

# Total antioxidants and antioxidant system microelements in the blood plasma of recreation saddle-horses after a ten-week-long working season

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### Summary

The aim of the study was to demonstrate that monotonous everyday work lasting for several hours a day and many weeks long in a commercial horse-riding centre may lead to chronic fatigue and consequently to increased susceptibility to oxidative stress in „recreation” horses used there. During the experiment, from the mid-June to the beginning of September, 16 half-bred horses – eleven geldings and five mares at the age of 4-8 years, were kept under uniform environmental conditions in a seasonal horse-riding seaside centre in Poland and used on the average four hours a day for six days each week. Before and after the season, their blood samples were collected, in which haematological indices, Mg, Fe, Cu, Zn and Mn concentrations, and total plasma reducing potential by the FRAP method were determined. The results showed a significant deterioration of peripheral blood morphology at rest and a decrease in plasma reducing potential. The product  $RBC \times HGB \times 10^{12} \text{ kg} \times \text{dm}^{-6}$  decreased from  $1.66 \pm 0.71$  to  $0.99 \pm 0.16$  ( $P_u < 0.005$ ). FRAP decreased from  $5.14 \pm 0.88$  to  $4.16 \pm 0.67 \times 10^{-4} \text{ mol/dm}^3$  ( $P_p < 0.001$ ), while Cu concentration increased to the upper normal range, i.e. to  $2.18 \pm 0.65 \times 10^{-5} \text{ mol/dm}^3$  ( $P_p < 0.06$ ). At the same time, Mg, Fe, Zn and Mn concentrations did not change significantly. The decrease of RBC and HGB values was acknowledged by us as a clinical symptom of chronic fatigue which, apart from general lesions such as weight loss, unwillingness to work and growing irritability, was accompanied by thrombocytopenia, hypoalbuminemia, dehydration, disturbance in microelement homeostasis and a drop in total plasma antioxidant potential. Consequently it is concluded that in some cases, at the end of working season, „recreation” horses should not be forced to an intensive effort, e.g. a race or jumps, because during intensive aerobic work both the oxygen transport system and antioxidant defence system undergo an overload, which is followed by the growing probability of damage to tissues by ROS.

**Keywords:** recreation horse, chronic fatigue, FRAP, trace elements

One often meets with the opinion that a „recreation” horse does not work hard. It is not always true because the meaning of equine recreation is very wide. Recreation horses in commercial horse-riding centres, which enjoy a great interest of customers increasing in the summer season, are in a particular situation. A horse kept there frequently works for several hours a day, under several riders with different experience levels, which in itself constitutes a discomfort for the animal. Even the horses that stay in stables with appropriate zoo-hygienic conditions, feeding and care can feel physical and psychical fatigue when overloaded with work. In the physiological classification of tiredness a division into fatigue and weariness is operative. Symptoms connected with long-lasting work are treated as fatigue. On

the other hand, a consequence of psychical activity is called weariness. Each of these forms may assume an acute, moderate (sub-acute) or chronic form. Acute and sub-acute fatigue is a physiological consequence of every work activity. They are connected with it causatively. In chronic fatigue, which is a sum of signs of prolonged fatigues that have not receded completely due to insufficient rest, many symptoms are of pathological character or at the border of physiology and pathology. The accumulation of changes developed in successive states of acute fatigue that occurs here does not allow for quick regeneration and sometimes even requires treatment. At a later time, during general fatigue (of a central origin), there occurs a reduction in the efficiency of those functions which originally have not been

overloaded with work, while a change in the character of work activity no longer restores the full efficiency of organism. If work activity is not stopped in order to regenerate the organism, general fatigue assumes the form of psychopathological lesions (11).

In such a situation one cannot exclude an increase in the susceptibility of the organism to the unfavourable effect of reactive oxygen species (ROS), being borne as by-products of oxygen transformations, and a development of oxidation stress in a borderline case. A high concentration of endogenous ROS is particularly dangerous for skeletal muscles and the heart muscle because the activity of antioxidant enzymes and the concentration of antioxidants are small in them (10). Moreover, it results from our studies that in the blood plasma of horses the antioxidant potential at rest (the concentration of antioxidants at rest) is very low (2), while the concentration of trace elements indispensable for the synthesis of tissue antioxidant proteins in recreation horses kept in commercial horse-riding centres tends to be unsatisfactory and lies below the normal range (5, 6).

