

Salivary cortisol levels in horses and their junior riders during show jumping

Katarzyna STRZELEC¹ , Sławomir PIETRZAK¹ , Tomasz PRÓCHNIAK^{2,*} ,
Iwona JANCZAREK¹ , Anna CYWIŃSKA³ , Andrzej BEREZNOWSKI⁴ 

¹Department of Horse Breeding and Use, Faculty of Animal Sciences and Bioeconomy,
University of Life Sciences in Lublin, Lublin, Poland

²Institute of Biological Basis of Animal Production, Faculty of Animal Sciences and Bioeconomy,
University of Life Sciences in Lublin, Lublin, Poland

³Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine,
Warsaw University of Life Science, Warsaw, Poland

⁴Laboratory of Veterinary Epidemiology and Economics, Warsaw University of Life Science, Warsaw, Poland

Received: 06.08.2019 • Accepted/Published Online: 28.01.2020 • Final Version: 06.04.2020

Abstract: The group of 22 warm-blooded half-breed horses (6 stallions, 6 mares, and 10 geldings) and their riders (12 women and 10 men) who ended jumping competition for juniors, were selected for a study. The horses were aged from 7 to 17 years, while the riders were 15 to 18 years old. The saliva samples were collected before and after each jumping round. The cortisol concentration was determined by using an immunoassay method. The following factors were considered: horses' sex, age, and breeds, riders' sex and age as well. In riders, significant correlation was found in the group of younger juniors (15–16 years old) between the level of cortisol concentration before and after jumping round. High significant correlation coefficient was found due to influence of sex and age of horses on salivary cortisol concentration, but only for stallions and mares. Individual horses' intensity of cortisol release in response to participation in jumping competition is found to vary greatly. Therefore, the salivary cortisol test seems to be a useful and efficient method to evaluate the horses' responses to competitions including the influence of the rider.

Key words: Horses, riders, saliva, cortisol, show jumping

1. Introduction

One of the main substances that indicate stress level is cortisol [1]. The concentration of cortisol depends on the natural circadian rhythm, which can be disturbed by physical as well as mental stress. The most effective way of determining cortisol concentration in animals seems to use their saliva. This is because taking saliva samples is a noninvasive and a low-stress procedure [2]. Therefore, the saliva testing is selected as the method of choice for sport horses and riders too. In humans, mental stress caused by participation in sport competitions was found to increase salivary cortisol level substantially [3]. Additionally, in horses, the salivary cortisol levels were found to reflect the stress level not only during various equestrian competition events [4,5] but also during training and road transport [6,7]. However it is known that not only stress, but also physical activity is a factor, which increases the hypothalamus-pituitary-adrenal axis and cortisol release [8].

Jumping round is not too long effort but it is really intensive for a horse and a rider because of turns, number

of fences, character and size of obstacles, and speed etc. [9].

The aim of the study was to compare the salivary cortisol levels in jumping horses and their junior riders during jumping competition.

2. Material and methods

Horses and riders: the study took place during international show jumping (Cavaliada Tour 2016 Lublin) for juniors (height of fences up to 130 cm) in Poland. Twenty two horses (10 Polish noble half-breed, 4 Hanoverian breed, 4 Wielkopolski breed, 2 Dutch warm-blood, 2 Westphalian breed) were taken for the study. The horses (6 stallions, 6 mares, 10 geldings) were aged from 7 to 17 years, while the riders (12 female and 10 male) were aged from 15 to 18 years. None of the horses had any clinical symptoms of health disorders. Each rider had trained and competed with his or her horse for at least 10 months prior to the study.

Saliva sampling from each horse and rider, 2 saliva samples were collected: 1/before warm up and start (in a

* Correspondence: tomasz.prochniak@up.lublin.pl

stable), 2/immediately after the end of a jumping round. The saliva samples were collected with a small piece of sponge, which was inserted into mouth of the horse with the use of tweezers. The riders were instructed to place and hold the sponge in their mouths until it became saturated. After soaking in saliva, the sponge was placed in a plastic tube, as described previously [10], and frozen. All procedures were performed in respect of the legislation on Animal Care (EU Directive 2010/63/EU) and the Internal Rules of University of Life Science in Lublin.

