result of its autonomous contractions in the initial phase of exercise, maintaining hypoxia, and even excess CO_2 in the blood during intense exercise (Bayly *et al.,* 1989; Jones *et al.,* 1989), and also the greater then in humans number of mitochondria in horse's myocytes (Kayar *et al.,* 1989).

During different forms of physical activity in horses, there is an increase in oxygen utilization by mitochondria in the active muscle cells, which generates more reactive oxygen species (ROS). Research results suggest that intense physical effort of horses may result in a high (even 60-fold) increase in oxygen consumption in energy processes, so these animals are particularly susceptible to excess ROS (oxidative stress) (Chiaradia *et al.,* 1998). This phenomenon induces damage to cell lipid membranes as a result of intensive flow of electrons through the mitochondrial respiratory chain, ischemia and reperfusion, as well as high concentration of haem-containing proteins (Ji, 1999; Evans, 2000). The increase in cell membrane permeability is also associated with muscle hypoxia during intense aerobic effort (Harris, 1998b). Another, so to speak secondary source of damage initiated by mechanical injuries are peroxidation processes induced by intensification of oxygen-dependent activity of phagocytes (Jackson and O'Farrell, 1993; Aoi *et al.,* 2004). These injuries, confirmed by histopathological examination, cause a release of many protein compounds (including CK) from the cytosol to the extracellular space (Valberg *et al.,* 1993; Kinnunen *et al.,* 2005b). The increase in ROS generation also results in changes in the antioxidant potential, which depend mainly on the duration and intensity of exercise, and on the degree of training, breed, sex, condition of horses, and the climatic conditions (Harris *et al.,* 1998b; Piccione *et al.,* 2007a, 2009; Lejeune *et al.,* 2010; Soares *et al.,* 2011; Janicki *et al.,* 2013d).

Activity of creatine kinase during exercise of horses: The results of many observations made in horses, indicate that the duration and intensity of physical activity are major determinants of exercise-induced CK activity in the blood (Art *et al.,* 1990; Stopyra, 2002; Jawor *et al.,* 2007; Krumrych, 2007). Research carried out 3-month training under controlled conditions on a treadmill 5 times a week showed that CK activity before and after exercise fell within the physiologically normal range (Boffi *et al.,* 2002). Standardized exercise test of horses on a treadmill submerged in water showed a significant increase in the plasma activity of this enzyme (Lindner *et al.,* 2012). In studies, Anderson (1975) confirmed that the post-exercise increase in CK activity is directly proportional to the duration of effort. This is confirmed by field observations, indicating that following a short-term effort increase in CK is smaller than in horses competing in endurance (Piccione *et al.,* 2007a; Serteyn *et al.,* 2009; Cywińska *et al.,* 2012; Jagrič *et al.,* 2012; Gondin *et al.,* 2013).

Own research (Krumrych, 2007) also confirmed the relationship of the physical load value with changes in the activity of this enzyme. Post-exercise increase in CK was relatively low (5%) in recreational horses and significantly different from the results recorded in the standard exercise training event (41%), race (31%) and jumping (23%) horses. Relatively high CK values were also found in racehorses, which suggests that muscle microinjuries may occur as a result of even short but intense exercise. The results obtained are correlated to the observations of other authors on horses subjected to different exercise loads (Art *et al.,* 1990; Stopyra, 2002; Jawor *et al.,* 2007), with the highest post-exercise increase in CK activity observed after long-distance rides (Kerr and Snow, 1991; Hargreaves *et al.,* 2002) – immediately after a 140-km ride, the activity of this enzyme increases as much as 10-fold (Marlin *et al.,* 2002). According to some authors, the increase in muscle enzyme activity in the blood plasma is common in endurance horses and should not be interpreted as a side effect, unless it is accompanied by clinical signs of myopathy, and the increased activity of this enzyme in the blood plasma lasting a long time (Nimmo and Snow, 1982). The study of Szarska (2001) and Cywińska *et al*. (2012) showed that the extent of change in the enzyme activity depends on the length of distance covered. The plasma activity of creatine kinase in horses after endurance exercise on the 120 km increased about 14-fold compared with before the exercise (Serteyn *et al.,* 2009). Another study (Cywińska *et al.,* 2012) showed that CK activity increased almost 18-fold after prolonged exercise. It should be noted that exercise-induced increase in CK activity is temporary. In horses, the activity of this enzyme may easily double without noticeable clinical consequences, and the values of 2,000-3,000 U/L post exercise are often observed in long-distance horses that complete the race. It seems that this phenomenon may reflect the adaptation of skeletal muscles to the exercise loads (Cywińska *et al.,* 2012) as a result of strengthening the muscle cell membranes and connective tissue, as well as conversion of type IIx fibres to tougher type IIB fibres and a decrease in oxidative stress by increasing the activity of antioxidant enzymes (Lindner *et al.*, 2006).

