

A comparison of haematological and biochemical blood indices between the Žemaitukai and Arabian horses participating in endurance competitions

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Abstract

The study was conducted on 30 clinically healthy Arabian horses and 28 Žemaitukai horses that competed in endurance race over the same distance (40 km). Blood samples were taken before and immediately after the exercise. The environmental conditions during the competitions varied, with a mean temperature of 22.5 °C and a mean relative humidity of 73.20%. The Žemaitukai horses showed lower haematological values and increased muscle catabolism after exercise. Arabian horses have higher aerobic capacity compared to the Žemaitukai horses which probably contributes to their superior low- to moderate-intensity exercise performance. Serum activity of muscle enzymes suggested that the muscle tissue of the Arabian horses has higher tolerance for exercise-induced muscle catabolism and lower muscle catabolism than that of muscle tissue of the Žemaitukai horses. Further studies need to be carried out to identify the differences in muscle tissue of both breeds.

Equine breeds, aerobic capacity, horse racing

It is acknowledged that certain horse breeds tend to dominate in certain types of competitions, although some of this dominance may actually reflect the history and development of that particular sporting event rather than the inability of other horse breeds to undertake this type of activity. It is also true that while certain horse breeds are considered to be well-suited for certain activities, this does not exclude their use in others (Prince et al. 2002).

The Žemaitukai is an ancient indigenous Lithuanian horse breed, known since the 6th–7th centuries. The Žemaitukai became particularly well-recognised in the 14th century as an excellent military horse breed used in the Lithuanian-Crusader battles. Later, the Žemaitukai became a utility horse breed. Back in the 18th century, the Žemaitukai mares contributed towards the formation of the Trakehner breed, and in the 20th century, the Žemaitukai breed became the foundation for the breeding of the Lithuanian Heavy Drought and large-type Žemaitukai. Recently, in consequence of unfavourable historical and economical circumstances, the numbers of the Žemaitukai have strongly declined (Juras et al. 2001). Arabian blood was added during the 19th century, giving the horse an Arab-type head, including the characteristic dish-shaped Arabian profile. The infusion of Arabian blood created two subtypes of the Žemaitukai: those with Arabian ancestry were considered suitable for riding, whereas the others, more closely related to the indigenous horses, were better adapted to farm work (Hendricks and Dent 1995).

The Arabian horse and Arabian crosses are the predominant breeds used for endurance riding. This distinction is probably based on the muscle fibre composition and preferential ability to utilize lipids during sub-maximal exercise (Wickler and Foss 2004). The Arab-based horses possess a flexible gene pool. Selection of genes favourable for particular environmental conditions under particular environmental pressures will produce the necessary physiological adaptations for a successful performance (Sneddon 1993).

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In Lithuania, the Arabian horses are mainly used in endurance sports, however, the Žemaitukai are becoming more popular in this area due to the uniqueness, availability, and importance of this breed in Lithuania.

Metabolic responses to exercise differ between the horse breeds, although changes in plasma enzymes have not been reported, and the most useful information is obtained from animals subjected to different training programmes (Muñoz et al. 2002). Long-term physical exertion may result in disturbances of homeostasis, such as energy depletion and changes in fluids, electrolytes and acid-base balance, with negative consequences for the horse's performance and health, which may lead to significant changes in various haematological and biochemical blood indices (Fielding et al. 2009). Hence, blood indices not only provide information on the animal's health, but can also serve as indicators of the physical condition of sport horses, enabling individual tailoring of training regimes (Padalino et al. 2007). There are few reports in the literature documenting physiological data for this breed (Mikniene et al. 2014).

Haematology and plasma or serum biochemistry are important tools for assessing the health of athletic horses (Kingston 2004). The aim of this study was to analyze selected haematological and biochemical analytes in the blood of clinically healthy Arabian and Žemaitukai horses, and to determine the magnitude of changes in blood indices as well as to evaluate the effect of the breed on the changes in these blood indices before and after endurance races.

