ACTA VET. BRNO 2019, 88: 177–185; https://doi.org/10.2754/avb201988020177

Responses for blood morphological indices in a 60-km horse endurance race depending on the season

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Received September 27, 2018
Accepted April 23, 2019

Abstract

The aim of this study was to find whether the magnitude of changes in blood before and after horse endurance competitions was the same at the beginning and the end of the season and if the studied indices could be useful in assessing the impact of such changes on the length of the endurance racing season. The study was conducted on 36 clinically healthy horses. Blood samples were taken during 60-km endurance competitions in 2014, three times in total. The study revealed an increase of lymphocytes (× 1.89–2.05; $P < 0.001$), red blood cell count (× 1.11–1.12; $P < 0.001$), and a decrease in the mean corpuscular haemoglobin concentration (× 1.20–1.27; $P < 0.001$), red cell distribution width (× 1.11; $P < 0.01$), platelet concentrations (× 1.33–1.40; $P < 0.05$), when compared at the beginning and in the end of the horse endurance racing season. During the entire season, haematocrit values significantly increased after the race but were at their lowest in the mid-season period. The results proved a significant increase in the white blood cell and granulocyte counts following the finish of a 60-km endurance race, when comparing the values at the beginning and in the end of the horse endurance racing season, and a significant reduction in lymphocytes when comparing the values at the beginning (35.7%) and at the end (29.7%) of the season. The endurance competition season highly influences haematology indices in horses' blood, however, to determine the season's impact on the results of the race, additional studies must be conducted.

Equine, sports, blood, haematology, competition

The effects of prolonged submaximal exercise have been reported on a number of physiological indices in the horse. Submaximal work, such as endurance racing, results in evaporative heat loss, with a loss of between 10–15 litres an hour, primarily in the form of sweat (McConaghy et al. 1995). Dehydration affects the capacity for evaporative heat loss, leading to elevated core temperatures in horses despite an unaltered sweat production (Flaminio and Rush 1998). Certain cardiovascular and haematological adaptations are necessary to guarantee the correct supply of oxygen and blood-borne substrates to active muscles during exercise, along with the release of metabolites. These systems could act as limiting factors to the aerobic potential and could, thereby, limit physical performance (Muńoz et al. 1997). The first factor that influences the athletic performance of a horse is its genetic background. Endurance competitions, stress, and fatigue are vividly shown by changes in circulating erythrocyte and leukocyte numbers and in the creatinine concentration in the horses (Lawan et al. 2010).

The cellular components of the blood reflect specific changes in an organ or body system or, more often, a general response by the individual to a particular physiological or pathological condition (Satué el al. 2012). Limited data are available regarding seasonal variations and the pre-race and post-race haematological indices of endurance horses in relation to the season. Undoubtedly, haematology and plasma or serum biochemistry are important tools when it comes to assessing the health of athletic horses (Waller et al. 2009). Therefore, it has been decided to investigate the changes in various haematological indices:

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white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), haematocrit level (HCT), mean corpuscular volume (MCV), haemoglobin concentration (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean platelet volume (MPV) in athletic horses during the competition season. The aim of this study was to find whether the magnitude of changes in blood before and after endurance competitions was the same at the beginning and at the end of the season, and if the indices studied could be useful in assessing the impact of the magnitude of such changes on the length of the horse endurance racing season.

Materials and Methods

Animals

The study was conducted on 36 (16 female and 20 male) clinically healthy Arabian horses (a clinical examination was conducted before and immediately after the competition according to the FEI vet gate protocol) at three endurance competitions in Lithuania and at the Lithuanian University of Health Sciences Veterinary Academy. All horses were 9 ± 2 years old, with an average body weight of 420 ± 40 kg.