The results of many studies suggest that increased activity of CK in the blood occurs during intensive exercise (Milne *et al.,* 1976; Pösö *et al.,* 1983; Harris *et al.,* 1990), while smaller loads do not cause a significant increase in the activity of this enzyme (Table 1). No changes in CK activity were found in foals as a result of standardized daily training during 140 days (Tateo *et al.,* 2008). In horses, prior to 14 days standardized exercise test, activity of CK in blood plasma was increased only slightly (and non-significantly) immediately after exercise (Escribano *et al.,* 2011). Slightly higher values of this enzyme were reported by Kędzierski (2009) for horses aged 2-4 years during standard exercise, done 5 days per week for one month. Another study found CK activity to gradually and significantly increase until 60 days of training and to decrease in the final stage of training (80 days) during standard training of horses aged 3-4 years (Fazio *et al.,* 2011). In a study by Janicki *et al*. (2013b) recorded a trend to a small, progressive increase in resting activity during of the 100-day performance test. Because the exercise load did not change towards the end of training, the muscle cells have probably adapted to the load applied (Lindner *et al.,* 2006). When the cyclic submaximal loads ended, CK activity in the plasma of horses was significantly higher than after a short test of

Table 1: Mean activity of CK in adult horses in physiological conditions depending on different types of exercise

	CK (IU/L)		
Characteristic of exercise	At rest	After exercise	References
Short, intense exercise on a treadmill	147	198	Oliveira *et al.* (2014)
Recreation exercise	219	229	Krumrych (2007)
Police horses on duty	165	224	Kruljc *et al.* (2014)
Polo competition	144	208	Zobba *et al.* (2011)
Jumping training	164	199	Jawor *et al.* (2007)
Jumping training	192	238	Krumrych (2007)
Jumping competition	120	195	Soares *et al.* (2011)
Jumping competition	179	269	Lejeune *et al.* (2010)
Race training (1000±200 m)	95	187	White *et al.* (2001)
Race competition (1800 m)	136	142	Piccione *et al.* (2007b)
Race trening	221	289	Krumrych (2007)
Trotters trening	238	271	Krumrych (2007)
Driving trening	207	293	Krumrych (2007)
Endurance training (30 km)	176	317	Mei *et al.* (2010)
Endurance rides (34-60 km)	180	335	Cywińska *et al.* (2012)
Endurance rides (40, 80, 120 km)	246	1,405	Adamu *et al.* (2012b)
Endurance rides (70, 100 km)	245	414	Teixeira-Neto *et al.* (2008)
Endurance rides (80 km)	277	611	Hargreaves *et al.* (2002)
Endurance rides (80 km)	275	568	Kinnunen *et al.* (2005a)
Endurance rides (91 km)	131	614	Muñoz *et al.* (2010)
Endurance rides (120 km)	191	2,667	Serteyn *et al.* (2010)
Endurance rides (121 km)	222	984	Castejon *et al.* (2006)
Endurance rides (140 km)	222	2,154	Marlin *et al.* (2002)
Endurance rides (120-160 km)	167	2,959	Cywińska *et al.* (2012)
Endurance rides (120, 160 km)	382	7,185	Lejeune *et al.* (2010)
Endurance rides (164 km)	318	1,189	Castejon *et al.* (2006)
Endurance rides (166 km)	155	1,309	Muñoz *et al.* (2010)

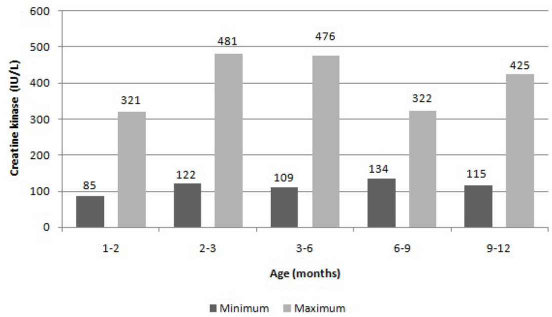

Fig. 1: The range of values CK (IU/L) in resting foals depending on month of age (Muñoz *et al.* 2012).