Materials and Methods

Horses

The study was conducted on 30 (13 females, 17 males) clinically healthy Arabian horses (weight 463 ± 33 kg) and 28 (12 females, 16 males) Žemaitukai horses (weight 410 ± 33 kg), of a mean age of 8–12 years, were used. Riders were asked to complete a pre-competition survey that detailed nutritional management, training regime, performance and medical history. These surveys were used to select horses kept, trained and fed under similar conditions. All studied animals were in good health condition and no major problems were identified during the physical examination at rest before the competitions according to the International Federation for Equestrian Sports (FEI) vet gate protocol. These animals had a broad range of previous competitive experience and were subjected to different diets during training and competition. Appropriate consent of the horse owner or rider was obtained prior to the study.

Experimental design

The research was carried out in accordance with the provisions of the Law of the Republic of Lithuania No VIII-500 on the Welfare and Protection of Animals of 6 November 1997 (The Official Gazette No 108-6595, November 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 Food and Veterinary Service of the Republic of Lithuania on the Use of Laboratory Animals for Scientific Tests. The study approval number was PK012868.

All horses competed in endurance races of the same distance (40 km). Blood samples were collected from each animal, by means of jugular venipuncture, into 1.6 ml vacutainer blood collection tubes with (for blood morphology) and without (used for blood biochemistry) EDTA (Terumo Europe, Leuven, Belgium), 30 min before the start and no later than 30 min after the sampled horse would reach the finish.

The investigated races were held in Lithuania, in accordance with the FEI rules. The competitions were carried out on a variable terrain with some muddy and some firm places and slight elevation changes (± 300 m). The environmental conditions during the competitions varied, with a mean temperature of 22.5 °C (withing the range of 12.50 – 26.5 °C) and a mean relative humidity of 73.20% (withing the range of 52 – 96%). The maximum permissible horse heart rate for the competitions was 56 and 64 beats/min.

Haematological analyses

All blood samples were stored at 18 °C and haematological testing was performed within 5 h after the collection of each sample. Haematological indices were analysed using an automatic cell counter, the Abacus Junior Vet Haematology Analyser (Diatron Messtechnik GmbH, Wien, Austria, 2006). The samples included white blood cell counts (WBC, $\times 10^3/\mu\text{l}$), lymphocytes (LYM, $\times 10^3/\mu\text{l}$), granulocytes (GRA, $\times 10^3/\mu\text{l}$), red blood cells (RBC, $\times 10^6/\mu\text{l}$), platelet count (PLT, $\times 10^4/\mu\text{l}$), haematocrit (HCT, %), mean corpuscular volume (MCV, fl), haemoglobin concentration (HGB g/dl), mean corpuscular haemoglobin (MCH, pg), mean corpuscular

haemoglobin concentration (MCHC, g/dl), mean platelet volume (MPV, fl), red cell distribution width (RDW, %) and platelet distribution width (PDW).

Serum urea (Urea, mmol/l), glucose (Glu, mmol/l), aspartate aminotransferase (AST, U/l), ferum (Fe, µg dl), creatinine (CREA, µmol/l), calcium (Ca, mmol/l), magnesium (Mg, mmol/l), phosphorus (P, mmol/l), total protein (TP, µmol/l), total bilirubin (TB, µmol/l), albumin (ALB, g/l), cuprum (Cu, µg/dl), zinc (Zn, µg/dl), gamma-glutamyl transferase (GGT, U/l) were measured using an automated analyser Hitachi 705 (Hitachi, Tokyo, Japan).

Statistical analysis

The statistical analysis of biochemical blood indices was carried out using the SPSS 25.0 software (SPSS Inc, Chicago, IL, USA). Having applied the method of descriptive statistics, normal distributions for all blood indices were assessed using Kolmogorov-Smirnov test. The results of the statistical analysis were expressed as the mean \pm standard error. The outcomes of the comparison of the mean figures were assessed using statistical hypothesis test which is sometimes known as Student's *t*-test. The linear relationship between the biochemical blood indices were evaluated using Pearson's correlation. Probability of < 0.05 was considered as being significant.

