Exercise-Induced Physiological Fatigue in Horses: Review

Atlarda Egzersize Bağlı Fizyolojik Yorgunluk

Berjan DEMİRTAŞ,[a]
Çağla PARKAN YARAMIŞ,[a]
Mukadder ATMACA[b]

[a]İstanbul University
Vocational School of Veterinary Medicine,
İstanbul
[b]Department of Physiology,
Dicle University Faculty of Medicine,
Diyarbakir

Geliş Tarihi/*Received:* 08.03.2016
Kabul Tarihi/*Accepted:* 17.03.2016

Yazışma Adresi/*Correspondence:*
Berjan DEMİRTAŞ
İstanbul University
Vocational School of Veterinary Medicine,
İstanbul,
TÜRKİYE/TURKEY
berjan@istanbul.edu.tr.

ABSTRACT Horses that become fatigued either stop exercising or continue exercising but at a lower intensity. The researches show that there are many causes of exercise induced fatigue. The depletion of energy stores and accumulation of metabolites during muscle contraction are considered as the important factors causing fatigue. Furthermore, generated heat during muscle contraction impairs the fluid, electrolyte and acid-base balances of internal environment of horse body. The occurrence of physiological fatigue differs according to the type of exercise the horse is performing. The intensity and the duration of the exercise are very important parameters during the onset of fatigue. The onset of fatigue in flat racing horses is completely different physiological phenomenon to fatigue in endurance horses performing with low intensity but long duration exercise. In this review we focus on the underlying physiological mechanisms of fatigue induced by exercise mainly observed in flat racing and endurance racing horses.

Key Words: Fatigue; horses; exercise; muscle fatigue; electrolytes; water-electrolyte balance; acid-base equilibrium

ÖZET Atlarda egzersiz sırasında yorgunluk oluştuğunda, at ya hiç egzersize devam etmek istemez ya da egzersizin şiddetini düşürür. Yapılan araştırmalarda egzersiz sırasında oluşan yorgunluğun birçok nedeninin olabileceği belirtilmiştir. Yorgunluğun nedenleri arasında iskelet kas hücrelerinde enerji depolarının azalması ve kas hücrelerinin kasılmaları sırasında oluşan metabolizma artıklarının birikimi önemli yer tutmaktadır. Ayrıca kasılma esnasında oluşan yüksek ısı vücudun sıvı, elektrolit ve asit-baz dengesinin bozulmasına yol açar. Egzersizin tipi fizyolojik yorgunluğun oluşum mekanizmasında önemlidir. Yüksek hız gerektiren düz yarışlar ile dayanıklılık öğesinin önemli olduğu endurans yarışlarında görülen yorgunluğun farklı fizyolojik temelleri bulunmaktadır. Bu derlemede özellikle düz yarış ve endurans atlarında görülen yorgunluğun oluşumunda rol oynayan fizyolojik nedenlere değinilmiştir.

Anahtar Kelimeler: Yorgunluk; atlar; egzersiz; kas yorgunluğu; elektrolitler; su elektrolit dengesi; asid-baz dengesi

Turkiye Klinikleri J Vet Sci 2015;6(2):60-6

Horses are considered as the most superior athlete within the animal kingdom. Horses are huge investments in terms of both time and money. Horses get trained to increase their exercise capacity and improve their overall performance.

Many factors compromise the athletic performance of the horses. Exercise induced fatigue is one of the limiting factor affecting the equine performance. Although fatigue in human athletes has been interested for more

doi: 10.5336/vetsci.2016-51263

than a century, fatigue in equine athletes is still considered as a new area which requires to be searched more.[1]

At a practical level in sport the term fatigue is described as the sensations of tiredness and the accompanying decrease in athletic performance.[2] More scientific definition describes fatigue as a complex and intricate physiologic response to exercise, leading to the inability to sustain further activity at the current intensity.[3] When fatigue occurs the horse either stops exercising or continues exercising at a lower intensities.[4]

Generally, exercise induced fatigue appears to involve psychological (or neurologic) and physiological contributions.[3] Psychological fatigue is mainly related to the motivation of the horse and generally caused by overtraining of horses, eventually becoming listless and sour and their performance will decline.[3,4] Since psychological fatigue is out of the subject of this review, it is not discussed here.

