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Research Article

Enumerating endogenous faecal glucocorticoid metabolites as indicators of stress in wild pigs interfering with agriculture adjoining forest regions correlating with conflict and meteorological factors - A Non invasive approach.

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Keywords

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Meteorological Factors
Conflict
Management

Abstract

The study was carried out to assess the faecal cortisol concentration in wild pigs entering the agricultural fields around the forest regions and in domestic pigs comprising of desi pigs and cross bred pigs, in order to arrive at the baseline values pertaining to stress factors. The adjoining areas of Mudumalai wildlife region, Sathyamangalam wildlife region and Anamalai wildlife region of Tamil Nadu, India were included in this study programme. The documentation of the quantifiable meteorological factors in the identified conflict areas was done. The mean faecal cortisol concentration in the case of wild pigs that entered in the agricultural fields adjoining the Mudumalai wildlife region, Sathyamangalam and Anamalai region were found using ELISA (Enzyme Linked Immuno Sorbent Assay). The mean faecal cortisol concentration of desi pigs as well as cross bred pigs was also documented to be used as primary and secondary control values. The wild pigs are always at conflict terms with agricultural production and cause unequalled damage. The occurrences of conflict were also classified as low, medium and high based on the intensity. Suitable management related measures were recommended pertaining to the findings observed in this study like increased mean faecal cortisol concentration in wild pigs, comparative wild pig- human conflicts pertaining to the variations in the meteorological factors of the areas were studied. The findings demonstrated that fecal glucocorticoid assays provide an index of physiological stress in wild pigs and may prove useful in addressing conservation and conflict issues. The application of such non-invasive techniques in the field of wildlife conservation would attribute to intense importance as invasive sampling cannot be practically applicable.

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 (glucocorticoid metabolites) 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 that helps in fixing baseline values (Mostl and Palme, 2002). Hormonal studies are currently being incorporated in wildlife research as a means to evaluate the health and physiology of individuals (Tarlow and Blumstein, 2007). Because stressful events have potential deleterious effects on animal reproduction and immune systems, it is of special concern to monitor the stress response

in free-ranging animals. Further, Weather is not the only source of unpredictable events. Other examples include sudden changes in social status, increased predator numbers, decreased food resources, and disease. Animals use environmental cues such as changing day length, temperature and rainfall to predict future events and adjust accordingly. However, responses to short lived perturbation factors require more rapid responses without possibility of anticipatory changes. This response has been collectively termed the emergency life history stage and serves to direct the individual away from normal life history stages into a physiological and behavioral state that will allow survival in the best condition possible (Wingfield and Kitaysky, 2002).Glucocorticoid metabolites are always excreted in feces, but the amount of different glucocorticoid metabolites varies according to species (Palme, 2005).

Early diagnosis of physiological changes may allow conservation and effective conflict management strategies. Noninvasive monitoring of the stress response of wild pigs might help to evaluate the factors that cause stress which in turn triggers m to fall at conflict with humans.

We aimed to validate ELISA for evaluating the stress response of these wild pigs and we were interested in determining whether increased or decreased faecal glucocorticoid metabolites in wild pigs interfering with agriculture in the adjoining forest regions ultimately led to human-wild pig conflict co-relating with the respective meteorological parameters of the adjoining areas . The faecal cortisol of desi pigs and cross bred pigs were also determined and taken as control units in order to fix baseline values.

Materials and Methods

The study area

Study on faecal cortisol and in wild pigs (*Sus scrofa*) interfering with agriculture was carried out in areas adjoining the Western Ghats (Mudumalai tiger reserve, Anamalai tiger reserve) and Eastern Ghats (Sathyamangalam region) of Tamil Nadu state in India during November, 2013 to May, 2014.

Number of samples examined

Fresh faecal samples were obtained from free-ranging wild pigs in agricultural fields of adjoining forest regions of Mudumalai, Sathyamangalam and Anaimalai Table 1.

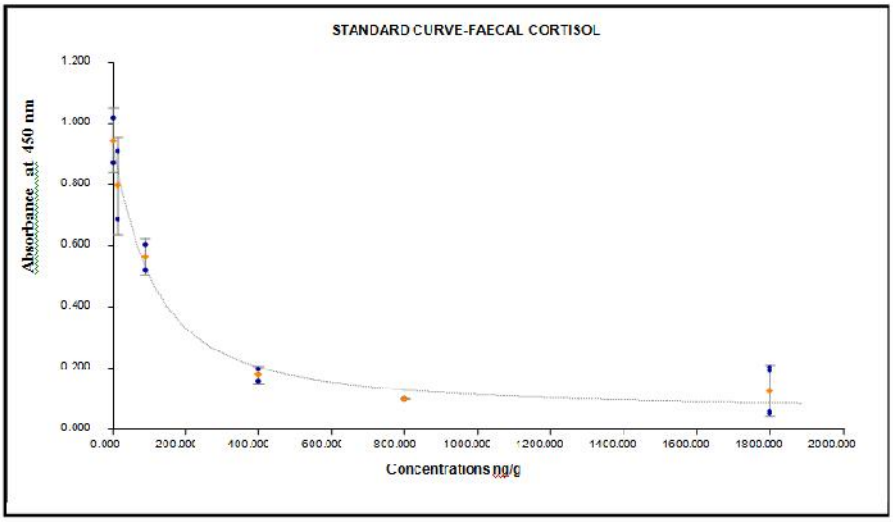
TABLE 1

FAECAL SAMPLES	WESTERN GHATS		EASTERN GHATS	TOTAL
	MUDUMALAI	ANAMALAI	SATHYAMANGALAM	
WILD PIG	10	10	10	30
DESI PIG	5	5	5	15
CROSS BRED PIGS	5	5	5	15

