University of Veterinary Medicine, Doctoral School

The impact of stress on the performance of race horses in rest and during exercise

Theses of PhD dissertation

Zsófia Nyerges-Bohák DVM

2017.

Supervisor and members of the scientific committee:

....
Prof. Dr. Ottó Szenci
candidate of sciences , teacher with habilitation
University of Veterinary Medicine, Department and Clinic of Food Animal Medicine
supervisor

....
dr. Levente Kovács
research fellow
MTA-SZIE Large Animal Clinical Research Group
Co-supervisor

....
dr. Ákos Hevesi
veterinarian
Pannon Lógyógyászati Szolgálat, Kaposvár
member of the scientific committee

....
dr. Tamás Veresegyházi
associate professor
University of Veterinary Medicine, Department of Physiology and Biochemistry
member of the scientific committee

Contents

Introduction and review of the literature **4**

Aims of the study **6**

Own studies **7**

1. Daily rhythm of cortisol concentration in rest **7**

Materials and methods *7*

Results *7*

Discussion *8*

2. Effect of temperament on HPA reactivity in racehorses **9**

Materials and methods *9*

Results and discussion *10*

3. Monitoring of the heart rate variability during exercise **11**

Materials and methods *11*

Results *12*

Discussion *12*

New scientific results **15**

Publications **16**

Acknowledgements **17**

Introduction and review of the literature

Nowadays, the growing number of competitions, the more frequent transport and the frequently changing environment make sport- and racehorses more and more stressful. Stress is a non-specific response of the body to any challenge or strain. Sport as physical exercise have a special role among the stressors, as it means stress induced by both, mental and physical origins.

Measurement of cortisol levels in humans and animals is one of the most common methods for assessing the psychophysiological response of the hypothalamo-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) to stress. Although examination of the level of glucocorticoids in rest and during exercise is a common topic in equine exercise physiology, results are still controversial.

The circadian rhythm of plasma- and salivary cortisol has been repeatedly demonstrated in rest with different sampling frequencies (1-6 hours) and different number of horses used (4-18 horses), but results are not consistent. Some have found relatively large differences between morning and evening plasma cortisol concentrations, whereas others could not confirm a difference or could only occasionally demonstrate circadian changes. For using cortisol levels in practice, it is indispensable clarifying these contradictions, which is only possible by repeated measurements on a greater study population.

There are also many questions posed by cortisol trials during exercise. However, it has been shown that ACTH and cortisol concentrations are also rised as a result of strenuous or prolonged physical work, there is no accepted reference for the degree of increase. It is also questionable whether the cortisol concentration increase depends on the extent of the load or rather on the duration of the work. Although several studies have described that the performance level of the horse can be measured by the cortisol response with lower HPA activity in well trained individuals, opposite results were also found. Due to the many contradictions, measurement of cortisol as a method of assessing equine performance has not yet spread. It is still questionable, whether the differences in the results arose from the lack of standards of experimental settings, from the use of predominantly stressed workloads (treadmill, race, etc.), or from unknown differences between individuals.

The examination of heart rate variability (HRV) is another possibility of measuring stress. HRV is the physiological phenomenon that heartbeats - in the case of healthy heart function - are followed by irregular intervals. This irregularity/variability is determined by the coordinated function of the efferent and afferent ANS branches, as well as by the coexistence of other mechanical, hormonal and physiological mechanisms. Traditionally used HRV parameters can be analyzed based on time differences and power distribution of RR distances. The most common indicators of the time domain analysis are:

- RMSSD: root mean square of the successive differences between interbeat intervals
- SDNN: the standard deviation of RR intervals
- pNN50: the proportion of the number of pairs of successive RRs that differ by more than 50 ms divided by total number of RRs
- SD1: Standard deviation of instantaneous IBI variability measured from axis 1 in the Poincaré plot
- SD2: Standard deviation of long-term continuous IBI variability measured from axis 2 in the Poincaré plot

The most common indicators of the requency domain analysis are:
- LF: low-frequency component of HRV
- HF: high-frequency component of HRV
- LF/HF: the ratio of the low- and the high-frequency components

From the physiological point of view, the current, the circadian, and the total HRV can be described. Although the practical use of HRV depends on many external conditions, generally, HRV is reduced in stress situations.