Having regard to the suggestions and observations presented above as well as the scarcity of knowledge on the relation of long-lasting work overload with susceptibility to oxidation stress in hard-working animals, we took up investigations aimed at demonstrating that monotonous everyday work lasting for several hours a day and many weeks long in a commercial horse-riding centre may lead to chronic fatigue and consequently to an increased susceptibility to oxidative stress in „recreation” horses used there.

### Material and methods

**Animals.** In the experiment, 16 half-bred horses – eleven geldings and five mares at the age of 4-8 years raised for the purpose of recreational horse-riding – were studied. During the experiment, in summer season (from the mid-June to the beginning of September), horses were kept under uniform environmental conditions in a horse-riding seaside centre in the Pomeranian Province in Poland, and used on the average four hours a day for six days each week. The daily dose of fodder given to the horses consisted of 3 kg pressed oats and 2 kg complete feed (Sukces Sport, Poland). Additionally, hay and water were given *ad libitum*.

**Sample collection.** From each horse, after the morning grooming, 3-5 ml of peripheral blood was collected from the jugular vein to test tubes with  $K_3EDTA$  (EQUIMED, Poland) at the beginning and after the end of the season. In order to limit the effect of atmospheric oxygen on antioxidants, the blood was placed immediately in a cooler in tightly closed and filled to capacity test tubes. The approval for blood collection was obtained from the Ethical Commission of the Agricultural University in Szczecin, Poland.

**Laboratory analyses.** In the blood samples blood platelet (PLT), white blood cell (WBC) and red blood cell (RBC) counts as well as haematocrit value (HCT), haemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were assayed and cal-

culated. The assays were made by the conductometric method by means of a haematological analyser Sysmex F-800 (ICN-Instruments-Poland; accuracy: WBC  $\pm 3.0\%$ , RBC  $\pm 2.0\%$ , PLT  $\pm 5.0\%$ ; reproducibility: HGB: C.V.  $\leq 1.0\%$ , WBC: C.V.  $\leq 1.5\%$ , RBC: C.V.  $\leq 1.0\%$ , PLT: C.V.  $\leq 4.0\%$ ). In blood plasma (3000 rpm, 10 min), magnesium, zinc, iron, copper, manganese and total antioxidants (reductants) were determined. Element concentrations were measured with the use of a Perkin-Elmer OPTIMA ICP-OES spectrophotometer. For the antioxidant assay, a FRAP method was used (3). Measurements were made with a PYE-Unicam SP1800 double-beam spectrophotometer at 37°C. The total concentration of reductants in the examined sample (FRAP) was determined by extrapolation method to FRAP $_{\infty}$  value (13, 19). The FRAP $_{\infty}$  value was a basis for reading, from the same progression line a value, of  $t_{1/2}$  (and  $t_{1/4}$ , if possible) (plasma full oxidation half/quarter-time). All the collected and calculated numerical data were transformed into SI units and processed statistically with MS Excel and MedCalc v4.15a computer software packages. The distribution of examined populations was presented as a mean value and population standard deviation.

The analysis of the distribution of each population showed that they fulfil homogeneity conditions, thus the t-Student test was applied for the comparison of mean values. It was assumed that two mean values of an unpaired variable or mean values of differences of a paired variable differ significantly when the calculated probability ( $P_u$  – unpaired;  $P_p$  – paired) is lower than 0.05.

### Results and discussion

The experimental data processed statistically are presented in tab. 1, 2ab and 3. The analysis of data compiled in tab. 1 shows a significant worsening of peripheral blood morphology at rest in the examined horses in the time period covered by the experiment, although it remained within the low normal range. Only white blood cell count and MCH did not change significantly, whereas other parameters decreased. Blood platelet count decreased below the low normal range. A statistically significant drop of RBC and HGB values being evidence of a decrease in total haemoglobin mass circulating in blood is worthy of particular attention. The product RBC  $\times$  HGB  $\times 10^{12}$  kg  $\times$  dm $^{-6}$  decreased from  $1.66 \pm 0.71$  to  $0.99 \pm 0.16$ , and this difference is statistically significant ( $P_u < 0.005$ ). At the same time, the MCV value increases, which when compared with a drop of MCHC value and a constant value of MCH is evidence of the erythrocyte swelling and a decrease in the concentration of their haemoglobin, while when compared with a drop of HCT value it is evidence of a decrease in the concentration of erythrocytes circulating in blood.

