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Article

Morning cortisol serum concentration in agricultural mules in the tropics

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Abstract:

This study evaluated the serum cortisol level and its relationship with the work type, age and sex of mules in tropical conditions. Each blood serum obtained from 97 mules was analyzed by a commercial ELISA kit specific to cortisol. The mean cortisol level was 96.3 ng/mL with no significant differences regarding the variables evaluated making it suitable for used as reference value. Also, this is lower than reported in previous studies in show and recreation horses from the same region (133.0 ng/mL). In summary, it is important to utilize species-specific values and conduct studies to determine the mules' ability to adapt and resist.

Key words: Well-being, Disease, Equines, Stress, Hybrids.

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Introduction

Mules are hybrids of horses and donkeys. There are produced worldwide and are known for their hardiness and effectiveness in agricultural work (e.g. dragging, hauling and loading). In Colombia, they are also used for riding, wrangling, showing, transport, tourism, equine therapy and even in policing[1,2]. Though widely used for many purposes, many aspects of mule disease epidemiology and general health are relatively unstudied compared to other equine species[2,3].

Cortisol is a hormone secreted by the adrenal gland cortex. It is a glucocorticoid with important functions in organic homeostasis: gluconeogenesis, proteolysis, lipolysis, hyperglycemia, and modulation of immunity and inflammation, among others[4,5]. It is also involved in pathological processes and is a valuable diagnostic tool in different equine gastrointestinal and respiratory disorders, as well as sepsis[6-9]. It is considered an adaptive hormone in stress-generating activities[10-13], and an indicator of physical conditioning and athletic performance in sport horses[14].

Cortisol is secreted in response to adrenocorticotropic hormone (ACTH), when the hypothalamic-pituitary-adrenal axis (HPA) is activated by physiological (glycemia, blood pressure), pathological, stress, environmental, climatological or seasonal factors[15,16]. ACTH is released in response to corticotropin-releasing hormone (CRH) from the hypothalamus, which acts on type 1 receptors located in the corticotrophs of the anterior pituitary; this, in turn, is activated by HPA and, simultaneously, is controlled by negative feedback[15].

The high variability in cortisol concentrations found in horses and donkeys is multifactorial[17-21]. As a result, reference values have been established according to physiological state, age group, activity type, and management and environmental conditions in different breeds. Cortisol has been extensively studied in horses, notably less so in donkeys, and almost not all in mules. The present study objective was to collect serum cortisol concentration data for a population of mules in the tropics, and relate it to age group, sex and agricultural activity.

Material and methods

The study design was approved by the Animal Experiments Ethical Committee of Antioquia University (Comité de Ética para la Experimentación Animal de la Universidad de Antioquia); Protocol No. 1222019. The sampled mules were from municipalities near the Medellín metropolitan area, Antioquia Department, Colombia. Average elevation in Antioquia Department is 2,055 m asl. Average annual rainfall ranges from 1,500 to 5,000

mm, with two dry and two rainy seasons. Relative humidity ranges from 63 to 73 %, and temperature from 18 to 28 °C. Based on the Köppen-Geiger system, climate is intertropical zone with tropical climates A (equatorial, monsoon and savannah climates) (IDEAM Cartografía Básica IGAC, 2018).

Animals

Experimental animals were 97 clinically healthy mules (65 males, 32 females), of 8.7 ± 4.4 yr of age, 290.5 ± 37.6 kg weight, and 5 ± 0.8 body condition[22]. They were grazed with supplementation with sugar cane byproducts, and were used in agricultural work such as hauling, wrangling and riding. The sampled mules were classified into three groups by age: young (<5 yr), adult (6-14 yr), and old (>15 yr); type of use was not considered in the classification. Each animal was physically examined and measurements taken of heart rate (HR), respiratory rate (RR), and body temperature (°C). Blood samples were taken to measure blood cortisol levels.

Blood samples

Blood samples were taken with minimal animal handling, in the morning (0700-1100 h) before their routine activities and after cleaning and sterilization of the puncture site. The samples were taken with a vacuum tube without anticoagulant, from the jugular vein. They were centrifuged at 210 xg for 10 min to separate out the serum, aliquots of which were placed in Eppendorf tubes and stored at -20° C until analysis.

Cortisol concentration

Cortisol quantification was done using an established protocol[23]. Briefly, a commercial sandwich ELISA kit was used (AccuBind®, Monobind Inc., USA), with a conventional 450-630 nm wavelength reader (Stat Fax 303® Plus Microstrip Reader, Awareness Technology Inc., USA). The kit was validated using six calibrators and three controls: Multiligan A: 07.7 ng/mL, Multiligan B: 97.4 ng/ml, and Multiligan C: 193.2 ng/mL (QSure® Multi-Ligand Control Tri-Level, Monobind Inc., USA). Serum cortisol concentrations were expressed as ng/mL. According to the manufacturer, the kit has 3.77 ng/ml sensitivity, and can detect concentrations from 4.0 to 950 ng/mL.

Statistical analyses

The number of individuals to evaluate was calculated using the conventional formula to find sample size in an infinite population, which generated a sample of 97 mules. The study design was descriptive and cross-sectional, taking a single sample from each individual. The resulting data were entered into Microsoft Excel®, and a Kolmogorov-Smirnov test run with the SAS® (ver. 9.2, USA) statistical software found the data not to have a normal distribution. The correlation analysis between age groups and activity was run using the Kruskal-Wallis test, and, for sex, the Mann-Whitney test was applied to identify significance level ($P<0.05$).