Throughout this study period, faecal samples were collected subsequent to the thorough mixing of the freshly voided wet fecal materials and were stored in 80 per cent methanol (Palme and Mostl 1997) 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 ^{o}C for subsequent use. Cortisol estimation was done using the ELISA KIT-DSI-EIA (Lupica and Turner, 2009). 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



Collection of meteorological parameters

The details of the meteorological parameters of each region were obtained from the Tamil Nadu Agricultural University (TNAU) portal. These meteorological parameters were ascertained to have direct or indirect effect on the wild pigs like seasonal migration, water availability, rainfall and radiation.

Conflict

The wild pig related conflict areas in the adjoining regions of Mudumalai Sanctuary were identified subsequent to the discussion with forest veterinary officer, officials of the forest department, villagers and other village level officers and the level of conflict pertaining to the three regions under study were recorded and correlated with the meteorological factors and the cumulative stress. Conflicts were graded as low, medium and high. Low - infrastructure damage, water source contamination, rooting of land and ecological damage, Moderate-agricultural crop raiding, livestock preying, damage to forest restoration and grasslands, High- injury to humans, causing fatalities

Management

Within the adjoining regions of wildlife areas taken under study suitable management measures were framed and recommended.

Statistical analysis

The statistical analysis of the data was carried out as per the guidelines furnished by Snedecor and Cochren (1980) using one way ANOVA, Chi square tests.

Results

Faecal cortisol

The faecal samples of wild pigs obtained from adjoining areas of Mudumalai, 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. Similarly, cortisol concentration was estimated from the faecal samples obtained from desi pigs, as well as cross bred pigs observed in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai regions, respectively

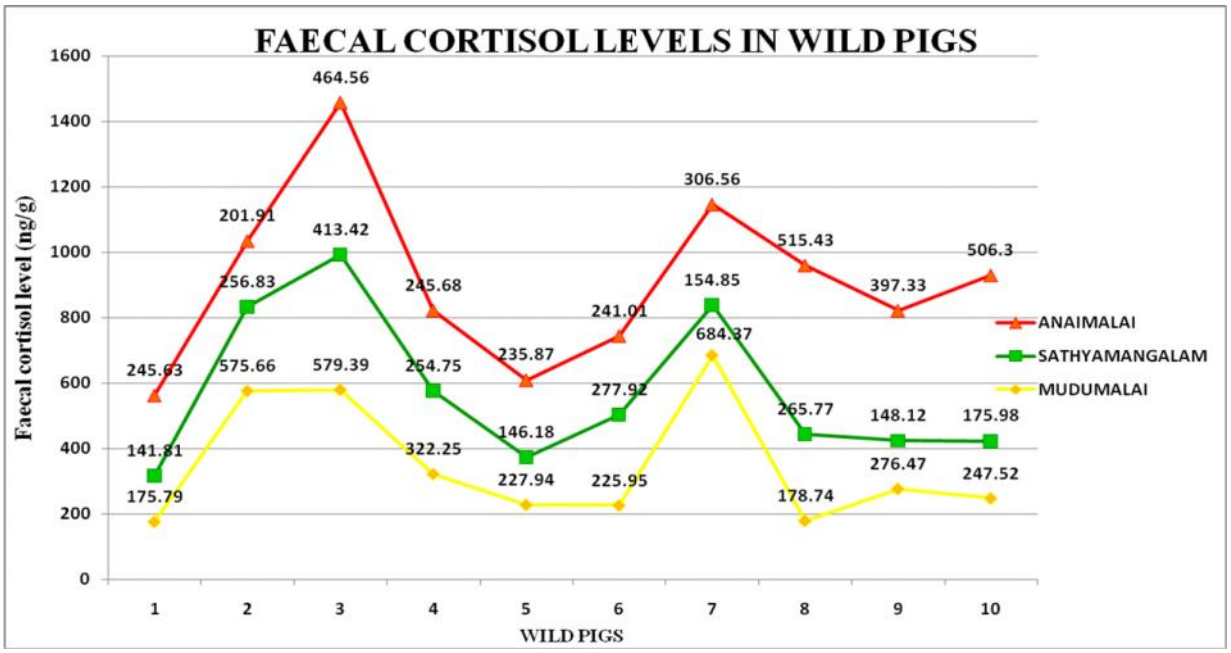
Wild pigs

Faecal cortisol concentration (Table 2) in ten fresh faecal samples (n=10) ranged from 175.79 to 684.37ng/g in adjoining areas of Mudumalai. The Faecal cortisol concentration in ten fresh faecal samples (n=10) ranged from 141.81 to 413.42ng/g in adjoining areas of Sathyamangalam. Faecal cortisol concentration in ten fresh faecal samples (n=10) ranged from 201.91 to 515.43ng/g in adjoining areas of Anaimalai. The mean ± S.E. values of faecal cortisol concentration of wild pigs in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai were 349.41±59.81, 223.57±27.53 and 336.03±38.83ng/g, respectively. The individual faecal cortisol values in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai were revealed in Figure 2.

