Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

ORIGINAL RESEARCH

Effects of reproductive seasonality on the excretion of fecal glucocorticoid metabolites in free-ranging Pampas Deer

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Keywords
cortisol; non-invasive endocrinology; physiological stress; reproduction; stress; Pantanal; *Ozotoceros bezoarticus*; corticosterone.

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Editor: Andrew Kitchener
Associate Editor: Isabel Barja

Received 31 August 2023; revised 29 January 2024; accepted 8 March 2024

doi:10.1111/jzo.13165

Abstract

Understanding how fecal glucocorticoid (GC) concentration is associated with reproduction in wild animals allows us to associate physiological stress with the costs of reproduction. Glucocorticoids are among the main stress-related hormones, and their secretion is strongly associated with reproductive seasonality. Using non-invasive methods (thereby avoiding causing stress), we used fecal GC metabolites (FGC) to test the hypothesis that the reproductive phase (mating, non-mating, gestation, and lactation) influences stress levels of the seasonally reproductive Pampas Deer (*Ozotoceros bezoarticus*). Furthermore, we compared FGC between sexes and between males of different antler statuses (velvet, hard, cast). During 1 year, in the Pantanal of Brazil, we collected 621 fresh fecal samples (327 from females, 294 from males) from which we estimated FGC using enzyme immunoassay (EIA). FGC concentrations varied by reproductive phase and antler status. Agonistic and courtship interactions associated with mating (i.e., fights between males, attempts to mount females), suggest that they influence FGC concentrations most strongly in both sexes. Females also had greater FGC concentrations during lactation, suggesting that this phase and parental care are also associated with increased physiological stress. In males, the association of FGC concentrations with antler status may be, in part, associated with photoperiod and testosterone secretion, both of which can trigger reproductive and agonistic behaviors. Finally, reproductive synchrony of the sexes causes similar FGC over time and suggests that environmental factors contribute as well. We show that non-invasive monitoring of glucocorticoid metabolites levels is an effective tool for detecting changes in the physiological stress response in Pampas Deer, suggesting that this tool will be useful for detecting changes in physiological stress caused by human disturbance, such as cattle ranching in Pantanal and similar disturbances elsewhere.

Introduction

Stress can be defined as the physiological response of an individual that must (1) focus energy on coping with a short-term threat to survival and (2) curtail long-term investments in functions, such as courtship, territorial defense, and reproduction (Busch & Hayward, 2009). Stress in animals can be the result of a wide variety of causes, and by measuring stress, we can better understand the challenges posed by the environment and how they are overcome. Glucocorticoids (GC – typically cortisol and corticosterone) are the main hormones that respond to stress, and they are regulated by the hypothalamic–pituitary–adrenal gland axis (Wasser et al., 2000). Measuring glucocorticoids is now a widely used method to monitor and study stress in a variety of species of wildlife (Beehner & McCann, 2008; Keay et al., 2006; Romero, 2002), and often includes examining reproductive, ecological, and anthropic sources of stress (Cavigelli, 1999; Creel et al., 2013; Dixon et al., 2021; Ewacha et al., 2017; Hammond et al., 2019; Pereira et al., 2006; Piñeiro et al., 2012; Romero, 2002; Spercoski et al., 2012; Szott et al., 2020). Measuring stress and identifying its sources and costs are important tools for animal welfare, wildlife management, and conservation programs (Creel et al., 2002; Foley et al., 2001; Konjević et al., 2011; Möstl & Palme, 2002).

Ecological and reproductive seasonality are important predictors of glucocorticoid secretion in wildlife (Corlatti et al., 2011; Eggermann et al., 2013; Fichtel et al., 2007; Gesquiere et al., 2008; Huber, Palme, & Arnold, 2003; Huber, Palme, Zenker, et al., 2003; Pokharel et al., 2019; Romero, 2002; Strier et al., 1999). Glucocorticoid levels can

change with reproductive phase, for example, due to social interactions and reproductive status (Barrett et al., 2002; Bergman et al., 2005; Cavigelli, 1999; Creel, 2001; Fichtel et al., 2007; Goymann et al., 2001; Muller & Wrangham, 2004; Ostner, Heistermann, et al., 2008; Ostner, Kappeler, et al., 2008; Spercoski et al., 2012), agonistic interactions during mate acquisition (Bergman et al., 2005; Creel et al., 1997; Crockford et al., 2008; Eggermann et al., 2013; Muller & Wrangham, 2004), and changes associated with lactation and parental care (Goymann et al., 2001; Weingrill et al., 2004). Greater concentrations of fecal cortisol metabolites in Red Deer (*Cervus elaphus*), for example, were associated with greater competition between males and females (Pavitt et al., 2015; Peña et al., 2021). Similarly, in red deer, cortisol metabolite concentrations varied with female reproductive status and were greatest for lactating females (Pavitt et al., 2016). Thus, glucocorticoid levels are often associated with increased metabolic and energetic demands associated with reproduction, such as milk production and let-down (Casey & Plaut, 2007), and so should be seasonal in those species that are seasonally reproductive (Romero, 2002).

Monitoring glucocorticoids is an important tool in conservation (Busch & Hayward, 2009; Millspaugh & Washburn, 2004; Romero, 2002), yet traditional methods for hormonal evaluation tend to require blood samples repeatedly taken over time and which may not be easily carried out in many species (Harper & Austad, 2001; Monfort et al., 1993). Non-invasive methods, on the contrary, provide an alternative that uses other biological material, including but not limited to feces, urine, or saliva, which do not require manipulation of or interaction with the animal under study (Möstl & Palme, 2002). Also, glucocorticoids measured in fecal metabolites (FGC) allow longitudinal studies of individuals, such that each individual can serve as its own control (Möstl & Palme, 2002), and which ensures the well-being of the wildlife under study in its natural habitat (Beehner & McCann, 2008; Keay et al., 2006; Rehnus et al., 2009), avoiding the need to capture the animal thereby avoiding stress and other issues associated with animal capture, such as capture myopathy in deer (Catão-Dias & Camargo, 2010).

The Pampas Deer (*Ozotoceros bezoarticus*) are medium-sized and tend to be found in open habitats, such as fields and savannas, from southern Brazil to central-northern Argentina (Eisenberg & Redford, 1999; Merino et al., 1997). Reproduction in this deer is seasonal, and which may be associated with resource availability or climate (Pereira et al., 2005; Ungerfeld, González-Pensado, et al., 2008). In the Pantanal of Brazil, the peak of births is in the rainy season, when food abundance increases and favors lactating females and newborn deer (Christofoletti, 2010; Lacerda, 2008; Merino et al., 1997). Seasonal variation in the requirements of reproduction may be associated with stress and reflected by seasonal variation in endocrine functions (Romero, 2002). For example, in the summer (when breeding), male Pampas Deer in the Brazilian *Cerrado* had greater concentrations of FGC than non-breeding males (Pereira et al., 2006). Given their reproductive synchrony and seasonality, Pampas Deer females also have seasonally variable FGC. Thus, examining FGC can indicate how reproductive physiology affects stress in both sexes and, which has not yet been studied in females. In addition, detailed endocrine studies over the breeding cycle will help us better understand reproductive cycles in tropical deer (Pereira, 2010).

