RESEARCH ARTICLE

Measuring faecal glucocorticoid metabolite concentrations as an indicator of stress in blue wildebeest (*Connochaetes taurinus*)

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In South Africa, blue wildebeest (*Connochaetes taurinus*) are routinely captured for relocation purposes. To monitor the stress caused by this practise, a non-invasive method assessing adrenocortical function as a measure of stress would minimize disturbance during sample collection. In our study, an adrenocorticotropic hormone challenge, and a mass-capture event were used to examine the suitability of five enzyme immunoassays (EIAs) for monitoring stress-related physiological responses using faeces as a sample matrix. The tested 11-oxoaetiocholanolone I EIA performed best, showing a 2126% increase above baseline after 22 h for a male, and a 474% increase for a female 23 h post-ACTH injection. Baseline faecal glucocorticoid metabolite (fGCM) concentrations did not differ between animals captured on either day 1 or day 2 of the capture event, indicating that the frequent presence of a helicopter during the two days did not influence fGCM concentrations. However, during capture-related restraint for up to 10 h, an overall 1.5-fold elevation in (fGCM) concentrations was found. Storage of faeces at ambient temperature post-defecation indicated a fair stability of fGCMs for up to 8 h. The ability to reliably assess adrenocortical function provides a solid basis to examine endocrine responses to putative stressful circumstances in blue wildebeest.

Keywords: capture, faecal glucocorticoid metabolites, ACTH challenge test, fGCM stability post-defecation.

INTRODUCTION

Over the last 25 years, private game farming has become an important sector of the bioeconomy in southern Africa because of the availability of suitable natural habitat, and the diversity of endemic wild animal species (Taylor, Lindsey & Davies-Mostert, 2015). The majority of such farms keep game for commercial use, including hunting, meat production and ecotourism (Luxmoore, 1985). Management and conservation purposes, such as reintroductions into suitable habitats, has resulted in an increased need to move animals between national parks and game reserves, with the result of an increased number of animals being captured, transported, and confined prior to release (Fischer & Lindenmayer, 2000; Griffith, Scott, Carpenter & Reed, 1989; Taylor *et al*., 2015). Although the capture and confinement of wild animals is routinely carried out, it is still associated with morbidity and mortality rates that can often be attributed to the subsequent effects of physiological and psychological stress (Knox, Hattingh & Raath, 1990). Studies in domestic and wild animal species have shown that capture,

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restraint, and transport are associated with a physiological stress response (Franceschini, Rubenstein, Low & Romero, 2008; Palme, Robia, Baumgartner & Möstl, 2000; Turner, Tolson & Hamad, 2002). A prolonged stress response during translocation and acclimatization to a new environment can have a long-term influence on the success of the translocation due to reduced reproduction or increased susceptibility to diseases (Boonstra, Hik, Singleton & Tinnikov, 1998; Hardy *et al.*, 2005; Sheriff, Dantzer, Delehanty, Palme & Boonstra, 2011).

