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Hormonal, biochemical, and hematological study of horses used in police patrol in an equatorial climate

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Abstract. This study aimed to evaluate cortisol secretion and biochemical and hematological parameters in police patrol horses in order to evaluate how patrolling stress could alter these parameters. The sample consisted of ten healthy male adult horses. Blood collection occurred before and after patrols, as well as 24 and 31 hours after the first patrol. The analyses included cortisol levels and secretion, lactate, lactate dehydrogenase, creatine and kinase rates, as well as red and white blood cell counts. Results showed no significant changes in cortisol concentrations but indicated a reduced circadian rhythm. Biochemical variables such as lactate, lactate dehydrogenase and creatine kinase were within reference values. Hematological parameters showed an altered neutrophil/lymphocyte ratio during the entire sampling period, with values below the ideal reference. In conclusion, urban patrolling did not cause temporary changes in the evaluated parameters, suggesting that the animals are adapted to this activity and that rest time is adequate. However, alterations in cortisol circadian rhythm rate and neutrophil:lymphocyte ratio indicate potential chronic wear on the well-being of these animals.

Keywords: Cortisol, patrolling, stress, well-being

Estudo hormonal, bioquímico e hematológico de cavalos utilizados em patrulhamento policial em clima equatorial

Resumo. Este estudo teve como objetivo avaliar a secreção de cortisol e parâmetros bioquímicos e hematológicos em cavalos de patrulha policial, a fim de avaliar como o estresse do patrulhamento poderia alterar esses parâmetros. A amostra foi composta por dez equinos machos, adultos e hígidos. A coleta de sangue ocorreu antes e depois das patrulhas, bem como 24 e 31 horas após a primeira patrulha. As análises incluíram níveis de cortisol e taxas de secreção, lactato, lactato desidrogenase, creatina quinase e contagem de glóbulos vermelhos e brancos. Os resultados não mostraram alterações significativas nas concentrações de cortisol, mas indicaram uma taxa de ritmo circadiano reduzida. Variáveis bioquímicas, como lactato, lactato desidrogenase e creatina quinase, estavam dentro dos valores de referência. Os parâmetros hematológicos mostraram relação neutrófilos/linfócitos alterada durante todo o período de amostragem, com valores abaixo da referência ideal. Conclui-se que o patrulhamento urbano não provocou alterações temporárias nos parâmetros avaliados, sugerindo que os animais estão adaptados a esta atividade e que o tempo de descanso é adequado. Entretanto, alterações na taxa de ritmo circadiano do cortisol e na relação neutrófilos/linfócitos indicam potencial desgaste crônico no bem-estar desses animais.

Palavras-chave: Bem-estar animal, cortisol, estresse, patrulhamento

Introduction

The relationship between humans and horses (*Equus caballus*) dates to the domestication of animals. Therefore, horses have been involved in various activities, such as transportation, sports, leisure, and therapy, which have changed their lifestyle. Currently, horses, like humans, work daily and are exposed to routines and social interactions (Hausberger et al., 2008). This demonstrates that these animals now live differently from their natural way of life and have had to adapt to new routines involving air/noise pollution, confinement, transportation, and solitude.

This scenario has led society to seek an understanding of the impact these activities both in compliance with laws and with increased awareness. If a horse develops behavioral or physiological mechanisms to cope with disturbances in its homeostasis, it experiences stress (Gonçalves et al., 2008; Molento, 2008). If these mechanisms fail or if adaptation becomes difficult, potential harm may occur, predisposing the horse to chronic stress and subsequent pathology (Rivera, 2006). Therefore, it is possible to state that, because of a stressful environment or routine, can lead to illness in animals.

The welfare of working animals, despite its impacts in the animals' efficiency, it's a moral and an ethical responsibility due to the social importance of the work they perform, as well as due to the emotional bonds that develop with the animals during the execution of the work (Merkies & Franzin, 2021). Thus, is crucial to understand the specific needs of these animals and provide them with appropriate care, housing, nutrition, and access to veterinary services. Additionally, efforts should be made to promote responsible breeding practices and discourage the use of practices that could cause harm to the animals. (World Organisation for Animal Health, 2011) emphasizes the need to monitor physiological, behavioral, and immunological changes caused by human-imposed management to ensure adequate health, comfort, nutrition, safety, and the absence of pain, fear, and distress in animals (Binder, 2005; Thiemann, 2015; World Organisation for Animal Health, 2011).