Here, we use fecal samples of individuals over time to evaluate FGC profiles as stress indicators to test the prediction that the reproductive phase is associated with FGC concentrations in free-living Pampas Deer. Considering seasonal reproduction (Pereira et al., 2005; Ungerfeld, González-Pensado, et al., 2008) and that reproduction is stressful (Reeder & Kramer, 2005; Romero, 2002), we predict that FGC is associated with the seasonal reproductive cycle (peaks at times of greatest stress, troughs at times of least stress). Because males fight for access to females, and females provide parental care (Merino et al., 1997; Pereira et al., 2005; Ungerfeld, González-Pensado, et al., 2008), we predicted that the greatest concentration of FGC for both sexes will be found during the mating phase of reproduction, as well as during lactation for females. In practice, male antler growth and condition will indicate breeding status and therefore stress (full, hard antlers during the time of greatest stress) and, in females, lactation should be associated with greatest stress (Goymann et al., 2001; Maestripieri et al., 2008; Pereira et al., 2010).

Materials and methods

Study area

Pampas Deer were studied at the Nhumirim/Embrapa (4310 ha; 18°59′19″ S, 56°37′10″ W) and Alegria (14 031 ha; 19°02′59″ S, 56°46′59″ W) ranches (in the central Brazilian Pantanal, locally known as *Nhecolândia*), in the municipality of Corumbá, Mato Grosso do Sul, Brazil (Fig. 1). Ranches are near each other (<7 km) and have similar biotic and abiotic characteristics (i.e., vegetation, wildlife, human and cattle perturbations). The climate is seasonal tropical, with a dry season from June to September and wet from November to March (and the remaining months are transitional). Annual average temperature is 26.4°C, with an average of 1152 mm annual precipitation (Rodela & Queiroz Neto, 2007). Local vegetation is a complex mosaic, including riparian forest to savanna and grasslands, where plant species used for forage by the deer are quite diverse (Ratter et al., 1988; Rodela et al., 2007).

Study animals and fecal sample collection

Pampas Deer are the only species in the genus *Ozotoceros* and are medium-sized, weighing, on average, 22–34 kg, with males somewhat larger than, but similar to, females (other than antlers in males). Deer tend to form small groups whose size and composition may vary with the breeding season and resource availability (González et al., 2010; Lacerda, 2008; Pereira et al., 2005; Rodrigues, 1996; Tomás et al., 2001).

We collected fecal samples from November 2008 to October 2009. Daily, we collected fresh feces immediately after defecation from individuals as they were encountered, with a goal of 25 samples per sex per month. When an individual was found and observable (and we were able to note coat color, size,

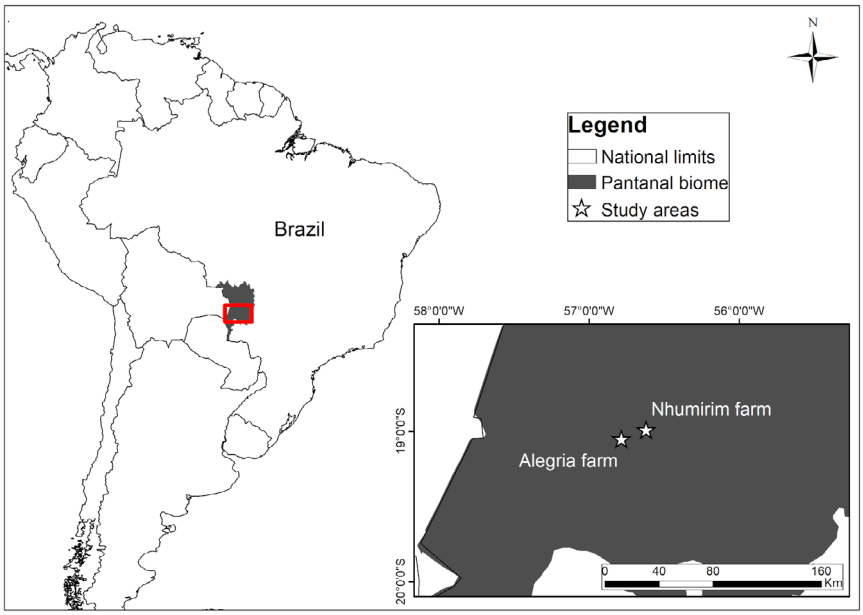

Figure 1 Map indicating study sites where fecal samples of the Pampas Deer (*Ozotoceros bezoarticus*) were collected in the Brazilian Pantanal in the state of Mato Grosso do Sul. The red square box highlights the study area (bottom right). The dark area in the large map indicates the Pantanal, and in the inset, the location of the study area.

antler condition), we followed that deer at a distance of no less than 70 m until it defecated, when we approached and collected the feces. We sampled for 3–5 days from different groups in different places during the same month to avoid collecting more than one fecal sample per individual during each collecting period, and so all fecal samples were considered independent. We standardized the time of collection (between sunrise and noon) to avoid circadian variations (Keay et al., 2006; Landys et al., 2006; Reeder & Kramer, 2005; Smith et al., 2012).

Upon collection, feces were placed in individual plastic bags noting sex, reproductive phase or antler condition, time, and date. The sample was stored in the field at 4°C until our return to the base camp (≤8 h) where it was then frozen at −20°C. All field procedures – fecal collections – were approved by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA/MMA (SISBIO no 16534-1) and ethics and animal welfare committee (CEBEA no 017687-08), and followed the American Society of Mammalogists guidelines (Sikes, 2016).

Reproductive phase and antler condition

Reproductive phase classification of the observed deer followed that in the published literature on the species (Christofoletti, 2010; Lacerda, 2008; Merino et al., 1997; Pinder, 1997; Rodrigues, 1996). Females in this population were either pregnant or had offspring (Christofoletti, 2010; Lacerda, 2008) and, so we assumed that all females were sexually mature and followed the same general temporal patterns of gestation, birth, and rearing. Reproductive phases were based on when births occurred, which was during August and September (Christofoletti, 2010; Merino et al., 1997). Thus, considering that gestation lasts 7 months (Eisenberg & Redford, 1999), mating occurred in January and February, gestation from March to July, and lactation from September to December. We excluded August to avoid sampling both pregnant and non-pregnant females.

Males were classified as mating in January and February, and not-mating for the rest of the year. In addition, males were classified by antler growth as follows: velvet – antlers visibly covered in velvet (from small to fully grown), hard – with no velvet and fully grown, and cast (absent).

Fecal metabolite extraction and immunoenzyme assays

Frozen fecal samples were transferred to a drying oven at 57°C for 72 h, after which the totally dry samples were triturated to uniformity (Barja et al., 2012; Millspaugh et al., 2001; Millspaugh & Washburn, 2004; Tanaka et al., 2019). Metabolite extraction of this triturate followed (Graham et al., 2001). This was carried out by mixing 0.5 g of the sample with 5 mL methanol 80%, then stirred for 30 s, after which the mixture remained in the horizontal agitator for 14 h and stirred again for 10 s. The samples were then centrifuged at 377 g for 20 min, after which the supernatant was separated and stored at −20°C until submitted to the assay.

Glucocorticoid concentrations were measured using the R4866 antibody (C. J. Munro, University of California, Davis, CA, USA). The antibody has a cross-reaction of 100% with cortisol, 9.9% with prednisolone, 6.3% with prednisone, 5.0%

with cortisone, 0.7% with corticosterone, and 0.5% with the remaining metabolites. This antibody was chosen because other studies indicated good reactivity with metabolites (cortisol) in the feces of our study species (Polegato, 2004). Coefficients of variation among assays were 6.8% for the high control (20.4% ligation) and 7.0% for the low control (57.0% ligation), while all intra-assay CVs were less than 10%.

