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Assessment of Faecal Cortisol Levels in Free-Ranging Nilgiri Tahrs (*Nilgiritragus hylocrius*) in Correlation with Meteorological Parameters: A Non-Invasive Study

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Abstract

The faecal glucocorticoid metabolites of a free-ranging small Nilgiri tahr population of Western Ghats, Tamil Nadu was studied to investigate contributing confounding influences of season, ambient temperature, rainfall and water level on the annual secretion pattern. The was done for a period of one year Oct 2013-Sep 2014. Individuals may cope with environmental challenges through the secretion of stress hormones (glucocorticoids) which allows the integration of environmental change as essential life events such as predator stress, food and water availability, resting cover, influence of tourists and life history events such as birth, death, maintenance of an essential population size by means of an adaptive feedback mechanism. Adaptation and eventually acclimatization to cyclic day-to-day activities, short-term environmental stressors or long-term ecological pressures have been observed with these animals. However, being a highly limited population the animals maintained an effective population size. A clear cut seasonal pattern of glucocorticoid metabolites excretion was detected, with increasing levels in summer and winter. The confounding factors such temperature, rainfall, relative humidity, solar radiation, soil temperature were recorded throughout the study period and did not have any correlation with the stress the animals exhibited. The observed pattern might be due to lack of feed availability both during summer and winter, a declining nutritional intake and reduction of metabolism during winter, clearly the animals were not in their "Thermo comfort Zone". However, broad retrospective studies are essential to identify potential contingent environmental stressors. This study reports the baseline cortisol level in Nilgiri Tahrs, with the relevant confounding factors correlating with their annual variation level.

Keywords: Faecal cortisol metabolites; Stress; Nilgiri Tahr; Meteorological parameters

Introduction

The Nilgiri Tahr (*Nilgiritragus hylocrius*) is an endangered mountain ungulate endemic to the southern part of the Western Ghats. The species is found in a roughly 400 km stretch in the Western Ghats which falls in the states of Kerala and Tamil Nadu. The local distribution of the species is attributed to the animal's preference for the habitat with grasslands with steep rocky cliff shelters. Owing to the disturbances in habitat and their degradation, fragmentation, predator pressure and co-inhabitation of other prey species sharing the same ecosystem. However, the factors that qualifies a particular habitat and owing the bioavailability of resources. It is an endangered mountain ungulate listed in Schedule-I of the Indian Wildlife Protection Act 1972. The IUCN lists Nilgiri Tahr as 'endangered' in the Red List 2010. Natural habitats to native animals are acclimatized environments where several situations, either predictable or capricious, may trigger an evident adaptive response in animals through behavioural, morphological or physiological modifications. On exposure to a stressful event, the adrenal cortex releases glucocorticoids into circulation, and their concentrations in the blood increase as part of the stress response that is mediated by an endocrine pathway and the glucocorticoid regulation level is dependent on either acute or chronic exposures. Glucocorticoids are also involved in metabolic regulations and may vary according to reproductive state and seasonal fluctuations adapting the organism to changing conditions and also govern these functions in a specific population. Acute stress enables animals to cope with unforeseen stress events which are favourable for the species survival, on the contrary chronic stress may lead to reduced survivability and reproductive success [1]. Glucocorticoids-either cortisol or corticosterone (glucocorticoid metabolites) are released during stressful situations, they can serve as an index of the stress response, and the development of non-invasive techniques to measure glucocorticoid metabolites in feces or urine has received increasing attention in field research. Such a technique has the advantage of keeping subjects undisturbed during collection of samples that helps in fixing baseline values and also exploiting the non-invasiveness of the technique. Glucocorticoids have been used as physiological indicators of stress in different species and prove as an index [2]. Our objective was to investigate seasonal variations in the Faecal Cortisol Metabolites (FCM) secretion of a free ranging population of Nilgiri Tahrs in response to potential sources of stress such as variations in temperature, rainfall, relative humidity, solar radiation, soil temperature.