HRV analysis during exercise and training associated HRV changes in rest are active areas of research in human sports medicine. Encouraging results suggest that it would be worthwhile to interpret this non-invasive approach for stress assessment in equestrian sports.

For example a significant correlation was found between HRV and some physiological threshold values (eg. ventilatory threshold) in human athletes during exercise. In case of a work intensity above the threshold, the vagus-like effect of the high respiratory frequency suppresses the cardiac vagal control, thus the spectral HRV parameters becomes undefinable. Hence, the disappearance of the meaningful HRV exactly determines the ventilatory threshold. This exact definition has not been described in horses so far. However, the basic phenomenon, that HRV is predominantly determinated by non-neural mechanisms above 120-130/min heart rate, has been shown in equine studies.

The HRV profile of fatigue has already been described in humans, and even in horses as well withcoincident results. The RMSSD (which is the most commonly used indicator of RR interval deviations) and SD2 (a geometric indicator reflecting the long-term fluctuation of RR modulation) has been lowered in skiing humans, equine trotters and endurance horses in parallel with the increase in the duration / repetition of the load. Moreover, the recovery of the heart rate in endurance horses after exercise was faster in horses with higher RMSSD, which is a priority in this sport due to the rules of competition. The RMSSD and SD2 values can indicate fatigue in horses, so regular HRV recordings can help to indentify poor performance in time and it can be useful to determine an effective training plan familiar to the horses' performance.

A series of human and equine experiments have been carried out to distinguish between the physical and psychological effects of a competition. The results are not consistent, and only Becker-Birck et al. (2013) used HRV parameters, while other experiments investigated only saliva and blood cortisol levels to estimate competition-related stress. In the experiment taking HRV into account, jumping and dressage horses participated in a three day long competition. Analyzes were performed only in the time domain, and the RMSSD and SDNN indicators were calculated. SDNN tended to decrease in response to competition. For RMSSD, a significant decrease was found during preparation of the horses for competition in the stable and during the actual competition on day 2 and was nearly reached on day 3. The appearance of competition-stress, as HRV deviations were not determined by the intensity of the load.

The relationship between HRV and competition-stress has not yet been studied in racehorses.

Aims of the study

Based on the literature on this subject I have realized that although both cortisol measurement and HRV analysis were used to investigate physical and mental stress, the physiological effect of exercise and the emotional influence of it are not well described. This scientific deficiency is no coincidence, as the clear separation of these two is not possible from the current knowledge. My main goal, however, was to take steps to separate mental and physical stress changes. I want to examine the mental stress of equine competition and to detect the importance of the overall emotional state of the horses in exercise physiology. In addition, I think that the presence, extent and correlation of the daily fluctuation of blood and saliva cortisol concentrations are not clearly described in horses, it is worth reconsidering it as a basic research.

1. My aim was therefore to describe the circadian rhythm of serum- and salivary cortisol concentrations in horses.

2. Then I intend to investigate the effect of the personality of horses on the exercise induced cortisol release.

3. I also consider to be important to examine "excitement" status of race horses. Although the presence of the anticipatory stress response in horses is not as clear as in human athletes,

there are many indications that horses are aware of the special nature of the competition day and the race is also a special mental stress in animals. My goal was to examine the presence or absence of this anticipation phenomenon by HRV data in horses.

4. I would like to study the relevance of HRV indicators measured during exercise, with the comparison of the HRV data recorded under slight and maximum workloads.

Own studies

1. Daily rhythm of cortisol concentration in rest

Materials and methods

I used 20 clinically healthy horses (n = 3 thoroughbred, n = 4 shagya-arab and n = 13 Hungarian sporthorse) in the experiment. The animals were between 3 and 13 years old, n = 8 mares, n = 7 geldings and n = 5 stallions. The horses were kept under the same conditions. Starting at 14:00 PM, blood and saliva samples were obtained from each of the 20 horses every 2 h for 24 h. Special care was used to minimize stress during the sampling.