Assay validation

We validated our assays (Brown et al., 2004) in three ways. First, the parallelism test, in which the pool dilution curve that is made using serial, random samples (1:2–1:8192) was immunologically similar to the standard curve at a dilution factor of 1:128, which approximates the central point of the standard curve. Next is the accuracy test, where the slope of the observed regression is parallel to the slope of the standard regression ($y = 0.975x–28.03$, $r^2 = 0.97$). Finally, we performed the physiological validation by adrenocorticotropic hormone (ACTH) stimulation of a captive male Pampas Deer at the Deer Research and Conservation Center (Brazilian acronym NUPECCE) of São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil. The animal was anesthetized with a combination of xylazine (1 mg kg^{-1}) and ketamine hydrochloride (7 mg kg^{-1}); anesthesia was induced and maintained for 2 h with isoflurane (3 V% diluted in 100% oxygen with a flow of 60 mL kg^{-1} min^{-1}). We administered a single dose of 0.5 mL ACTH (Synacthen 0.25 mg mL^{-1}, Novartis, Linz, Austria) intravenously. Then, after it recovered, we monitored the animal in individual stall every 2 h to check the defecations. Thus, we collected fresh fecal samples beginning 12 h before ($n = 4$) to 72 h ($n = 28$) after ACTH application. A FGC baseline was established through an iterative process with the samples collected before injecting ACTH and was calculated excluding points greater than a certain threshold (mean + 2 SD) until no points fall above threshold (Palme, 2019). The ACTH challenge demonstrated biologically meaningful elevations in FGC in Pampas Deer, with a peak of excretion (653.0 ng g^{-1}) 251% greater than baseline (259.9 ng g^{-1}) approximately 33 h after ACTH injection. This indicates that the assay antibody was efficient for measuring glucocorticoids metabolites in feces. In addition, results demonstrate that the time between the stimulus and the increase in FGM levels is about 33 h. All animal experimentation procedures were approved by Secretaria de Infraestrutura e Meio Ambiente de São Paulo (SMA no 7569).

Statistical analyses

We tested that FGC concentrations varied by reproductive phase in both male and female Pampas Deer. We divided the female year into three periods: (1) mating (January, February), (2) gestation (March–July), (3) lactation (September–December), and compared FGC between periods with analysis of variance (ANOVA, Tukey test). Similarly, we divided the male year into three categories based on antlers (velvet, cast, hard) and breeding (January, February) and non-breeding (the rest of the year) intervals and compared FGC (ANOVA and *t*-test, respectively). We estimated monthly FGC by sex and tested for correlations between the sexes by month. FGC concentration was $\log_{10}$ transformed to normalize residuals in all analyses.

Fecal glucocorticoid metabolite concentrations are expressed as mean of nanograms FGC per gram of dry feces (ng g^{-1}). We used an alpha of 0.05 for significance, and all analyses were carried out in R (ver. 4.0.4, R Development Core Team, 2021).

Results

A total of 621 fecal samples were gathered (327 female samples, 25–35 samples $month^{-1}$; 294 male samples, 20–27 samples $month^{-1}$). In females, FGC concentration (*N*, mean, lower and upper 95% confidence interval) was greatest during mating (63, 7177.6, 6323.9–8146.4) and during lactation (109, 6734.8, 6064.0–7479.8) was greater than during gestation (131, 5025.5, 4538.4–5564.9; $F_{2,300} = 17.05$, $P < 0.001$, Fig. 2). Similarly, in males, FGC concentrations was greater during mating (47, 7053.0, 6332.9–7854.9) than non-mating (247, 5335.5, 5006.9–5685.5; unequal variance $t = 4.47$, d.f. = 83.5, $P < 0.001$, Fig. 3).

In males, FGC concentration was greatest when antlers were hard (196, mean = 6295.6, 5865.1–6757.7), followed by cast (12, 5099.9, 3521.2–7386.5), and then lowest in velvet (86, 4289.0, 3750.3–4905.0; $F_{2,291} = 20.4$, $P < 0.001$, Figs 4 and 5).

Timing of FGC levels, and thus time intervals of stress, were correlated between the sexes as well ($r = 0.92$; d.f. = 10; $P < 0.001$, Fig. 6).

Discussion

Fecal glucocorticoid metabolite levels demonstrate that stress varies over the year associated with reproductive activities in the Pampas Deer. Also, even though the sexes are behaviorally and physiologically different, the annual cycle of stress is similar between them. Variation in fecal glucocorticoid metabolite concentrations allows us to better understand when and how stress might be important. Clearly, as expected, both sexes produce greater concentrations of FGC during the mating period. At that time (which may last days before copulation occurs), males are courting females and vying with other males for access to those females (Merino et al., 1997; Pereira et al., 2005). In the Brazilian *Cerrado*, urine and anogenital sniffing, flehmening, chasing, fighting, and mounting were frequent behaviors for breeding males (Pereira et al., 2006). Other interactions may also become more common at that time, such as agonistic interactions between females and young males that increase just prior to female receptiveness (Lacerda, 2008). Clearly, the reproductive period is the most stressful time of the year because other species of deer, such as Red Deer, also have similar patterns of timing and stress (Pavitt et al., 2015; Peña et al., 2021).

Females had greater FGC concentrations during lactation, as observed in other mammals (Cavigelli, 1999; Goymann et al., 2001; Maestripieri et al., 2008; Spercoski, 2007), which suggests that lactation is an energetically costly and stressful,

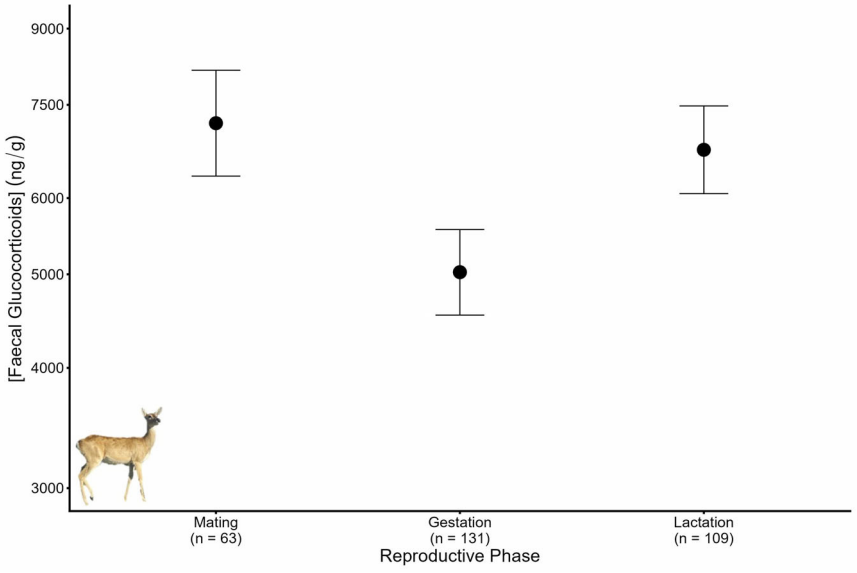

Figure 2 Comparasions of fecal glucocorticoid metabolite concentrations (mean, 95% confidence interval) in female Pampas Deer (*Ozotoceros bezoarticus*) among reproductive phases (mating, $n = 63$, gestation, 131, lactation, 109) in the Brazilian Pantanal. Mating and lactation have greater concentrations than gestation (Tukey test, $P < 0.001$).