Table 2: Mean activity of CK in adult horses with exertional rhabdomyolysis

Conditions	CK (IU/L)	References
Affected	> 1,500	McEwen and Hulland (1986)
Day 1 after competition	10,340	Muñoz *et al.* (2013)
1.5 hrs after stopping exercise	12,430	
Mild	56,103	El-Ashker (2011)
Severe	267,906	
Survivors	73,999	El-Ashker (2012)
Non- survivors	241,164	

high intensity. The period of preparation for increasing exercise loads has a significant effect on CK activity in the blood while reducing the horse's response to effort (Siciliano *et al.,* 1995). The differences in post-exercise CK activity reflect different exercise load of horses, depending on the type of use. Higher mean activity of this enzyme was observed after exercise in event and racing horses. More than 60% of the animals of these groups demonstrated significantly higher after exercise (Krumrych, 2007), but in the range of physiological standards (Muñoz *et al.,* 2012) CK activity (60-340 IU/L), compared with the other horses. This result implies a relationship of intensity of damage muscle cells, as in the whole course of the training session event horses registered the highest mean value of $\%VO_{2max}$, while the racehorses were characterized by far the largest mean value of this ratio in the phase of intensive effort.

On the resting CK activity in horses affected, except to the intensity and duration of exercise, many other physiological factors such as age, breed or circadian rhythm. In foals, aged between 3 and 6 months was observed the largest resting activity of CK in blood plasma (Fig. 1). It is considered to be associated with increased muscle activity during the weaning period, although foals generally have higher CK levels than adult horses (Muñoz *et al.,* 2012). In a study by Janicki *et al.* (2013b), mean resting activity of CK in the serum of 3- to 4-year-old stallions during a 100-day performance test fell within the physiologically normal range for the species. Jawor *et al*. (2007) showed the mean resting levels of CK activity in the blood of endurance horses to be significantly higher than in jumping, race and event horses. It was also found that in the developing countries, cold-blooded horses working in adverse conditions are characterized by higher CK activity in blood compared to warm-blooded horses in the developed countries (Pritchard *et al.,* 2009). A reliable assessment of CK activity should also account for the circadian rhythm. The acrophase (time of peak) during daily rhythm of CK activity was observed in horses during the photophase (period of light) before exercise (11:02-10:26 AM) and post exercise (2:51-3:56 PM), and during the scotophase (the dark phase) (7:08-6:25 PM) immediately after exercise (Piccione *et al.,* 2009).

Diagnostic usefulness of determining creatine kinase during physical effort of horses: After intense exercise, the blood concentration of CK peaks within 4 to 6 hours and its half-life is 90-120 minutes, which makes CK a reliable and relatively sensitive indicator for detection and monitoring of muscle damage in the horse (Siciliano *et al.,* 1995; Harris *et al.,* 1998b; Boffi *et al.,* 2002; Chaney *et al.,* 2004). This enzyme is thus relatively quickly removed from the blood, and the high CK values return to normal in a short period of time (Harris *et al.,* 1998b; Aoki and Ishii, 2012). Interpretation of the results is complicated by large variation in the activity of this enzyme as a result of different exercise intensity and duration regimens used, together with varying sampling intervals and the possibility that the studies may have included horses with possible muscular problems (Muñoz *et al.,* 2002; 2010; 2013). It is suggested that the sampling at least 24 h after exercise can be helpful in the diagnosis of skeletal muscle damage. This allows to distinguish horses showing normal physiological response to exercise and those with abnormal or pathologic response to the applied load (Harris *et al.,* 1990). The blood is most commonly collected directly before and after (up to 5 min.) exercise and 15, 30, 60 minutes or 24 hours after the exercise (Kanter *et al.,* 1988; Räsänen, 1995; Castejon *et al.,* 2006; Krumrych, 2007; Jawor *et al.,* 2007; Piccione *et al.,* 2007a, 2010; Escribano *et al.,* 2011; Soares *et al.,* 2011; Zobba *et al.,* 2011; Janicki *et al.,* 2013b). It should be noted, however, that a delay in analyzing CK activity in the blood collected from horses may cause an additional increase in CK induced by adenylate kinase being released from erythrocytes and free hemoglobin. This enzyme may also disturb the assessment of the activity of other analytes and adversely affect the diagnostic interpretation of the results obtained (Rendle *et al.,* 2009).

