

## Monitoring of Cortisol Levels in Hog Deer with Varying Environment Exposure

Original  
Article

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Hog deer (*Axis porcinus*) is one of the least studied animal species in Pakistan. It belongs to Order Artiodactyla and the family Cervidae. IUCN classified *Axis porcinus* as an endangered species in 2008. The present study was conducted to investigate the effects of varying environmental exposure, genders, and seasonal changes on captive hog deer (*A. porcinus*) at Lahore Zoo, Safari Zoo, and Jallo Park in Lahore, Pakistan. Non-invasive techniques were used to monitor stress levels in hog deer. For sample collection, four definite months belonging to two seasons' winter and summer were considered. A total of 48 urine and faecal samples were collected from both male and female hog deer. Seasonal fluctuations have been found to have a significant impact on faecal and urinary cortisol levels. Higher cortisol levels were found in both male and female hog deer in the summer season at all three visited sites. Fluctuations in environmental exposure at three research sites had a significant impact on faecal and urinary cortisol levels. Higher levels of cortisol were found in both male and female hog deer at Jallo park and Lahore Zoo, as compared to Safari Zoo. It was concluded that lower cortisol levels at Safari Zoo might be due to better environmental conditions and more flexible enclosure size and interaction of various species of deer. Temperature affected hog deer cortisol levels in summer, as higher levels were measured in summer compared to winter. In addition, sex did not predict any stress levels in hog deer. It has been suggested that a large enclosure size can control levels of cortisol in hog deer.

**Keywords:** Captive Environment; Glucocorticoids; Cortisol; Stress levels; Hog deer.

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### Author's Contribution.

All of my co-authors contributed to this research in the form of data collection, write-up, and improvement.

### Conflict Of Interest:

The author(s) declare that the publication of this article has no conflict of interest.

**Project details.** Nil

## Introduction

In Pakistan, Hog deer (*Axis porcinus*) is one of the least studied animal species. It belongs to Order Artiodactyla and the family Cervidae. Hog deer is a little deer with a massive large body with dark brown coloration and short thin legs[1]. The hind limbs of the hog deer are longer than that of the forelimbs. The face is wedge-shaped and small in size[2]. The Hog Deer (*Axis porcinus*) is an endemic species, geographically kept to South and Southeast Asia[3]. In Pakistan, hog deer is present in woodlands along the waterways, meadows, and particularly in those spaces having thick grass[4]. Hog Deer (*A. porcinus*) is financially significant because of its meat, skin, and tusks for which they are pursued and cultivated[5].

The Hog Deer (*Axis porcinus*), went through a drastic decline during the mid and late twentieth Century[6]. It was once widespread; however, the population has declined quickly across its geographic range. It is reported that the global decrease rate of this species is very high almost 50% in the last two decades and 90% inside its Southeast Asian range. IUCN classified *Axis porcinus* as an endangered species in 2008[3]. In the Indian Wildlife Protection Act, 1972 hog deer is protected under Schedule I. *A. porcinus* faces a genuine reduction in number, particularly in Pakistan because of habitat destruction and hunting pressure. Although, *A. porcinus* is an endangered species it is one of the least studied and ignored mammalian species[6].

Human-induced environmental changes have resulted in an overall decrease in the number of this species[7]. However, Zoos provide a good environment to help wildlife activities around the world. The wild animal feels a high level of frustration in the presence of visitors[8]. Various investigations have shown that visitor's presence is related to animal social and physiological changes[9]. It is, therefore, necessary for health experts to understand the possible influence of zoo visitors on the well-being of animals.

In living organisms' levels of hormones can be estimated via blood, salivation, urine, faeces, milk, and hair[10]. The glucocorticoids are steroid hormones that are released from the adrenal cortex in the condition of stress and are the essential effectors of the hypothalamic-pituitary-adrenal (HPA) axis. The levels of cortisol have been related to stress, particularly in primates, rodents, and canines[11]. Non-invasive techniques, for example, gathering urine and faeces to evaluate the stress levels in organisms are getting popular nowadays [12].

Extremely restricted data is available on the impact of temperature, varying environmental exposures, humidity seasonal variations, sex, and visitor numbers on cortisol levels[13]. This study will be the first one to investigate the effects of various environmental and seasonal changes on male and female hog deer (*A. porcinus*) at Lahore Zoo, Safari Zoo, and Jallo Park in Lahore, Pakistan. Faecal and urinary cortisol levels were assessed as a physiological response to various stresses. The findings of this investigation may be helpful for the administration to establish better management of wild and captive fauna.

