

Physiological measure of animal welfare in relation to semi-captive African Elephant (*Loxodonta africana*) interaction programs

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Abstract

Elephant interaction programs, specifically ones that provide elephant back riding, have come under public scrutiny, and little information exists to show whether these activities affect animal welfare. This study examined the impact of human interactions and ride-based activities on physiological stress-related indicators in African elephants. Fifteen trained semi-captive elephants, as well as free-ranging elephants roaming under the same ecological conditions, were monitored. Faecal samples were collected over a nine-month period from both groups and these were analysed using an enzyme immunoassay detecting faecal glucocorticoid metabolites (fGCMs) with a $5\beta\text{-}\alpha\text{-ol-11-one}$ structure. Elephants that participated in elephant-back-safari (EBS) activities showed significant decreases in fGCM concentrations when EBS were discontinued. Similarly, fGCM concentrations of the trained semi-captive individuals that did not participate in EBS showed decreased steroid concentrations over the same time. Overall, fGCM concentrations of the trained semi-captive herd and the free-ranging herd did not differ significantly. The collected data will help to better understand the physiological and behavioural requirements of semi-captive elephants with frequent exposure to humans. The findings will also help to optimise management strategies for wild elephant populations and elephants living in controlled environments on reserves exposed to wildlife tourism.

Keywords: conservation, elephant, endocrinology, faecal, physiology, stress

African elephants (*Loxodonta africana*) are a socially complex species, dependent on herd members for comfort, protection and raising of young (Estes 1991). Threats against the species have led to increased conservation efforts through sanctuaries (Lee and Graham 2006; Herbig and Minnaar 2019). Elephants attract tourists (Duffy and Moore 2010), with some seeking out close-up animal experiences, like elephant back safari rides (EBS). In South Africa alone, approximately 38 independent sites host interactions in the form of riding, touching, petting, or feeding (Lama 2017). Although these interactions have been met with huge popularity and demand by tourists, they are criticised as controversial practices (Millsaugh et al. 2007; Vries et al. 2014).

To assess animal well-being, physiological markers, such as body condition, blood pressure, and stress related hormone concentrations can be used (Ilies et al. 2010). Stress is the physiological and behavioural effects that occur in response to a stimulus that alters the homeostasis of an individual (Selye 1936; Wielebnowski 2003). An essential component of

the response to stress is the activation of the hypothalamic–pituitary–adrenocortical axis, which results amongst others in an increase in glucocorticoid secretion (Möstl et al. 2002). Although glucocorticoid responses can be adaptive in the short-term, prolonged periods of elevated glucocorticoid levels can have damaging and long-lasting effects (Sapolsky et al. 2000; Möstl et al. 2002).

Using non-invasive approaches to measure physiological stress responses have become preferred, typically when dealing with free-roaming wildlife (Ganswindt et al. 2012). Here, faeces have become a popular sample matrix, because of ease of collection and minimal impact to the animal (Wasser et al. 2000; Millspaugh and Washburn 2004). For African elephants, a group-specific enzyme immunoassay (EIA), detecting faecal glucocorticoid metabolites (fGCM) with a 5β - 3α -ol-11-one structure has proven to be suitable for monitoring stress-related steroid concentrations (Ganswindt et al. 2003; Ganswindt et al. 2010).

Thus far, very few studies focused on the effects of human interaction/presence on semi-captive or wild elephant welfare. Elephants in South Africa are mainly contained in fenced sections on reserves, where their populations are consistently monitored and managed (Selier et al. 2018). Multiple generations of elephants have been born in these managed parks, where they have been exposed to human presence and safari rides since birth. Therefore, the aims of this study were: a) to compare fGCM concentrations in semi-captive elephants with their free-roaming counterparts living in the same ecological environment, and b) to examine the stress-related effects of human-interaction programs, including EBS, on a trained semi-captive group of African elephants.