The measurement of CK activity before and after exercise is helpful in diagnosing some pathological conditions of the muscles and can be a reliable indicator of overtraining in horses (Padalino *et al.,* 2007; Aoki and Ishii, 2012). According to many authors, plasma activity of CK in clinically healthy horses ranges between 90 and 275 U/L (Siciliano *et al.,* 1995; Muñoz *et al.,* 2002), although Ostaszewski *et al.* (2012) believe that the maximum value should not exceed 200 U/L. As a result of intense exercise, serum activity of CK may increase as much as 35-fold (Siciliano *et al.,* 1995). It should be noted at the same time that this reaction is often unrelated to any external clinical signs of myopathy in horses. Post-exercise increase in the activity of CK in blood is mainly due to a change in cell membrane permeability rather than muscle cell necrosis. It was found that only very high levels of CK activity in blood plasma (>10,000 U/L) reflect a considerable disintegration of skeletal muscles in horses (Siciliano *et al.,* 1995). One such disease is recurrent exertional rhabdomyolysis a hereditary disorder of calcium metabolism with concurrent changes in gait due to muscle cramping and stiffness (Castejon *et al.,* 2006; Chaney *et al.,* 2009), during which serum CK activity may increase as much as 10- to 900-fold (Table 2). A significant increase in resting values of CK activity was found in draught horses diagnosed with exertional rhabdomyolysis, which may be indicative of muscle cell degradation (El-Ashker, 2012). In endurance horses with metabolic disorders, CK activity was significantly higher compared to horses without metabolic disorders that completed the race (Adamu *et al.,* 2012a). Measurement of serum CK activity after maximal exercise may be also useful for diagnosing polysaccharide storage myopathy (PSSM**)**. This disease, which is mainly found in Quarter Horses, is related to abnormal glycogen metabolism in skeletal muscles. CK activity in the serum of these horses increases considerably 4 h after maximal and submaximal exercise and is significantly higher in affected compared to healthy horses of this breed. However, submaximal exercise is more commonly used in sport and recreation and can be, according to Annandale *et al*. (2005), more effective in inducing increase in muscle enzymes in horses with PSSM than maximal exercise. Therefore, the measurement of CK activity after physical exercise may be a more effective toolin the monitoring of this disease in horses (Evans, 2007).

It has recently been shown (Ostaszewski *et al.,* 2012) that β-hydroxy-β-methylbutyrate (HMB) may strengthen the muscle cell membrane during intensive exercise in horses. Giving horses 15 g CaHMB/day caused a significant decrease in exertion-induced increase of CK activity in blood plasma. Another effective antioxidant that reduces the release of CK during exercise in horses is γ-oryzanol, which, as a potent inhibitor of reactive oxygen species generation, protects lipid cell membranes from peroxidation, thus reducing their permeability.

Conclusion: Determine the activity of creatine kinase may be a reliable indicator of the impact assessment exercise in horses. The CK activity during exercise depends largely on the duration and intensity of exercise. These factors may affect different CK activity during exercise in horses as well as age, breed or circadian rhythm. According to many authors, plasma activity of CK in clinically healthy horses ranges between 90 and 275 U/L. However, the increase above this range, the activity of CK in the blood plasma is a frequent phenomenon in endurance horses and should not be interpreted as an undesirable symptom, unless it is accompanied by clinical signs of myopathy or persisting over a longer period of time to increase the activity of this enzyme in the blood. Dispersion of the results on CK activity shown in the literature may also result from different time of blood sampling from horses after exercise. It is recommended to collect blood from the horse directly before and after (up to 5 min.) exercise and 15, 30, 60 minutes or 24 hours after the exercise. Due to the various analytical methods applied and various units in which the results are presented, it seems that it would be appropriate to present variability of post-exercise CK activity in percentages, which will facilitate the assessment of this phenomenon in different individuals, often subjected to very different loads. In addition, further research on CK in horses should include determining the activity of the muscle isoform of creatine kinase (M-CK), which is only released from skeletal muscle cells. This will allow a precise assessment of the impact of the applied loads on the status of skeletal muscle cells than total CK activity in the organism of horses.

REFERENCES

Adamu L, MA Noraniza, A Rasedee and A Bashir, 2012a. Metabolic responses in endurance horses during racing in relation to uric acid profile, leucocytes, heart rate and plasma biochemical parameters. Vet Med (Praha), 57: 591-596.

Adamu L, NM Adzahan, R Abdullah and B Ahmad, 2012b. Alterations in biochemical, hematological and physical parameters in endurance horses with metabolic crisis. J Anim Vet Adv, 11: 4108-4114.

Anderson MG, 1975. The influence of exercise on serum enzyme levels in the horse. Equine Vet J, 7: 160-165.

Annandale EJ, MS Stephanie, J Valberg and B Essén-Gustavsson, 2005. Effects of submaximal exercise on adenine nucleotide concentrations in skeletal muscle fibers of horses with polysaccharide storage myopathy. Am J Vet Res, 66: 839-845.

Aoi W, Y Naito, Y Takanami, Y Kawai, K Sakuma, H Ichikawa, N Yoshida and T Yoshikawa, 2004. Oxidative stress and delayed-onset muscle damage after exercise. Free Radic Biol Med, 37: 480-487.

Aoki T and M Ishii, 2012. Hematological and biochemical profiles in peripartum mares and neonatal foals (Heavy Draft Horse). J Equine Vet Sci, 32: 170-176.

Art T and P Lekeux, 1995. Ventilatory and arterial blood gas tension adjustments to strenuous exercise in Standardbreeds. Am J Vet Res, 56: 1332-1337.

Art T, H Amory, D Desmecht and P Lekeux, 1990. Effect of show jumping on heart rate, blood lactate and other plasma biochemical values. Equine Vet J, 22 (Suppl 9): 78-82.

