HAEMATOLOGICAL AND BIOCHEMICAL VALUES IN RELATION TO TRAINING PROGRAMME IN THOROUGHBRED RACEHORSES*

H. Härtlová[1], R. Rajmon[1], D. Řehák[2], M. Petrtýl[1], V. Luka[1]

[1]Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Department of Veterinary Sciences, Prague, Czech Republic
[2]Research Institute of Animal Production, Prague, Czech Republic

Seven healthy thoroughbred horses were under observation for eight months at regular one-month intervals. Biochemical and haematological analyses were carried out in relation to various types of exercise – low-, moderate- , and high-intensity.A significant higher activity of LD (lactate dehydrogenase) was found in the period of maximum exercise. Individual excesses of CK (creatine kinase) activity appeared in the same period. The activities of CK and LD reflected the instantaneous state of the muscle damage. Stable activity of AST during the entire monitored period means adequacy requirement for monitored horse. The concentrations of Na^+ ions were significantly lower in the period of moderate-intensity exercise with a subsequent intense increase soon after the beginning of the high-intensity exercise period. The K^+ concentration dropped twice – at the beginning of the whole training program and after the beginning of the high-intensity exercise period. Maximum attention should be given to mineral supply in these phases of the training programme. Haematological parameters first indicated an organism adaptation to training process just at the beginning, later under high intensity of exercise both the electrolyte values and the haematological parameters were obviously influenced by the onset of hypervolemy. For evaluation of these indicators it is necessary, not only to correct the obtained values with respect to the actual TP (total protein) concentration, but also to reflect this in the interpretation of the results.

horse; training period; biochemical-haematological profile; adaptation

INTRODUCTION

The activities of plasmatic aspartate aminotransferase (AST) and creatine kinase (CK) are used as indicators of skeletal muscle overtraining or damage during exercise because they increase when cells are damaged (Lindholm, 1987; Noakes, 1987; Harris et al., 1998). Evaluation of changes activities of CK and AST can respect fact that half–life of activity CK in circulation is very short – 2 hours and even marked elevations in CK may returned to normal within 12 to 24 hours after a single muscle insult. On the other hand activity of AST is relatively long and elevation may persist for a long time after episode of muscle damage (Duncan, Prasse, 1994). Van Der Muelen et al. (1991) showed increased serum activities of AST, CK, and also LD (lactate dehydrogenase) in rats with histological findings of damaged muscle cells, too. However, indicated damage to skeletal muscles in this way are not always accompanied by clinical signs (Rej, 1977). Siciliano et al. (1995) compared the changes in AST and CK activities in horses in relation to their fitness. A comparison of trained and untrained horses after exercise revealed lower AST and CK activities in the trained horses. Exercise stress in untrained horses increases the permeability of sarcolemma, and muscle cells are thus more prone to damage (Clarkson, Tembley, 1988). Valberg et al. (1993) studied the changes in AST and CK activities during various types of exercise. These activities were higher after submaximal exercise. This may be due to the fact that submaximal exercise, i.e. increasing oxidative metabolism, results in the increase of oxygen radicals and may be a potential cause of damage (Sjodin et al., 1990). Guy and Snow (1977) studied the activities of LD and its isoenzymes in relation to the training period. The activities increased in the second half of this period. Harris et al. (1990), on the contrary, observed very small changes in enzyme activities in relation to various types of exercise. Similarly, Rej et al. (1990) studied AST activity in 2–3-year-old thoroughbred horses during the period of their high-intensity exercise. They found only a moderate increase of AST activity induced by AST cytosol isoenzymes.

The intensity and duration of exercise also influence the electrolyte balance (Wilkerson et al., 1982). During a strong muscle contraction, potassium is released from the working muscle into the extracellular fluid (Sjøgaard et al., 1985). Nevertheless, hyperkalemia promptly returns to normal when exercise stops (Carlson, Nelson, 1976; Kjellmer, 1965). The release of potassium from cells during the exercise depends on the rate of acidosis. This is very important with horses, in which high lactate concentrations are reached during exercise (Snow et al., 1985). The loss of in-cellular potassium, together with a water shift, result in changes in the membrane potential and play a role in overall exhaustion. The

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changes in Na^+ concentration are not as marked as those in K^+. However, these values must be corrected according to the total protein (TP) concentration in order to obtain the values of circulating sodium (Sjøgaard et al., 1985). According to Snow (1987), increase in the TP and haematocrit value (PCV) indicates a decrease in plasma content. However, the haematocrit values could be influenced by the horses' stress during the collecting of blood. The variability depends on the spleen tonus controlled by the alpha adrenoreceptors of the symphaticus. Every change in symphaticus activity also influences the values of haemoglobin and haematocrit in the rest period (Lucke, Hall, 1980; Persson, 1983). Van Heerden et al. (1990) did not find any significant differences in haematocrit values in healthy thoroughbred horses with regard to differences in their exercising and age. Significant differences were found between mares and stallions and between the haematocrit of calm and easily excited horses.

