Research Article

Assessment of Cortisol Levels in Captive Blackbucks (*Antilope cervicapra*)

Mubashar Ahmad, Roheela Yasmeen*, Aisha Waheed Qurashi, Muhammad Hassan Ali Shah

Department of Biology, Lahore Garrison University, Sector C, Phase VI, DHA, Lahore.

Abstract | Wildlife is decreasing due to loss of habitat, hunting, killing, poaching and availability of contaminated food. To save animals formation of Zoos is years old practice. The animals are placed in captivity for recreation, education and economic benefits. The physical needs of animals are even met in captivity however the conditions of confinement and exposure to humans can result in physiological stress. The stress response consists of the suite of hormonal and physiological reactions which are helpful in an animal's survival. Cortisol levels are frequently used to determine the stress in animals. The elevation in cortisol levels are associated with increased stress levels. The present study was aimed to find the reliability of the faecal and urine samples for the determination of the stress levels in animals. A non-invasive technique was used to measure the cortisol levels. Urine and faecal samples of male and female blackbucks (*Antilope cervicapra*) were collected from Lahore Zoo, Safari Zoo and Jallo Zoo in winter and summer season to determine the stress levels. Both faecal and urine samples were prepared and further analyzed by radioimmunoassay (RIA) kit to determine the faecal and urine cortisol levels. Higher concentrations of cortisol were recorded in both males and females of blackbuck in the Jallo Park and Lahore Zoo as compared to Safari Zoo. Moreover, higher cortisol levels were recorded in the blackbuck in summer season as compared to winter season. Results of ANOVA showed significant differences in cortisol levels among different sites at <0.05 p-value. Temperature was positively correlated with the cortisol levels. While humidity levels were negatively correlated with the faecal and urine cortisol concentration in blackbuck. It was concluded that the faecal and urine samples can be used to determine the stress levels in captive blackbuck (*Antilope cervicapra*).

Keywords | Cortisol, captive sites, seasons, radioimmunoassay (RIA), blackbuck

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***Correspondence** | Roheela Yasmeen, Department of Biology, Lahore Garrison University, Sector C, Phase VI, DHA, Lahore; **Email:** roheelayasmeen@lgu.edu.pk
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INTRODUCTION

Wild animals inhabit different Earth's ecosystems such as grasslands, forests, plains and deserts. Although these animals live in their natural environment, however, literature showed a global decline in wildlife is due to disturbances of natural ecosystems and increased anthropogenic activities. Continued loss of these species in the wild has accelerated the need for wildlife management outside their natural environment in captivity as an effort for conservation (Russello and Jensen, 2018).

Zoos play a significant role in the conservation and protection of different species through captive breeding, education and research (Hutchins et al., 2003). However, many Zoos all over the world are not able to stimulate the captive surroundings to mimic the extensive wild habitat of the species that are kept in captivity (Mellor et al., 2018).

Approximately six billion wild animals including about 10,000 species all around the world have been kept in captive Zoological Parks and breeding centers for their conservation (Mason, 2010). It is reported that animals even in captivity are not safe as in these environments, animals are exposed to a variety of external and internal stressors such as visitors's impact and abiotic factors (Hosey, 2005). Stress in large sense has great impact on wild animals particularly captive animals that produced diverse effects on reproduction, immune system and behavior of animals. Physiological assessment of stress parameters is necessary to improve the health and reproduction of animals. Stress in large sense has great impact on different types of body functions. It has diverse effects on reproduction, immune system and behavior of vertebrates (Von Holst, 1998).

Scientists showed great interest in hormones in animals because hormones are involved in all body functions like development, homeostasis, reproduction and behavioral expression. Large number of endocrine factors are directly interlinked to the genetics, environment and social changes that animals face during their lifetime which leads to the stimulation of adrenal and gonadal steroids, growth factors, pituitary peptides and other amines. Particularly these hormones are called stress hormones (Touma and Plam, 2005). A stressor hormone facing by an individual usually showed a stress response like behavioral and physiological changes to cope with these hormonal changes. One of the main negotiators of this stress response is the hypothalamic-pituitary-adrenal. These are not only confined to stress response but also many other responses like emotional arousal of sex and courtship behavior (Spolasky et al., 2000). After perceiving stress, the adrenal cortex starts to secrete glucocorticoids (GC) within a few minutes. These glucocorticoids mainly contain cortisol or corticosterone (Romero, 2004). The main GC (glucocorticoid) in primates, ungulates and carnivores is cortisol. Cortisol is responsible for the organism's response due to its effect on different organs like brain to harmonize behavior and physiology (Palme, 2005).

