

Cortisol Measurements in Animals



Introduction

Homeostasis is a complex dynamic equilibrium kept by living organisms.¹ Homeostasis must be kept in order for an organism to stay alive. This equilibrium is influenced by stressors. A stress response occurs when homeostasis is threatened, or when an animal perceives that there is a potential threat to them. When an animal becomes stressed, the hypothalamic-pituitary-adrenal axis (HPAA) is activated.² Glucocorticoids are the final outputs of the HPAA, with cortisol as the main output in mammals.² By examining cortisol levels, it can be determined what factors influence acute (short term) or chronic (long term) stress, and how it can affect the health of animals. Cortisol can be measured in biological samples, such as plasma, saliva, urine, feces, milk, hair, nails, and sweat.² Blood, saliva, sweat, and milk cortisol levels are used to determine acute stress, while cortisol in urine and feces accumulates over several hours. Hair and nail samples can provide a look at chronic stress or an animal's past exposure of stress.

Goals

The purpose of this research was to determine the cortisol levels in 35 domestic animals. By doing this, we could determine whether these samples were a reliable way to test cortisol levels in animals. We also wanted to compare two different conditions in order to see if there was a significant difference in cortisol levels between the two conditions.

Sample Preparation

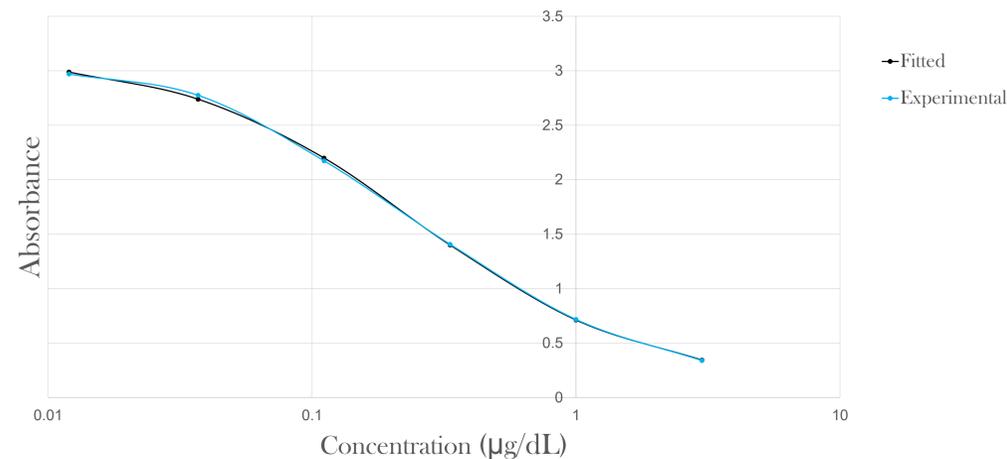
The samples were collected by Dr. Contreras and her team at Long Island University. The samples were washed four times with isopropanol in order to rid of any external contamination. The samples were thoroughly dried in a 55 °C water bath under a stream of nitrogen. The samples were then weighed for future analysis.

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Standard Curve

Standard Curve showing the Experimental vs. Fitted Data



Results

The average cortisol concentration found in condition 1 was 1.938 pg/mg, while the average concentration in condition 2 was 2.617 pg/mg. The average concentration of cortisol in condition 2 was significantly higher than that found in condition 1 ($t_{3,5} = 2.26$, $P = 0.027$). One sample was determined to be an outlier and was not included in the analysis. The limit of the blank (LOB)³ was found to be 0.0151 microg/dL. One sample was found to be below the LOB. The limit of detection (LOD)³ was found to be 0.0206 microg/dL. Three samples were found below the LOD.

Discussion

Our method was found to be a reliable way to determine cortisol levels in animals. This presents a new way to determine cortisol levels in these domestic animals. These methods could be applied in veterinary medicine to diagnose and treat animals with various conditions. This could lead to a further understanding of chronic stress in animals.



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Experimental

A 1000 µL portion of methanol was added to the samples, and the samples were vortexed. The samples were then incubated in a 55 °C oven overnight. The methanol portion was removed from the samples and put into another vial, and a 1000 µL portion of acetone was added to the samples. The samples were incubated at room temperature for five minutes. The portion of acetone was removed and stored in the supernatant vial. Another portion of methanol was added to the sample and again was incubated at 55 °C overnight. There was a total of three overnight extractions. The supernatant was then thoroughly dried and stored at 4 °C.

Enzyme immunosorbent linked assay (ELISA):

A 1000 µL portion of acetone was added to the dried supernatant, then dried again. A 150 µL portion of assay dilutant was added, then heated in a 55 °C water bath for 5 minutes and centrifuged at 4000 rpm for 15 minutes. The sample was then pipetted into the ELISA plate. A high sensitivity salivary cortisol enzyme immunoassay kit was used to analyze the samples in duplicate. The absorbance was read at 450 nm.

Acknowledgments

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References

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