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Original article

The influence of selected factors and sport results of endurance horses on their saliva cortisol concentration

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Abstract

The aim of the study was to define the influence of the selected factors (gender, age, transportation time, riding distance and air temperature during the ride) on the cortisol secretion and finding a correlation between the hormone level and the horses' sport results (veterinary parameters and the ride route parameters). The research was performed on 38 Arabian pure breed horses taking part in the endurance rides. The cortisol level was measured with enzyme-immunological method in saliva samples, taken four times from each horse. In order to verify the differences between the mean results the repeated measures design was applied. The significance of the differences between the mean values was determined by the Tukey test. To evaluate the interrelations between the analysed attributes Pearson's correlation analysis was applied. The cortisol level at rest was not affected by any of the analysed factors. In case of other results, the most significant influence ($P \le 0.05$) was related to the gender, as well as the ride distance and air temperature during the ride. Higher cortisol level was noted in mares, horses running the longest distances and at the highest temperatures. A significant increase in the cortisol level was noted when the ride distance was longer. There were no clear correlation between the adrenal cortex activity and the veterinary parameters at different riding speed. High cortisol concentration can negatively affect the heart rate (HR) by increasing it, but it can simultaneously stimulate the body to fight dehydration.

Key words: endurance horses, stress, cortisol, sport results

Introduction

Stress triggers increased secretion of cortisol which is a steroid hormone produced in the adrenal cortex. As a result the body is stimulated to become less dependent on the external energy sources used for the preparation for threatening situations. The cells start to produce enzymatic protein, needed for the glucogenesis process, using amino acids as the substrates (Desmecht et al. 1996, Schmidt et al. 2010a,

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b, c, d). These reactions allow us to treat cortisol level as an indicator of stress-related reactions (Dorn et al. 2007). Cortisol level can be measured in blood or in saliva (Peeters et al. 2010). The level in saliva is directly proportional to its concentration in the unbound fraction in blood, the presence of which results from poor solubility of steroids in water. Therefore, after secretion by the adrenal cortex, 85-98% of steroids bind to the plasma globulins. The remaining corticosteroids, unbound to the plasma proteins, circulate in the blood and affect the body cells. Only the unbound fraction can infiltrate into saliva, as the cortisol bound to the plasma proteins does not leave the vascular bed. Determination of cortisol level in saliva provides information on changes in the biologically active unbound fraction of this hormone in the blood and constitutes a reliable method of studying stress (Kirschbaum 2000, Dorn et al. 2007, Peeters et al. 2010). The stress phenomenon is mainly related to the sport use of horses in equestrian competitions, during which they are subject to different types of physical and mental factors that can negatively influence their final scores, as well as their well-being or the riders' safety (Peeters et al. 2010). According to the research, the stress level increase during the competitions is related to the horses' young age and resulting lack of experience (Nogueira and Barnabe 1997), transport conditions (Schmidt et al. 2010d) or even staying in an unfamiliar stable. The last factor was identified in a study on American trotting horses awaiting the start (Irvine and Alexander 1996). Insufficient training and too intensive exercise are also important considerations (Mircean et al 2007). According to Lassourd et al. (1996) not necessarily intensive but long lasting physical exercise in endurance horses increases the activity of the adrenal cortex, resulting in triplicating the cortisol level in comparison to the rest levels. As the current knowledge on different factors affecting the cortisol level as a stress indicator concerns mostly the horses doing short and intensive exercise, we decided to identify the factors enhancing cortisol secretion in endurance horses and to investigate whether the hormone level correlates with the horses' condition during the ride and the competition results.