The noninvasive methods are used to investigate reproductive activities by observing behavior, and monitoring faecal and urine steroids like cortisol in blackbucks. A complete hormonal profile of a captive species can be used for better communication and reproductive management of blackbucks in captivity (Schwarzenberger, 2007).

The blackbucks (*Antilope cervicapra*) are antelope. These are elegant gazelle-like animals which are native to South-East Asia and are known as the most attractive members of the order Artiodactyla. Mature male blackbucks have a black and white color which is very different from the reddish yellow hue of immature males and females (Jhala and Isvaran, 2016). Several studies reported that the loss of habitat, killing, hunting, contaminated food and poaching creates massive ruckus in habitable zones of the blackbuck (Meena et al., 2017). All these factors are responsible for the disturbance of the blackbuck's population in various parts of the world. Animals are now kept in captivity to breed and to increase their population (Aleem, 1978; Chaudhary and Maharjan, 2017; Bist et al., 2021).

In the Antilopini family, there is only one alive and stout species that is blackbuck (*Antilope cervicapra*) which have spirally horned and are considered as the sole living species of the late Miocene (Gentry, 2003). These animals are listed as vulnerable in the IUCN red list according to report of June 2016 (Chaudhary and Maharjan, 2019). This antelope is native to and found mainly in India, while it is locally extinct in Pakistan and Bangladesh. In Pakistan now it is only available in captivity.

The study was aimed to determine the seasonal effect (temperature, humidity) on cortisol levels by investigating the faecal and urine samples in captive male and female blackbuck (*Antilope cervicapra*) at Lahore Zoo, Safari Park and Jallo Park Lahore.

MATERIALS AND METHODS

Study area

The considered study sites were Lahore Zoo, Lahore Safari Zoo and Jallo Park Lahore (Figure 1). All three sites were provided with various wildlife species such as bear, sambar, woofing deer, Nilgai, chinkara, elephant, lion, fox, jackal, wolf, Indian gaur, tiger, panther, spotted deer, hoard deer and blackbuck, among a couple of others.

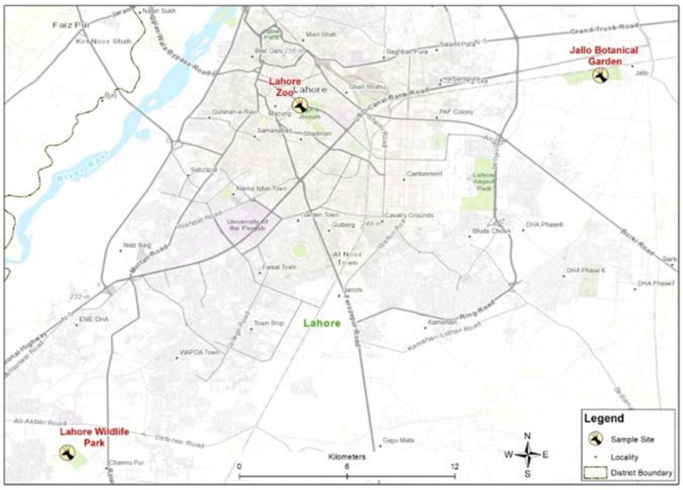

Figure 1: A map showing the location of Lahore Zoo, Safari Zoo and Jallo Park

Selection of animals

One antelope species of order Artiodactyla blackbuck was selected for the present study from all three visited sites. At the time of study, there were total 17 blackbucks at the

Lahore Zoo. Three of them were male, 12 were females, and rest were juveniles. In Safari Zoo, there were total 18 blackbucks, 8 of them were males and rest were females. In Jallo Zoo there were total of 20 blackbucks three of them were males and rest of them were females. The three selected study sites were different in its environmental exposure to animals as in Lahore zoo there are only blackbucks in their cage while, Lahore Safari is more natural with different deer species kept at one place and Jallo park was open but neglected site.