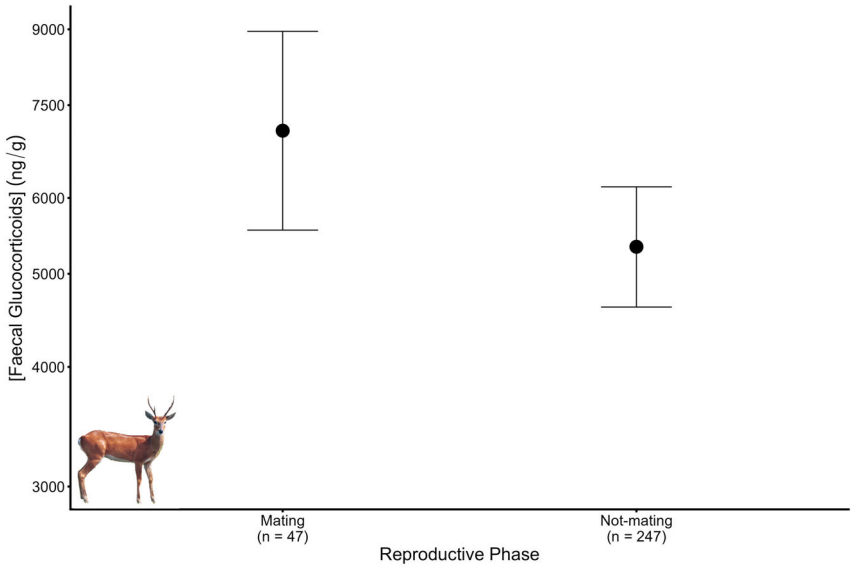

Figure 3 Comparisons of fecal glucocorticoid metabolite concentrations (mean and 95% confidence interval) of reproductive phases demonstrating that fecal glucocorticoid metabolite concentrations are greater during the mating ($n = 247$) than the non-mating (47) periods in male Pampas Deer (*Ozotoceros bezoarticus*) in the Brazilian Pantanal ($P < 0.001$).

time period (Feldhamer & McShea, 2012; Goymann et al., 2001; Robbins, Oftedal, & O'Rourke, 1987). Also, GCs, in conjunction with progesterone, are involved in secretory activation and milk production (Casey & Plaut, 2007). Furthermore, the challenges of maternal care in vigilance, protection, and feeding of young, are reflected in FGC levels (Cavigelli, 1999; Feldhamer & McShea, 2012; Lacerda, 2008; Maestripieri et al., 2008; Monclús et al., 2011; Spercoski, 2007). Pampas Deer mothers with young offspring tend to stay away from other individuals and behave in ways (more alert, less tolerant of others) that reduce predation likelihood (Lacerda, 2008; Rodrigues, 1996). This behavior pattern was illustrated in GPS-collared females in the Pantanal, where they tended to move around more during lactation (Nievas, 2012).

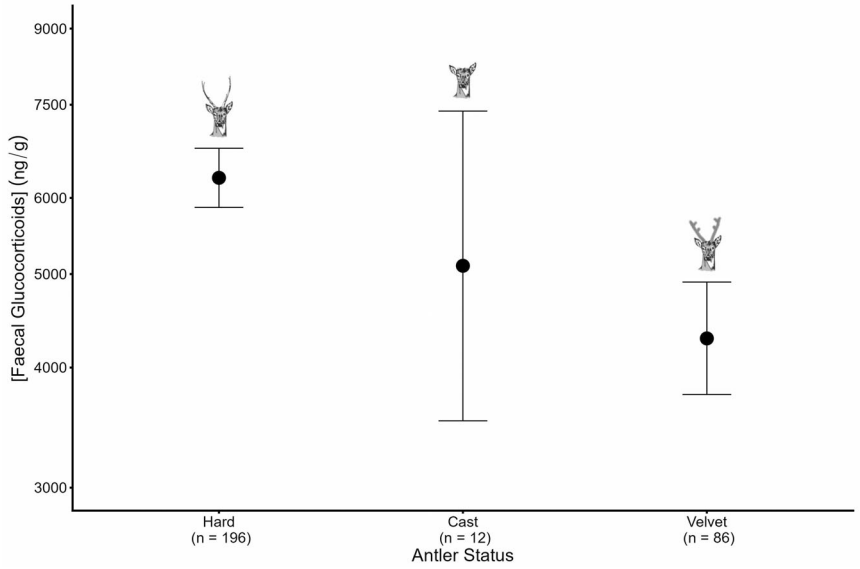

Figure 4 Comparisons of fecal glucocorticoid metabolite concentrations (mean and 95% confidence interval) among antler status in Pampas Deer (*Ozotoceros bezoarticus*) showing that concentrations are greatest (Tukey test, $P < 0.001$) when males have hard antlers versus velvet, in the Brazilian Pantanal.

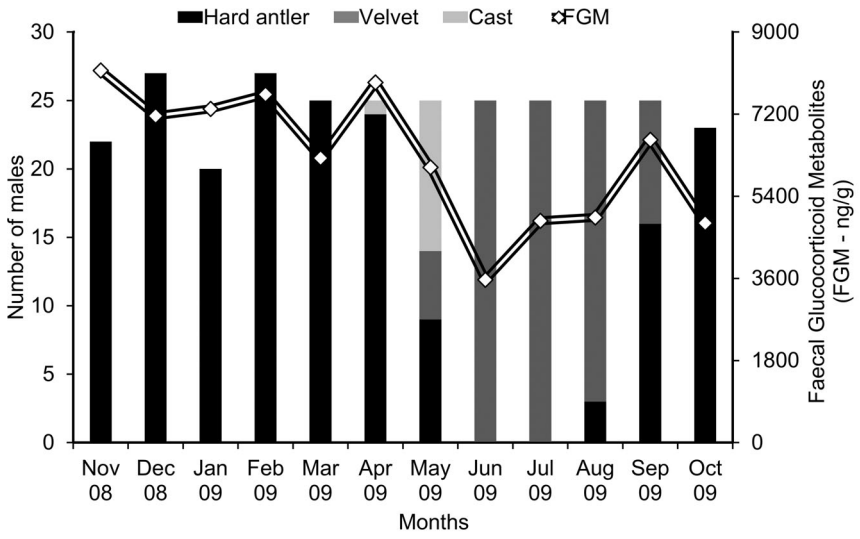

Figure 5 Monthly comparisons of the stage of antler growth (bars) and FGC concentrations (line) in male Pampas Deer (*Ozotoceros bezoarticus*) in the Brazilian Pantanal.

Thus, greater FGC in female Pampas Deer during lactation indicates the important physiological role of GC associated with lactation and increased vigilance to avoid predators.

Different from most mammals, pregnancy is similar to lactation in terms of metabolic costs and so in the transition from one to the other there may be no change in cortisol levels in ungulates (Edwards & Boonstra, 2018). Studies have shown that the pattern of cortisol excretion during pregnancy will only increase on the day of delivery (not included in our samples) when levels increase significantly (Brunet & Sebastian, 1991; Comline et al., 1974; Edwards & Boonstra, 2018; Patel et al., 1996; Wintour et al., 1976).

