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Fitness improvement of show jumping horses with deep water treadmill training

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ABSTRACT: Athletes, including equine athletes need high intensity training in order to achieve high performance. However, continuous high intensity training often results in injuries to the locomotive system. The buoyancy of water reduces the force born on joints; therefore, training in water has been used for rehabilitation purposes both in humans and horses. The few studies dealing with water treadmill training of horses suggest that the fitness of horses can be improved using this training method, but none tested the subsequent performance of horses after water training. Therefore, the aim of this study was to test the effect of water training of varying intensity on the fitness-related parameters of show jumpers during training and after competition. Four similarly trained show jumper sport horse (aged between 7–11 years) competing at the same level (110 cm) were selected. Horses were subjected to 44-min deep water treadmill training with three intensities (9, 11, 13 km/h maximum speed) three times a week in addition to their normal training. At the conclusion of the week, horses participated in a two-day indoor show jumping event. Blood samples (4 ml) were taken from the jugular vein during the third water training and before and after the completion of the show jumping course on each day. From the blood plasma, lactate dehydrogenase (LDH), creatine kinase (CK) and aspartate aminotransferase (AST) activities, as well as lactate, glucose and triglyceride levels were determined. Data analysis was carried out with SAS (SAS Inst. Inc., Cary, USA) using the GLM procedure and Duncan's new multiple range test. Pearson correlation coefficients were calculated between the same blood parameters from different sampling times. No interactions were detected between training intensity and sampling time during water training. Plasma lactate and glucose levels were decreased during the water training, while values increased afterwards. In contrast, heart rate, triglyceride and cortisol levels were elevated as a result of water training. Increasing the maximum speed of the water treadmill had no influence on the average heart rate of horses subjected to the training. Plasma lactate levels decreased with the increased maximum speed of the water trainer. Activities of AST, CK, LDH, and levels of cholesterol, cortisol and bilirubin decreased when the maximum speed of the treadmill was set to 11 km/h compared to the 9 km/h training. Water training resulted in lower heart rate measured right after completing the show jumping course when horses were subjected to medium intensity water training. The AST, CK and LDH activities measured before and after water training had only weak to moderate positive correlations with values measured after competition. In conclusion, our results indicate that deep-water training alters the biochemical processes and can improve the aerobic energy supply of show jumpers. Water training is a strenuous exercise, which initially leads to increased muscle damage. However, this initial phase is followed by subsequent adaptation.

Keywords: equine; water training; show jumping; haematochemical indicators

The physical condition achieved by regular training is very important for every athlete, including equine athletes. Horses have a great capacity for physical work; however, physical exercise induces various physiological responses and metabolic adaptations (Arfuso et al. 2016). Specifically, depend-

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ing on the intensity, duration and different types of physical exercise, equine metabolism has to adapt to nervous, cardiovascular, endocrine and respiratory system requirements in order to restore homeostatic equilibrium (Casella et al. 2016). In equine sport medicine, the effects of stress related to exercise are generally associated with poorly understood disorders. Monitoring of alterations in the haemogram and plasma or serum biochemistry has been used to assess the health status and fitness level, as well as to investigate cases of poor performance in athletic horses (Piccione et al. 2015). Training in water was first used in the rehabilitation of human athletes. The buoyancy of water decreases the static load on the joints, but allows the muscles to develop anaerobic capacity. The training of horses in water is not a novel concept, but recently there has been increased interest in the possible use of water treadmills for horses, based on the belief that they could be used both for rehabilitation and for training purposes. Several studies have been performed to test the effect of a water treadmill on metabolism using mainly heart rate and lactate as indicative variables (Nankervis et al. 2008; Lindner et al. 2010; Lindner et al. 2012). The swimming training of eventing horses led to a significant elevation in oxygen-transport parameters and lactate production (Knudsen and Jorgensen 2000). Horses are generally exercised on an open field or indoors, and are exposed to numerous other factors such as the rider, other horses, weather, spectators, decorations, terrain, etc. (Serrano et al. 2001). Several studies have shown that physiological responses to treadmill exercise do not replicate responses to field exercise. Plasma lactate concentrations in Standardbred horses pulling a 10-kilopond draught load were lower on the treadmill than on the racetrack (Gottlieb-Vedi and Lindholm 1997), while blood lactate in trotters was lower during exercise on a level treadmill than during exercise on a racetrack (Courouce et al. 2000). In sport horses, it has also been found that blood lactate concentrations were lower on a level treadmill compared with during exercise over ground (Sloet van Oldruitenborgh-Oosterbaan and Barneveld 1995). Therefore, it is important to find indicative variables which allow the monitoring of progress in training as well as close correlation with performance indicators measured during competition. The literature regarding equine athletes are relative abundant with respect to Thoroughbreds, endurance and eventing horses. The few field tests with show jumpers dealt with more experienced horses competing in 130–150 cm classes (Art et al. 1990a; Art et al. 1990b). Thus, the aim of this study was to test the effect of increasing water treadmill training intensity on the heart rate and several plasma biochemical parameters (as indicators of fitness) of lower level show jumpers during water training and after competition.