When an animal experiences a stressful situation, two major systems in the body are involved to cope with the challenge. The hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adrenomedullary (SAM) system. The SAM system is the rapid reaction of the body facilitated by the release of adrenaline and noradrenaline (Nelson, 2011). In addition, the simultaneous activation of the HPA axis leads to a release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH is a relatively small protein hormone and is therefore rapidly inactivated (Reith & Neidle 1981). The ACTH release leads to a temporal increase in glucocorticoid (GC) production, such as cortisol or corticosterone, to help restore homeostasis (Sapolsky, Romero & Munck, 2000). The release of GCs stimulates gluconeogenesis, as well as protein and fat metabolism, to facilitate a temporary hyperglycaemia and energy supply (Möstl & Palme, 2002; Sheriff *et al.*, 2011; Touma & Palme, 2005). Thus, alterations in GC concentrations have been shown to be reliable physiological indicators in monitoring the level of disturbance of an individual (Palme, Rettenbacher, Touma, El-Bahr & Möstl, 2005; Sheriff *et al.*, 2011). GC concentrations can be measured in various biological matrices, such as blood, urine, saliva or faeces (Hodges, Brown & Heistermann, 2010). The use of blood is the most commonly used method; however, blood samples in wild animals can only be obtained when the animals are restrained or immobilized, which is often not possible in free-roaming species on a frequent basis. Additionally, the capture procedure itself has a rapid effect on blood GC concentrations and can therefore introduce unknown variables into the measurement (Schwarzenberger, 2007; Touma & Palme, 2005). Moreover, blood samples reflect hormonal levels within a relatively narrow time window, whereas steroid hormones that accumulate in the faeces are metabolized over several hours and may thus provide a better reflection of the general hormonal status of an animal (Schwarzenberger, 2007; Touma & Palme, 2005). Therefore, non-invasive hormone monitoring, using faeces as a hormone matrix, has become a widely accepted tool for measuring stress responses in wildlife (for reviews see Palme, 2019; Touma & Palme, 2005). The native biologically active steroid, that is normally measured in blood samples, is metabolized by the liver and then excreted as metabolites *via* the bile into the gut (Palme, Fischer, Schildorfer & Ismail, 1996; Taylor, 1971). As steroid metabolism is a highly species-specific process, methods determining glucocorticoid metabolite (GCM) concentrations therefore need to be validated for each species, especially when monitored for the first time (Palme *et al.*, 2005; Touma & Palme, 2005).

Capture and translocation procedures are essential in the conservation and management of wildlife, and it is therefore crucial, from a welfare perspective, to understand the related effects to these procedures. In addition to welfare concerns, improving the success of conservation and management efforts have stimulated more studies investigating the stress-associated reactions in response to capture and translocation (*e.g.* Franceschini *et al.*, 2008; Hämäläinen, Heistermann, Fenosoa & Kraus, 2014; Turner *et al.*, 2002).

The blue wildebeest (*Connochaetes taurinus*) is a common game ranching species, occurring on approximately 70% of private game ranches in South Africa. They are of commercial value due to the breeding and sale of colour morphs, as well as for trophy hunting (Taylor *et al.*, 2015). For the closely related species, the white-bearded wildebeest, it has been shown that anthropogenic disturbance and seasonal environmental changes have a significant effect on their physiological stress response (Stabach, 2015). However, no non-invasive technique to reliably monitor alterations in faecal glucocorticoid metabolite concentrations using an enzyme immunoassay, has been established for blue wildebeest. Therefore, the overall aim of our study was to examine the suitability of five enzyme immunoassays (EIAs) to non-invasively monitor adrenocortical function in blue wildebeest, using faeces as a matrix. To achieve this aim, an adrenocorticotropic hormone stimulation test (ACTH challenge test) was used as a physiological validation. Additionally, the effect of capture and confinement was used to biologically validate the adrenocortical

stress response. Finally, a storage test was conducted to investigate the stability of faecal glucocorticoid metabolite (fGCM) levels post-defecation.

MATERIALS AND METHODS

Study animals

For the ACTH challenge and stability of fGCM concentrations post-defecation test, one subadult male (20 months of age, 160 kg) and one adult female (40 months of age, 180 kg) from the same free-roaming herd ($n = 12$ animals) were captured at Emerald Casino Game Park, Gauteng, South Africa, by means of chemical immobilization. The animals were darted with a combination of etorphine hydrochloride (2 & 3 mg, M99, Novartis South Africa (Pty) Ltd, Johannesburg), thiafentanil oxalate (2 & 3 mg, Thianil, Wildlife Pharmaceuticals, White River), xylazine hydrochloride (10 & 15 mg, Xylavet 2%, MSD Animal Health SA, Isando) and azaperone (40 & 50 mg, Stresnil, Janssen Pharmaceutica, Sandton). Once immobilized the two animals were loaded onto a utility vehicle and transported within 20 min to enclosures approximately 3 km from the capture site. After arrival, the effects of the immobilization were reversed by administering diprenorphine (4 & 6 mg, M50/50, Novartis South Africa (Pty) Ltd, Johannesburg) and the animals were monitored until full recovery. The two animals were housed in separate, but adjacent enclosures. The pens (82.5 m^2 each) allowed the animals to have visual and olfactory contact with each other throughout the study. Individuals were fed fresh teff hay and 0.5 kg of concentrate pellets (Wildlife Pellets, Epol™, Rustenburg) daily. Each enclosure provided adequate fresh water and shelter.