Results

The impact of breed on morphological blood indices during endurance competitions

The study results are presented in Table 1. The analysis of blood morphological indices before endurance competitions revealed that the values of MCV, MCH, MCHC, RDW, PLT, and PDW in horses were significantly different in both groups and depended on the breed ($P < 0.01$). After endurance competitions, the breed showed a significant ($P < 0.01$) effect on a higher number of blood morphological indices – WBC, GRA, LYM%, GRA%, RBC, HGB, MCV, MCH, MCHC, and MPV. A significantly lower level of MCHC (2.82%; $P = 0.014$), RBC (7.60 %; $P = 0.022$), and PDW (17.12%; $P = 0.021$) before endurance competitions was found in the Žemaitukai horses compared to the same values of the Arabian breed. A significantly lower level of blood RDW (4.06%; $P = 0.034$), MCH (5.47%; $P = 0.003$), MCV (8.12%; $P = 0.001$), and MPV (9.10%; $P = 0.001$) before endurance competitions was observed in the Arabian horses, compared to the same values of the Žemaitukai breed.

The same tendency for the differences in blood morphological parameters of the studied breeds according to RBC variables (the difference was 13.17%; $P = 0.028$), MCV (10.15%; $P = 0.001$), MCH (6.30%; $P = 0.032$), MCHC (3.38%; $P = 0.003$), and MPV (18.63%; $P = 0.003$) after endurance competitions was observed. For both breeds, the values of blood GRA and GRA% (19.17–36.55%; $P = 0.001$), HGB (13.88–24.36%; $P = 0.001$), HCT (16.57–20.63%; $P = 0.001$ –0.05), MCHC (2.38–2.98%; $P = 0.008$ –0.05) significantly increased, and the values of LYM (27.85–42.56%; $P = 0.001$ –0.006), and RDW (5.92–8.44%; $P = 0.007$ –0.008) significantly decreased.

The analysis of correlations (Fig. 2) between the evaluated blood morphological indices before and after endurance competitions showed the same tendency of change. The highest positive correlation coefficients between both breeds were found for MON, MCV, MCH, RDV, and MPV (in the range of 0.729–0.962; $P < 0.01$), the lowest positive correlation coefficients between both breeds were found for the values of PCT in the Arabian breed ($r = 0.112$).

The effect of breed on biochemical blood indices during endurance races

The study revealed differences in biochemical blood indices before and after endurance competitions. The study results (Table 2) showed a significant increase in CREA (1.22–1.23 \times , $P = 0.001$ –0.05) and TB (1.27–1.79 \times , $P = 0.001$ –0.008) in both breeds of horses after endurance races. Compared to the Arabian breed, in the Lithuanian Žemaitukai horses significantly lower levels of CREA ($P = 0.001$), Ca ($P = 0.001$ –0.002), TB ($P = 0.001$ –0.006), ALB ($P = 0.001$ –0.013) before and after endurance competitions was observed. Compared to the Žemaitukai breed, in the Arabian horses we observed

Table 1. Morphological blood indices before and after endurance competitions.