To determine whether there was either diurnal variation or circadian rhythm in the measured serum and salivary cortisol concentrations, we used the Cosinor program of Refinetti et al. Circadian variables such as acrophase (the clock time of the maximum value of the curve), MESOR (true mean of the oscillating variable over its entire period), and amplitude (half of the difference between the maximal and minimal value in the curve) were extracted from the cosinor analysis. To assess the association between salivary and serum cortisol concentrations, the Pearson correlation test was used in the R 2.12.2. statistics software. The significance level was set at $P< 0.05$.

Results

Cosinor analysis of group mean data confirmed a significant circadian component for both serum and salivary cortisol concentrations ($P<0.001$ in both cases). The serum cortisol circadian rhythm had an acrophase at 10:50 AM (95% CI, 10:00 AM–11:40 AM), a MESOR of 22.67 ng/mL, and an amplitude of 11.93 ng/mL. The salivary cortisol circadian rhythm had an acrophase at 10:00 AM (95% CI, 9:00 AM–11:00 AM), a MESOR of 0.52ng/mL, and an amplitude of0.12ng/mL. We found a significant but weak association between salivary and serum cortisol concentrations; the Pearson correlation coefficient was 0.32 ($P<0.001$).

Discussion

Our present study confirmed the existence of a cortisol circadian rhythm in horses. In agreement with previous reports, the maximum cortisol concentrations occurred in the morning and the minimum values occurred in the evening. It also should be mentioned, that cosinor analysis and determination of a circadian rhythm is most appropriately performed on data of more than one cycle of data. This is a limitation of this study, and should be examined further involving more cycles.

Salivary cortisol also showed a significant circadian rhythm, however only a weak correlation between serum and salivary cortisol concentration was observed. In contrast, Peeters et al. (2011) found a strong but nonlinear association between the salivary and total serum cortisol concentrations, while Elsaesser et al. (2001) and Pell et al. (1999) could not find a significant relationship between the salivary and serum cortisol concentrations.

The discrepancy suggests a number of interpretations. In most horses in the studies, the saliva and blood samples were obtained simultaneously. However, a study using the adrenocorticotropic hormone (ACTH) stimulation test clearly demonstrated that an increase in the serum cortisol level was followed by an increase in the salivary cortisol level about 20 min later. Without a stimulation test, the follow-up period may increase or may become considerably diversified at rest. The correlation between the serum and salivary cortisol circadian rhythms may be disturbed or even masked by this phenomenon.

The discrepancies might also be caused by the difference between the bound and free cortisol levels. A fraction of serum cortisol is bound to corticosteroid binding globulin (CBG), but the free cortisol is responsible for the biological effect of the hormone. It is very difficult to measure the real bound to free cortisol ratio in the plasma because free cortisol in the serum is not proportional to the total serum cortisol. CBG concentrations are not constant and the saturation state of CBG is also variable. How the level of salivary free cortisol is affected by increased or reduced saliva production could also be an interesting investigation.

Several other physiological effects may therefore influence the ratio between the free cortisol concentrations in saliva and plasma, so the relationship between the two is difficult to model.

With respect to the daily highest and lowest concentrations in serum and saliva, other researchers have obtained similar results. The onset times of the cortisol peak and trough are notable because this is inconsistent with the timing of maximum and minimum serum cortisol concentrations in people.

Despite the identified discrepancies, the present study can justify the use of salivary cortisol concentration for examining HPA-axis activity in horses.

2. Effect of temperament on HPA reactivity in racehorses

Materials and methods

From twenty-seven healthy horses, twenty healthy Thoroughbred stallions (n = 10) and mares (n = 10), aged three years were selected for the study based on a 25-item rating questionnaire survey. Based on the questionnaire, horses were classified as ‘calm’ (scores 0–2, means ± SD = 1.2 ± 0.3), ‘average’ (scores 3–4, means ± SD = 3.4 ± 0.2) and ‘temperamental’ (scores 5–7, means ± SD = 5.2 ± 0.3). Average horses (n = 7) were excluded. Eight temperamental (five stallions and three mares) and twelve calm horses (five stallions and seven mares) took part in the experiment.