Data collection took place at the Kapama Game Reserve (KGR), an approximately 130 km² wildlife reserve, which is part of the greater Kruger National Park, South Africa. The focal animals were 15 trained semi-captive elephants that reside within the reserve. The elephants were trained to perform EBS ($n = 9$; males: 5; females: 4) for lodge guests. In April 2017, EBS was discontinued and a new elephant interaction program implemented, involving four of the elephants that previously participated in EBS ($n = 4$; males: 2; females: 2). The herd members that do not participate ($n = 11$; males: 3; females: 8) browse in the nearby bush during the interaction period. Female and male herd members receive a Gonadotropin-releasing hormone (GnRH) vaccine form of contraception every five to six months. The semi-captive individuals are housed in a fenced enclosure during the night, to ensure no contact with free-ranging elephants.

The free-roaming elephant population within the KGR consists of approximately 58 females and young and several bulls following closely behind the herd (Tigre, Camp Jabulani, pers. comm. 2018). The elephants' only forms of human exposure are related to game drives and recreational vehicles.

Faecal sample collection took place for one month (March 2017), while EBS was occurring. Weekly samples were collected from each of the 15 trained semi-captive elephants and from the wild population on two to three occasions over the month. Sample collection continued for eight months for both groups after discontinuation of the EBS. Samples were collected once a week during April, May, and October, and once a month for the remaining time from each of the 15 trained semi-captive elephants. Samples from the wild population were collected on two to three occasions within each month *ad libitum*, except for July. Faecal samples were collected within 5 minutes after defecation to prevent alterations in fGCM

composition post-defecation (Webber et al. 2018). To avoid cross contamination, material was removed from the middle of the boli using gloves. Samples were put on ice immediately after collection, frozen within 2 h post-collection, and kept frozen until reaching the Endocrine Laboratory, University of Pretoria, South Africa. In total, 603 faecal samples (467 samples from the semi-captive elephants, and 136 samples from the free-ranging elephants) were collected for fGCM analysis.

Previously established faecal hormone extraction techniques were used as described in Ganswindt et al. (2010). Immunoreactive fGCM concentrations were determined using an enzyme immunoassay (EIA) already established to monitor fGCM alterations in African elephants (Ganswindt et al. 2003; Viljoen et al. 2008a). Detailed assay characteristics, including full descriptions of the assay components and cross reactivities were previously described by Möstl et al. 2002. Assay sensitivity was 1.2 ng g^{-1} dry weight (DW) at 90% binding. Intra- and inter-assay coefficient of variation of high- and low-concentration quality controls were 4.24% and 5.31% (intra-assay CV), as well as 12.06% and 15.43% (inter-assay CV), respectively. Serial dilutions of faecal extracts gave displacement curves that were parallel to the respective standard curve, with relative variation of the slope of respective trendlines of <5% (Ganswindt et al. 2003).

To examine differences in fGCM concentrations of the trained semi-captive and the free-ranging elephants, a linear mixed-effects model was used.

A Kruskal–Wallis test was used to determine whether fGCM concentrations varied between the three subgroups (elephants that either participated in EBS, that did not participate in EBS, or belong to the group of free-ranging elephants) in a) March, when EBS still took place, b) April, when EBS was discontinued, and c) the remaining months of the study. A Wilcoxon rank sum test was used *post hoc* for multiple pairwise-comparison between subgroups, if required. A paired *t*-test was used to examine differences in individual fGCM concentrations of EBS and no-EBS semi-captive elephants between March and April. A Wilcoxon rank sum test was used to examine differences in fGCM concentrations of free-ranging individuals between March and April.

If more than one fGCM concentration was available for an individual within a given period, the individual's median fGCM concentration was used for comparison. All datasets were tested for normality using the Shapiro–Wilk normality test. A Bonferroni correction was applied to *post hoc* tests. Statistical analysis was performed using the programming language R (version 3.5.0) (R Core Team 2013).

