

The effect of 60 km endurance exercise on serum electrolytes and acid–base balance in the Žemaitukai horses

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Abstract

The Lithuanian Žemaitukai horse breed is one of the oldest in Europe. Currently, there is a lack of information about the effect of endurance competition on blood indices and acid–base balance in the Žemaitukai horses. The aim of this study was to evaluate the effect of endurance exercise on the acid–base balance and electrolyte indicators of the Žemaitukai horses in an official endurance competition. In total, 48 horses of the Žemaitukai breed competed in endurance competitions over the same distance (60 km). Samples were taken before and immediately after the exercise. The following indicators were analysed: the hydrogen potential (pH), partial carbon dioxide pressure (pCO₂), partial oxygen pressure (pO₂), base excess in blood (BE), base excess in the extracellular fluid [BE (ecf)], HCO₃⁻ (bicarbonate), cSO₂ (oxygen saturation), tCO₂ (blood total carbon dioxide), haematocrit (HCT), haemoglobin concentration (cHgb), sodium (Na⁺), potassium (K⁺), calcium (Ca), chlorides (Cl), and lactate (Lac). Increase in pH showed adaptation of the horse metabolism to exercise. Blood changes were caused by the development of dehydration and metabolic alkalosis. According to Lac results, all horses were fit and tolerated well physical activity. The exercise did not significantly affect the demand for minerals. Based on the results obtained, it can be stated that horses of the Žemaitukai breed are suitable for endurance competing.

Equine metabolism, blood indices, competition

The Lithuanian Žemaitukai horse breed is one of the oldest in Europe. During the 19th–21st centuries the breed was on the verge of extinction four times. Now it is recognized as an internationally watched breed. The Žemaitukai is a multipurpose pony-type horse breed used for work, driving, tourism, children's pastime, hippotherapy, and sports. Their height at withers ranges from 128 to 142 cm, the chest girth from 154 to 187 cm, the cannon bone girth from 17 to 19 cm, and the weight from 360 to 420 kg (Macijauskienė and Jatkauskienė 2007).

Electrolytes play an important role in the capability of a competition horse. Dietary electrolytes, such as chloride, sodium, and potassium are needed for basal processes in the body, including maintaining osmotic pressure, movement of electrical impulses, and muscle contraction (Assenza et al. 2014). During exercise, water and electrolytes are excreted from the body through sweat, resulting in fatigue and muscle weakness (Walker and Collins 2017). Horses may develop exhaustion during prolonged exercise due to loss of water and electrolytes in sweat with hypovolaemia and electrolyte changes (Schott et al. 2006), heat accumulation and intramuscular glycogen depletion. A number of descriptive field studies of endurance competition have been conducted over the last decades (Robert et al. 2010).

Endurance horses are subjected to substantial modifications in their internal homeostasis due to prolonged exercise and water and electrolyte loss (Muñoz et al. 2010). Most breeds have been tested and used for endurance races; the most competitive are Arabian or Arabian crosses due to their muscle fibre composition, nevertheless, other breeds, including the Thoroughbred, Quarter Horses, Mustangs, Appaloosas, Morgans, Standardbred,

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and even Mules, have been used successfully (Duren 2000). Currently, there is a lack of information on the effect of endurance competition on the acid–base balance in the Lithuanian Žemaitukai horses. It has been hypothesized that horses in endurance exercise undergo significant changes in blood serum electrolytes and acid–base balance. Therefore, the aim of this study was to characterize and evaluate the effect of endurance exercise on the Žemaitukai horses as regards their acid–base balance and biochemical indicators in a 60 km endurance exercise competition.

Materials and Methods

Horses

The study was conducted on 48 male clinically healthy horses of the Žemaitukai breed. Clinical examination of the horses was conducted before and immediately after the competition (according to the FEI vet gate protocol) at three endurance competitions in Lithuania. All horses were 8–12 years old, with an average body weight of 420 ± 14 kg.

Experimental design

The horses competed in endurance competitions over the same distance (60 km) at an average ambient temperature of 19.2–20.9 °C and air humidity of 66–77%. The horses that completed the race with an average speed of 11.5 kph (± 1.5 SEM) on arrival were tested.

