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Kaposvári Egyetem, Állattudományi Kar, Kaposvár
Kaposvár University, Faculty of Animal Science, Kaposvár

Effect of age and event on post exercise values of blood biochemical parameters in show jumping horses

A. Vincze[1], Cs. Szabó[1], Á. Hevesi[2,3], S. Veres [3], D. Ütő [3], L. Babinszky[1]
[1]Kaposvár University, Faculty of Animal Science, 7400 Kaposvár, Guba S. u. 40., Hungary
[2]Szent István University, 2301 Gödöllő, Páter K. u. 1., Hungary
[3]Hungarian Equine Rehabilitation and Health Service Ltd., 7400 Kaposvár, Guba S. u. 40., Hungary

ABSTRACT

Fitness is a certain metabolic status – as a result of training – which makes the equine athletes capable of good results in sport. Assess the fitness status in field conditions is therefore very important. Rather limited information is available on low class show jumpers, therefore the aim of this trial was study the effect of age and event on the biochemical blood parameters. During the winter indoor competition tournament (from October to February) blood samples were collected right after the first class of the last day of the event from all together thirty show jumpers (five, six and seven years old horses, five randomly selected horse from each year category at the first and at the last competition of the tournament). From the blood samples taken from the jugularis vein the plasma were separated and the lactate, LDH, CK, AST, glucose, cholesterol, triglyceride, total billirubin and cortisol level were measured for evaluating the training status of the horses. Five year old horses had significantly lower lactate level compared to six and seven year old horses. At the end of the tournament horses had lower glucose and higher cortisol level in the plasma right after show jumping class. It can be concluded, that show jumping competitions on up to 100 cm height and a minimum of 300 m/min average speed do not involve substantial anaerobic energy supply. The frequent stress situation can elevate the cortisol response given. Multivariate methods applied on several post exercise blood parameter can be useful to detect slight differences in fitness.
(Keywords: show jumping, horse, training, fitness)

INTRODUCTION

The condition as a result of training is very important for every athlete including equine athletes. Standard exercise tests provide the possibility of to run the horse under controlled conditions, however data collected from a treadmill test do not reflect the horse's response to a sport event. Horses are generally exercised on an open field or indoor, having exposed to numerous other factors such as the rider, other horses, weather, spectators, decorations, terrain, etc. (*Serrano et al.,* 2001). Therefore, testing the biochemical and physiological changes due to field training or competition is important. The literature of equine athletes are relative abundant on data about Thoroughbreds, endurance and eventing horses. The few field test with show jumpers deal with more experienced horses and 130–150 cm high classes (*Art et al.,* 1990a,b). No information was found in the literature about the post exercise biochemical values of young horses competing on lower levels. Therefore, the aim of this study was to examine the effect of age and event on post exercise blood biochemical and enzymatic activity parameters.

MATERIALS AND METHODS

Experimental animals: During the winter period (from October to February) the Indoor Show Jumping Championship is organized in Hungary. One location of the tournament is the Pannon Equestrian Academy at the Kaposvár University. Fifteen horses were randomly selected in three age categories (five, six and seven years old, five animal/age group) at the first (October 2009) and at the last (February 2010) event of the tournament (30 horse all together). Gender was not considered in the selection of animals to be tested.

Blood sampling: On the last day of the events, immediately after the first course two times 4 ml blood sample was taken from the jugular vein into the sampling tubes containing NaF-oxalate and Na-heparine. The blood samples were stored on ice until we spanned them. The samples were spanned at 4000 rpm for 3 minutes. Plasma were pipetted to an eppendorf tube and stored at a temperature -18 ^{o}C until the analysis.

Laboratory analysis: From the blood plasma samples lactate, lactate dehidrogenase (LDH), creatine kinase (CK), aspartate amino transferase (AST), glucose, cholesterol, triglyceride, total-billirubin and cortisol level were determined in the laboratory of the Kaposi Mór Teaching Hospital (Kaposvár, Hungary) using Roche Modular SWA (Hoffmann-La Roche Ltd.) measuring sytem.

Statistical analysis: The experimental data were evaluated by the SAS 9.2 (SAS Institute Inc., Cary, NC, USA) statistical software package with GLM procedure. Interaction of age and event effect was not significant in the case of any parameter; therefore it had been left out from the general model and results presented as pooled. In case of significant main effect, the differences between the group means were tested by Tukey-test. Discriminant analysis used to test the hypothesis that treatments can be separated based on the blood parameters.