Study design

Samples of four months and two definite seasons: winter and summer were considered. A total 48 urine and faecal samples were collected from both male and female blackbucks during the study period (May and June 2020 (summer season); December 2020 and January 2021 (winter season). General observations such as temperature, humidity were also made at the time of visit and collection of samples regarding visitor's presence on each site.

Sample collection and processing

A total of 48 samples, 24 of faecal and 24 of urine samples were gathered during the study period (four month). Six urine and faecal samples both from male and female blackbucks were collected once in each month (December, January, May and June) from three visited sites. A pilot study was done to understand the defecation pattern and for the gender-wise differentiation of faecal material in blackbucks. The faecal and urine samples were collected in morning on second Sunday of each month. Samples were immediately collected after defecation and urination and stored aseptically within two 2 hours. Faecal samples were collected into plastic zipper bags that were closed firmly and stuffed into cool boxes containing ice packs. Samples were properly labelled according to visited study site, gender and date of collection. By following, samples were transported from the enclosures to the lab and at that point kept under - 20°C refrigerator.

Preparation of faecal samples

Faecal samples were treated as the method described by Wasser et al. (2004), and Fanson et al. (2017). Took 0.5 g of wet faeces from the sample into 1.5 mL centrifuge tubes containing 5 mL of ethanol (80%). Samples were placed on a shaker (Add Model) for 30 min and centrifuged at 2000 rpm for ten minutes. Then 2 ml polypropylene was added to supernatant and prepared samples were stored at -20 °C for Immunoassay.

Preparation of urine sample

Before to applying Immunoassay the urine samples were centrifuged at 4,000 rpm for 15 min in the laboratory. Then the supernatant was separated and was diluted 400 times with buffer assay prior to cortisol assay.

Radioimmunoassay (RIAs)

Faecal and urine samples were prepared and processed for radioimmunoassay kit in vet and pet laboratory DHA, Lahore (Calbiotec Cortisol Elisa kit). RIAs have few advantages, such as high precision, stoutness, and a long lasting in act.

Statistical Analysis

A software SPSS version 21.00 was used to perform all statistical analyses. Levene's test was used to test normality of data when variances were not found equal then data was normalized and later on ANOVA a collective data of blackbucks was used to determine differences in cortisol levels among different visited sites. While, Independent sample T test was used to see cortisol levels differences in gender and season wise. Correlation was used to determine the relationship between temperature, humidity, faecal and urinary cortisol levels. The level of significance was determined at $p \leq 0.05$.

RESULTS

In the current studies total of 48 urine and faecal samples were collected at three different sites i.e., Safari, Jallo Park Zoo and Lahore Zoo per month. Faecal and urine samples were collected both from male and female blackbucks.

Levels of cortisol in faecal and urine samples of blackbuck were studied for Lahore Zoo, Safari Zoo and Jallo Park. A higher cortisol levels were recorded in the faecal samples for both the male and female blackbucks at Jallo Park as compared to Lahore and Safari Zoo (Table 1). ANOVA was applied and statistically significant differences were noticed in the mean cortisol levels of faecal ($p = 0.01$) and urine ($p = 0.03$) samples of blackbuck at varying environmental exposures. An overall higher levels were recorded for females from all studied sites as compared to male blackbucks (Table 1). Independent sample t test showed male and female cortisol levels were significantly different for both faecal and urine cortisol levels ($p=0.031$).

Abbreviations: n= number of samples per sex

The comparison of faecal and urine cortisol levels was made with winter and summer seasons (Table 2). Overall a higher concentrations of cortisol levels were recorded in summer as compared to winter at all the studied sites (Lahore Zoo, Safari Zoo, Jallo Park).