2.1. Analyses

Before the examination, the saliva samples were melted, warmed up to room temperature, and centrifuged at $500 \times g$ for 15 min at room temperature. Afterwards, the sponge was removed and the saliva was transferred into test tubes. The concentration of cortisol in saliva samples was measured by the enzyme-immunoassay method [11] using the Cortisol ELISA kit SLV-4635 (DRG Instruments Company, Marburg, Germany). All assays were based on polyclonal rabbit antibody directed towards an antigenic site on the cortisol molecule. The analytical sensitivity of the assay was 0.012 ng/mL. The absorbance was measured using a Multiscan reader (Labsystem, Vantaa, Finland) equipped with a GENESIS V 3.00 program. The results were expressed as nmol/L. The minimal detectable concentration was 0.3 nmol/L.

2.2. Statistics

The results including standard deviation (SD) were analysed using the common SAS software (Version 9.4, 2013) and the ANOVA–GLM method for analyzing repeated measures and Tukey test. The following factors and effects were considered: 1/ rider-age group and sex 2/ horse- age group, sex, and breed. The Pearson correlation coefficient was also assessed to compare the analysed data. The statistical significance was accepted at 2 levels of $P \leq 0.05$ and $P \leq 0.01$.

3. Results

The results of the salivary cortisol concentration in the studied riders are shown in Table 1. The results obtained for the samples before the start of women (14.20 nmol/L) were significantly higher than those obtained by men (9.98 nmol/L). The obtained data did not differ statistically for men and women immediately after finishing a jumping round. However, the differences between the level of salivary cortisol concentration (before starting and after finishing the round) in both cases (men and women) were statistically significant. In women's case, this level increased more than twice that of men's case (respectively woman 14.20–31.33; man 9.98–24.31 nmol/L).

Interesting data are shown in Table 2, which presents an average salivary cortisol concentration in 2 age groups of riders (15–16 and 17–18). In the group of the

Table 1. Average values of saliva cortisol concentration of riders due to sex.

Sex	Number		Rest	Effort
Female	12	\bar{x}	14.20 ^{ax}	31.33 ^{ay}
		sd	12.40	20.41
Male	10	\bar{x}	9.98 ^{bx}	24.31 ^{ay}
		sd	7.22	18.51

Averages with different letters (a, b in columns, x, y in rows) differ significantly at $P \leq 0.05$.

Table 2. Average values of saliva cortisol concentration of riders due to age.

Age	Number		Rest	Effort
15–16	12	\bar{x}	9.87 ^{ax}	27.40 ^{ay}
		sd	7.61	21.60
17–18	10	\bar{x}	16.50 ^{ax}	29.69 ^{ay}
		sd	13.62	16.51

Averages with different letters (a, b in columns, x, y in rows) differ significantly at $P \leq 0.05$.

younger juniors (15– 16), the average salivary cortisol concentration measured before the start was at the level of 9.87 but in the older one it was higher (16.50 nmol/L). The differences were not statistically significant. In the group of younger juniors, after the end of jumping rounds, cortisol concentration was nearly 3 times higher in comparison to the level before the start. The differences were statistically significant. Table 3 includes average cortisol concentration of studied horses within their sex. The level of mares' cortisol concentration was higher than stallions' and geldings' not only after exercise but also before it. The differences were statistically significant between mares and other gender groups only after effort. All studied horses were divided into 2 groups by their age (up to 10 years old and older than 10) to see the differences of saliva cortisol concentration (Table 4). There were no significant statistical differences between the age groups before the effort and after it as well, but they were present in both groups between the level of values before and after a jumping round. Data included in Table 5 show that there are no significant statistical differences between the studied horse breeds due to cortisol concentration before the start of a jumping competition and after it. A statistically important correlation was found between the level of cortisol concentration in riders before and after

Table 3. Average values of saliva cortisol concentration of horses due to sex.

Gender	Number		Rest	Effort
Stallion	6	\bar{x}	0.36 ^{ax}	5.43 ^{ay}
		sd	0.12	0.61
Mare	6	\bar{x}	0.51 ^{ax}	7.85 ^{by}
		sd	0.44	0.94
Gelding	10	\bar{x}	0.33 ^{ax}	5.48 ^{ay}
		sd	0.09	2.13

Averages with different letters (a, b in columns, x, y in rows) differ significantly at $P \leq 0.05$.

