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The effect of induced training on selected equine blood plasma indicators on treadmill trained horses

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Abstract: The aim of this study was to evaluate the effect of induced training on the horses' metabolism during an experiment lasting nine weeks where we continually scaled up the load on the horses by three defined stages. Blood was obtained from eighteen horses – two stallions, eight mares and eight geldings. In the experiment, we focused on the biochemical analysis of the blood plasma on multiple mineral profile indicators – Ca, P, Mg, K, Cl and Na, and some other variables (energy, nitrogen, AST, ALT, glucose, urea, creatinine kinase, total proteins). The result showed significant changes between the groups in most indicators. A significant increase in the potassium, phosphorus and calcium and a decrease in the concentrations of magnesium over the course of the experiment were found. For the other indicators, a significant increase in the activities of the AST and ALT out of the other indicators and the fluctuating values in the total proteins were noticed. Summarised, significant changes of multiple indicators were observed in different stages of the experiment. These changes had no visible effect on the horses' organisms throughout entire duration of the experiment and were most probably caused by the muscular work and possible muscular damage during training.

Keywords: stress; horse; biochemical profile; blood; training

Over the course of last several years, horse breeding has mostly focused on sports and leisure activities. Their use as a towing animal has significantly decreased. Large amounts of money have to be invested to obtain individuals with the best performance. The best specimens are utilised in sports as well as in the use of biotechnological methods during reproduction (Halo et al. 2017).

It is important to follow the health status of the horses during the training process, as different types of exercise that suit some animals might not suit other individuals. Training is an extremely important part of the daily routine of sports horses as it maintains the individual's condition and keeps the muscles in shape. Training involves the use of regular periods of exercise to promote

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changes in the structure and function of the animal to improve the overall performance (Jones 2005). Repetitive exercise results in a multitude of changes in the body at the cellular, tissue, organ and whole organism level. The main method used to assess the efficacy of training is to verify the modification of the blood indicators relative to the competition (Hinchcliff and Geor 2004).

The aim of this study was to observe the effect of induced training stress on the horses' metabolic indicators during training on a Horse Gym 2000 regulator and to evaluate the changes in the blood indicators of the horses including the mineral profile, energy and nitrogen profiles. The Horse Gym 2000 training regulator is important in eliminating the effect of adverse weather and to simulate uphill training with its possibility to set an elevation percentage.

MATERIAL AND METHODS

In our study, we analysed the effect of training stress on the heart frequency of the horses during the simulated stress on a Horse Gym 2000 training regulator (Horse Gym 2000 GmbH, Harburg-Großsorheim, Germany). The training regulator is computer assisted with the possibility of setting up the length of the travelled route, the time, the speed and the elevation percentage (Mlynekova et al. 2016). The Horse Gym 2000 training regulator is specifically important for simulating uphill training in an area of a stable, which rules out the effects of bad weather, thus, the horses can be trained indoors as well to maintain the muscularity of the horse. The Horse Gym 2000 training regulator is also used to maintain the speed and prevent an accident during RHR (real horseback riding) (Kim et al. 2016).

The horses were stabled and tested in the Experimental Centre of the Department of Animal Husbandry (Slovak University of Agriculture, Nitra). The experiment was realised from May 2019 until July 2019.

Eighteen Slovak warmblood horses – two stallions, eight mares and eight geldings, all being in the range of 6–14 years of age with an average body mass of 550 ± 50 kg were used in the experiment. All the tested horses were stabled in individual boxes with an average interior temperature of 20–24 °C.

They were fed three times per day at 7:00, 12:00 and 18:00 in a ratio of 25 : 25 : 50 percent. The feed dose consisted of 10 kg of hay per horse per day, 3.5 kg of oats per horse per day and 1 kg of supplementary compound feed per horse per day. All the horses had previously been trained on the training regulator (Table 1).

The stress testing experiment was split into three stages, each taking three weeks.