Materials and Methods

The study area Valparai is a Taluk and hill station in the Coimbatore district of Tamil Nadu, India. It is located 3,500 feet (1,100 m) above sea level on the Anaimalai hills range of the Western Ghats at co-ordinates 10°22′12″N, 76°58′12″E. It has an average elevation of 3,914 feet (1,193 m). The study population of Nilgiri Tahr that is endemic to the Anaimalai hill range showed a density of about 10 individuals/1000 ha. Forestry activities like timber logging, firewood collection, social forestry are no longer carried out within this range other than the human disturbance that potentiated by tourism and the road being the only source of connecting route. Glucocorticoid metabolites can be measured as a parameter of adrenal activity in faecal samples, which offer the advantage of being easily collected and feedback free [2,3]. The study was carried out from December 2013 to November 2014, 10 fresh faecal samples were collected randomly each month from the group containing 30 animals and immediately stored in 80% ethanol to initiate steroid extraction immediately after collection [4]. All samples at the fringe areas and near roadways where these animals were usually sighted. Post collection, well-mixed wet faeces (0.6 g) was placed in a capped tube, containing 2.00 mL 80% methanol, vortexed for 30 min and then the tubes were carefully centrifuged for 20 min at 2500 rpm. The supernatant material was diluted in Phosphate Buffer Saline and stored at -80°C for subsequent use. Cortisol estimation was done using the ELISA KIT-DSI-EIA. The calibration curve with the mean absorbance on Y-axis and the calibrator concentration on X-axis was obtained using a 4-parameter curve by immuno assay software. The value of cortisol concentration of the unknowns was read directly from the calibration curve. The data for the meteorological parameters were obtained TNAU weather portal and monthly averages were ascertained for temperature, rainfall, relative humidity, solar radiation, soil temperature and wind speed throughout the study period. The monthly variations of glucocorticoids with these predictor variables were compared and their intra monthly, inter monthly variations were subjected statistical analysis using SPSS. The water sources for this particular region were also noted as these animals basically migrated based on their needs for establishing a permanent contact with the water source.

Results

The results suggest a significant variation in the cortisol level between the months and these variations were attributed to the temperature, relative humidity, wind speed, soil temperature, rainfall and solar radiation (Table 1). The maximum cortisol level was recorded 255.02 ng/g of faeces during May and the minimum was 169.84 ng/g of faces during July. The highest temperature was recorded during May and the least was during December. The relative humidity percentage was highest during December and lowest during May. Highest wind speed was recorded in the month of April and the lowest in the month of December. Rainfall was highest during the month of July. The solar radiation and soil temperature recorded was highest in the month of May and the lowest in January (Table 1). There about eight descriptive water sources in the study area namely, Sholayar, Azhiyar, Parambikulam, upper Nirar, Lower Nirar, Kadamparai and Upper Azhiyar.

Season	Months	Cortisol	Temperature	Relative humidity (%)	Wind speed (kmph)	Soil temperature (°c)	Rainfall (mm)	Solar radiation (cal/cm^2)
Winter	Nov	200.51	11.2	85.65	2.2	8.2	0	221.65
Winter	Dec	205.23	2.1	91.25	1.8	0	0	197.45
Winter	Jan	195.87	5.3	88.21	3.3	0	2	159.22
Winter	Feb	180.45	15.25	85.66	2.5	11.33	3	190.22
Summer	March	175.54	20.32	83.25	4.3	18.03	6.08	412.84
Summer	April	219.87	28.02	69.38	6.9	22.22	0	535.05
Summer	May	255.02	37.81	61.11	6.6	35.96	2.9	590.22
Summer	June	210.38	24.45	89.23	4.1	18.5	13.57	530.06
Rainy	July	169.84	27.54	87.56	2.5	20.16	15.9	380.25
Rainy	Aug	180.78	28.66	81.37	4.2	19.65	12.96	280.45
Rainy	Sep	175.13	22.21	70.65	5.1	19.12	6.21	370.16
Rainy	Oct	179.03	18.58	79.15	5.7	13.98	7.54	419.55

Table 1: Results variation in the cortisol level between temperature, relative humidity, wind speed, soil temperature, rainfall and solar radiation.

The statistical analysis by Pearson's correlation revealed no significant co-relations between the cortisol level and the abiotic factors ($p<0.001$, $p<0.05$) such as temperature, relative humidity, rainfall and revealed significant co-relations for wind speed, soil temperature, and solar radiation. However there was a significant correlation ($p<0.05$) between temperature and relative humidity, wind speed and highly significant correlations ($p<0.001$) between temperature and soil temperature, solar radiation.

Discussion

Huber et al., [5] found a clear seasonal pattern of Glucocorticoid metabolites secretion in captive red deer (*Cervus elaphus*) population, with higher level in winter and lower level in summer. The same variation has been reported by other studies on deer species in temperate climates, like white-tailed deer *Odocoileus virginianus*, [6] and mule deer *Odocoileus hemionus*, [7], this was not coinciding with our findings. We detected a clear pattern in the seasonal level of FCM, with highest concentrations in May and lowest concentration in July, (Figure 1). The data analysis shows no significant correlation between cortisol and other corresponding variable factors that were taken into consideration during the period of study. The high level of secretion of glucocorticoids in summer might be due to drastic change in the temperature and also adding to increased anthropogenic pressures that is because of tourism [8-10]. However individual animal variations and physiology also plays a major role contributing to the cumulative stress quotient, the increased in stress during summer might be due to the non-availability of feed and grazing grounds rising a physiological concern. The temperature, rainfall, relative humidity, solar radiation, soil temperature and wind speed recorded established no influence in the secretion of glucocorticoids. The observed pattern of secretion might be due to various factors including nutritional intake, predator density, tourism [7,11,12]. The meteorological variables like temperature, soil temperature and solar radiation were also high during the month of May but had no contribution to the cumulative stress quotients that increase glucocorticoid secretion additively. The environmental conditions have seen to have played a greater portion in the variations in stress in this small group of animals.