MATERIAL AND METHODS

Experimental animals. Four similarly trained show jumper horses with ages between seven and 11 years, and competing at the same level (110 cm), were selected. Horses were housed in individual boxes (3 × 3 m). The daily feed allowance consisted of 12 kg meadow hay and 2.6 kg concentrate which provided 134.5 MJ DE and 1042 g crude protein.

Training program. Selected horses were competing during the winter period (from October to February) at the Indoor Show Jumping Championship at the Pannon Equestrian Academy (Kaposvar, Hungary). Test horses participated in three experimental training programs, which were preceded by a two-week standardised training program. During the first training period, no water training was applied before the competition (conventional training served as control). During the subsequent three experimental periods horses were subjected to water training of increasing intensity three times in the week before competition (Table 1). During the event, horses completed one course daily, where the height of the obstacles was 110 cm, the length of the course varied between 400–450 m (was the same for all horses in each particular competition), and the required speed was 350 m/min. The training program was discussed with the riders and trainers in order to assure that water training did not cause unacceptable disturbances to the usual daily program. Therefore, it was decided that horses should be water-trained

Table 1. Training program of the 14-day (Day 1 = Monday) experimental periods

	Day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Normal training	x	x		x		x		x	x			x		
Jumping training			x		x					x				
Water training								x	x		x			
Competition													x	x

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between the morning and noon feeding. Horses were trained according to the schedule presented in Table 1. Normal training was one hour with the rider, while jumping training consisted of half an hour of warming up and half an hour of jumping with the rider. The protocol of the 44-min long water training (performed three times a week) and blood sampling is reported in Table 2. The enrolled horses participated in a previous trial, and, thus, no adaptation was required. The water trainer was filled with water of a temperature of 21 °C, the level of water was set to 85% of the height of the withers. Heart rate was monitored using a Polar Equine RS800cx. This program lasted 44 min and after the training the horses were dried under infra-red lamps. Afterwards the horses were taken back to the stable.

Blood sampling. Blood samples of 4 ml were taken during the third day of the water treadmill training program at the time intervals indicated in Table 2. These samples were taken from the jugular vein via catheters and placed in sampling tubes containing NaF-oxalate or in Na-heparine BD Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Additional blood sampling was carried out on the morning of the competition days and right after the completion of the show jumping course with jugular venipuncture within the same period of one hour for all horses. The blood samples were stored on ice until centrifugation. The samples were centrifuged at 2000 × *g* for 3 min. Plasma was pipetted to an Eppendorf tube and stored at a temperature of −18 °C. Laboratory analyses were carried out in the week following the competitions.

Laboratory analysis. All feed components fed to the horses were sampled and analysed for crude protein (93/28/EEC), crude fibre (92/89/EEC), crude fat (98/64/EC), sugar (71/250/EEC) and starch (99/79/EC) content. From the blood plasma samples activities of lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), concentrations of lactate, glucose, total bilirubin, total cholesterol, triglyceride and cortisol were determined using the Roche Modular SWA (Hoffmann-La Roche Ltd.) measuring system. The individual measuring principles are the following: bilirubin – photometric, Dichlorophenyl-Diazonium (DPD) method; triglycerides – photometric, GPO-PAP method; cholesterol – photometry, enzymatic CHOD-PAP method; LDH – photometric, according to the DGKC recommendations, optimised standard method; AST – photometry, according to IFCC recommendations, optimised kinetic UV test; CK – photometric, IFCC reference method, UV test; cortisol – electrochemiluminescence immunoassay (ECLIA), biotinylated polyclonal anti-cortisol antibodies.