In the first stage (E1), the observed load was:

- 1st week (W1) – 50 min walking, 0% elevation, 5 km/h velocity;
- 2nd week (W2) – 50 min walking, 0% elevation, 5.5 km/h velocity;
- 3rd week (W3) – 50 min walking, 0% elevation, 6.0 km/h velocity.

Table 1. Scheme of the experiment

Week/activity	Walking (minutes)	Elevation (%)	Velocity (km/h)	Walking (minutes)	Elevation (%)	Velocity (km/h)
Experimental group E1						
W1	50	0	5.0	–	–	–
W2	50	0	5.5	–	–	–
W3	50	0	6.0	–	–	–
Experimental group E2						
W4	50	0	5.5	15	3	5.5
W5	50	0	5.5	15	3	5.5
W6	50	0	5.5	15	4	5.5
Experimental group E3						
W7	50	0	6.0	15	3	6.0
W8	50	0	6.0	15	3	6.0
W9	50	0	6.0	15	4	6.0

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In the second stage (E2), the experimental load was:

- 4th week (W4) – 50 min walking, 0% elevation, 5.5 km/h velocity + 15 min walking, 3% elevation, 5.5 km/h velocity;
- 5th week (W5) – 50 min walking, 0% elevation, 5.5 km/h velocity + 15 min walking, 3% elevation, 5.5 km/h velocity;
- 6th week (W6) – 50 min walking, 0% elevation, 5.5 km/h velocity + 15 min walking, 4% elevation, 5.5 km/h velocity.

In the third part (E3), the experimental load was:

- 7th week (W7) – 50 min walking, 0% elevation, 6.0 km/h velocity + 15 min walking, 3% elevation, 6.0 km/h velocity;
- 8th week (W8) – 50 min walking, 0% elevation, 6.0 km/h velocity + 15 min walking, 3% elevation, 6.0 km/h velocity;
- 9th week (W9) – 50 min walking, 0% elevation, 6.0 km/h velocity + 15 min walking, 4% elevation, 6.0 km/h velocity.

Blood samples were collected from the *vena jugularis* at the end of each experimental stage immediately after the animals finished training. The samples were later centrifuged and the blood plasma was separated.

Throughout the entire duration of the experiment, there were no visual changes to the health state of the horses. The blood of the horses was analysed by a Randox RX Monza biochemical analyser (Randox Laboratories, Crumlin, United Kingdom) using commercial DiaSys kits (Diagnostic Systems GmbH, Holzheim, Germany) (Massanyi et al. 2014; Halo et al. 2017; Kovacik et al. 2017; Kovacik et al. 2019).

Statistical analysis

The data were analysed using GraphPad Prism v6.1 (GraphPad Software Inc., San Diego, USA). The values of the experimental groups were analysed using one-way factor analyses with the application of Tukey's test. All the statistical tests were carried out at the significance levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$ and the results were interpreted as the means expressed with the SD.

RESULTS

A significant increase in the calcium in group E3 compared to E1, as well as a significant increase in the Ca in the E3 group in comparison to the E2 group was found. The highest Ca concentration was found in the samples from group E3. The phosphorus concentrations were also significantly changed. A significant increase in the E3 samples compared to the E1 group was noticed. Intriguingly, the magnesium concentration were the highest in the samples from the first collection – a significant decrease in the magnesium concentration after 6 weeks (E2) compared to the group collected after 3 weeks (E1) was found. The sodium values showed a significant decreasing trend, where the E1 group had a significantly higher sodium concentration than the E2 group, and the E2 group also had a significantly higher sodium concentration than the E3 group. We observed the exact opposite trend in the potassium concentrations where the E1 group showed the lowest concentration. The E2 group had a potassium concentration significantly lower than the E3 group and the E1 group had a significantly