Fecal glucocorticoid metabolites in males were greatest during mating, which occurs during the summer in the Brazilian *Cerrado* (Pereira et al., 2006). During mating season, males compete with each other in disputes over females (Creel, 2005; Fichtel et al., 2007; Girard-Buttoz et al., 2009). In the White-tailed Deer (*Odocoileus virginianus*), FGC levels are

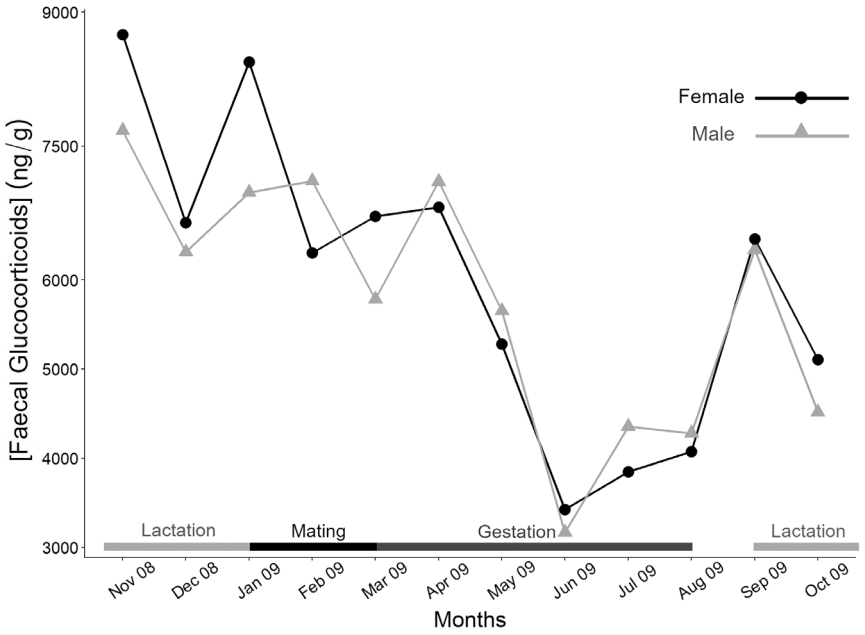

Figure 6 Annual profile of the mean FGC concentrations in female (black line) and male (gray line) Pampas Deer (*Ozotoceros bezoarticus*), with a horizontal bar indicating reproductive state (black – courtship and mating for both sexes, other months – non-mating for males, dark gray – gestation, and light gray – lactation for females), in the Brazilian Pantanal.

greatest during periods of intense aggression against other males over females (Mccoy & Ditchkoff, 2012). While Pampas Deer males tend to be more tolerant of others than the White-tailed Deer, they are most aggressive to other males during courtship and copulation (Lacerda, 2008; Merino et al., 1997; Rodrigues, 1996; Ungerfeld, González-Pensado, et al., 2008). At that time, males produce more testosterone associated with reproductive behaviors (Li et al., 2001; Mccoy & Ditchkoff, 2012; Pereira et al., 2005). Thus, as in other deer (Mccoy & Ditchkoff, 2012), the increase in FGC during mating associated with increased testosterone (Pereira et al., 2005), together prepare males for mate competition (Eggermann et al., 2013; Fichtel et al., 2007; Girard-Buttoz et al., 2009; Mccoy & Ditchkoff, 2012; Ostner, Kappeler, et al., 2008).

Antler growth seasonality in this study was similar to that in others (Pereira et al., 2005; Rodrigues, 1996; Tomas, 1995; Ungerfeld, González-Sierra, et al., 2008), and is associated with FGC and aggression and disputes among males (Jackson & Langguth, 1987; Lincoln, 1992; Pereira et al., 2005, 2006; Rodrigues, 1996). During the cast phase, males have a large variation in FGC, and we believe this is a result of the small sample size. Males with hard antlers in this study tended to have the greatest FGC concentrations. In contrast, in the *Cerrado*, males in velvet had the greatest concentrations (Pereira et al., 2006). This variation in FGCs by antler status among studies may be attributed to several factors, including climate and environment. For example, resource availability and temperatures are both associated with GC secretions in deer (Corlatti et al., 2011; Huber, Palme, & Arnold, 2003). Antlers of Pampas Deer are in velvet during the dry season, when food is more likely to be limiting, which may explain greater FGC concentrations in the dry season in that study (Pereira et al., 2006). In contrast, at the same time of year, flood waters are receding, and grasslands increasing in the Pantanal (Mamede & Alho, 2006). These flood and drought oscillations can affect spatial ecology and animal movement patterns (Grotta-Neto et al., 2019; Mamede & Alho, 2006; Tomas et al., 2001). The Pampas Deer is strictly found in grasslands and avoids flooded areas and forests (Pinder, 1997). Thus, the reduced availability of grassland in the summer, and estrous, may cause conflicts among males over territory and mating opportunities, resulting in greater FGC levels at that time.

Fecal GC variation in wildlife can be influenced by factors other than reproduction. Anthropic, social, climate, and other environmental factors influence GCs in cervids. For example, minimum ambient temperature and snow increased FGC levels of red deer, suggesting a catabolic action for adaptation in periods of limited resources (Corlatti et al., 2011; Huber, Palme, & Arnold, 2003; Huber, Palme, Zenker, et al., 2003). In Pampas Deer males, FGC concentrations were associated with human disturbance, season, and breeding (Pereira et al., 2006). We suggest that flooding and droughts in the Pantanal also influence FGC levels. As discussed above, reduced grassland area concurrent with mating can increase agonistic interactions and contribute to the secretion of FGC. Also, over time, FGC profiles are correlated between the sexes, indicating that similar environmental factors influence the secretion of FGC in both sexes. Future studies should consider multiple factors, such as habitat and resource availability,

extreme temperatures, climate change, and more in generalized models to determine the relative importance of each variable on stress (FGC) in the Pampas Deer.

From a conservation perspective, Pampas Deer is stressed by anthropic perturbations (Pereira et al., 2006). The Pantanal is currently suffering extensive environmental modification to favor cattle rearing, which alters food availability and increases the likelihood of transmission of zoonotic illnesses (Merino et al., 1997; Pinder, 1997). We recommend that FGC be continuously monitored under these conditions to determine when and how these perturbations influence stress levels, and consequently, population dynamics in the deer. In addition, considering the stress caused by capture (Munerato et al., 2015), we suggest only capturing deer (and only if necessary) in periods of the lowest stress (i.e., low FGC concentration). Here, we demonstrate that FGC is associated with reproductive seasonality in temporally similar ways in both sexes of Pampas Deer. Monitoring seasonality and stress through fecal samples is an effective, non-invasive way that avoids stressful capture and recaptures in other methods. Because population dynamics are tied to reproductive patterns and success, the use of FGC analysis should be an important tool for all monitoring and conservation studies in the remaining populations of neotropical deer.

Acknowledgments

We thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for their financial support to carry out the project (Proc. N° 2007/59562-7) and for the grants to FG-N (2009/09386-3), MDC (2007/06178-5), and CMH-R (2014/18954-3), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for supporting JMBD (302368/2018-3), the EMBRAPA Pantanal and Alegria ranche for their logistical support and help in the field, and James J. Roper for English review.

Conflict of interest

There are no conflicts of interest.

Author contributions

Mauricio D. Christofoletti, Ubiratan Piovezan, and José M. B. Duarte conceived the idea for the study and delineated the methodology used. Mauricio D. Christofoletti collected the data. Francisco Grotta-Neto, Mauricio D. Christofoletti, and Cláudia M. Ribas analyzed the data. Francisco Grotta-Neto and Mauricio D. Christofoletti wrote the manuscript. José M. B. Duarte was advisor for the research and he read and revised the manuscript. All the authors contributed critically to the drafts and gave final approval for publication.

References

Barja, I., Escribano-Avila, G., Lara-Romero, C., Virgos, E., Benito, J., & Rafart, E. (2012). Non-invasive monitoring of adrenocortical activity in European badgers (*Meles meles*) and effects of sample collection and storage on faecal cortisol metabolite concentrations. *Animal Biology*, **62**, 419–432.

Barrett, G. M., Shimizu, K., Bardi, M., Asaba, S., & Mori, A. (2002). Endocrine correlates of rank, reproduction, and female-directed aggression in male Japanese macaques (*Macaca fuscata*). *Hormones and Behavior*, **42**, 85–96.