Police patrol horses stand out due to the limited information available on their biological parameters in response to work demands Thus, the aim of this study was to evaluate cortisol secretion, biochemical parameters, and hematological parameters in police patrol horses.

Materials and methods

Ethical considerations

This project was approved by the Ethics Committee on Animal Use of the Federal University of Western Pará, CEUA-UFOPA, under protocol number 10014/2014.

Animal sample

Ten male police horses from the Santarém, Pará police regiment were selected for this study. The horses had no defined breed, were between 11 and 23 years old, had an average height of 1.55 meters, and an average weight of 501 kg. All horses were clinically healthy after a physical examination, which included heart rate measurements using a stethoscope and respiratory rate by counting thoracic movements visually and using a stethoscope according to Gontijo et al. (2018) methodology.

Animal housing and patrolling routine

The horses were housed in a common 450 m² sandy area with water available *ad libitum*. Daily feeding consisted of 9 kg of concentrate feed, divided equally into three mealtimes (6:00, 11:30, and 17:00), offered in troughs. Each horse worked 6 hours of nighttime urban patrolling for every 42 hours of rest. The patrol distance covered was approximately 10 km, primarily at a walking pace, without offering water throughout the route. Each hour of riding was followed by a 15-minute break. All horses had been patrolling for at least 3 years.

Data collection and biological samples collection

Data was collected during the same period, monitoring three groups of horses. Each group completed two patrols, with a 42-hour interval between patrols. Blood collection was performed at the following times: one hour before the first patrol (M0 – 18:00), up to one hour after the patrol (M1 – between 01:00 and 02:00), 24 hours after the patrol (M2 – 01:00), 31 hours after the patrol (M3 – 08:00), one hour before the second patrol after a 41-hour rest (M4 – 18:00), and up to one hour after the last patrol (M5 – between 01:00 and 02:00). Blood samples were collected by venipuncture of the external jugular vein using a needle and syringe after local disinfection. A maximum of 15 mL of blood was drawn within 30 seconds. The horses were appropriately restrained, and the same experienced police professional performed all blood collections.

Laboratory analysis

Blood cortisol analysis was conducted using the electrochemiluminescence method (IMMULITE® 1000, Siemens Healthcare Diagnostics, Germany). The circadian rhythm rate of cortisol (RCC) was calculated according to (Douglas, 1999). Biochemical parameters, including lactate, lactate dehydrogenase, and creatine kinase concentrations, were determined using an automatic biochemical analyzer (RX Daytona®, Randox© Laboratories Ltd., United Kingdom).

Hematological parameters evaluated included erythrocyte count, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, total leukocyte count, and differential leukocyte count. These parameters were established using an automated counter (BC-2800Vet®, Mindray Bio-Medical Electronics, China), and the differential count was performed using blood smears prepared using Romanowsky's method (Panótico Rápido LB®, Laborclin, Brazil) with manual counting as described by (Pereira et al., 2015). All procedures performed during the analyses adhered to the instructions provided by the manufacturers.

Statistical methods

The data distribution was checked using the Shapiro-Wilk test. The comparison between the mean values at time zero (M0) and all subsequent time points was verified using paired t-tests for normally distributed data and the Wilcoxon test for non-normally distributed data. BioEstat 5.2 statistical software was used for data analysis, with a significance level of 5%.

Results

Table 1 shows the mean values and standard deviation of cortisol concentration and biochemical parameters. Cortisol levels fluctuated between 3.7 (M0) and 4.3 (M3), with the highest elevation occurring at M3, although no significant statistical differences were observed between these time points. However, comparing results before and immediately after urban patrolling (M0 x M1) revealed a significant increase in lactate concentration. During rest periods (M0 and M5), the mean plasma lactate levels were 0.6 mmol/L and 0.9 mmol/L, respectively. The LDH enzyme activity concentration ranged from M0 (139.1) to M5 (159.1), staying within the reference values. The CK enzyme concentration evaluation indicated that the mean values throughout the sampling times were in accordance with those reported in the literature.