. **TABLE 2FAECAL 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 2



Desi pigs

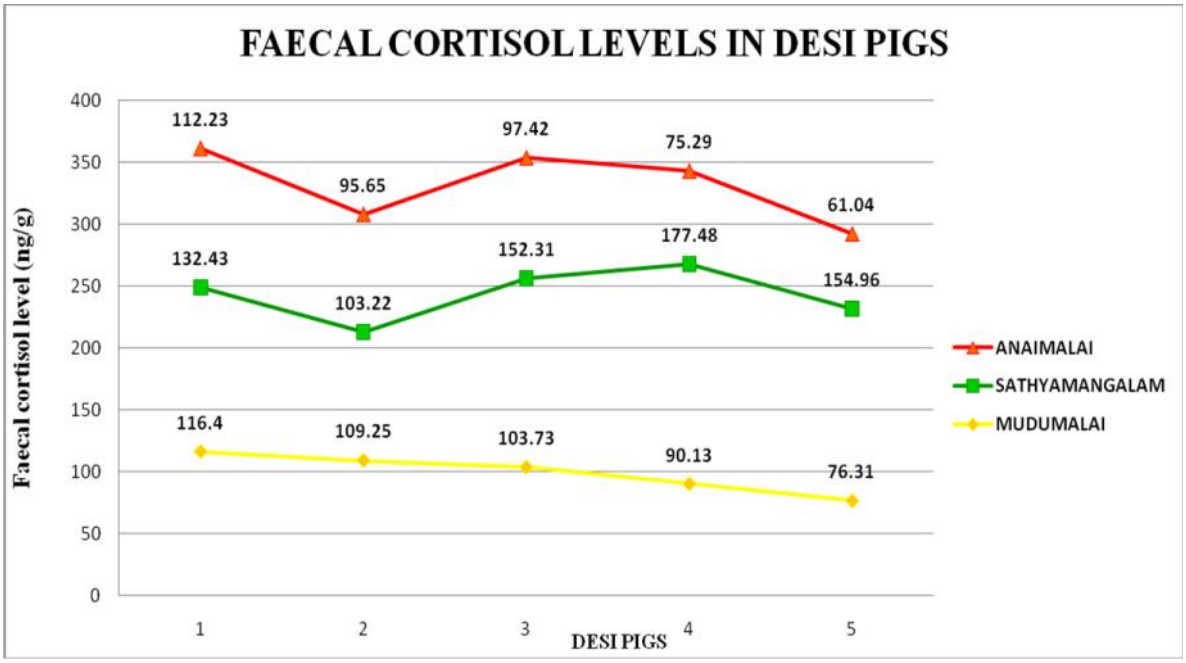
In desi pigs of adjoining areas of Mudumalai (n=5), the faecal cortisol level ranged from 76.31 to 116.40ng/g (Table 3). Similarly, the desi pigs of adjoining areas of Sathyamangalam (n=5) revealed faecal cortisol concentration ranging from 103.22 to 177.48ng/g and in case of adjoining areas of Anaimalai (n=5) the range of faecal cortisol concentration varied from 61.04 to 112.23ng/g. The mean ± S.E. values of faecal cortisol concentration of desi pigs in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai were 99.17±7.16, 144.08±12.46 and 88.32±9.00ng/g, respectively. The individual faecal cortisol values in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai were revealed in Figure 3.

TABLE 3

FAECAL CORTISOL LEVEL IN DESI PIGS (ng/g)

S.NO	ADJOINING REGIONS MUDUMALAI (n=5)	ADJOINING REGIONS SATHYAMANGALAM (n=5)	ADJOINING REGIONS ANAIMALAI (n=5)
1	116.40	132.43	112.23
2	109.25	103.22	95.65
3	103.73	152.31	97.42
4	90.13	177.48	75.29
5	76.31	154.96	61.04
MEAN	**99.17±7.16**	**144.08±12.46**	**88.32±9.00**