Physiological fatigue is mainly related to energy metabolism and contracting mechanisms of muscle cells and consequent effect of muscle exercise on the internal environment of the horse.[1]

Physical exercise occurs when muscles contract and generate force. During exercise some biochemical changes occur in muscle and affect the equilibrium of muscle cells. Increased muscle metabolites and the generated heat by muscle contraction during exercise may decline not only the force of muscle but also affect the equilibrium of internal environment of other body systems of the horse. All these different complex mechanisms create sensation of fatigue and finally, a reduction or complete cessation of exercise occurs. Depending on the type of exercise, sooner or later fatigue will occur.[1]

Many factors can influence the onset of fatigue in horses such as the age, the fitness, and the environmental factors. Apart from these factors, the intensity and the duration of exercise are very important parameters on the onset of physiological fatigue. Fatigue in response to high intensity exercise (e.g. flat racing) has different underlying physiological mechanisms from fatigue during prolonged submaximal exercise (e.g. endurance racing).[2]

In this review we will focus on fatigue caused by changes in muscle cell and fatigue caused by changes in internal environment of the horse body during exercise.

FATIGUE CAUSED BY CHANGES IN SKELETAL MUSCLE CELLS (MUSCULAR FATIGUE)

The energy source for contraction of muscle cells (muscle fibres) is adenosine triphosphate (ATP).[1] The amount of ATP is low and will only support exercise for several seconds of muscular activity.[2] When ATP is broken down it should be regenerated again. The major pathways for ATP regenerations in the muscle are:[1,5]

1. A rapid production of ATP from creatine phosphate (CP) in sarcoplasm of muscle cells. The stores of CP are limited and can only support exercise for a few seconds.[2]

2. A somewhat slower production of ATP by anaerobic glycolysis in sarcoplasm of muscle cells. Muscle glycogen is broken down to lactic acid. The generation of ATP by anaerobic glycolysis is also very limited and can support exercise for 1-2 minutes.[4] This pathway is essential at the onset of exercise before aerobic systems have come up to speed.[2]

3. A slower but very effective production of ATP by aerobic pathways of carbohydrate (muscle glycogen or blood glucose) and fat metabolism in mitochondria of muscle cells. This pathway can support the exercise for hours (by aerobic metabolism of carbohydrates) and even days (by aerobic metabolism of fats). This pathway is fundamental for endurance horses which have lower intensity but longer duration of exercise compared to flat racing horses.[2,4]

Repeated, intense use of muscles leads to changes in many muscle properties including energy stores, intracellular metabolites, extracellular and intracellular ions and related action potential of muscle cells that cause fatigue during exercise.

DEPLETION OF ENERGY STORES

Aerobic pathway for energy production is used in exercise intensities below the anaerobic threshold (blood lactate < 4 mmol/l). Aerobic energy pathway utilises free fatty acids and glycogen. Lipid stores supply enough fuel for days to sustain low grade exercise. Whilst it is possible to deplete muscle and liver glycogen, it is almost impossible to deplete lipid stores in a single bout of exercise. Even a thin endurance horse is estimated to have enough fat to complete 5X100 mile race.[2] The limiting factor for endurance horses is the availability of glucose (from glycogen within liver and active muscle) rather than free fatty acids. During endurance exercise muscle glycogen stores decrease slowly and the muscle tissue gradually increases its consumption of blood glucose.[1] Finally, the availability of glucose is smaller than the glucose consumption and the concentration of blood glucose may even decrease.[6] It is reported that horses at the end of 100 mile endurance competition have very low blood glucose concentration (< 2.5mmol/l), indicating considerable depletion of liver glycogen stores.[2] In endurance horses when this occurs, horse can maintain exercise in very low intensity since free fatty acids can be used as a fuel. Glycogen replacement after depletion may take 24-72 hours.[4] Depletion of muscle glycogen is also seen in human marathon runners and is referred to as 'hitting to wall'.[1] When this point is reached, the marathon runner obtains the energy from aerobic metabolism of free fatty acids but is limited as to maximum speed and unable to accelerate.[7] The same scenario for endurance horse might occur.