Table 1. Mean values and standard deviation of cortisol concentration and biochemical parameters of horses used in police patrolling

Parameters evaluated	MO	M1	M2	M3	M4	M5	Reference values
Cortisol (μg/dL)	3.7±0.8a	3.2±0.7a	3.8±0.9a	4.3±1.5a	3.5±1.1a	3.5±0.8a	1.1-14.3*
Lactate (mmol/L)	0.6±0.2a	1.5±0.5b	1.8±0.9b	0.8±0.3a	0.9±0.2a	1.8±0.7b	2.0**
LDH (U/L)	139.1±54.4a	137.0±35.6a	151.5±65.2a	134±35.7a	158.2±64.0a	159.1±41.8a	150-450***
CK(U/L)	42.7±12.2a	39.9±8.2a	40.7±11.0a	45.4±15.7a	44.6±11.7a	43.9±9.3a	10-135***

Different lowercase on the same line indicates a significant ($p<0.05$) between MO and the respective experimental moment. LDH – lactate dehydrogenase; CK – creatinaquinase; *Hart & Barton (2011), **Southwood (2013), ***Paciello et al. (2006).

The mean value found in this study for the circadian rhythm rate of cortisol was 0.27, as can be seen in table 2.

Table 2. Cortisol circadian rhythm (RCC) rate of horses used in police patrols

	Cortisol (µg/dL)		Cortisol circadian rhythm (RCC)
	Morning (M3)	Afternoon (M4)	
Animal 1	3.79	2.77	0.27
Animal 2	2.87	2.26	0.21
Animal 3	4.33	4.01	0.07
Animal 4	4.84	4.03	0.25
Animal 5	4.37	3.90	0.11
Animal 6	3.62	3.80	0.05
Animal 7	2.66	4.70	0.43
Animal 8	7.44	4.90	0.34
Animal 9	3.08	2.14	0.31
Animal 10	5.73	2.03	0.65
Mean and Standard deviation			0.27±0.18

Regarding the hematological parameters, when comparing the results before urban patrolling with subsequent time points, significant changes were observed in mean corpuscular volume (MCV), total leukocyte count, and neutrophil count ($p < 0.05$) (Table 3). These changes may indicate physiological adjustments in response to the physical demands and stress associated with the patrolling activity.

Table 3. Mean values and standard deviation of hematological parameters of horses used in police patrols

Parameters evaluated	MO	M1	M2	M3	M4	M5	Reference Values
HEM(x10^6µL)	8.2±0.5^a	8.1±0.4^a	7.9±0.4^a	7.8±0.5^a	7.9±0.6^a	8.0±0.3^a	6.6 – 11*
VG (%)	42.7±4.5^a	42.1±3.2^a	41.3±3.3^a	40.7±4.3^b	41.2±4.6^a	41.6±2.6^a	30 – 44*
HGB (g/dL)	13.7±1.4^a	13.5±1.0^a	13.1±1.0^a	13.1±1.1^a	13.3±1.5^a	13.2±0.7^a	11 –16*
VCM (fL)	51.6±3.3^a	51.8±3.3^a	51.9±3.2^a	51.7±3.3^a	51.6±3.5^a	51.9±3.7^a	33 –51*
HCM (pg)	16.2±0.8^a	16.1±1.1^a	16.2±1.0^a	16.2±1.1^a	16.2±1.1^a	16.1±1.0^a	13 – 19*
CHCM (g/dL)	33.9±6.1^a	32.0±1.0^a	31.7±1.2^a	32.2±1.2^a	33.0±3.2^a	31.7±1.0^a	35 – 39*
PQT (x10^3 µL)	215.0±42.6^a	240.4±127.6^a	176.4±48.0^a	204.2±47.9^a	246.8±83.5^a	220.6±65.9^a	100 – 303*
LEU (x10^3 µL)	9.6±0.9^a	8.7±1.5^b	9±1.1^a	8.5±1.5^b	9.4±1.1^a	9.1±1.1^a	5.6-11.6*
LINF(x10^3µL)	4.8±1.2^a	5.0±0.9^a	5.5±0.9^a	4.9±1.2^a	4.5±1.0^a	5.1^a	1.1 – 5.7*
MONO	0.4±0.2^a	0.4±0.1^a	0.4±01^a	0.3±0.1^a	0.3±0.1^a	0.4±0.1^a	0 – 0.7*
NEU	4.0±1.1^a	2.9±0.9^b	2.8±0.8^b	3.0±1.1^b	4.2±1.2^a	3.2±1.3^a	2.6 – 6.7*
EOS	0.4±0.2^a	0.3±0.1^a	0.3±0.2^a	0.3±0.1^a	0.4±0.2^a	0.4±0.2^a	0 – 0.6*
N/L	0.83	0.58	0.51	0.61	0.93	0.63	1.5**