For monitoring fGCM concentrations during capture and confinement, a population of blue wildebeest ($n = 71$) was sampled for two consecutive days in June 2015 during a planned mass-capture at the Mokala Game Reserve, Northern Cape, South Africa. The landscape consisted of numerous rocky ridges and outcrops, surrounded by expansive plains.

This study was conducted with the approval of the University of Pretoria Animal Ethics committee (Reference V055-14).

ACTH challenge test

The two animals stayed in their individual enclosures for ten days for acclimatization before each animal received 400 IU (1–2 IU/kg) of synthetic ACTH (Synacthen Depot©, Novartis South Africa (Pty) Ltd, Johannesburg) intramuscularly in the gluteus muscle using a pressurized dart syringe (Dan-Inject, Skukuza) at 08:30 on day 11.

During the 16 days of the study, both individuals were observed from 07:00 to 18:00 each day and all faecal samples voided during this time were collected within 20 min post-defecation. As enclosures could not be accessed at night, only samples that were warm to the touch were collected the next morning at 07:00. All samples ($n = 142$ in total, $n = 79$ for the male and $n = 63$ for the female) were immediately frozen and stored at –20°C until analysis. On day 16 of the study, the enclosure gates were opened and the animals were released to join their herd mates in the game park.

Stability of faecal glucocorticoid metabolite concentrations post-defecation

Three fresh faecal samples, that were also collected from the penned individuals at Emerald Casino Game Park, were mixed thoroughly and divided into 24 subsamples, which were stored at room temperature (21–24°C). Three of these subsamples were collected and immediately frozen at time = 0 h (control). Thereafter, three subsamples were collected at time points 1 h, 2 h, 4 h, 8 h, 16 h, 24 h, and 48 h, respectively. Collected material was stored at –20°C until analysis.

Faecal collection from mass-captured blue wildebeest

The sample collection took place during a two-day mass-capture event. Animals were chased with a helicopter from within a 12 km radius into a collapsible man-made temporary mass-capture boma. This boma was constructed from double layered plastic sheets and shade cloth and was designed as a large funnel, in which the animals remained for several minutes. The neck of the funnel, the chute, was connected to a steel handling passage with a ramp through which the animals were loaded onto a truck. All defecated faecal samples found in the chute were collected into individual plastic containers and were marked with the time and date of collection. On day 1, 39 wildebeest were captured (26 samples collected) and directly transported to the town of Loxton in the Karoo, Eastern Cape. On day 2, another 32 wildebeest were captured (37 samples collected).

These animals were not transported immediately and were thus left in the transport trucks overnight. Sixteen samples were collected immediately after capture, three fresh samples after the animals spent 3 h on the truck, and another 18 fresh samples were collected after the animals spent 10 h on the truck. Collected faecal material was placed on ice immediately and stored at –20°C within 20 min of collection until further processing.

Faecal steroid extraction and analysis

Frozen faecal samples were lyophilized, pulverized, and sieved through a mesh to remove fibrous material (Fieß, Heistermann & Hodges, 1999). Following this, 0.10–0.11 g of the faecal powder was vortexed for 15 min with 3 ml of 80% ethanol. Following centrifugation for 10 min at 1500 *g*, supernatants were transferred and stored at –20°C until analysis (Ganswindt *et al.*, 2010).