Experimental design

The horses competed in endurance competitions of the same distance (60 km). Blood samples were taken three times during the endurance season of 2014: at the beginning of the season, in April (with an average ambient temperature of 5.8–7.5 °C); at mid-season in July (with an average weather temperature of 19.2–20.9 °C); and at the end of the season, in October (with an average ambient temperature of 4.7–8.6 °C). Blood samples were collected from each animal by jugular venipuncture into 2.0 ml vacutainer blood collection tubes containing 3.6 mg of ethylenediaminetetraacetic acid (Terumo Europe, Belgium), 30 min before the start of each of the season's endurance competitions and no later than 30 min after the horse reached the finish line, comprising a total of three data recordings. None of the horses showed any stress reaction during the blood sampling which was carried out within less than 30 s for each sample, thus excluding an excitement-induced spleen contraction (Satue et al. 2012).

Haematological analyses

All blood samples were stored at an 18 °C temperature, and haematological testing was carried out 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, Austria, 2006). The samples included WBC (× 10^3/µl), lymphocytes (LYM, × 10^4/µl), granulocytes (GRA, × 10^4/µl), RBC (×10^6/µl), PLT (×10^4/µl), HCT (%), MCV (fl), Hb (g/dl), MCH (pg), MCHC (g/dl), MPV (fl) and red cell distribution width (RDW, %).

Statistical analysis

A statistical analysis of the data was carried out using the SPSS 20.0 software (SPSS Inc, Chicago, IL, USA). Using the descriptive statistics, normal distributions for all variables were assessed by the Kolmogorov-Smirnov test. The results were expressed as the mean ± standard deviation. The outcomes of the comparison of the mean figures were evaluated using statistical hypothesis test which is sometimes known as Student's *t*-test. A probability of less than 0.05 was considered significant ($P < 0.05$).

Results

The study revealed changes in blood indices during the racing season (Table 1). A significant increase between the beginning and end of the season was observed for LYM (× 1.89–2.05; $P < 0.001$) (Fig. 1), LYM% (× 1.76–2.02; $P < 0.001$), RBC (× 1.11–1.12; $P < 0.0001$) (Fig. 2), and a decrease in MCHC (× 1.20–1.27; $P < 0.0001$) (Fig. 3), RDW (× 1.11; $P < 0.01$) (Fig. 4), and PLT (× 1.33–1.40; $P < 0.05$) (Fig. 5).

During the entire season, HCT values were found to be significantly increased after each race but were at their lowest at the mid-season (Fig. 6).

The results presented in Table 1 show a significant increase in WBC, GRA, and GRA% levels following each 60-km endurance race at the beginning and at the end of the season (starting between 9.5–13.5% for WBC, and reaching between 26.1–32.4% for GRA), and there was also a significant reduction in LYM (Fig. 1) at the beginning of the season (35.7%, $P < 0.008$) and at the end of the season (29.7%, $P < 0.000$). However, there was a significantly reliable lower level of RDW (1.3%; $P < 0.033$) after the beginning of the season (see Fig. 4) in comparison with the level identified in this trait before the beginning of the season.

Table 1. Responses for blood morphological indices.