Horses were kept under the same conditions and the same trainer – who fulfilled the questionnaire – dealt with all the horses since the first mounting of a rider. Data were collected between 10 and 25 June, daily between 05:00 and 07:00 a.m., ensuring that the temperature remained between 18–23°C during the test. Horses were equipped with a Polar Equine RS800CX Multi device (Polar Ltd.; New York, USA) for recording their heart rate, distance traveled, and velocity. Horses had a 10-min warm-up trot with a rider, and then galloped 2,300 m on a rounded sand track. To avoid the psychical influence of the rider-instructions, horses were allowed to gallop without restraint or encouragement. In all cases, the rider was the familiar jockey of the horse. All of the horses galloped alone during the test.

Blood was collected by jugular venipuncture into heparinized tubes (S-Monovette, Sarstedt, Nümbrecht-Rommelsdorf, Germany) during the following periods:

- in the morning at rest between 05:00 and 06:00 a.m. (S0),
- after the warm-up period (S1),
- after the galloping period (S2),
- after the 30-min recovery period (S3).

Samples were cooled to and stored at 4°C until centrifugation at 2000 g for 10 min. Plasma was removed and stored at –18°C until cortisol assay. Cortisol analyses were performed in duplicates using direct RIA method validated for horses. The analyses were carried out using R 3.2.3 statistical software. For hypothesis testing, a linear mixed model was fitted. For multiple comparisons, we tested contrast and applied the Tukey method to avoid the accumulation of Type 1 errors.

Results and discussion

Based on the speed and heart rate, I found that the horses tested, received significantly the same workload without the influence of riders. A distinct increase in serum cortisol concentrations was observed in both temperamental and calm horses in response to the exercise. In our study, maximal cortisol levels were observed 30 min after the exercise has finished in temperamental group, and immediately after exercise in calm horses. The peak level of cortisol were 105.1 ± 15.3 and 119.9 ± 16.9, with 22.6% and 43.1% higher than baseline levels in calm and temperamental horses, respectively. Our results contradict the results of Nagata et al. (1999), who found an early peak in cortisol levels already during the warm-up phase. However, the mentioned results have possibly been influenced by the anticipatory stress of the use of treadmills in the experimental design.

In line with a recent observation in cattle, we found no difference in basal serum cortisol concentrations (S0) between temperament groups ($P = 0.888$) reflecting that human presence prior to the exercise did not cause higher stress for temperamental horses than for calm ones. Despite it was non-significant, the difference between groups was already visible at S1 and S2 samplings ($P = 0.084$ and $P = 0.141$, respectively), while 30 min after the gallop phase (S3), cortisol levels were significantly higher for temperamental than calm horses ($P = 0.036$).

Exercise means not only physiological stress for horses. Horses are also exposed to environmental, social, and psychological factors during the daily training. Based on our results, responsiveness to these effects might independent of the exercise load but may affect the strength of the cortisol response; however, only the exercise was followed. It is possible, that comparison of areas under the cortisol response curves or time to return to baseline levels between temperament groups would have been provide more information on the magnitude of the HPA response; however, cortisol levels did not return to baseline in the duration of the sampling to enable such analysis. It is a limitation of our study, that cortisol was not measured at least 2-h following the exercise has completed. On the other hand, by setting a relatively short sampling period we eliminated the possible confounding effects of diurnal rhythms of serum cortisol secretion on our results.

3. Monitoring of the heart rate variability during exercise

Materials and methods

The measurement was carried out with seven Standardbred horses (n = 7 stallions). The horses were in perfect state of health during the experimental period, even at least half a year before. The horses were kept under the same conditions in Kincsem Park. The horses were between 3 and 4 years of age at the time of the study. All seven of the horses lived in the same stable and were trained by the same trainer. During 4 months prior to the trial, horses had a training session 5 days a week (3 times milder, 2 times stronger). The tests were carried out at Kincsem Park and the horses were not transported during the experimental period. There was no relevant difference between the ambient temperature and the humidity during the test. To measure the heart rate, RR data, and speed, the horses were equipped with Polar Equine RS800CX Multi device (Polar Ltd., New York, USA). The measurements were carried out under three different conditions:

- at rest: a total of 20 minutes RR data was recorded on an average rest day in the morning (08:00 to 09:00), while the horses were in their own box. No feeding was due either 2 hours prior to the recordings, or even after 2 hours. All the other confusing circumstances were tried to exclude.
- during a steady state training session: Horses receive a mild training every Monday, Tuesday and Friday. They trot 8-9000 m with the usual driver at 20-25 km/h. On those days, the tests were performed always in the morning between 8:00 and 10:00.