In the semi-captive elephant group, overall individual median fGCM concentrations were $0.27 \mu\text{g g}^{-1}$ DW (range: $0.19\text{--}0.44 \mu\text{g g}^{-1}$ DW) in the months of April through November. Median fGCM values were comparable between the sexes (females: $0.27 \mu\text{g g}^{-1}$ DW, range: $0.19\text{--}0.31 \mu\text{g g}^{-1}$ DW; males: $0.28 \mu\text{g g}^{-1}$ DW, range: $0.21\text{--}0.44 \mu\text{g g}^{-1}$ DW) and between age groups (adults: $0.27 \mu\text{g g}^{-1}$ DW, range: $0.20\text{--}0.44 \mu\text{g g}^{-1}$ DW; subadults: $0.25 \mu\text{g g}^{-1}$ DW, range $0.19\text{--}0.29$). Within the free-ranging elephant herd, the overall median fGCM concentration was $0.23 \mu\text{g g}^{-1}$ DW (range: $0.19\text{--}0.33 \mu\text{g g}^{-1}$ DW) and were comparable between the sexes (females: $0.26 \mu\text{g g}^{-1}$ DW, range: $0.19\text{--}0.32 \mu\text{g g}^{-1}$ DW; males: $0.23 \mu\text{g g}^{-1}$ DW, range: $0.20\text{--}0.36 \mu\text{g g}^{-1}$ DW) and age groups (adult: $0.25 \mu\text{g g}^{-1}$ DW, range $0.06\text{--}0.73 \mu\text{g g}^{-1}$ DW; subadults: $0.23 \mu\text{g g}^{-1}$ DW, range $0.07\text{--}0.73 \mu\text{g g}^{-1}$ DW). Overall individual median fGCM concentrations of the captive individuals did not differ significantly from the

fGCM concentrations determined for the free-ranging population ($F_{(14,107)} = 1.021$; $p = 0.18$).

For the semi-captive elephants that participated in the elephant back safari rides, individual median fGCM concentrations were significantly higher in March, when elephant back safari rides were still occurring, compared with April, when elephant back safari rides were discontinued ($t = 4.86$, $df = 8$, $p = 0.001$, Figure 1). The semi-captive elephants that did not participate in elephant back safari rides also showed a significant decrease in individual median fGCM concentrations when comparing fGCM values from March and April ($t = 2.94$, $df = 4$, $p = 0.032$, Figure 1). Free-ranging elephant fGCM concentrations did not vary significantly when comparing values for March and April ($T_{(24,14)} = 328$, $p = 0.099$, Figure 1).

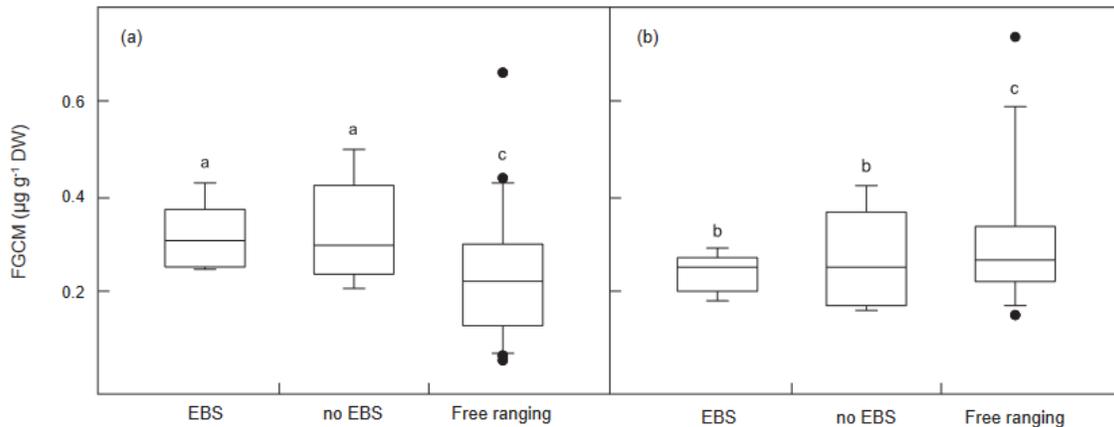


Figure 1: fGCM concentrations for the three subgroups (EBS, non-EBS and free ranging) of African elephants during March (a) and April (b). Significant differences between the two months within a group are indicated by different letters