Tab. 2ab presents the results of assays concerning the concentration of magnesium and four trace elements related to antioxidant system in the blood plasma of the examined horses. Magnesium is related to the energy production apparatus in the cell. In the blood plasma it is present, in its aqueous part, in the ionised state. It was assumed that magnesium ( $Mg^{2+}$ ) concentration in the blood plasma water of the examined horses was constant. This is confirmed by the studies of Szarska

**Tab. 1. Peripheral blood morphology from recreation saddle-horses prior to and after ten-week long working season**

Parameter	Before season (n = 14)*	After season (n = 16)
PLT × 10 <sup>11</sup> (dm <sup>-3</sup> )	1.79 ± 0.45 <sup>a</sup>	1.07 ± 0.41 <sup>b</sup>
WBC × 10 <sup>9</sup> (dm <sup>-3</sup> )	8.19 ± 2.07 <sup>a</sup>	7.72 ± 1.01 <sup>a</sup>
RBC × 10 <sup>13</sup> (dm <sup>-3</sup> )	1.03 ± 0.23 <sup>a</sup>	0.78 ± 0.07 <sup>b</sup>
HGB × 10 <sup>-1</sup> (kg × dm <sup>-3</sup> )	1.56 ± 0.34 <sup>a</sup>	1.27 ± 0.10 <sup>b</sup>
HCT × 10 <sup>-1</sup> (dm <sup>3</sup> × dm <sup>-3</sup> )	4.39 ± 0.60 <sup>a</sup>	3.83 ± 0.33 <sup>b</sup>
MCV × 10 <sup>-14</sup> (dm <sup>3</sup> )	4.38 ± 0.77 <sup>a</sup>	4.93 ± 0.34 <sup>b</sup>
MCH × 10 <sup>-14</sup> (kg)	1.54 ± 0.31 <sup>a</sup>	1.63 ± 0.11 <sup>a</sup>
MCHC × 10 <sup>-1</sup> (kg/dm <sup>3</sup> )	3.54 ± 0.50 <sup>a</sup>	3.31 ± 0.05 <sup>c</sup>

Explanations: \* – two samples were rejected due to haemolysis; a, b – means with different superscript letters differ significantly at  $P_u \leq 0.05$  (a, c – at  $P_u \leq 0.10$ )

**Tab. 2a. Concentration of magnesium and antioxidant system microelements in peripheral blood plasma from recreation saddle-horses prior to and after ten-week long working season**

Element	Before season (n = 16)	After season (n = 16)
Mg × 10 <sup>-4</sup> (mol/dm <sup>3</sup> )	7.80 ± 0.68 <sup>a</sup>	8.26 ± 1.89 <sup>a</sup>
Fe × 10 <sup>-5</sup> (mol/dm <sup>3</sup> )	3.75 ± 1.08 <sup>a</sup>	3.88 ± 1.66 <sup>a</sup>
Cu × 10 <sup>-5</sup> (mol/dm <sup>3</sup> )	1.84 ± 0.22 <sup>a</sup>	2.18 ± 0.65 <sup>b</sup>
Zn × 10 <sup>-5</sup> (mol/dm <sup>3</sup> )	1.16 ± 0.30 <sup>a</sup>	1.24 ± 0.28 <sup>a</sup>
Mn × 10 <sup>-6</sup> (mol/dm <sup>3</sup> )	1.77 ± 0.56 <sup>a</sup>	1.82 ± 0.56 <sup>a</sup>

Explanations: a, b – means with different superscript letters differ significantly at  $P_p \leq 0.05$  ( $P_p = 0.0586$ )

**Tab. 2b. Molar concentration ratio of antioxidant system microelements against magnesium in peripheral blood plasma from recreation saddle-horses prior to and after ten-week long working season**

