Baseline corticosterone levels of a migratory bird in brazilian cerrado

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Keywords: Bird hormones; stress levels; neotropical savanna.

ABSTRACT – The main physiological parameter used in birds stress analyses is the corticosterone level. That hormone has been the main tool used to infer physiological state, but little is known about those levels in the tropical birds. Lesser Elaenia, *Elaenia chiriquensis albivertex*, is a migratory flycatcher at the cerrado and this study aimed to measure baseline corticosterone levels during the reproductive phase. The release of baseline corticosterone of Lesser Elaenia was 12.96 ± 5,07 ng/mL (n = 43). There was a positive correlation between corticosterone and body mass (y = 4.2329x - 54.844; R^2 = 0.7839). Our study shows a important record of basal corticosterone of a tropical bird during migration with a potential to evaluate individuals health such as in animal rehabilitation and release programs.

Níveis basais de corticosterona de uma ave migratória do cerrado brasileiro

Palavras-chave: Hormônios de aves; níveis de estresse; cerrado neotropical.

RESUMO – O principal parâmetro fisiológico utilizado em análises de estresse em aves é o nível de corticosterona. Esse hormônio tem sido a principal ferramenta utilizada para correlacionar o estado fisiológico, mas pouco se sabe sobre esses níveis nas aves tropicais. A *Elaenia chiriquensis albivertex* é um tiranídeo migratório do cerrado brasileiro, e o objetivo deste estudo foi medir os níveis basais de corticosterona durante a fase reprodutiva. A liberação de corticosterona basal de *E. chiriquensis* foi de 12,96 ± 5,07 ng/mL (n = 43). Houve uma correlação positiva entre corticosterona e massa corporal (y = 4.2329x - 54.844; R^2 = 0.7839). O nosso estudo mostra um registro raro da corticosterona basal de uma ave tropical durante a migração, com potencial para avaliar a saúde dos indivíduos, por exemplo, em programas de reabilitação e realocação de animais.

Niveles basales de corticosterona en un ave migratoria del cerrado brasileño

Palabras clave: Hormonas de las aves; niveles de estrés; sabana neotropical.

RESUMEN – El principal parámetro fisiológico utilizado para analizar el estrés en aves de corral es el nivel de corticosterona. Esta hormona ha sido la principal herramienta utilizada para correlacionar el estado fisiológico, pero se sabe poco sobre estos niveles en aves tropicales. Elaenia chiriquensis albivertex es un papamoscas migratorio del cerrado brasileño y el objetivo de este estudio fue medir los niveles basales de corticosterona durante la fase de cría. La liberación basal de corticosterona de E. chiriquensis fue de 12,96 ± 5,07 ng/mL (n = 43). Existe una correlación positiva entre la corticosterona y la masa corporal (y = 4.2329x - 54.844; R^2 = 0.7839). Nuestro estudio muestra un registro poco frecuente de corticosterona basal en un ave tropical durante la migración, con potencial para evaluar la salud de los individuos, por ejemplo en programas de rehabilitación y reubicación de animales.

Introduction

Physiological studies have advanced the understanding of life history theory and the responses of organisms to their environment [1][2]. Hormones have been the main tool used to correlate physiological state with environmental changes, body condition, time of breeding, reproductive success, among others. Many studies have shown the effect of stress (as measured by glucocorticoid levels in the bloodstream) on the survival and reproductive fitness of vertebrates [3]. Stress is assumed to be the result of the release of glucocorticoid hormones into the bloodstream controlled by the hypothalamic-pituitary-adrenal (HPA) axis according to internal and external stimuli to the organism. Cortisol and corticosterone, which are better known, can be present in different animal groups and species, but in birds corticosterone predominates [4][5]. Birds have two types of corticosterone that can be collected: basal (or circulating) corticosterone and induced corticosterone [6][7][8].

There are differences in corticosterone releases due to different aspects of birds' biology, environmental site and survival pressures. Tropical birds, for example, have slow development, low metabolism, low reproductive rate and high longevity [9][10]. As long-lived birds, they are expected to invest more in their own preservation (survival), especially when they reach high corticosterone concentrations, which indicates the management of stressful situations [11]. However, little is known about the stress physiology of tropical birds.

This study addresses the baseline corticosterone levels in a free-ranging population of the migratory bird Lesser Elaenia, *Elaenia chiriquensis albivertex* (Tyrannidae; Lawrence, 1865), at the reproductive site in a Neotropical Savanna environment. The Lesser Elaenia, is a small passerine (15.5 g) with little sexual dimorphism, which occurs from Costa Rica to Argentina [12], breeds in central and south Brazil and migrates to several regions [13][14]. This study aimed to measure the baseline corticosterone and body mass of Lesser Elaenia (Figure 1) in the reproductive sites of migration, to show especially the baseline levels during the reproductive performance.

Figure 1 – Lesser Elaenia, *Elaenia chiriquensis albivertex* (Tyrannidae). Photo by André de Camargo Guaraldo.

