

FAST BLOOD PLASMA REDUCTANTS IN WELL-TRAINED STANDARDBRED TROTTERS BEFORE AND AFTER INTENSIVE RACE

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Abstract. Concentrations of haematological parameters in five Standardbred mares were determined at rest and after race, as well as blood plasma dehydration factor D_f , TAS and $TAS \times D_f$, and fast plasma reductants (FPR) and $FPR \times D_f$. An increase in PLT, WBC and RBC values was observed after intensive race, as well as a significant growth in HGB and HCT concentrations values. The $RBC \times HGB$ values at rest observed in this study are characteristic of good horse preparation to aerobic exercise. The observed post-exercise drop in the TAS concentration was significant, while that in the FPR concentration was insignificant. It is apparent that post-exercise horse blood parameters should be examined in association with organism dehydration degree.

Key words: dehydration factor, fast plasma reductants, horses, TAS

INTRODUCTION

Of horse breeds, most loaded during a race, trotters are distinguished by high maximum breathing capacity. Increased respiration rate enlarges the formation of reactive oxygen species (ROS). Under extreme conditions, oxygen consumption may increase even 100 times, which means more or less a 100-fold increase in superoxide anion radical production. The latter is much more reactive than oxygen and may result in development of oxidative stress in muscles [Jenkins et al. 1984, Ji 1999, Viña et al. 2000, Leeuwenburgh and Heinecke 2001, Szarska 2002a]. In the course of evolution, aerobic organisms developed efficient defensive mechanisms protecting them against ROS formation and their reaction with cell and tissue

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components. It was demonstrated that the ratio of oxidised glutathione to reduced glutathione concentrations (GSSG/GSH), as well as that of allantoin concentration, increases in mammalian muscles after a sub-maximal effort [Hellsten et al. 2001].

In our recent experiments, we observed that the level of blood plasma reductants significantly decreased after intensive race in two-year-old Standardbred trotters of both sexes, as well as in recreation saddle-horses after a ten-week long working season [Ogoński et al. 2008, Ogoński and Cieśla 2009]. For assays, we used a TAS (Total Antioxidant Status) analytical kit (Randox, UK), following the procedure recommended by its manufacturer. However, subsequent experiments and a thorough analysis of the results obtained have led us to a conclusion that TAS values do not describe proper antioxidative abilities of horse blood plasma and require additional interpretation through application of fast plasma reductants (FPR). The aim of the present study is an attempt to use these two assays, which define the antioxidative status in horses, in evaluation of their physical capacity.

MATERIAL AND METHODS

Animals

For the experiment, five two-year-old well-trained Standardbred mares were chosen which had been prepared for a racing season at a Horse Training Centre in Bonin in the Western Pomeranian Province, Poland. The experiment was carried out after an eight-month-long initial training (226 days) on a day with the conditions promoting water depletion (shade temperature 25°C; relative humidity 40%; 1010 hPa). From each horse, 3–5 ml of the peripheral blood from the external jugular vein was collected twice to test tubes with Na₄EDTA, the first time at rest (after morning grooming) and the second time immediately (\pm 30 seconds) after the training race over a distance of 3200 m at a pace of 550 m·min⁻¹. The approval for blood collection was obtained from the Ethical Commission of the Agricultural University in Szczecin, Poland.

Laboratory analyses

The following parameters were determined in blood samples: blood platelet (PLT), white blood cell (WBC) and red blood cell (RBC) counts, as well as the haematocrit value (HCT) and the haemoglobin concentration (HGB). The obtained values were a basis for calculating red cell indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), as well as blood plasma dehydration factor (D_f). The assays

were performed conductometrically using a Sysmex F-800 haematological analyser. The blood samples were centrifuged (3000 rpm, 10 min.) and the Randox reducing ability (TAS), as well as fast antioxidant concentration (FPR) were determined of the blood plasma. For assays, a TAS (Total Antioxidant Status) analytical kit (Randox, UK) was used. Measurements were made using a PYE-Unicam SP1800 double-beam spectrophotometer at 37°C, recording the increase of absorbance in samples at a wavelength of 600 nm against water in a time interval of 0 to 5 minutes. Calibrations were carried out based on a set of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid standards, ranging from 0.206 to 1.650 mmol·dm⁻³. The lag time in signal recording was between 2 and 4 seconds. The reading of TAS values was made directly from the kinetic curve exactly after 180 seconds from plasma sample injection into a measuring cuvette, whereas that of FPR values was performed by assigning sample oxidation lag time limit which is proportional to fast reductant concentration in the sample.