RESULTS AND DISCUSSION

Five-year old horses had significantly lower lactate level than the 6 or 7 years old horses (*Table 1*). The average levels of the young horses were within the usual range (ref. value 0.5–2.0 mmol/l) indicated for resting animals, while older horses developed a characteristic of post exercise level with the mean value of 3.5 mmol/l. *Art et al.* (1990a,b) measured post competition lactate level between 6–9 mmol/l for horses competing on 130–150 cm obstacles with above 350 m/min speed. *Davie and Evans* (2000) demonstrated that exercise over 800 m at speeds 780–960 m/min results in blood lactate concentrations in the range of 4–19 mmol/l measured right after the exercise. The maximum lactate concentration determined by *Munoz et al.* (2008) after exercise in draft horses were 13.7 mmol/l, in racing horses were 12.8 mmol/l and in the endurance horses it was 2.9 mmol/l. The high intensity training programme consisting of gallop or trot at high speed resulted in a blood lactate concentration of more than 7 mmol/l in less than 3 min (*Guy and Snow,* 1977). These results indicate that lower class show jumping (up to 120 cm height) is not a strenuous exercise to horses. At the outset of any excercise energy is provided by the ATP stores and the ATP-creatine phosphate pathway. However these energy supplies deplete rapidly (up to one minute) and as the excercise intensity increasing the lactic acid pathway take over until the aerobic pathways can provide the energy requirement (1–2 minute) (*Ellis and Hill,* 2005). *Lindner* (1997) suggested that there is always enough oxigen available for the aerobic pathways, even during intensive excercise.

More likely the low energy providing capacity over time of the aerobic pathways is the answer why lactic acid pathway is used. The results of older show jumping horses support that theory. The warm up period usually last for about 30 minutes and involves light work and than riding the course takes 60–90 seconds as intensive work. The different results of the 5 years old horses can be answered by the difficulty of challange they are facing with: the maximum height of the obstacles is 100 cm and the average minimum speed is 300 m/min, while for the 6 and 7 years old horses it is 110 cm, 325 m/minute and 120 cm, 325 m/min, respectively. The oxygen consumption during exercise is known to be close correlation with the energy expenditure during vork. *Eaton et al.* (1995) and *Pagan and Hintz* (1986) estimated the energy expenditure based on oxygen uptake. They estimated similar values at the speed of 300m/min but markedly different at 400 m/min. These results suggest that up to 100 cm class competitions the aerobic energy providing pathway can meet the energy need of the horse.

Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with parallel interconversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased, thus lactate to piruvate is preferred. In this respect the elevated level (reference value 74–206 U/l, *Lumsden et al.,* 1980) of LDH is the result of anaerobic energy supply, however the differences in lactate level of age groups did not reflected in mean LDH values. Interestingly, *Art et al.* (1990a,b) found lower LDH levels in spite of higher post exercise lactate values, however horses were competing on higher classes.

The elevated level of ckreatine kinase reflects the intensity of actual workload and it can be easily double as seen in endurance horses and in our trial (*Table 1*). Again, *Art et al.* (1990a,b) measured much lower post exercise CK levels in horses competing in higher classes. It has been documented, that overtraining is not resulted in elevated CK activity (*Harris et al.,* 1997; *Hamlin et al.,* 2002). Nevertheless *Pritchard et al.* (2009) determined a 210 U/l reference value for Lahore working horses (Pakistan). They stipulated that the high levels of CK in working horses may indicate a low-grade, chronic muscle damage rather than a short-term and reversible effect of overwork. This assumption was based on the fact that the reference population was working for short periods daily. Elevated AST and total billirubin levels can indicate muscle damage due to muscle strain from use, training. Our results fall in the wide reference ranges set for both light and working horses (*Lumsden et al.,* 1980; *Pritchard et al.,* 2009), but *Art et al.* (1990a,b) measured noteworthy lower AST values in high class show jumpers. Nevertheless, *Harris et al.* (1990) found persistently elevated AST (above 300 U/l) in thoroughbred racehorses with good race results. *Art et al.* (1990b) concluded that a single blood sampling could lead to a misinterpretation of post exercise biochemical values due to high interindividual variations. This regards particularly to blood lactate, LDH and CK. This phenomenon implies that the ability to compare different research results based on these biochemical parameters are limited.

All horses had normal serum cholesterol level, however it is tended to be higher at the end of the season. It has been shown that cholesterol level in blood plasma increases as a result of exercise but not due to conditioning (*Hambleton et al.,* 1980). The low serum triglyceride levels indicate that fat metabolism was not affected by show jumping. Plasma adrenaline, noradrenaline, b-endorphin and cortisol concentrations were increased by exercise in cool dry conditions (cortisol: 90 ng/ml) and were further increased by the same exercise in hot humid (cortisol: 130 ng/ml) conditions (*Williams* *et al.,* 2002). The degree of increase in plasma cortisol concentration appears to better reflect duration of workload rather than work intensity. Maximum plasma concentration was observed about 30 min after the end of a high intensity exercise (*Marc et al.*, 2000). It has been also revealed that discrimination based on cortisol net increase (due to excercise) between endurance and dressage plus jumping trained horses are possible. *Covalesky et al.* (1992) demonstrated that more experienced horses has lower cortisol concentrations than less experienced ones after riding the course. In our study we were not able to detect this difference. Most probably the reason is that even young (5 year old) horses were already accustomed to the show environment.