Materials and Methods

Design of the study

The research was conducted on 38 Pure Breed Arabian horses which took part in the endurance rides organized by one of the leading equestrian organization in Poland within the last two years. The competitions were organized according to the official endurance rides regulations, provided by the International Federation for Equestrian Sports. The group consisted of 11 stallions, 12 mares and 15 geldings (gender factor: 1 – stallions, 2 – mares, 3 – geldings) aged between 70 and 180 months (age factor: 1 – up to 100 months, 2 – between 101 and 120 months, 3 – over 120 months). The horses were transported to the competition venue a day before the start, between 6:00 and 10:00 a.m. The transportation time ranged between 2 and 11 hours (transportation time factor: 1 – up to 3h, 2 – between 3 and 6h, 3 – over 6 hours). At the venue, the horses stayed in two box stables of the same parameters. The riding distance ranged from 80 to 160 km (distance factor: 1 – 80-100 km, short distance; 2 – 120-130 km, medium distance; 3-160 km, long distance). All the horses started the competition between 6:00 and 6:30 a.m. on the next day after arriving to the venue. The rider's rank and weight carried by the horses were similar. The research included also air temperature measurements taken during the ride. They were the means of six separate measurements taken with an external electronic thermometer by the competition's technical support team. The measurements were taken every 3 hours, starting at 6:00 a.m. (temperature factor: 1 – 19.33°C, 2 – 28.04°C). In order to evaluate the correlation between the saliva cortisol level and the official competition score the information about each horse's condition was also taken into consideration. It was obtained from the official veterinary files completed at the veterinary gates after each phase of the ride, so called "loop" and accompanied by technical parameters describing the speed during ride parts, ride routes, average ride speed, the time the horse and the rider entered the veterinary gates, partial sport results (technical parameters). Due to the fact that the veterinary parameters, like gut sounds, mucus membrane condition and the gait are not measurable, a unified scoring system, designed especially for this occasion, was used. This study was based on the results of horses whose speed at the particular phases (loops) was similar. Description of the veterinary and technical parameters, measurements, own scores for some cases, mean values and standard deviations are shown in Tables 1 and 2.

Sample collection

The saliva samples were collected in order to determine the level of cortisol as a stress indicator, mirroring the adrenal cortex secreting activity. A cubical sponge 2x2 cm in size soaked in 1% acetic acid was

Table 1. Veterinary parameters of horses examined at the veterinary gates.

Parameter	Test method	Measurement or marking	Own score in case of no metrics available	After the first loop		After the loop preceding the first test cortisol level related to exercise		After the last loop	
				mean	s	mean	s	mean	s
HR during first test	checked with phonendoscope in the heart area for 1 min. after entering veterinary gate	bpm	–	59.11	4.18	59.33	4.65	56.75	5.84
Gut sounds	checked with phonendoscope on the right and left abdominal area	N-normal	5	4.21	1.18	3.45	1.51	3.97	1.49
		↓ or ↑ – slightly deviated	2.5						
		↓↓ – deviated	1						
Dehydration	pulling the skin on the neck or shoulder blade, when the horse is dehydrated the skin doesn't flatten immediately	s	–	1.17	0.43	1.68	0.87	1.89	0.75
Mucus membranes	checking the colour of the third eye lid, which changes from light pink into dark red when the horse becomes more and more exhausted	A: Light pink	5	3.38	1.81	2.91	1.72	1.97	1.45
		AB: colour...	2.5						
		B: colour...	1						
Capillary refill	pressing the non--pigmented gum membrane of the top incisor, filling the white spot created by pressing should not take longer than 3 seconds	s		1.24	0.41	1.44	0.46	1.72	0.66
Gait	evaluated during a trot on the hard surface, aimed at catching all irregularities and lameness	regular movement	5	4.18	1.43	3.27	1.39	3.32	1.32
		irregular movement	2.5						
		Lameness	0						
HR during second test	checked with phonendoscope in the heart area for 1 min. directly after quality of movement checking	bpm	–	57.18	4.22	58.73	5.81	56.62	6.81

s – standard deviation

used to collect the samples (Pell and McGreevy 1999). The sponge was inserted into the horse mouth with metal tweezers and rubbed against its tongue, inner cheeks and palate. The sponge was then placed in a centrifugation tube, where a 2 cm long piece of plastic straw separated it from the bottom. The samples were stored in -18°C until the cortisol level determination took place.

Laboratory analysis

After defrosting all samples were centrifuged (500 x g; 15 min). During centrifugation the plastic straw separating the sponge from the bottom of the tube prevented the saliva from soaking back into the sponge. Cortisol level in the supernatant was determined with the enzyme-immunological method using

Table 2. Technical parameters of competitions considered in the study.