Bayly WM, DR Hodgson, DA Schultz, JA Dempsey and PD Gollnick, 1989. Exercise-induced hypercapnia in the horse. J Appl Physiol, 67: 1958-1966.

Boffi FM, J Cittar, G Balskus, M Muriel and E Desmaras, 2002. Training-induced apoptosis in skeletal muscle. Equine Vet J, 34 (Suppl 34): 275-278.

Brancaccio P, N Maffulli and FM Limongelli, 2007. Creatine kinase monitoring in sport medicine. Br Med Bull, 81: 209-230.

Butler PJ, AJ Woakes, K Smale, CA Roberts, CJ Hillidge, DH Snow and DJ Marlin, 1993. Respiratory and cardiovascular adjustments during exercise of increasing intensity and during recovery in Thoroughbred racehorses. J Exp Biol, 179: 159-180.

Castejon F, P Trigo, A Muñoz and C Riber, 2006. Uric acid responses to endurance racing and relationships with performance, plasma biochemistry and metabolic alterations. Equine Vet J, 38 (Suppl 36): 70-13.

Chaney K, JM MacLeay, RM Enns, F Al-Sobayil, C Kawcek and D Frisbie, 2004. Effects of induced lameness via carpal osteochondral fragmentation on plasma creatine kinase activity in horses. J Equine Vet Sci, 24: 531-534.

Chiaradia E, L Avellini, F Rueca, A Spaterna, F Porciello, MT Antonioni and A Gaiti, 1998. Physical exercise, oxidative stress and muscle damage in racehorses. Comp Biochem Physiol B, 119: 833-836.

Cywińska A, E Szarska, R Górecka, L Witkowski, M Hecold, A Bereznowski, A Schollenberger and A Winnicka, 2012. Acute phase protein concentrations after limited distance and long distance endurance rides in horses. Res Vet Sci, 93: 1402-1406.

El-Ashker MR, 2011. Acute kidney injury mediated by oxidative stress in Egyptian horses with exertional rhabdomyolysis. Vet Res Commun, 35: 311-320.

El-Ashker MR, 2012. Diagnostic efficiency of selected biochemical variables to predict the clinical outcome of exertional rhabdomyolysis in Egyptian draft horses. Comp Clin Pathol, 21: 1103-1108.

Escribano BM, El Agüera, MD Rubio, R Santisteban, R Vivo, S Agüera and P Tovar, 2011. Utility of the discriminant analysis to categorize untrained Spanish Pure Bred horses on the basis of biochemical and muscle variables before and after a standardized exercise test. Res Vet Sci, 91: 144-148.

Evans DL, 2007. Physiology of equine performance and associated tests of function. Equine Vet J, 39: 373-383.

Evans WJ, 2000. Vitamin E, vitamin C, and exercise. Am J Clin Nutr 72 (Suppl 2): 647-652.

Fazio F, Assenza, F Tosto, S Casella, G Piccione and G Caola, 2011. Training and haematochemical profile in Thoroughbreds and Standardbreds: A longitudinal study. Livestock Sci, 141: 221-226.

Gondin MR, NSB Foz, MC Pereira, JJ Flagliari, CAG Orozco, FHF D'Angelis, A Queiroz-Neto and GC Ferraz, 2013. Acute phase responses of different positions of high-goal (elite) polo Ponies. J Equine Vet Sci, 33: 956-961.

Grzyb K and EF Skorkowski, 2008.Izoenzymy kinazy kreatynowej - charakterystyka i funkcje w komórce. [Creatine kinase isoenzymes - characterization and functions in cell]. Postepy Biochem, 54: 274-283 [in Polish].

Gu J, DE Machugh, BA McGivney, SDE Park, LM Katz and EW Hille, 2010. Association of sequence variants in *CKM* (creatine kinase, muscle) and *COX4I2* (cytochrome c oxidase, subunit 4, isoform 2) genes with racing performance in Thoroughbred Horses. Equine Vet J, 42 (Suppl 38): 569-575.

Hargreaves BJ, DS Kronfeld, JN Waldron, MA Lopes, LS Gay, KE Saker, WL Cooper, DJ Sklan and PA Harris, 2002. Antioxidant status of horses during two 80-km endurance races. J Nutr, 132: 1781-1783.

Harris PA, DH Snow, TR Greet and PD Rossdale, 1990. Some factors influencing plasma AST/CK activities in thoroughbred racehorses. Equine Vet J, 22 (Suppl 9): 66-71.

Harris PA, DJ Marlin and J Gray, 1998b. Plasma aspartate aminotransferase and creatine kinase activities in Thoroughbred racehorses in relation to age, sex, exercise and training. Vet J, 155: 295-304.