Different lowercase on the same line indicates a significant ($P < 0.05$) between MO and the respective experimental moment. HEM - Red cells; VG – volume globular; HGB – hemoglobin; VCM – mean corpuscular volume; HCM – hemoglobin corpuscular mean; CHCM – mean corpuscular hemoglobin concentration; PQT – platelets; LEU – total leukocytes; LNF – lymphocytes; MONO – monocytes; NEU – neutrophils; EOS – eosinophils; N/L – neutrophil/lymphocyte ratio, **Harvey (2012), ***Kingston (2008).

Discussion

It is interesting to note that the parameters evaluated remained almost unchanged, except for the circadian rhythm rate of cortisol (RCC) and the neutrophil-to-lymphocyte ratio, the evaluated parameters in this study were within the reference intervals reported in the literature for equines and duly highlighted in the tables as comparative.

The cortisol concentration variables in the animals of this study remained within the reference standards, without any statistically significant difference compared to the results before patrolling at all subsequent times. These findings are like those reported by Kruljc et al. (2014), who analyzed 14 animals and observed no elevation after patrolling. However, they found higher mean cortisol concentrations during rest (18.7 ± 4.1 µg/dL) and after patrolling (20.5 ± 4.2 µg/dL), suggesting that the cause may be pre-duty excitement and later due to the stress caused by transporting the animals to the patrolling location for a 2-hour period. As the animals in this study did not present elevated cortisol levels at any time, it is suggested that these animals may be accustomed to patrolling activities, unlike those evaluated by Kruljc et al. (2014), which may be more predisposed to the effects of stress, mainly due to being transported to the patrol location. It should also be noted that the patrols accompanied in this study had habitual characteristics, such as simple approaches, absence of occurrences involving large crowds, gunshots, and animal galloping.

Regarding the RCC rate, there was no statistical difference in the data; however, the mean was below 0.3, which, according to Douglas (1999) and Leal et al. (2011), indicates an alteration in cortisol. This may suggest that even though the police horses were accustomed to the patrolling activity, there could still be some degree of influence on their stress levels and circadian rhythm.

Similar results were founded when studying police horses subjected to patrolling and different housing conditions (Douglas, 1999; Leal et al., 2011). Horses performing urban patrols part-time or full-time had a mean RCC rate of 0.20 and 0.22, respectively. A reduced RCC rate indicates a smaller variation between morning and evening serum cortisol concentrations (Alexander & Irvine, 1998), which is an indicator that the horse is not coping with the stressful situation imposed on it. That chronic stress tends to abolish the circadian rhythm of cortisol (Möstl & Palme, 2002).

It is clear that the management of police horses imposes an environment adverse to the natural conditions of the species, including their restriction to urban areas with reduced locomotion space, modified feeding, and a work routine that requires physical effort and a direct and constant relationship with humans. All these factors, combined with the region's climate characteristics, could be the reason for changes in the RCC of these animals because an inadequate environment and deficient communication between man and horse are the main causes of stress in the animal (Beerda et al., 1997; Carretón et al., 2017; Munsters et al., 2013).

The RCC of police horses and found no RCC alterations in 52% of the animals evaluated (Gontijo et al., 2018). However, they observed a significant incidence of behavioral changes (stereotypies) indicative of stressed animals. They believe that animals with a stereotypy pattern associated with normal cortisol concentrations or no change in the RCC rate may already be adapted to the situation, with stereotypy being part of the adaptive process.

Regarding lactate concentration, a significant variation occurred after the animals started the activity. During rest, lactate plasma averages varied, with values below 1.0 nmol/l; however, this value tends to increase when animals are subjected to different stressors, such as exercise (McGowan, 2013). It is believed that, in the present study, the increase in lactate concentration at time M1 (1.5 mmol/L) occurred due to the characteristic of patrolling as a reasonably long exercise (about five hours), where a slower pace predominated. Similar results in military police animals, but without statistical differences were founded (Kruljc et al., 2014; Munsters et al., 2013). Any increase was recorded in lactate concentration after police patrols (Moreira et al., 2015). It is suggested that these differences may be due to the animals' conditioning status since. Plasma lactate is an indicator of the horse's athletic capacity. Animals with high aerobic capacity usually have low elevations in lactate concentrations in response to exercise or have more efficient lactate removal. We highlight that these horses were used in patrols for three years, therefore it may influence the results.