METABOLIC PRODUCTS AND CHANGES IN ION CONCENTRATIONS OF MUSCLE CELL

When muscle cells are contracting many intramuscular metabolites form and intramuscular and extra muscular changes occur which affect the muscle force causing the sensation of fatigue. These are mentioned below:

Accumulation of Phosphate Ions (PI)

Independent of which pathway is dominating, muscle contraction will always be associated with an increase in adenosine diphosphate (ADP) and inorganic phosphate (Pi).[1] However, the breakdown of CP during high intensity exercise accumulates more Pi and increased Pi can depress the contractile functions of muscle fibres. Accumulation of Pi in the sarcoplasm causes a decrease in contractile force due to the inhibition of cross bridge interaction between actin and myosin filaments.[8] Most studies imply that accumulation of Pi is the most important cause of fatigue during high intensity exercise both in human and equine athletes.[1,2,8] Therefore, fatigue caused by Pi accumulation in muscle cell is an important factor for flat race horses but not for endurance horses which have lower intensities of exercise.

Accumulation of Lactic Acid and Hydrogen Ions (H+)

When the intensity of exercise reaches a certain level, energy is supplied by anaerobic glycolysis. Muscle glycogen is broken down to lactic acid and furthermore lactic acid dissociates into lactate ions (LA^-) and hydrogen ions (H^+). LA^- ions form a salt with sodium (Na^+) or potassium (K^+) ions hence do not pose a threat to the muscle cells.[4] Accumulation of H^+ ions in the sarcoplasm causes a decrease in the contractile force due to the inhibition of cross bridge interactions of actin and myosin filaments. In addition, the accumulation of H^+ may cause a depression of calcium ion (Ca^{2+}) reuptake in the sarcoplasmic reticulum and this might prolongs the relaxation time and cause fatigue.[1] Increasing concentrations of H^+ decline the pH of muscle and cause intramuscular acidosis. Intramuscular acidosis may be responsible for the occurrence muscular fatigue due to disturbance in mitochondrial function, impairment of glycolysis and consequent decline in muscle ATP concentration, swelling of mitochondria and sarcoplasmic reticulum.[9,10]

Inhibition of Calcium Ions (Ca^{2+}) Release

With accumulation of Pi and H^+ ions, Ca^{2+} release is incited by the precipitation of calcium phosphate within the lumen of sarcoplasmic reticulum.[1] Declined pH in muscle cell also disturbs morphological structure of sarcoplasmic reticulum and the ability of sarcoplasmic reticulum to release Ca^{2+}. Reduced Ca^{2+} concentrations in sarcoplasm result

in a less powerful contraction of muscle cell. The uptake of Ca^{2+} by mitochondria also reduces the efficiency of ATP regeneration.[2,11] Hence, intramuscular Ca^{2+} disturbance may be an important factor in fatigue for endurance horses.[2]

Increased Efflux of Potassium Ions (K^+)

In rested muscle, the intracellular K^+ concentration is 160 mmol/l and extracellular K^+ concentration is 4 mmol/l.[12] During muscle contraction, very small amount of K^+ (approximately 2-10 μmol/l) is lost from muscle cells to extracellular compartment.[13] High intensity exercise may lead to K^+ concentration in excess of 10 mmol/l in extracellular compartment.[14] Activity induced changes in ion concentration of K^+ are not compensated by the activity of Na^+- K^+ pumps and other means and lead to reduction in membrane potential and consequent muscle excitability and force.[11] It has been reported that increased K^+ concentration in extracellular compartment induces muscle fatigue by decreasing membrane potential and causing neuromuscular conduction disturbance.[9]

Accumulation of Magnesium Ions (Mg^{2+})

During muscle activation an increase in Mg^{2+} concentration occurs in sarcoplasm. In high intensity exercise, while ATP concentration decreases, Mg^{2+} concentration rises because ADP, AMP and IMP all have much lower affinity for Mg^{2+} than ATP.[10,15]

Mg^{2+} ions play important roles in the function of sarcoplasmic reticulum. Increased Mg^{2+} concentration in sarcoplasm reduces Ca^{2+} fluxes across the membrane of sarcoplasmic reticulum and causes fatigue by reducing muscle force.[16,17]

Accumulation of Reactive Oxygen Species (ROS)

Exercise enhances the production of reactive oxygen species (ROS). [9] The increased demand for energy activates mitochondrial respiration as a consequence, increases oxygen uptake in the muscle.[18] The source of ROS is the partial reduction of oxygen in mitochondria during oxidative phosphorylation and the xanthine oxidase during degradation of purine nucleotides.[19] About 5% of the total amount of oxygen in mitochondria is converted into reactive oxygen species such as superoxide anion ($\cdot O^{2-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$).[20-22]

