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# Assessment of changes in the saliva cortisol level of horses during different ways in recreational exploitation

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**Abstract:** The aim of this study was to evaluate the saliva cortisol levelsof recreational horses dependent on the type of horse activity. A total of 68 horses were included in the experiment and divided into groups according to recreational activity, type of horse, age, term of saliva collection, and sex. Saliva samples were obtained from each horse at 3 measurement points: in the morning (6 a.m.), in the evening (6 p.m.), and immediately after work. The overall analysis shows that the term of sample collection, recreational activity, and age influence cortisol concentration. The diurnal concentration of saliva cortisol does not differ between the types of horses and between mares and geldings. However, the concentration of cortisol in saliva after recreational riding differs significantly in comparison to the morning and evening levels. The analysis showed that age differentiates the saliva cortisol level significantly in young horses (3–5 years old). In all groups, exercise increased the level of cortisol. Horses performing dressage were characterized by the highest level of cortisol. Driving and lunging exercises resulted in a significant increase in the cortisol concentration after work. The lowest level of cortisol was obtained in horses used in reining.

**Key words:** Horse, recreational exploitation, stress, cortisol

## 1. Introduction

Since domestication, horses have been exposed to a variety of anthropogenic stress factors [1]. Their traditional forms of utilization have changed over the centuries. Nowadays, horses are used in developing countries for drought power and transportation [2], but in affluent countries, horses are predominantly used for recreation, sport, and as companions [3].

In recent times, numerous research studies have been undertaken to estimate the well-being and factors disrupting the state of welfare in horses in many aspects of their utilization [4–6]. The welfare of horses used for recreation is becoming an increasingly important issue [7]. Recreational riding is a very special kind of physical activity due to the objectives pursued by the rider; his/her experiences are based on the presence of the horse. This is related to the need to ensure adequate welfare of horses in an increasingly growing number of equestrian centers. To this end, it is necessary to possess knowledge about the physiology and behavior of these animals. However, too few studies have focused exclusively on recreational horses. Up to now, well-being indices have been described as body and hoof condition [8], stereotypical behaviors [9,10], as well as the relationship between horse owners and animals that affect their well-being [7].

Many studies concerning the measurement of well-being in horses have examined concentrations of cortisol as an indicator of stress, which is a response to the environmental determinants that disturb the organism's homeostasis [11]. Cortisol is one of the organic compounds classified into natural steroid hormones produced by the adrenal cortex under the stimulation of the activity of the sympathetic nervous system [12]. Stress cortisol levels have been studied in horses in many aspects of their welfare: in transportation [1], during the initial training of 3-year-old sport horses [13], during racing training [14], foaling mares [15], and foals at weaning [16].

There are several major types of recreational use of riding horses such as lunging, riding in a group, individual riding, and riding out. During rational leisure exploitation,

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assuming optimal conditions for maintenance, a working horse is not exposed to excessive physical stress. However, stress may occur when numerous negative psychological factors are present, including disruption of natural biological rhythm by changing the hours of training, a high monotony level of work, and unprofessional handling and grooming, which can cause discomfort and pain. Furthermore, the need for cooperation with a number of riders, who are often at very different levels and who often use inappropriate and exaggerated help, are indicated as major stressors [17]. Therefore, the aim of this study was to evaluate the saliva cortisol level in recreational horses depending on the type of horse activity.

## 2. Materials and methods

### 2.1. Animals

A total of 68 horses were included in the experiment. The animals were divided into groups according to recreational activity (jumping, dressage, lesson, lunging, riding out, reining, driving, hippotherapy), type of horse (primitive vs. warmblood), age group [group 1 (3–5 years old), group 2 (6–10 years old), and group 3 (11–18 years old.)], term of sample collection, and sex. Detailed information about the horses is shown in Table 1.

All horses were ridden by riders at the same skill level (riding over three gaits in a controlled manner). All horses were stabled in individual boxes and were fed according to demand, including oats, concentrates, and mineral supplements 3 times a day; good quality hay was provided twice a day. Water was available at libitum. The horses were working five days a week, 2h per day, with 2 days off. The horses from individual activity groups worked during similar hours each working day. All animals included in the experiment had access to paddocks all year round.

All procedures were performed in compliance with the legislation on Animal Care (EU Directive 2010/63/EU) and the Internal Rules of the University of Agriculture in Cracow, Poland.