Hill EW, SS Eivers, BA McGivney, RG Fonseca, J Gu, NA Smith, JA Browne, DE MacHugh, and LM Katz, 2010. Moderate and high intensity sprint exercise induce differential responses in *COX4I2* and *PDK4* gene expression in Thoroughbred horse skeletal muscle. Equine Vet J, 42 (Suppl 38): 576-581.

Hornemann T, S Kempa, M Himmel, K Hayeß, DO Fürst and T Wallimann, 2003. Muscle-type creatine kinase interacts with central domains of the M-band proteins myomesin and M-protein. J Mol Biol, 332: 877-887.

Jackson MJ and SO O'Farrell, 1993. Free radicals and muscle damage. Br Med Bull, 49: 630-641.

Jagrič MS, SA Nemec, P Zrimšek, P Kramarič, KV Kos, T Vovk and S Kobal, 2012. Plasma malondialdehyde, biochemical and haematological parameters in Standardbred horses during a selected field exercise test. Acta Veterinaria (Beograd), 62: 53-65.

Janicki B, A Kochowicz, D Cygan-Szczegielniak and W Krumrych, 2013d. Wysiłkowa indukcja stresu oksydacyjnego u koni oraz antyoksydacyjna ochrona organizmu. [Strain induction of oxidative stress in horses and anti-oxidative protection of the organism]. Med Weter, 69: 213-218 [in Polish].

Janicki B, A Kochowicz, M Buzała and W Krumrych, 2013b. Variability of selected biochemical parameters in young stallions during the 100-day performance test. J Equine Vet Sci, 33: 1136-1141.

Janicki B, A Kochowicz, M Buzała and W Krumrych, 2013c. Variability of selected clinical and haematological indices in young stallions during 100-day performance test. Bull Vet Inst Pulawy, 57: 91-96.

Janicki B and M Buzała, 2013a. The role of creatine in the organism of pigs and its effect on the quality of pork: a review. Ann Anim Sci, 13: 207-215.

Jawor P, T Stefaniak, J Borkowski, M Przewoźny, H Wierzbicki and JH Swagemakers, 2007. Przydatność wybranych parametrów krwi w monitorowaniu prawidłowości treningu koni dyscypliny skoków przez przeszkody. [Utility of selected blood parameters in monitoring the accuracy of show jump-horse training]. Med Weter, 63: 213-218 [in Polish].

Ji LL, 1999. Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med, 222: 283-291.

Jones JH, KE Longworth, A Lindholm, KE Conley, RH Karas, SR Kayar and CR Taylor, 1989. Oxygen transport during exercise in large mammals. I. Adaptive variation in oxygen demand. J Appl Physiol, 67: 862-870.

Kanter MM, GR Lesmes, LA Kaminsky, J La Ham-Saeger and ND Nequin, 1988. Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Eur J Appl Physiol, 57: 60-63.

Kayar SR, H Hoppeler, SL Lindstedt, H Claassen, JH Jones, B Essen-Gustavsson and CR Taylor, 1989. Total muscle mitochondrial volume in relation to aerobic capacity of horses and steers. Pflugers Archiv Europ J Physiol, 413: 343-347.

Kerr MG and DH Snow, 1991. Plasma enzyme activities in endurance horses. Equine Exerc Physiol, 3: 432-440.

Kędzierski W, 2011. Correlation of plasma creatine kinase activity and glucose level in exercised Purebred Arabian horses. Med Weter, 67: 541-544.

Kędzierski W, D Bergero and A Assenza, 2009. Trends of hematological and biochemical values in the blood of young race horses during standardized field exercise tests. Acta Veterinaria (Beograd), 59: 457-466.

Kinnunen S, M Atalay, S Hyyppä, ALehmuskero, O Hänninen and N Oksala, 2005a. Effects of prolonged exercise on oxidative stress and antioxidant defense in endurance horse. J Sports Sci Med, 4: 415-421.

Kinnunen S, S Hyyppä, J Lappalainen, N Oksala, M Venojärvi, C Nakao, O Hänninen, CK Sen and M Atalay, 2005b. Exercise-induced oxidative stress and muscle stress protein responses in trotters. Eur J Appl Physiol, 93: 496-501.

Kruljc P, N Čebulj-Kadunc, R Frangež and A Nemec Svete, 2014. Changes in blood antioxidant, biochemical and haematological parameters in police horses on duty. Slov Vet Res, 51: 119-129.

Krumrych W, 2007. The effect of physical exercise on oxygen metabolism of neutrophils and antioxidative potential of blood in horses. [Wpływ wysiłku fizycznego na metabolizm tlenowy neutrofilów oraz potencjał antyoksydacyjny krwi koni]. Ed. PIWet-PIB, Pulawy, Poland [in Polish].

