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# Non-Invasive Monitoring of Stress in Large Captive Herbivores: A Review

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***Abstract:*** ***A potential indicator of animal welfare is the absence of stress. The unpredictable components of life result in changes in the endocrine and metabolic status of an organism. The numbers of mega vertebrates are declining rapidly in their native habitat, therefore, the demand for more complete measures of the impact of conservation interventions and the effects of captive environments on animal health and welfare has risen. Measuring an individual's physiological arousal provides a window into how that individual animal is coping with its environment, whether in the wild or captivity. Non-invasive monitoring of Fecal glucocorticoid metabolites (FGM) has become a popular measure of physiological function and welfare in a variety of species because they reflect adrenocortical activity over a certain time period and can therefore be used to answer a wide variety of research questions. The present study reviews the role of FGM as a stress indicator in large captive herbivores by measuring fecal glucocorticoid metabolites (FGM) and suggests measures to bring back these animals from the bark of extinction.***
***Keywords: Non-invasive monitoring, fecal glucocorticoid metabolites (FGM), Conservation, Stress, captive***

## I. INTRODUCTION

Scientists internationally argue that the 6th phase of mass extinction has already begun, threatening mammals, birds, reptiles, amphibians, fish, invertebrates, plants, and ultimately, humankind. Especially for our large terrestrial mammals in Asia and Africa, the future looks bleak[1]. For example, the overall population of giraffes (Giraffa camelopardalis spp.) has plummeted by 40% during the past 30 years over the entire African range[2]. The Asian rhino species, especially, show significantly low numbers. There are probably only 80 Sumatran rhinos(Dicerorhinus sumatrensis), around 70 javan rhinos(Rhinoceros sondaicus), and 3900 Indian rhinos (Rhinoceros unicornis) left in the wild[3]. As a result, captive populations are regularly supplemented with wild ones, adding further pressure on the struggling wild population. The understanding of endocrine activity provides unparalleled insight into animal and species biology because hormones affect all tissues in the body.

The benefits of a noninvasive approach to endocrinology have been reviewed previously for avian(Goymann, 2005), amphibian(Narayan, 2013), and mammalian(Hodges et al., 2010)species. The major purpose of this review is to highlight the role of non-invasive measurement of stress hormone by measuring fecal glucocorticoid metabolites(FGM) and to highlight the application of these approaches in the research of mammals threatened with extinction. The attempt is to study the stress hormone and its management in large captive herbivores, especially, giraffe, and Asian elephants with the help of published studies, standard methods, and data or materials that supports the potential application of the approach to threatened species research.

Stress- a physiological mechanism There is no standard definition of stress and no single biochemical assay system to measure stress[4]. The discovery of metabolic, immunological, and neuroendocrine mechanisms makes it possible to describe the stress reaction in physiological terms[5]. Hormones like ACTH, glucocorticoid, catecholamines, prolactin, etc. are involved in the stress response. The adrenal glands have a key role in hormonal reactions to stress as they are involved both in the hypothalamic-pituitary-adrenocortical axis and the sympathetic-adreno-medullary system[6].

Stress is not inherently bad. For example, glucocorticoids are released in response to situations that are normally regarded as stressful, including courtship, copulation, and hunting [7]. Short-term stress increases fitness as glucocorticoids improve energy mobilization and may change behavior [8]. However, prolonged periods of high cortisol may decrease individual fitness by immunosuppression and atrophy of tissues [9].

### *A. Hormones as indicators of stress*

Animal welfare is of increasing importance and the absence of chronic stress is one of its prerequisites. During stress, various endocrine responses are involved in improving the fitness of an individual. Glucocorticoids and catecholamines are the two front-line hormones to overcome a stressful situation. These hormones are parameters of adrenal activity and thus disturbance.

The concentration of glucocorticoids (or their metabolites) can be measured in various body fluids or excreta. Above all, fecal samples can be easily collected and this procedure is feedback free[10] .To enable the measurement of groups of cortisol metabolites in animal feces, recently EIA(enzyme immunoassays) have been developed and successfully tested. Metabolite determination in fecal samples is one of the most practical methods to monitor glucocorticoid production.