- real race: The horses ran 2300 meters in the morning in a real race with their usual drivers. I started to record the HRV data in the box before the preparation of the horses.

The analysis of the RR data was performed by the Kubios 2.2 HRV analysis software developed by Tarvainen and Niskanen (2008). To remove the faults, I used the 'custom' filter of the program, then I examined the RR curve and the remaining artifact sections were not used for further analysis. The RR data recorded during rest, light work and competition was analyzed based on 1 minute intervals, also with time- and frequency-domain analysis. The frequency ranges were determined on the basis of a previous experiment carried out on trotters during high workload: LF: 0.04-0.2 Hz and HF: 0.2-2 Hz.

I calculated the HRV indices for the sum of the power spectral density (PSD) within the LF and the HF spectral ranges, in ms^2/Hz. The LF and HF values were then normalized and given as a percentage of total short-term spectral energy (TP).

In the time domain analysis, I calculated and compared the "HF-type" RMSSD and the SD2 value derived from geometric analysis, which has much more "LF-nature", as they have been found to be indicators of the fatigue in previous studies. It is important, however, that the SD2 value should be treated with reservations due to the short data set, as some authors argue that geometric analysis will only provide a certain result over a longer period.

Results

Although the RMSSD and SD2 values did not show significant differences in the morning of the competition compared to an average morning rest period, LFn (P <0.001) and LF / HF (P = 0.009) were significantly higher, while HFn (P <0.001) was significantly lower before the race.

On the training day, the lactate value did not exceed the lactate threshold of 4 mmol/L, ie horses actually performed an aerobic work, while on the racing day the lactate level rose dramatically after warm-up and each horse carried out anaerobic exercise.

My further result is that the LF / HF value is with 95% chance between 0.98 and 1.11 (95% CI for mean: 0.98-1.11), and between 0.0046 and 0.24 (95 % CI for mean: 0.00 - 0.24) (P <0.001) in the steady-state aerobic exercise and at maximum workload, respectively. It is another interesting result that the resting LF / HF was never less than 1 (P <0.001) in the investigated horses.

Discussion

The HRV indicators of pre-competitive stress were only studied in horses by Becker-Birck et al. (2013), but in this experiment analyzes were performed only in time domain: RMSSD and SDNN indicators were analyzed. The horses were sport horses, that were transported from their familiar environment to a foreign stable to the jumping or dressage competition. Significant reductions in RMSSD were observed in the morning of the second and third day of the competition, but on the first day no sign of extra stress was noticeable. I did not calculate SDNN, based on the short length of the analyzed interval. I calculated but RMSSD, and in the present experiment the RMSSD did not differ in the morning of the race compared to an average morning value. The reason for this is probably not the lack of anticipatory stress, rather than the fact that this indicator is not suitable for detecting such "latent" stress situations. The RMSSD, according to other authors, may be a marker of fatigue. In my opinion, the RMSSD decrease on Day 2 and Day 3 in the 3-day competition described above is due to fatigue rather than mental stress.

Based on the parameters measured by spectral analysis, the pre-competitive stress was significantly detectable. Higher LFn (P <0.001), higher LF / HF (P=0.009) and lower HFn (P <0.001) were measured in the morning of the competition. Our experimental horses were kept and trained on the racetrack, so environmental changes and transportation were no influencing factors before the race. However, the trotting races in Kincsem Park are organized on a regular basis (every Wednesdays and Saturdays), so horses, based on their internal biological rhythm can easily be prepared to the race. It is a good question, whether those horses that did not start in the experimented competition, would also show changed HRV indicators in the morning, or only the individuals who started the race will have a special feel about the distinctive nature of the day. As trainers reported, from the changed feeding (plus energy in the evening food) and from the extra rest before the day of the race, most horses clearly understand what comes, and respond positively or negatively, but mostly with changed behavior. Also based on my results, it seems that the anticipatory stress phenomenon can be measured in trotters.