Krumrych W, 2010. Blood antioxidant defence in horses during physical exercise. Bull Vet Inst Pulawy, 54: 617-624.

Lejeune JP, C Sandersen, D Votion, I Caudron, L Vander Heyden, T Franck, J Ceusters, A Mouithys-Mickalad, A Niesten, G de la Rebiere de Pouyade and D Serteyn, 2010. Effect of intensive exercise on plasmatic neutrophil elastase level in eventing and endurance horses. Equine Vet J, 42 (Suppl 38): 12-16.

Lindner A, A Signorini, L Brero, E Arn, R Mancini and A Enrique, 2006. Effect of conditioning horses with short intervals at high speed on biochemical variables in blood. Equine Vet J, 38 (Suppl 36): 88-92.

Lindner A, S Wäschle and HHL Sasse, 2012. Physiological and blood biochemical variables in horses exercising on a treadmill submerged in water. J Anim Physiol Anim Nutr, 96: 563-569.

Lohmann K, 1934. Űber die enzymatische aufspaltung der kreatin phosphorsaure zugleich ein Beitragzum Chemismus der Muskelkontraktion. Biochem Z, 271: 264-277.

Malinowski K, CL Betros, L Flora, CF Kearns, KH McKeever, 2002. Effect of training on age-related changes in plasma insulin and glucose. Equine Vet J, 34:147-153.

Marlin DJ, K Fenn, N Smith, CD Deaton, CA Roberts, PA Harris, C Dunster and FJ Kelly, 2002. Changes in circulatory antioxidant status in horses during prolonged exercise. J Nutr, 132: 1622-1627.

McEwen SA and TJ Hulland, 1986. Histochemical and morphometric evaluation of skeletal muscle from horses with exertional rhabdomyolysis (tying-up). Vet Pathol, 23: 400-410.

McLeish MJ and GL Kenyon, 2005. Relating structure to mechanism in creatine kinase. Crit Rev Biochem Mol Biol, 40: 1-20.

Mei LS, BAF Mohamed, NM Adzahan and R Abdullah, 2010. Changes in blood parameters of endurance horses in 30-km training. 5th Proceedings of the Seminar in Veterinary Sciences, 5-8 January, 36-42.

Milne DW, RT Skarda, AA Gabel, LG Smith and K Ault, 1976. Effects of training on biochemical values in Standardbred horses. Am J Vet Res, 37: 285-290.

Muñoz A, C Riber, P Trigo, and F Castejón, 2012. Age- and gender-related variations in hematology, clinical biochemistry, and hormones in Spanish fillies and colts. Res Vet Sci, 93: 943-949.

Muñoz A, C Riber, P Trigo, C Castejón-Riber and FM Castejón, 2010. Dehydration, electrolyte imbalances and renin-angiotensin-aldosterone-vasopressin axis in successful and unsuccessful endurance horses. Equine Vet J, 42 (Suppl 38): 83-90.

Muñoz A, C Riber, R Santisteban, RG Lucas and FM Castejón, 2002. Effect of training duration and exercise on blood-borne substrates, plasma lactate and enzyme concentrations in Andalusian, Anglo-Arabian and Arabian breeds. Equine Vet J, 34 (Suppl 34): 245-251.

Muñoz A, RGM Rodríguez, C Riber, P Trigo, M Gómez-Díez and F Castejón, 2013. Subclinical *Theileria equi* infection and rhabdomyolysis in three endurance horses. Pak Vet J, 33: 257-259.

Nimmo MA and DH Snow, 1982. Time course of ultrastructural changes in skeletal muscle after two types of exercise. J Appl Physiol, 52: 910-913.

Oliveira CAA, JF Azevedo, ACT Miranda, BG Souza, MT Ramos, APD Costa, CD Baldani, VP Silva and FQ Almeida, 2014. Hematological and blood gas parameters' response to treadmill exercise test in eventing horses fed different protein levels. J Equine Vet Sci, 34: 1279-1285.

Ostaszewski P, A Kowalska, E Szarska, P Szpotański, A Cywińska, B Bałasińska and T Sadkowski, 2012. Effects of β-Hydroxy-β-Methylbutyrate and γ-Oryzanol on blood biochemical markers in exercising Thoroughbred race horses. J Equine Vet Sci, 32: 542-551.

Padalino B, G Rubino, P Centoducati and F Petazzi, 2007. Training versus overtraining: evaluation of two protocols. J Equine Vet Sci, 27: 28-31.

Piccione G, C Giannetto, F Fazio, S Casella and G Caola, 2009. A comparison of daily rhythm of creatinine and creatine kinase in the sedentary and athlete horse. J Equine Vet Sci, 29: 575-580.

Piccione G, F Fazio and E Giudice, 2007a. Oxidative stress in standardbred horses during official races of 1600 and 2000 meters. Med Weter, 63: 1554-1557.