*B. Non- invasive Hormone Analysis*

Non-invasive methods provide a more practical approach for assessing ovarian, testicular and, and more recently, adrenocortical activity in captive animals. The concentration of cortisol in the blood is widely used as an indicator of stress whereas, sample collection, which often involves confinement or handling of animals may by itself be stressful and may confound the results(Hopster al., 1999; cook et al., 2000) To overcome these problems researchers have investigated non-invasive sampling procedures such as corticoid(metabolites)in the urine(Hay and Mormede, 1998), saliva(Cooper et al., 1989) or milk(Verkerket.,1998). However, due to certain drawbacks related to each, these samples can be used to a limited extent in free-moving animals. Above all, fecal samples offer the advantage that they can be easily collected without stressing the animals. Fecal steroid metabolites of placental and gonadal origin are a well-established method to evaluate reproductive function.

Several studies have been performed to evaluate the biological relevance of fecal cortisol metabolites in captive mega vertebrates.

*C. Glucocorticoid and its Metabolism (Fig. 1)*

Basic knowledge of the metabolism and excretion of glucocorticoids is necessary for the development of non-invasive techniques to monitor adrenocortical activity. Glucocorticoids are a family of steroid hormones that are essential to life. The primary glucocorticoid is cortisol. Without the secretion of cortisol during stress, an animal could not survive. In the situation of stress, the hypothalamus sends the corticotropin-releasing hormone(CRH), a peptide of 41 amino acids, to the pituitary where the hormone ACTH(adreno-corticotropin hormone) is released into the circulatory system and delivered to the adrenal cortex's zona fasciculata. This then results in the activation of steroidogenesis which produces cortisol[15].