Blood morphological indices	Point in the season	Before the start		After the finish		Change	
		Mean	SD	Mean	SD	Difference	*P*
WBC	Beginning	10.38	1.90	11.76	2.52	-1.4	0.021
	Middle	5.30	1.05	5.63	1.22	-0.3	0.215
	End	10.54	2.09	11.50	2.25	-1.0	0.001
LYM	Beginning	1.96	0.49	1.24	0.36	0.7	0.008
	Middle	0.87	0.38	0.73	0.18	0.1	0.275
	End	3.70	1.69	2.55	1.42	1.1	0.000
GRA	Beginning	8.04	2.03	10.19	2.57	-2.1	0.001
	Middle	4.15	0.92	4.73	1.23	-0.6	0.110
	End	6.48	1.76	8.57	2.43	-2.1	0.001
LYM%	Beginning	19.58	6.50	11.08	4.50	8.5	0.004
	Middle	16.19	5.47	13.44	4.25	2.7	0.282
	End	34.55	12.71	22.41	11.66	12.1	0.000
GRA%	Beginning	76.71	7.14	86.10	4.68	-9.4	0.004
	Middle	78.81	6.52	83.41	6.28	-4.6	0.157
	End	62.01	12.51	74.09	11.41	-12.1	0.000
RBC	Beginning	8.51	0.90	8.93	0.81	-0.4	0.171
	Middle	4.92	0.57	5.11	0.56	-0.2	0.143
	End	9.51	2.84	9.95	2.57	-0.4	0.123
Hb	Beginning	130.75	14.42	138.00	17.06	-7.3	0.214
	Middle	148.78	19.16	157.11	20.56	-8.3	0.112
	End	145.42	41.19	151.63	35.32	-6.2	0.204
HCT	Beginning	38.74	4.10	40.45	4.70	-1.7	0.002
	Middle	23.12	2.50	24.36	3.19	-1.2	0.003
	End	31.80	30.93	32.26	31.42	-0.5	0.005
MCV	Beginning	45.75	3.88	45.05	4.65	0.7	0.103
	Middle	47.11	3.14	47.67	2.96	-0.6	0.095
	End	54.41	5.04	54.43	4.50	0.0	0.915
MCH	Beginning	15.38	0.82	15.31	1.05	0.1	0.758
	Middle	30.28	1.92	30.69	1.87	-0.4	0.237
	End	15.40	1.02	15.38	0.93	0.0	0.851
MCHC	Beginning	360.63	71.51	341.13	14.88	19.5	0.466
	Middle	642.67	30.18	644.67	18.81	-2.0	0.721
	End	284.47	16.97	283.26	11.11	1.2	0.650
RDW	Beginning	22.90	0.75	22.60	0.56	0.3	0.033
	Middle	21.88	0.65	22.13	0.45	-0.3	0.177
	End	20.52	2.96	20.31	2.73	0.2	0.126
PLT	Beginning	145.25	46.32	130.13	59.11	15.1	0.190
	Middle	133.56	36.76	146.11	20.53	-12.6	0.293
	End	103.84	44.12	97.53	46.33	6.3	0.574
MPV	Beginning	6.99	0.46	6.80	0.21	0.2	0.263
	Middle	7.66	0.79	7.78	0.29	-0.1	0.701
	End	4.06	3.77	3.92	3.73	0.1	0.251

White blood cell counts (WBC, $\times 10^3/\mu l$), lymphocytes (LYM, $\times 10^4/\mu l$), granulocytes (GRA, $\times 10^4/\mu l$), red cell counts (RBC, $\times 10^6/\mu l$), platelet counts (PLT, $\times 10^4/\mu l$), haematocrit (HCT, %), mean corpuscular volume (MCV, fl), haemoglobin concentration (Hb, g/dl), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, g/dl), mean platelet volume (MPV, fl) and red cell distribution width (RDW, %). SD - standard deviation

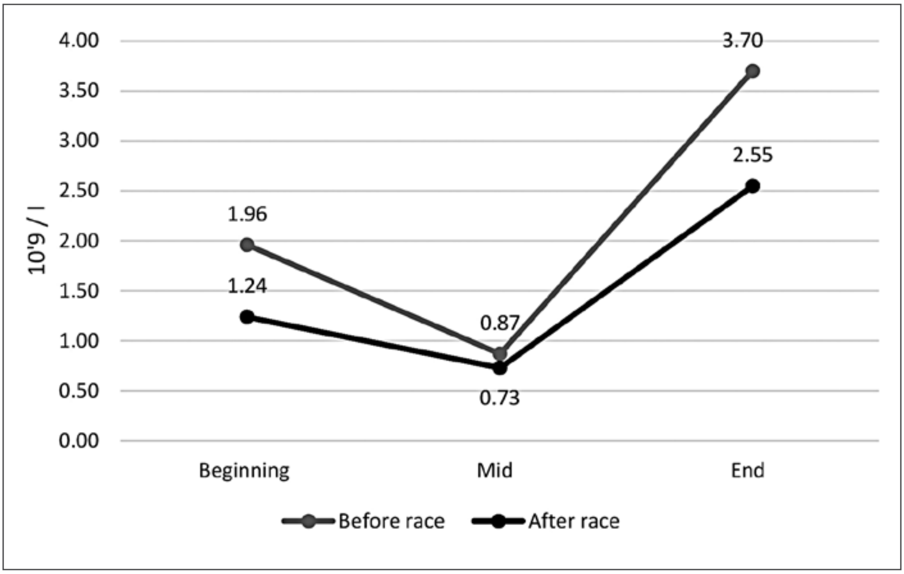

Fig. 1. Lymphocyte changes in horse blood during the competition season.