Beehner, J. C., & McCann, C. (2008). Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (*Theropithecus gelada*). *Physiology and Behavior*, **95**, 508–514.

Bergman, T. J., Beehner, J. C., Cheney, D. L., Seyfarth, R. M., & Whitten, P. L. (2005). Correlates of stress in free-ranging male chacma baboons, *Papio hamadryas ursinus*. *Animal Behaviour*, **70**, 703–713.

Brown, J., Walker, S., & Steinman, K. (2004). *Endocrine manual for the reproductive assessment of domestic and non-domestic species* (2nd ed.). Smithsonian Institution.

Brunet, A. G., & Sebastian, A. L. (1991). Effect of season on plasma concentrations of prolactin and cortisol in pregnant, non-pregnant and lactating ewes. *Animal Reproduction Science*, **26**(3–4), 251–268.

Busch, D. S., & Hayward, L. S. (2009). Stress in a conservation context: A discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation*, **142**, 2844–2853.

Casey, T. M., & Plaut, K. (2007). The role of glucocorticoids in secretory activation and milk secretion, a historical perspective. *Journal of Mammary Gland Biology and Neoplasia*, **12**, 293–304.

Catão-Dias, J. L., & Camargo, C. M. S. (2010). Capture myopathy. In J. M. B. Duarte & S. González (Eds.), *Neotropical cervidoloy: Biology and medicine of Latin American deer* (pp. 324–329). Funep/IUCN.

Cavigelli, S. a. (1999). Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. *Animal Behaviour*, **57**, 935–944.

Christofoletti, M. D. (2010). *Caracterização da gestação em veado-campeiro (Ozotoceros bezoarticus) no Pantanal por meio de progestinas fecais*. Thesis, Universidade Estadual Paulista. Jaboticabal, São Paulo, Brasil.

Comline, R. S., Hall, L. W., Lavelle, R. B., Nathanielsz, P. W., & Silver, M. (1974). Parturition in the cow: Endocrine changes in animals with chronically implanted catheters in the foetal and maternal circulations. *Journal of Endocrinology*, **63**, 451–472.

Corlatti, L., Palme, R., Frey-Roos, F., & Hackländer, K. (2011). Climatic cues and glucocorticoids in a free-ranging riparian population of red deer (*Cervus elaphus*). *Folia Zoologica*, **60**, 176–180.

Creel, S. (2001). Social dominance and stress hormones. *Trends in Ecology & Evolution*, **16**, 491–497.

Creel, S. (2005). Dominance, aggression, and glucocorticoid levels in social carnivores. *Journal of Mammalogy*, **86**, 255–264.

Creel, S., Christianson, D., & Schuette, P. (2013). Glucocorticoid stress responses of lions in relationship to group composition, human land use, and proximity to people. *Conservation Physiology*, **1**, cot021.

Creel, S., Creel, N. M., Mills, M. G. L., & Monfort, S. L. (1997). Rank and reproduction in cooperatively breeding african wild dogs: Behavioral and endocrine correlates. *Behavioral Ecology*, **8**, 298–306.

Creel, S., Fox, J. E., Hardy, A., Sands, J., Garrott, B., & Peterson, R. O. (2002). Snowmobile activity and glucocorticoid stress responses in wolves and elk. *Conservation Biology*, **16**, 809–814.

Crockford, C., Wittig, R. M., Whitten, P. L., Seyfarth, R. M., & Cheney, D. L. (2008). Social stressors and coping mechanisms in wild female baboons (*Papio hamadryas ursinus*). *Hormones and Behavior*, **53**, 254–265.

Dixon, G., Marriott, A. S., Stelfox, G., Dunkerley, C., & Batke, S. P. (2021). How do red deer react to increased visitor numbers? A case study on human-deer encounter probability and its effect on cortisol stress responses. *Nature Conservation*, **43**, 55–78.

Edwards, P. D., & Boonstra, R. (2018). Glucocorticoids and CBG during pregnancy in mammals: Diversity, pattern, and function. *General and Comparative Endocrinology*, **259**, 122–130.

Eggermann, J., Theuerkauf, J., Pirga, B., Milanowski, A., & Gula, R. (2013). Stress-hormone levels of wolves in relation to breeding season, oack size, human activity, and prey density. *Annales Zoologici Fennici*, **50**, 170–175.

Eisenberg, J. F., & Redford, K. H. (1999). Order Artiodactyla (even-toed ungulates). In *Mammals of the neotropics* (3rd ed., pp. 332–355). The University of Chicago Press.

Ewacha, M. V. A., Roth, J. D., Anderson, W. G., Brannen, D. C., & Dupont, D. L. J. (2017). Disturbance and chronic levels of cortisol in boreal woodland caribou. *Journal of Wildlife Management*, **81**, 1266–1275.

Feldhamer, G. A., & McShea, W. J. (2012). *Deer: The animal answer guide*. The Johns Hopkins University Press.

Fichtel, C., Kraus, C., Ganswindt, A., & Heistermann, M. (2007). Influence of reproductive season and rank on fecal glucocorticoid levels in free-ranging male Verreaux's sifakas (*Propithecus verreauxi*). *Hormones and Behavior*, **51**, 640–648.

Foley, C. a. H., Papageorge, S., & Wasser, S. K. (2001). Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging african elephants. *Conservation Biology*, **15**, 1134–1142.

Gesquiere, L. R., Khan, M., Shek, L., Wango, T. L., Wango, E. O., Alberts, S. C., & Altmann, J. (2008). Coping with a challenging environment: Effects of seasonal variability and reproductive status on glucocorticoid concentrations of female baboons (*Papio cynocephalus*). *Hormones and Behavior*, **54**, 410–416.

Girard-Buttoz, C., Heistermann, M., Krummel, S., & Engelhardt, A. (2009). Seasonal and social influences on fecal androgen and glucocorticoid excretion in wild male long-tailed macaques (*Macaca fascicularis*). *Physiology and Behavior*, **98**, 168–175.

González, S., Cosse, M., Braga, F. G., Vila, A. R., Merino, M. L., Dellafiore, C., Cartes, J. L., Maffei, L., & Dixon, M. G. (2010). Pampas deer *Ozotoceros bezoarticus* (Linnaeus 1758). In J. M. B. Duarte & S. González (Eds.), *Neotropical cervidoloy: Biology and medicine of Latin American deer* (pp. 119–132). Funep/IUCN.

Goymann, W., East, M. L., Wachter, B., Höner, O. P., Möstl, E., Van't Hof, T. J., & Hofer, H. (2001). Social, state-dependent and environmental modulation of faecal corticosteroid levels in free-ranging female spotted hyenas. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 2453–2459.

Graham, L., Schwarzenberger, F., Möstl, E., Galama, W., & Savage, A. (2001). A versatile enzyme immunoassay for the determination of progestogens in feces and serum. *Zoo Biology*, **20**, 227–236.

Grotta-Neto, F., Peres, P. H. F., Piovezan, U., Passos, F. C., & Duarte, J. M. B. (2019). Influential factors on gray brocket deer (*Mazama gouazoubira*) activity and movement in the Pantanal, Brazil. *Journal of Mammalogy*, **100**, 454–463.

Hammond, T. T., Vo, M., Burton, C. T., Surber, L. L., Lacey, E. A., & Smith, J. E. (2019). Physiological and behavioral responses to anthropogenic stressors in a human-tolerant mammal. *Journal of Mammalogy*, **100**, 1928–1940.

Harper, J. M., & Austad, S. N. (2001). Effect of capture and season on fecal glucocorticoid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). *General and Comparative Endocrinology*, **123**, 337–344.

