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Faecal Cortisol as A Marker of Stress in Wild and Captive Fishing Cats and Jungle Cats

Tania Chatterjee[1], Rudradev Mukherjea[2], Apratim Maity[2], Joydip Mukherjee[2], Guru Prasad Mandal[2], Mrityunjoy Mondal[2*] and Md. Habib[2]

[1]Zoo Biologist, Alipore Zoological Garden, Kolkata- 700027, West Bengal, India
[2]Department of Veterinary and Animal Science, West Bengal University of Animal and Fishery Sciences, Kolkata–700 037, West Bengal, India

**Corresponding author*

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A B S T R A C T

Animals in wild habitat and in captive condition undergo various natural stressful situations like physical harm, inclement weather, sickness *etc*. The purpose of this study was to investigate the relationship between such long-term natural stress and cortisol levels in fishing cats and jungle cats in wild and captive condition through noninvasive method of fecal cortisol estimation. Cortisol levels in cat faeces were measured (ng/g of feces) as a physiological long-term stress marker. Cortisol in cats can be used to accurately evaluate adrenocortical activity. It ranged between 195 and 238 in captive jungle cats with a mean of 212; whereas in wild jungle cats the same has been between 147 and 231 with a mean of 179. The analysis of data suggested no significant difference in the levels of faecal cortisol in jungle cats in captive and wild condition. In case of fishing cats, the high and low of faecal cortisol concentrations were 199 and 328 in captive condition with a mean of 254 while the same has been between 151 and 237 with a mean of 184 in wild condition. Statistical analysis of data revealed that the level of faecal cortisol in fishing cats is significantly higher in captive condition compared to wild condition.

Introduction

The reproductive function of mammals is reduced by many factors including stress. Faecal concentrations of cortisol metabolites (FCM), an indicator of stress, had been considered as an integrative aspect of management for both in situ and ex situ African wild dog populations (Van der Weyde *et al.*, 2016). Therefore, the measurement of levels of stress and welfare can be assessed by measuring cortisol. Cortisol measurement from blood, saliva, urine faeces and hair has been extensively used as a stress indicator (Novak *et al.*, 2013). Collection of blood from wild animal is very

difficult as well as risky. There are a variety of species whose glucocorticoids, including cortisol and corticosterone, can be monitored in a non-invasive manner to determine adrenocortical activity and physiological stress load (Nemeth *et al.*, 2016). Measuring faecal cortisol metabolites can be useful to identify factors for better health management of animals in captive and wild condition. It is well known that stressful stimuli can induce ACTH release, which increases the synthesis and secretion of cortisol by the adrenal glands (Jones, 1979). Cortisol analysis has been widely used as an index of stress. Available report suggested that faecal and salivary glucocorticoid measurements provide a more accurate indicator of physiological stress levels in guinea pigs compared to circulating plasma levels in the short and long-term (Nemeth *et al.*, 2016). Moreover, noninvasive method is easy and do not disturb the animal and provide an accurate assessment of stress. The present study was undertaken to compare faecal cortisol as a parameter to evaluate long-term natural stress in a noninvasive manner in fishing cats and jungle cats between wild and captive condition.

Materials and Methods

Cortisol metabolites are stable in faecal samples at room temperature for up to 12 hours before freezing (Van Meter *et al*., 2009). The faecal samples can be stored in a household freezer (−8◦C) after defecation and transported in a thermal cooler to the laboratory for storage at −20◦C until prepared for analysis (Ramos *et al*., 2013).

Sample collection

Faecal samples were collected both from wild animals during rescue/transport and in captive condition at the Alipore Zoological Garden, Kolkata. Necessary permission for collection of faecal samples was duly obtained from the authority of Alipore Zoological Garden, Kolkata. Fresh faecal samples were collected from mature animals (apparently of more than 3 years age) of both sex in clean plastic container and immediately placed in the ice box. The samples were collected with the help of their handlers in the zoo and rescue centre without disturbing them. Faeces from normal evacuation were collected in fresh condition. Moreover, the animals were not made to defecate forcefully or by the application of any purgatives. Five samples in each category were collected and analyzed from wild and captive fishing cats and jungle cats. The faecal samples were frozen at -20^{0}C within one hour of collection and stored until analysis (Biswal *et al*., 2018). As per the practice, jungle cats and fishing cats were offered chicken and lata fish as their normal diet in the zoo.