## Materials And Methods

**Selection of study site:** The present study was carried out at Safari Zoo, Lahore Zoo and Jallo Forest, and Wildlife Park Lahore. These three sites are available with several animals such as the Bengal tiger, emu, lion, ostrich, common pheasant, blackbuck, and hog deer along with many others.

## Selection of Animal

An Indian hog deer (*Axis porcinus*) was selected for the purposed study. It belongs to the order Artiodactyla and the family Cervidae. The details about the number, gender, and age of Hog deer at Lahore Zoo, Lahore Safari Zoo, and Jallo Park are shown in Table 1. Animals were given the right amount of water and food as indicated by their caretakers. The taking care time was covered from 10 am to 12 pm every day.

**Table 1.** Number and age of animals at three different studied sites

Site	Total Number	Gender		Age (Mean $\pm$ S.D)
		Males	Female	
LHR Zoo	20	06	14	4.2 $\pm$ 0.55
SFR Zoo	15	04	11	4.5 $\pm$ 0.45
Jallo Park	24	05	19	5.1 $\pm$ 0.65

## Collection of Faecal Sample

A pilot study was conducted to understand the defecation patterns of hog deer. Twenty-four faecal samples were collected at three locations from both male and female hog deer. Samples were collected once a month in the morning (Sunday). Samples were taken after defecation and stored within 2-3 hours. All of the collected samples were put into tightly sealed plastic bags and preserved in ice boxes containing ice cubes before they took into a lab. Samples were adequately marked by the species, site, sex, and date of collection. Samples were transported from the confined area to the laboratory and stored below -20 °C.

## Pre-Treatment of Faecal Sample

Processing of Faecal samples was done by the process described by [14] along with the directions given by the EIA kit maker. Wet faeces from the sample in the quantity of 0.5 grams were placed into centrifuge tubes having ethanol (80%) in the quantity of 5 ml. After 30 min of shaking, samples were centrifuged at 2000 rpm for ten minutes. The supernatant was moved into a polypropylene tube after those samples were stored at -20 °C for immunoassay. All the results for fecal cortisol levels were recorded in ng/g.

## Collection of Urine Sample

Like faecal samples, 24 urine samples were collected in the morning (Sunday) once in a month. The samples were collected immediately after urination and stored within two hours. Each sample was collected with the help of a syringe right after urination. Then urine samples were shifted into a clean tube. Samples were adequately marked by the species, site, sex, and date of collection. Later on, the samples were transported from the enclosures to the lab and filtered to remove the dust particles and any impurities. At that point, samples were stored at under -20°C. All the results for fecal cortisol levels were recorded in ng/ml.