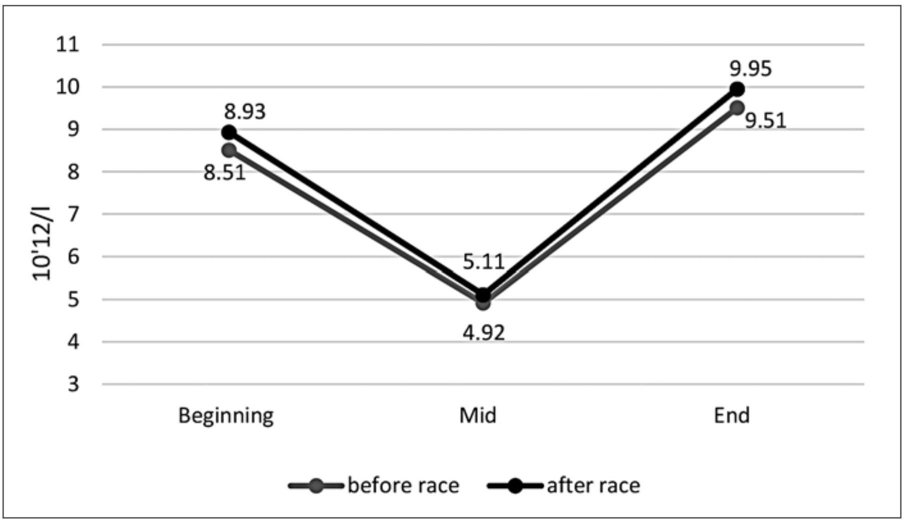

Fig 2. Red blood cell count changes in horse blood during the competition season.

Discussion

Exercise has variable effects on haemogram levels, depending on work intensity, fitness, training levels, environmental conditions, and the breed of horse (Satue et al. 2012).

Apart from the inherent characteristics, the performance of the horses during races is closely associated with cardiac function and tissue oxygenation. These are dependent on

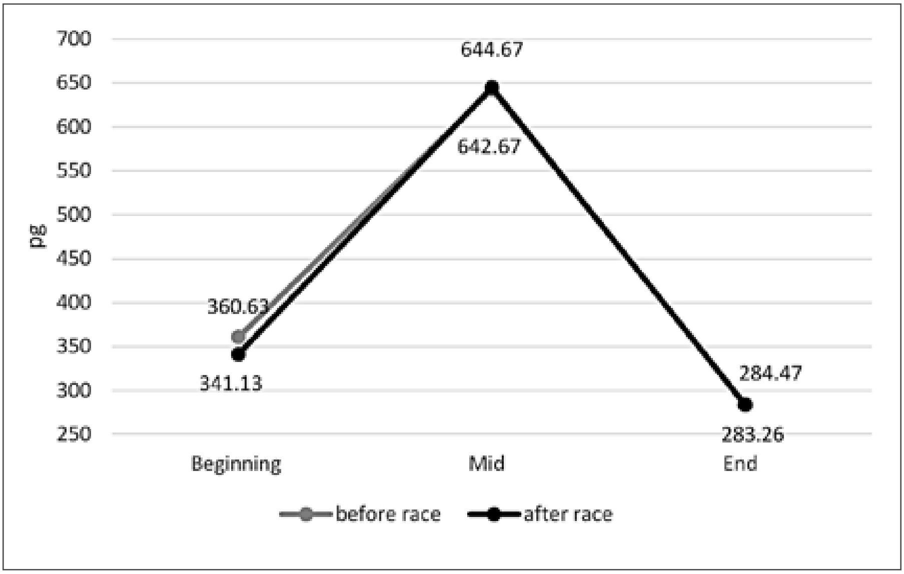

Fig 3. Mean corpuscular haemoglobin concentration changes in horse blood during the competition season.