Blood samples were taken from each animal by applying the technique of jugular venipuncture into 1.6 ml heparinized vacutainer blood collection tubes (Terumo Europe, Belgium), 30 min before the start and no later than 30 min after the horse reached the finish. All of the horses showed no stress reaction during the blood sampling that was carried out in less than 30 s for each sample, excluding an excitement-induced spleen contraction (Satuė et al. 2012).

The samples were taken before and immediately after the exercise, then they were identified and stored in ice bath for a maximum period of 30 min until processing. With the use of the epic blood gas analysers (EPOC, Canada, Ottawa), the following indices were analysed: hydrogen potential (pH), partial carbon dioxide pressure ($p\text{CO}_2$), partial oxygen pressure ($p\text{O}_2$), base excess in blood (BE), base excess in the extracellular fluid [BE (ecf)], cHCO_3^- (bicarbonate), sO_2 (oxygen saturation), tCO_2 (blood total carbon dioxide), HCT (haematocrit), cHgb (haemoglobin concentration), Glu (glucose), Na (sodium), K (potassium), Ca (calcium), Cl (chloride), and Lac (lactate). The research was carried out in accordance with the provisions of the Law of the Republic of Lithuania No. 8-500 on Protection, Keeping and Use of Animals of 6 November 1997 (“Valstybės žinios” (official gazette) No. 108-6595 dated 28/11/1997), the Order No. 4-361 of 31 December 1998 of the State Veterinary Service of the Republic of Lithuania on Breeding, Care, Transportation of Laboratory Animals, and the Order No. 4 of 18 January 1999 of the State Veterinary Service of the Republic of Lithuania on the Use of Laboratory Animals for Scientific Tests. The study approval number was PK012868.

Data analysis and statistics

The data on horses were classified into two groups according to clinical examination (group BC – before competition, $n = 48$; group AC – after competition, $n = 48$).

The data on the tested horses were analysed using the IBM SPSS statistics Version 20.0 for Windows. The distributions of the clinical examination traits have been used to carry out the assessment according to the Kolmogorov-Smirnov test. The mean (M), the standard error of the mean (SEM) and the linear relationship between normal distribution indicators (Pearson correlation coefficient) were estimated. In order to compare the estimated indicators between the groups before and after the competition, one-factor analysis of variance (ANOVA) was applied. The differences between the means were estimated according to the Fisher’s LSD criterion. The results were considered to be significant at a P level < 0.05 .

Results

The average values of the horse blood gasometrical indicators after the competition demonstrated a significant decrease in $p\text{CO}_2$ ($P = 0.05$) and $p\text{O}_2$ ($P = 0.036$), whereas the value of BE (b) increased significantly ($P = 0.048$) compared to the values determined before the competition (Table 1). In this study, no significant alteration of cHCO_3^- , cSO_2 , cTCO_2 and BE (ecf) concentration caused by the competition was observed. A significant increase ($P < 0.05$) in HCT, cHgb, pH and Lac was observed in the horses. No significant difference was observed for the Glu concentration in their blood after the competition (Table 2). A decrease ($P < 0.05$) in blood electrolyte concentrations (Na^+ , K^+ , Ca^{++}) was observed after the competition.

Table 1. Indices of acid–base balance before (BC) and after (AC) competition.

Indices		M	SEM	P
pH	BC	7.48	0.007	0.001
	AC	7.53	0.013	
pCO ₂	BC	36.20	1.033	0.050
	AC	32.53	1.541	
pO ₂	BC	155.96	14.030	0.036
	AC	115.31	12.492	
cHCO ₃	BC	26.51	0.501	0.900
	AC	26.40	0.651	
cSO ₂	BC	98.03	0.579	0.057
	AC	94.86	1.512	
cTCO ₂	BC	27.62	0.525	0.797
	AC	27.39	0.691	
BE (efc)	BC	2.93	0.470	0.190
	AC	5.25	1.681	
BE (b)	BC	2.95	0.389	0.048
	AC	4.06	0.382	

pH - hydrogen potential, pCO₂ - partial dioxide the carbon pressure, pO₂ - partial oxygen pressure, BE (b) - base excess in blood, BE (efc) - base excess in the extracellular fluid, cHCO₃ - bicarbonate, sO₂ - oxygen saturation, tCO₂ - blood total dioxide the carbon

Table 2. Concentration of haematological, biochemical, and electrolyte indices before (BC) and after (AC) competition.