The purpose of this study was to evaluate the influence of increasing exercise load during the training period on AST, CK, and LD activities, electrolyte concentrations, and haematological parameters together.

MATERIAL AND METHODS

A total of 7 English thoroughbred horses weighing 500 ± 30 kg were used in our studies. Feeding doses were related to the rate of the workload (Table 1). Training records of each horse under study were kept by its trainer (Table 2). Examinations were performed at regular one-month intervals from December, always in the morning following the day with standard exercise corresponding to each period. Blood samples for biochemical and haematological examinations were collected at 8 a.m., 1.5 hours after feeding, before that day exercising and were immediately processed in the laboratory. They were collected from the *v. jugularis* into a vacutainer system. Biochemical analyses were carried out using commercially produced sets and an automatic Hitachi 911 analyzer. AST was determined by a two-step method. The rate of NADH oxidation was proportional to the catalytic AST activity. The total activity rate LD was demonstrated using the transformation of pyruvate to lactate. The rate of NADH oxidation was proportional to LD activity. The determination of CK was made using the principle of a three-step enzyme reaction. The rate of NADPH formation was proportional to creatine kinase activity. The concentrations of Na^+, K^+, and Cl^- ions were determined by ion-selective electrodes. A coulter CBC-5 analyzer was used for haematological examinations. A statistical evaluation was made by a mixed linear model with repeated measures at the 95% significance level.

RESULTS AND DISCUSSION

The activities of CK and AST (Table 3) were higher than those considered by Harris (2000) to be normal (CK lower than 1,7 $\mu kat.l^{-1}$ and AST lower than 5.0 $\mu kat.l^{-1}$) even during the resting period and low-intensity exercise at the beginning of the training period. Nevertheless, the cited reference values appear rather low. Some other

Table 1. Composition of feeding doses during experiment

Nutriments	Resting period	Training period – intensity of exercise		
		low	moderate	high
Dry matter (kg/day)	8.0	8.76	8.8	9.7
N-substances (g/day)	762.8	846.2	940.8	988.3
Sek (MJ/day)	87.31	93.33	101.27	120.29
Na (g/day)	15.23	7.96	10.06	9.06
K (g/day)	86.5	99.0	85.0	85.0

Table 2. Training workload of individual horses

	Month	XII	I	II	III	IV	V	VI	VII
Horse	1	R	L	L	M	M	–	–	H
	2	R	L	L	M	M	H	H	H
	3	R	L	L	M	M	H	H	H
	4	R	L	L	M	M	H	–	–
	5	R	L	L	M	M	H	H	–
	6	R	L	L	M	M	H	H	H
	7	–	–	–	M	M	H	H	H

R – resting period
L – low-intensity exercise: gallop up to 500 $m.min^{-1}$, trot to 20 min
M – moderate-intensity exercise: gallop up to 700 $m.min^{-1}$, trot to 40 min, regular training (at least 6 times per week)
H – high-intensity exercise: gallop up to 1000 $m.min^{-1}$, trot to 20 min, regular training, run

Table 3. Biochemical and haematological parameters in racehorses at different training workloads