Steady state exercise means any activity that is performed at a relatively constant speed and heart rate for an extended period of time and a balance develops between energy required and energy available, so it is performed with persistent physiological parameters. Steady state exercise typically occurs at a moderate intensity, and it is an aerobic training, since lactate and carbon dioxide accumulating during the anaerobic energy supply makes it impossible to keep physiological parameters unchanged. From my results, it is clear that both lactate and heart rate were at constant values during the training, so our experimental horses performed real aerobic steady state workload. Only on the basis of a heart rate - although many trainers use this method - this steady state condition can no doubt be justified. There may be a lactate elevation at a constant pulse rate. However, due to the invasive nature of lactate measurement, it is difficult to measure lactate level during all workouts, i.e. the non-invasive detection of steady state status is not yet possible in horses.

In present study, during steady-state aerobic exercise, LF / HF was between 0.98 and 1.11 (P <0.001), meaning that steady-state exercise can be verified by HRV analysis. LFn and HFn are stable to equal about 50, which results in an LF / HF value of around 1 at this workload.

The LFn-HFn equilibrium observed in the steady-state training, and the LF / HF stabilization to 1 may indicate a complete equilibrium of the autonomic nervous system, but can also be originate from the increase in HFn caused by the steady-state hyperventilation. From a certain point of view, it is not essential to separate these two possibilities: if we can prove that the value of LF / HF is always 1 (real or as a respiratory artifact) in steady state condition, we could help the trainers to find the exact extent of the workload with non-invasive method for an aerobic steady state training program.

At maximum workload (during the race) LFn and LF / HF was 0 (95% CI 0.00-0.24; P <0.001), so the hyperventilation induced HF elevation suppressed the evaluable HRV. The heart rate of our experimental horses was much higher than 130 / min at race, which is the max. value according to Physick-Sheard et al. (2000) where HRV is still measurable. Taking this into account, our LF / HF result was expected. However, the fact, that the LF / HF value is 1 at aerobic steady state condition while it is 0 at anaerobic energy supply, supports the possibility that falling LF / HF below 1 can be the indicator of the respiratory threshold even in horses. Based on an experiment performed with increased number of horses and with measurement of the respiratory function, this scientific finding would be a novelty in the field of equine exercise physiology. My plan is to perform such an experiment.

It is interesting to note that in my measurements the resting LF / HF was not below 1 (P <0.001), but even 2-3 could be measured in the examined horses. The strong parasympathetic overweight of horses in rest did not manifest as decisively as in humans. Kuwahara et al. (1999) have demonstrated that a good exercise program in horses increases the resting LF value while the HF remains unchanged. That is, the strong parasympathetic tones of horses can no longer be strengthened by training, which leads to a relative increase in LF / HF in well trained horses. The results of the examined trotters also prove this phenomenon: in a well-trained state the parasympathetic overweight decreases and a mild sympathetic dominance will be detectable.

New scientific results

1. In order to clarify controversial results in the subject, I described with a higher number of individuals (n = 20 horses) and with more frequently sampling (2 hourly) that the serum and salivary cortisol concentration show daily circadian pattern in horses. A weak correlation between serum and salivary cortisol concentrations was found.

2. I firstly examined the relationship between horse temperament and changes in cortisol levels during exercise. I have found that temperament is an important factor in cortisol response. Based on my results I questioned the usefulness of cortisol hormone in assessing the degree of acute workload.

3. I was the first who described the pre-competition mental stress using HRV indicators in racehorses.

4. My studies suggest that both the steady-state aerobic exercise and the anaerobic threshold are detectable based on HRV analysis. Based on these results, it would be worth carrying out an experiment with a larger number of individuals.

Publications

Publications and conference proceedings related to the dissertation

Bohak Zs., Beckers J.F., Melo de Sousa N., Kutasi O., Nagy K., Szenci O.: Monitoring the circadian rhythm of serum and salivary cortisol concentrations in the horse, Dom. Anim. End., 45:1. 38-42. 2013.

Bohák Zs., Kutasi O., Szenci O.: A kortizol hormon sportélettani szerepe lovakban: The role of cortisol hormone in equine exercise physiology, Magyar Állatorvosok Lapja 138. 643-652. 2016.