Indicator	Breed	Before endurance competitions		After endurance competitions		Evaluation of difference before and after endurance competitions
		mean \pm SE	<i>P</i>	mean \pm SE	<i>P</i>	<i>P</i>
WBC $\times 10^3/\mu\text{l}$	A	10.93 \pm 0.56	n.s	11.29 \pm 0.45	0.050	0.005
	Z	9.76 \pm 0.52		10.00 \pm 0.46		n.s
LYM $\times 10^4/\mu\text{l}$	A	4.43 \pm 0.42	n.s	2.54 \pm 0.23	n.s	0.001
	Z	4.05 \pm 0.39		2.92 \pm 0.23		0.006
MON	A	0.31 \pm 0.04	n.s	0.32 \pm 0.03	n.s	n.s
	Z	0.32 \pm 0.03		0.32 \pm 0.03		0.001
GRA $\times 10^4/\mu\text{l}$	A	6.19 \pm 0.32	n.s	8.45 \pm 0.42	0.006	0.001
	Z	5.42 \pm 0.30		6.72 \pm 0.43		0.001
LYM%	A	40.29 \pm 2.42	n.s	22.64 \pm 1.82	0.027	0.001
	Z	39.94 \pm 2.27		28.66 \pm 1.87		n.s
MON%	A	2.96 \pm 0.29	n.s	3.02 \pm 0.25	n.s	n.s
	Z	3.21 \pm 0.27		3.20 \pm 0.25		0.001
GRA%	A	56.75 \pm 2.47	n.s	74.00 \pm 1.83	0.022	0.001
	Z	56.85 \pm 2.32		67.75 \pm 1.87		0.001
RBC $\times 10^6/\mu\text{l}$	A	6.42 \pm 0.15	0.022	8.25 \pm 0.33	0.028	0.001
	Z	5.93 \pm 0.14		7.17 \pm 0.34		0.050
HGB g/dl	A	114.07 \pm 2.64	n.s	141.86 \pm 3.33	0.002	0.001
	Z	110.65 \pm 2.48		126.00 \pm 3.42		0.001
HCT %	A	0.37 \pm 0.01	n.s	0.44 \pm 0.02	n.s	0.001
	Z	0.37 \pm 0.01		0.43 \pm 0.02		0.05
MCV fl	A	57.21 \pm 0.79	0.001	54.25 \pm 1.03	0.001	n.s
	Z	61.86 \pm 0.75		59.76 \pm 1.06		n.s
MCH pg	A	17.78 \pm 0.22	0.003	17.35 \pm 0.34	0.032	0.05
	Z	18.75 \pm 0.21		18.45 \pm 0.35		n.s
MCHC g/dl	A	311.07 \pm 2.46	0.014	320.33 \pm 2.40	0.003	0.008
	Z	302.29 \pm 2.31		309.50 \pm 2.46		0.05
RDW %	A	17.25 \pm 0.23	0.034	15.79 \pm 0.43	n.s	0.007
	Z	17.95 \pm 0.22		16.89 \pm 0.44		0.008
PLT $\times 10^4/\mu\text{l}$	A	94.93 \pm 8.82	n.s	177.14 \pm 33.21	n.s	n.s
	Z	92.71 \pm 8.28		130.00 \pm 34.03		n.s
MPV fl	A	8.97 \pm 0.16	0.001	8.00 \pm 0.33	0.003	n.s
	Z	9.78 \pm 0.15		9.49 \pm 0.34		n.s
PCV	A	0.08 \pm 0.01	n.s	0.49 \pm 0.27	n.s	n.s
	Z	0.09 \pm 0.01		0.10 \pm 0.27		n.s
PDW	A	38.67 \pm 1.98	0.021	33.21 \pm 3.17	n.s	0.002
	Z	32.05 \pm 1.86		30.02 \pm 3.24		n.s

WBC - white blood cell counts; LYM - lymphocytes; MON - monocytes; GRA - granulocytes; RBC - red blood cells; PLT - platelet count; HCT - haematocrit; MCV mean corpuscular volume; HGB - haemoglobin concentration; MCH - mean corpuscular haemoglobin; MCHC - mean corpuscular haemoglobin concentration; MPV - mean platelet volume; PCV - packed cell volume; RDW - red cell distribution width; PDW - platelet distribution width

Table 2. Biochemical blood indices before and after endurance competitions.