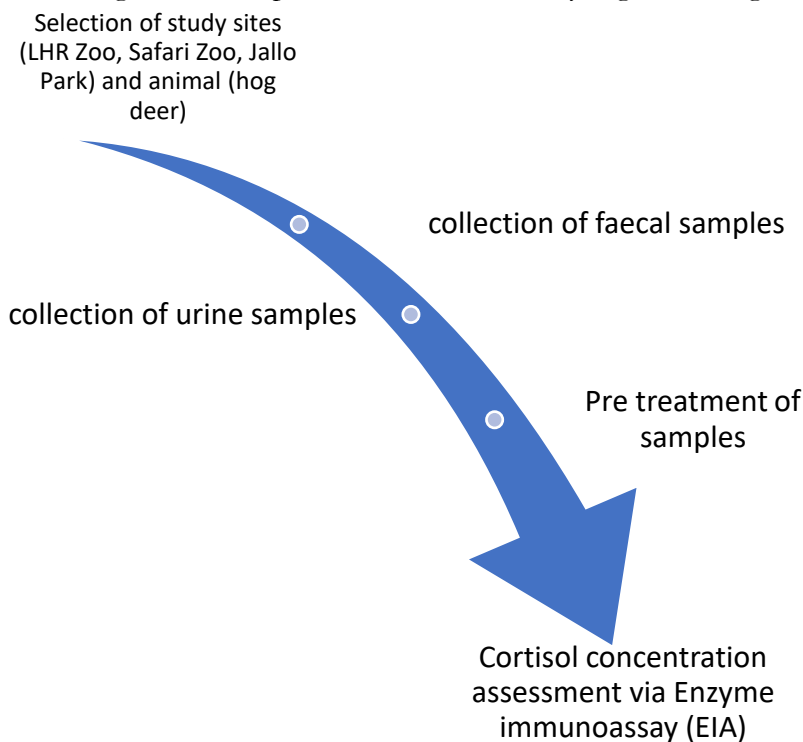
## Pre-Treatment of Urine Sample

Urine samples were centrifuged in the laboratory at 4,000 rpm for 15 minutes at room temperature. Then, at this point, the supernatant was diluted several times with assay buffer before the cortisol test and the results were within the respective standard curves (a pilot assay was used to determine the dilution ratio).

## Enzyme immunoassay (EIA)

Cortisol concentrations were assessed using a commercially available EIA kit (index # K003-H5) from Arbor Assays (Ann Arbor, MI, USA). As the manufacturer states, the cross-reactivity of cortisol antibodies is 100% used for cortisol. The coefficient of variation within the assay was 6.5% and 7.8% for high and low fixed quality control, respectively. The CV between assays was 9.3% (n = 5) and 10.2% (n = 5) for high- and low-quality controls,

respectively. The analytical protocol relied on item guidelines, but the standard for urinary cortisol analysis was 3,200, 1,600, 800, 400, 200, and 100 pg/mL, but in testing fecal samples to improve results it has been halved. Optical density was checked at 450 nm using a plate reader (DNM 9602, Beijing Pulang New Technology Co., Ltd, Beijing, China). Cortisol levels were determined using a 4-parameter online logistic curve fitting project provided by the manufacturer. A diagrammatical presentation of the study is given in Figure. 1.



**Figure. 1. Representation of data in the form of a flow chart**

**Statistical Analysis**

SPSS 22. A version was used to perform all statistical analyses. Independent sample t-test, correlation, and one-way ANOVA were applied to all collected data.

**Results**

In this study, we investigated faecal and urinary cortisol levels in hog deer exposed to varying environmental exposures. Overall, a higher concentration of fecal and urine cortisol levels was recorded in hog deer of Jallo Park as compared to Lahore Zoo and Safari Zoo. The concentration of faecal and urine cortisol levels was recorded minimum at Lahore Safari Zoo as compared to Lahore Zoo and Jallo Park (Table 2). It was noticed in faecal samples the cortisol levels were found higher as compared to urine samples.

Table 2. A comparison of faecal (ng/g) and urine (ng/ml) cortisol levels in hog deer at different captive sites

Types of Samples	LHR Zoo (Mean ± SD)	LHR SAF Zoo (Mean ± SD)	Jallo Park (Mean ± SD)
Faecal Sample	463.05±186.92	223.66±84.42	578.06±167.06
Urine Sample	70.55±34.21	29.40±27.05	132.37±93.30

The faecal and urine samples of Hog deer were also analyzed by one-way ANOVA and statistically significant differences were noticed at all three captive sites at (p = .008) for faecal and urine cortisol levels of hog deer at varying sites.

A higher concentration of fecal and urine cortisol levels was recorded in females as compared to males (Table 3).

Table 3. A comparison of cortisol levels in fecal (ng/g) and urine (ng/ml) samples of adult males and females at different study sites

Types of samples	Gender	LHR Zoo (Mean ± SD)	Safari Zoo (Mean ± SD)	Jallo Park (Mean ± SD)
Faecal Sample	Male	441.47±201.76	210.50±78.76	543.32±162.11
	Female	484.62±198.94	236.82±99.82	612.80±188.74
Urine Sample	Male	69.50±36.25	22.10±20.65	112.67±73.58
	Female	71.60±37.59	36.70±33.75	152.07±117.74

Adult males and females of hog deer had not a significant difference ( $p > 0.05$ ) for both fecal and urine cortisol levels at all three sites (Lahore Zoo, Safari Zoo, Jallo Park). The significance value was determined by using an independent t-test. The recorded value was  $p = .600$  for faecal cortisol and  $p = .53$  for urine cortisol.

All three sites surveyed (Lahore Zoo, Safari Zoo, and Jallo Park) found higher levels of cortisol in faeces and urine in the summer than in the winter (Table 4).