There is strong evidence that ROS contribute to the process of fatigue during exercise.[9,11] It has been suggested that exercise induced fatigue may be related to the modification of muscle cell membrane caused by ROS.[23,24]It has been also indicated that increased amounts of ROS cause decline in force by damaging the contractile proteins and the Na^+-K^+ pumps of muscle cells.[11]

FATIGUE CAUSED BY CHANGES IN INTERNAL ENVIRONMENT OF THE HORSE BODY

Apart from muscle, many other systems are involved to maintain homeostasis during exercise. While the steady state of muscle cell is deteriorated during exercise, some changes occur in other systems of horse body as well. Exercise causes many changes in internal environment of horse body such as increased production of heat, consequent increase in sweat loss, increased production of CO_2, hydrogen ions, lactate, ammonia in blood and increased consumption of oxygen and nutrients (glycogen, glucose and fatty acids). The larger workload the larger the effects of these variables are seen in the internal environment. A deterioration of steady state of internal environment by exercise can induce sensation of fatigue and have devastating effect on exercise performance.[1] Since changes in internal environment is very integrated and involve wide range of body systems we will mention only the most involved ones that has been suggested as fatigue factors in exercising horses.

HYPERTHERMIA

During intense exercise heat production by muscles increases up to 50 times that of the resting value. The body temperature rises from 37-38°C to 43°C.[4] Thus, exercise cause heat load in the internal environment.[1] This high temperature disturbs not only the structural, physiological and biochemical properties of muscle cells but also all other cell types (including brain cells) in whole body system.

Hence, high body temperature can be life threatening unless body's own cooling system gets involved and works properly. The success of horse delaying fatigue caused by high temperature relies on the ability to dissipate the heat during exercise which it does mostly by sweating.

DISTURBANCES IN FLUID-ELECTROLYTE AND ACID-BASE BALANCES

During exercise, fluid-electrolyte balance of the body should be considered together with the acid base balance. Changes in pH are closely linked to the changes in electrolyte balance between intracellular fluid (ICF) and extracellular fluid (ECF).[25]

The changes in pH during exercise can be respiratory and metabolic origin. An increase in blood pCO_2 (> 45 mmHg) causes decline in pH (pH< 7.35) resulting with acidemia while a decrease in pCO_2 (< 35 mmHg) causes increase in pH (pH> 7.45) resulting with alkalemia.[26] Apart from CO_2, strong ion difference (SID) are considered as independent variables to affect pH changes in blood during exercise.[25,26] Na^+, K^+, Cl^-, LA^- are the major strong ions determine the pH changes during exercise.[25] Stewart equitation formula of plasma acid base balance is referred as strong ion difference (SID) and adapted for exercise as SID= $([Na^+]+[K^+])-([Cl^-]-[LA^-])$. According to this formula, when SID decreases pH also decreases.[27-29] The SID should be in constant value to maintain pH in normal range (pH 7.35-7.45). If SID is different from the constant value the body try to compensate the changes via dependent variables such as H^+ and HCO_3^- ions.[25]

There have been many papers published on the effect of acute exercise on whole body acid base status in the horse.[28,29] In the horse, short duration with high intensity exercise creates a respiratory and metabolic acidosis resulting a decrease in blood pH from resting values of 7.4 to levels 6.8.[25] During high intensity exercise, pCO_2 increases and the rate of elimination is inadequate because the horse does not hyperventilate during exercise as do humans.[25] Consequently hypercapnea occurs and dramatic decrease in pH is observed.[29] The decrease in SID value also contributes to a decrease in pH. The decrease in SID is caused by lactic acid efflux from muscle fibres to the blood in high intensity exercises.[9] High concentrations of LA^- in blood cause a decrease in SID and blood pH. Plasma K^+ and Na^+ concentrations have also been increased but not as much as LA^-.[25] Interestingly it has been reported that plasma Cl^- concentration has not been changed after high intensity of exercise.[27-29] The increase in plasma LA^- concentration is higher than the increase in plasma K^+ and Na^+ concentrations in high intensity exercise.[25] As a consequence metabolic acidosis will be inevitable during high intensity exercises. The decrease pH in both muscle and blood are one of the factors causing fatigue in high intensity exercise.