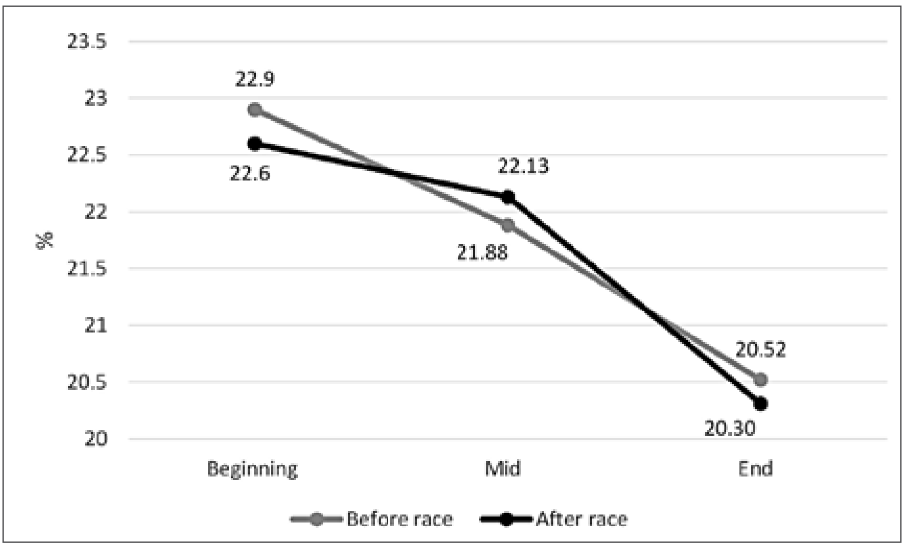

Fig 4. Red cell distribution width changes in horse blood during the competition season.

the oxygen-carrying capacity of the blood, which itself is dependent upon the erythrocyte and haemoglobin concentrations (Lawan et al. 2010). A significant increase ($P < 0.001$) in the RBC count was found during the season. Similar results were identified by Fan et al. (2002) during a five-month horse endurance training season, where it was discovered that RBC increased to a plateau during the second and third month of endurance training.

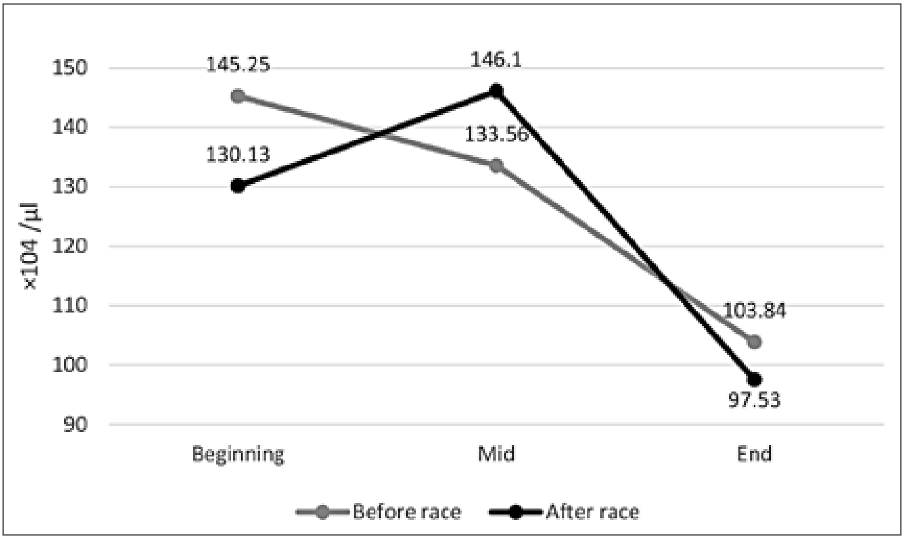

Fig 5. Platelet count changes in horse blood during the competition season.