A detailed comparison of faecal and urine cortisol levels according to different months have shown in Table 3. Overall a higher concentrations of cortisol levels were recorded in the month of June as compared to other months. Moreover, a decreased levels of cortisol were recorded in

Table 1: Faecal cortisol ng/g and Urine cortisol µg/ml levels in blackbucks at three different sites (Lahore Zoo, Safari Zoo, Jallo Park)

Samples	Lahore Zoo		Safari Zoo		Jallo Park	
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Faecal cortisol	362.67±49.19	434.45±117.50	276.07±50.30	301.47±53.91	473.17±144.03	483.10±196.60
Urine cortisol	153.37±47.89	166.52±52.01	106.20±26.66	157.37±167.00	167.97±112.40	217.80±127.14

December at Lahore Zoo, Safari and Jallo Park in both males and females.

Table 2: Seasonal faecal cortisol ng/g and urine cortisol levels µg/mg in blackbucks

Samples	Winter (Mean±SD)	Summer (Mean±SD)
Faecal cortisol	278.64±83.87	513.88±136.69
Urine cortisol	38.84±8.70	159.10±45.18

Faecal and urine cortisol levels of blackbuck in different months such as December, January, May and June were compared. It was noticed faecal and urine cortisol levels of December and January, and May and June were not significantly different from each other. However, cortisol levels of December and January were significantly different at p value 0.00 from cortisol levels of May and June for both faecal and urine cortisol levels.

Table 3: Month-wise cortisol levels in Faecal and Urine samples of blackbucks

Sample	December	January	May	June
	(Mean ±SD)	(Mean ±SD)	(Mean ±SD)	(Mean ±SD)
Faecal cortisol (ng/g)	331.18 ±74.14	349.88 ±140.53	364.85 ±58.59	508.05 ±166.41
Urine cortisol (µg/mg)	153.20 ±158.15	114.83 ±41.28	131.26 ±56.47	228.20 ±62.38

Table 4: Environmental data used to correlate faecal and urine cortisol levels in blackbucks

Months	Temperature °C	Relative Humidity
December	7-10°C	92-100%
January	11-13°C	75-83%
May	28-32°C	52-54%
June	38-41°C	56-58%

Effect of temperature, and humidity on faecal and urine cortisol levels in blackbucks

During sample collection abiotic factors such as temperature, and humidity were also monitored (Table 4) and later on statistically analyzed for the correlation. There was a weak but positive correlation between faecal (p-value=0.244) and urine cortisol levels (p-value=0.373) with temperature. However, a significant negative correlation was noticed between faecal (p-value = -0.737) and urine cortisol levels (p-value= -0.737) with the humidity.

DISCUSSION

In present study faecal and urine cortisol levels of blackbuck were determined at three captive facilities Lahore Zoo, Lahore Safari Zoo, and Jallo Park. Higher cortisol levels were recorded in Jallo Park followed by Lahore and Lahore Safari Zoo (Table 1). A higher levels at Lahore Zoo and Jallo Park in blackbucks might be due to the stress which was caused by the restricted enclosure size. Moreover, an improper management system was recorded for Jallo Park that was also reported by Van Waeyenberge et al. (2018). A number of literature studies also showed that enclosure size is very much important for the health, and bodyweight of the captive animals (Terranova and Coffman, 1997; Hutchins and Kreger, 2006; Marinath et al., 2019). Van Waeyenberge et al. (2018) reported on feed stress in snakes that were kept in captivity. An overall higher cortisol levels were recorded in both male and female blackbucks as compared to levels that reported by Nikhil (2020).

In present study cortisol levels were compared between male and female for urine and faecal samples of blackbuck and higher levels were recorded for females. However, Bubenik et al. (1998) and Huber et al. (2003) explained in their studies that adult male and female reindeer and blackbucks did not have significant difference in faecal and urinary cortisol concentration by exposure of both males and females to similar environmental conditions and activities throughout the study period. It can then be inferred that species received similar treatments and stress levels which did not lead to variation of faecal and urinary cortisol. A higher cortisol levels were found in females as compared to males in present findings and similar results were reported in musk deer by He et al. (2014). While, a higher cortisol levels in males as compared to females were reported by Rangel-Negrin et al. (2009). The observed results could be due to the fact that the collection of all urine produced by an animal during the full 24 hours in a day was difficult and impractical in general one sample a day was obtained. The amount of urine produced in one day is not constant and the concentration of any solute present in it is modified according to the amount of water and inorganic salts filtered through the kidney. About 1% of this circulating cortisol is excreted in urine without being any change in it. This excreted volume of cortisol is known as the urinary cortisol. This reflects the non-protein bounded cortisol. The catabolic breakdown takes place in liver and cortisol is excreted into the urine. Urine cortisol have short lag phase as compared to faecal cortisol levels. Faecal cortisol levels were high because steroid that were found in the intestine are transported with the ingesta, the passage speed from the duodenum to the rectum can give an estimate of the time they appear in feces (Wasser, 2004).