Statistical analysis

All collected results were processed statistically using computer software packages MS Excel and MedCalc v4.15a. The structure of examined populations was presented as a mean value with population standard deviation $S(x)$. Statistical analysis was performed using the Student's *t*-test (for paired variables $P_p \leq 0.05$). For better data interpretation, real P_p values are presented.

RESULTS AND DISCUSSION

Experimental data, calculations and their selected statistical descriptions are presented in Tables 1 and 2, as well as Figure 1. Analysis of the structure of respective data populations showed that they satisfied a homogeneity condition for the examined traits:

- 1) with a slight trend towards left-sided asymmetry and platykurtic distribution “at rest” and leptokurtic distribution “after race” for the haematological data; and
- 2) with a slight trend towards right-sided asymmetry and platykurtic distribution for the redox data.

The comparison of assay results confirmed our previous findings [Ogoński et al. 2008], showing a statistically significant increase in HGB and HCT values and increase in PLT, WBC and RBC values (Table 1), as well as no statistically significant changes in the indices calculated based on them, i.e. MCV ($P_p = 0.5199$), MCH ($P_p = 0.1611$) and MCHC ($P_p = 0.3868$).

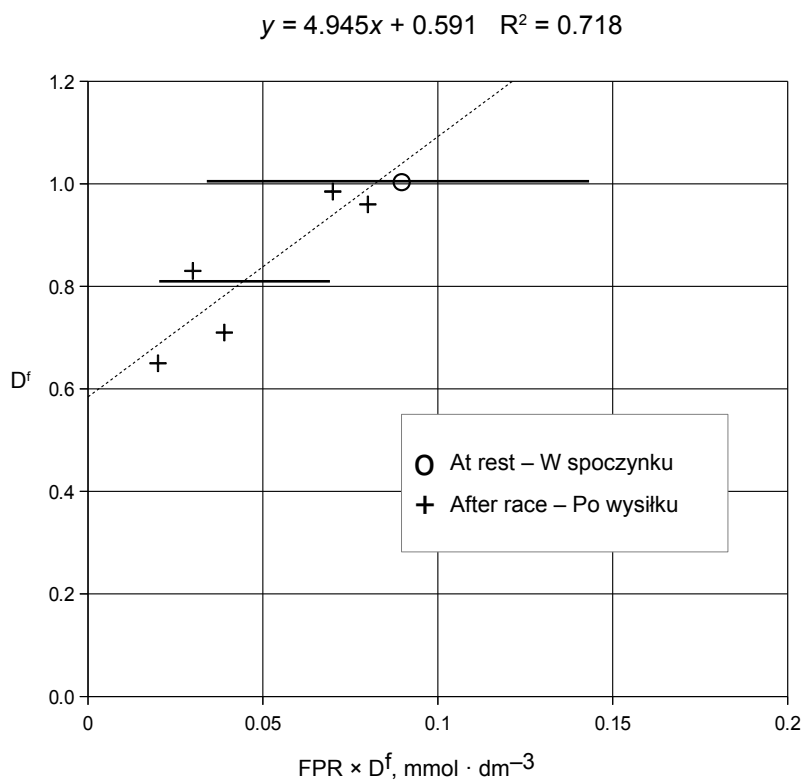


Fig.1. The antioxidant ability of peripheral blood plasma in two-year old Standardbred mares before and after race; FPR quantity in the blood plasma of mares which lost more water during the race is lower

Rys. 1. Potencjał antyoksydacyjny surowicy 2-letnich klaczy Standardbred przed i po wysiłku; FPR surowicy klaczy bardziej odwodnionych jest niższa

Haematological data were the basis for evaluating the degree of horse training progress, as well as calculating a race-dependent plasma dehydration factor. The former was assessed assuming that the race endurance of a given animal is the greater, the higher is its current oxygen limit defined as a maximum amount of oxygen taken up by organism during one minute. Therefore, it is accepted that one of the results of properly conducted training of sport horses is high erythrocyte count at rest and high haemoglobin content in them. This enables more efficient distribution of oxygen in the organism and thereby extension of the work time under full oxygen saturation conditions [Szarska 1994, Sitarska et al.