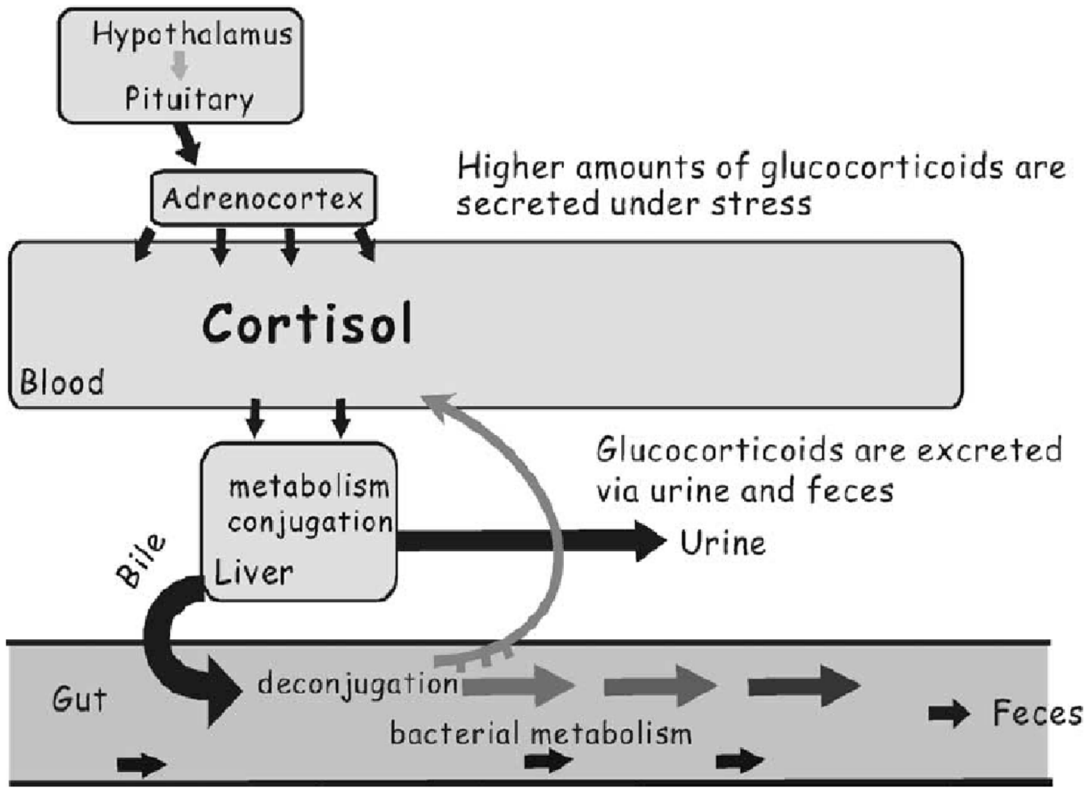


Fig. 1 scheme of secretion, metabolism, and excretion of glucocorticoid
Source; hormones as indicators of stress (E. Mostl, R. Palme) Domestic animal endocrinology 23(2002).

*D. Consequences of Stress in Animals*

As per evidence, short-term stress is not at all bad for the animal's health. Whereas, if the stress persists for a longer duration of time it may affect the overall health of an animal, especially in large captive animals who are continuously forced to live in a restricted area or enclosures and exposed to visitors for an unlimited period. The concentration of fecal cortisol metabolites increases during adverse situations and results in decreased activity and altered behavioral patterns. The reproductive physiology of the animal gets disturbed and the frequency to produce offspring reduces or the overall fertility rate decreases among the stressed animals. These situations can be overcome by monitoring the health of an animal by implementing noninvasive techniques of sample collection (the fecal sample collection) and analyzing the presence of different cortisol metabolites with the help of standard assay methods discussed by several researchers.

## II. STUDY POPULATIONS

*A. Captive Populations*

With more than 1200 mammalian species threatened with extinction (IUCN, 2013), there is not a shortage of species to study but assessing a population is very limited due to the degradation, fragmentation, and overall loss of habitat. Opportunities to conduct endocrine studies in situ are few, therefore captive environments provide investigators the intimacy needed to collect endocrine data on animals with known histories[16]. In the present study, we have focussed on two mega-vertebrates, giraffe(Giraffa Camelopardalis spp) and Asian elephants(Elephas maximus) in a captive environment, and the samples collected from these animals when analyzed for different cortisol metabolites clearly reflected the high stress levels due to several factors such as environment, social, physical, physiological and age related factors.

*B. Sampling*

To study the biology of an animal observations and measures must be taken without disrupting that biology, for which non-invasive sampling emerged as the ideal means to assess without perturbation. Therefore, sampling is not about invasiveness or lack thereof, but more about non- perturbation [17] . Although blood is the predominant sample substrate to assess endocrine activity, repeated samples are required thereby increasing the handling and restraint imposed on animals. Alternatively, non-invasive sampling like a collection of urine and fecal samples requires no, or minimal, contact with the animal, obviating the need for veterinary or trained personnel to collect samples and permitting long-term sample collection. Furthermore, because the rate of excretion is proportional to the amount of hormone in circulation, endocrine values derived from the excreta are a reflection of endocrine changes in the body [18]. Among the secreted hormones, steroids are the most resistant to degradation and abundant for quantification. The adaptation of fecal and urinary hormone assessment to threatened species endocrine studies(Lasley and Kirkpatrick, 1991) opened up new opportunities to research biological mechanisms that otherwise would have gone undiscovered(Schwarzenberger and Brown, 2013)[21]. Therefore once regarded as a waste product now has become a valuable 'research resource' and a virtual pool of biological information on reproductive cyclicity, pregnancy, and adrenocortical(stress) status.

*C. Extraction and Immunoassay methods used to determine FGCM from faecal samples;*

In the lab, feces were freeze-dried, pulverized, and sieved through a thin metal strainer for the removal of fibrous material (Fieß et al., 1999). For steroid extraction, 0.10 - 0.11 g of fecal powder was vortexed for 15 min. with 80% ethanol in water (3 ml). After centrifugation for 10 min. at 1500 g, the supernatants were aliquoted and stored at -20˚C until the assay procedure (Seeber et al., 2013). Extraction efficiency was determined by adding a known amount of $^{3}H$ labeled steroids to the faecal sample before extraction(Umapathy et al.,2013).