Table 4. Average values of saliva cortisol concentration of horses due to age.

Age	Number		Rest	Effort
Up to 10 years	8	\bar{x}	0.37 ^{ax}	6.21 ^{ay}
		sd	0.27	1.67
Over 10 years	14	\bar{x}	0.40 ^{ax}	6.06 ^{ay}
		sd	0.23	2.01

Averages with different letters (a, b in columns, x, y in rows) differ significantly at $P \leq 0.05$.

the start (0.41) Table 6. For the horses it was not found a similar dependence. Negative statistically significant correlation coefficient (-0.70) between the level of cortisol concentration, measured before the start of a jumping competition and after it, was found in the group of male juniors (Table 7). In the case of age, statistically significant difference was found for younger juniors (0.64). Another statistically significant correlation coefficient between cortisol concentration in horses before the start and after it, was found in the group of stallions (0.95) Table 8. The same situation was observed in the group of older horses (0.72) as well as in the group of polish half-breed horses (0.72). High value of correlation coefficient (0.80) was noticed also for Hanoverian horses, but due to the low number, it was not considered as statistically significant.

4. Discussion

Results obtained in this study proved a significant influence of the analysed factors (sex and age of riders and also sex, age, and breed of horses) on saliva cortisol concentration before and after exercise. Significantly higher cortisol concentration in the group of women, before the start of

Table 5. Average values of saliva cortisol concentration of horses due to breeds.

Breed	Number		Rest	Effort
Hanoverian	4	\bar{x}	0.56 ^{ax}	6.36 ^{ay}
		sd	0.40	2.29
Royal Dutch sport	2	\bar{x}	0.36 ^{ax}	5.07 ^{ay}
		sd	0.08	0.04
Polish half-breed	10	\bar{x}	0.32 ^{ax}	6.16 ^{ay}
		sd	0.15	2.23
Westphalian	2	\bar{x}	0.56 ^{ax}	7.62 ^{ay}
		sd	0.54	0.14
Wielkopolski	4	\bar{x}	0.32 ^{ax}	5.51 ^{ay}
		sd	0.06	1.01

Averages with different letters (a, b in columns, x, y in rows) differ significantly at $P \leq 0.05$.

Table 6. Correlation between cortisol concentration of horses and riders before and after jumping round.

	Horse	Rider
	Rest	Rest
Effort	0.22	0.41 ^{**}

^{**}Correlation significant at $P \leq 0.01$.

Table 7. Correlation between cortisol concentration of riders due to sex and age.

Sex		Female	Male
		Rest	Rest
	Effort	0.28	-0.70^{**}
Age		15-16 years	17-18 years
		Rest	Rest
	Effort	0.64 [*]	-0.15

^{*}Correlation significant at $P \leq 0.05$; ^{**}Correlation significant at $P \leq 0.01$.

a jumping competition, can prove their higher emotional sensitivity, when compared to men in show jumping. This observation is not consistent with the results of other studies, in which males showed greater cortisol response to stress than females [12]. Similar results to Franco et al. [12] were shown by Strzelec et al. [13], where females

Table 8. Correlation between cortisol concentration of horses due to sex, age, and breed.

Sex		Stallion		Mare		Gelding
		Rest		Rest		Rest
	Effort	0.95**		0.53		0.40
Age		Up to 10 years			Over 10 years	
		Rest			Rest	
	Effort	-0.11			0.74**	
Breed		Hanoverian	Royal Dutch	Polish half-breed	Westphalian	Wielkopolski
		Rest	Rest	Rest	Rest	Rest
	Effort	0.80	0.01	0.72*	0.02	0.33

*Correlation significant at $P \leq 0.05$; **Correlation significant at $P \leq 0.01$.

participating in eventing had significantly lower level of cortisol concentration than males.

Significant differences between cortisol concentration before and after the effort found in the group of men are interesting. About 3 times increase of level of cortisol concentration after finishing a jumping round was probably an effect of mental stress and also physical effort. This observation is consistent with the results of Garwin et al. [14], Franco et al. [12], and Strzelec et al. [13], in which males showed greater activity of the hypothalamus-pituitary-adrenal axis and higher cortisol release than females.