1997, Szarska 2000, 2002b]. It results from our observations that the $RBC \times HGB$ ($\times 10^{-12} \cdot dm^{-6}$) index may be used for evaluation of the degree of horse readiness to aerobic exercise. We assumed that the $RBC \times HGB$ values at rest within the 1–2 range are characteristic of the efficient system of blood oxygen transport and of a good preparation of horse to aerobic exercise. Since there is the output of erythrocytes from the spleen in horses during an effort [Szarska 1994, 2002b], the $RBC \times HGB$ values increase significantly. We believe that the $RBC \times HGB$ values after race could be assumed to be a measure of training progress degree. In well-trained and maximum-effort-effective horses, these values shift to the 3–5 range. In the group of horses examined by us, the $RBC \times HGB$ ($\times 10^{-12} \cdot dm^{-6}$) index amounted respectively to 1.3 ± 0.12 (at rest) and 1.7 ± 0.23 (after race).

Table 1. Mean of haematological parameters in two-year-old Standardbred mares at rest and after race

Tabela 1. Średnie wartości współczynników hematologicznych 2-letnich klaczy Standardbred w spoczynku i po wysiłku

Examination Badanie	Haematological parameters – Wskaźniki hematologiczne				
	PLT $10^9 \cdot dm^{-3}$	WBC $10^9 \cdot dm^{-3}$	RBC $10^{12} \cdot dm^{-3}$	HGB $10^{-2} kg \cdot dm^{-3}$	HCT %
At Rest (n = 5) W spoczynku	315 ± 68	9.9 ± 2.1	9.5 ± 0.4	13.6 ± 1.4	38.7 ± 7.5
After race (n = 5) Po wysiłku	390 ± 107	12.0 ± 1.4	10.3 ± 0.4	16.8 ± 1.9	43.7 ± 8.1
Probability (P_p) Prawdopodobieństwo	0.0806	0.0841	0.0517	0.0225	0.038

A post-exercise statistically significant increase in PLT, WBC, RBC, HGB and HCT values is a direct evidence of water loss during the race. The D_f factor, defined as a quotient of the concentrations of selected blood elements (PLT and WBC) after race and at rest, may serve as a measure of blood plasma dehydration in a given animal, adopting an assumption that there are no significant changes in the volume of blood cells during the experiment [Ogoński et al. 2008]. We obtained the following values: PLT ($D_f = 0.82 \pm 0.128$) and WBC ($D_f = 0.82 \pm 0.147$). The D_f value calculated based on a set of values obtained for PLT and WBC ($D_f = 0.82 \pm 0.138$; $n = 10$) was adopted as a mean measure of blood plasma dehydration in the examined horses. Since one should be prepared for cell volume reduction and blood plasma cellular water shift in the hypertonic blood plasma, the D_f values presented in this paper should be treated as maximum values, while

the amount of water being lost by a single horse may be estimated with caution at 3–7% of the blood volume at rest.

Table 2. The reducing ability of blood plasma in two-year-old Standardbred mares at rest and after race

Tabela 2. Potencjał redukcyjny surowicy 2-letnich klaczy Standardbred w spoczynku i po wysiłku

Horse Konia	Antioxidant indices – Wskaźniki antyoksydacyjne					
	TAS mmol · dm ⁻³			FPR mmol · dm ⁻³		
	At rest W spoczynku	After race Po wysiłku		At rest w spoczynku	After race Po wysiłku	
		without D _f	with D _f		without D _f	with D _f
1	1.387	1.349	1.276	0.117	0.082	0.077
2	1.419	1.364	1.347	0.111	0.070	0.069
3	1.433	1.372	0.952	0.173	0.058	0.040
4	1.379	1.362	0.890	0.033	0.029	0.019
5	1.377	1.378	1.158	0.030	0.035	0.029
Mean – Średnia	1.399ab	1.365a	1.125b	0.093	0.055	0.047
S _(n)	0.023	0.010	0.178	0.054	0.020	0.023
Probability (P _p) Prawdopodobieństwo	0.0443	0.0373		0.1437	0.1165	

S_(n) – population standard deviation – odchylenie standardowe. The same small letter denotes significant differences at P ≤ 0.05 – te same małe litery oznaczają różnicę istotną przy P ≤ 0,05.

