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Research Article



Non-invasive enumeration of cortisol metabolite level to evaluate stress response in gaurs (*Bos gaurus*) to predict fitness.

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Abstract

Gaurs are docile animals and keep clear far distances from people and habitations but now there is a chain in the scenario, gaurs are moving into human habitations and agricultural lands. The study was carried out to assess the faecal cortisol concentration in gaurs entering the agricultural fields around the forest, in order to arrive at the baseline values contributing to the stress factors that predict the fitness parameters. The adjoining areas of Bandipur wildlife region, Sathyamangalam wildlife region and Anamalai wildlife region of Tamil Nadu, India were included in this study programme. Faecal samples were collected from gaurs in each of these areas. The mean faecal cortisol concentration in the case of gaurs that entered in the agricultural fields adjoining the Bandipur wildlife region, Sathyamangalam and Anamalai region were found using Enzyme Linked Immuno Sorbent Assay (ELISA). The occurrences of conflict, feed availability, water, co existence of predators and other competitive species were also taken into account and anthropogenic stressors as well play an important role in these animals. Suitable management related measures were recommended pertaining to the findings observed in this study like increased mean faecal cortisol concentration in gaurs. The findings demonstrated that fecal glucocorticoid assays provide an index of physiological stress in gaurs. These techniques may prove useful in addressing conservation and conflict issues and also directly interpret the degree of fitness of the individuals.

Keywords: Gaurs-Faecal Cortisol-stressors-fitness

Introduction

Stress physiology has been a vital concern in non invasive animal studies these days. The adrenal cortex releases glucocorticoids into circulation, and their concentrations in the blood increase as part of the stress response. 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 the environmental factors. Because 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 becomes warranting. Such a technique has the advantage of keeping subjects undisturbed during collection of samples that helps in fixing baseline values unlike blood sampling. Hormonal studies are currently being incorporated in wildlife research as a means to evaluate the health, fitness and physiology of individuals. The factors that drive animals into human habitations, their varying feeding ecology and water

availability had to be studied. Crop raiding is a major conflict term that has to be addressed. Their roughage intake varies around 50-60 kg/ animal. Also in the absence of predator the population keeps escalating leading to sharing of biomass than the carrying capacity. The gaurs at various geographical and meteorological areas have been included to draw concrete conditions contributing to stress ultimately conflict and also to potentiate the attributing meteorological factors. Because stressful events have potential deleterious effects on animal population, it is of special concern to monitor the stress response in free-ranging animals and ultimately predict their fitness.

Materials and Methods

The study area

Study on faecal cortisol and in gaurs (*Bos gaurus*) interfering with agriculture was carried out in areas adjoining the Western Ghats (Bandipur tiger reserve, Anamalai tiger reserve) and Eastern Ghats (Sathyamangalam region) of Tamil Nadu state in India during february, 2012. The samples were collected from animals by foot transect. All samples were collected from the different regions at the same time period.