Within the month of March, when elephant back safari rides were occurring, a significant difference between the three groups (Kruskal–Wallis $\chi^2 = 6.03$, $df = 2$, $p = 0.049$, Figure 1) was found. However, *post hoc* pairwise comparisons proved to be not significant (EBS vs no EBS: $p = 1.00$, EBS vs Wild: $p = 0.091$, no EBS vs Wild: $p = 0.189$). Within the month of April, when elephant back safari rides were discontinued, no significant difference was found between the three groups of elephants (Kruskal–Wallis $\chi^2 = 1.3834$, $df = 2$, $p = 0.501$, Figure 1). Likewise, no significant difference was found between the three groups of elephants for each of the remaining months of the monitoring period (May–November, Kruskal–Wallis $\chi^2 = 2.3071$, $df = 2$, $p = 0.316$).

In our study, we looked at stress-associated fGCM concentrations of semi-captive elephants in relation to the occurrence of EBS and also compared those values to fGCM concentrations of free-ranging elephants living under the same ecological conditions.

No significant differences in fGCM concentrations were found between semi-captive elephants that participated in EBS and individuals that did not participate. This is similar to findings by Kumar et al. (2019), where elephants also remained in their natural habitats and were allowed to express natural behaviours, and also received supplemental nourishment with open access to resources.

Although the semi-captive elephants showed slightly higher fGCM concentrations, overall median fGCM values were comparable between the free-ranging and the semi-captive individuals within the month of March, when elephant back safari rides were occurring. This is in line with another study, looking at the impact of human interaction on elephant fGCM levels (Pretorius 2004). In contrast, a large influx in tourism numbers resulted in significantly higher fGCM values in wild elephants on another Game Reserve (Szott et al. 2020).

Our results show that individual median fGCM levels significantly decreased amongst the nine semi-captive elephants that participated in EBS after discontinuation of the program. Interestingly, the five trained semi-captive elephants that did not participate in EBS showed the same decreasing trend in fGCM concentrations. In contrast, fGCM concentrations of the free-ranging elephants did not vary between those months of transition. A possible explanation for the observed decrease in fGCM values amongst the semi-captive elephants could be the change in routine/stimuli for the herd. Once EBS was discontinued, four of the elephants were transitioned to a new interaction activity; which included different commands and behavioural displays for the elephants, like blowing air out of their trunks or raising their feet. Changes in environment or routine, such as a transition from EBS to a new interaction activity, could allow individuals to utilise their drive to explore and benefit from a new stimulus. Animals in captivity that were exposed to various stimuli, both intrinsic and novel to the animal's nature, were found to have a significant improvement in welfare conditions (Wells 2009). Another explanation could be that elephants that had previously participated in the EBS activity were now able to spend more time with their non-participating herd members than what was allotted before. In a study conducted by Schulman et al. (2014), they found that in broodmares fGCM values increased significantly when social structure was disturbed as an effect of breaking up social groups during events, such as sale consignment. Although fGCM levels were significantly higher in the month during EBS, compared with the month after discontinuation for both groups, median fGCM values for each month were comparable to monthly median fGCM values throughout the study period.

In our study, fGCM concentrations of the semi-captive or the free-ranging herd appear comparable for factors like age or sex. Similarly, Viljoen et al. 2008b found no significant differences between different age classes. The physiology of males and females differ in various aspects, including glucocorticoid metabolism (Touma et al. 2003; Touma and Palme 2005), and therefore any sex-related differences in fGCM concentrations found have to be interpreted with caution (Wielebnowski et al. 2003; Hunnack et al. 2017). However, fGCM concentrations appear similar between the sexes in our study population and could, amongst others, be a result of a GnRH suppressing birth control in both sexes.