Indicator	Breed	Before endurance competitions			After endurance competitions			Change (before – after endurance competitions)	
		mean ± SE	Difference (%) between breeds (A-Z)	<i>P</i>	mean ± SE	Difference (%) between breeds (A-Z)	<i>P</i>	%	<i>P</i>
Urea	Arabian	6.36 ± 0.25			7.48 ± 0.35			-17.56	0.001
mmol/l	Žemaitukai	7.19 ± 0.26	-13.00	0.024	7.74 ± 0.36	-3.54	n.s.	-7.73	n.s.
Glu	Arabian	2.40 ± 0.23			2.41 ± 0.27			-0.29	n.s.
mmol/l	Žemaitukai	2.67 ± 0.24	-11.42	n.s.	3.14 ± 0.28	-30.64	n.s.	-17.59	n.s.
AST (GOT)	Arabian	306.88 ± 16.42			343.54 ± 16.44			-11.95	0.046
U/l	Žemaitukai	403.57 ± 17.02	-31.51	0.001	406.62 ± 17.03	-18.36	0.010	-0.76	n.s.
Fe	Arabian	163.60 ± 6.69			175.60 ± 7.45			-7.34	0.050
µg/dl	Žemaitukai	148.59 ± 6.94	9.17	n.s.	151.41 ± 7.72	13.78	0.028	-1.89	n.s.
CREA	Arabian	134.34 ± 3.59			164.97 ± 3.95			-22.79	0.001
µmol/l	Žemaitukai	112.19 ± 3.72	16.49	0.001	136.59 ± 4.10	17.20	0.001	-21.76	0.050
Ca	Arabian	3.28 ± 0.05			3.35 ± 0.05			-2.11	n.s.
mmol/l	Žemaitukai	3.05 ± 0.05	7.19	0.002	3.03 ± 0.05	9.77	0.001	0.73	n.s.
Mg	Arabian	0.70 ± 0.04			1.02 ± 0.23			-46.03	n.s.
mmol/l	Žemaitukai	0.73 ± 0.04	-5.33	n.s.	0.73 ± 0.24	27.73	n.s.	-0.20	n.s.
P	Arabian	13.99 ± 5.88			14.68 ± 6.00			-4.94	0.050
mmol/l	Žemaitukai	20.96 ± 6.09	-49.88	n.s.	21.09 ± 6.21	-43.73	n.s.	-0.63	n.s.
TP	Arabian	75.48 ± 1.21			81.46 ± 1.47			-7.91	0.001
µmol/l	Žemaitukai	77.41 ± 1.26	-2.55	n.s.	77.97 ± 1.52	4.28	n.s.	-0.72	n.s.
TB (bilirub)	Arabian	26.66 ± 2.04			47.73 ± 3.02			-79.05	0.001
µmol/l 1	Žemaitukai	18.31 ± 2.12	31.30	0.006	23.20 ± 3.13	51.39	0.001	-26.69	0.008
ALB	Arabian	40.38 ± 0.64			42.59 ± 0.60			-5.47	0.001
g/l	Žemaitukai	38.00 ± 0.67	5.88	0.013	38.52 ± 0.62	9.55	0.001	-1.36	n.s.
Cu	Arabian	69.43 ± 4.09			72.63 ± 4.66			-4.60	n.s.
µg/dl	Žemaitukai	75.93 ± 4.24	-9.36	n.s.	74.56 ± 4.83	-2.65	n.s.	1.81	n.s.
Zn	Arabian	177.36 ± 3.51			176.97 ± 2.45			0.22	n.s.
µg/dl	Žemaitukai	183.19 ± 3.64	-3.29	n.s.	175.76 ± 2.54	0.68	n.s.	4.06	0.003
GGT	Arabian	15.31 ± 2.66			15.53 ± 2.77			-1.46	n.s.
U/l	Žemaitukai	29.56 ± 2.76	-93.11	0.001	28.30 ± 2.87	-82.22	0.002	4.26	n.s.

Urea - serum urea; Glu - glucose; AST - aspartate aminotransferase; Fe - ferum; CREA – creatinine; Ca - calcium; Mg - magnesium; P - phosphorus; TP - total protein; TB - total bilirubin; ALB - albumin; Cu - cuprum; Zn - zinc; GGT - gamma-glutamyl transferase

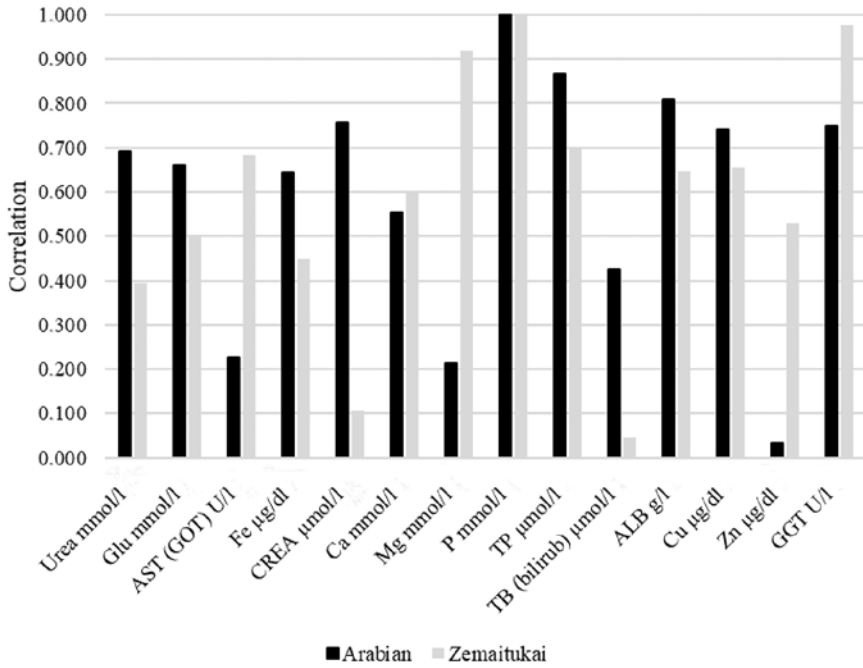


Fig 1. Correlations between blood indicator values before and after endurance competitions.