Indices		M	SEM	P
HCT	BC	34.38	0.914	0.003
	AC	38.08	0.756	
cHgb	BC	11.68	0.309	0.003
	AC	12.95	0.259	
Glu	BC	5.49	0.188	0.099
	AC	6.07	0.287	
Lac	BC	1.55	0.167	0.049
	AC	2.11	0.217	
Na ⁺	BC	139.29	0.397	0.013
	AC	137.46	0.593	
K ⁺	BC	3.64	0.102	0.017
	AC	3.27	0.109	
Ca ⁺⁺	BC	1.40	0.021	0.009
	AC	1.31	0.023	

HCT - haematocrit, cHgb - haemoglobin concentration, Na⁺ - sodium, K⁺ - potassium, Ca⁺⁺ - calcium, Lac - lactate

derangements seen in horses are due to the physiological process or due to administration of supplements by riders.

The first finding that can be drawn from this study is that the resting values of the indicators under investigation are within the range of normal values as reported by specialised literature on the horse (Piccione et al. 1999).

The results of the experiment showed that the blood indices most affected by the competition were the following (Fig. 1): BE (efc) (-79.18%), BE (b) (-37.63%), and Lac (-36.13%) According to the data of the correlation analysis, a positive relationship was estimated between all blood indices tested before and after the competition ($r = 0.061$; $P = -0.55$), except for cSO₂ ($r = -0.249$; $P = 0.05$). The highest ($P = 0.001$) positive paired Pearson's correlation coefficients were determined for blood Glu concentration ($r = 0.550$), HCT ($r = 0.490$) and cHCO₃ ($r = 0.481$).

Discussion

Following the current common practice in Lithuania, the horses were not administered any electrolyte supplementation during the rides or training. This may be controversial as electrolyte supplementation during endurance rides is advised by many authors; however, the study results show that the Žemaitukai horses can finish 60 km rides without electrolyte supplementation. Above all, the research allowed to evaluate how horses really cope with long distance race, whereas, in most field studies, authors are unable to obtain information about electrolyte administration (Fielding et al. 2009) and it is impossible to determine whether the electrolyte

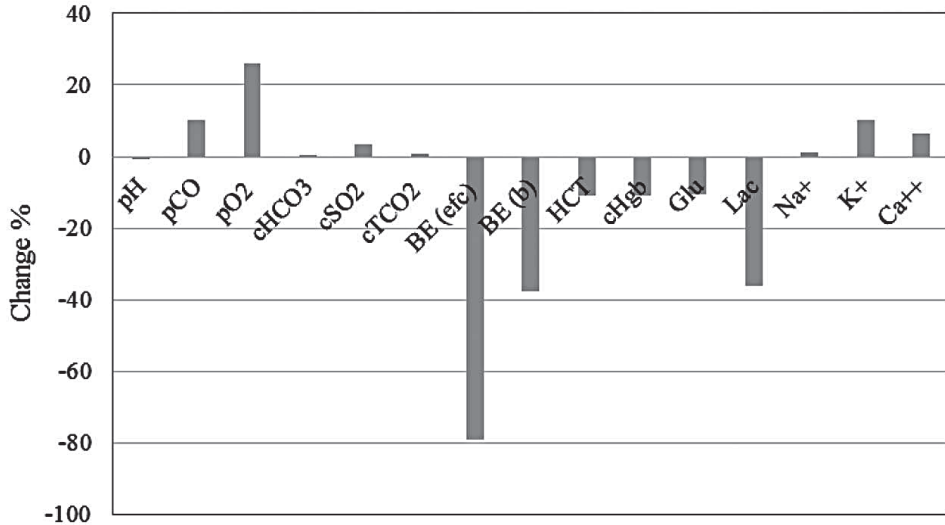


Fig. 1. Change (%) in blood indices after competition

pH - hydrogen potential, pCO₂ - partial carbon dioxide pressure, pO₂ - partial oxygen pressure, BE (b) - base excess in blood, BE (efc) - base excess in the extracellular fluid, cHCO₃ - bicarbonate, cSO₂ - oxygen saturation, tCO₂ - blood total dioxide the carbon, HCT - haematocrit, cHgb - haemoglobin concentration, Na⁺ - sodium, K⁺ - potassium, Ca⁺⁺ - calcium, Lac - lactate.