Element	Before season (n = 16)	After season (n = 16)
Fe/Mg × 10 <sup>-2</sup> (mol/mol)	4.82 ± 1.37 <sup>a</sup>	4.83 ± 1.98 <sup>a</sup>
Cu/Mg × 10 <sup>-2</sup> (mol/mol)	2.36 ± 0.29 <sup>a</sup>	2.62 ± 0.36 <sup>b</sup>
Zn/Mg × 10 <sup>-2</sup> (mol/mol)	1.48 ± 0.35 <sup>a</sup>	1.53 ± 0.30 <sup>a</sup>
Mn/Mg × 10 <sup>-3</sup> (mol/mol)	2.26 ± 0.68 <sup>a</sup>	2.28 ± 0.77 <sup>a</sup>

Explanations: a, b – means with different superscript letters differ significantly at  $P_p \leq 0.05$

**Tab. 3. Ferric reducing ability of peripheral blood plasma from recreation saddle-horses prior to and after ten-week long working season**

Parameter	Before season (n = 16)	After season (n = 16)
FRAP <sub>∞</sub> × 10 <sup>-4</sup> (mol/dm <sup>3</sup> )	5.14 ± 0.88 <sup>a</sup>	4.16 ± 0.67 <sup>b</sup>
t <sub>1/4</sub> × 10 <sup>1</sup> (s)	1.21 ± 0.80 <sup>a</sup>	1.37 ± 0.89 <sup>a</sup>
t <sub>1/2</sub> × 10 <sup>2</sup> (s)	1.58 ± 0.81 <sup>a</sup>	1.84 ± 0.75 <sup>a</sup>

Explanations: a, b – means with different superscript letters differ significantly at  $P_p \leq 0.001$

(20), who reported that magnesium concentration in the blood plasma of sport horses ranged from 7.0 to 8.0 × 10<sup>-4</sup> mol/dm<sup>3</sup> and the exercise did not affect significantly the concentration of that element. If this assumption is correct, the mean water loss in the blood of examined horses calculated on the basis of the experimental data showed in tab. 2a is up to 6.2% (0.0619 ± 0.239; n = 16).

The analysis of data compiled in tab. 2a shows that the mean concentration of Fe and Mn in the blood plasma of examined horses was higher than the values describing the upper normal range for these elements in horses (15, 23). Mean Cu concentration was within the normal range, whereas that of Zn was below the lower range and/or within the normal range given by Danek and Wiśniewski (7), who reported that Zn concentration in the blood plasma of mares ranging from 0.81 to 1.19 × 10<sup>-5</sup> mol/dm<sup>3</sup> could be acknowledged as deficient, while a decrease in plasma Zn concentration was evidence of a deficit of that element in the organism.

In the period covered by the experiment, mean Cu concentration increased up to the upper normal range ( $P_p < 0.06$ ), whereas that of Fe, Zn and Mn did not undergo a significant change. Nevertheless, the fact that in all examined cases the mean values of concentrations of the assayed elements reached higher numerical values in the blood plasma collected at the end of season cannot be left uncommented. One cannot exclude that this regularity has an external cause and may be related to anhydraemia in the examined horses during the experiment. Such a conclusion is strengthened by the results of statistical analysis of the molar ratios of respective elements and magnesium assayed at the same time in each blood plasma before and after the experiment (tab. 2b). Such a linking of data diminishes the effect of changes in the volume of dispersion medium (water and proteins) in the blood plasma collected in the remote stages of the experiment.

Tab. 3 presents the results of total reducing (antioxidant) ability of blood plasma. The analysis was made by a kinetic method using the TPTZFe<sup>3+</sup> complex in acidic environment as an oxidising agent. The recorded progression lines were a basis for reading the value of total concentration of FRAP<sub>∞</sub> (mol/dm<sup>3</sup>), reaction quarter- and half-times t<sub>1/4</sub> (s) and t<sub>1/2</sub> (s), and the constant rate of the second phase of reduction (t<sub>0</sub> = 180 s) k<sub>180</sub> (s<sup>-1</sup>). The obtained results showed that the reducing potential of the blood plasma of horses after the end of season decreased significantly, whereas quarter- and half-times t<sub>1/4</sub> and t<sub>1/2</sub> did not differ significantly. This means a drop in the concentration of reductants of the second phase of reduction (so-called slow reductants) and the lack of significant differences in the concentration of reductants of the first phase of reduction (so-called fast reductants). Since the constant rates of reduction of the second phase k<sub>180</sub> × 10<sup>-3</sup> (s<sup>-1</sup>) for blood plasma before (2.05 ± 0.69; n = 16) and after (1.83 ± 0.41; n = 16) do not differ significantly ( $P_p > 0.05$ ),

it can be acknowledged that in both cases we deal with the same reaction. The numerical value of the  $k_{180}$  constant under similar conditions corresponds to that determined in our laboratory for the reaction with bovine serum albumin (BSA; Sigma;  $k_{180} = 1.79 \pm 0.51 \times 10^{-3} \text{ s}^{-1}$ ;  $n = 5$ ).