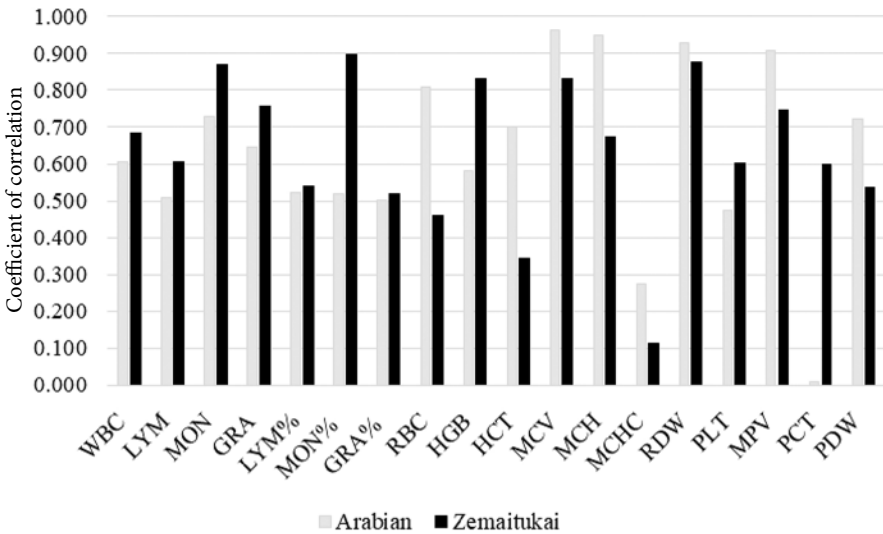


Fig. 2. Correlations between blood morphological indices before and after endurance competitions

a significantly lower level of blood urea ($P = 0.024$), AST (GOT) ($P = 0.001$), and GGT ($P = 0.001$) before endurance competitions, and lower AST (GOT) ($P = 0.01$) and GGT ($P = 0.002$) after endurance competitions. A significant increase in blood urea ($P = 0.001$), AST (GOT) ($P = 0.046$), Fe ($P = 0.050$), CREA ($P = 0.001$), P ($P = 0.050$), TB ($P = 0.001$), and ALB ($P = 0.001$) of the Arabian breed was observed between the beginning and end of endurance competitions. An increase in the values of CREA ($P = 0.050$), TB ($P = 0.008$) and Zn ($P = 0.003$) in the end of endurance competitions was observed in the Žemaitukai breed.

The analysis of correlations showed a high relationship (in the range of 0.647–0.999; $P < 0.01$) in P, Cu, TP, ALB, and GGT values in both breeds before and after endurance competitions. For the Arabian breed, significant correlations before and after endurance competitions were established with the values of blood urea, Glu, Fe, Crea, Ca (in the range of 0.554–0.752; $P < 0.01$), and TB ($r = 0.423$; $P < 0.05$), and for the Žemaitukai breed – with those of Glu, AST, Ca, Mg, Zn (in the range of 0.498–0.918; $P < 0.01$) and between urea and Fe (in the range of 0.395–0.450, $P < 0.05$). The correlations of the blood test results by breed are shown in Fig. 1. Correlation analysis of blood indicators before and after endurance competitions revealed a higher relationship in blood urea, Glu, Fe, Crea, TP, ALB, and Ca in the Arabian breed, compared to those in the Žemaitukai horses. On the other hand, a higher relationship in blood Ca, Mg, Zn, AST, and GGT values was estimated for the Žemaitukai breed before and after endurance competitions.

Discussion

The main limiting factor in this study was the lack of information in the reference sources available on this topic, which encumbered the comparison of the results obtained in the study with those reported by other authors, as well as the explanation of some of the differences observed.