A number of studies have described about the cortisol levels which was recorded higher in summer months as compared to the winter (Leining et al., 1980; Kennaway et al., 1981; Lincoln et al., 1982; Feher et al., 1994; Ingram et al., 1999; Monfort et al., 2004; Rangel-Negrin et al., 2009, Nikhil 2020). It means cortisol levels increased in captive animals by an increase of temperature. It is quite possible, however, that the photoperiod has no particular effects on the regulation of cortisol secretion. Instead, the variations of the "cortisol set-point" in the hypothalamic feed-back regulatory system may be associated with the prominent control of reproductive functions by the photoperiod. This view is supported by the finding that in lambs, pineal ectopy prevented the photoperiod-induced changes in cortisol levels, as it prevents the changes of prolactin and testosterone (Brinklow and Forbes, 1984).

In this study it was noticed that summer season have great impact on faecal and urine cortisol levels while effect of winter season on cortisol levels were reported as compared to October month heat in blackbucks by Nikhil (2020). Faecal and urinary cortisol levels of blackbucks are greatly affected by temperature, and humidity at Lahore Zoo, Safari Zoo and Jallo Park. Similar was reported for winter season by Nikhil (2020). Moreover, the humidity increased and the cortisol concentration decreased. This decrease in urine and faecal cortisol concentration might be due the production of more urine and decrease in reabsorption of water from large intestine due to much availability of humidity in air. So, an increased in relative humidity leads to decrease in cortisol concentration (Hodges, 1996).

CONCLUSION

It was concluded that variation in environmental exposure at three captive facilities had significant impact on faecal and urine cortisol levels. A higher concentrations of cortisol levels were recorded in the Jallo park and Lahore Zoo as compared to Safari Zoo. However, overall an increased cortisol levels were noticed in females as compared to males. Seasonal variations have great impact on the faecal and urine cortisol levels as higher levels were recorded for summer as compared to winter. Moreover, non-invasive methods have potential to study cortisol levels in captive animals although, faecal samples showed higher cortisol levels as compared to urine sample.

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

NOVELTY STATEMENT

Study is novel as no data on cortisol levels of blackbuck was reported from different captive sites of Lahore Pakistan.

AUTHORS CONTRIBUTION

Mubashar Ahmad: M.Phil student who did research work Roheela Yasmeen: Supervisor, plan of study, write up of manuscript, corresponding author. Aisha Waheed Qurashi: Co-supervisor Muhammad Hassan Ali Shah: Facilitate in collection of samples

REFFRENCES

Aleem A (1978). Re-introduction of blackbuck in Pakistan. Pak. J. Forest. 28: 111-115.

Bist BS, Ghimire P, Poudyal LP, Pokharel CP, Sharma P, Pathak K (2021). From extinction to recovery: The case of blackbuck *Antilope cervicapra* from Nepal. Mammal. Res. 1-5. https://doi.org/10.1007/s13364-021-00576-5

Brinklow BR, Forbes JM (1984). Effect of pinealectomy on the plasma concentrations of prolactin, cortisol and testosterone in sheep in short and skeleton long photoperiods. J. Endocrinol. 100(3): 287-294.

Bubenik GA, Schams D, White RG, Rowell J, Blake J, Baztos L (1998). Seasonal levels of metabolic hormones and substrates in male and female reindeer (*Rangifer tarandus*). Comp. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol. 120(2): 307-315. https://doi.org/10.1016/S0742-8413(98)10010-5

Chaudhary RB, Maharjan M (2019) 'Parasitic infection in blackbuck (*Antilope cervicapra*) of Blackbuck Conservation Area, Bardiya and Shuklaphanta Wildlife Reserve.