Significant differences were not found between cortisol concentrations before exercise in both age groups of riders. Higher level of older juniors can be influenced by the atmosphere of rivalry before competition as well as after jumping round because of a bigger emotional engagement of more experienced athletes [13].

The effect of horse's sex was the highest in the group of mares. Average cortisol concentration in this group was higher than those of stallions and geldings. The obtained results are similar to the other authors' [1,6,7,9]. According to Strzelec [15], this situation can be influenced by physical effort of jumping and the atmosphere of the show, but also of mares' sex hormones activity. Different results were found by Fazio et al. [16], where the level of cortisol concentration of stallions was higher than that of mares.

Age of horses was not a factor which determined saliva cortisol concentration. Slightly lower level of cortisol after exercise suggests that older horses have more experience and release of the hormone is influenced mostly by physical effort. According to Nogueira et al. [17], the proper training of sport horses can decrease stress during the competition. After coming to the show, horses feel the atmosphere of rivalry so the level of cortisol concentration measured before the start shows

their mental stress [1,18].

In the studied horses, participation in the jumping competition caused cortisol release; however, between the horses' breeds, the changes in the mean salivary cortisol concentration did not reach the level of statistical significance. Probably, the relatively small number of horses, and the high individual variability of salivary cortisol levels from horse to horse, which is expected, decreased the possibility of observing statistically significant differences. This observation is not consistent with the results of Strzelec [15] in which German warm-blood breeds of horses (very well known in world equestrianism) were characterized by higher level of cortisol concentration in comparison to the other breeds. High variability of salivary cortisol level from horse to horse was also observed by other researchers [4,10].

There was a significant correlation between the level of cortisol concentration before the start and after it in the group of riders but not in the horses. It may suggest that young riders are more stressed before the start than horses. It could be explained by their age and lack of experience. Similar results were shown by Peeters et al. [4] and Strzelec et al. [13]. Significant negative correlation was found in the sex group of male but positive significant correlation in the group of younger juniors. Therefore, the age of the riders cannot be influenced by saliva cortisol concentration. According to the other researches this situation depends more on the level of physical effort and individual immunity to stress of riders and horses [3,19].

High significant correlations were found due to the influence of sex, age, and breed of horses on salivary cortisol concentration, but only for stallions and mares. It means that high level of cortisol concentration before exercise in the case of stallions and mares is also high after exercise. Similar results were shown by Schmidt et al. [6,7] and Strzelec [15] where high cortisol concentration before effort, especially in the group of mares, was present

also after exercise. These researches explain this fact by the excessive excitability of stallions and specific psychic traits of mares which can influence their behaviors during performance tests.

In conclusion, individual horses are found to vary greatly in their intensity of cortisol release in response

to participation in jumping competition. In the context of such individual variability, the horse's response to a jumping round is found to be repetitive. Therefore, the salivary cortisol test seems to be a useful and efficient method to evaluate the horse's response to competitions which include the influence of the rider.