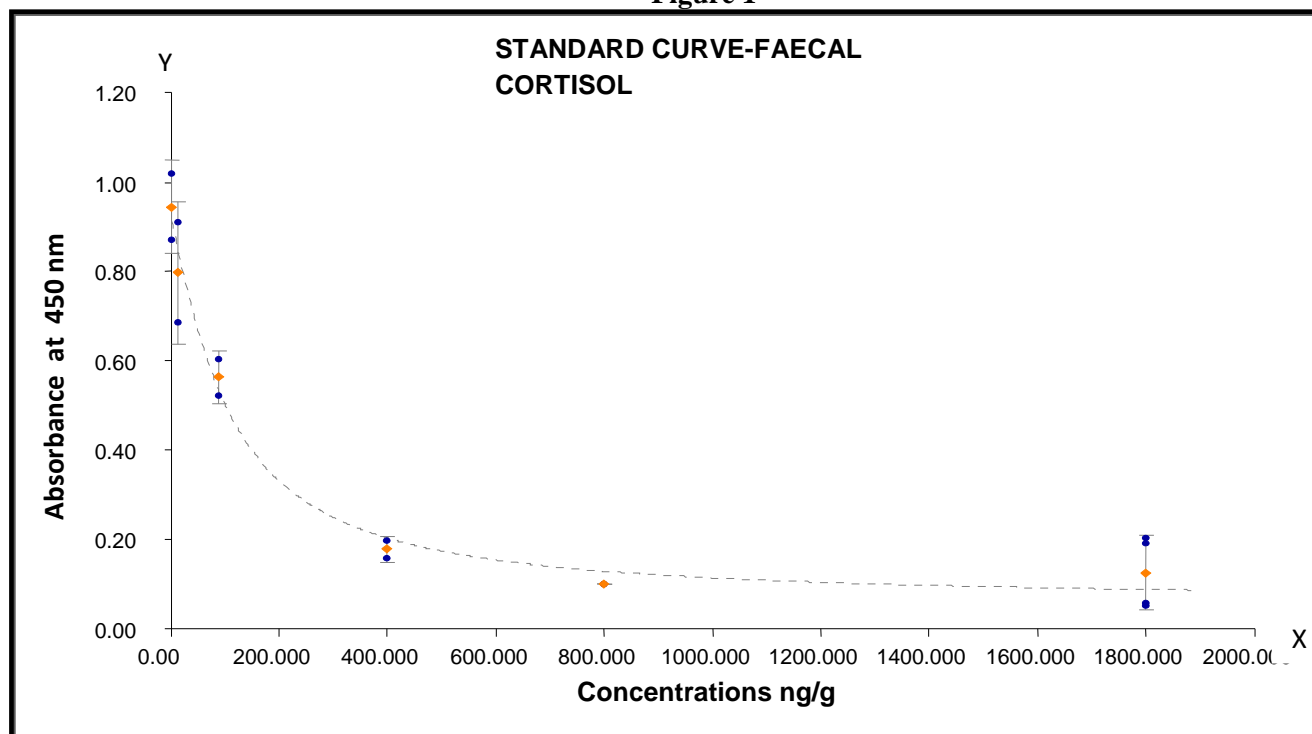
Number of samples examined

Fresh faecal samples were obtained from free-ranging gaurs in agricultural fields of adjoining forest regions of Bandipur (n=10), Sathyamangalam (n=10) and Anaimalai (n=10).

Throughout this study period, faecal samples were collected subsequent to the thorough mixing of the freshly voided fecal materials and were stored in 80 per cent methanol for steroid extraction pertaining to the estimation of cortisol.

Well-mixed wet feces (0.6gram) was placed in a capped tube, containing 2.00 ml 80 per cent methanol, vortexed for 30 minutes and then the tubes were carefully centrifuged for 20 minutes 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. Figure 1.

Figure 1



The statistical analysis of the data was carried out as per the guidelines furnished by Snedecor and Cochren (1989), using one way ANOVA.

Results

Faecal cortisol of gaurs

The faecal samples of gaurs obtained from adjoining areas of Bandipur, Sathyamangalam and Anaimalai wildlife regions were processed and subsequently

subjected to estimation of cortisol concentration by using the ELISA kit (DSI-EIA- STEROID-CORTISOL EHE-151). Using the ELISA reader, the absorbance values of standards as well as the samples were analyzed and standard curve was obtained using standard techniques.