Steroid extracts from the ACTH challenge samples were analysed for immunoreactive fGCM concentrations using five different enzyme immunoassays (EIA), namely: (1) 11-oxoaetiocholanolone I EIA (detecting 11,17-dioxoandrostane), (2) 11-oxoaetiocholanolone II EIA (detecting fGCMs with a 5β-3α-ol-11-one structure), (3) 5α-pregnane-3β,11β,21-triol-20-one EIA (detecting fGCMs with a 5α-3β-11β-diol structure), (4) cortisol EIA, and (5) corticosterone EIA. Detailed assay characteristics, including cross-reactivities, are described by Palme & Möstl (1997) for the 11-oxoaetiocholanolone I, cortisol, and corticosterone EIA, by Möstl, Maggs, Schrötter, Besenfelder & Palme (2002) for the 11-oxoaetiocholanolone II EIA, and by Touma, Sachser, Möstl & Palme (2003) for the 5α-pregnane-3β,11β,21-triol-20-one EIA. Subsequently, samples related to the investigations of fGCM concentration stability post-defecation and of the effects of mass-capture and constraint were analysed using only the 11-oxoaetiocholanolone I EIA.

Sensitivity of the assays at 90% binding were 0.6 ng/g dry weight (DW) for the 11-oxoaetiocholanolone I, the 11-oxoaetiocholanolone II and the cortisol EIA, 1.5 ng/g DW for the 5α-pregnane-3β,11β,21-triol-20-one EIA, and 1 ng/g DW for the corticosterone EIA. Intra-assay coefficients of variation (CV), determined by repeated measurements of high- and low-value quality controls (n = 19 QC high and n = 18 QC low), was 5.5% and 6.2% for the 11-oxoaetiocholanolone I EIA, 6.1% and 8.7% for the 11-oxoaetiocholandone II EIA, 4.9% and 6.3% for the 5α-pregnane-3β,11β,21-triol-20-one EIA, 9.5% and 11.4% for the cortisol EIA, and 4.5% and 7.9% for corticosterone EIA. Inter-assay CV, also determined by repeated measurements of high- and low-quality controls (n = 21 each), was 10.7% and 15.3% for the 11-oxoaetiocholanolone I EIA.

Hormone analysis were performed at the Endocrine Research Laboratory, University of Pretoria, South Africa, following already established protocols (Ganswindt, Heistermann, Borragan & Hodges, 2002).

Data analysis

To determine alterations in fGCM concentrations following ACTH administration an iterative approach was used to establish individual baseline fGCM values pre-injection (Brown, Bellem, Fouraker, Wildt & Roth, 2001). Therefore, all fGCM concentrations of an individual's dataset exceeding the mean plus 2 standard deviations (S.D.) were excluded. The average was then recalculated, and the elimination process repeated until no hormone concentrations exceeded the mean plus 2 S.D. The peak in fGCM concentration post-injection was expressed as percentage (%) increase using the individual baseline levels as 100%.

To determine changes in fGCM concentration post-defecation, the relative change (%) of fGCM concentration post-defecation were calculated for each subsample separately, using the mean fGCM value determined at t = 0 as 100%. Differences in relative alteration rate between samples stored at t = 0 h and 1–48 h post-defecation were examined by one-way repeated measures ANOVA, followed by *post hoc* analysis using a *t*-test, with application of Bonferroni correction.

Comparison between the two study groups, the influence of the day of capture and the influence of prolonged confinement, was made by using a Mann-Whitney *U*-test. Respective data subsets were tested for normality using Shapiro-Wilk test. Data were statistically analysed using SigmaPlot 12.5.