Fecal glucocorticoid metabolite (FGM) concentrations were determined using an 11-oxo etiocholanolone enzyme immunoassay (antibodies raised in rabbits against 5β-androstane-3α-ol-11- one-17-CMO: BSA; Möstl et al., 2002) which has been shown to reliably monitor fGCM alterations in a variety of mammals including giraffes (Bashaw et al., 2016; Palme, 2012 for review)

faecal glucocorticoid metabolites were measured using cortisol polyclonal antibody (R4866) diluted to 1:9000, HRP-conjugated cortisol 1:250,000 (C. Munro, University of California, Davis) and cortisol standards (1000–1.95 pg/well). This cortisol assay was successfully validated for other animals to provide reliable quantitative information regarding glucocorticoid output (Young et al., 2004, 2001) and found to cross-react with cortisol 100%(Young et al., 2004). The significant increase of fecal glucocorticoid levels following public processions and temporary injury could be considered as biological validation regarding the reliability of the assay used(Young et al.,2004; Ganswindt et al., 2010b)[27]

*D. Fecal Glucocorticoid Metabolites in GIRAFFES*

Giraffes occur in a highly flexible fission-fusion social system and group compositions can change on a daily basis, with bulls establishing an age-related dominance hierarchy and showing a roaming strategy in the search for fertile females. It was found that fGCM levels in giraffe bulls are age-class dependent, as well as associated with changes in the social environment.

*1)* Among all-male groups, subadult/juvenile bulls have the highest fGCM levels.

*2)* Older sexually active adult bulls have the highest fGCM levels, in the presence of females.

*3)* Sexual activity leads to higher fGCM levels, but only in adult giraffe bulls.

Stressful situations may arise from a variety of internal and external stimuli, such as environmental factors (e.g. extreme weather), social stimuli (e.g. mating or dominance conflicts), or physiological challenges (e.g. injuries or food scarcity) (Nelson, 2011; Touma et al., 2003; Touma and Palme, 2005). However, one of the primary sources of perceived stress is the social environment of an animal and its associated interaction with conspecifics (Creel et al., 2013)[30].

*E. Fecal Glucocorticoid Metabolites in Elephants*

Asian elephant(Elephas maximus) survival is under threat because of continuous poaching and loss of habitat and corridors due to rapid habitat fragmentation. Captive Asian elephants constitute 22-30% of the total Asian elephant population(Lair, 1997; Sukumar, 2003), and need immediate attention to be bred so as to meet the increasing demand for captive elephants. Endocrine profiling of reproductive hormones(progestagens and androgens) and the stress hormone(glucocorticoids) could facilitate better management and breeding strategies.

Males during musth showed increased fecal androgen metabolites and a slight increase in fecal . glucocorticoid metabolites levels. Elephants used in public festivals and processions showed a significant increase in fecal glucocorticoid metabolite levels.

Studies conducted on 12 captive Asian elephants using a non-invasive method based on steroid analysis of 1700 dung samples revealed that on average, elephants in musth showed a three to four times increase in faecal androgen metabolites concentrations compared to individual basal levels and remained elevated during the period of musth. Also, fecal glucocorticoid increased prior to 2-3 weeks and during musth in male elephants. Females which were frequently used for public procession exhibited very high levels of glucocorticoid metabolites concentrations(2-10 folds increase from the individual basal level)[32].

Fecal glucocorticoid metabolites concentration is related to age and facility also, where the lowest was observed in the oldest female and the highest in one of the younger females.

A large portion of animals represent threatened species and not studying them would be counterproductive for advancing the field of endocrinology. The present study reports on non-invasive stress monitoring of large captive herbivores using dung samples. In assessing an animal's adrenal status and stress physiology, which can be beneficial to in situ and ex-situ conservation, non-invasive hormone analysis is a vital tool. Faecal measures of hormone concentration are becoming widely used as it minimizes the impact of sampling on the animals and can be collected easily and non-invasively. The higher concentrations of faecal glucocorticoid metabolites evidence the presence of stress in captive animals for many reasons over a long time. Measuring the stress hormone cortisol in faeces of animals helps to monitor the health status both in captivity and wild.