Parameter	Description	Measurement	Mean	s
Speed in the first loop	from the start to the entrance of the first veterinary gate	km/h	16.57	0.54
The speed in the loop preceding the first cortisol level check	from the start to the following loop until entering the mid-distance veterinary gate	km/h	15.16	0.7
Speed in the last loop	from the start of the last loop until the finish line	km/h	14.92	1.13
Average ride speed.	sum of all the loop speeds multiplied by the number of loops	km/h	15.63	1.09
Time of entering the first veterinary gate	from finishing the first until entering the veterinary gate	s	331.50	94.69
Time of entering the veterinary gate after the first cortisol level check	from crossing the finish line in mid-distance until entering the veterinary gate	s	382.50	148.64
Time of entering the last veterinary gate	after crossing the finish line until entering the veterinary gait	s	518.20	134.59

s – standard deviation

CORTISOL EIA kit (for saliva) DSL-10-67100 made by Diagnostic System Laboratories Inc (USA). The light absorbance wavelength was 450 and 620 nm. The measurement was conducted in Multiscan reader produced by Labsystems, Helsinki, equipped with GENESIS V 3.00 software. The results were expressed in nmol/l. Four samples were collected from each horse at different time: late at time (between 8:00 p.m. and 10:00 p.m.), a day before the ride, early in the morning (between 5:00 a.m. and 6:00 a.m.) the following day, 3 minutes after entering the veterinary gate in mid-distance and 3 minutes after entering the veterinary gate at the end of the ride. The collected saliva samples were used to determine the following cortisol level values: 1 – evening sample at rest – evening level at rest, 2 – morning sample collection – morning level at rest, 3 – first exercise sample collection in mid-distance – first exercise level, 4 – second exercise sample collection at the end of the ride – second exercise level.

The study was accepted by the Local Ethical Committee for Animal Experimentation and conducted according to the European Community regulations concerning the protection of experimental animals.

Statistical analysis

The statistical calculations were performed with SAS software. In order to verify the differences between the average results repeated measures design was applied (sigma-restricted model, active hypothesis decomposition), taking into consideration the following factors: gender, age, transportation time, ride's distance, air temperature during the ride, cortisol level measurement and the common interactions between all the factors. The choice of this type of analysis was based on the fact that huge variability of the analysed factors would not yield reliable results using ANOVA – GLM variance analysis (Littel et al. 2002).The significance of the differences between the mean values was determined by the Tukey test. To evaluate the relationships between the analysed attributes Pearson's correlation was applied.

Results

The investigated factors did not affect the evening and morning cortisol levels at rest (Table 3). The mean values for each factor level ranged from 0.62 to 1.59 nmol/l. A different situation was observed when analysing the mean values for the first and second cortisol level after exercise. The mid-distance test revealed a significantly higher cortisol level among the stallions aged over 120 months, transported for 3-6 hours, taking part in medium distance ride in the lower air temperatures. Similarly, the results of the second test correlated with the ride distance and air temperature, and the lowest cortisol level was observed in the group of horses taking part in the medium distance ride at lower temperatures. In the case of other factors, the lowest values were shown for stallions and geldings and for horses transported for up to 3 and over 6 hours. No correlation was observed

Table 3. The results of variance analysis for the repeated measures of cortisol level (nmol/l) in the horse saliva.

Cortisol level		Evening at rest	Morning at rest	First after the exercise	Second after the exercise
			Sex factor		
Stallions	$\bar{x}$	1.59aa'	2.07aa'	19.63ab'	25.07ab'
	s	1.91	1.36	26.76	30.36
Mares	$\bar{x}$	0.66aa'	2.42a'	31.64bb'	39.87bc'
	s	0.64	2.26	32.23	36.08
Geldings	$\bar{x}$	0.62aa'	6.12aa'	30.31bb'	30.10ab'
	s	0.60	9.13	27.21	28.28
			Age factor		
Up to 100 months	$\bar{x}$	1.23aa'	4.84aa'	21.83 a b'	30.94ab'
	s	1.79	8.55	16.43	26.79
From 101 to 120 months	$\bar{x}$	0.67aa'	2.72aa'	20.24ab'	27.44ab'
	s	0.49	2.67	24.52	33.81
Over 120 months	$\bar{x}$	0.77aa'	3.53aa'	39.85bb'	34.57ab'
	s	0.75	5.23	38.91	35.11
			Transportation time factor		
Up to 3 h	$\bar{x}$	0.67aa'	2.91aa'	37.66ab'	25.74ac'
	s	0.68	2.50	40.45	34.11
From 3 h to 6 h	$\bar{x}$	0.74aa'	4.04aa'	15.35bb'	37.08bc'
	s	0.51	5.71	13.67	37.24
Over 6 h	$\bar{x}$	1.31aa'	4.41aa'	28.49cb'	28.82ab'
	s	1.86	8.80	22.27	16.48
			Ride distance factor		
80-100 km	$\bar{x}$	1.21aa'	6.78aa'	25.92ab'	31.93ab'
	s	2.03	11.83	19.03	24.38
120-130 km	$\bar{x}$	0.63aa'	2.62aa'	15.32bb'	21.38bb'
	s	0.47	2.38	12.73	24.47
160 km	$\bar{x}$	1.12aa'	3.68aa'	42.45cb'	45.74cb'
	s	1.33	5.28	38.06	46.26
			Air temperature factor		
19.33°C	$\bar{x}$	1.04aa'	1.84aa'	7.27aa'c'	11.09ab'c'
	s	1.09	0.84	3.19	6.7
28.04°C	$\bar{x}$	0.83aa'	5.53aa'	46.08bb'	57.06bc'
	s	1.31	8.07	28.81	28.99