Chaudhary RB, Maharjan M (2017). Parasitic infection in blackbuck (*Antilope cervicapra Linnaeus*, 1758) of Blackbuck Conservation Area, Bardiya and Shuklaphanta Wildlife Reserve, Kanchanpur, Western Nepal. Nepal. J. Environ. Sci. 5: 9-17. https://doi.org/10.3126/njes.v5i0.22710

Fanson KV, Best EC, Bunce A, Fanson BG, Hogan LA, Keeley T, Narayan EJ, Palme R, Parrott ML, Sharp TM, Skogvold K (2017). One size does not fit all: monitoring faecal glucocorticoid metabolites in marsupials. Gen. Comp. Endocrinol. 244: 146-156. https://doi.org/10.1016/j.ygcen.2015.10.011

Feher T, Zomborszky Z, Sandor E (1994). Dehydroepiandrosterone, dehydroepiandrosterone sulphate, and their relation to cortisol in red deer (Cervus elaphus). Comparative Biochemistry and Physiology Part C: Pharmacol. Toxicol. Endocrinol., 109(3), 247-252.

Gentry A, Clutton-Brock J, Groves CP (2003). The naming of wild species and their domestic derivatives. J. Archaeol. Sci. 31:645–651. https://doi.org/10.1016/j.jas.2003.10.006

He L, Wang WX, Li L, Liu BQ, Liu G, Liu SQ, Hu DF (2014). Effects of crowding and sex on fecal cortisol levels of captive forest musk deer. Biol. Res. 47(1): 1-6. https://doi.org/10.1186/0717-6287-47-48

Hodges K (1996). Comparative aspects of the metabolism and excretion of cortisol in three individual nonhuman primates. Gen. Comp. Endocrinol. 117: 427–438. https://doi.org/10.1006/gcen.1999.7431

Hosey GR (2005). How does the zoo environment affect the behaviour of captive primates? Appl. Anim. Behav. Sci. 90(2): 107-129. https://doi.org/10.1016/j.applanim.2004.08.015

Huber S, Palme R, Arnold W (2003). Effects of season, sex, and sample collection on concentrations of faecal cortisol metabolites in red deer (*Cervus elaphus*). Gen. Comp. Endocrinol. 130(1): 48-54. https://doi.org/10.1016/S0016-6480(02)00535-X

Hutchins M, Kreger MD (2006). Rhinoceros behaviour: implications for captive management and conservation. International Zoo Yearbook. 40(1): 150-173. https://doi.org/10.1111/j.1748-1090.2006.00150.x

Hutchins M, Smith B, Allard R (2003). In defense of zoos and aquariums: the ethical basis for keeping wild animals in captivity. J. Am. Vet. Med. https://doi.org/10.2460/javma.2003.223.958-2

Ingram JR (1999). Measurement of stress. In: Moberg GP, Mench JA, editors. The biology of animal stress. CABI Publishing,. p. 123–46

Jhala YV, Isvaran K (2016) 'The Ecology of Large Herbivores in South and Southeast Asia', 225. https://doi.org/10.1007/978-94-017-7570

Kennaway DJ, Obst JM, Dunstan EA, Friesen HG (1981). Ultradian and seasonal rhythms in plasma gonadotropins, prolactin, cortisol, and testosterone in pinealectomized rams. Endocrinology,. 108(2): 639-646.

Leining H, Allen Tucker JS, Kesner (1980). Growth Hormone, Glucocorticoid and Thryoxine Response to Duration, Intensity and Wavelength of Light in Prepubertal Bulls, J. Anim. Sci. 51(4): 932–942. https://doi.org/10.2527/jas1980.514932x

Lincoln GA, Almeida OFX, Klandorf H, Cunningham RA (1982). Hourly fluctuations in the blood levels of melatonin, prolactin, luteinizing hormone, follicle-stimulating hormone, testosterone, tri-iodothyronine, thyroxine and cortisol in rams under artificial photoperiods, and the effects of cranial sympathectomy. J. Endocrinol., 92(2): 237-250.