In endurance races, acid base balance is related to the tremendous amount of sweat lost by horse during low intensity but prolonged exercise.[25] The heat during exercise must be dissipated through mainly sweating. In hot conditions a horse competing in endurance race may lose up to 10% of its body weight.[25] The water for sweating is derived from both extracellular and intracellular fluids, and represents 15 % of total body water.[1] The loss of that amount of water by sweat lead to dehydration. The sweat of horse is hypertonic compared with the plasma.[4,9,25] Therefore, its production is accompanied by a loss of electrolytes such as Na^+, K^+, Ca^{2+} and especially Cl^-, with a consequent reduction of these ions in plasma.[9,30] Therefore, the amount of electrolytes lost by sweating is very high in horses. This affects SID value and consequently acid base status of blood. In endurance race the horse encounters a disproportional loss of Cl^- by sweat.[9,25,30] The loss of high amount of Cl^- causes Cl^- deficit in plasma (hypochloremia). Hypochloremia increases SID value.[25] Consequently, pH rises up to 7.8 by retention of HCO_3^- from kidney to the blood.[25,26]

Electrolyte losses also cause an imbalance in the distribution of electrical charges on either side of cell membrane and this can disrupt the normal function of excitable tissues such as muscle and nerve.[4] Especially loss of Ca^{2+} and Mg^{2+} can lead to sensitisation of the phrenic nerve of diaghragm.[4,26] At sensitised state it begins to fire at the same time with heart since this nerve passes through heart

route. This is known synchronous diaphragmatic flutter (SDF) or thumbs and considered as elimination factor from endurance races as considered as severe fatigue syndrome.[4] All these alterations in fluid, electrolyte and acid-base status impairs performance capacity of horse and may even be life threatening.[31]

CONCLUSION AND RECOMMENDATION

Many physiological changes occur in horse body when horses are exercising. Fatigue in horses mainly occurs due to the physiological and biochemical changes in muscle cells and in the internal environment of horse body. Sooner or later fatigue will occur in all exercising horses. The causes of fatigue are very complex, integrated and depend on the intensity and duration of exercise. Fatigue in response to high intensity exercise (e.g. flat racing horses) is mainly due to the depletion of ATP, CP, accumulation of lactate in muscle cells and subsequent decrease in intramuscular and extracellular pH. During prolonged, lower intensity exercise (e.g. endurance horses) fatigue mainly develops due to the depletion of muscle and liver glycogen, hyperthermia, subsequent dehydration and electrolyte imbalance of horse body. A good knowledge of exercise-induced fatigue, its physiological mechanisms may help trainers minimize its occurrence by appropriate training and competition programmes, acclimatization methods, nutrition and diet supplements and help veterinarians to apply preventative and emergency treatments before and after fatigue occurs. There is still more to discover for the physiological mechanisms of fatigue in exercising horse.