The content of reductants in the blood plasma of horses taking part in this experiment, grouped into TAS reductants (antioxidants, other low-molecular reductants and part of structural reductants) and fast reductants (FPR) is presented in Table 2. When calculating the content of reductants in the blood plasma collected from horses after race completion, a drop in the blood volume caused by water loss during the race was taken into account. This enabled a comparison of the results and their statistical analysis. The data presented in Table 2 show that intensive aerobic exercise in all examined horses leads to a decrease in blood plasma redox potential and that the concentration of fast reductants, i.e. the defensive ones (FPR), is very low in them. At rest, it is only about 7% of the concentration of “total reductants”, while it decreases to about 4% after race. It should be noticed that the FPR value corresponds to the content of ascorbic acid found in the blood plasma of horses [Balogh et al. 2001]. A post-exercise decrease in the concentration of “total reductants” is statistically significant (TAS, P_p = 0.0373). However, differences in the FPR values are not significant, the concentration of fast reductants decreases. Nevertheless, it is likely that high oxygen supply during the race contributes to a decrease in the concentration of active plasma defensive reduc-

tants (antioxidants) due to identical direction of changes in that value in particular horses (FPR, mean difference = -0.0459), high correlation with corresponding TAS values ($R^2 = 0.688$) and relatively low probability of the null hypothesis in the statistical examination. We have assumed in the analysis of findings that the D_f value calculated is a measure of the amount of energy expended during the race. If such an assumption is correct, the D_f value should be connected with the FPR value. In Figure 1 the relationship linking these values is presented. Horizontal lines show a range in FPR value sets before and after race. In three out of five horses, the FPR values at rest were considerably higher than after race. Horses marked with numbers 4 and 5 are an exception which the FPR values at rest were low and did not change distinctly after race. This may be evidence of the individual variability or the training progress of these animals (high D_f value).

Ascorbic acid is a main plasma antioxidant (but not necessarily the only one). Low concentration of ascorbic acid at rest in the blood plasma of horses means that this compound is preserved in a reduced state under physiological conditions owing to efficient mechanisms of the transfer of reducing power from tissues (blood cells, liver) to the blood plasma. It should be stated that outcomes of the presented experiment point to a disturbed balance between the rate of oxidation and regeneration of this compound during intensive exercise. Consequently, there are conditions for development of oxidation stress and for oxidation of tissue structures, which is attested by a post-exercise drop in the TAS values.

CONCLUSIONS

It results from our observations that the $RBC \times HGB$ index may be used in evaluation of the degree of horse readiness to aerobic exercise. The observed post-exercise changes in the TAS and FPR concentrations suggest that they may be applied to assess training progress degree of a horse. In the light of the study, it is evident that post-exercise antioxidant and haematological parameters of the horse blood should be examined in association with organism dehydration degree. The obtained results may be evidence of direction of the changes which should be confirmed in further research.

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REDUKTORY FAZY SZYBKIEJ W SUROWICY KŁUSAKÓW STANDARDTBRED PRZED I PO INTENSYWNYM WYSIŁKU

Streszczenie. U pięciu klaczy Standardbred, w spoczynku i po intensywnym wysiłku, określono koncentrację wskaźników hematologicznych, a także współczynnik odwodnienia surowicy (D_f), wartość TAS oraz stężenie reduktorów fazy szybkiej (FPR) oraz

obliczono współczynniki $TAS \times D_f$ oraz $FPR \times D_f$. Po intensywnym wysiłku zanotowano wzrost liczby PLT i WBC oraz RBC oraz potwierdzony statystycznie wzrost stężenia HGB i HCT. Zaobserwowany w niniejszych badaniach spoczynkowy wzrost wartości $RBC \times HGB$ jest charakterystyczny dla koni prawidłowo przygotowanych do treningu aerobowego. Po wysiłku stwierdzono istotny spadek wartości TAS, podczas gdy stężenie FPR nie zmieniło się istotnie. Uzyskane wyniki sugerują, że powyższe parametry krwi u koni powinny być rozpatrywane w odniesieniu do stopnia odwodnienia organizmu.

Słowa kluczowe: konie, reduktory fazy szybkiej, TAS, współczynnik odwodnienia

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