Marinath L, Vaz J, Kumar D, Thiyagesan K, Baskaran N (2019). Drivers of stereotypic behaviour and physiological stress among captive jungle cat (*Felis chaus* Schreber, 1777) in India. Physiol. Behav. 210: 112651. https://doi.org/10.1016/j.physbeh.2019.112651

Mason GJ (2010). Species differences in responses to captivity: stress, welfare and the comparative method. Trends. Ecol. Evol. 25(12): 713-721. https://doi.org/10.1016/j.tree.2010.08.011

Meena R, Saran RP, Chourasia V (2017). Assessment of threats to blackbuck, *Antilope cervicapra* (Linn) in sorsan grassland, Rajasthan, India. Int. J. Zool. Stud. 2(6): 194-198.

Mellor E, McDonald Kinkaid H, Mason G (2018). Phylogenetic comparative methods: Harnessing the power of species diversity to investigate welfare issues in captive wild animals. Zoo Biol. 37(5): 369-388. https://doi.org/10.1002/zoo.21427

Monfort SL, Wildt DE (2004). Rapid extraction of faecal steroids for measuring reproductive cyclicity and early pregnancy in free-ranging yellow baboons (*Papio cynocephalus cynocephalus*). Reproduction. 92(2): 415-423. https://doi.org/10.1530/jrf.0.0920415

Nikhil SB (2020). Faecal Cortisol Metabolites as an Indicator of Stress in Captive Spotted Deer (*Axis Axis*) and Blackbuck (*Antilope cervicapra*) in India (Doctoral dissertation, University of Nairobi).

Palme R (2005). Measuring faecal steroids: guidelines for practical application. Ann. N.Y. Acad. Sci. 1046: 75–80. https://doi.org/10.1196/annals.1343.007

Rangel-Negrín A, Alfaro JL, Valdez RA, Romano MC, Serio-Silva JC (2009). Stress in Yucatan spider monkeys: effects of environmental conditions on fecal cortisol levels in wild and captive populations. Anim. Conserv. 12(5): 496-502. https://doi.org/10.1111/j.1469-1795.2009.00280.x

Romero LM (2004). Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19: 249–255. o

Russello MA, Jensen EL (2018). Ex Situ Wildlife Conservation in the Age of Population Genomics. https://doi.org/10.1007/13836_2018_44

Sapolsky RM, Romero LM, Munck AU (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparativeactions. Endocr Rev 21: 55–89. Smith TE, French JA. 1997. Psychosocial stress and urinary cortisol excretion in marmoset monkeys (*Callithrix kuhli*). Physiol. Behav. 62:225–232. https://doi.org/10.1016/S0031-9384(97)00103-0

Schwarzenberger F (2007). The many uses of non- invasive faecal steroid monitoring in zoo and wildlife species. Int. Zoo Yearbook. 41(1), pp.52-74. https://doi.org/10.1111/j.1748-1090.2007.00017.x

Terranova CJ, Coffman BS (1997). Body weights of wild and captive lemurs. Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association. 16(1): 17-30. https://doi.org/10.1002/(SICI)1098-2361(1997)16:1%3C17::AID-ZOO4%3E3.0.CO;2-E

Touma C, Palme R (2005). Measuring faecal glucocorticoid metabolites in mammals and birds: the importance of validation. Annal. NY. Acad. Sci. 1046(1): 54-74. https://doi.org/10.1196/annals.1343.006

Van Waeyenberge J, Aerts J, Hellebuyck T, Pasmans F, Martel A (2018). Stress in wild and captive snakes: quantification, effects and the importance of management. Vlaams Diergeneeskundig Tijdschrift. 87(2): 59-65. https://doi.

org/10.21825/vdt.v87i2.16082

Von Holst D (1998). The concept of stress and its relevance for animal behavior. Adv. Study Behav. 27: 1–131. https://doi.org/10.1016/S0065-3454(08)60362-9

Wasser SK, Monfort SL Wildt DE (2004). Rapid extraction of faecal steroids for measuring reproductive cyclicity and early pregnancy in free-ranging yellow baboons (*Papio cynocephalus cynocephalus*). Reprod. 92(2): 415-423. https://doi.org/10.1530/jrf.0.0920415