Explanations:
mean values referring to subsequent levels of particular factor designated with the same letters are not significantly different: a – in columns, a' – in versus (in other cases, the difference significance at $P \le 0.05$).

between the age factor and cortisol level after the second exercise.

The results showed significant differences in cortisol concentration at rest for all the discussed factors. The differences were also noticed for the consecutive exercise values of mares, horses transported up to 6 hours and those competing at the highest temperatures. In most cases the cortisol level at the end of the ride was higher than at mid-distance. In other cases, where the consecutive cortisol exercise values were compared, as well as the values at rest, the average results were similar.

Most significant correlations between the cortisol level in saliva during consecutive measurements and the veterinary parameters were recorded during the test conducted at the end of the ride (Table 4). Significant factors were usually positive, when we compared the evening test at rest and the second test after exercise with the HR taken at the second test, both exercise tests, with the mucus membranes condition and the gait quality. Negative correlations were found for the morning cortisol levels at rest and the state of dehydration and capillary refill time. The latter veterinary parameter also negatively correlated with the

Table 4. Correlation between cortisol level and the veterinary parameters..

Cortisol level	Evening at rest	Morning at rest	First after the exercise	Second after the exercise
After the first loop				
HR during the first test	-0.298	-0.048	0.121	0.284
HR during the second test	-0.093	-0.030	0.171	0.205
Gut sounds	-0.302	0.090	-0.083	0.050
Dehydration	-0.077	-0.065	-0.313*	-0.065
Mucus membranes	-0.286	0.312	0.201	0.050
Capillary refill	0.105	0.004	0.303	0239
Gait	0.029	-0.033	-0.221	-0.092
After the loop preceding the first cortisol level check				
HR during the first test	0.206	0.285	0.074	0.155
HR during the second test	0.077	0.138	0.140	0.376*
Gut sounds	0.072	-0.011	-0.593*	-0.595*
Dehydration	-0.202	-0.150	-0.052	0.061
Mucus membranes	-0.051	0.134	0.146	-0.181
Capillary refill	-0.081	0.016	-0.269	-0.298
Gait	-0.075	0.086	0.162	-0.030
After the last loop				
HR during the first test	0.113	-0.247	0.009	0.390*
HR during the second test	0.315*	-0.275	0.106	0.384*
Gut sounds	-0.198	-0.249	0.008	0.097
Dehydration	0.193	-0.370*	0.281	0.031
Mucus membranes	0.136	-0.127	0.442*	0.371*
Capillary refill	0.057	-0.313*	-0.350*	-0.302
Gait	0.176	-0.136	0.402*	0.485*

* – correlation significant at $P \leq 0.05$

Table 5. Correlation between cortisol level and the individual technical parameters.