The analysis of pH, pCO₂ and HCO₃ in venous blood samples is a useful way to evaluate the acid–base balance of the body. A previous study reported a significant effect of training on pH, pO₂, and pCO₂ in Italian saddle horses, informing that physiological alterations in blood gas profile due to training exercise are common in horses (Casella et al. 2012).

A significant decrease in venous pCO₂ was reported after the exercise and after show jumping (Aguilera-Tejero et al. 2001; Piccione et al. 2004). The detected decrease could be due to hyperventilation, the primary physiological response to muscular exercise, the contemporary increase in pH supports this hypothesis. Training could affect the degree of hyperventilation.

The obtained results showed a significant increase in pH after the competition, reiterating the non-occurrence of metabolic acidosis, unlike the results found for high intensity exercises in thoroughbred horses undergoing supramaximal exercises (Bayly et al. 2006); in other words, the exercise intensity is probably related to the development of hypo- or hypercapnia. This finding could be due to the adaptation of the horse metabolism to physical exercise and energy production that occurred by means of aerobic pathways during the training. This exercise induced alkalosis in the horses as an effect of hyperventilation and increase in Hb as a result of mobilisation of the splenic reserve. Exercise is correlated with changes in the acid–base status as a consequence of muscular anaerobic metabolism.

Same as in human sports medicine, a strong relationship between exercise performance and lactate-related variations exists, so that the lactate response to supramaximal exercise is a sensitive indicator of adaptation to sprint training (Jacobs 1986). Beneke and von Duvillard (2003) demonstrated that, in different sport modalities, blood lactate concentration can vary depending upon the skeletal muscle mass recruited during exercise (Beneke and von Duvillard 2003). In the research, a significant variation during the rest and after exercise was observed. Aguilera-Tejero et al. (2001) found that intense exercise results in significant changes in the acid–base balance with a significant increase in lactate

concentrations after the exercise. These changes are caused by the release of muscular energy, mainly by anaerobic metabolism, and can result in metabolic acidosis, with lactate values highly increased at the end of the exercise. Additionally, leakage of lactate from muscle into blood is another possibility (Hyypä and Pösö 1998). Furthermore, a blood lactate concentration of 4 mmol/l or more is used to indicate that a horse is unfit during training protocols (Lindner 2010). Immediately after the study trial, there was a significant BE (ecf) and BE (b) increase that could be correlated to lactate excess with consequent inadequate buffering (Aguilera-Tejero et al. 2001).

Exercise increases the sympathetic activity in horses, thus increasing haematocrit values. The number of red blood cells released from the spleen in response to exercise is not “all-or-none,” but rather it is related to the extent of the increase in sympathetic activity that is related to exercise intensity (Steven et al. 2000). The degree of spleno-contraction depends on the oxygen required by the active muscles (Muñoz et al. 1999). There is fluid loss due to sweating, a shift of plasma water into the extracellular space due to the accumulation of osmotically active metabolites, and filtration as a consequence of an increased capillary hydrostatic pressure (Convertino 1987). Haematocrit and cHgb are influenced by both the intensity of exercise and the individual excitation caused by the competitive environment (Voss et al. 2002) and are considered good indices of cardiovascular and metabolic adjustments to physical effort (Padalino et al. 2005).

Decreases in plasma Na^+ , K^+ , and Ca^{++} are usually found at the end of the ride as compared to the pre-ride values (Barton et al. 2003). Exercise did not affect mineral requirements greatly, despite a possible increase in the requirements for minerals associated with the need of more energy to the muscles (K^+) and production of saliva and sweat (Na^+) (Pearson and Dijkman 1994). The authors associated this result with the loss of sodium from sweating. In addition to major sweat loss, substantial losses of electrolytes occur. Electrolytes are essential for controlling the membrane potential, muscle contraction, nerve conduction, and enzyme reactions, and they play a central role in the physiological process of exercise. The thermoregulatory system of the horse is capable of maintaining homeothermy during prolonged exercise when they lose substantial sweat in order to maintain heat loss. Heat is transferred from the body surfaces to the environment by conduction, radiation, and evaporation, with sweat evaporation being the main way of losing heat (McConaghy 1994). Besides the intensity of the exercise, the concentration of these ions in sweat depends on the environmental temperature and relative air humidity (Meyer et al. 2010). Sodium provides the greatest part of the osmotically active solute in the plasma and can influence the concentration of water in both intracellular and extracellular fluid compartments (Rose 1986).