Material and Methods

Study area

Birds were captured on a 100-ha grid, at the conservation unit Estação Ecológica de Águas Emendadas (ESECAE) in the Distrito Federal (15° 29' and 15° 36' S, 47° 31' and 47° 41' W, 1,040 m asl), from August to December 2012. ESECAE, in the *cerrado*, a savanna-like biome, is a 10,500-ha reserve surrounded by small patches of natural vegetation, farms and houses [15][16]. The reserve vegetation ranges from natural grasslands, open and dense *cerrado*, to patches of forest areas [17]. Climate of the region is seasonal, with a rainy season from September to April, and a dry season in the other months [18].

Capture, marking and collection of blood samples from individuals in nesting

To collect blood, marked females were captured close to their nests monitored from the beginning. Captures started in September, when the first nests of the species are usually found, with weekly expeditions of capture and banding and finished on December. A mist net was opened in the morning next to each nest (8 m) to capture the female flying from-to the nest. In birds, baseline corticosterone is obtained up to three min after the capture of the individual, while the material collected after this time represents induced corticosterone, as it results from the stress arising from capture and manipulation [7][2]. Thus, to allow baseline corticosterone analyses, a blood sample from each female was collected under three min of capture by perforation of the right-wing brachial vein with a hypodermic needle and heparinized capillary. After the blood samples were taken, the other ringing activities were carried out, such as measurements, weight, etc. Females were already individually marked with CEMAVE rings and with color bands, from another long-term capture and marking project carried out in the study area since mid-2004.

According to the ethical indication of wild animal manipulation, the volume of blood collected was approximately 40 μL and did not exceed the recommended limit of 1% of the mass of each individual bird [19][20]. After collection, the capillaries were closed at only one side (non-heparinized). During transportation to the lab, capillaries were deposited in vertical position on ice. The time between collection and centrifugation in the laboratory was approximately four hours (253 ± 15 min) of capture. In the laboratory, samples were centrifuged for 10 min in a microhematocrit centrifuge at 12,000 rpm. Then, the plasma part of each sample was separated and stored at -80 °C for further hormonal analyses.

Hormonal analyses

Hormonal analyses of each assay lasted a total duration of 18 min and followed two phases of incubation sample. First, 20 μL of plasma sample were incubated with a specific biotinylated antibody antitussive and a ruthenium complex labeled baseline corticosterone derivative. Depending on the concentration of baseline corticosterone in the sample and the formation of the respective immune complex, the binding site of the labeled antibody was in part occupied by baseline corticosterone and partly with ruthenium-labeled hapten. In the second incubation, after the addition of the streptavidin-coated microparticles, the complex formed binds to the solid phase through the interaction of biotin and streptavidin.

The reaction mixture was aspirated into the reading cell, where the microparticles are magnetically attached to the surface of the electrode. The unbound elements are then removed with ProCell/ProCell M. Subsequently, the application of an electric current to the electrode induces a chemiluminescent emission that is measured by a photomultiplier. The results are determined based on a calibration curve generated specifically by the analyzer, through a 2-point calibration, and on a main curve included in the reagent bar code. Kit reagents for baseline corticosterone detection were included in a ready-to-use unit that cannot be separated. All necessary information for correct operation was inserted in the analyzer from the respective reagent bar codes. The analyzer automatically calculated baseline corticosterone concentration of each sample in μg/dL and for the purpose of standardizing data of bird physiology, and the values obtained in the equipment were divided by 100 to obtain data in ng/mL.

The normality of the data was verified by the Shapiro-Wilk test and the t-test was used to verify differences in each parameter. The normal blood baseline corticosterone range for Lesser Elaenia was established by the arithmetic mean with a 95% confidence level.

Results

The breeding population had mean baseline corticosterone values of 12.96 ± 5,07 ng/mL (*n* = 43) with a minimum value of 3.96 ng/mL and a maximum of 19.50 ng/mL. The 43 females sampled in reproduction had an average body mass of 16.2 ± 1.06 g (*n* = 43), with a minimum value of 13.9 g and maximum of 17.7 g. There was a fluctuation in corticosterone dosages between individuals of Lesser Elaenia during the reproduction phase (Figure 2).

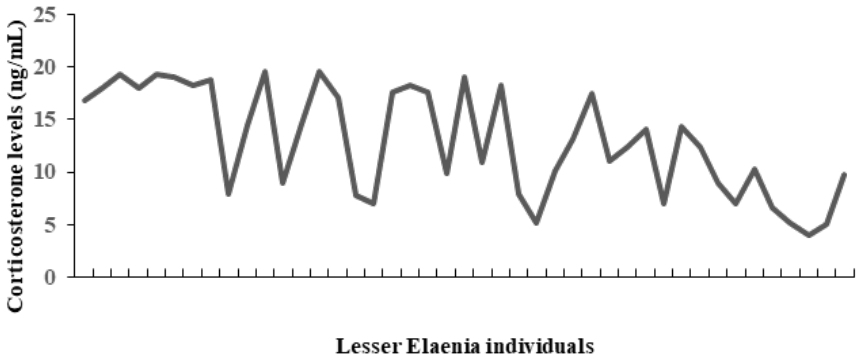

Figure 2 – Fluctuation graph of corticosterone dosage among Lesser Elaenia individuals (*Elaenia chiriquensis*) during the reproductive phase in the cerrado of central Brazil.