Table 2. Mineral profile during the experiment

Indicator (units)	E1	E2	E3	P-value^{+}
Calcium (mmol/l)	3.06 ± 0.21^{A}	2.79 ± 0.4^{B}	$3.66 \pm 0.44^{A,B}$	ns;$^{***A;***B}$
Phosphorus (mmol/l)	0.79 ± 0.16^{A}	0.91 ± 0.13	0.96 ± 0.25^{A}	ns;^{*A}
Magnesium (mmol/l)	1.08 ± 0.22^{A}	0.94 ± 0.07^{A}	0.98 ± 0.14	ns;^{*A}
Sodium (mmol/l)	142.0 ± 2.49^{A}	141.1 ± 1.76^{B}	$139.2 \pm 1.80^{A,B}$	ns;$^{***A;*B}$
Potassium (mmol/l)	3.70 ± 0.54^{A}	3.73 ± 0.50^{B}	$4.17 \pm 0.38^{A,B}$	ns;$^{*A;*B}$
Chlorides (mmol/l)	103.1 ± 2.57	103.0 ± 1.48	101.8 ± 1.92	ns

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

$^{+}$Within the same row, the means with different letters differ significantly; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{A,B,C}The significance between the individual groups

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lower concentration than the E2 group. The chloride levels did not show any significant changes and stayed within a similar concentration over the course of the entire experiment (Table 2, Figures 1–6).

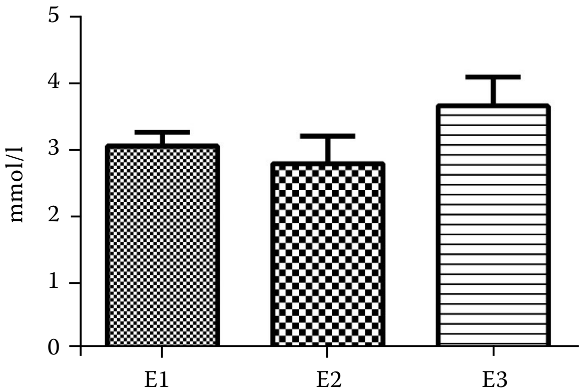

Figure 1. Blood calcium (Ca) concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

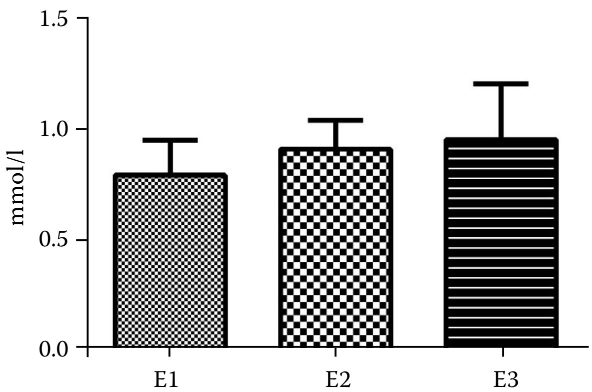

Figure 2. Blood phosphorus (P) concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

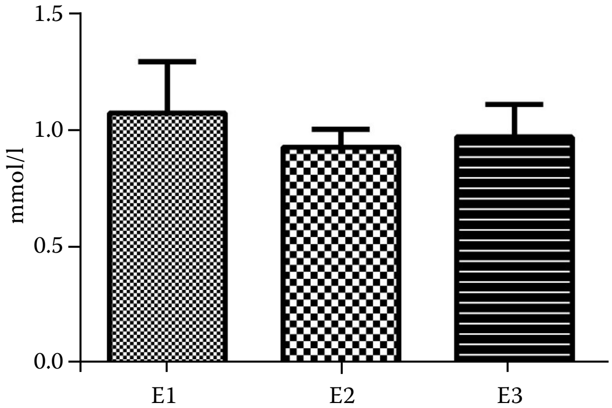

Figure 3. Blood magnesium (Mg) concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