No seasonal effect was found on fGCM concentrations within the trained semi-captive group or amongst the free-ranging herd. This is in contrast with other studies on free-ranging African elephants, which found higher fGCM values during the dry season (Foley et al. 2001; Rasmussen et al. 2008; Viljoen et al. 2008b; Ganswindt et al. 2010). One reason could be that our dataset did not include hormone values from the peak wet season.

Looking forward, facilities that own trained semi-captive African elephants should consider related welfare implications when running or implementing human interaction programs. It is highly recommended that facilities implement an fGCM monitoring system. Measuring fGCM concentrations on even a biweekly to monthly basis would greatly improve insight into the animal's health and welfare condition. Providing adequate levels of stimulation, enrichment, encouraging socialization between family groups, and allowing animals to perform natural behaviours are critical to maintaining elephant welfare and the safety of both animals and

humans alike.

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Ethics declaration

This study was conducted in accordance with the guidelines of the Animal Ethics Committee of the University of Pretoria, permit V065-17.

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References

Duffy R, Moore L. 2010. Neoliberalising nature? Elephant back tourism in Thailand and Botswana. *Antipode* 42: 742–766. doi:10.1111/j.1467-8330.2010.00771.x.

Estes R. 1991. The behaviour guide to African mammals, including hoofed mammals, carnivores, primates. Berkeley, USA: University of California Press.

Foley CAH, Papageorge S, Wasser SK. 2001. Non-invasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. *Conservation Biology* 15: 1134–1142. doi:10.1046/j.1523-1739.2001.0150041134.x.

Ganswindt A, Palme R, Heistermann M, Borragan S, Hodges JK. 2003. Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to musth. *General and Comparative Endocrinology* 134: 156–166. doi:10.1016/S0016-6480(03)00251-X.

Ganswindt A, Münscher S, Henley M, Palme R, Thompson P, and Bertschinger H. 2010. Concentrations of faecal glucocorticoid metabolites in physically injured free-ranging African elephants *Loxodonta africana*. *Wildlife Biology* 16: 323–332. doi:10.2981/09-081.

Ganswindt A, Brown J, Freeman E, Kouba A, Penfold L, Santymire R, Vick M, Wielebnowski N, Willis E, Milnes M. 2012. International Society for Wildlife Endocrinology: the future of endocrine measures for reproductive science, animal welfare, and conservation biology. *Biology Letters* 8: 695–697. doi:10.1098/rsbl.2011.1181.

- Herbig F, Minnaar, A. 2019. Pachyderm poaching in Africa: interpreting emerging trends and transitions. *Crime, Law, and Social Change* 71: 67–82
<https://doi.org/10.1007/s10611-018-9789-4>. doi:10.1007/s10611-018-9789-4.
- Hunnick L, Ringstad I, Jackson C, May R, Fossøy F, Uiseb K, Killian W, Palme R, Røskaft E. 2017. Being stressed outside the park—conservation of African elephants (*Loxodonta africana*) in Namibia. *Conservation Physiology* 6: doi:10.1093/conphys/cox080.
- Ilies R, Dimotakis N, de Pater I. 2010. Psychological and physiological reactions to high workloads: Implications for well-being. *Personnel Psychology* 63: 407–436.
doi:10.1111/j.1744-6570.2010.01175.x.
- Kumar V, Pradheeps M, Kokkiligadda A, Niyogi R, Umapathy G. 2019. Non-Invasive Assessment of Physiological Stress in Captive Asian Elephants. *Animals (Basel)* 9(8): 553. doi:10.3390/ani9080553.
- Lama T. 2017. Botswana's Elephant-Back Safari Industry - Stress-Response in Working African Elephants and Analysis of their Post Release Movements. MSc thesis. University of Massachusetts, Amherst, USA. Lee PC, Graham MD. 2006. African elephants *Loxodonta africana* and human elephant interactions: implications for conservation. *International Zoo Yearbook* 40(1): 9–19.
- Millspaugh JJ, Burke T, Van Dyk GUS, Slotow R, Washburn BE, Woods RJ. 2007. Stress response of working African elephants to transportation and safari adventures. *The Journal of Wildlife Management* 71(4): 1257–1260. doi:10.2193/2006-015.
- Millspaugh JJ, Washburn BE. 2004. Use of faecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *General and Comparative Endocrinology* 138(3): 189–199. doi: 10.1016/j.ygcen.2004.07.002.
- Möstl E, Maggs JL, Schrötter G, Besenfelder U, Palme R. 2002. Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications* 26: 127–139.
- Pretorius Y. 2004. Stress in the African Elephants on Mabula Game Reserve, South Africa. MSc thesis. University of KwaZulu-Natal, South Africa.
- Rasmussen HB, Ganswindt A, Douglas-Hamilton I, Vollrath F. 2008. Endocrine and behavioural changes in male African elephants: linking hormone changes to sexual state and reproductive tactic. *Hormones and Behaviour* 54: 539–548.
- R Core Team. 2013. A language and environment for statistical computing. Vienna, Austria: R Core Team.
- Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21: 55–89.