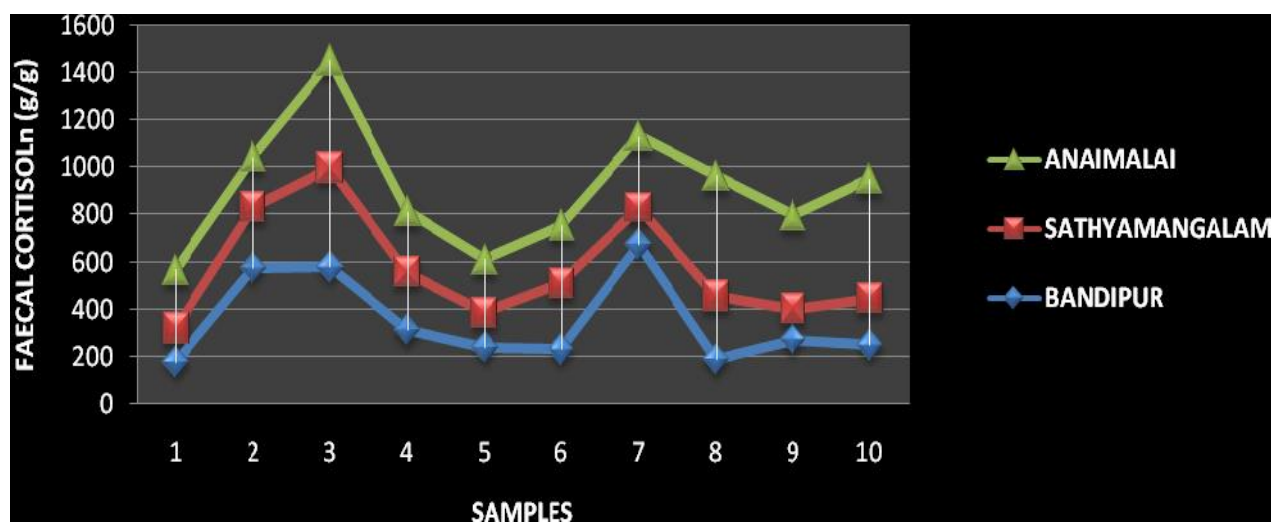
On Comparison of mean faecal cortisol among gaurs of adjoining regions, the statistical analysis revealed absence of variations between the gaurs of all the three different adjoining areas (Table 1).The mean over all faecal cortisol level in gaurs (n=30) was found to be 302.62ng/g. (Figure 2)

Table 1. Faecal cortisol level in gaurs (n=30)

S.no	Bandipur	Sathyamangalam	Anaimalai
1	175.79	141.81	247.63
2	575.66	254.83	211.91
3	579.39	417.42	454.56
4	312.25	244.75	255.68
5	237.94	146.18	225.87
6	230.95	277.92	245.01
7	675.37	154.85	301.56
8	188.74	265.77	510.43
9	272.44	128.12	395.33
10	249.55	194.98	506.30
Mean	349.80±13.62	222.86±9.42	335.42±10.92
F value	2.453^{NS}		

NS- NON SIGNIFICANT

Figure 2. Faecal cortisol level of gaurs



The mean cortisol concentrations in the faecal samples obtained from the gaurs, between the adjoining areas of Western Ghats (Bandipur and Anaimalai regions) and Eastern Ghats (Sathyamangalam region). It was estimated that the gaurs of the Western Ghats showed a higher level of faecal cortisol 342.61 ng/g to the Eastern Ghats 222.66 ng/ g of faeces.

Discussion

Faecal cortisol

In general, there is a scarcity of technical information pertaining to the faecal cortisol in bovines, especially the gaurs. However, considering the utilities of cortisol assessment in this study comprising of gaurs, the cortisol level was estimated in this research programme to predict survival or fitness quotients. This was in coinciding agreement with the report presented by Carlsson *et al.* (2007) who opined that non-invasive sampling method; based on the quantification of stress sensitive molecules that were important in the objective assessment of animal welfare as an alternative to the quantification of such molecules in blood. This was supported by Touma and Palme (2005) who opined that the non invasive monitoring of the steroid hormone metabolites in faeces of mammals had become an increasingly popular technique in the recent years, since it has offered several advantages. In this regard, it is warranted to mention the reports presented by Borell and Schaffer (2005) who revealed that non invasive measurements of stress indicating metabolites in saliva, faeces or urine had been recently developed and validated and were useful parameters with regard to the legal requirements and assessment of stress and welfare in animals. Enzyme Linked Immunosorbent Assay (ELISA) was used throughout this study with faecal samples obtained from gaurs. The usage of ELISA technique as carried out in the study was in agreement with the report furnished by Sink *et al.* (2008) who opined that when compared with radio immunoassays, the usage of ELISA technique for the detection of cortisol level had merits in terms of elimination of health hazards and costs of handling radio isotopes. Usage of ELISA technique as done in this study was supported by Lupica and Turner (2009) who opined that the results of validation of enzyme linked immunosorbent assay revealed ELISA as an efficient, sensitive and reliable method for cortisol

measurements in faecal extracts with regards to assessment of stress.