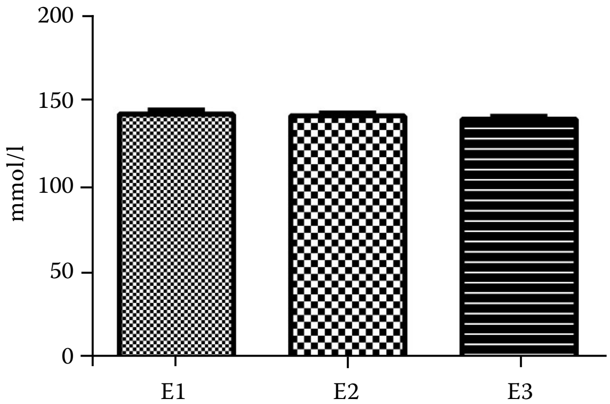

Figure 4. Blood sodium (Na) concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

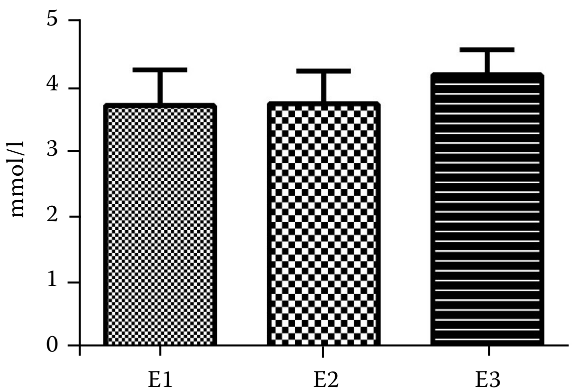

Figure 5. Blood potassium (K) concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

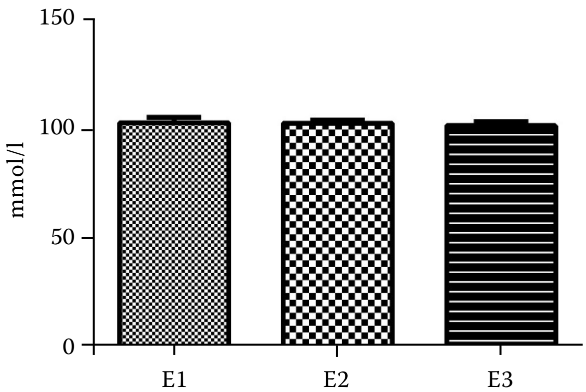

Figure 6. Blood chloride (Cl) concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

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The ratio of the elements was different during the experiment – Ca : P 3.87 (E1), 3.06 (E2) and 3.81 (E3). Also, we checked the Na : K ratio which was different in the experimental groups – 38.38 (E1), 37.83 (E2) and 33.62 (E3), but the differences were not significant (Table 2, Figures 1–6).

The urea concentrations did not change significantly over the course of the entire experiment.

Table 3. Profile of the other indicators during the experiment

Indicator (units)	E1	E2	E3	*P*-value⁺
Urea (mmol/l)	3.91 ± 0.58	4.42 ± 0.79	4.45 ± 0.76	ns
Total proteins (g/l)	62.86 ± 4.37^{A}	57.67 ± 9.08^{A,B}	64.12 ± 3.68^{B}	ns$^{*A;**B}$
Glucose (mmol/l)	5.88 ± 0.33	5.86 ± 0.26	5.50 ± 0.84	ns
AST (μkat/l)	4.60 ± 0.47	4.19 ± 0.43^{A}	4.96 ± 0.70^{A}	ns^{***A}
ALT (μkat/l)	0.14 ± 0.05^{A,B}	0.21 ± 0.06^{A,C}	0.28 ± 0.07^{B,C}	$^{**A;***B;**C}$
CK (μkat/l)	4.48 ± 1.83	4.96 ± 1.80	4.82 ± 1.92	ns

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatinine kinase; E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

⁺Within the same row, the means with the different letters differ significantly; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{A,B,C}The significance between the individual groups