### 2.2. Sampling procedures and hormone analysis

Saliva samples were obtained from each horseat 3 measurement points: in the morning (6 a.m.), in the evening (6 p.m.), and immediately after work. All horses taking part in the experiment were clinically healthy (no veterinary interference in recent months; no injuries or disorders that could cause pain or discomfort during exploitation), and the mares were beyond estrus. Saliva samples were collected on cotton swabs held with surgical clams placed in the horse's jaws. The swab was centrifuged at 4500 g for 25 min to recover the saliva, placed in Eppendorf tubes, and stored frozen at –20 °C until the assay[18]. Salivary cortisol levelwas measured in duplicate using the immunoenzimatic ELISA test (DRG International, Inc., Springfield, NJ, USA) according to the manufacturer's instructions. The principle of the test was based on competitive binding of the polyclonal rabbit antibody directed towards an antigenic site on the cortisol molecule; endogenous cortisol of a donor sample competes with a cortisol–horseradish peroxidase. The absorbance ratio was determined at 450 nm (BioTek Instruments, Inc., Winooski, VT, USA). The range of the assay was 0.537–80 ng/mL. The intraassay coefficient of variation was 4.52%, the interassay coefficient of variation was 7.47%.

### 2.3. Statistical analysis

Data were analyzed using the GLM procedure. The final model was: $x_{ijklmn} = \mu + t_i + b_j + a_k + s_l + g_m + e_{ijklmn}$,where $x_{ijklmn}$–observation, $\mu$ – overall average, $t_i$–effect of time of testing (morning, evening, after work), $b_j$–fixed effect of type (primitive vs. noble), $a_k$–fixed effect of recreational activity (jumping, dressage, lesson, lunging, riding out, reining, driving, hippotherapy), $s_l$–fixed effect of sex (gelding vs. mares), $g_m$– fixed effect of age group [group 1 (3–5 years old), group 2 (6–10 years old), and group 3 (11–18 years old)], and finally $e_{ijklmn}$– the random error.

Tukey's test was used to estimate the significance between means. A P value ≤0.05 was considered as significant. All results were shown as means ±SE. Statistical analysis was performed using the SAS package v. 9.4 (SAS Institute, Inc., Cary, NC, USA).

## 3. Results

The results of the performed analysis are shown in Table 2. The overall analysis shows that the term of saliva collection, recreational activity, and age influence cortisol concentration. The diurnal concentration of saliva cortisol does not differ between the types of horses. Mares and geldings also do not differ significantly. However, the concentration of cortisol in saliva after recreational riding differs significantly ($P \leq 0.05$) between the morning and evening levels. When considering the obtained results in relation to groups according to age [group 1 (3–5 years old), group 2 (6–10 years old), and group 3 (11–18 years old)], the analysis showed that age differentiates the saliva cortisol level significantly in young horses ($P \leq 0.05$).

There was no statistical difference between morning and evening levels of the investigated hormone between horses belonging to all groups. In all groups, exercise increased the level of cortisol ($P \leq 0.05$). Horses performing dressage were characterized by the highest level of cortisol after work ($P \leq 0.05$). Driving and lunging exercises resulted in a significant increase in the cortisol concentration after work ($P \leq 0.05$). The lowest level of cortisol was obtained in horses used in reining.

## 4. Discussion

The influence of type—measurement time, type of activity, and sex and age—on saliva cortisol concentration in

**Table 1.** Characteristics of horse groups included in the study.

| Group/usability | Number (n) | Age group | | | Sex | | Type |
|---|---|---|---|---|---|---|---|
| All | 68 | 1 | 2 | 3 | Geldings | Mares | |
| A - Dressage | 6 | 0 | 5 | 1 | 4 | 2 | Warmblood |
| B - Jumping | 11 | 0 | 11 | 0 | 6 | 5 | Warmblood |
| G - Reining | 12 | 0 | 9 | 3 | 3 | 9 | Warmblood |
| M - Lesson | 9 | 5 | 0 | 4 | 1 | 8 | Primitive |
| L - Lunging | 9 | 5 | 0 | 4 | 1 | 8 | Primitive |
| T - Riding out | 9 | 5 | 0 | 4 | 1 | 8 | Primitive |
| Z - Driving | 6 | 3 | 2 | 1 | 0 | 6 | Primitive |
| H - Hippotheraphy | 6 | 0 | 4 | 2 | 5 | 1 | Primitive |

**Table 2.** Cortisol saliva concentration (ng/ml) in accordance to tested factors.

| Factor | Levels | Number of observations | Cortisol (ng/ml) * |
|---|---|---|---|
| Type | Primitive | 117 | 1.7188[a] |
| | Warmblood | 87 | 1.5005[a] |
| Sex | Geldings | 63 | 1.6910[a] |
| | Mares | 141 | 1.5966[a] |
| Term of collection | After work | 68 | 2.1597[a] |
| | Morning | 68 | 1.3864[b] |
| | Evening | 68 | 1.3311[b] |
| Exploitation | Dressage | 18 | 2.7276[a] |
| | Driving | 18 | 2.1589[ab] |
| | Lunging | 27 | 1.9434[ab] |
| | Riding out | 27 | 1.8069[abc] |
| | Lesson | 27 | 1.6406[bcd] |
| | Jumping | 33 | 1.6064[bcd] |
| | Hippotherapy | 18 | 0.9272[cd] |
| | Reining | 36 | 0.7899[d] |
| Age group | 3–5 years | 54 | 2.3343[a] |
| | 6–10 years | 93 | 1.4533[b] |
| | 11–18 years | 57 | 1.2358[b] |