Throughout the study carried out, fresh faecal samples subjected to thorough mixing were obtained from all the gaurs under study and cortisol assessment was carried out with these faecal samples only. Schwarzenberger *et al.* (1996) opined that the faecal samples had the advantage that they could be easily be collected without stressing the animals. Washburn and Millspaugh (2002) opined that the environmental conditions might influence the faecal glucocorticoid metabolite measurements, if the samples could be collected immediately after the deposition and the faecal samples exposed to rainfall might not be suitable for faecal glucocorticoid analysis, because it might lead to artificial elevation of faecal glucocorticoid measurements.

Obtaining of samples from well mixed faecal materials of the gaurs under this study was in agreement with the findings reported by Millspaugh and Washburn (2003) who opined that since using only a few pellets from a faecal mass might lead to a biased interpretation of the assay, homogenization of the faecal mass before removing a sub-sample of faecal material for analysis was effective. In this regard, it becomes essential to mention the report of Palme and Mostl (1997) who opined that the faecal steroids might be unevenly distributed in the faecal balls of horses, swine and elephants. Hence, throughout the study, more care was undertaken for obtaining the homogenized faecal samples from gaurs. Throughout the collection procedure, 80 per cent of methanol was used towards storage of fresh and well homogenized faecal materials of the gaurs. This was supported by the finding reported by Palme and Mostl (1997) who revealed highest recoveries of faecal glucocorticoids during the storage of wet faeces in 80 per cent methanol.

Similarly, the faecal samples in 80 per cent methanol were stored at -20°C prior to subjecting the faecal samples to ELISA technique and this was in agreement with the report presented by Khan *et al.* (2002) who emphasized on the stability of faecal glucocorticoids when stored at -20°C in these preservatives. The mean faecal cortisol level in each of the adjoining areas of Bandipur, Sathyamangalam and Anaimalai wildlife regions were revealed in Table 1 in addition to the cortisol values of each sample from

gaurs. The mean faecal cortisol levels were higher in gaurs in the Western Ghats and the variations in stress levels due to the existence of multifaceted etiological factors including the variations in the climatic causal factors for encountering of such differences in the levels of faecal cortisol concentrations pertaining to the gaurs. The overall mean cortisol level was found to be 302.62ng/g in case of gaurs. During the statistical analysis (Table 1), in this study, the mean faecal cortisol concentration in gaurs revealed no significant variations ($P < 0.01$), in the adjoining areas of Bandipur, Sathyamangalam and Anaimalai wildlife regions implementing that the level stress was the same pertaining to any wildlife region. The existence of no significant variations in terms of increase of faecal cortisol level in gaurs might be attributed to the various biotic as well as abiotic factors, like reduced availability of feed materials including water for drinking, adverse change in the environmental conditions, proximity of various species of predators and visitors agonistic encounters social challenges, lack of highly palatable and easily available feed resources etc that were similar in the three regions. Invariable of the geographical location the animals exhibited the same level of stress. The prevalent faecal cortisol level as encountered in gaurs of this study might be due to the stress factors operating on this species. This was in agreement with the report presented by Touma and Palme (2005) who opined that disturbances caused by the presence of humans, agonistic encounters, social challenges etc. might lead to the influence of faecal glucocorticoid metabolite in various species of mammals. In this context, it is important to mention the report furnished by Pride (2005) 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 (2006) opined that elevation of cortisol observed at emergence might facilitate the acquisition of anti-predator behaviors. The encountering of elevated level of faecal cortisol concentrations in majority of individual gaurs indicated the existence of stress causing factors pertaining to the gaurs belonging to Bandipur, Sathyamangalam and Anaimalai wild life regions. Hence it could logically assumed that the gaurs get involved in human-animal conflicts by interfering the agriculture field developed by farming community inhabiting the immediate adjoining areas of these three wildlife regions. Though it might be