Individual technical parameter/ Cortisol level	Evening at rest	Morning at rest	First after the exercise	Second after the exercise
Speed in the first loop	-0.265	0.296	0.100	-0.023
Speed in the loop preceding the first cortisol level check	-0.383*	0.133	-0.225	-0.507*
Speed in the last loop	-0.167	0.257	-0.201	-0.259
Average ride speed.	-0.439*	0.302	0.005	-0.012
Time of entering the first veterinary gate	-0.046	-0.050	-0.167	0.308
Time of entering the veterinary gate after the first cortisol level check	0.127	0.101	0.108	0.480*
Time of entering the last veterinary check gate	-0.553*	0.074	-0.186	-0.155

* – correlation significant at $P \leq 0.05$

cortisol level measured at mid-distance. Veterinary parameters defined at the veterinary gate after the loop preceding the first exercise test significantly correlated with three investigated factors. First of all, negative correlation was found between the gut sounds and both exercise tests cortison levels and, secondly, HR positively correlated with the second exercise test cortisol level. After the first loop a significant negative correlation was confirmed only for dehydration and cortisol level in the first exercise test.

Cortisol level measured in the evening at rest and after the second exercise correlated with the individual technical parameters of the horses (Table 5). In the case of the first measurement negative interrelation was observed for the parameter defining the speed on the loop preceding the first exercise test of cortisol level, average ride speed and the time of entering the final veterinary gait. However, cortisol level from the second exercise test negatively correlated with the speed on the loop preceding the first exercise and positively with the time of entering the veterinary gate after this loop.

Discussion

Proper training schedule, adjusted to the planned exercise, and the control over undesirable factors protects the horse biochemical homoeostasis from stress-related disturbances. Cortisol secretion depends on the animal experience in competitions (Nogueira et al. 2002), ride duration, transport conditions (Schmidt et al. 2009), animal age (Nogueira and Barnabe 1997), character (Fazio et al. 2011) and staying in new environment before the start (Alexander and Irvine 1998a).