- Schulman M, Becker A, Ganswindt S, Guthrie A, Stout T, Ganswindt A. 2014. The effect of consignment to broodmare sales on physiological stress measured by faecal glucocorticoid metabolites in pregnant Thoroughbred mares. *BMC Veterinary Research* 10: 25. doi:10.1186/1746-6148-10-25.
- Selier SJ, Slotow R, Balfour D. 2018. Management of African elephant populations in small fenced areas: Current practices, constraints and recommendations. *Bothalia* 48(2): 1–3. doi:10.4102/abc.v48i2.2414.
- Selye H. 1936. A syndrome produced by diverse nocuous agents. *Nature* 138(3479): 32. doi:10.1038/138032a0.
- Szott I, Pretorius Y, Ganswindt A, Koyama N. 2020. Physiological stress response of African elephants to wildlife tourism in Madikwe Game Reserve, South Africa. *Wildlife Research* 47(1) 34–43. <https://doi.org/10.1071/WR19045>.
- Touma C, Sachser N, Möstl E, Palme R. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *General and Comparative Endocrinology* 130: 267–278.
- Touma C, Palme R. 2005. Measuring Fecal Glucocorticoid Metabolites in Mammals and Birds: The Importance of Validation. *Annals of the New York Academy of Sciences* 1046: 54–74. doi:10.1196/annals.1343.006.
- Viljoen JJ, Ganswindt A, Du Toit JT, Langbauer Jr WR. 2008a. Translocation stress and faecal glucocorticoid metabolite levels in free-ranging African savanna elephants. *South African Journal of Wildlife Research* 38(2):146–152. doi:10.3957/0379-4369-38.2.146.
- Viljoen JJ, Ganswindt A, Palme R, Reynecke HC, Du Toit JT, Langbauer WR Jr. 2008b. Measurement of concentrations of faecal glucocorticoid metabolites in free-ranging African elephants within the Kruger National Park. *Koedoe* 50: 18–21. doi:10.4102/koedoe.v50i1.129.
- Vries de L. 2014. An elephant is not a machine, a survey into the welfare of private captive elephants in Sauraha, Chitwan National Park, Animal Nepal, Kathmandu, Nepal.
- Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, Millspaugh JJ, Larson S, Monfort SL. 2000. A generalized faecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *General and Comparative Endocrinology*, 120(3): 260–275. doi:10.1006/gcen.2000.7557.
- Webber JT, Henley MD, Pretorius Y, Somers MJ, Ganswindt A. 2018. Changes in African Elephant (*Loxodonta africana*) faecal steroid concentrations post-defaecation. *Bothalia* 48(2): 1–8. doi:10.4102/abc.v48i1.2312.
- Wells DL. 2009. Sensory stimulation as environmental enrichment for captive animals: A review. *Applied Animal Behaviour Science* 118(1–2): 1–11. doi:10.1016/j.applanim.2009.01.002.

Wielebnowski N. 2003. Stress and distress: evaluating their impact for the well-being of zoo animals. *Journal of the American Veterinary Medical Association* 223(7): 973–977.
doi:10.2460/javma.2003.223.973.