RESULTS

ACTH challenge test

All five EIAs performed adequately for both sexes when using 100% as a set minimum increase. The 11-oxoaetiocholanolone I EIA performed best for the male (2126% increase from 0.19 µg/g DW baseline concentration, Table 1),

Table 1. Baseline and peak faecal glucocorticoid metabolite (fGCM) concentrations, percentage increase and time taken to attain peak fGCM concentration for one male and one female blue wildebeest after adrenocorticotropic hormone (ACTH) administration for each of the five enzyme immunoassays tested.

	11-oxoaetio-cholanolone II	Cortisol	5α-pregnane-3β,11β,21-triol-20-one	Corticosterone	11-oxoaetio-cholanolone I
Female					
Baseline fGCM concentration (μg/g DW)	1.53	0.04	0.57	0.95	0.87
Peak fGCM concentration (μg/g DW)	2.45	0.15	2.07	1.64	4.99
Hours after injection to reach peak concentration	23	23	23	23	23
Response (%)	60.13	310.35	263.16	72.63	473.56
Male					
Baseline fGCM concentration (μg/g DW)	0.57	0.02	0.3	0.33	0.19
Peak fGCM concentration (μg/g DW)	6.09	0.08	1.67	1	4.23
Hours after injection to reach peak concentration	22	22	22	22	22
Response (%)	968.42	252.79	456.67	202.03	2126.32

and for the female (474% increase from 0.87 μg/g DW baseline concentration). The 11-oxoaetiocholanolone II EIA performed second best for the male, showing an increase of 968% from baseline concentrations (0.57 μg/g DW), but only a 60% increase from baseline concentrations (1.53 μg/g DW) was found in the female. Overall, the cortisol, 5α-pregnane-3β,11β,21-triol-20-one, and corticosterone EIA also performed suitably in both male and female wildebeest, with the cortisol EIA showing an increase of 253% and 310% in baseline fGCM concentrations (0.02 μg/g DW and 0.04 μg/g DW, respectively). Both, the corticosterone and the 5α-pregnane-3β,11β,21-triol-20-one EIA resulted in increases above the baseline concentrations from 73% to 457% for both males and females (Table 1). Peak fGCM concentrations were present in faecal samples voided 22 h post-ACTH administration across all five assays in the male and at 23 h post-injection in the female (Table 1). Subsequently, only the 11-oxoaetiocholanolone I EIA was used to analyse samples from the steroid stability post-defecation experiment, as well as to look at capture- and restraint-induced alterations in fGCM levels.

Stability of fGCM concentration post-defecation

Faecal GCM concentrations from samples collected and frozen at time points 2, 4 and 8 h were slightly elevated (up to 12% at 2 h) compared to fGCM concentrations determined for time = 0 (Fig. 1). Respective fGCM concentrations of samples collected and frozen between 16–48 h post-defecation were significantly lower (F = 35.72, $P < 0.001$, *post hoc* analysis: P-values varied between $P < 0{,}001$ and $P = 0{,}011$ for 16, 24 and 48 h, respectively). Mean fGCM concentrations dropped to 71% after 16 h, and to 44% and 30% of the initial fGCM concentration, after 24 h and 48 h post-defecation, respectively. Distribution of fGCM concentration across sampling subsets ranged from 2–8% for respective measuring points in time.

Alterations in fGCM concentrations in relation to capture and constraint

No significant differences in fGCM concentrations were found when comparing values of samples collected from the chute on either day 1 or day 2 of the mass-capture event ($t_{16,26} = 357$, P = 0.75). Mean fGCM concentrations measured for day 1 were 0.134 μg/g DW (range: 0.056–