Table 4. Cortisol levels in male and female faecal (ng/g) and urine (ng/ml) samples in the winter and summer seasons

Types of samples	Gender	Winter (Mean ± SD)	Summer (Mean ± SD)
Faecal Sample	Male	300.43±151.07	496.43±211.13
	Female	347.95±195.66	541.55±220.85
Urine Sample	Male	35.58±27.73	100.71±65.25
	Female	35.16±27.47	138.25±92.44

Seasonal variation had a significant effect on faecal and urinary cortisol levels in both males and females at three captive sites (Lahore Zoo, Safari Zoo, and Jaro Park) ( $p < 0.05$ ). The value of significance that was determined using the independent sample t-test was  $p = 0.019$  and  $0.02$  for faecal and urine cortisol levels of hog deer during the winter and summer seasons.

Hog deer faecal and urine samples were collected in December, January, May, and June. In June, cortisol levels in faeces and urine were higher than in other months. There was also an increased concentration of cortisol recorded in May in both males and females but it was less than that of the values of June. In addition, in December, cortisol hormone levels were reduced in both males and females at Lahore Zoo, Safari, and Jallopark. Table 5 shows a detailed comparison of faecal and urinary cortisol levels in different months.

Table 5. Comparison of cortisol levels with different months at different study sites

Sites	Types of Samples	December (Mean ± SD)	January (Mean ± SD)	May (Mean ± SD)	June (Mean ± SD)
LHR Zoo	Faecal Sample	274.50±34.22	332.65±24.96	530.55±39.66	714.50±23.19
	Urine Sample	36.00±0.28	41.60±0.98	97.70±2.82	106.90±1.83
SAF Zoo	Faecal Sample	155.65±7.56	142.70±3.67	269.60±29.98	326.70±33.23
	Urine Sample	5.50±0.14	8.75±1.48	47.85±32.03	55.50±7.63
Jallo Park	Faecal Sample	587.10±103.52	452.55±27.64	452.30±9.19	820.30±56.14
	Urine Sample	72.25±12.65	48.65±8.69	149.45±51.97	259.15±63.42

ANOVA was used and statistically significant differences were found in different months from all three sites with faecal cortisol at  $p = 0.032$  and urinary cortisol levels at  $p = 0.013$ .

## Discussion

Faecal and urine samples of hog deer from Safari Zoo, Lahore Zoo, and Jallo Park were collected and analyzed for cortisol levels. Results of our findings showed, that there were higher cortisol levels in Lahore Zoo and Jallo Park as compared to Safari Zoo. It was noticed that each site has its environment that varies in respect to its physical, social, ecological, and behavioral properties. Several animals showed behavioral change against different practices that results in and around captivity and may result in higher cortisol levels[15]. This means all of these conditions had a significant impact on captive hog deer. It was noticed during the study the captive environment of Lahore Safari Zoo was comparatively better and provided with more enriched enclosures as compared to other sites. So, enriched enclosures at Lahore Safari Zoo might be a reason for the decreased cortisol levels. Environmental enhancement is a common way to improve zoo animal welfare. Enrichment is a simple and practical change in the captive sites where animals are provided in a way that produced highly valuable effects on their behavior and physiology[16]. Providing hidden food and manipulable objects to bears has been found to improve behavioral activity and exploration[17]. In one study, it was observed that providing walrus with a realistic foraging opportunity, that is, looking for food between concrete blocks, significantly reduced the amount of stereotyped swimming.

As the results of this study show, the sex of hog deer did not significantly affect faecal and urinary cortisol levels. Adult males and females of species showed relatively similar faecal and urinary cortisol levels irrespective of different sites or seasons (winter or summer). This might be attributed to the exposure of both males and females to similar environmental conditions and activities throughout the study period. It is also reported that species that received similar treatments and stress levels did not show variation in faecal and urine cortisol levels[18]. Additionally, coexistence between captive male and female hog deer in the same enclosure during the non-breeding season might be led to a lack of variation in faecal and urine cortisol levels in both genders during the study period. The findings of this study were supported by that of [18], [19] who reported that adult male and female reindeer and red deer did not have a significant difference in faecal and urine cortisol levels in the studies conducted in Alaska and Austria. Similarly, findings of [19] reported that male and female reindeer have similar cortisol levels during non-breeding seasons also supports the results of this study as data was collected during the non-breeding season. A study on red deer (*Cervus elaphus*), have also reported that no significant difference was noticed in cortisol levels between male and females[18].