Statistical analysis. The experimental data were evaluated in the SAS 9.1 (SAS Institute Inc., Cary, USA) statistical software package using the GLM procedure. Heart rate and plasma parameters measured at rest (T0) were used as covariance factor. The interaction was not significant ($P > 0.05$) in any instance; therefore, it was left out of the model and the data are presented as pooled. In cases of significant treatment effects mean differences were tested using Duncan's new multiple range test. The parameters measured before water training (T0), after water training (T4) and one hour after water training (T5) were correlated to the same parameters measured after the competition. Pearson correlation coefficients were calculated.

RESULTS

Water training

Increasing the maximum speed of the water treadmill had no influence on the average heart

Table 2. Protocol of the water training and blood sampling

Phase	Time (min)	Speed of water trainer (km/h)	Blood sampling (min (code))	Activity
0	0	–	0 (T0)	standing, preparation
1	0–10	4.5	10 (T1)	walking, filling up the water trainer
2	10–40	9/11/13	40 (T2)	trotting in water
3	40–44	4.5	44 (T3)	walking, emptying the water trainer
4	44–60	–	60 (T4)	standing under infrared lamps
5	60–120	–	120 (T5)	resting in the box

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rate of horses subjected to the training (Table 3). Plasma lactate levels decreased with increasing maximum speed of the water-trainer. Activities of AST, CK, LDH, and concentrations of cholesterol, cortisol, and bilirubin decreased when the maximum speed of the treadmill was 11 km/h compared to 9 km/h. Interestingly, when the maximum speed was further increased to 13 km/h, this resulted in similar or sometimes higher values to those observed at the lowest training intensity. The levels of glucose and triglyceride increased when the maximum speed was increased from 9 to 11 km/h. However, when the speed was further increased to 13 km/h, glucose concentrations remained similar, while triglyceride values were lower than at the lowest intensity water training. Heart rate was elevated according to the speed of the treadmill and returned to normal levels after the training (Table 3). Plasma lactate and glucose concentrations decreased significantly during water training and increased after the training. The triglyceride concentrations were elevated only at the end of the trotting phase and returned to resting levels one hour after the training. The increased plasma cortisol concentrations during water training shows that the horses were undergoing a stressful situation. Other plasma biochemical indicators such as AST, CK, LDH, cholesterol and bilirubin exhibited no response to water treadmill training.

Competition

Medium intensity (11 km/h) water training resulted in significantly lower heart rates after competition compared to the control measurements (Table 4). A further increase in training intensity had no effect on heart rate. Lactate concentrations measured after competition were unaffected by water training intensity; however, the measured values were about two times higher compared to those detected after one hour of water training. Activities of AST, CK, LDH, and the concentrations of glucose and triglyceride were elevated in response to the lowest intensity of water training compared to values measured in response to conventional training (control). Increasing the water training intensity to 9 and 11 km/h resulted in decreased activities of AST, CK and LDH after competition, while the concentrations of glucose and triglyceride remained unchanged. Water training had no influence on plasma cholesterol, cortisol and bilirubin concentrations measured after competition. Competing on consecutive days resulted in elevated AST and CK activities.

Table 3. Effect of water training and its intensity on the average heart rate and plasma biochemical parameters of show jumpers