Measurement of fecal cortisol

Frozen samples were thawed at room temperature before extraction of glucocorticoid by vortexing the faecal samples as per the procedure described by (Wasser *et al.*, 2000). A quantity of 0.6 gm well mixed wet faeces was placed in a capped tube containing 2 ml 90% methanol. The tubes were vortexed for 30 minutes for proper mixing of the sample in methanol using Tarson 3004 spinix vortex shaker. This was followed by careful centrifugation of the tubes at 2500 rpm for 20 minutes. The supernatant was then collected in 2 ml microcentrifuge tubes and stored at -30^{0}C for further analysis. Cortisol ELISA kit was purchased from LDN®, Germany. The concentration of cortisol in faecal extracts (ng/g of feces) was measured using ELISA technique as per manufacturer's protocol.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to test the effect of environmental stress on faecal cortisol level, and Duncan's multiple range tests with $P < 0.05$ was used to detect the significance of differences among all groups (Snedecor and Cochran, 1967).

Results and Discussion

The values of faecal cortisol concentrations (ng/g of faeces) in wild and captive jungle cats and fishing

cats from the present study have been presented in Table 1. It ranged between 195 and 238 in captive jungle cats with a mean of 212; whereas in wild jungle cats the same has been between 147 and 231 with a mean of 179 (Fig. 1). The analysis of data suggested no significant difference in the levels of faecal cortisol in jungle cats in captive and wild condition. In case of fishing cats, the high and low of faecal cortisol concentrations were 199 and 328 in captive condition with a mean of 254 while the same has been between 151 and 237 with a mean of 184 in wild condition (Fig. 2). Statistical analysis of data revealed that the level of faecal cortisol in fishing cats is significantly higher in captive condition compared to wild condition. The purpose of this study was to investigate the relationship between long-term natural stress and faecal cortisol levels in fishing cats and jungle cats in wild and captive condition through noninvasive method of faecal cortisol estimation.

Table.1 Mean faecal cortisol concentrations (ng/g of faeces) in wild and captive Jungle cat and Fishing cat

Jungle cat		Fishing cat	
Captive	**Wild**	**Captive**	**Wild**
224	177	273	197
195	231	328	156
197	159	207	151
209	147	199	179
238	179	265	237
Mean±SE 212±8.2	Mean±SE 179±14.4	Mean±SE 254±23.7*	Mean±SE 184±15.6

*Means with different superscripts vary significantly ($p<0.05$) within a row

There are many studies on effect of stress in domestic animals. But apart from the effects of captivity on wild animals, there have been scanty reports on effects of stress on wild animals. As a result, scientific study has probably understated the magnitude and number of stresses that influence wild animals.

Daily challenges that wild animals must deal with include physical harm, sickness, inclement weather, food scarcity, confrontations with other members of their species or herd and so on. On the other hand, there are a variety of factors that might cause stress in animals kept in captivity, including cage confinement, human proximity, unfamiliar surroundings, and more mild stressors such as artificial lighting conditions (Morgan and Tromborg, 2007).

Cortisol levels in cat faeces were measured as a physiological stress marker. Since they reflect an average amount of circulating glucocorticoids over time rather than a point sample, faecal glucocorticoid metabolite (FGM) assays offer a more precise evaluation of long-term glucocorticoid levels (Harper and Austad, 2000). In the present study, increased cortisol in fishing cats in captive condition might be attributed to stress due to cage confinement, human proximity, unfamiliar surroundings etc. The faecal cortisol levels in fishing and jungle cat both in wild as well as captive conditions are in consonance with the study of Carlisle *et al*., (2021).

Although inferences about the differences in stress levels between wild and captive jungle and fishing cats cannot be made due to the limited sample size, the approach offered by this study can be used as a springboard for further research into this crucial topic for the welfare of wild and captive cats.

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