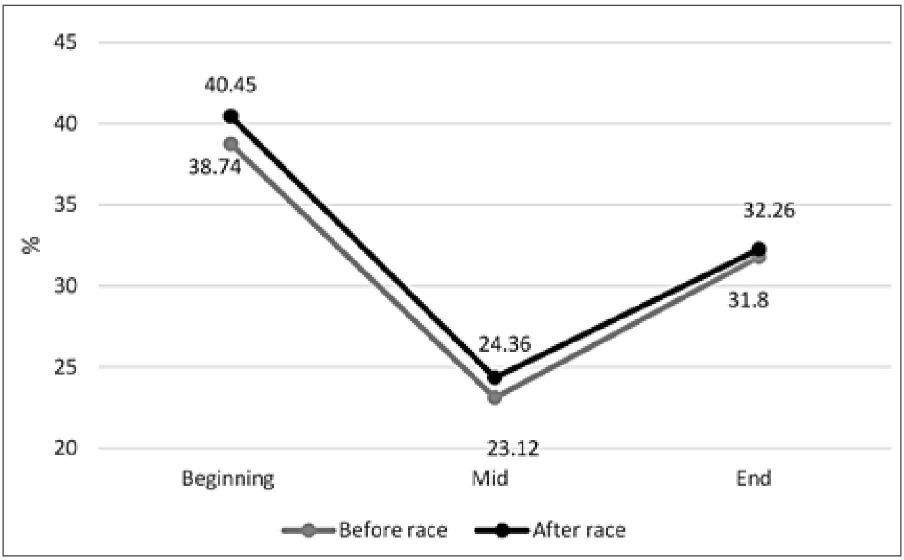

Fig 6. Haematocrit changes in horse blood during the competition season.

Horses that are conditioned for high-intensity athletic bouts are characterised by an increase in RBC counts, Hb concentrations, and blood volumes (Tseliou et al. 2014). The increase in the value of erythrocyte indices in horses is caused, most of all, by a release of erythrocytes from the spleen (Satue et al. 2012). In endurance efforts, several factors can contribute to an elevation in the HCT percentage figure. In a more prolonged exercise, splenic contraction due to an adrenergic stimulus and sweating will lead to extensive loss

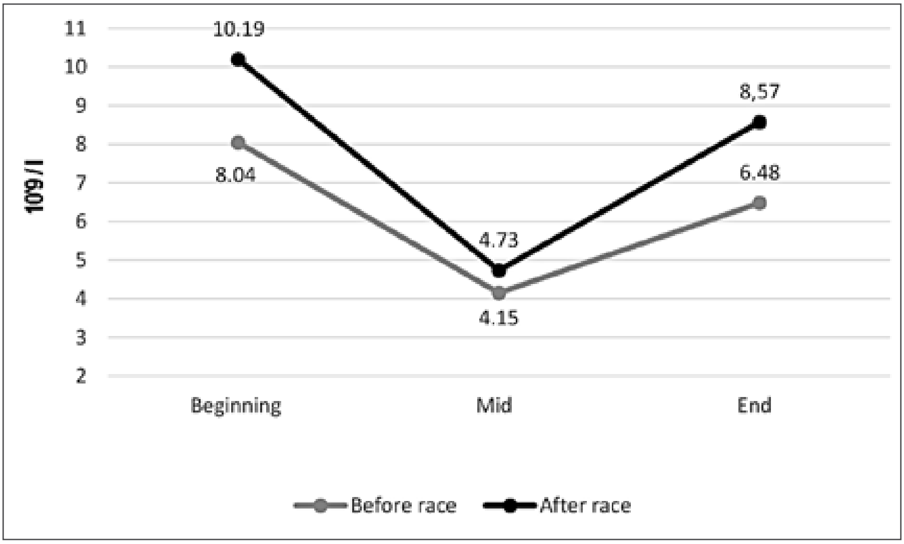

Fig 7. Granulocyte changes in horse blood during the competition season.

of body fluid. Thermoregulation leads to intense levels of sweating with an evident loss of body fluid and haemoconcentration (Waller et al. 2009). Therefore, it may be concluded that the elevation of HCT and Hb levels may be due to splenic contraction which is not related to dehydration and body water loss. Prolonged exercise, such as endurance riding, is associated with leukocytes which result from neutrophilia and lymphopaenia (Rose and Hodgson 1982). This is probably due to an increase in circulating corticosteroids (Dybdal et al. 1980).