REFERENCES

1. Ament W, Verkerke GJ. Exercise and fatigue. Sports Med 2009;39(5):389-422.
2. Marlin DJ. The physiology of fatigue in horses during exercise. http://flairstrips.com/education-and-research/education-articles/. Date accessed: 15 January 2016.
3. Gerard MP, De Graff-Roelfsema E, Hodgson DR, Van der Kolk JH. Energetic considerations and exercise. In: Hodgson DR, McKeever KH, McGowan CM, eds. The Athletic Horse. 2nded. China: Elsevier Saunders; 2013. p.19-33.
4. Marlin D, Nankervis K. Energetics of exercise. Chapter 2. Aspects of physiological stress and fatigue. Chapter 11. In: Marlin D, Nankervis K, eds. Equine Exercise Physiology. 1sted. U.K.: Blackwell; 2003. p.1-132.
5. Murray RK. Muscle. Chapter 58. In: Murray RK, Granner DK, Mayes PA, Rodwell VW, eds. Harper's Biochemistry. 23rded. Norwalk (VA): Appleton & Lange; 1993. p.647-64.
6. Powers SK, Howley ET. Exercise Physiology: Theory and Application to Fitness and Performance. 2nd ed. Madison (WI) and Dubuque (IA): WCB Brown and Benchmark Publishers; 1994. p.1-30.
7. Mayes PA. Integration of metabolism and the provision of tissue fuels. Chapter 29. In: Murray RK, Granner DK, Mayes PA, Rodwell PA, eds. Harper's Biochemistry. 23rd ed. Norwalk (VA): Appleton & Lange; 1993. p.279-92.
8. Westerblad H, Allen DG, Lännegren J. Muscle fatigue: lactic acid or ınorganic phosphate the major cause? News Physiol Sci 2002;17:17-21.
9. Art T, Lekeux P. Exercise-induced physiological adjustments to stressful conditions in sport horses. Livest Prod Sci 2005;92(2):101-11.
10. Westerblad H, Allen DG. Myoplasmic free Mg2+ concentration during repetitive stimulation of single fibresfrom mouse skeletal muscle. J Physiol 1992;453:413-34.
11. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev 2008;88(1):287-332.
12. Sejersted OM, Sjøgaard G. Dynamics and consequences of potassium shifts in skeletal muscle and heart during exercise. Physiol Rev 2000;80(4):1411-81.
13. Clausen T. Na-K pump regulation and skeletal muscle contractility. Physiol Rev 2003;83(4):1269-324.
14. Harris P, Snow DH. Plasma potassium and lactate concentrations in thoroughbred horses during exercise of varying intensity. Equine Vet J 1992;24(3):220-5.
15. Blazev R, Lamb GD. Adenosine inhibits depolarization-induced Ca(2+) release in mammalian skeletal muscle. Muscle Nerve 1999;22(12):1674-83.
16. Lamb GD, Stephenson DG, Stienen GJ. Effects of osmolality and ionic strength on the mechanism of Ca2+release in skinned skeletal muscle fibres of the toad. J Physiol 1993;464:629-48.
17. Westerblad H, Allen DG. Myoplasmic free Mg2+ concentration during repetitive stimulation of single fibres from mouse skeletal muscle. J Physiol 1992;453:413-34.
18. Avellini L, Chiaradia E, Gaiti A. Effect of exercise training, selenium and vitamin E on some free radical scavengers in horses (Equus caballus). Comp Biochem Physiol B Biochem Mol Biol 1999;123(2):147-54.
19. Sen CK. Oxidants and antioxidants in exercise. J Appl Physiol (1985) 1995;79(3):675-86.
20. Barja de Quiroga G. Brown fat thermogenesis and exercise: two examples of physiological oxidative stress? Free Radic Bio Med 1992;13(4):325-40.
21. Davies KJA, Quintanilha AT, Brooksand GA, Packer L. Free radicals and tissue damage produced by exercise. Biochem Bioph Res Commun 1982;107(4):1998-2005.
22. Phung CD, Ezieme JA, Turrens JF. Hydrogen peroxide metabolism in skeletal muscle mitochondria. Arch Biochem Biophys 1994;315(2):479-82.
23. Matsuki N, Tamura S, Ono K, Wtari T, Goitsuka R, Yamanobe A, et al. Exercise-induced phospholipid degradation in equine skeletal muscle and erythrocytes. J Vet Med Sci 1991;53(6):1001-7.

24. Valberg S, Häggendal J, Lindholm A. Blood chemistry and skeletal muscle metabolic responses to exercise in horses with recurrent exertional rhabdomyolysis. Equine Vet J 1993;25(1):17-22.

25. McKeever KH, Lehnhard RA. Physiology of acid-base balance and fluid shifts with exercise. Chapter 6. In: Hodgson DR, McKeever KH, McGowan CM eds. The Athletic Horse. 2nd ed. China: Elsevier Saunders; 2013. p.69-87.

26. Özaydın İ. [Disorders of fluid, electrolyte and acid-base balance]. Veteriner Acil Klinik: İlkyardım-Transport-İlk Müdahale. Erzurum: Eser Offset Matbaacılık; 2004. p.35-51.

27. Constable PD. A simplified strong ion model for acid-base equilibria: application to horse plasma. J Appl Physiol (1985) 1997;83(1):297-311.

28. Kingston JK, Bayly WM. Effect of exercise on acid-base status of horses. In: Hinchcliff KW, ed. Vet Clinics North America Equine Practice: Fluids, Electrolytes and Thermoregulation in Horses. 1st ed. Philadelphia: WB Saunders; 1998. p.61.

29. Lindinger MI, Waller A. Muscle and blood acid-base physiology during exercise and in response to training. Chapter 6. In: Hinchcliff K, Kaneps A, Geor R, eds. Equine Exercise Physiology. 1st ed. Philadelphia: Elsevier; 2008. p.350.

30. Bergero D, Assenza A, Caola G. Contribution to our knowledge of physiology and metabolism of endurance horses. Livest Prod Sci 2005;92(2):167-76.

31. McConaghy F. Thermoregulation. In: Hodgson DR, Rose RJ, eds. The Athletic Horse: Principles and Practice of Equine Sports Medicine. 1st ed. Philadelphia: W.B. Saunders; 1994. p.181-202.