Traits	Time of sampling (min after starting water training)						Water training intensity (max. speed of treadmill, km/h)			RMSE	Sam	Int	Cov
	0	10	40	44	60	120	9	11	13				
Heart rate (bpm)	55.4^{c}	79.9^{b}	107.7^{a}	56.3^{c}	–	–	76.3	76.5	71.7	8.9	***	ns	***
Lactate (mmol/l)	0.63^{b}	0.41^{c}	0.38^{c}	0.40^{c}	0.68^{b}	1.11^{a}	0.66^{a}	0.58^{ab}	0.56^{b}	0.14	***	*	*
AST (U/l)	308	298	299	294	290	293	307^{a}	279^{b}	305^{a}	30.0	ns	**	***
CK (U/l)	232	217	208	205	200	208	220^{a}	184^{b}	231^{a}	28.6	ns	***	***
LDH (U/l)	668	635	609	611	603	613	672^{a}	543^{b}	655^{a}	60.9	ns	***	***
Glucose (mmol/l)	4.80^{b}	3.93^{d}	3.93^{d}	3.99^{d}	4.38^{c}	5.41^{a}	4.21^{b}	4.49^{a}	4.52^{a}	0.44	***	*	**
Triglyceride (mmol/l)	0.36^{bc}	0.36^{bc}	0.43^{a}	0.44^{a}	0.38^{ab}	0.31^{c}	0.37^{b}	0.44^{a}	0.33^{c}	0.07	***	***	***
Cholesterol (mmol/l)	2.07	2.00	1.97	1.96	1.97	1.91	1.98^{b}	1.85^{c}	2.10^{a}	0.16	ns	***	***
Cortisol (nmol/l)	155^{c}	183^{b}	219^{a}	216^{a}	200^{ab}	132^{c}	191^{a}	154^{b}	207^{a}	33.1	***	***	***
Bilirubin (mmol/l)	15.0	14.9	16.0	15.9	16.4	15.9	16.3^{a}	12.7^{b}	18.1^{c}	1.56	ns	***	***

Cov = probability of the effect of covariate (parameter value at rest), Int = probability of the effect of water training intensity, ns = not significant, RMSE = root mean square error, Sam = probability of the effect of sampling time

^{a,b,c,d}Means with the same letter are not significantly different ($P > 0.05$)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

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Table 4. Heart rate and plasma biochemical parameters of show jumpers after competition as affected by water training intensity and days in competition

Traits	Water training intensity (max. speed of treadmill, km/h)				Days in competition		RMSE	Int	Day	Cov
	Control	9	11	13	1	2				
Heart rate (bpm)	89.3[a]	82.6[ab]	76.9[b]	75.4[b]	81.8	80.3	9.43	*	ns	**
Lactate (mmol/l)	2.31	2.77	2.42	2.03	2.31	2.46	0.99	ns	ns	***
AST (U/l)	327[c]	374[a]	356[b]	337[c]	341[b]	356[a]	15.6	***	**	***
CK (U/l)	221[c]	328[a]	245[bc]	267[b]	251[b]	279[a]	26.4	***	**	***
LDH (U/l)	633[b]	833[a]	676[b]	695[b]	686	732	94.2	**	ns	*
Glucose (mmol/l)	3.60[b]	4.51[a]	4.38[a]	4.60[a]	4.20	4.35	0.45	***	ns	ns
Triglyceride (mmol/l)	0.34[b]	0.45[a]	0.46[a]	0.43[a]	0.42	0.42	0.057	**	ns	*
Cholesterol (mmol/l)	2.30	2.14	2.31	2.26	2.22	2.28	0.16	ns	ns	***
Cortisol (nmol/l)	199	172	164	193	183	181	40.1	ns	ns	**
Bilirubin (mmol/l)	16.7	16.0	17.1	17.9	16.7	17.1	2.00	ns	ns	***

Cov = probability of the effect of covariate (parameter value at rest), Day = probability of the effect of days on competition, Int = probability of the effect of water training intensity, ns = not significant, RMSE = root mean square error

[a,b,c]Means with the same letter are not significantly different ($P > 0.05$)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Correlation between values of plasma parameters measured before and after water training and after competition

It was hypothesised that water training can improve the fitness of horses during competition. Therefore, plasma parameters measured after water training may correlate with the same parameter measured after competition. To test this idea, Pearson correlation coefficients were calculated between the same parameters measured before water training (at rest, T0), after water training (standing under infrared lamps, T4) and one hour after water training (resting in the box, T5), with the values measured after competition. Lactate concentrations measured after competition had a weak positive correlation only with plasma values obtained one hour after water training (Table 5). AST, CK and LDH activities had weak to moderate correlation with the resting and post-training values. The values of cholesterol and bilirubin had a weak correlation with resting (T0) values. Cortisol concentrations found immediately after and one hour after water training exhibited a weak correlation with values obtained after competition.