Bohak Zs., Szenci O., Harnos A., Kutasi O., Kovács L.: The effect of temperament on the cortisol response to single exercise bout in Thoroughbred racehorses, Acta Veterinaria Hungarica - accepted

Nyerges-Bohák Zs., Kutasi O., Szenci O.: Sportélettan és teljesítmény- Az endokrin rendszer – túledzettség, avagy jóból is megárt sok, XXIV. Lógyógyászati Kongresszus, Telki, Magyarország, 2016.12.2-3.

Bohák Zs., Kutasi O., Szenci O.: Terhelésre adott szívfrekvencia-variabilitás és vér kortizol válasz összevetése angol telivér lovakban In: Akadémiai beszámoló: Klinikumok. Budapest, Magyarország, 2016.01.25-28.

Bohák Zs., Kutasi O., Harnos A., Bartesch C., Szenci O.: Fizikai munkavégzés, illetve stressz hatása a kortizol hormon elválasztására ügető lovakban, In: Akadémiai beszámoló: Klinikumok. Budapest, Magyarország, 2015.01.26-29.

Bohák Zs., Kutasi O., Harnos A., Bartesch C., Szenci O.: Szívfrekvenciavariabilitás vizsgálata ügető lovakon, In: Akadémiai beszámoló: Klinikumok. Budapest, Magyarország, 2014.01.27-30.

Bohák Zs., Szabó F., Nagy K., Boros B., Kutasi O., Melo de Sousa N., Beckers J.F., Szenci O.: Kortizol koncentráció napi ingadozásának vizsgálata lovakban, In: Akadémiai beszámoló: Klinikumok, Budapest, Magyarország, 2012.01.16-19.

Bohák Zs., Csepi G., Kutasi O., Tóth P., Izing S., Szenci O.: Angol telivér versenylovak teljesítményvizsgálata, In: Akadémiai beszámoló: Klinikumok, Budapest, Magyarország, 2012.01.16-19.

Bohák Zs., Kutasi O., Hevesi Á., Szenci O.: Teljesítménycssökkenés tüneteivel érkezett klinikai beteg diagnosztikai vizsgálata, In: Akadémiai beszámoló: Klinikumok, Budapest, Magyarország, 2011.01.24-27.

Acknowledgements

I would like to thank my supervisor, Prof. Dr. Ottó Szenci, the many critical comments and suggestions made in connection with my scientific publications and dissertation which contributed greatly to the final form of my work. I am grateful to my co-supervisor Dr. Levente Kovács (MTA-SZIE Large Animal Clinical Research Group) for his assistance in designing and implementing experiments and for his extensive support during the writing of the dissertation.

I thank Prof. Dr. Ottó Szenci for giving the possibility to be a member of the MTA-SZIE Large Animal Clinical Research Group, where I was able to discuss my results more widely, and to receive inspirations and advice from the research team.

I thank dr. Kinga Joó, dr. Constanze Bartesch, dr. Gábor Csepi Pál, dr. Bálint Boros and Viczena Bettina, my friends and colleagues to help me with data collection and processing.

Thanks for the work of Erzsébet Sípos and Erzsébet Tani (former) laboratory workers (University of Veterinary Medicine, Department and Clinic of Equine Medicine) and Dr. Huszeniczáné Dr. Margit Kulcsár (University of Veterinary Medicine, Department of Obstetrics and Reproduction, Endocrinology Laboratory) for his assistance in the cortisol hormone analysis.

I thank Bábolna National Stud Farm Ltd. and Emil Csordás for providing the racehorses, and the drivers and jockeys from racetrack Alag and Kincsem Park, for the help in practical implementation of the experiments.

I would like to thank Dr. András Székely for the thorough grammatical and stylistic re-examination of my publication in English and for the help with the final drafting of the papers.

I thank Dr. Krisztina Nagy for giving me a lot of useful advice in the use of the RR-recorders and the HRV analysis program at the beginning of my experiments.

I thank her, Dr. Andrea Harnos and my brother andrás Bohák for the help with the statistical analysis.

I would like to thank my 3-year-old daughter, Nyerges Lotti, for her endless patience during the data collection and the writing of the dissertation. I also thank my husband and parents for their personal support in the successful implementation of my doctoral research.