Parameter	Resting period ($n = 6$)		Training period – intensity of exercise					
			low ($n = 12$)		moderate ($n = 14$)		high ($n = 23$)	
			$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
LD ($\mu kat.l^{-1}$)			7.98^{a,b}	± 1.61	7.79^{a}	± 1.59	9.37^{b}	± 1.97
CK ($\mu kat.l^{-1}$)	3.84	± 1.13	5.04	± 3.54	7.24	± 7.86	10.02	± 13.14
AST ($\mu kat.l^{-1}$)	5.80	± 0.92	5.07	± 0.57	5.67	± 1.03	5.69	± 1.01
Na^+ ($mmol.l^{-1}$)	140^{a}	± 1.41	138.6^{a}	± 1.16	134.2^{b}	± 3.19	140.3^{a}	± 3.71
K^+ ($mmol.l^{-1}$)	3.56^{a}	± 0.46	3.11^{b}	± 0.23	3.52^{a}	± 0.34	3.53^{a}	± 0.37
Cl^- ($mmol.l^{-1}$)	103.5^{a}	± 1.76	100.9^{b}	± 2.19	101^{b}	± 1.04	104.4^{a}	± 2.81
TP ($g.l^{-1}$)	62.22	± 2.20	62.51	± 3.70	61.79	± 2.69	61	± 4.23
RBC ($T.l^{-1}$)	7.49^{a}	± 0.65	8.63^{b}	± 0.45	8.12^{ab}	± 0.92	7.86^{a}	± 0.57
Hb ($g.l^{-1}$)	13.72	± 1.13	14.45	± 0.85	14.56	± 0.97	14.01	± 1.13
PCV ($l.l^{-1}$)	0.377^{a}	± 0.044	0.441^{b}	± 0.033	0.415^{ab}	± 0.048	0.405^{a}	± 0.036

The values of each parameter signed with a different index differ at the significance level $P = 0.05$

authors mention borderline values of these enzyme activities as higher, for example Malý and Sekaninová (1995). Maximum exercise resulted in a significant increase in LD activities (Table 3). The average activity of CK increased only insignificantly, but in individual cases some values above 30.0 $\mu kat.l^{-1}$ appeared during this period, accompanied by LD activity values above 9.0 $\mu kat.l^{-1}$. These changes in CK and LD activities reflected higher demands on the muscle cells (Rej, 1977; Guy, Snow, 1977; Valberg et al., 1993). It can be assumed that the greater permeability of the cell membranes was caused by increasing oxidative stress (Davies et al., 1982; Jackson et al., 1985). According to Valberg (1998), the increase in CK activity up to 35 times after the exhausting load does not yet indicate myopathy. On the other hand, Harris (2000) state that 2–6 hours after the workload CK activity should not exceed twice the resting values. This corresponds with the fact that most of the CK activity values obtained during the high workload period did not differ from the data of the earlier periods. But the excesses in CK activity mentioned above indicate an abnormal situation, although overloading rather than damage to the muscle cells was probably involved (Valberg, 1998; Volfinger et al., 1994). AST activities (Table 3) did not differ significantly among the individual types of exercise. Similarly Harris et al. (1998) also demonstrated very small changes in AST activities in relation to the type of exercise, and according to Hamlin et al. (2002), plasma concentrations of creatine kinase and aspartate aminotransferase showed no clear-cut change with overtraining.

The start of the training program and increasing workload markedly influenced the serum electrolyte levels – concentrations of all three determinate ions were significantly lower during the low or moderate exercise intensity periods (Table 3); Na^+ and K^+ concentrations close to the minimal values considered to be normal (Judson et al., 1983; Snow, 1987). Nevertheless, the dynamism of the changes was rather different in the individual ions (Fig. 1) – potassium dropped immediately after the beginning of the training programme in January. In March – the beginning of the moderate-intensity exercise period – its concentration returned to the original level, but another decrease followed again in May at the beginning of the high-intensity exercise period. The following months' values were over the initial ones. The lowered K^+ concentration might appear curious with respect to a standard increase of this kation plasma concentration during exercise (Sjøgaard et al., 1985). But it has to be kept in mind that a rapid transfer of K^+ into the intracellular medium after termination of the exercise, and thus to the resting value, is usual (Katz et al., 1985). Coenen (2005) states that temporarily higher K^+ extracellular levels lead to its higher renal excretion and the same author recommends to balancing the K^+ deficit after exercise.

Na^+ concentration decreased gradually with the continuation of the training programme. Minimum values were obtained in April and May around the time of the change from moderate- to high-exercise intensity. Nevertheless, a sharp increase then followed to the upper limit of reference values, i.e. above the initial value; later on it decreased slightly. The chloride values fluctuation showed the similar pattern as the K^+ and Na^+ concentration changes – minimum values in February and April – but in June and July, on the contrary, its values increased above the initial one. TP concentration (Fig. 1) remained constant in the same period, or an opposite trend of fluctuation appeared compared to ion concentration, so that the changes in electrolyte levels described above were probably not induced by plasma volume shift (Harris, Snow, 1988). The cause of the Na^+, Cl^- ion decrease might be changes in the internal environments of the organism, when the production of acid metabolites (lactate, keto substances, phosphoric acid) induces a transfer of sodium and chloride ions to the cells (Judson et al., 1983; Hyyppa, Poso, 1998). Nevertheless, changes in the gastrointestinal tract and renal function also cannot be excluded (McKeever et al., 2002; Lindner et al., 1983). The electrolyte concentration increase observed in our experiment during the high-intensity exercise period was accompanied by a si-