References

- Cravana C, Giovani FD, Medica P, Fazio E, Ferlazzo A. Cortisol response of therapeutic riding horses to hippotherapy and horse riding sessions. In: Conference on Equine Sports Medicine and Science; 2008. pp. 153-158. doi: 10.3920/978-90-8686-643-4
- Aardal E, Holm A-C. Cortisol in saliva-reference ranges and relation to cortisol in serum. *Clinical Chemistry and Laboratory Medicine* 1995; 33 (12): 927-932. doi: 10.1515/cclm.1995.33.12.927
- Marc M, Parvizi N, Ellendorff F, Kallweit E, Elsaesser F. Plasma cortisol and ACTH concentrations in the warmblood horse in response to a standardized treadmill exercise test as physiological markers for evaluation of training status. *Journal of Animal Science* 2000; 78 (7): 1936-1946. doi: 10.2527/2000.7871936x
- Peeters M, Closson C, Beckers J-F, Vandenheede M. Rider and horse salivary cortisol levels during competition and impact on performance. *Journal of Equine Veterinary Science* 2013; 33 (3): 155-160. doi: 10.1016/j.jevs.2012.05.073
- Peeters M, Sulon J, Serteyn D, Vandenheede M. Assessment of stress level in horses during competition using salivary cortisol: preliminary studies. *Journal of Veterinary Behavior: Clinical Applications and Research* 2010; 5 (4): 216. doi: 10.1016/j.jveb.2009.10.043
- Schmidt A, Aurich J, Möstl E, Müller J, Aurich C. Changes in cortisol release and heart rate and heart rate variability during the initial training of 3-year-old sport horses. *Hormones and Behavior* 2010; 58 (4): 628-636. doi: 10.1016/j.yhbeh.2010.06.011
- Schmidt A, Biau S, Möstl E, Becker-Birck M, Morillon B et al. Changes in cortisol release and heart rate variability in sport horses during long-distance road transport. *Domestic Animal Endocrinology* 2010; 38 (3): 179-189. doi: 10.1016/j.domaniend.2009.10.002
- Desmecht D, Linden A, Amory H, Art T, Lekeux P. Relationship of plasma lactate production to cortisol release following completion of different types of sporting events in horses. *Veterinary Research Communications* 1996; 20 (4): 371-379. doi: 10.1007/BF00366544
- Cartmill JA, Thompson Jr DL, Storer WA, Gentry LR, Huff NK. Endocrine responses in mares and geldings with high body condition scores grouped by high vs. low resting leptin concentrations. *Journal of Animal Science* 2003; 81 (9): 2311-2321. doi: 10.2527/2003.8192311x
- Strzelec K, Kankofer M, Pietrzak S. Cortisol concentration in the saliva of horses subjected to different kinds of exercise. *Acta Veterinaria Brno* 2011; 80 (1): 101-105. doi: 10.2754/avb201180010101
- Barrou Z, Thomopoulos P, Luton JP. Assay of salivary cortisol. An interesting method for exploring the adrenal cortex. *Presse Medicale (Paris, France)* 1997; 26 (7): 329-331.
- Franco C, Paris JJ, Wulfert E, Frye CA. Male gamblers have significantly greater salivary cortisol before and after betting on a horse race, than do female gamblers. *Physiology & Behavior* 2010; 99 (2): 225-229. doi: 10.1016/j.physbeh.2009.08.002
- Strzelec K, Kędzierski W, Bereznowski A, Janczarek I, Bocian K et al. Salivary cortisol Levels in horses and their riders during three-day-events. *Bulletin of the Veterinary Institute in Pulawy* 2013; 57 (2): 237-241. doi: 10.2478/bvip-2013-0042
- Garwin AW, Koltyn KF, Morgan WP. Influence of acute physical activity and relaxation on state anxiety and blood lactate in untrained college males. *International Journal of Sports Medicine* 1997; 28 (06): 470-476. doi: 10.1055/s-2007-972666
- Strzelec K. Cortisol concentration in saliva as an indicator of the stress reaction in horses studied during different types of utility. *Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie* 2013; (374): 87.
- Fazio E, Medica P, Aronica V, Grasso L, Ferlazzo A. Circulating β -endorphin, adrenocorticotrophic hormone and cortisol levels of stallions before and after short road transport: stress effect of different distances. *Acta Veterinaria Scandinavica* 2008; 50 (1): 6. doi: 10.1186/1751-0147-50-6
- Nogueira G de P, Barnabe RC, Bedran-de-Castro JC, Moreira AF, Fernandes WR et al. Serum cortisol, lactate and creatinine concentrations in Thoroughbred fillies of different ages and states of training. *Brazilian Journal of Veterinary Research and Animal Science* 2002; 39 (1): 54-57. doi: 10.1590/S1413-95962002000100010
- Ferlazzo A, Medica P, Cravana C, Fazio E. Endocrine changes after experimental showjumping. *Comparative Exercise Physiology* 2009; 6 (2): 59-66. doi: 10.1017/S1755254009990110
- Möstl E, Palme R. Hormones as indicators of stress. *Domestic Animal Endocrinology* 2002; 23 (1-2): 67-74. doi: 10.1016/S0739-7240(02)00146-7