

Serum cortisol in mules intended for agricultural work

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Cortisol is a hormone secreted by the adrenal glands that is stimulated by physiological and pathological factors and has been studied widely in equids, but not in mules. The objectives of this study were to obtain a reference value for serum cortisol in mules destined for agricultural activities and to identify age- and gender-related differences in the value. The concentration was obtained in blood samples using a commercial sandwich ELISA specific for cortisol. The concentration was 96.3 ± 40.6 ng/ml and similar between age groups and genders.

Key words: agricultural work, mule, serum cortisol, stress

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Cortisol is a hormone secreted by the cortex of the adrenal glands and is a glucocorticoid with roles in such things as gluconeogenesis, proteolysis, lipolysis, hyperglycemia, and modulation of immunity and inflammation [10, 12]. Likewise, it is considered the hormone of adaptation to different stress-generating activities in equines [3, 9, 13].

The high variability in cortisol concentrations in horses and donkeys has led to the determination of reference values according to physiological status, age group, type of activity, management, and environmental conditions in different breeds around the world [5, 11, 13]. Despite extensive cortisol studies in equines and to a lesser extent donkeys, there is no information in mules.

In mules, many aspects of the epidemiology of diseases and health are unknown, as their resistance to disease leads to the alterations, they can present not being evident to their owners and administrators. Therefore, this study aimed to determine the serum cortisol concentration in a population of mules used for agricultural activities.

This work was approved by the Ethics Committee for Animal Experimentation of the University of Antioquia (protocol No. 1222019). In addition, informed consent was obtained from the owners of the mules.

A total of 97 clinically healthy mules of both genders

(65 males and 32 females) with an average age of 8.7 ± 4.4 years, average body weight of 290.5 ± 37.6 kg, and average body condition of 5 ± 0.8 [7] were selected for this study; all animals were kept on pasture, supplemented with by-products of sugar cane, and destined for agricultural work (riding, pack, and ranch work) in the Department of Antioquia, Colombia. The mules were categorized according to age as young (≤ 5 years old), adult (6 to 14 years old), and geriatric (≥ 15 years old) and according to gender (male and female).

With minimal manipulation of the mules, blood samples were collected into vacuum tubes without additives in the morning (7:00 AM–11:00 AM) by venipuncture of the jugular vein after cleaning and antiseptic preparation of the site. The samples were centrifuged at 201 g for 10 min to obtain the serum, aliquoted into Eppendorf tubes, and then frozen at -20°C until analysis.

For the analysis and measurement of serum cortisol, the commercial sandwich ELISA kit (AccuBind[®], Monobind Inc., Lake Forest, CA, U.S.A.) was used. ELISA plates were analyzed on a conventional wavelength reader at 450–630 nm (Stat Fax 303[®] Plus Microstrip Reader, Awareness Technology Inc., Palm City, FL, U.S.A.). Validation of the kit was performed using six kit calibrators and three commercial cortisol controls: Multi-ligand control A, 7.7 ng/ml; Multi-ligand control B, 97.4 ng/ml; and Multi-ligand control C, 193.2 ng/ml (QSure[®] Multi-Ligand Control Tri-Level, Monobind Inc.). Serum cortisol concentrations were expressed in ng/ml, and the cortisol AccuBind[®] ELISA Test System has a sensitivity of 3.7 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainly) statistic to

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calculate the minimum dose. This range was validated with known concentrations. This test was compared with a coated tube radioimmunoassay method. Biological specimens from low, normal, and high cortisol level populations were used. The values range from 4 ng/ml to 950 ng/ml according to information from the manufacturer.

The number of individuals to evaluate was determined using the conventional formula for finding the sample size in an infinite population. Initially, a descriptive cross-sectional study was carried out, taking a single sample of the individuals. The data were recorded using Microsoft Excel® (Microsoft Office 2013®, Microsoft Corp., Redmond, WA, U.S.A.) and subjected to the Kolmogorov-Smirnov test using the SAS® statistical software (Version 9.2, SAS Institute, Cary, NC, U.S.A.), which revealed that the data did not have a normal distribution. The difference between the age groups was analyzed using the Kruskal-Wallis test, and the Mann-Whitney test was used to identify the level of significance for gender.

The median serum cortisol concentration, minimum and maximum values, and first and third quartile are shown in Table 1, and the serum cortisol concentrations by age and gender are shown in Tables 2 and 3, respectively. Overall, the serum cortisol concentration was 96.3 ng/ml, and the values were similar between the age groups and genders according to the Kruskal-Wallis and Mann-Whitney tests, respectively, with no significant statistical differences ($P>0.05$).

The serum cortisol concentration determined in the present study (96.3 ng/ml) was higher than those in most of the reports in other equines (horses and donkeys), which have ranged between 29 and 66 ng/ml [4, 6, 8]; although the value was less than those reported overall (133 ± 74 ng/ml), for females (145 ± 93 ng/ml), for males (126.0 ± 51.9 ng/ml), and for different age group in Colombian Creole Horse (CCH) managed under similar climatic and geographic conditions but with different management and working systems in which the horses were kept in stables and maintained with established physical activity or work routines [13], the mules in the present study were kept on grass, and they had long periods of work and fasting.

The range of the serum cortisol concentration was extreme among the individuals, which had all been declared clinically healthy after clinical examinations before the study. Therefore, the higher cortisol concentration was like more likely to be associated with factors of each individual (temperament) and the effect of the tropics [2]. Additionally, 75% of the mules had cortisol concentrations that were less than or equal to 134.4 ng/ml, which represented the quartile closest to the median, and this was considered during the analysis of the reference value for the mules kept under the conditions of this study. The development of new studies to determine the rhythmicity and response of the circadian cycle of cortisol in mules is important, as only morning concentrations were measured in the present study and afternoon values are needed to quantify the level of

Table 1. Serum cortisol concentration (median) and percentile distribution of the general population of working mules (n=97)

Median (ng/ml)	Minimum (ng/ml)	Maximum (ng/ml)	First quartile (ng/ml)	Third quartile (ng/ml)
96.3	21.2	248.2	78.6	134.4

Table 2. Serum cortisol concentrations according to age group

Age group	N	Cortisol (ng/ml)	Standard deviation (\pm SD)	Significance
Young	17	93.1	36.5	0.368
Adult	67	80.0	41.7	
Geriatric	13	98.3	40.5	

Table 3. Serum cortisol concentrations according to gender

Gender	N	Cortisol (ng/ml)	Standard deviation (\pm SD)	Significance
Females	32	98.4	33.9	1
Males	65	93.1	43.7	

chronic stress by determining the cortisol index [5], since mules are subjected to stressors due to the particularities of the agricultural activities they carry out. Therefore, it is necessary to detect the degree of adaptation and level of welfare of these animals [1].

In conclusion, the present study found that the serum cortisol concentration of mules used as riding, pack, and ranch animals under tropical conditions was around 96.3 ± 40.6 ng/ml.

Conflict of Interest

The authors declare no conflict of interest.

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