Significant influence of these factors on cortisol secretion and release of the unbound fraction to the endurance horses' saliva was found. However, the described situation concerns only cortisol levels after the exercise. Comparison of cortisol levels in the evening the day before the competition and in the morning on the day of the start showed no significant differences for the analysed factors. Even though, the morning test revealed a tendency to elevated cortisol level in comparison to the test conducted in the evening on the previous day. It is worth emphasizing that cortisol levels at these moments only sporadically reached 1 nmol/l, which means that none of the horses showed visible signs of stress in pre-start conditions (Irvine and Alexander 1996). The results, however, do not exactly correspond to the later research performed by the Alexander and Irvine (1998b). According to them, staying in a new environment and waiting for the start induces stress. Another study claims that low cortisol level is linked to the horse's experience (Nogueira et al. 2002) which is confirmed by our results. All the horses taking part in the research were experienced in competitions. The results we obtained might have also be influenced by the balanced temper of the endurance horses which, according to Fazio et al. (2011), modifies the cortisol secretion from the adrenal cortex and is a key factor shaping the stress reactions. Different situation was observed when evaluating the influence of the analysed factors on the cortisol level after exercise. The saliva cortisol level during the ride was lower in the stallions than in the mares and geldings. The results for the mares revealed an interesting tendency – the highest cortisol levels were observed in mid-distance and at the end of the ride. It is worth mentioning that the most visible cortisol level increase during the second exercise test was not recorded in the group of geldings. The age of endurance horses only slightly affected cortisol levels in their bodies. The differences were only seen in the mid-distance test, in the group of the oldest horses, which had significantly higher hormone levels. No increase in adrenal cortex activity was noticed in this case in relation to the duration of the ride in any of the groups. Some authors claim that the age factor and lack of experience significantly boost the stress hormone secretion. Cortisol level falls as the animal gets older (Lassourd et al. 1996, Nogueira and Barnabe 1997). This is probably related to gaining more experience or to the phenomenon of chronic stress. The results obtained in this study do not fully confirm those presented in the cited publications. It may be due to the fact that all the horses, even the youngest ones, had enough competition experience. Another factor – the transportation time, significantly affected the cortisol levels after exercise in the focus group of the endurance horses. The mid-distance result analysis showed the highest cortisol levels in horses transported for the shortest and the longest time. This situation changes at the end of the ride, where the highest cortisol levels were observed in horses transported for between 3 and 6 hours. Many authors (Shanahn 2003, Szarska 2003, Schmidt et al. 2009, Schmidt et al. 2010d) claim that the transportation negatively influences the physical and mental condition of the sport horses. Our results for the mid-distance seem to corroborate the findings presented by Schmidt et al. (2010d) and Ohmura et al. (2006). According to those authors the increased activity of the hypothalamic-pituitary-adrenal axis during transportation appears already at the first stage of the travel. Cortisol secretion during a short travel is practically the same as during longer one. It seems however, that our results contradict those of Fazio et al. (2008), who stated that cortisol level and therefore stress level at the end of the travel do not depend on the distance. It is worth investigating why there is not a similar relationship for cortisol level in the discussed groups at the end of the ride. Most probably, the transportation time is a cortisol releasing factor, but only to a certain point of the ride, after which other factors become more important. Another analysed aspect was the ride distance. The highest cortisol level after exercise was observed in horses covering 160 km (maximum distance for a one-day ride). Most likely, high cortisol level in this case is directly related to the amount of exercise the horse has to do in order to complete the ride. The lowest and the medium values were observed in horses on medium and short distances, respectively. It seems that the distance under 100 km is connected with the horse excitement related to the very participation in the competition and not directly related to body exhaustion. The lowest hormone values on the distance of 120-130 km might indicate that the excitement is fading away as the competition continues and the animal is not very exhausted yet. This situation can also be explained by better physical preparation of the horses taking part in the rides longer than 100 km. According to Urhausen et al. (1995) exhausting physical exercise significantly lowers the top cortisol levels which is considered to be a defence reaction of the body. It can also be assumed that the horses evaluated in our research, which covered the distance of 160 km, were simply not exhausted. It is worth mentioning that the results of this research correspond to the ones described by Lassourd et al. (1996), who noted maximum triple level of cortisol after exercise as compared to the level at rest. The cortisol levels after exercise described in this study were even ten times higher than at rest. It may be due to a different ride distance. The last of the analysed factors – air temperature during the competition, also revealed substantial changes in cortisol level, high concentration of which was linked to high temperatures. Comparable results, but concerning the transported horses, were published by Fazio et al. (2011). Similarly, Schmidt (2010d) and Stull and Rodiek (2000) noticed the effect of long travels in high temperatures or sunlight on the body weight loss and substantial dehydration. Another aspect describing the value of a horse is the link between cortisol level and the results of the compulsory veterinary check-ups and its way of covering the distance shown by the partial and total speed and the time of entering the veterinary gate. The shorter the time of entry the better the shape of the horse, which can quicker start the next loop. The results obtained for this topic show that this does not work for all the veterinary parameters and in some cases there is no direct correlation at all. It becomes more visible only at the end of the ride. Positive correlation between the cortisol level and HR becomes more explicit. Simultaneous analysis of those two parameters is considered justified, as heart rate variability reflects the balance of sympathetic and parasympathetic tone and provides information on the stress response of the autonomic nervous system (von Borell et al. 2007, Schmidt et al. 2010 c). The mucus membranes condition and the quality of gait are also related to the cortisol level in saliva collected at the end of the competition. However, in this case the dependency is completely different than for the HR, which might show that the body reactions to cortisol secretion are different for particular veterinary parameters. Therefore, it is quite a challenge to define the most beneficial cortisol level from the point of view of physiological parameters observed during competitions. It should also be noted that cortisol level at rest is linked to three different veterinary parameters. In the future we may be able to determine the range of the saliva cortisol level at rest, during which specific physiological states could be expected. Cortisol concentration in the saliva samples collected in the evening of the day preceding the start and its concentration at the end of the ride also depends on individual technical parameters. Negative correlation with the ride speed indicates the effect of cortisol secretion on the horses' scores. Shanahn (2003) and Krumrych (2008) share this view claiming that a stressful situation significantly reduces the body's immunity responses and makes the horses more susceptible to disease and achieve worse results due to worse condition.

Conclusions

Increased cortisol secretion during and after exercise in endurance horses can be expected during competitions, mostly in the case of mares, horses covering longest distances and during competitions taking place at high temperatures. Other factors do not seem to be clearly related to the cortisol levels which may be due to the their interactions. The correlation between cortisol level and sport results is rarely visible and there is no clear answer to the question how the increase in the adrenal cortex activity affects the horses' veterinary parameters or their speed during the competition. The results presented in this study suggest that high level of cortisol can negatively raise the horses' heart rate, but at the same time it stimulates the body to fight dehydration. It does not shorten the time needed to cover the ride distance. Considering the practical applications the correlation between the scores and cortisol level at rest the day before the start looks rather promising. In the future a pre-start saliva cortisol concentration might be included into parameters examined in horses participating in endurance competition.

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