Huber, S., Palme, R., & Arnold, G. W. (2003). Effects of season, sex and sample collection on concentrations of faecal cortisol metabolites in red deer (*Cervus elaphus*). *General and Comparative Endocrinology*, **130**, 48–54.

Huber, S., Palme, R., Zenker, W., & Mostl, E. (2003). Non-invasive monitoring of the adrenocortical response in Red Deer. *Journal of Wildlife Management*, **67**, 258–266.

Jackson, J. E., & Langguth, A. (1987). Ecology and status of the pampas deer in the Argentinian pampas and Uruguay. In C. M. Wemmer (Ed.), *Biology and management of the Cervidae* (pp. 403–409). Smithsonian Institute Press.

Keay, J. M., Singh, J., Gaunt, M. C., & Kaur, T. (2006). Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: A literature review. *Journal of Zoo and Wildlife Medicine*, **37**, 234–244.

Konjević, D., Janicki, Z., Slavica, A., Severin, K., Krapinec, K., Božić, F., & Palme, R. (2011). Non-invasive monitoring of adrenocortical activity in free-ranging fallow deer (*Dama dama* L.). *European Journal of Wildlife Research*, **57**, 77–81.

Lacerda, A. C. R. (2008). *Ecologia e estrutura socialdo veado-campeiro (Ozotoceros bezoarticus) no Pantanal*. Dissertation, Universidade de Brasília. Brasília, Brazil.

Landys, M. M., Ramenofsky, M., & Wingfield, J. C. (2006). Actions of glucocorticoids at a seasonal baseline as compared

to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology*, **148**, 132–149.

Li, C., Jiang, Z., Jiang, G., & Fang, J. (2001). Seasonal changes of reproductive behavior and fecal steroid concentrations in Pére David's deer. *Hormones and Behavior*, **40**, 518–525.

Lincoln, G. A. (1992). Biology of antlers. *Journal of Zoology*, **226**, 517–528.

Maestripieri, D., Hoffman, C. L., Fulks, R., & Gerald, M. S. (2008). Plasma cortisol responses to stress in lactating and nonlactating female rhesus macaques. *Hormones and Behavior*, **53**, 170–176.

Mamede, S. B., & Alho, C. J. R. (2006). Response of wild mammals to seasonal shrinking-and-expansion of habitats due to flooding regime of the Pantanal, Brazil. *Brazilian Journal of Biology*, **66**, 991–998.

Mccoy, J. C., & Ditchkoff, S. S. (2012). Patterns of fecal hormones in a fenced population of white-tailed deer. *Wildlife Society Bulletin*, **36**, 641–646.

Merino, M. L., González, S., Leeuwenberg, F., Rodrigues, F. H. G., Pinder, L., & Tomas, W. M. (1997). Veado-campeiro (*Ozotoceros bezoarticus*). In J. M. B. Duarte & S. González (Eds.), *Biologia e Conservaçao de Cervídeos Sul-Americanos: Blastocerus, Ozotoceros e Mazama* (p. 238). FUNEP.

Millspaugh, J. J., & Washburn, B. E. (2004). Use of fecal glucocorticoid metabolite measures in conservation biology research: Considerations for application and interpretation. *General and Comparative Endocrinology*, **138**, 189–199.

Millspaugh, J. J., Woods, R. J., Hunt, K. E., Raedeke, K. J., Brundige, G. C., Washburn, B. E., Wasser, S. K., Millspaugh, J. J., Woods, R. J., Hunt, K. E., Kenneth, J., Brundige, G. C., Washburn, B. E., & Wasser, S. K. (2001). Fecal glucocorticoid assays and the physiological stress response in elk. *Wildlife Society Bulletin*, **29**, 899–907.

Monclús, R., Tiulim, J., & Blumstein, D. T. (2011). Older mothers follow conservative strategies under predator pressure: The adaptive role of maternal glucocorticoids in yellow-bellied marmots. *Hormones and Behavior*, **60**, 660–665.

Monfort, S. L., Brown, J. L., & Wildt, D. E. (1993). Episodic and seasonal rhythms of cortisol secretion in male Eld's deer (*Cervus eldi thamin*). *Journal of Endocrinology*, **138**, 41–49.

Möstl, E., & Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, **23**, 67–74.

Muller, M. N., & Wrangham, R. W. (2004). Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). *Behavioral Ecology and Sociobiology*, **55**, 332–340.

Munerato, M. S., Marques, J. A., Caulkett, N. A., Tomás, W. M., Zanetti, E. S., Trovati, R. G., Pereira, G. T., & Palme, R. (2015). Hormonal and behavioural stress responses to capture and radio-collar fitting in free-ranging pampas deer (*Ozotoceros bezoarticus*). *Animal Welfare*, **24**, 437–446.

Nievas, A. M. (2012). *Padrão de atividade de femeas de veado-campeiro (Ozotoceros bezoarticus) nas diferentes fases reprodutivas, no Pantanal Sul- Mato-Grossense*. Thesis, Universidade de São Paulo. Piracicaba, São Paulo, Brasil.

Ostner, J., Heistermann, M., & Schülke, O. (2008). Dominance, aggression and physiological stress in wild male Assamese macaques (*Macaca assamensis*). *Hormones and Behavior*, **54**, 613–619.

Ostner, J., Kappeler, P., & Heistermann, M. (2008). Androgen and glucocorticoid levels reflect seasonally occurring social challenges in male redfronted lemurs (*Eulemur fulvus rufus*). *Behavioral Ecology and Sociobiology*, **62**, 627–638.

Palme, R. (2019). Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & Behavior*, **199**, 229–243.

Patel, O. V., Takahashi, T., Takenouchi, N., Hirako, M., Sasaki, N., & Domekis, I. (1996). Peripheral cortisol levels throughout gestation in the cow: Effect of stage of gestation and foetal number. *British Veterinary Journal*, **152**(4), 425–432.

Pavitt, A. T., Pemberton, J. M., Kruuk, L. E. B., & Walling, C. A. (2016). Testosterone and cortisol concentrations vary with reproductive status in wild female red deer. *Ecology and Evolution*, **6**, 1163–1172.

Pavitt, A. T., Walling, C. A., Möstl, E., Pemberton, J. M., & Kruuk, L. E. B. (2015). Cortisol but not testosterone is repeatable and varies with reproductive effort in wild red deer stags. *General and Comparative Endocrinology*, **222**, 62–68.

Peña, E., Barja, I., & Carranza, J. (2021). Social environment with high intrasexual competition enhances the positive relationship between faecal testosterone and cortisol metabolite levels in red deer. *Mammalian Biology*, **101**, 207–215.

Pereira, R. J. G. (2010). Male reproduction. In J. M. B. Duarte & S. González (Eds.), *Neotropical cervidoloy: Biology and medicine of Latin American deer* (pp. 39–50). Funep/IUCN.

Pereira, R. J. G., Duarte, J. M. B., & Negrão, J. A. (2005). Seasonal changes in fecal testosterone concentrations and their relationship to the reproductive behavior, antler cycle and grouping patterns in free-ranging male Pampas deer (*Ozotoceros bezoarticus bezoarticus*). *Theriogenology*, **63**, 2113–2125.

Pereira, R. J. G., Duarte, J. M. B., & Negrão, J. A. (2006). Effects of environmental conditions, human activity, reproduction, antler cycle and grouping on fecal glucocorticoids of free-ranging pampas deer stags (*Ozotoceros bezoarticus bezoarticus*). *Hormones and Behavior*, **49**, 114–122.