FIGURE 3



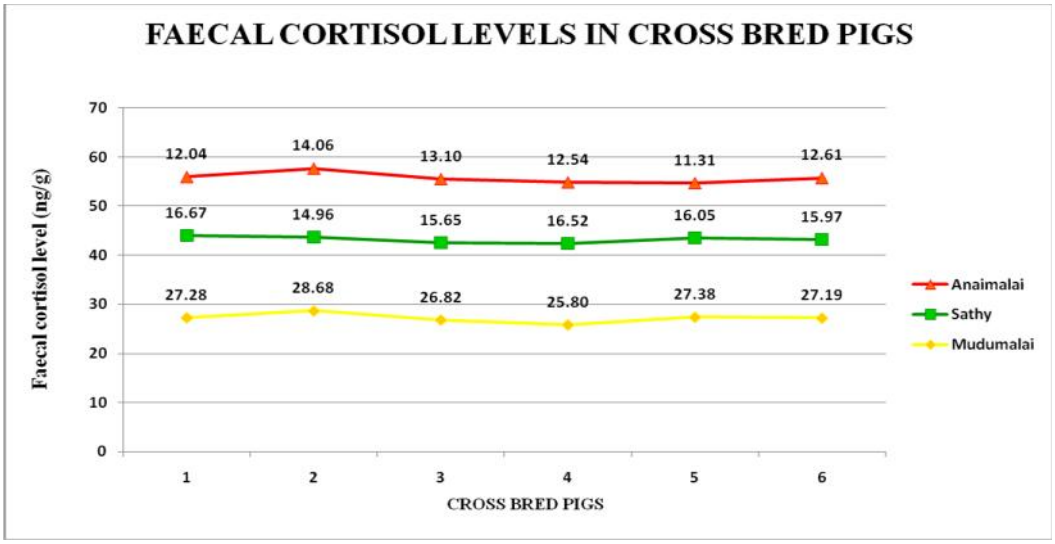
Cross bred pigs

The cortisol level in the faecal samples (Table 4) obtained from cross bred pigs (n=5) of the adjoining areas of Mudumalai ranged from 25.80 to 28.67ng/g. Similarly, the faecal (n=5) cortisol concentration had the range from 14.96 to 16.67ng/g in adjoining areas of Sathyamangalam. The range of faecal (n=5) cortisol concentration was from 11.31 to 14.06ng/g in adjoining areas of Anaimalai. The mean ± S.E. values of faecal cortisol concentration of desi pigs in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai were 27.96±0.46, 15.97±0.31 and 12.61±0.47ng/g, respectively. The individual faecal cortisol values in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai were revealed in Figure 4.

TABLE 4 FAECAL CORTISOL LEVEL IN CROSS BRED PIGS (ng/g)

S.NO	ADJOINING REGIONS MUDUMALAI (n=5)	ADJOINING REGIONS SATHYAMANGALAM (n=5)	ADJOINING REGIONS ANAIMALAI (n=5)
1	27.28	16.67	12.03
2	28.67	14.96	14.06
3	26.82	15.64	13.10
4	25.80	16.52	12.54
5	27.38	16.05	11.31
MEAN	**27.19±0.46**	**15.97±0.31**	**12.61±0.47**

FIGURE 4



Comparison of mean faecal cortisol among pigs of adjoining regions

The mean concentration of cortisol in faecal samples obtained from wild pigs, desi pigs and cross bred pigs were also compared statistically (Table 5) in each of different adjoining areas. Highly significant (P≤0.01) variations were encountered among the wild pigs, desi pigs and cross bred pigs in each of the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai respectively. The mean faecal cortisol concentration in desi pigs however differed significantly, when compared to the mean faecal cortisol concentration in cross bred pigs.

TABLE 5 COMPARISON OF MEAN FAECAL CORTISOL LEVEL AMONG PIGS OF ADJOINING REGIONS (ng/g)

ADJOINING REGIONS	PIGS	MEAN(ng/g)	F-VALUE
MUDUMALAI	WILD PIGS(n=10)	349.41 ± 59.81 ^{b}	**11.120****
	DESI PIGS(n=5)	99.17 ± 7.16 ^{a}	
	CROSS BRED PIGS(n=5)	27.19± 0.46 ^{a}	
SATHYAMANGALAM	WILD PIGS(n=10)	223.56 ± 27.52 ^{c}	**17.167 ****
	DESI PIGS(n=5)	144.08 ± 12.46 ^{b}	
	CROSS BRED PIGS(n=5)	15.97 ± 0.31 ^{a}	
ANAIMALAI	WILD PIGS(n=10)	336.03 ± 38.83 ^{b}	**26.123 ****
	DESI PIGS(n=5)	88.32 ± 9.00 ^{a}	
	CROSS BRED PIGS(n=5)	12.61 ± 0.46 ^{a}	

Means bearing different superscripts in the group differ significantly
NS-NOT SIGNIFICANT
**** HIGHLY SIGNIFICANT (P≤0.01)**

Comparison of mean faecal cortisol in pigs among adjoining regions

The statistical analysis revealed absence of variations between the wild pigs of all the three different adjoining areas (Table 6). However, highly significant variations (P≤0.01) were encountered in the faecal cortisol concentration of desi pigs at adjoining areas of Sathyamangalam, when compared with the faecal cortisol level in desi pigs at adjoining areas of Mudumalai and Anamalai. Highly significant (P≤0.01) variations were noticed among the faecal cortisol concentrations in case of cross bred pigs of these areas.

TABLE 6 COMPARISON OF MEAN FAECAL CORTISOL LEVEL IN PIGS AMONG ADJOINING REGIONS (ng/g)

ANIMALS	ADJOINING REGIONS	MEAN (ng/g)	F-VALUE
WILD PIGS	MUDUMALAI (n=10)	349.41±59.81	2.453^{NS}
	SATHYAMANGALAM (n=10)	223.57±27.53	
	ANAIMALAI (n=10)	336.03±38.83	
DESI PIGS	MUDUMALAI (n=10)	99.17±7.16 ^{a}	9.115 **
	SATHYAMANGALAM (n=5)	144.08±12.46 ^{b}	
	ANAIMALAI (n=5)	88.32±9.00 ^{a}	
CROSS BRED PIGS	MUDUMALAI (n=5)	27.19±0.46 ^{c}	329. 82**
	SATHYAMANGALAM (n=5)	15.97±0.31 ^{b}	
	ANAIMALAI (n=5)	12.61±0.47 ^{a}	

Means bearing different superscripts in the group differ significantly
NS-NOT SIGNIFICANT
**** HIGHLY SIGNIFICANT (P≤0.01)**

Overall and region wise mean faecal cortisol values in pigs

The mean over all faecal cortisol level in wild pigs (n=30) was found to be 302.99ng/g and it was found to be 110.52ng/g in case of desi pigs (n=15). Similarly, the mean faecal cortisol concentration in cross bred pigs (n=15) was 18.59ng/g. (Figure 5). Mean faecal cortisol values in pigs of different adjoining regions were revealed in Figure 6.