In the case of LDH enzyme concentration, the mean values presented by the animals showed no significant difference when comparing the sampling times in studies involving equines practicing classical dressage, who also found no statistically significant differences before and after exercises (Dittrich et al., 2010). This may indicate that the animals had good physical conditioning and that the exercise did not cause significant muscle damage. Again, we highlight that these horses were used in patrols for three years, therefore it may influence the results.

Regarding the evaluation of creatine kinase (CK) enzyme concentration, no significant variations were observed between the time points during animal rest (52.0 ± 13.4 U/L) (Dittrich et al., 2010), but with a significant increase after eight hours of patrolling (88.4 ± 41.8 U/L). The discrepancy between these studies might be attributed to the occurrence of occasional trots or gallops during the police patrols in Fonseca's (2008) study. Higher mean resting values (165.5 ± 15.5 U/L) and a significant increase after patrolling (223.7 ± 21 U/L), indicating a difference in exercise intensity (Kruljc et al., 2014) compared to the present study. Some horses may have a higher physiological production of CK enzyme (Harris et al., 1990), or their enzymes are removed more slowly from circulation, or they may even have greater muscle cell membrane permeability compared to other animals when faced with similar stimuli.

Hematological parameters showed a decrease in globular volume over time, remaining within the reference values and with significant values only 31 hours after the patrol (M3). Total leukocytes showed a significant decrease, also within the reference values, one hour after the patrol (M1) and after 31 hours (M3); while neutrophils showed lower values one hour after the patrol (M1) and after 24 (M2) and 31 (M3) hours of rest. These findings do not correlate with the stress patterns found in the literature.

According to Beerda et al. (19970 and Carretón et al. (2017), animals respond to exposure to a stressor by mobilizing the neural, neuroendocrine, and metabolic systems. Each stressor produces a specific neurochemical signature, involving central and peripheral mechanisms. Acute stress is mediated by catecholamines, usually resulting in the mobilization of erythrocytes from the spleen, while chronic stress is mainly mediated by cortisol and specifically affects the white blood cell count. Leukocytosis, neutrophilia, eosinophilia, and lymphocytosis constitute the so-called physiological leukocytosis pattern, which is common in situations causing acute stress, such as fear, excitement, or venipuncture; while leukocytosis, neutrophilia, eosinopenia, and lymphopenia characterize the stress leukogram commonly found in chronic stress situations, such as persistent pain or stressful environments (Fam et al., 2010). When comparing values before and after urban patrolling, they observed significant increases in packed cell volume (PCV), red blood cell count (RBC), total leukocytes, neutrophils, and significant decreases in lymphocytes and eosinophils. The difference between the studies might be justified by the different conditions of the patrolling activities.

The final hematological parameter analyzed was the neutrophil-to-lymphocyte ratio (N/L), which remained below the adequate value throughout the study. The N/L ratio is closely linked to cortisol secretion (Davis et al., 2008). Given the normal blood cortisol concentrations associated with an RCC rate indicative of chronic stress and an alteration in the N/L ratio, a study on the same target population is suggested, raising the hypothesis of subclinical hypocortisolism. Hypocortisolism is commonly associated with chronic stress as a response following a period of hyperactivity of the HPA (hypothalamus-pituitary-adrenal) axis (Waller et al., 2014). The subclinical classification is because there has not yet been a depletion in basal cortisol concentration (Betterle et al., 1983). Finally, it is worth highlighting hypocortisolism is a protective response that cushions the chronic HPA axis activity (Waller et al., 2014), helping to reduce the harmful effects of the glucocorticoid response to a daily stressor. Lastly, the difficulty in deepening the comparative analysis between studies involving animals subjected to patrolling is emphasized, as differences in daily management and work dynamics exist.

Conclusion

Urban patrolling did not cause temporary changes in the evaluated parameters, indicating that the animals are adapted to this activity and that the rest time is adequate. On the other hand, the alteration in the circadian rhythm rate of cortisol and the neutrophil/lymphocyte ratio suggests chronic wear on the well-being of these animals. It is highly recommended that police patrol animals are regularly evaluated with cortisol tests to check whether the patrol service is compromising the animal's well-being and whether there are changes in these indices in the long term.

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