Piccione G, F Fazio, C Giannetto, A Assenza and G Caola, 2007b. Oxidative stress in thoroughbreds during official 1800-metre races. Vet Archiv, 77: 219-227.

Piccione G, S Casella, C Giannetto, V Messina, V Monteverde, G Caola and S Guttadauro, 2010. Haematological and haematochemical responses to training and competition in standardbred horses. Comp Clin Pathol, 19: 95-101.

Pösö AR, T Soveri and HE Oksanen, 1983. The effect of exercise on blood parameters in Standardbred and Finnish-bred horses. Acta Vet Scand, 24: 170-184.

Pritchard JC, CC Burn, ARS Barr and HR Whay, 2009. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Res Vet Sci, 87: 389-395.

Räsänen LA, 1995. Exercise induced purine nucleotide degradation and changes in myocellular protein release. Equine Vet J, 27 (Suppl 18): 235-238.

Rendle DI, J Heller, KJ Hughes, GT Innocent and AE Durham, 2009. Stability of common biochemistry analytes in equine blood stored at room temperature. Equine Vet J, 41: 428-432.

Rojo M, R Hovius, R Demel, T Wallimann, HM Eppenberger and K Nicolay, 1991. Interaction of mitochondrial creatine kinase with model membranes. A monolayer study. FEBS Letters, 281: 123-129.

Schröder W, A Klostermann and O Distl, 2011. Candidate genes for physical performance in the horse. Vet J, 190: 39-48.

Schuback K and B Essen-Gustavsson, 1998. Muscle anaerobic response to a maximal treadmill exercise test in Standardbred trotters. Equine Vet J, 30: 504-510.

Serteyn D, C Sandersen, JP Lejeune, G de la Rebiere de Pouyade, J Ceusters, A Mouithys-Mickalad, A Niesten, A Fraipont, E van Erck, AG Goachet, C Robert, JL Leclerc, DM Votion and T Franck, 2010. Effect of a 120 km endurance race on plasma and muscular neutrophil elastase and myeloperoxidase concentrations in horses. Equine Vet J, 42 (Suppl 38): 275-279.

Siciliano PD, LM Lawrence, K Danielsen, DM Powell and KN Thompson, 1995. Effect of conditioning and exercise type on serum creatine kinase and aspartate aminotransferase activity. Equine Vet J, 27 (Suppl 18): 243-241.

Soares JCM, R Zanella, C Bondan, LP Alves, MR de Lima, AC da Motta and EL Zanella, 2011. Biochemical and antioxidant changes in plasma, serum, and erythrocytes of horses before and after a jumping competition. J Equine Vet Sci, 31: 357-360.

Stopyra A, 2002. Wskaźniki gospodarki tlenowej i aktywność wybranych enzymów surowicy krwi koni w warunkach ekstremalnego wysiłku. [Gasometry indices and the activity of selected enzymes in horse serum during extreme exertion]. Med Weter, 58: 543-548 [in Polish].

Szarska E, 2001. Zmiany wybranych parametrów krwi koni rajdowych w zależności od długości dystansu. [The changes of selected blood parameters after different distances covered by endurance horses]. Med Weter, 57: 522-526 [in Polish].

Tateo A, E Valle, B Padalino, P Centoducati and D Bergero, 2008. Change in some physiologic variables induced by Italian traditional conditioning in Standardbred yearling. J Equine Vet Sci, 28: 743-750.

Teixeira-Neto AR, GC Ferraz, ARC Moscardini, GM Balsamão, JCF Souza and A Queiroz-Neto, 2008. Alterations in muscular enzymes of horses competing long-distance endurance rides under tropical climate. Arq Bras Med Vet Zootec, 60: 543-549.

Valberg S, J Häggendal and A Lindholm, 1993. Blood chemistry and skeletal muscle metabolic responses to exercise in horses with recurrent exertional rhabdomyolysis. Equine Vet J, 25: 17-22.

Wallimann T, M Wyss, D Brdiczka, K Nicolay and HM Eppenberger, 1992. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem J, 281: 21-40.

Westerblad H, JD Bruton and A Katz, 2010. Skeletal muscle: energy metabolism, fiber types, fatigue and adaptability. Exp Cell Res, 316: 3093-3099.

White A, M Estrada, K Walker, P Wisnia, G Filgueira, F Valdés, O Araneda, C Behn and R Martínez, 2001. Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. Comp Biochem Physiol Part A, 128: 99-104.

Zobba R, M Ardu, S Niccolini, F Cubeddu, C Dimauro, P Bonelli, CD Dip, S Visco and MLP Parpaglia, 2011. Physical, hematological, and biochemical responses to acute intense exercise in Polo Horses. J Equine Vet Sci, 31: 542-548.