In conclusion, stress can be detrimental if it remains unnoticed. Cortisol can also be a potent inhibitor of the immune system. Overproduction of cortisol may result in several diseased conditions and thus reducing the overall efficiency of an individual. Non-invasive monitoring of faecal glucocorticoid metabolites has opened a new gateway toward the management and assessment of overall health status and environmental factors responsible for stress in captive animals.

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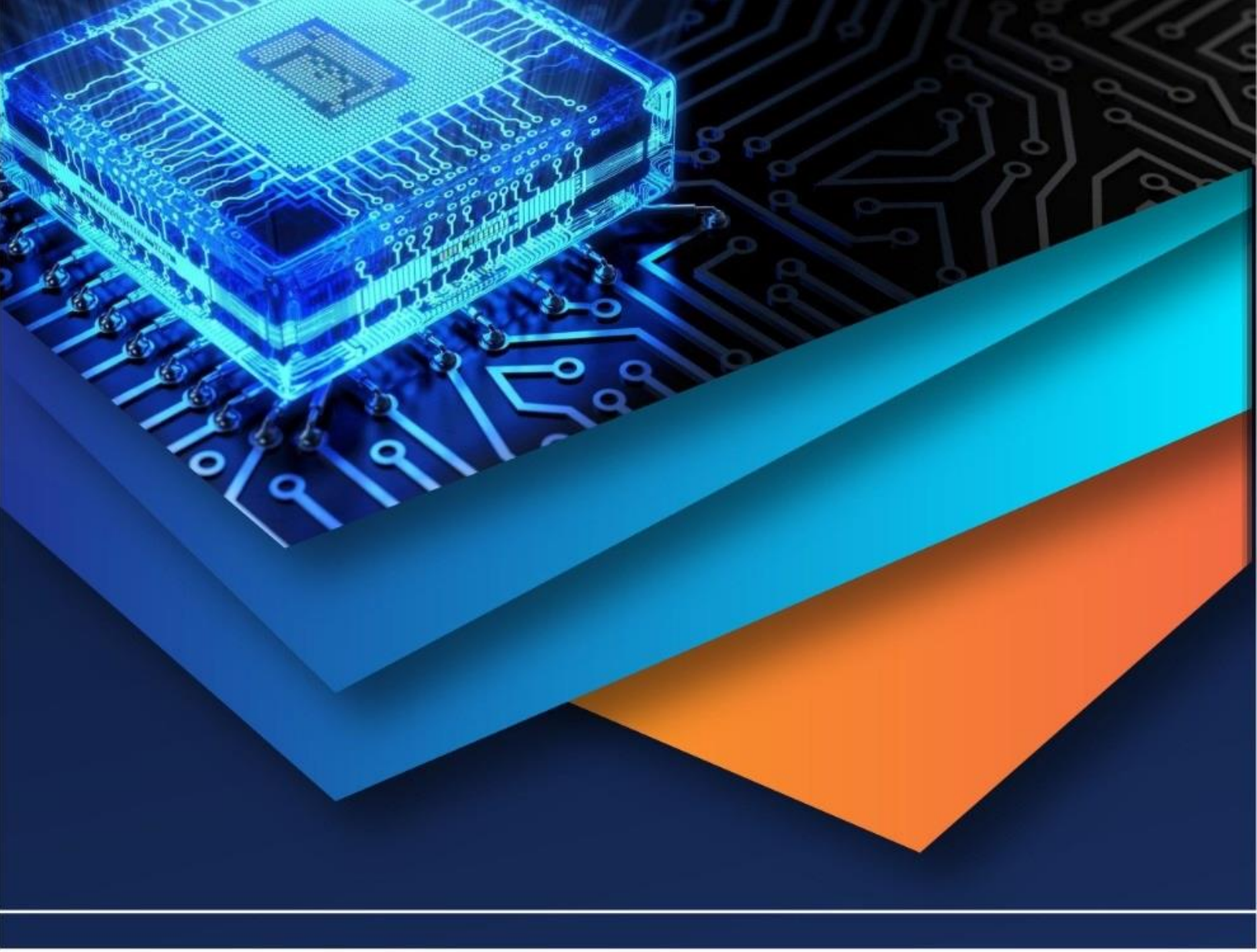

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