* Experimental groups marked with different letters differ significantly for $P \leq 0.05$.

recreational horses has been analyzed in the present study. Recreational riding is a very special kind of physical activity that interconnects objectives and experiences pursued by the rider based on the presence of another living creature—the horse. To date, little research has been undertaken to establish recreational horses' welfare [7]. Cortisol concentration is the most commonly used marker of welfare in domestic animals[19–22].

The circadian rhythm of saliva cortisol concentration in horses has been well established [23]. Salivary cortisol concentrations are lowest before noon (10 a.m.), with the highest value during the night [24]. However, the daily rhythm of cortisol could be easily affected by the weather [25], stable management procedures [26], and transport [1,13]. In the present study, all factors that could disrupt cortisol concentration have been excluded. The obtained

results show that diurnal saliva concentration does not differ significantly between mornings (1.39 ng/mL) and evenings (1.33 ng/mL), and the obtained results were similar to those reported by Aurich et al. [25] for adult horses.

Cortisol saliva may vary during the breeding season (December to June) according to the reproduction state in mares induced by photoperiod. However, none of the mares were covered and the mean values of cortisol were slightly higher (1.69 ng/mL) than reported by Aurich et al. [25] for nonpregnant mares. In our study, this discrepancy may be the result of the mares' usability rather than the reproduction state. It is worth mentioning that in the peripartum period, the diurnal rhythm of cortisol release is absent and during late pregnancy it is suppressed by increased fetal cortisol [27].

Previous studies have shown that age has an effect on cortisol release in horses [28,29], especially when taken into account in the context of exercise. In the present study, young horses had a higher level of saliva cortisol concentration in all groups performing a recreational activity and maintained at a high level up to the evening. This result may suggest that with age and experience, horses acquire the skills to cope with stress caused by working in recreational riding and/or the type of work performed with increasing age is integrated into the natural daily rhythm of the animal, especially if schooling lessons are performed at regular hours and cyclically.

Equestrian exercise is one of the stressors influencing cortisol release [30,31] and its level depends on exercise duration [32]. In the present study, the overall means show that primitive horses have higher cortisol concentrations after work compared to warmbloods. Moreover, the results suggest that the type of recreational exercise can affect cortisol release due to the fact that horses have worked during similar time periods. Introduction of equestrian training elements to recreational riding such as dressage, jumping, harness, and reining differentiate cortisol release, whereas lunging, schooling in and out, and hippotherapy do not significantly affect cortisol levels in measurement points. This may be associated with the basic skills to work the horse in the basic three gaits and sustainability. Elements of targeted training could affect the stress level and thus affect cortisol levels in horse saliva (Table 2). Increased levels of stress may be due to deficiencies of training of both the horse and the rider and the interactions between them. Furthermore, dressage style exercises themselves are commonly controversial in terms of well-being and stress response due to hyperflexion (head-neck-positions) [33,34] being 'on the bit', and hence shifting the horse's center of gravity caudally [35]. Moreover, research conducted on dressage and jumping horses indicates that dressage horses are more susceptible to a wider range of stressors, e.g., transport, surroundings, and adoption of abnormal posture [36]. Little information is available ondriving exercised horses. In this study, the significant increase in saliva cortisol concentration was found after work and these results are in accordance with the results obtained by Kędzierski [37] for driving working horses. However, Vergara and Tadich [38] claim that cortisol levels in tourism carriage horses return to basal values 10 min after work, and in their investigation, they showed a nonsignificant cortisol level increase.

In conclusion, recreational riding does not disturb the circadian rhythm of saliva cortisol concentration in horses. Young horses have a higher level of saliva cortisol concentration in all groups performing a recreational activity, and it is maintained at a high level up to the evening. Thus, our results suggest that with age and experience, horses acquire the skills to cope with stress caused by working in recreational riding. Especially so when the type of work performed with increasing age is integrated into the natural daily rhythm of the animal, particularly if schooling lessons are performed at regular hours and cyclically. Furthermore, the type of recreational exercise can affect cortisol release. Introduction to recreational riding equestrian training elements such as dressage, jumping, harness, and reining differentiate cortisol release, whereas lunging, schooling in and out, and hippotherapy do not significantly affect cortisol levels at measurement points, which may be associated with the basic skills to work the horse in the basic three gaits and sustainability.

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