While sport horses are objects of many researches, the horses used for recreational purposes are of minor importance for scientists and horse trainers. Usually, the horses prepared for a professional event are put to a rational long-lasting training, which prepares selected animals for an intensive effort. The monitoring of a horse trainability degree is based in such cases on a periodic evaluation of the values of haematological parameters and numerous biochemical indicators, including the concentration of chemical elements (Na, K, Mg, P) and metabolites (total protein, glucose, lactic acid) as well as the activity of enzymes (AST, CPK), while the efficiency of antioxidant defence system is evaluated by measuring the concentration of plasma antioxidants (TBARS, TAR, PAOC, vitamin E and Se) and the activity of antioxidant enzymes (SOD, GPx, GR). Studies carried out in this field in the country and abroad covered mostly the horses used for sport purposes such as long-distance tours, jumps and the Three-Day Event (9, 20, 21) and races (1, 2, 16, 22).

In case of recreation horses it is different. Their selection is frequently random and preparation for the season is generally not preceded by a period of gradual work training and metabolic efficiency examination. It can be only presumed that in horses of that type efficiency indices will be lower and less stable than in sport horses. Even less is known on the status of antioxidant system in recreation horses.

The findings of the present study showed that arduous ten-week-long work activity in a commercial, recreational horse-riding centre contributed to a clear worsening of the health status of horses used there. At the biochemical level, this manifested itself in the secondary thrombocytopenia, decrease of total weight of haemoglobin circulating in blood, dehydration and finally in a decrease of plasma total antioxidant potential, with undisturbed Mg, Fe, Zn and Mn homeostasis and elevated Cu concentration. The observed drop in blood platelet count below the standard is difficult to interpret. Excluding an analytical error, it can be presumed that this acquired thrombocytopenia (secondary thrombocytopenia) is connected with a reduced production and/or excessive destruction of platelets, which may result from the activity of exogenous physicochemical factors, viral infections or auto-allergic response to stress (!). On the other hand, a drop in RBC  $\times$  HGB and HCT can be acknowledged as symptoms of anaemia, which is defined as quantitative or qualitative blood deficit. Usually, it concerns a reduced red blood cell count or a reduced haemoglobin concentration. All types of anaemia lead to fatigue, a decrease in strength and apathy. The symptoms can appear long before the ex-

amination of blood shows that its composition does not correspond to the standards. This means that the examined blood shows a deviation only when changes are advanced enough to induce a discernible pathology. Changes in RBC  $\times$  HGB and HCT were observed by many authors during the training of sport horses. It is said that the increase of RBC  $\times$  HGB and HCT values at rest should be an effect of rational, properly conducted training, whereas a training overload produces the reverse effect (20, 21).

The increase of erythrocyte volume in the blood of horses after the end of season may be explained by the development of hypoalbuminemia, the symptom of which is a shift of water from the vascular bed to tissues. The results of plasma total antioxidant potential measurements as well as the lack of significant changes in the concentration of transient metals, Fe, Zn and Mn, bound in blood with specialised transport globulins, which determine their toxicity or utilisation, corroborate such a conclusion. There may be many reasons for hypoalbuminemia (hypoproteinemia). It is, however, beyond doubt that a negative nitrogen balance in adult individuals is a signal pointing at progressive emaciation. A particularly dangerous consequence of hypoalbuminemia and anhydraemia may be an increase of concentration of transient metal ions present in blood, i.e. Fe, Cu, Zn, and Mn. For they bound non-specifically to albumin and in this combination are transported in blood. In certain situations, ions of transient metals may manifest antioxidant properties, e.g. when catalysing superoxide anion-radical dismutation or reacting with  $\text{OH}^{\cdot}$  radical. In most biological situations, however, transient metals are definitely pro-oxidants.