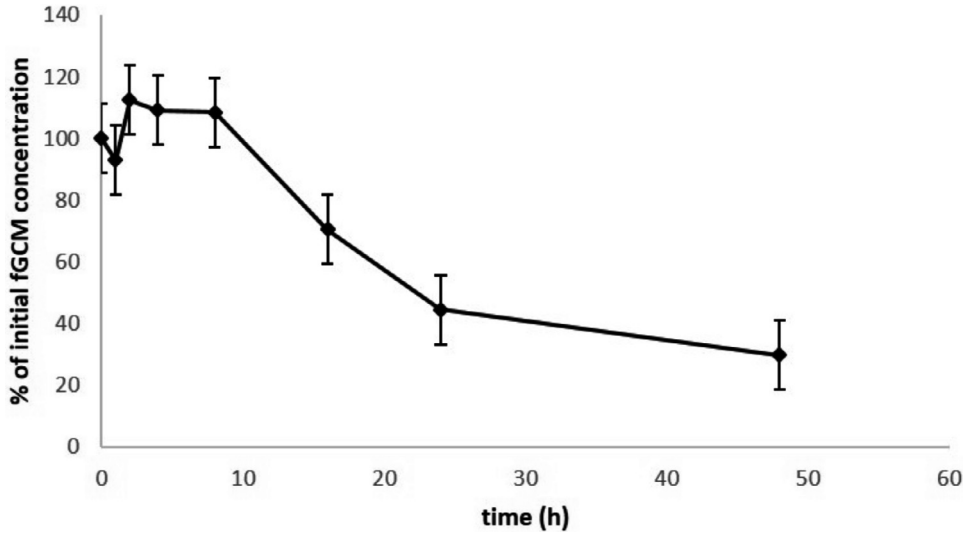

Fig. 1. Relative change (%) in faecal glucocorticoid metabolite (fGCM) concentrations (mean ± S.E.M) in blue wildebeest faeces over time (0, 1, 2, 4, 8, 16, 24, 48 h post-defecation).

0.210 µg/g DW) and 0.139 µg/g DW (range: 0.066–0.288 µg/g DW) for day 2 in the same population and capture site.

Faecal GCM concentrations from samples collected in the chute directly after capture (day 2) differ significantly from fGCM concentrations determined after subsequent confinement for 3–10 h ($t_{16,21}$ = 176, P <0.001, Fig. 2). Mean fGCM concentrations increased from 0.139 µg/g DW (range 0.066–0.288 µg/g DW) to 0.527 µg/g DW (range 0.104–1.720 µg/g DW).

DISCUSSION

In our study, we validated an EIA for monitoring fGCM concentrations in blue wildebeest *via* ACTH challenge and biological validation (temporal confinement) and determined a time window deemed suitable for faeces collection to determine the stress response non-invasively. We also obtained insight into the effect that capture and confinement procedures have on this species by comparing different capture days.

The ACTH challenge test resulted in suitable

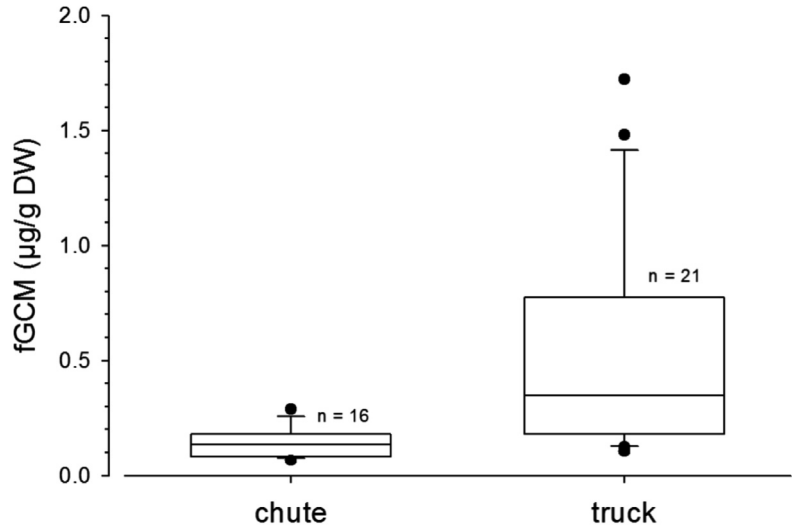

Fig. 2. fGCM concentrations from blue wildebeest collected directly after capture (n = 16) while standing in the chute, and subsequently after confinement for 3–10 h on a vehicle (n = 21).

responses in all five EIAs tested, with elevations in fGCM concentrations above 100% of fGCM concentrations pre-administration, in the male. However, only three EIAs showed an adequate response in the female. The enzyme immunoassay detecting 11,17 dioxoandrostane showed the most distinct response to the ACTH challenge in both study animals, and thus seems to be the most suitable for monitoring fGCM concentrations in blue wildebeest.