Results

Median serum cortisol concentration was 96.3 ng/mL, with a minimum of 6.8 ng/mL and a maximum of 248.2 ng/ mL; the 25% percentile distribution was 78.6 ng/mL and the 75% was 134.4 ng/mL (Table 1). In other words, serum cortisol concentration was 96.3 ng/mL (variance: 165.3 ng/mL) with no differences between sex, age group and activity ($P>0.05$). Additionally, 75 % of the population had a cortisol value less than or equal to 134.4 ng/mL, with a distribution range between 6.8 and 248.2 ng/mL.

Table 1: Serum cortisol concentration (median), by sex, age and activity in a sample of 97 mules

	N	Cortisol (ng/mL)			HR (bpm)	RR (bpm)	Temp (ºC)
		Mean	SD	*P*-value			
Age group[1]							
Young	17	93.1	36.5		40.0	20.0	37.6
Adult	67	80.0	41.7	0.368	38.0	24.0	37.6
Old	13	98.3	40.5		36.0	20.0	37.8
Sex[2]							
Female	32	98.4	33.9	1.0	37.5	20.5	37.7
Male	65	93.1	43.7		38.0	22.0	37.6
Activity[1]							
Hauling	63	108.2	45.6		39.0	21.0	37.4
Wrangling	28	82.0	24.4	0.564	38.0	27.0	37.5
Riding	6	105.9	29.2		41.0	22.0	37.7

SD= standard deviation; HR= heart rate; RR= respiratory rate.

[1]Kruskal-Wallis test, [2]Mann-Whitney test.

Discussion

Mules are known for their resistance and efficiency, and are therefore used, at times overexploited, in myriad activities[1,2]. The present study provides valuable data on serum cortisol levels in mules, but does not address diseases caused by abuse, neglect and others disorders related to animal welfare[24]. The mule specimens sampled in the present study were representative of the region.

In contrast to mules, cortisol concentrations have been widely studied in horses in different types of samples (plasma, saliva, tears, fecal matter and hair) using different laboratory techniques, and including variables such as physiological state, health condition, age, sex, breed, management strategy, zootechnical use, and stress response[25-29]. This has allowed comparisons to be made between environmental conditions and geographic region[18,19]. Though studied less than horses, reference values reported for donkeys are similar to horse baseline concentrations[30,31,32]. Unlike in horses, there are no reports of cortisol levels responding to climatic season[33], although, given the seasonality of ACTH production, some effect is likely to exist. No such studies have been done in mules, leaving no recourse other than to compare the present results with previous studies of other equine species.

The 96.3 ng/mL serum cortisol concentration observed here is higher than the 29.0 and 66.0 ng/mL reported for horses and donkeys in non-tropical countries[30-33]. However, it is lower than the 133.0 ± 74.0 ng/mL reported in Colombian Criollo Horses (CCC), in a study using the same commercial kit, and animals managed in similar climatic and topographical conditions, but under a different use regime (stabling and established exercise routines)[13]. These discrepancies may be explained by evolutionary differences between species, adaptation level, hardiness and degree of physiological response to stress factors and adversity.

The outlier serum cortisol concentrations observed here in mules (minimum: 6.8 ng/mL, maximum: 248.2 ng/mL) were lower in both instances than those reported in CCC (minimum: 42.0 ng, maximum: 481.0 ng)[13]. In the present results and the study of CCC, the animals were clinically normal (physiological constants within species parameters), suggesting that the higher cortisol levels in the CCC may be due more to factors inherent to each individual (temperament) and the effect of the tropics[26]. Of particular note is that, despite their working long and demanding days, with prolonged fasting, cortisol values in the studied mules were lower than in the CCC. Absence of stress or adaptation level cannot be inferred from a single cortisol value, without a control group and without characterizing animal workload, and would require morning and evening blood samples to calculate the cortisol index[17].

The lack of statistical difference in serum cortisol concentrations between males and females, age groups and type of activity, was also similar to that reported in the CCC[13]. However, this lack of difference was more homogeneous in the variables of the mules than in those of the CCC. This could indicate some level of stability in cortisol concentration, suggesting it might function as a reference value for mules, considering the individual and external responses reflected in the minimum and maximum values[17,34]. Any comparison of cortisol concentrations between mules and CCC would require simultaneous sampling of horses and mules from the same region and in similar environmental conditions.

Differences between techniques have been reported when determining cortisol concentration, especially between chemiluminescence and ELISA; differences of up to 15.7 ng/mL are reported, with the former being more accurate[7]. No such differences have been reported when quantifying cortisol concentration with radioimmunoassay and ELISA[8]. Sample type can also influence cortisol concentration results. Several studies have used different substrates to quantify cortisol concentration, some reporting no differences between substrates[14]. Serum was used in the present study since no values have been reported previously for this substrate. Measures were taken here to minimize results alteration from external factors, for example, minimal manipulation of animal when taking samples, and technique validation with commercial controls.

Conclusions and implications

Serum cortisol concentrations in mules (96.3 ng/mL) used in extensive agricultural activities under tropical conditions were lower than reported for horses in the same geographic region and under less stressful conditions. Further research is needed to confirm if these differences are due to adaptation and stress resistance.

Acknowledgements and conflicts of interest

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