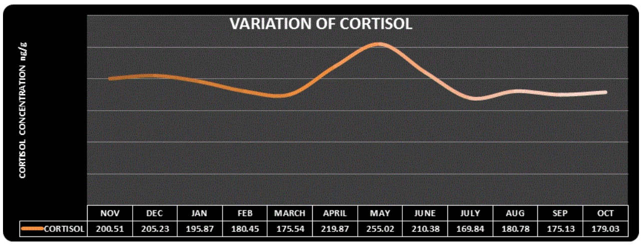

Figure 1: Variation of cortisol in the different months.

In this regard, it becomes essential to mention the report furnished by Pride [13], who quoted that glucocorticoid measures could be useful predictors of individual survival probabilities in the wild populations and existence of high glucocorticoid levels indicated the lowered individual fitness or even population variability. Mateo [14], mentioned that elevation of cortisol observed at emergence might facilitate the acquisition of anti-predator behaviors, with a conclusion that the minimum level of stress operating on the species. The encountering of elevated level of faecal cortisol concentrations in the population of Nilgiri Tahr could be directly attributed to the physiological status of the particular animals. However, it might be impossible to conclude whether it was acute stress or chronic stress that operated in these animals under study.

Schwarzenberger et al., [15] stated that the delayed between the circulation of steroids and their appearance in urine samples was rather short but the lag time of faecal steroids was about 12-24 h in ruminants. This is a baseline data that provide the level of quantifiable stress that prevail in these animals and also owing to the non-invasive tools of its assessment and the ease of sampling. Lesser disturbances in terms of number of visitors might be, however, assigned as the reason for the encountering of lesser faecal cortisol concentration level during the other months of July, August and September. This might be due to increased availability of feed materials including water for drinking, adequate environmental conditions, absence of various species of predators and most importantly decrease in tourism and no visitors reduce the social challenges that they have meet out, may be putting them in a comfort zone. How the environmental conditions (Meteorological parameters) did not impacted this rhythm still remains unclear. Cavigelli [16] stated that fecal cortisol levels were relatively high corresponding with the end of dry season when high intensity anti predatory behaviour and estimates of feeding effort were high, which is coinciding with the study. Harper et al., [17] observed a clear temporal pattern variation in fecal glucocorticoid levels and it was lowest during October, which was coincident with shortening day length and decreasing ambient temperatures.

The faecal glucocorticoid excretion varied seasonally with a response to cold stress and the parameters such as minimum ambient temperature and snow proved to be the only factors exerting significant effects on fecal glucocorticoid excretion, also the mean daily cortisol concentrations were not significantly different between seasons, but cortisol displayed a circadian rhythm only during the summer. The absence of a circadian rhythm of cortisol during the winter might have been a result of the limited amount of daylight as well as the continual need to produce metabolic heat as a by-product of gluconeogenesis [3,5,18,19]. The increase in the concentration of fecal cortisol was influenced by the days of rainfall and temperature, season and humidity index [20-24].

The mean minimum ambient temperature and mean temperature humidity index values had a significant positive correlation with mean fecal cortisol values [25], however, these values had no correlation with stress quotient of the animal and were individually a physiological response in our study. So in conclusion it is the adrenocortical activity that plays an important role in the seasonal and daily regulation of their physiological states, individually dependant on the animal and not the parameters [26].

Interestingly all the animals are in the same area, with the constant meteorological factors, so the stress acting upon each of the subjects should that are aided by these parameters are constant. The variations in the cortisol level that was observed with the animals may be due to the individual physiological states of the subjects. The quotients of the attributed stress by the meteorological parameters are present but the individual variations observed may be due to, predator pressure, dominance, aggression, reproductive status, competition from co-existing herbivores and finally anthropogenic activities. The conclusion of this study projects a baseline data on glucocorticoid metabolites and their variation and statistically provides proof that the meteorological factors show no correlation with the cortisol, which provides reasons of stress, may be of the physiological conditions of the individual animals such as starvation, pregnancy aggression both intra specific and inter specific. Intense individual animal studies are further required to come to concrete conclusions.

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