Mean faecal cortisol level between adjoining areas of Western and Eastern Ghat regions.

The mean cortisol concentrations in the faecal samples obtained from the wild pigs, desi pigs and cross bred pigs between the adjoining areas of Western Ghats (Mudumalai and Anaimalai regions) and Eastern Ghats (Sathyamangalam region) were presented in Figure 7

.

TABLE 7METEOROLOGICAL PARAMETERS OF THE VARIOUS REGIONS DURING THE SEASONS

PLACES	SEASON	TEMPERATURE°C		RELATIVE HUMIDITY %	WIND SPEED (Kmph)	SOIL TEMP °C	RAIN FALL (mm)	SOLAR RAD (cal/cm2)
		MAX	MIN					
MUDUMALAI	WINTER	28.32	10.73	91.25	4.2	9.65	12.96	280.45
	SUMMER	30.32	18.74	83.25	4.3	20.03	6.08	388.84
SATHYAMANGALAM	WINTER	29.33	14.55	70.65	5.1	25.12	6.21	370.16
	SUMMER	35.87	22.34	69.38	6.9	32.22	0	510.05
ANAMALAI	WINTER	28.66	15.45	91.25	4.2	9.65	12.96	280.45
	SUMMER	37.42	19.14	79.15	5.7	23.98	7.54	419.55