The highest cortisol levels were found in summer due to long photoperiods and high temperatures, and the low levels found in winter due to short photoperiods and low temperatures were found in hog deer. Monthly fluctuations in faecal and urinary cortisol levels were recorded, with the highest recorded in May and June, especially in June under extreme summer conditions in Pakistan. Several past and present investigations explained the outcomes of our study. In ruminant species, occasional or photoperiodic variety of cortisol levels has been found in some however not in all studied animals[20]. The variations in cortisol levels also depend on the age and sex of the living beings or the social interaction of different animals[21].

The photoperiod may not have a specific effect on cortisol release. However, fluctuations in "cortisol levels" may be associated with the control of fertility by photoperiod. This has been analyzed in a study conducted on sheep. There, a pineal

resection was performed to stop the progression of prolactin and testosterone, which suppressed changes in cortisol levels mediated by photoperiodism[22].

The results of this study showed that temperature and humidity significantly affect the faecal and urine cortisol level of hog deer. An increase in temperature results in enhanced faecal and urinary cortisol levels in hog deer. The positive relationship reported in this study could be due to seasonal variation in response to parameters such as maximum temperature and heat stress[23]. In this study, heat stress and ambient temperature were some of the factors leading to significant effects on the faecal and urine cortisol levels in the hog deer during summer. The observed results might be due to drastic changes in weather conditions within a short period which can interfere with adrenocortical activity and also reported by[24]. The adrenocortical hormones were known to help in the daily regulation of the physiological status of the body[23]. The changes in adrenocortical activity result in an increased production of faecal and urine cortisol which could be explained by the concept reported by[25] that environmental factors are stressors for Zoo animals and affects them by altering their adrenocortical hormones.

Besides, the elevated concentration of faecal cortisol may be attributed to the process of adapting to harsh environmental conditions especially the hot weather during summer[19]. The observation was very similar to our findings as higher cortisol levels were recorded in hog deer which may be due to the warm and harsh environment of Pakistan. However, the increased levels of faecal cortisol in hog deer during hot seasons could also be due to the shift in body metabolism as reported by[23]. Several vertebrate species modulate faecal cortisol concentrations seasonally [18]with higher levels during adverse weather conditions[26]. The results of our study were in line with that of [18], [19], [27] who reported that the drastic change in weather conditions effects on the production of faecal cortisol.

This is the first study of faecal and urine cortisol concentration on hog deer at Safari Zoo, Lahore Zoo, and Jallo Park as affected by temperature, humidity, and wind Speed. Similar variations in faecal cortisol concentration being significant during the summer season have been reported on mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and reindeer (*Rangifer tarandus*). Other studies however have reported these results, including a study conducted on North American elk (*Cervus elaphus*) who reported a significant change in faecal cortisol concentration in summer and not in winter.

There was a measurably huge distinction in the mean of faecal and urine cortisol levels of hog deer in our examination. The observed results may be because it was impractical to collect all the urine produced by an individual 24 hours a day. A total of one sample was taken each day. The amount of urine produced per day is not constant, and the concentration of all solutes depends on the amount of water and mineral salts sifted by the kidney. Faecal cortisol levels were high because the steroid metabolites found in the intestine were carried with the ingested substances, the passage speed from the duodenum to the rectum can give an estimate of the time they appear in feces. In faecal samples, there is no impurity record, as creatinine is present in urine.

Non-invasive measurements of glucocorticoid concentrations, including cortisol and corticosterone, serve as reliable indicators of adrenocortical activities and physiological stress loads in a variety of species[28]. This non-invasive technique of glucocorticoid assessment is very attractive allowing sample collection for research that does not stress animals,

particularly the endangered ones[29]. There is a constant practice of faecal samples collection of wild animals and later use of these samples to monitor glucocorticoid metabolites[30].

### Conclusion.

The study concluded that non-invasive methods could study cortisol levels in captive animals and that both faecal and urine samples have the potential to monitor cortisol levels in hog deer. As higher levels of cortisol were measured at Jallopark and Lahore Zoo compared to Safari Zoo, that might be due to reason of availability of more attention and resources as compared to Jallopark. Higher levels of cortisol have been found in females compared to men. In addition, higher values were recorded in summer compared to seasonal fluctuations in winter.

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