Monitoring cortisol metabolites in the faeces of captive fallow deer (Dama dama L.)

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ABSTRACT

Monitoring of the faecal glucocorticoid metabolites (fGCM) of various wildlife species has become an important non-invasive tool for wildlife managers that enables them to understand the influences of the season, sex, age and physiological status on the animal's organism and to discover potential stressors in order to adjust management practices and thus minimize their negative impact. Here we present a one-year study on fallow deer kept in extensive captive breeding in inland Croatia. We measured fGCM by 11-oxoaetiocholanolone enzyme immunoassay. The obtained results confirmed the seasonal pattern of cortisol release with the highest concentrations of 11.17-dioxoandrostanes (11,17-DOA) during the winter period (950; 430-2385 ng/g faeces, expressed as median, min. and max. values), followed by early summer (864; 186-3271 ng/g) and spring (610; 129-2896 ng/g). Significantly lower concentrations were determined during the late summer period (306; 95-2071 ng/g). Compared with fGCM levels in free-ranging fallow deer, concentrations in captive animals followed the same pattern, but with lower values in every season. This may be attributed to habituation and to the less challenging and more predictable environment under captive conditions.

Key words: fallow deer, cortisol metabolites, 11,17-DOA, faeces

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Introduction

Glucocorticoids (GC) are steroid hormones that participate in the regulation of several different systems and processes. These include the immune system through regulation of expression of inflammatory proteins, and the development and homeostasis of T-cells (PAZIRANDEH et al., 2002); metabolism through glucose regulation; response to environmental conditions (MATTERI et al., 2000) and participation in regulation of foetal development (both closely related to glucose management), and homeostasis of body fluids (LIU et al., 2010 and 2011). Furthermore, they are an important part of stress response through HPA axis (hypothalamo-pituitary-adrenocortical). The GC levels can be measured in various samples, such as blood, saliva, urine, faeces, hair, feathers, milk, eggs and even water in the case of fish (for a review see SHERIFF et al., 2011). Due to the differences in invasiveness of these methods, the ability to trace the effects of shortterm or only chronic stressors, and the fact that collecting certain samples (i.e. blood) is difficult and sometimes even impossible in wildlife, monitoring of GC metabolites in faeces (fGCM) offers several advantages over other methods. This non-invasive technique has been applied in numerous wildlife species, such as roe deer (DEHNHARD et al., 2001), elk and white-tailed deer (MILLSPAUGH et al., 2001 and 2002; TAILLON and CÔTÉ, 2008), wolves and elk (CREEL et al., 2002), red deer (HUBER et al., 2003a,b; CORLATTI et al., 2011), alpine chamois (CORLATTI et al., 2012), fallow deer (KONJEVIĆ et al., 2011), capercaillie (THIEL et al., 2011), camelids (ARIAS et al., 2013), Mountain hare (REHNUS et al., 2014), etc. Since pronounced species differences in GC metabolism and excretion were found, their measuring requires previous method validation for each species to ensure that the measured fGCM adequately reflects the increase in adrenal cortex activity so the results will be biologically meaningful (PALME, 2005; TOUMA and PALME, 2005). In the case of fallow deer, validation was performed by exogenous stimulation of adrenocortical activity, and it proved the physiological relevance of fGCM analysis in fallow deer (KONJEVIĆ et al., 2011). Levels of GC are frequently used to monitor stress responses, despite the fact that their secretion may be influenced by various conditions besides stress (MÖSTL and PALME, 2002). However, if other environmental conditions, habituation, influence of season and sex are known, monitoring the levels of fGCM can assist wildlife managers to recognize stressors and develop strategies to minimise their potential impact on the animals' well-being and health (MILLSPAUGH and WASHBURN, 2004; SHERIFF et al., 2011).

This study is part of a larger project that investigated for the first time adrenocortical activity in fallow deer (*Dama dama*) through non-invasive monitoring of fGCM (KONJEVIĆ, 2009). The aim of this study was to determine the annual pattern of glucocorticoid excretion in the faeces of captive fallow deer, to obtain referral values in routine management, and to compare the obtained values with those in free-ranging populations.