Time of measurement (effect of event) had significant effect only on the blood glucose and cortisol level. One of the primarily function of cortisol is to increase blood sugar level for instance in case of physical exertion. That can be the reason why we found significantly higher value while blood sugar level was lower. Cortisol plays important role in adaptation to stress. As summarized by *Coenen* (2005) different metabolic stresses – like jumping – can double the level of plasma cortisol. The significantly higher level at the last competition of the tournament can be the result of higher metabolic stress. Most horses did compete every second week during the winter season which together with the frequent transportation can cause higher reaction to these stress. Blood lactate concentration tended to be higher at the last event. *Art et al.* (1990b) found no difference in post competition lactate level over five competitions. Therefore, the slight difference in our case maybe accounted to the individual variation, as not the same horses were tested at the last event.

Table 1

Blood parameters of different aged show jumpers at the beginning and at the end of winter competition period (mean values)

Blood parameter	Age (year)			Event		RMSE	P values (age)	P values (event)
	5 (n=10)	6 (n=10)	7 (n=10)	First (n=15)	Last (n=15)			
Lactate (mmol/l)	1.5^{a}	3.5^{b}	3.5^{b}	2.3	3.4	1.6	0.010	0.059
LDH (U/l)	539.7	589.4	572.5	577.9	556.5	115.0	0.623	0.614
CK (U/l)	241.5	243.8	211.8	231.5	233.3	39.5	0.151	0.902
AST (U/l)	281.5	317.7	275.5	283.3	299.8	58.7	0.239	0.449
Glucose (mmol/l)	3.9	4.6	4.4	4.7^{a}	3.8^{b}	0.9	0.171	0.007
Cholesterol (mmol/l)	2.3	2.3	2.4	2.3	2.5	0.3	0.607	0.098
Triglyceride (mmol/l)	0.36	0.30	0.42	0.37	0.35	0.12	0.100	0.651
Total billirubin (µmol/l)	27.7	29.8	32.2	28.2	31.4	7.1	0.376	0.220
Cortisol (nmol/l))	197.7	199.6	170.8	167.0^{a}	211.7^{b}	42.2	0.252	0.007

[a.b] Means within a row lacking a common superscript differ significantly $P<0.05$; RMSE: route mean square error

Since few significant differences were found with analyses of variance, we hyphotetized that treatment groups may be still can be distinguished based on all the traits measured. Therefore, discriminant analysis was applied on the data set. We are aware that in this study we had too few observations compared to the number of traits to make firm conclusions on the result of this statistical analysis. Moreover statistically proven outliers were not excluded, because those values were physiologically sound. Therefore, even significant results interpreted as a tendency.

Three data were misclassified from the event groups of data (*Table 2*), and Pillai' Trace statistics show week discriminant function. None of the data were misclassified from age groups (*Table 3*) and statistics indicates a strong discriminating function. It is a general view that only post exercise plasma lactate level and heart rate are good indicators of fitness, and other biochemical and enzymatic activity analyses are of limited usefulness (*Art et al.,* 1990a, *Art et al.,* 1994; *Couroucé,* 1999). However, our results indicate that the difference in fitness and metabolic status based on various biochemical parameters of different aged show jumpers could be possible in spite of few significant differences.

Table 2

Classification results of sampling time groups

From event group	Into event group				Total		Pillai' Trace	
	1		2					
	count	%	count	%	count	%	value	P
1[a]	14	93.33	1	6.67	15	100		
2[b]	2	13.33	13	86.67	15	100		
Total	16	53.33	14	46.67	30	100	0.55	0.030

[a]first event; [b]ast event

Table 3

Classifications results of age groups

From age group	Into age group						Total		Pillai' Trace	
	5		6		7					
	count	%	count	%	count	%	count	%	value	P
5	10	100	0	0	0	0	10	100		
6	0	0	10	100	0	0	10	100		
7	0	0	0	0	10	100	10	100		
Total	10	33.33	10	33.33	10	33.33	30	100	0.93	0.040

CONCLUSIONS

Show jumping competitions on up to 100 cm height and a minimum of 300 m/min average speed do not involve substantial anaerobic energy supply. The frequent stress situation can elevate the cortisol response given. Multivariate methods applied on several post exercise blood parameter can be useful to detect slight differences in fitness.

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Corresponding authors:

Csaba Szabó
Kaposvár University, Faculty of Animal Science
H-7400 Kaposvár, Guba S. u. 40. Hungary
Tel.: +36 82 505 800; fax: +36 82 320 175
e-mail: szabo.csaba@ke.hu