The results observed for costicosterone and mass follow normal disbribution for Shapiro-Wilk test ($p < 0{,}0001$); and the corticosterone and mass data showed a positive distribution according to the regression graph (Figure 3).

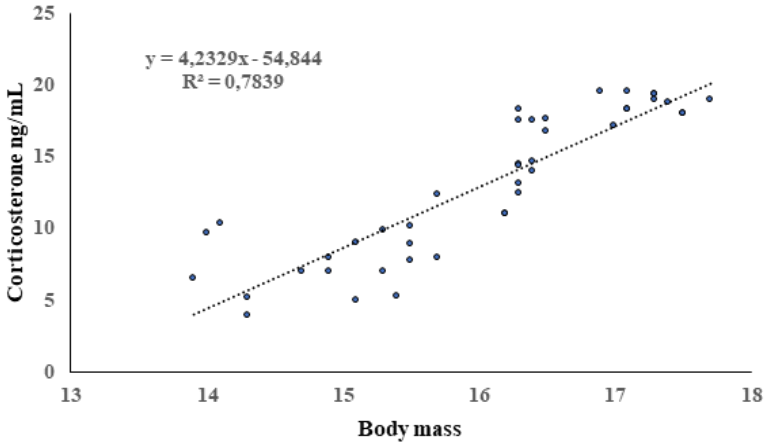

Figure 3 – Relationship between corticosterone levels and body mass of Lesser Elaenia (*Elaenia chiriquensis*) during the reproductive phase in the cerrado of central Brazil.

Therefore these findings may be related to each individual's stage of reproduction, i.e. whether they are incubating eggs or close to the hatching stage.

Discussion

For Lesser Elaenia, the now known levels of baseline corticosterone can imply mechanisms that promote the first insights into their hormonal stress

physiology. The passerine baseline corticosterone concentrations range from 0.7 to 57 ng/mL [21]. Lesser Elaenia is within that limit, besides other tropical birds show lower values such as Lance-tailed Manakin (*Chiroxiphia lanceolata*) with 3.28 ng/mL [22]; Florida scrub jay (*Aphelocoma coerulescens*) with 1.93 ng/mL [23] and Norther monkingbird (*Mimus polyglottos*) 3.75 [24]. More studies with tropical birds from the same family could help to compare the results.

The positive relationship between corticosterone levels and body mass in Lesser Elaenia was overwhelming, suggesting a possible dependence between these variables for this species. This has been observed before in some other birds [3][11] [21] and for Lesser Elaenia similar condition was observed on arrival and departure from migration, when hematocrit and body mass were positively related [25]. Nevertheless, a recent global study comparing resident and migratory birds showed that migratory status alone may not predict corticosterone levels, indicating the role of environmental conditions effects [26], which needs to be better investigated in the future for Lesser Elaenia.The characteristics of this migratory species, though abundant at our study site, shows that when studying free-ranging species there are limitations intrinsically related to their biology. For a sub-tropical bird, the African stonechats, *Saxicola torquata axillaris*, for example, parents presented high levels of baseline corticosterone (> 5 e < 20 ng/mL) during the feeding period of the nestlings [27]. These differences among species deserve more detailed studies to verify phenotypic, regional or even pace-of-life differences between corticosterone release [2] [28][29][30]. It is important to observe other aspects of reproduction, in addition to predation pressure and survival risk; such as nest parasitism, which for Common terns, *Sterna hirundo*, show more corticosterone stress in individuals that have been parasitized than birds that have not been parasitized [31].

It has been suggested that life-history strategy can be a major determinant of glucocorticoid effects on fitness, with long-lived species favoring corticosterone-tradeoff hypothesis, but short-lived species favoring a corticosterone-adaptation hypothesis [21][11]. Tropical birds, like the Lesser Elaenia, suffer varying survival pressures, generally raise few nestlings per clutch, but need to survive with high predation pressure, what would explain those levels of baseline corticosterone [32][11]. There are some factors that may cause stress to Lesser Elaenia at the breeding site, such as high nest predation pressure [33][14] and the physiological preparation (including hematological changes) to migrate back to wintering areas [34][25], which potentially results and explain the levels of baseline corticosterone of tropical species [28][24]. It is well known that corticosterone promotes emergency, alert and escape responses, but it is a physiological parameter that expresses much about the evolutionary history of a particular species, especially when associated with other variables and ecological questions [2][35][36]. This study recorded, for this tropical bird, the usefulness of baseline corticosterone levels during the reproductive phase and may contribute as a parameter for evaluating individuals, including in animal rehabilitation and release programs.

Conclusion

This study contributes with, the now known, baseline levels of the stress hormone corticosterone for Lesser Elaenia (*Elaenia chiriquensis*) during the reproductive phase of its migration in the Brazilian Savanna. The levels permeate almost 13 ng/mL and are within the limits already identified for other passerines, even those from temperate regions. There was also a marked relationship between this parameter and individual body mass. Future studies could be carried out in areas where Lesser Elaenia is resident (Amazon region), to verify discrepancies or similarities in these levels, as well as the adaptive factors that influence their release.

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