A simple evaluation of the concentration and correlation of magnesium and four trace metals bound with antioxidant system proteins, i.e. Fe, Cu, Zn and Mn, in the blood plasma of examined horses is difficult due to much variance in information available on that subject in the scientific literature. Adopting as a reference point the ranges of physiological concentrations given by other authors (7, 15, 23), it was determined that Fe and Mn concentrations in the blood plasma of examined horses did not exceed the upper normal range and did not change significantly during the experiment. The concentration of Zn was relatively low and did not change either. On the other hand, Cu concentration increased significantly within the normal range. A reason for high Fe, Cu and Mn concentrations may be a low concentration of Zn. For it is known that this element always occurs on the second oxidation degree and is competitive to  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  ions in reactions with chelating compounds (24). The lack of statistical differences in Mg, Fe, Zn, and Mn concentrations indicates that homeostasis of these elements during the experiment was not upset. On the other hand, the increase of Cu concentration may be a consequence of a rise in the concentration of ceruloplasmin ( $\beta_2$ -globulin) as a response to high iron concentration and/or a response to

increased ROS concentration in blood. Ceruloplasmin may play an important part in the defence against ROS – it removes  $\text{Fe}^{2+}$  ions, preventing at the same time the development of superoxide anion-radical (8, 17).

These conclusions corroborate in part the reports of other authors. The study on horses used recreationally (4) showed significant changes in the concentration of Mg (decrease) and Zn (increase), the values of which were initially within the range of physiological standards but changed significantly during the holiday season (when horses worked intensively), as well as no effect of season on Fe and Cu values. In the examined horses, a low concentration of Cu was found simultaneously, amounting on average to  $7.7 \times 10^{-6}$  mol/dm<sup>3</sup>, thus being considerably below the low normal range (15, 23). As these authors state (4), Cu deficiencies are frequently connected with an excess of Zn in fodder. However, in the blood plasma of stallions fed with a Zn-deficient fodder Danek and Wiśniewski (7) found a significant decrease in the concentration of Zn to  $1.15 \times 10^{-5}$  mol/dm<sup>3</sup> as well as that of Cu to  $2.06 \times 10^{-5}$  mol/dm<sup>3</sup>. A similar relationship is given by Róžański and Saba (18), who, when examining correlations between selected biochemical components in the blood plasma of young horses, found a highly significant positive correlation between Zn and Cu as well as Fe and Mg ( $P \leq 0.001$ ) and no correlation between Zn and Mg, Zn and Fe, Cu and Mg, and Cu and Fe. Reports on the correlation between the concentration of chemical elements connected with antioxidant system in horse blood and their supply in the fodder inspire to look for other factors that could have an effect on the assimilation and metabolism of those elements. These factors may include a seasonal change of environment, change of climate and psycho-physical overload. In our earlier studies (5), no effect of breed was found on the concentration of Cu and Mn in the blood plasma of horses kept in some breeding centres, but mean concentrations of those elements were lower than the low normal range for horses (15) and amounted to  $1.38 \times 10^{-5}$  mol/dm<sup>3</sup> for Cu and  $9.00 \times 10^{-8}$  mol/dm<sup>3</sup> for Mn. There were, however, numerous differences in the concentrations of both elements between respective horse-riding centres. Kabata-Pendias and Pendias (12) report that Mn metabolism is mainly affected by genetic factors. On the other hand, according to Kośła and Anke (14) the concentration of Mn in the horse organism is significantly affected by environment, whereas the breed, age and sex are of no effect.

Summing up the results of the described experiment, it should be stated that monotonous everyday work lasting for several hours a day and many weeks long in a commercial horse-riding centre may lead to chronic fatigue in the horses used there. Apart from general lesions, such as weight loss, unwillingness to work and irritability, clear clinical lesions occur: anaemia, thrombocytopenia, hypoalbuminemia, dehydration, disturbance in homeostasis of certain microelements and

a decrease of plasma total antioxidant potential. As a matter of fact, the latter lesion does not mean a direct exposure to oxidation stress, however when combined with anaemia and a defective distribution of microelements to tissues it indicates a reduction in the work abilities of the organism. In such a situation, horses should not be forced to an intensive effort, e.g. to gallop or jumps, because during intensive aerobic work both oxygen transport system and antioxidant defence system undergo an overload, which is followed by the growing probability of damage to tissues by ROS.

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