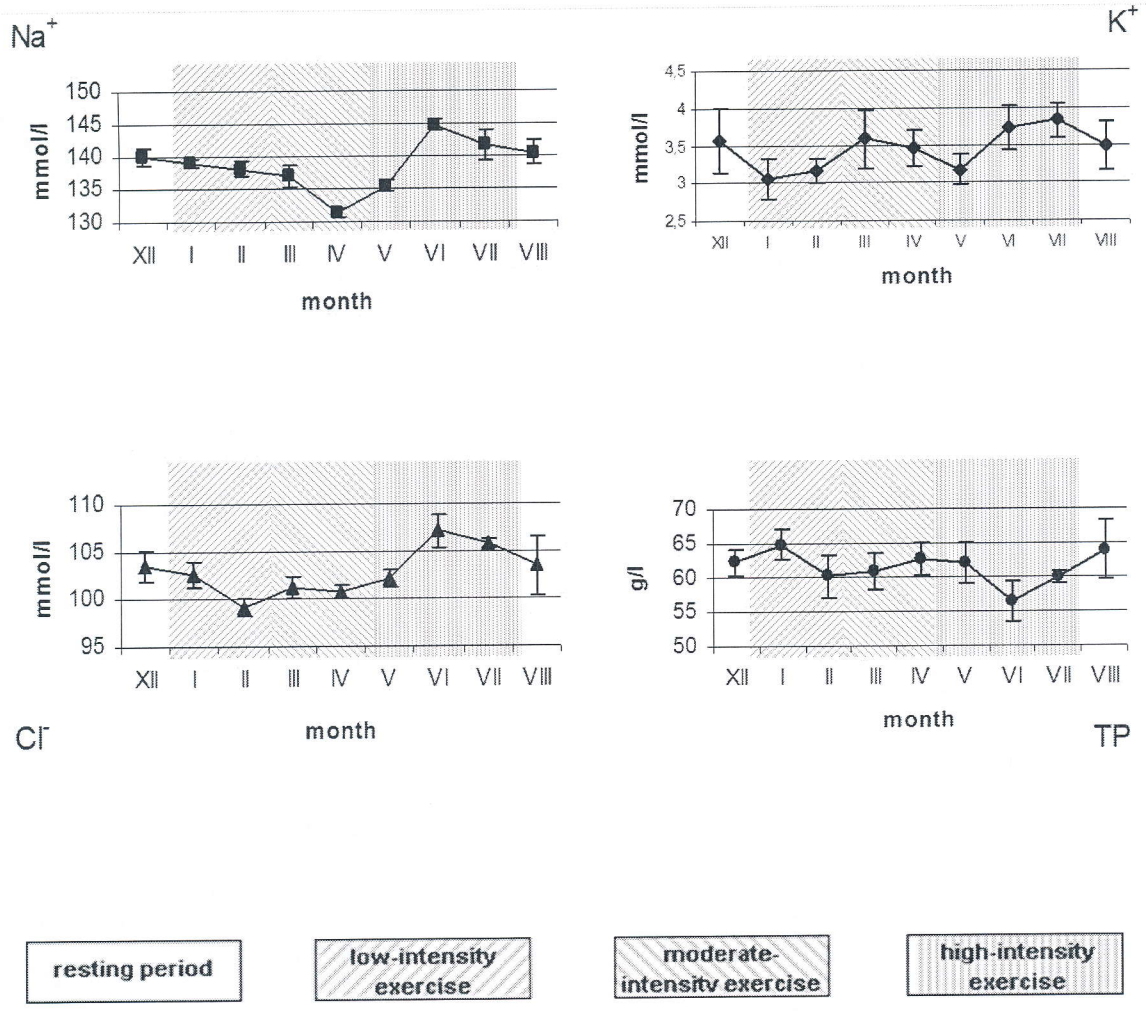

Fig. 1. Dynamism of electrolytes and TP concentrations changes

multaneous drop in TP concentration. The TP decrease could be a result of a training-induced hypovolemy seen in horses (McKeever et al., 1987). This hypothesis is also supported by the results of haematological tests. In such a case the observed changes in Na^+ and Cl^- concentrations might be secondary with regard to blood normoosmolality maintenance. Nevertheless, our data are not sufficient to prove this hypothesis significantly. The second explanation of TP decrease might consistin insufficient intake of N-substances in diet during the critical period.