It was found that for both studied breeds the values of blood GRA, HGB, HCT, MCHC, CREA, and TB significantly increased and the values of LYM and RDW decreased after endurance competitions. According to the literature, in more prolonged exercises, splenic contraction due to an adrenergic stimulus and sweating leads to extensive body fluid losses. Thermoregulation leads to intense sweating with evident body fluids losses and haemoconcentration (Snow et al. 1992; Waller et al. 2009). Over 5% body weight loss occurs in horses after a 30 km ride and this may cause a change in certain blood indices (Lawan et al. 2012). Endurance horses are able to perform prolonged submaximal exercise by increasing their metabolic rate 10- to 20-fold, with the production of major amounts of energy. Approximately 70–80% of this energy is eliminated in the form of heat to avoid substantial core body temperature increases. The main mechanisms of thermolysis in the horse are sweat evaporation from the skin (65%) and respiratory evaporation (25%) (Muñoz et al. 2010), with sweating rates of 10–15 l/h in hot environmental conditions. As a result of sweating, water is lost mainly from the extracellular fluid, resulting in decreases in blood and plasma volumes (Larsson et al. 2013). Up to 50% of the blood cells are stored in the spleen at rest (Fan et al. 2002). Exercise induces the production of epinephrine, which causes spleen contraction and consequent release of RBC and PLT in circulating blood (auto transfusion) (McKeever 2004) resulting in a significant increment of these indices (Golland et al. 2003). These adaptations induce a great augmentation of the oxygen carrying capacity of blood and of the aerobic capacity (Kearns et al. 2002).

The results of this study revealed that both before and after endurance competitions the RBC count was significantly lower in the Lithuanian Žemaitukai horse breed. The differences in haematological values among breeds are reported in literature, emphasizing erythrocyte value differences between Thoroughbreds and Coldbloods (Morris 2000).

Blood indices of the Žemaitukai horses and the Arabian horses can be classified as those of the “warm-blooded” horse blood type (Mikniene et al. 2014). Studies with the Žemaitukai horses showed higher haematological values (Mikniene et al. 2014) compared to this study, suggesting that the specific endurance training process generated physiological changes. In Standardbred horses studied in Australia, there was actually a significant reduction in the measured red blood cell volume and maximal packed cell volume (PCV) in over-trained horses compared to the controls (Golland et al. 2003). The reduction of the RBC volume in these over-trained horses was only weakly reflected as small but non-significant decreases in PCV, RBC number and Hb compared to the controls (Tyler-McGowan et al. 1999).

The increase in RBC, HB, and PCV varies with the relative exercise intensity that determines the degree of splenocontraction, which in turn depends on the need for oxygen in the active muscles (Muñoz et al. 1999a). Additionally, exercise induces alterations in blood water content, because of changes in blood pressure, with intercompartmental shift of fluids between the vascular and extracellular compartments and/or loss of water and electrolytes from the evaporation of sweat to control the core temperature. Therefore, the modifications in blood water content also determine the degree of increased RBC, HGB, and PCV during exercise (Muñoz et al. 1999b). Having taken into account the importance of increased HGB in the oxygen transport capacity, it is plausible to consider that increased PCV, HGB, and RBC lead to higher aerobic capacity and therefore, exercise performance (Muñoz et al. 1997).

According to the literature (Masini et al. 2000) no decrease of erythrocytes was observed during exercise tests, only an increase as measured by the MCV. Evidence of exercise-induced variations in the MCV suggested that RBC volume is altered by splenic contraction (Hanzawa et al. 1995). The research on the effect of excitement on the RBC values before endurance competitions compared to the resting and pre-racing values, showed a highly significant increase in PCV, HGB, and RBC count, but no difference in the MCV (Masini et al. 2000). In another study, the evaluation of erythrocyte indices after spleen emptying induced by either exercise or adrenaline injection showed no significant change in the MCV after adrenaline injection, whereas the MCV after exercise was significantly higher than basal value (Snow and Martin 1990). Some authors have stated that endurance training causes a decrease of the MCV (Santhiago et al. 2009; Rietjens et al, 2005) whereas high-intensity training results in an increase of the MCV (Santos 2019).