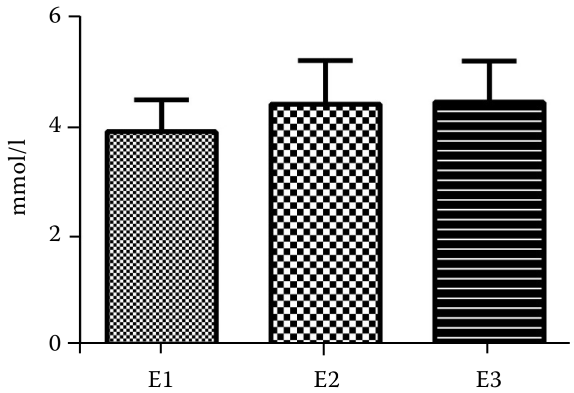

Figure 7. Blood urea concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

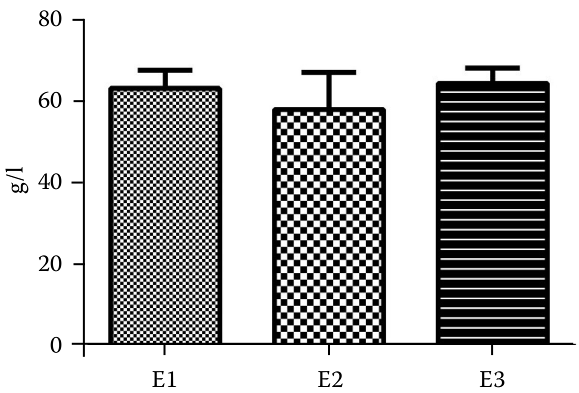

Figure 8. Blood total protein (TP) concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

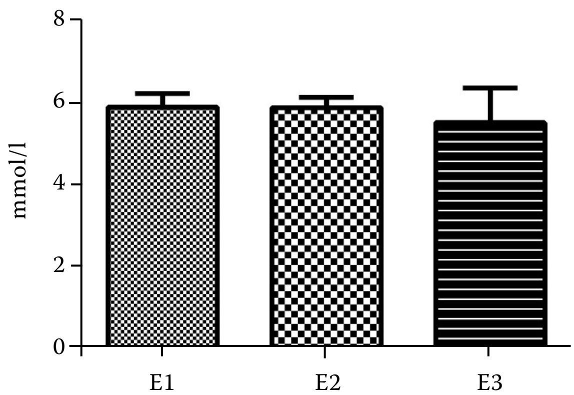

Figure 9. Blood glucose (Glu) concentrations during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

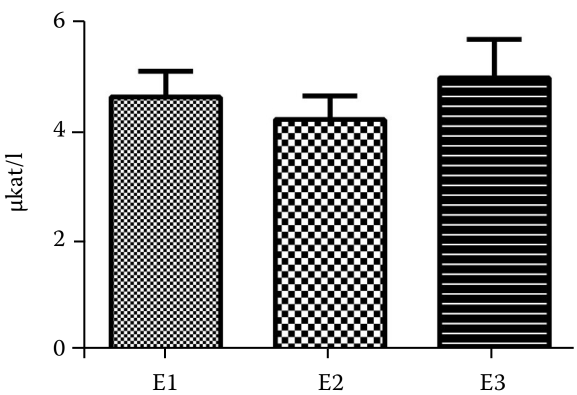

Figure 10. Blood aspartate aminotransferase (AST) activities during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

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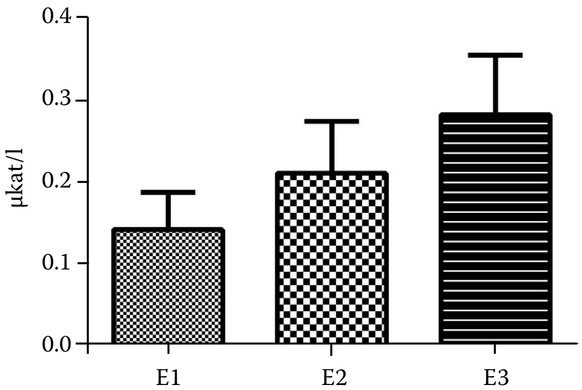

Figure 11. Blood alanine transferase (ALT) activities during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