Although the obtained values of checked haematological parameters were always in published reference intervals (Snow, 1987), significant differences from pre–training values were found in the number of erythrocytes (RBC) and haematocrite values (PCV). There was a progressive increase in these parameters immediately at the beginning of the low-intensity exercise; later a slow decrease to almost resting values appeared. Splenic contraction is dependent on the workload, as this determines the increase in sympathetic activity (Lucke, Hall, 1980;Persson, 1983). An increased proportion of circulating red blood cells results in an increased capacity for oxygen transportation to working muscles. (Snow, 1987). The higher values observed, then, reflect an adaptation process in the horses in relation to the training workload (Snow, 1987). The later value decrease might be at least partially connected with hypovolemy mentioned above.

CONCLUSION

In conclusion, the exercise intensity level influenced the LD activity more than it did all the traced enzymes. The CK activity is sensitive indicator of the instantaneous state of the organism. Stable activity of AST during the entire monitored period means adequacy requierement for monitored horse. The increase of the training workload induced a drop in the electrolyte concentration, so maximum attention should be given to mineral supply as soon as the early phase of the training programme. Haematological parameters indicated an organism adaptation to higher demands on the oxygen transport system in the L-period. During the later phase of the training programme under high-intensity exercise, both the electrolyte values and the haematological parameter ones were influenced

by the onset of hypovolemy. For evaluation of these indicators it is necessary, then, not only to correct the obtained values with respect to the actual TP concentration, but also to reflect this in the interpretation of the results.

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HÄRTLOVÁ, H. – RAJMON, R. – ŘEHÁK, D. – PETRTÝL, M. – LUKA, V. (Česká zemědělská univerzita, Fakulta agrobiologie, potravinových a přírodních zdrojů, katedra veterinárních disciplín, Praha; Výzkumný ústav živočišné výroby, Praha, Česká republika):

Hematologické a biochemické hodnoty plnokrevných koní ve vztahu k zátěži.

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Bylo sledováno sedm plnokrevných koní po dobu osmi měsíců. Krev byla odebírána v pravidelných měsíčních intervalech. Hodnoty zjištěné při prvním odběru byly považovány za referenční. Biochemicko-hematologické analýzy byly hodnoceny ve vztahu ke třem typům zátěže – mírné , střední a intenzivní.

Signifikantně vyšší aktivity enzymu laktátdehydrogenázy (LD) byly zjištěny v období intenzivní zátěže. Ve stejném období byla zaznamenána individuální zvýšení aktivit enzymu CK (kreatinkinázy), zatímco aktivity enzymu AST (aspartátaminotransferázy) se během narůstající zátěže významně neměnily. Aktivity enzymů LD a CK odrážejí momentální změny v pracujícím svalu, které zátěž navozuje. Stabilní aktivita enzymu AST, s výrazně delším poločasem rozpadu v cirkulaci, poukazuje na adekvátnost zatížení sledovaných koní. Také koncentrace elektrolytů (Na^+, Cl^-, K^+) byla ovlivněna zátěží, což se projevilo ve snížení sodíkových, chloridových i draslíkových iontů při střední zátěži. Následně došlo ke konsolidaci hladin elektrolytů. Domníváme se, že zvláště v období nástupu zátěže je třeba sledovat suplementaci dostihových koní minerálními látkami. Hematologické parametry prokázaly na počátku tréninkové periody signifikantní vzestup, který koreluje s adaptací organismu na zátěž. Hodnoty hematokritu na počátku maximální zátěže jsou ovlivněny jednak individuálními výkyvy a jednak zjištěnou hypervolémií. Hematologické parametry je vhodnější sledovat přísně individuálně a jako referenční použít klidové hodnoty před zahájením tréninkové periody.

koně; zátěž v tréninkovém období; biochemicko-hematologické parametry; adaptace

Contact Address:

MVDr. Helena Härtlová, CSc., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, katedra veterinárních disciplín, Kamýcká 129, 165 21 Praha 6-Suchdol, Česká republika, e-mail: hartlova@af.czu.cz