EVALUATION OF ENDOGENOUS FAECAL CORTISOL AS A NON INVASIVE ASSESSMENT OF STRESS IN FREE RANGING WILD PIGS *(Sus scrofa)*

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ABSTRACT

The study was carried out to assess the faecal cortisol concentration in wild pigs entering the agricultural fields around the adjoining areas of Mudumalai Tiger reserve (MTR), Sathyamangalam Tiger reserve (STR) and Anamalai tiger reserve (ATR) of Tamil Nadu, India, in order to arrive at the baseline values contributing to the stress factors. Faecal samples collected from wild pigs in each of these areas were subjected to Enzyme Linked Immuno Sorbent Assay (ELISA) for estimation of faecal cortisol level and the mean faecal cortisol concentration of wild pigs were arrived. The occurrences of conflict, lack of food and water availability, temperature, rainfall and prey prevalence played an important role to their stress quotients. Suitable management related measures were recommended. The findings demonstrated that fecal glucocorticoid assays provide an index of physiological stress in wild pigs. These techniques may prove useful in addressing conservation issues and additionally being non invasive.

Keywords: Faecal Cortisol, Stress, Wild pigs

INTRODUCTION

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. Glucocorticoids are also involved in metabolic regulations and may vary according to reproductive state and seasonal fluctuations adapting the organism to changing conditions (Romero, 2002). Because glucocorticoids—either cortisol or corticosterone are released during stressful situations, they can serve as an index of the stress response, and the development of noninvasive 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 and helps in fixing baseline values (Mostl and Palme, 2002). Hormonal studies are currently being incorporated in wildlife research to evaluate stressful events that have potential deleterious effects on animal reproduction and immune systems, it is of special concern to monitor the stress response in free-ranging animals.

MATERIALS AND METHODS

Fresh faecal samples were obtained from free-ranging wild pigs interfering with agriculture areas adjoining in the Western Ghats [Mudumalai tiger reserve (n=10), Anamalai tiger reserve (n=10)]

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and Eastern Ghats [Sathyamangalam tiger reserve (n=10)] of Tamil Nadu in India from November, 2013 to May, 2014.

Freshly voided faecal samples were collected, thoroughly mixed and stored in 80 per cent methanol for steroid extraction of cortisol metabolite by Enzyme Linked Immuno Sorbent Assay (ELISA).

Well-mixed wet feces (0.6g) were placed in a capped tube containing 2.00 ml 80 per cent methanol (Palme and Mostl 1997), vortexed for 30 minutes and then centrifuged for 20 minutes at 2500 rpm. The supernatant material was diluted in Phosphate Buffer Saline (PBS) and stored at-80ºC for subsequent use. The cortisol estimation was done using the ELISA KIT-DSI-EIA EH-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. 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). The statistical analysis of the data was carried out by one way ANOVA using Statistical Analysis in Social Science (SPSS) 13.0 software.

RESULTS

Faecal cortisol concentration in ten fresh faecal samples (n=10) ranged from 175.79 to 684.37 ng/g in adjoining areas of MTR. Whereas in adjoining areas of STR, the faecal cortisol concentration were obtained for ten fresh faecal samples (n=10) ranged from 141.81 to 413.42 ng/g and faecal cortisol concentration from fresh faecal samples (n=10) ranged from 201.91 to 515.43 ng/g in adjoining areas of ATR (Table 1). The mean ± S.E. values of faecal cortisol concentration of wild pigs in the adjoining areas of MTR, STR and ATR were 349.41±59.81, 223.57±27.53 and 336.03±38.83ng/g, respectively and there was no statistical difference in faecal cortisol concentration between different study areas. The mean over all faecal cortisol level in wild pigs (n=30) was found to be 302.99 ng/g. Wild pigs of the Western Ghats had higher levels of cortisol metabolites (342.70 ng/g) in relation to those of the Eastern Ghats (223.57 ng/ g).

DISCUSSION

The overall mean cortisol level was found to be 302.99 ng/g in case of wild pigs, much higher than the value recorded by Williams 2006 who recorded the baseline faecal cortisol value as 65.8 ng/ mg ± 12.6. The mean faecal cortisol concentration in wild pigs did not reveal any significant variations ($P \leq 0.01$) in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai wildlife regions. The faecal cortisol level thus recorded in wild pigs 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 and other analytical factors (Palme, 2012). The increased faecal cortisol level encountered in wild pigs 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 regard, it becomes noteworthy 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 wild pigs indicated the existence of stress causing factors pertaining to the wild pigs belonging to Mudumalai, Sathyamangalam and Anaimalai wild life regions. Hence it could logically be assumed that the wild pigs get involved in human-animal conflicts by interfering with the agriculture field adjoining areas of these three wildlife regions in addition to environmental stressors as well. Due to the encountering of enhanced faecal cortisol level in the faecal samples of majority of the individual wild pigs, it could 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 habitat, meteorological factors etc. might be the causal factors for the existence of chronic type of stress. However, in order to arrive at a concrete conclusion, it is warranted that undertaking of further research comprising of more number of wild pigs 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 lesser mean faecal concentration level in wild pigs of the adjoining areas of Sathyamangalam region as compared to the Western Ghats. The increased number of visitors, varying types of habitat, variations in climatic factors, increased tourist activities etc. might be assigned as the reason for encountering elevated mean cortisol concentration in samples from adjoining areas of Western Ghats.

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TABLE 1

FAECAL CORTISOL LEVEL IN WILD PIGS (ng/g)

S. No.	ADJOINING REGIONS MUDUMALAI (n=10)	ADJOINING REGIONS SATHYAMANGALAM (n=10)	ADJOINING REGIONS ANAIMALAI (n=10)
1	175.79	141.81	245.63
2	575.66	256.83	201.91
3	579.39	**413.42**	464.56
4	322.25	254.75	245.68
5	227.94	146.18	235.87
6	225.95	277.92	241.01
7	**684.37**	154.85	306.56
8	178.74	265.77	**515.43**
9	276.47	148.12	397.33
10	247.52	175.98	506.30
MEAN	**349.41±59.81**	**223.57±27.53**	**336.03±38.83**

Figure - 1