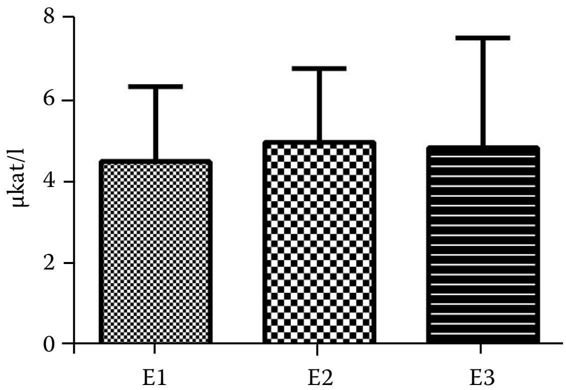

Figure 12. Blood creatine kinase (CK) activities during the training stages of the horses

E1 = experimental group 1; E2 = experimental group 2; E3 = experimental group 3

An increasing tendency was detected during the experiment which may have been caused by the changing water to urea ratio in blood during the training load. The total protein concentration significantly decreased in the samples from the second sample collection compared to the ones gathered after the first stage. The E2 group also had a significantly lower total proteins (TP) concentration compared to the E3 group. We did not spot any significant changes in the glucose concentrations over the duration of the experiment. The same trend was observed for the creatinine kinase. For the additional variables – the aspartate aminotransferase (AST) values significantly increased in the E3 samples compared to the E2 group. The alanine aminotransferase activities were the highest in the E3 group – significantly higher than the E1 and E2 groups; also, the E2 group had a significantly higher concentration than the E1 group (Table 3, Figures 7–12).

The animals did not suffer any injuries over the duration of the experiment that could have affected results.

DISCUSSION

The statement by McKeever et al. (1987) that the potassium concentrations in the plasma significantly decreased during training differs from the values that we obtained. Nielsen et al. (1998) found that there was an increase in the calcium retention in Quarter Horses and it proved beneficial in facilitating the bone remodelling process and overall skeletal growth. We also measured a significant increase in the calcium concentrations during the training process. During the recovery phase – the Na, Cl, and Ca concentrations and TP values were still higher than the basal values (Lindinger et al. 1995). We also found a similar tendency in our samples except for chloride where no changes were observed.

Halo et al. (2009) observed that the concentrations of the minerals in sport horses during training was 0.96 mmol/l for phosphorus, whereas, in our experiment, it varied from 0.79 mmol/l to 0.96 mmol/l, which are both within the reference values for horses. The same authors also observed magnesium concentrations of 0.26 mmol/l, where our samples varied from 0.94 to 1.08 on the other hand. The reference range for magnesium (Johansson et al. 2003) in the blood serum of horses is 0.69–1.08, thus, our samples are within the reference range.

In a different study by Halo et al. (2008), they measured the glucose concentrations during horse training and found values of 4.101 mmol/l and 3.126 mmol/l in different groups. With a higher load, the glucose concentrations decreased to 3.677 mmol/l and changed to 5.085 mmol/l in the last stage. In our experiment, higher concentrations were found compared to this study ranging from 5.50 mmol/l to 5.88 mmol/l. In the same study, alanine aminotransferase activities were also measured – ranging from 0.077 µkat/l to 0.142 µkat/l with an increased load. In our experiment, some higher values of 0.14 µkat/l to 0.28 µkat/l were found compared to the increased load stages.

Octura et al. (2014) observed the elevation of blood creatine kinase and selected blood indicators after exercise in thoroughbred racehorses and found that the activity of creatine kinase was 2.75 µkat/l before

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the exercise and 9.50 µkat/l after the exercise. Our values do not correspond with those after training, as they were much lower in all the stages of the experiment – ranging from 4.48 µkat/l to 4.96 µkat/l.