DISCUSSION

Elevated activities of AST and CK can be associated with muscle damage (Balogh et al. 2001). A phase of increase in the activities of these enzymes can be explained by the increase in the anaerobic threshold. The higher rate of glycolytic metabolism can cause permeability changes in muscle fibre membranes (Harris et al. 1990). However, if the anaerobic threshold value stabilises and adaptation occurs, decreased AST and CK activities can result. This theory is supported by the data of Fazio et al. (2011), who observed a reduction in

Table 5. Correlation between plasma biochemical parameters measured after competition with the same parameters determined before, after and one hour after water training

	Plasma biochemical parameters								
	lactate	AST	CK	LDH	glucose	triglyceride	cholesterol	cortisol	bilirubin
Before water training (T0)	0.18	0.68***	0.73***	0.52**	−0.37	0.21	0.45*	0.10	0.41*
After water training (T4)	−0.17	0.54**	0.41*	0.51*	−0.24	0.19	0.12	0.55**	0.12
1 h after water training (T5)	0.52**	0.61**	0.79***	0.64***	−0.02	0.15	0.11	0.52**	0.18

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

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AST and CK activities after a phase of increase in these values at the end of a prolonged exercise period (80 days). Dry training of horses or training in a low water level (< 77% of withers height) treadmill was described to result in linear increases in lactate accumulation in plasma (Lindner et al. 2010; Lindner et al. 2012) at low training intensities. However, experimental results (Lindner et al. 2012) demonstrated that lactate levels were decreased when the level of water was increased from 63 to 77% of the height of withers. In our study the level of water was adjusted to about 85% of the height of withers (deep water treadmill) from the beginning of the exercise test, and the plasma lactate concentrations decreased even in spite of training (Table 3). Deep water training (where the level of water is around 80% of the height of withers) results in lower body temperatures (Nankervis et al. 2008). It was reported that low temperature water (13–16 °C vs 19 °C) can also reduce the heart rate response to treadmill exercise. The temperature of water in our study was 21 °C, which is still well below normal body temperature. Thus, the cooling effect of water on muscles was certainly present in our study as well. It can be suspected that this effect is behind the unusual lactate response compared to dry or low water level treadmill training. This is supported by the results of Kang et al. (2012), where the slow (3.6 km/h) swimming training of riding horses resulted in significantly lower heart rates as well as glucose and lactate concentrations. The importance of this phenomenon lies in the fact that it is common in equine conditioning studies to use the relationship between blood lactate and exercise speed, such as V_4 (the speed at which a blood lactate level of 4 mmol/l is reached) to define exercise intensity, and in order to decide on an adequate stimulus to improve performance (Trilk et al. 2002). Since water training attenuated the effect of exercise on lactate production, this value cannot be correctly calculated. Thus, the question still remains, whether the energy metabolism of muscles was really altered. Low lactate accumulation is a sign of aerobic energy metabolism and the use of slow-contracting Type I muscle fibres. Plasma glucose and free fatty acids are the most important energy sources during submaximal exercise (Lawrence 1994). Lowered plasma glucose levels indicate its more intensive utilisation, while elevated triglyceride levels are indicative of an increased mobilisation of fat stores. These data indicate that aerobic energy generation was more intensive during water training. Show jumping competition including warming up and riding the course encompasses about 40–50 min of moderately intensive work (maximum speed is about 24 km/h); thus, it falls into the aerobic energy supply range (Ellis and Hill 2005). Therefore, it can be hypothesised that deep water training can improve the fitness of show jumpers.

It has been proven that the effect of training should be monitored in competition environments as well (Fazio et al. 2011). Heart rate is closely linked with oxygen uptake and energy expenditure during exercise (Coenen 2005). However, horses competing at higher classes had lower post-performance heart rate values. Higher heart rate can be associated with higher lactate concentrations, more faults, lower technical score and closer take-off distance (Harris et al. 2014). These results indicate that heart rate measured after show jumping can reflect differences in fitness. In our experiment, the medium level water training (maximum speed 11 km/h) reduced the heart rate measured after the show jumping course, indicating that the training resulted in improved fitness. The unaffected lactate concentrations indicate that the anaerobic capacity was not affected by water training. Lactate values were around the anaerobic threshold value, suggesting that the energy requirement of the performance was mainly met by aerobic energy sources. Interestingly, in another study, show jumpers competing at similar or even lower obstacle heights developed higher lactate concentrations (Sloet van Oldruitenborgh-Oosterbaan et al. 2006). The minimum speed of the horses was similarly 350 m/min, but the course length was 700 m. Show jumping courses with 12 obstacles (13–15 efforts) rarely exceed 500 m in length. These results show that the length of the course can significantly affect the plasma lactate concentrations. Therefore, in order to ensure the applicability of conclusions, field tests should be run under competition conditions.