The mild decrease observed in the Ca concentration in the blood samples of the tested horses could have been caused by their loss with the sweat during the competition. Calcium bonds with troponin molecules for actin-myosin activation, permitting efficient muscle contraction during exercise (Dumont et al. 2012). The reduction of Ca^{++} after exercise can also be related to complexation of these ions with organic and inorganic anions and linkage with albumin (Foreman et al. 2004). Decrease in Ca^{++} has been reported during a polo competition by Zobba (2011).

The natural diet of adult horse contains large quantities of potassium (Johnson et al. 1991). Potassium is the major intracellular cation and is important for osmosis and normal balance of water and cellular biochemical functions. The study data showed a decrease in serum K during the ride, remaining in the normal range. High electrolyte losses are usually observed in poorly trained horses which get tired easily (Kupczyński and Spitałniak 2015).

The present study aimed to describe the effect of endurance exercise on the Žemaitukai horses. An increase in pH shows the adaptation of the horse metabolism to physical

exercise and energy production. Major changes were the development of dehydration and metabolic alkalosis. According to Lac results before and after the race, all horses were fit and tolerated well the physical activity. The exercise did not significantly affect the demand for minerals. Based on the results obtained, it can be stated that horses of the Žemaitukai breed are suitable for endurance competing.

References

- Aguilera-Tejero E, Bas S, Estepa, JC, López I, Mayer-Valor R, Rodriguez M 2001: Acid–base balance after exercise in show jumpers. Conference on equine sports medicine and science (CESMAS), pp. 43-45
- Assenza A, Bergero D, Congiu F, Tosto F, Giannetto C, Piccione G 2014: Evaluation of serum electrolytes and blood lactate concentration during repeated maximal exercise in horse. *J Equine Vet Sci* **34**: 1175-1180
- Bayly WM, Kingston JK, Brown JA, Keegan RD, Greene SA, Sides RH 2006: Changes in arterial, mixed venous and intraerythrocytic concentrations of ions in supramaximally exercising horses. *Equine Vet J* **36**: 294-297
- Barton MH, Williamson L, Jacks S, Norton N 2003: Body weight, hematological findings, and serum and plasma biochemical findings of horses competing in a 48-, 83-, or 159-km endurance ride under similar terrain and weather conditions. *Am J Vet Res* **64**: 746-753
- Beneke R, von Duivillard S 1996: Determination of maximal lactate steady state response in selected sport events. *Med Sci Sports Exerc* **28**: 241-246
- Casella S, Alberghina D, Giannetto C, Piccione G 2012: Response to training and standardized exercise test in the athlete horse: changes in blood gas profile. *Comp Clin Pathol* **21**: 611-614
- Convertino VA 1987: Fluid shifts and hydration state: effects of long-term exercise. *Can J Sport Sci* **12**: 136S-139S
- Dumont CBS, Leite CR, Moraes JM, Moreira M, Moscardini ARC, Godoy RF, Lima EMM 2012: Osmolality, anion gap, percentage hydrogen (pH) and the measurable plasma ions in purebred Arabian horses which were finalists in 90 km endurance races. *Pesq Vet Bras* **32**: 542-546
- Duren SE 2000: Feeding the endurance horse. In: Pagan JD (Ed.): *Advances in Equine Nutrition*. Nottingham University Press, Nottingham, pp. 351-363
- Fielding CL, Magdesian, KG, Rhodes DM, Meier CA, Higgins JC 2009: Clinical and biochemical abnormalities in endurance horses eliminated from competition for medical complications and requiring emergency medical treatment: 30 cases (2005-2006). *J Vet Emerg Crit Care* **19**: 473-478
- Foreman JH, Waldsmith JK, Lalum RB 2004: Physical, acid–base and electrolyte changes in horses competing in training, preliminary and intermediate horse trials *Equine Comp Exerc Physiol* **1**: 99-105
- Hyypä S, Pösö AR 1998: Fluid, electrolyte, and acid–base responses to exercise in racehorses *Vet Clin North Ame Equine Pract* **14**: 121-136
- Jacobs I 1986: Blood lactate. Implications for training and sports performance. *Sports Med* **3**: 10-25
- Johnson PJ, Goetz TE, Foreman JH 1991: Effect of whole-body gluteal muscle potassium concentration of healthy, adult horses. *Am J Vet Res* **52**: 1676-1683
- Kupczyński R, Spitalniak K 2015: Analysis of acid–base balance as well as hematological and biochemical parameters in horses of combined driving discipline. *Arch Anim Breed* **58**: 221-228
- Lindner AE 2010: Maximal lactate steady state during exercise in blood of horses *J Anim Sci* **88**: 2038-2044
- Macijauskienė V, Jatkauskienė V 2007: Effect of blood infusion from other breeds on the phenotype and genotype of the žemaitukai horse breed. Proceedings of the 13th Baltic Animal Breeding Conference. Tartu, pp. 113-117
- McConaghy F 1994: Thermoregulation. In: Hodgson DR, Rose RJ (Eds): *The athletic horse: principles and practice of equine sports medicine*. W.B. Saunders, Philadelphia. 497 p.
- Meyer ND, Bayly WM, Sides RH, Wardrop KJ, Slinker BK 2010: Changes in arterial, mixed venous and intraerythrocytic ion concentrations during prolonged exercise *Equine Vet J* **42**: 185-190
- Muñoz A, Riber C, Trigo P, Castejon-Riber C, Castejon FM 2010: Dehydration, electrolyte imbalances andrenin-angiotensin-aldosterone-vasopressin axis in successful and unsuccessful endurance horses. *Equine Vet J* **38**: 83-90
- Muñoz A, Riber, Santisteban R, Rubio MD, Agüera EI 1999: Cardiovascular and metabolic adaptations in horses competing in cross-country events. *J Vet Med Sci* **61**: 13–20
- Padalino B, Frate A, Siniscalchi M, Quaranta A 2005: Valuation of condition athletics preparation of standardbred trotting on straight race course through determination of lactate, haematocrit and some physiological parameters. *Ippologia* **16**: 31-33
- Pearson A, Dijkman JT 1994: Nutritional implications of work in draught animals. *Proc Nutr Soc* **53**: 169-179
- Piccione G, Fazio F, Caola G 1999: Cronoperformance nel cavallo saltatore: andamento circatrigintano di alcuni parametri emogasanalitici. *Atti So Fi Vet* **3**: 44-48
- Piccione G, Ferrantelli V, Fazio F, Percipalle M, Caola G 2004: Blood–gas profile in the show jumper undergoing increasing workloads during a 2-day event. *Comp Clin Pathol* **13**: 43-50
- Robert AG, Goachet A, Fraipont DM, Votion E, Van Erck JL 2010: Hydration and electrolyte balance in horses during an endurance season. *Equine Vet J* **42**: 98-104
- Rose RJ 1986: Endurance exercise in the horse: a review. *Br Vet J* **142**: 531-552