Only three assays showed an adequate response in the female wildebeest. Not only were there sex differences in the number of assays suitable, the male also showed an overall higher response post-injection compared to the female (2126% and 474% above baseline values in the male and female, respectively). One reason could be potential individual or sex-related differences in the adrenal responsiveness to the ACTH injection, as it has been observed in other studies (Ganswindt, Myburgh, Cameron & Ganswindt, 2014; Ludwig *et al*., 2013; Palme, Robia, Messmann, Hofer & Möstl, 1999). As males and females differ in some physiological aspects, sex differences may also occur due to differences in glucocorticoid metabolism or different excretion routes. These differences have been shown for example in mice (*Mus m. domesticus*), with males excreting more corticosterone metabolites into the faeces than females (Touma *et al*., 2003). Furthermore, differences in the types of excreted metabolites in males and females have been found in several other species (rat, Cavigelli *et al*., 2005; quail and domestic chicken, Hirschenhauser, Spreitzer, Lepschy, Kotrschal & Möstl, 2012; mouse, Touma *et al*., 2003).

In our study, peak fGCM concentrations were present in faecal samples voided 22 h post-ACTH administration in the male and at 23 h post-injection in the female. This is comparable with other large herbivores, such as giraffes (*Giraffa camelopardalis*) and elephants (*Loxodonta africana*) (Bashaw *et al*., 2016; Ganswindt, Palme, Heistermann, Borragan & Hodges, 2003). However, this result is later than the peak in another study on blue wildebeest where the peak appeared within the first 12 h after ACTH injection (Laubscher, 2015). These time variances could be due to differences in the specificity of the antibody utilized for fGCM quantification, the fibre content of the offered feed, as high fibre contents are thought to have an effect on faecal mass, gut transit time or altered steroid hormone metabolites due to changed microbial activity within the gut (Dantzer, McAdam, Palme, Boutin & Boonstra, 2011; Palme 2019; Touma & Palme 2005; von der Ohe & Servheen, 2002). Reduced food intake before the procedure or the use of anaesthetics could also have influenced gut transit time and dropping production, and therefore lead to a faster measurable peak in fGCM concentrations (Goymann, 2012).

In unpreserved material the quantity and composition of fGCMs can change over time (Heistermann, 2010). Bacterial enzymes present in faeces may continue to metabolize GCs and the resulting metabolites may more or less cross-react with the antibodies used in a particular enzyme immunoassay (Touma & Palme, 2005; Washburn & Millspaugh, 2002). A study by Lexen, El-Bahr, Sommerfeld-Stur, Palme & Möstl (2008) showed that the hormone concentrations in sheep faeces distinctly decreased or increased after storage at room temperature, depending on the EIA used. Another study on domestic livestock indicated an alteration in fGCM concentrations after only 1 and 4 h in bovines and equines, respectively (Möstl, Messmann, Bagu, Robia & Palme, 1999). In contrast, a study in leopards (*Panthera pardus*) (Webster, Burroughs, Laver & Ganswindt, 2018) showed that fGCM concentrations remained relatively stable for up to six days post-defecation. When testing the stability of steroid hormone concentrations post-defecation using the 11-oxoaetiocholanolone I EIA, our study revealed only minor changes of up to 12% after 8 h, indicating a fair stability of these metabolites for that time interval. However, after 16 h post-defecation, fGCM concentrations dropped by 71%, making an interpretation of other impacting variables rather challenging. Therefore, taking faecal samples greater than 8 h after defecation may not provide an accurate measure of fGCM concentrations in blue wildebeest. A test of the other suitable EIAs might help to find one that provides a longer time window for sample collection in blue wildebeest.