Research carried out on human athletes showed that there appear to be quite extensive seasonal variations in HCT (with a relative change of up to 15%) with lower values in summer than in winter which may result in season-to-season changes from ~42% in summer and 48% in winter, as identified amongst several thousand participants of the study. Seasonal changes depend upon climatic effects (Thirup 2003). Studies of seasonal changes in the HCT of athletes are few and far between, but they do indicate that HCT may be decreased by another 1–2% in summer by the addition of a training effect (Mairbäurl 2013). This could explain the haematocrit decrease in the mid-season period in the blood of endurance horses, prior to taking part in the competition.

The RDW value was found to be reduced after exercise compared to the pre-exercise period during the season. Red cell distribution width (RDW) is calculated as the percentage of the standard deviation of RBC size/mean corpuscular volume (Tseliou et al. 2014). In studies of healthy subjects, a decrease in the RDW level after exercise has been reported (Tseliou et al. 2014). Tayebi et al (2005) reported a significant decrease in RDW and a significant increase in MCV in ten students of physical education after a session involving circuit resistance training. In this study, it has been observed that a significant decrease in RDW levels in endurance horses after exercise can be caused by increased resistance and adaptation of horses to regular exercise.

At the beginning and at the end of the season, there was a significant elevation in the WBC in the horses' blood. This is probably due to a combined effect of increased circulation of corticosteroids and splenic contraction. Horses which completed an endurance event at a faster speed have higher levels of LYM than do slower horses (Trigo et al. 2010). Additionally, it has been demonstrated that exhausted endurance horses had a left shift in the neutrophils (NEU) and significant lymphopaenia (Trigo et al. 2010). There was a significant decrease of LYM at the end of the season, after a race. According to Trigo et al (2010), this may occur due to exhaustion. In the study, a simultaneous increase in the number of GRA was defined. According to the study by Art et al (2009), intense exercise induces activation of blood GRA, with degranulation of NEU and a release of myeloperoxidase. However, minimal variations in WBC indices are indicative of good levels of performance in the horse (Adamu et al. 2012).

The significant decrease in PLT which was observed in horses at the end of the season contrasts with the changes which have been described in the literature. The platelets, as is similar to erythrocytes, are subject to an increase in the number of them which are circulating due to the release of catecholamine, splenic contraction, and haemoconcentration (Hideo et al. 1999). One possible explanation for this decrease in thrombocytes may be related to blood loss. Indeed, studies of marathon runners have shown significant gastrointestinal blood loss (Larsson et al. 2013). Most likely, endurance may have the same effect in horses, and could in this way explain this decrease. This theory is confirmed by a decrease in RBC and WBC in the middle of the season.

A decrease in MCHC during the competition season was observed; this finding could be explained by the cell swelling phenomenon, something that is further suggested by the evidence of constant MCH values during the test as a whole. The red blood cells appear to act as a buffer against any exercise-associated 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 (Weiss and Evanson 1997). 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 (Weiss and Smith 1998).

Based on the study results, it can be concluded that the endurance competition season highly influences the haematology indices in horse blood. There was a significant decrease in all blood haematological indices, except for the MCHC, which decreased in the mid-season and increased at the end of the season. The results proved that there was a significant increase in WBC, GRA, and GR% levels following the finish of a 60-km endurance race at the beginning and at the end of the racing season. To determine the season's impact on the results of the race (speed, heart rate recovery time), additional studies must be conducted.

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