- Satue K, Hernandez A, Muñoz A, Charles Lawrie 2012: Physiological factors in the interpretation of equine hematological profile. In: Hematology - Science and Practice Intech, Rijeka, Croatia, pp. 573-596
- Schott H, Marlin DJ, Geor RJ, Holbrook TC, Deaton CM, Vincent T, Dacre K, Schroter RC, Jose-Cunilleras E, Cornelisse CJ 2006: Changes in selected physiological and laboratory measurements in elite horses competing in a 160 km endurance ride. *Equine Vet J* **36**: 37-42
- Valstybės žinios 1997: Naminių gyvulių laikymo reikalavimai (Official requirements for keeping animals) No. 108-6595
- Voss B, Mohr E, Krzywanek H 2002: Effects of aqua-treadmill exercise on selected blood parameters and on heart-rate variability of horses. *J Vet Med* **49**: 137-143
- Walker E, Collins S 2017: The effect of exercise intensity and use of an electrolyte supplement on plasma electrolyte concentrations in the Standardbred horse. *Can J Anim Sci* **97**: 668-672
- Wickler SJ, Anderson PT 2000: Hematological changes and athletic performance in horses in response to high altitude (3,800 m). *Am J Physiol Regulatory Integrative Comp Physiol* **279**: R1176–R1181
- Zobba, R, Ardu M, Niccolini S, Cubeddu F, Dimauro C, Bonelli P, Dip CD, Visco S, Parpaglia ML 2011: Physical, hematological, and biochemical responses to acute intense exercise in polo horses *J Equine Vet Sci* **31**: 542-548