The elevated activities of AST, CK and LDH after training indicate that deep water training is a strenuous exercise for horses. However, excessive exercise does not necessarily result in an increase in CK activity (Hamlin et al. 2002). Considerably lower CK values were measured in show jumpers performing in higher class competitions (Art et al. 1990a; Art et al. 1990b), suggesting successful adaptation. Nevertheless, the role of AST and CK in

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signalling muscle damage is confirmed by the observation that even a muscle biopsy can increase the activities of these enzymes (Soares et al. 2013). Together with our results this suggests that water training causes increased muscle damage in the initial phase of training, but that then the horses successfully adapt to the situation. The major oxidative energy sources for oxidation of muscle cells are free fatty acids and glucose via anaerobic glycolysis. Plasma glucose concentrations remain stable during short and low-intensity exercise, but increase even in moderate intensity exercises by about 2–4 mmol/l (Hyyppa 2008). In contrast to those observations, in our case we observed decreased glucose concentrations after competition (Table 4) compared to the resting values (5.06, 5.02, 4.93 and 5.09 mmol/l for control, 9, 11 and 13 km/h water-trained groups, respectively). The decrease was about 0.5 mmol/l when horses were water-trained, while there was a 1.4 mmol/l difference when horses were trained conventionally. Art et al. (1990b) reported similar results with show jumpers. The decrease in plasma glucose concentrations is due to the depletion of liver glycogen, which is observed in endurance horses after prolonged low intensity exercise of three to four hours in duration (Hyyppa 2001). However, careful inspecting the figures reveal that in the first half an hour of the exercise, plasma glucose concentrations decrease by about 1 mmol/l, and that the levels start to increase after that. The warm-up period and the completion of the show jumping course takes about 30–40 min, which can explain why we and others found decreased plasma glucose concentrations after show jumping tests. Since water-trained horses had lower decreases in plasma glucose and increased plasma triglycerides, it can be assumed that the capacity of horses to generate oxidative energy is improved.

It is widely believed that the fitness of horses can be improved as a result of training. Therefore, it is an obvious assumption that haematochemical parameters measured after training should correlate with the same parameter measured after competition. In that sense, our results, which demonstrate only a few weak to moderate correlations, are disappointing. One of the main shortcomings of experiments performed with different conditioning programs is that none or not well documented comparisons were made to other conditioning programmes (Werkmann et al. 1996). Furthermore, many studies have not determined performance in a competition situation after the condition programme. For this reason, we could not locate any data for direct comparison. However, identification of appropriate markers to test the effectiveness of training programs is of great importance. Some studies indicate that Thoroughbred horses with superior competition performances have lower heart rates during trotting and slow gallops and lower lactate levels after treadmill exercise tests (Evans et al. 1993). As fitness increases, the post-ride lactate level decreases in endurance horses (Lindner 2010). In the case of show jumpers, it is very difficult to accurately determine differences in the level of performance in the same class. Penalty points at the same class are not reflected in the blood biochemical parameters measured after competition due to the fact that numerous other factors such as neuromuscular coordination, experience, motivation, respect of the fence, the rider's skill and the warming-up process, all have an influence (Art et al. 1990b). However, when the penalty points are examined over several competitions, a good correlation ($r = 0.75$) can be found between the results and the blood lactate concentrations. In this study, the weak to moderate correlation between some pre- and post-training plasma biochemical values and post-competition concentrations indicates that these markers are not robust enough to predict fitness to compete. Therefore, it cannot be stated that the measurement of plasma lactate or other biochemical variables after training will help trainers to predict the performance of show jumpers, and further research is needed to identify useful markers for the training of show jumpers.

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