Pereira, R. J. G., Zanetti, E. D. S., & Polegato, B. F. (2010). Female reproduction. In J. M. B. Duarte & S. González (Eds.), *Neotropical cervidoloy: Biology and medicine of Latin American deer* (pp. 51–63). Funep/IUCN.

Pinder, L. (1997). *Niche overlap among Brown brocket deer, pampas deer, and cattle in the Pantanal of Brazil*. University of Florida.

Piñeiro, A., Bárja, I., Silvn, G., & Illera, J. C. (2012). Effects of tourist pressure and reproduction on physiological stress response in wildcats: Management implications for species conservation. *Wildlife Research*, **39**, 532–539.

Pokharel, S. S., Seshagiri, P. B., & Sukumar, R. (2019). Influence of the number of calves and lactating adult females in a herd on the adrenocortical activity of free-ranging Asian elephants. *Wildlife Research*, **46**, 679–689.

Polegato, B. F. (2004). *Validação de método endócrino não invasivo para o monitoramento da fisiologia reprodutiva e da atividade dos glicocorticoides em cervídeos neotropicais*. Universidade Estadual Paulista. Jaboticabal, São Paulo, Brazil.

R Development Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. *R version 4.0.4*. https://www.R-project.org/

Ratter, J. A., Pott, A., Pott, V., Cunha, C. N., & Haridasan, M. (1988). *Observations on woddy vegetation types in the Pantanal and at Corumbá, Brazil*. Notes of the Royal Botanical Garden, Edinburg 45, 503–526.

Reeder, D. A. M., & Kramer, K. M. (2005). Stress in free-ranging mammals: Integrating physiology, ecology, and natural history. *Journal of Mammalogy*, **86**, 225–235.

Rehnus, M., Hackländer, K., & Palme, R. (2009). A non-invasive method for measuring glucocorticoid metabolites (GCM) in mountain hares (*Lepus timidus*). *European Journal of Wildlife Research*, **55**, 615–620.

Robbins, C. T., Oftedal, O. T., & O'Rourke, K. I. (1987). Lactation, early nutrition, and hand-rearing of wild ungulates, with special reference to deer. In C. M. Wemmer (Ed.), *Biology and management of the Cervidae* (pp. 429–442). Smithsonian Institute Press.

Rodela, L. G., & Queiroz Neto, J. P. (2007). Climate seasons on Nhecolândia Pantanal, Mato Grosso do Sul State, Brazil. *Revista Brasileira de Cartografia*, **59**(1), 101–113.

Rodela, L. G., Queiroz Neto, J. P., & Santos, S. A. (2007). *Classificação das pastagens nativas do Pantanal da Nhecolândia, Mato Grosso do Sul, por meio de imagens de satélite*. Anais XIII Simpósio Brasileiro de Sensoriamento Remoto. 4187–4194.

Rodrigues, F. H. G. (1996). *História natural e biologia comportamental do veado-campeiro Ozotoceros bezoarticus em Cerrado do Brasil Central*. Programa de Pós-graduação em Ecologia.

Romero, L. M. (2002). Seasonal changes in plasma glococorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology*, **128**, 1–24.

Sikes, R. S. (2016). 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy*, **97**, 663–688.

Smith, J. E., Monclús, R., Wantuck, D., Florant, G. L., & Blumstein, D. T. (2012). Fecal glucocorticoid metabolites in wild yellow-bellied marmots: Experimental validation, individual differences and ecological correlates. *General and Comparative Endocrinology*, **178**, 417–426.

Spercoski, K. M. (2007). *Monitoramento a longo prazo da excreção de corticóides fecais em Lobos-Guará (Chrysocyon brachyurus, Illiger 1811) de cativeiro e vida livre: Uma contribuiçao para o manejo e a conservação da espécie*. 68p. Thesis, Universidade Federal do Paraná. Curitiba, Paraná, Brazil.

Spercoski, K. M., Morais, R. N., Morato, R. G., de Paula, R. C., Azevedo, F. C., May-Júnior, J. A., Santos, J. P., Reghelin, A. L., Wildt, D. E., & Songsasen, N. (2012). Adrenal activity in maned wolves is higher on farmlands and park boundaries than within protected areas. *General and Comparative Endocrinology*, **179**, 232–240.

Strier, K. B., Ziegler, T. E., & Wittwer, D. J. (1999). Seasonal and social correlates of fecal testosterone and cortisol levels in wild male muriquis (*Brachyteles arachnoides*). *Hormones and Behavior*, **35**, 125–134.

Szott, I. D., Pretorius, Y., Ganswindt, A., & Koyama, N. F. (2020). Physiological stress response of African elephants to wildlife tourism in Madikwe game reserve, South Africa. *Wildlife Research*, **47**, 34–43.

Tanaka, Y., Sandoval, E. D. P., & Duarte, J. M. B. (2019). Non-homogeneous distribution of steroids in fecal pellets: An example in brown brocket deer (*Mazama gouazoubira*) with progesterone metabolites. *General and Comparative Endocrinology*, **282**, 113206.

Tomas, W. M. (1995). Seasonality of the antler cycle of pampas deer (*Ozotoceros bezoarticus leucogaster*) from the Pantanal wetland, Brazil. *Studies on Neotropical Fauna and Environment*, **30**, 221–227.

Tomas, W. M., De Salis, S. M., Da Silva, M. P., & De Miranda Mourão, G. (2001). Marsh deer (*Blastocerus dichotomus*) distribution as a function of floods in the Pantanal wetland, Brazil. *Studies on Neotropical Fauna and Environment*, **36**, 9–13.

Tomás, W. M., Mcshea, W., De Miranda, G. H. B., Moreira, J. R., Mourão, G., & Borges, P. L. (2001). A survey of a pampas deer, *Ozotoceros bezoarticus leucogaster* (Arctiodactyla, Cervidae), population in the Pantanal wetland, Brazil, using the distance sampling technique. *Animal Biodiversity and Conservation*, **1**, 101–106.

Ungerfeld, R., González-Pensado, S., Bielli, A., Villagrán, M., Olazabal, D., & Pérez, W. (2008). Reproductive biology of the pampas deer (*Ozotoceros bezoarticus*): A review. *Acta Veterinaria Scandinavica*, **50**, 16.

Ungerfeld, R., González-Sierra, U. T., & Bielli, A. (2008). Seasonal antler cycle in a herd of pampas deer (*Ozotoceros bezoarticus*) in Uruguay. *Mammalian Biology*, **73**, 388–391.

Wasser, S. K., Hunt, K. E., Brown, J. L., Cooper, K., Crockett, C. M., Bechert, U., Millspaugh, J. J., Larson, S., & Monfort, S. L. (2000). A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *General and Comparative Endocrinology*, **120**, 260–275.

Weingrill, T., Gray, D. A., Barrett, L., & Henzi, S. P. (2004). Fecal cortisol levels in free-ranging female chacma baboons: Relationship to dominance, reproductive state and environmental factors. *Hormones and Behavior*, **45**, 259–269.

Wintour, E. M., Blair-West, J. R., Brown, E. H., Coghlan, J. P., Denton, D. A., Nelson, J., Scoggins, B. A., Whipp, G. T., & Wright, R. D. (1976). The effect of pregnancy on mineralo- and gluco-corticoid secretion in the sheep. *Clinical and Experimental Pharmacology & Physiology*, **3**(4), 331–342.

Comline, R. S., Hall, L. W., Lavelle, R. B., Nathanielsz, P. W., & Silver, M. (1974). Parturition in the cow: endocrine changes in animals with chronically implanted catheters in the foetal and maternal circulations. *Journal of Endocrinology*, **63**, 451–472.