The average Ca : P ratio of horses is approximately 3 : 1 in equine blood (Massanyi et al. 2014). In our experiment, the values ranged from 3.1 : 1 to 3.8 : 1 which is slightly higher, but the measured values are within the reference ranges. A similar trend in the Na : K ratio, where the ratio is 34 : 1 in horse blood (Massanyi et al. 2014) was detected and, in our samples, it ranged from 33.3 : 1 to 38.3 : 1, where the measured values were within the reference ranges for horses. Changes in magnesium in the blood may be caused by muscular damage or by the dilution ratio of the water to magnesium in the blood (Massanyi et al. 2014).

Tyler-McGowan et al. (1999) found that the AST activity in their experiment significantly changed in the horses that underwent overload training only and they interpreted the results that they were due to the muscle damage. In our experiment, a significant increase occurred in the AST activity in the E3 group compared to the E2 group. It has been stated that overtraining may be associated with multiple types of injuries as well as prolonged fatigue and poor performance (Kibler et al. 1992). The creatinine kinase activity changes following training (muscular exercise) as well as after suffering muscle damage (Siciliano et al. 1995; Valberg et al. 1999; Balogh et al. 2001). In humans, the highest urea serum concentrations have been measured in the competitive phase when the performance level is the highest (Manna et al. 2010). Most studies have shown that the increase in the TP in horses is a results of racing exercises, competitions and training programmes (Rose et al. 1983; Art et al. 1990; Jablonska et al. 1991; Sommardabl et al. 1994; Dahalborn et al. 1996; Schott et al. 2006). The increase in the TP can be explained by a loss of the extracellular fluid, a decrease in the blood volume and haemoconcentration. All the concentrations of minerals were within the reference values over the entire duration of the experiment and the changes could have been caused by muscle work during the training. Jumpers tend to exceed the reference mineral values compared to recreationally used horses, most probably because of their metabolism and high stamina. This could be a reason for the changes in the blood profile (Winnicka 2011). Equine sweat is rich in potassium and profuse sweating can cause hypokalaemia. Also, the blood potassium might be diluted by the fatigued animals drinking water, who require a high water intake after a performance (Larsson et al. 2013). The TP values also increase with physical activity in show jumping horses and racing horses compared to recreational horses (Szarska 2003). Thus, the changes in our horses' blood can be caused by the higher training load.

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in our experiment did not change too much from the control values and are all within the reference ranges. AST and ALT are hepatic enzymes that indicate the proper function of the liver (Szarska 2003). Miglio et al. (2020) stated that long-term training has a significant effect on the metabolism. In their experiment, they found similar decreasing tendencies in the blood sodium concentrations and an increasing trend in the calcium concentrations that we did in our experiment. Generally, the measured values reached very similar values to the ones measured in our experiment.

In another experiment, an anti-inflammatory state in Arabian horses introduced to endurance training was observed. In their study, the total protein concentrations ranged from 62–70 g/l and were slightly increasing with an increasing training duration, which is the same as in our experiment, where the values ranged from 57.67–64.12 g/l (Witkowska-Pilaszewicz et at. 2019a). In another study, Witkowska-Pilaszewicz et al. (2019b), stated that changes in the TP occur mostly in endurance horses, but not in race horses due to the total osmolar increase in the plasma to remain constant during the plasma volume expansion due to training-induced hypervolemia. The TP levels were increasing over the entire duration of the 7-month long training programme in endurance horses during the first training season. Our changes might have been caused by the current health state of the animals over the course of the experiment.

The goal of this study was to observe the effect of induced training stress on the equine blood profile. Specific biochemical analyses were selected for the evaluation of the individual profiles. Using these techniques, we can conclude that multiple significant changes in the blood indicators were observed during the experiment.

From our results, it can be concluded that most of the physiological parameters, which confirm

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the increased stress on a horse's organism and many of the parameters could possibly be increased due to the muscular work or muscular damage.

To summarize, it can be concluded that the training stress affects the physiological parameters of the blood. Multiple changes in blood profile can be caused by muscular work or even muscular damage training. Especially the parameters of mineral profile which especially when depleted should be replenished in animals before additional training.

Conflict of interest

The authors declare no conflict of interest.

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