It has been shown in a variety of studies that the capture, restraint and translocation of wild animals is associated with increased glucocorticoid concentrations (Dehnhard, Clauss, Lechner-Doll, Meyer & Palme, 2001; Hämäläinen *et al*., 2014; Viljoen, Ganswindt, du Toit & Langbauer, 2008). Wildlife capture on an individual or population level depends on factors like season, time of day, species, and the area of occupation, therefore capture operations often need to be conducted over a number of days. Consequently, animals in

the vicinity of a capture site may also be disturbed, especially if a helicopter or other vehicles are used. However, when comparing fGCM concentrations of animals during the capture event, wildebeests that were captured on the second day did not have elevated fGCM concentrations just after capture. Since there is a lag time from determining stress-related changes in GCM concentrations in plasma and GCM concentrations in faeces due to gut passage time, a stress response is usually only detected in faeces several hours post a stressor (Palme *et al.*, 1996). In our study, the peak post-ACTH injection occurred after 22 to 23 h and it can therefore be assumed that the concentrations measured in the faeces represent plasma concentrations from the previous day. The animals appear to have tolerated the noise and presence of a helicopter in their environment. This finding is in contrast to a study in brood mares where individuals not being captured showed elevated fGCM concentrations as well, probably due to a disruption in the social order (Schulman *et al.*, 2014). A possible explanation for the lack of elevated fGCM concentrations caused by the capture procedure itself, could be that the wildebeest developed only a minor and short-lived stress response. Helicopters are routinely used to manage animals and therefore the study animals might have been acquainted with them without being the subject of a mass-capture themselves. A more frequent sample collection or a prolonged exposure to such a stressor would be needed to further elucidate the effects of the capture procedures. Further, it cannot be completely ruled out that some of the individuals captured on day 2 came from a different herd or from a different location within the reserve and were therefore not being disturbed by the procedures of the previous day.

Wildebeest being confined for up to 10 h on a transporter showed a distinct and significant elevation in fGCM concentrations. A study in cattle showed a distinct increase in fGCM concentrations in cows that were transported, and although to a lesser extent, an increase was also found in individuals that were loaded onto a stationary truck, suggesting that the restraint and loading itself represents a stressful event (Palme *et al.*, 2000). The animals in that study were used to being handled and transported, and it can therefore be assumed that the prolonged confinement of the wild blue wildebeest on the truck induced a strong stress response. In translocated Grevy's zebra (*Equus grevyi*) it has been shown that the time of confinement before the release increased fGCM levels even higher than the capture procedure itself (Franceschini *et al.*, 2008). Similar increases in fGCM have been found in white (*Ceratotherium simum*) and black rhinoceroses (*Diceros bicornis*) following restraint and transportation (Turner *et al.*, 2002). Another possible explanation for the increase in fGCM concentrations could be a disruption of social bonds during the capture process, as it is possible that some herd members have been separated (Schulman *et al.*, 2014).

Despite blue wildebeest being commonly captured, the physiological effects of capture in this species has not been sufficiently studied. Our study attempts to determine which enzyme immunoassay is the most suitable to assess a stress response in this species. A potential limitation of the current study is the limited sample size and the number of capture events. Future studies with a higher sample size and a prolonged sample collection can therefore help improving animal welfare during capture by investigating methods that reduce the stress response in these animals. Further research is also needed to investigate the actual pathophysiological effects caused by the perceived stress, such as susceptibility to disease or reproductive success. The ability to reliably assess adrenocortical function in blue wildebeest now provides a solid basis to further examine endocrine responses to putative stressful circumstances in this species.

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