difficult to say whether it was acute stress or chronic stress that operated in the gaurs under study, it becomes noteworthy to mention the report furnished by Schwarzenberger *et al.* (1996) who 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 hours in ruminants and about 24 hours to over 48 hours in animals that were hind gut fermentors like horse, elephants, pigs, rhinoceros and primates. Due to the encountering of enhanced faecal cortisol level in the faecal samples of majority of the individual gaurs, it might be assumed that the nature of stress factors related with such an elevation might probably be a chronically existing stress than the acute type of stress. Further variations in the different types of habitat, meteorological factors etc. might be assigned as the causal factors for the existence of chronic type of stress as revealed by enhanced faecal cortisol concentration in majority of gaurs. However, in order to arrive at a concrete conclusion, it is warranted that undertaking of further research comprising of gaurs inhabiting especially the core areas selected wildlife regions.

Lesser disturbances in terms of number of visitors might be however assigned as the reason for the encountering of comparatively lesser mean faecal concentration level in gaurs of the adjoining areas of Sathyamangalam region. Comparison of overall mean faecal cortisol concentration in gaurs of Western Ghats comprising of regions adjoining Bandipur and Anaimalai with that of the Eastern Ghats comprising of regions adjoining Sathyamangalam revealed elevated mean faecal cortisol concentrations in case of faecal samples obtained from Western Ghats. The increased number of visitors, varying types of habitat, variations in climatic factors, increased in tourist activities etc. might be assigned as the reason for encountering of elevated mean cortisol concentration in samples from adjoining areas of Western Ghats

The mean faecal cortisol concentrations in the case of gaurs were found to be increased. Hence, a systematic research programme is highly warranted in the protected regions like Bandipur tiger reserve, Sathyamangalam tiger reserve and Anaimalai tiger reserve with regard to the identification of stress causing factors like tourists or visitors entry, availability of the routinely consumed feed varieties, availability of the highly palatable feed varieties,

increase in predator-density, increase in competition among the co-existing herbivores and omnivores in the concerned wildlife region, carrying capacity of the region extensive activities or manipulation by human beings in the forest regions, persons entering the wildlife regions for the purpose of collection of plants, fallen wood, leaves etc. Hence the guidelines pertaining to the identifying of stress related factors through systematic individual research programme need to be strengthened or modified accordingly by the concerned authorities. The mixing of gaurs with non descriptive cattle that often graze in the adjoining areas of the wildlife regions should be avoided to a great extent, in order to prevent the spread of diseases between the gaur and domestic cattle. Measure pertaining to the confinement of the gaurs within the wildlife region need to be strengthened and similarly, the control measures with regard to avoiding of straying activities need to be strictly implemented, so as to minimize the contact between the non descriptive cattle and gaurs. The plantations of crops that are avoided by gaurs can be planted at the immediate outside areas of the wildlife region and additionally, some space shall be left between the borders of the wildlife regions. However, frequent monitoring by the concerned farmers especially in the apt season in which the feed-crops were highly sought by the gaurs should be maintained by the concerned farming community. Further, it could be concluded that the conflicts between gaurs and humans encountered might also be due to the adaptation behavior of the concerned gaurs in the wake both of its natural habitat and progressive decline of its natural wild food-base. The easy access to more energy rich food resources or highly palatable food resources might be associated as one more feature pertaining to the occurrence of conflicts between the gaurs and humans.

Conclusion

The encountering of mean faecal cortisol concentration in gaurs that entered agricultural fields adjoining the wildlife regions indicated the existence of possible stress factors affecting the gaurs. Additional guidelines with regard to prevention or minimizing of the human casualties especially when people enter into forests for the collection of fuel wood, fodder, medicinal plants, grazing of the livestock ultimately reducing conflict. It becomes a need to increase the number and type of awareness programmes among the farming community in areas

adjoining the Bandipur, Sathyamangalam and Anamalai wildlife regions. All these in addition to the variations in the planting, ripening or fruiting or harvesting activities might get associated with occurrence of conflicts with gaurs throughout the year. The suggested management measures put together can aid in decreasing conflict, maintaining a balance and improving conservation. Gaurs' also being a prime prey for the tigers, then wandering into villages then there is going to be an increased note of Human animal conflict on the rise. Also the predator quotient in the areas studied should be considered to comment on the carrying capacity to narrow down to individual stress factors.

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