FIGURE 5

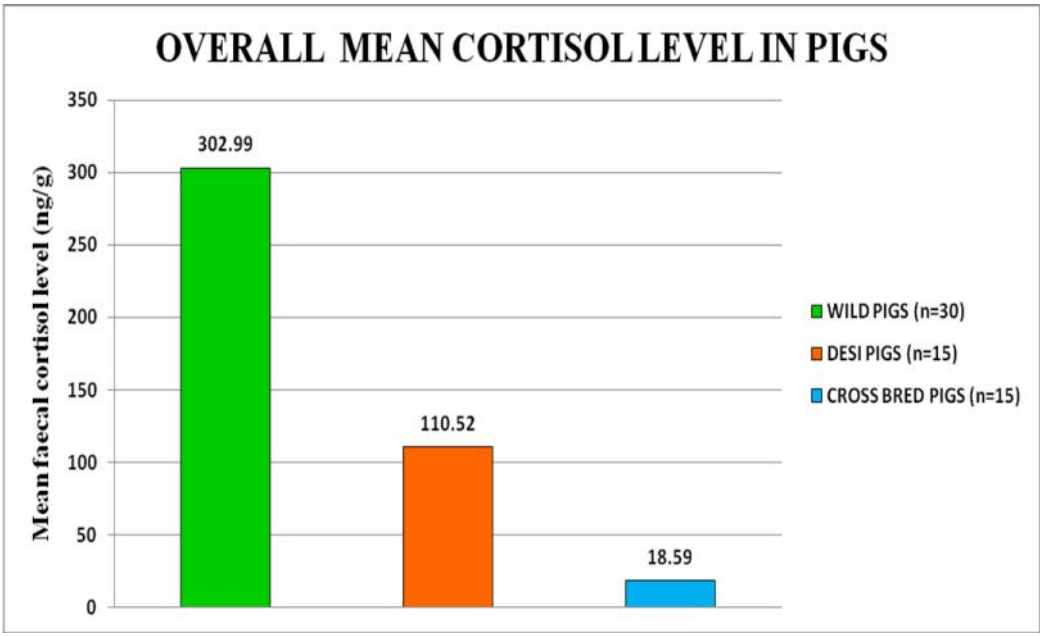


FIGURE 6

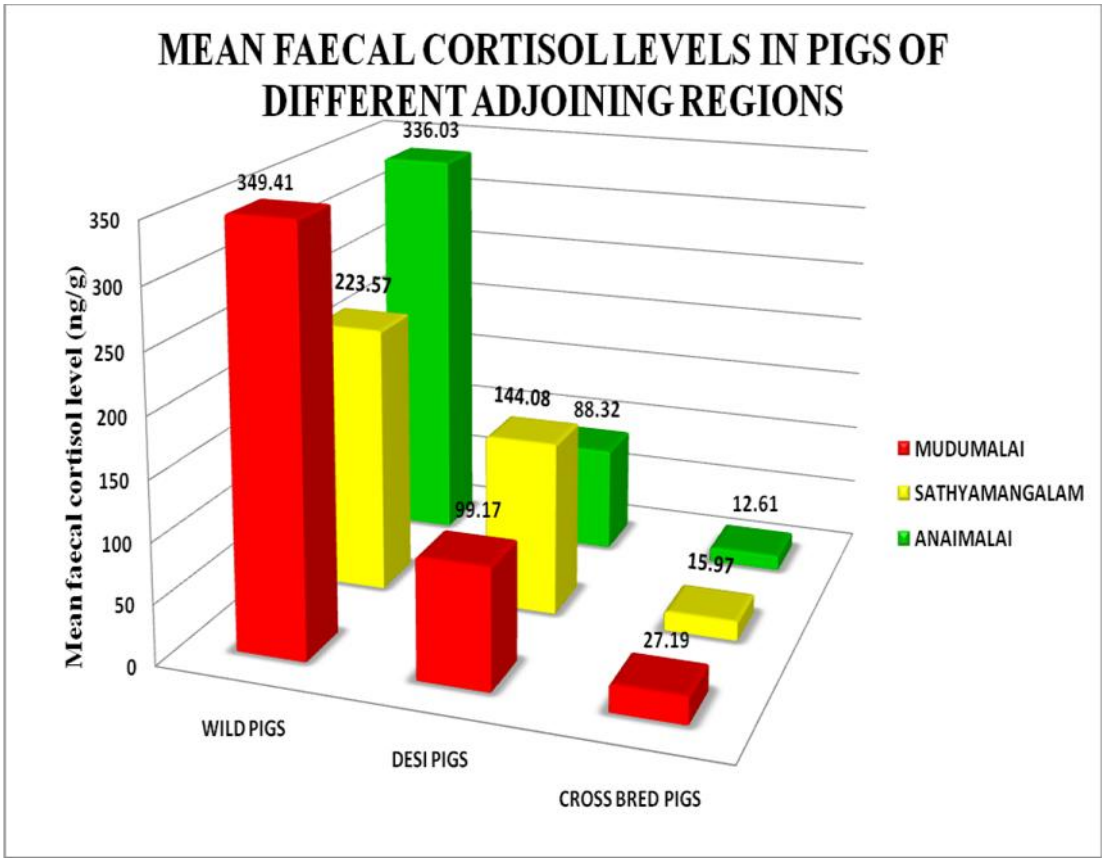
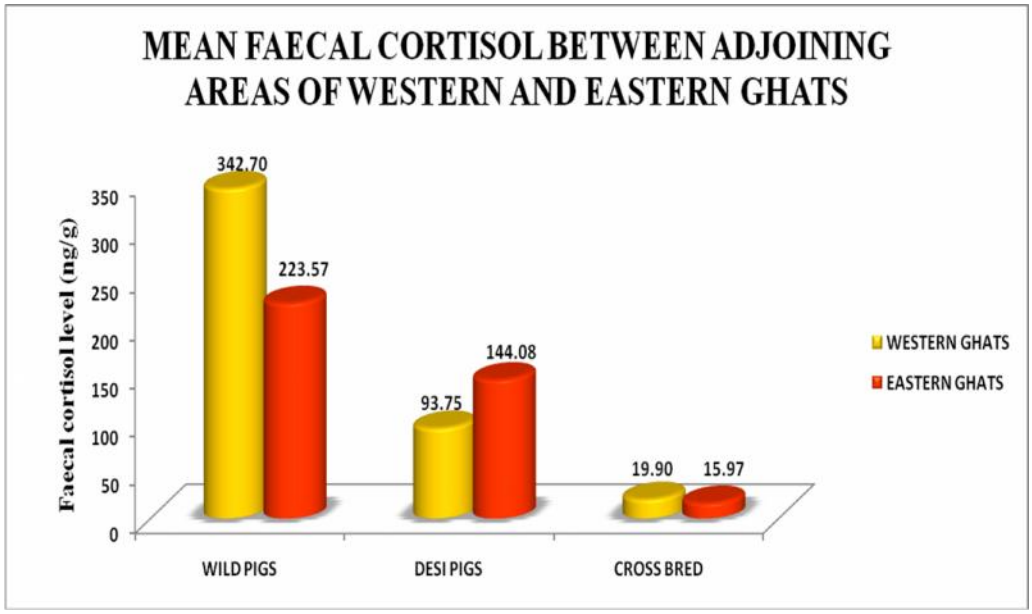


FIGURE 7



Meteorological parameters

The different meteorological parameters comprising of temperature (degree centigrade), relative humidity (per cent), wind speed (Kmph), soil temperature (degree centigrade), rainfall (mm) and solar radiations (cal/cm^2) were presented in Table 7. The range of values for each of the meteorological parameters was furnished.

Conflict

Conflicts were recorded as mentioned as low, medium and high. Low - infrastructure damage, water source contamination, rooting of land and ecological damage, Moderate-agricultural crop raiding, livestock preying, damage to forest restoration and grasslands, High- injury to humans, causing fatalities. Highly significant variations were recorded on occurrences of conflict among the three study regions during winter (Table 8). Similarly, highly significant variations were recorded on occurrences of conflict among the three study regions during summer (Table 9). Interestingly there were no seasonal variations in conflict occurrence except in areas adjoining Mudumalai showing significant variations (Table 10).

TABLE 8 REGIONAL VARIATION OF CONFLICT IN WINTER

REGION	LOW	MEDIUM	HIGH	X^2
MUDUMALAI	32(**48.48%**)	27(**40.90%**)	7(**10.6%**)	**15.45****
SATHYAMANGALAM	16 (**45.71%**)	18(**51.42%**)	1(**2.86%**)	
ANAMALAI	4(**16%**)	21(**84%**)	**NIL**	

NS- NOT SIGNIFICANT
***-SIGNIFICANT**
****-HIGHLY SIGNIFICANT**

TABLE 9 REGIONAL VARIATION OF CONFLICT IN SUMMER

REGION	LOW	MEDIUM	HIGH	X^2
MUDUMALAI	23(**79.31%**)	5(**17.24%**)	1(**3.44%**)	**16.48****
SATHYAMANGALAM	2(**20.00%**)	8(**80.00%**)	**NIL**	
ANAMALAI	4(**33.33%**)	8(**66.66%**)	**NIL**	