Breeds that are ancestrally closer have minor differences in HGB, MCH, and MCHC (Chikhaoui et al. 2018). An increase in the MCHC during the competition season was observed in our previous study (Poškienė et al. 2018) and it was explained by the cell swelling phenomenon. The RBC appear to act as a buffer against any exercise-related increase in plasma potassium and any decrease in blood pH since the uptake of potassium into the cell and the exchange of bicarbonate with chloride may be beneficial to performance but can lead to erythrocyte swelling (Masini et al. 2000). The RBC swelling also reduces the surface-to-volume ratio, thereby decreasing cell deformability, which is expected to increase capillary wall shear stress, possibly contributing to that capillary rupture that is associated with exercise-induced pulmonary haemorrhage (Morán and Folch 2013).

The increase in the RDW values observed in the course of the study indicates that, since an increase in MCV was observed, there are erythrocytes in the bloodstream characterised by different volumes, and that a change in MCV is a phenomenon that does not occur homogeneously.

In this study, the significantly higher CREA and AST activity found in the Žemaitukai horses resulted in a considerably higher reference interval than that in the Arabian horses.

It was found that muscle catabolism in the Žemaitukai horses increased after exercise. According to the literature, urea is the ultimate catabolite of endogenous protein breakdown,

while creatinine is the final catabolite of muscular robust metabolism (Carlotti et al. 2008). In both breeds, urea was not induced by exercise, although in the Žemaitukai horses creatinine levels were higher and they also increased after exercise, leading to significant decreases in the CREA ratio. Abnormal fluid balance associated with sweating and dehydration is commonly reported during endurance rides (Piccione et al. 2007) and intercompartmental fluid shifts (McGowan 2008) result in the decrease in plasma volume and an increase in TP, ALB, urea, and CREA concentrations (Robert et al. 2010).

The study revealed that AST values increased immediately after exercise in both breeds. Compared to the Arabian breed, the AST activity was higher in the Žemaitukai horses before the races and AST levels also increased more in the Žemaitukai breed after the exercise. Short-term changes in the CREA and AST levels might be caused by great effort, and sometimes may be the first symptom of over-training. Under normal conditions, serum AST activity in horses should not exceed 300 U/l (Szarska 2002). Elevated activity of AST may also (in addition to skeletal muscle overload) result from cardiac overload and liver dysfunction. The Australian study (McGowan and Whitworth 2008) revealed that serum activity of the muscle enzymes was significantly elevated in over-trained horses, and the New Zealand study (Hamlin et al. 2002) revealed that it was also elevated at the beginning of the intensified training period. The increase in serum AST concentration was interpreted as caused by subclinical muscle damage, and may be representative of delayed onset muscle soreness (DOMS) or low-grade muscle tear. The equine rhabdomyolysis syndrome or 'tying-up' can also result in increased serum AST (McGowan and Whitworth 2008). According to Trigo et al. (2010), Vergara and Tadich (2015), and Padalino et al. (2007), over-training leading to skeletal muscle damage may also be evidenced by an increase in AST and LDH activity and the decrease of bilirubin secretion.

By analysing the concentration of bilirubin, a significant increase in the concentration of this substance was observed in both groups after the race. Bilirubin at low concentrations according to the reference values has potent antioxidant properties, as it is involved in the removal of free radicals. It seems that an increase in the total bilirubin concentration was probably caused by a reaction to increased production of free radicals caused by stress and physical activity factors (Bartosz 2004). This fact may be of great importance in horses since physiological concentration of this antioxidant in serum is several times higher than in many other animal species (Winnicka 2004). This result corresponds to the observations of other authors that despite the fact that it is rarely used, bilirubin can serve as an index in the course of physical effort (Kuwabara et al. 1996). Bilirubin levels increase during the training period (Judson et al. 1983).

In conclusion, studies with the Žemaitukai horses showed higher haematological values (Mikniene et al. 2014) compared to this study, suggesting that the specific endurance training could process the generated physiological changes. In this study, Žemaitukai horses showed lower haematological values and increased muscle catabolism after exercise. Arabian horses have higher aerobic capacity compared to the Žemaitukai horses which probably contributes to their superior low- to moderate-intensity exercise performance. Serum activity of muscle enzymes suggests that the muscle tissue of the Arabian horses has higher tolerance for exercise-induced muscle catabolism and lower muscle catabolism than that of the muscle tissue of the Žemaitukai horses. Further studies need to be carried out to identify the differences in the muscle tissue of both breeds.

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