NS- NOT SIGNIFICANT
***-SIGNIFICANT**
****-HIGHLY SIGNIFICANT**

TABLE 10 SEASONAL VARIATION OF CONFLICT

S.NO	SEASON	LOW	MEDIUM	HIGH	X^2
MUDUMALAI	**WINTER**	32(**48.48%**)	27(**40.90%**)	7(**10.6%**)	**7.48***
	SUMMER	23(**79.31%**)	5(**17.24%**)	1(**3.44%**)	
SATHYAMANGALAM	**WINTER**	16 (**45.71%**)	18(**51.42%**)	1(**2.86%**)	**2.67^{NS}**
	SUMMER	2(**20.00%**)	8(**80.00%**)	**NIL**	
ANAMALAI	**WINTER**	4(**16%**)	21(**84%**)	**NIL**	**0.53^{NS}**
	SUMMER	4(**33.33%**)	8(**66.66%**)	**NIL**	

NS- NOT SIGNIFICANT
***-SIGNIFICANT**
****-HIGHLY SIGNIFICANT**

Discussion

Faecal cortisol

In general, there is paucity of technical information pertaining to the faecal cortisol in swine, especially the wild pigs. However, considering the utilities of cortisol assessment in this study comprising of wild pigs and desi pigs, in addition to the cross bred pigs, the cortisol level was estimated in this research programme. This is in 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 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 offered several advantages. In this regard, it becomes noteworthy 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 pigs. Enzyme Linked Immunosorbent Assay (ELISA) was used throughout this study with samples obtained from wild pigs, desi pigs and cross bred pigs. 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.

Further, usage of ELISA technique as done in the 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 pigs under study and cortisol assessment was carried out with these faecal samples only. In this regard, 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 pigs 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 noteworthy 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 wild pigs, desi pigs and cross bred pigs.

Throughout the collection procedure, 80 per cent of methanol was used towards storage of fresh and well homogenized faecal materials of the wild pigs, desi pigs and cross bred pigs. 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^{o}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^{o}C in these preservatives.

The mean faecal cortisol level in each of the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai wildlife regions were revealed in Table 2 to 4 and Figure 2 to 4 in addition to the cortisol values of each sample from these pigs. Comparatively cortisol levels in faecal samples were observed to be lower in cross bred pigs then the desi as well as wild pigs. Similarly, the mean faecal cortisol levels were higher in wild pigs than the desi pigs, as well as the cross bred pigs. The variations in stress levels due to the existence of multifaceted etiological factors including the differences in the management related measures might be assigned as the causal factors for encountering of such differences in the levels of faecal cortisol concentrations pertaining to the wild pigs, desi pigs and cross bred pigs. The overall mean cortisol level was found to be 302.99ng/g in case of wild pigs, whereas the overall mean cortisol level in desi pigs was 110.52ng/g and it was 18.59ng/g in cross bred pigs (Figure 5). During the statistical analysis (Table 5 and Figure 6), in this study, the mean faecal cortisol concentration in wild pigs was found to be higher, followed by desi pigs and cross bred pigs. The mean cortisol level of wild pigs was found to reveal highly significant variations ($P \leq 0.01$), when compared to desi pigs, as well as cross bred pigs in the adjoining areas of Mudumalai, Sathyamangalam and Anaimalai wildlife regions. The existence of such highly significant variations in terms of increase of faecal cortisol level 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 etc. The increased faecal cortisol level as encountered in wild pigs of this study when compared to desi pigs or cross bred pigs might be due to the stress factors operating on this species. This was in agreement with the report presented by Touma and Palme[8] 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, when compared to the maximum range of (177.48ng/g) of faecal cortisol in desi pigs indicated the existence of stress causing factors pertain to the wild pigs belonging to Mudumalai, Sathyamangalam and Anaimalai wild life regions. Hence it could logically assumed that the wild pigs 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 wild pigs 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 wild pigs, 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 wild pigs. However, in order to arrive at a concrete conclusion, it is warranted that undertaking of further research comprising of more number wild pigs inhabiting especially the core areas selected wildlife regions.

Similarly, the mean faecal cortisol level of desi pigs revealed (Table 5) highly significant variations ($P \leq 0.01$) when compared with the mean faecal cortisol level of cross bred pigs in case of adjoining areas of Sathyamangalam regions only. The reasons for encountering of highly significant rise in the mean faecal cortisol level in desi pigs might be attributed to the lack of availability of feed resources, drinking water, inconvenient housing arrangements made by the owners, lesser health-care related measures in the areas of studied.

The mean faecal cortisol level within pigs of different regions however failed to reveal any significant variations within the wild pigs of adjoining areas (Table 6 and Figure 6) of Mudumalai, Sathyamangalam and Anaimalai. 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. Similarly, the different types of housing arrangements, variations in feeding regiment, variations in the husbandry and management related practices, variations in the health-care related measures, variations in the environmental conditions etc. might be assigned as the causal factors for the encountering of highly significant variations ($P \leq 0.01$)

pertaining to the mean faecal cortisol concentrations in case of cross bred pigs.

Comparison of overall mean faecal cortisol concentration in wild pigs of Western Ghats comprising of regions adjoining Mudumalai and Anaimalai with that of the Eastern Ghats comprising of regions adjoining Sathyamangalam (Figure 7) 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

Meteorological parameters and conflict

From the discussion with veterinary officer of forest department, village personnel and farmers, it was understood that wild pig-human conflicts in terms of entry into their agricultural fields were found to occur almost throughout the year the. Though the extremes of temperature including the

solar radiations in both the summer season and winter season, varying levels humidity (Table 7) and other related factors could lead to stress in case of wild pigs of the wildlife regions, it was also equally true that most of the stress causing factors like existence of feed resources related competitions among co-existing species belonging to different taxonomic classes, presence of the predators or the hunting type of carnivore species like tigers and leopards etc. were found to be almost persistent type of stress related factors in case of wild pigs. Further, variable crops were planted in both the summer and winter seasons by the related farming community. Hence, logically it could be stated that conflicts between wild pigs and humans in terms of entry into the agriculture fields, in particular might occur throughout the year, regardless of the occurrence of variations in the meteorological parameters documented in summer as well as in winter seasons (Table 8, 9 and 10). The variations in the number of conflict related events pertaining to wild pigs might be dependent on the following factors:

- The variations in the type of crops preferred at various levels by the wild pigs.
- Variations in the fruiting or ripening or harvesting stage of the crops in the agricultural fields luring the wild pigs.
- Variations in the period of plantations of such crops in the agricultural fields of adjoining areas of wild life.
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However, it could be logically assumed that lack of adequate feed materials and lack of watering resources might become the additional stress causing factors in case of the wild pigs during summer season. The sampling in wild pigs could not subjected to the season wise grouping because of the practical difficulties encountered towards the collection of faecal samples in fresh condition during this study.

It becomes noteworthy to mention the report furnished by Watve (1992) who encountered higher helminthic loads in elephants during the dry season, when compared to the wet season in the wild life region. Similarly, the variations in wind speed as evidenced in the Table 6 could be associated with the movement of wild pigs and this was in agreement with the findings furnished by Wobeser (2007) who quoted that abnormal weather over a short or long time might allow population of animals to expand their home range and further, Busch and Hayward (2009) revealed the linkage between the climatic change and feed abundance for the wild animals. Hence, all these were logically linked to the movement of the wild animals like wild pigs. Further Ananthasubramaniam (1989) also linked the environment related factors like temperature, humidity, atmosphere and light including the predators and the competitors, with the movement of wild animals.

The water level in pykara dam of Mudumalai, Aliyiar dam of Anamalai and Bhavanisagar dam of Sathyamangalam, in addition to the natural as well as artificial water holes in these wildlife regions could be overall associated with the movement pattern of different wild animal species, including wild pigs and water could be one of the significant factors pertaining to the conflict. This was in agreement with the report furnished by Desai *et al.* (1996) who opined that areas close to water like the human-settlement areas were the preferred areas for wild animals.

Management measures

The mean faecal cortisol concentrations in the case of wild pigs were found to be increased, when compared to these values in case of either the desi pigs or the cross bred pigs. Hence, a systematic research programme is highly warranted in the protected regions like Mudumalai tiger reserve, Sathyamangalam tiger reserve and Anamalai 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 if plants, fallen wood, leaves etc. Hence the guidelines pertaining to the identified stress related factors through systematic

individual research programme need to be strengthened of modified accordingly by the concerned authorities. The mixing of wild pigs with desi pigs that often stray 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 wild pigs and the domestic pigs like desi pigs. Measure pertaining to the confinement of the wild pigs 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 desi pigs and wild pigs. The plantations of crops that are deserted by wild pigs shall 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 wild pigs should be maintained by the concerned farming community. In this regard, it becomes noteworthy to mention the report furnished by Gopakumar *et al.* (2012) who stated that in Kerala, crops like ginger were found to be deserted by the wild pigs and were profitably planted in the fields bordering the forests and this helped to eliminated, the entry of wild boars into the agricultural fields- electrical or solar powered fencing, wild boar-proof fences using of trained dogs as effective deterrents for wild pigs and field patrolling by the farmer groups on a regular rotation basis etc., might be adopted and adhering to the existing legal aspects of wildlife conservation in India. Further, it could be stated that the conflicts between wild pigs and humans encountered in the areas adjoining the wildlife regions might also be sue to the adaptation behaviour of the concerned wild pigs in the woke 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 wild pigs and humans. Hence, the management plan in the protected regions shall focus in the measures that help to prevent the deterioration of feed-resources for the wild pigs. Similarly, appropriate crop insurance schemes might be strengthened pertaining to the wild pig associated high risk croplands identified and all these might definitely help to mitigate the conflict problems between the wild pigs and humans in the areas adjoin the wildlife region.

Conclusion

The encountering of increased mean cortisol concentration in wild pigs that entered agricultural fields adjoining the wildlife regions when compared with the value in desi pigs or cross bred pigs indicated the existence of possible stress factors affecting the wild pigs. 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 Mudumalai, Sathyamangalam and Anamalai wildlife regions. The meteorological parameters that were varying in different months, with regard to the range of temperature, relative humidity, wind speed, soil temperature, rainfall and solar radiation contributed to the conflict arousal in haphazard manner. All these in addition to the variations in the planting, ripening or fruiting or harvesting activities might get associated with occurrence of conflicts with wild pigs throughout the year. The suggested management measures put together can aid in decreasing conflict without tragic outcomes and maintaining a balance.

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