Materials and methods

Study site and animals. For the purpose of whole-year monitoring of adrenal activity in captive fallow deer, we collected faecal samples in the Radobojski Orehovec breeding site. Radobojski Orehovec is a small village situated in the Krapinsko-zagorska County. It is a hilly terrain habitat, with altitudes ranging from 250 to 450 m a.s.l. The climate is middle European continental, with subalpine characteristics. According to Köppen's classification, the climate belongs to Cfb (temperate humid climate with warm summer; ŠEGOTA and FILIPČIĆ, 2003), while according to Lang's rain factor, the area has a humid climate, with average annual precipitation of 1026 mm. The dominant forest community is Illyrian sessile oak and common hornbeam forest (*Epimedio-Carpinetum betuli* / Ht.938/Borh.963) (VUKELIĆ, 2012). The studied area is a breeding site for fallow deer and mouflon (*Ovis gmelini musimon* Pall.).

Sample collection. Faecal samples were collected on an anonymous base, trying to minimize potential sampling/sample identification errors (fallow deer are herd animals). Samples were not divided according to the sex of the animal, as no significant differences in glucocorticoid production between males and females have been observed in other deer species (BUBENIK et al., 1998; HUBER et al., 2003a). The animals were monitored from a distance without disturbing them, and the defecation site was noted on a map of the sampling area. After defecation, faecal samples were collected on the basis of this map, individually marked, immediately stored in an ice chest, and later frozen at - 20 °C. Only fresh samples were considered suitable and were collected. This protocol was followed in order to minimize possible bacterial influence on fGCM concentrations in the samples (as noted by PALME, 2005; TOUMA and PALME, 2005; LEXEN et al., 2008). Besides that, samples were only collected in dry conditions, as rainfall may decrease metabolite levels in exposed faeces (REHNUS et al., 2009).

Steroid extraction and EIA. In the lab, after thawing, the samples were homogenized and processed according to the standard protocol (PALME et al., 2013). We extracted 0.5 g faeces with 5 mL of 80 % methanol. After 30 minutes shaking in a multivortex, and centrifugation (2.500 g, 15 min) we determined the amounts of cortisol metabolites (11.17-dioxoandrostanes - 11,17-DOA) in the supernatant, by a group specific 11-oxoaetiocholanolone EIA. The EIA applied including cross-reactions is described in detail by PALME and MÖSTL (1997).

Statistical analysis. The obtained data were analysed using Statistica 12.0. (StatSoft, Inc., 2013). Normality of distribution was tested using Kolmogorov-Smirnov and Lilliefors tests. Due to the lack of normality of the original data, log-transformation of the data was performed. Levene's and Sheffé's post hoc tests were used to assess differences.

Results

A total of 106 faecal samples were collected during the one-year sampling period at the Radobojski Orehovec breeding site. The highest concentrations of 11,17-DOA in fallow deer faeces were recorded during the winter period (950; 430-2385 ng/g, mid-November, presented as median, min. and max. values) which was closely followed by the summer period (864; 186-3271 ng/g, June). Lower fGCM concentrations were recorded during the spring period (610; 129-2896 ng/g, March).

According to Levene's test, no differences in variances between the seasons were found (F = 2.390; p = 0.07). According to Sheffé's post hoc test (Table 1) significantly lower concentrations of 11,17-DOA in fallow deer faeces were recorded during the late summer-autumn season (306; 95-2071 ng/g). No significant differences were observed between the other three seasons. Levels of fGCM, according to the season, are presented in Fig. 1.

Table 1. Descriptive statistics and statistical test for differences in concentrations of 11,17-DOA in fallow deer faeces according to seasons. Different letters within a column denote significant differences between concentrations (P<0.05)

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Seasons	N	Median	Min	Max	
Spring	17	610 ^a	129	2896	
Early Summer	35	864ª	186	3271	
Late Summer/Autumn	29	306 ^b	95	2071	
Winter	29	950ª	430	2385	

Discussion

The results of the one-year study at the Radobojski Orehovec breeding site follow the pattern of adrenocortical activity observed in fallow deer from Brijuni Island (KONJEVIĆ et al., 2011) and that of other deer species that originate from a temperate climate (e.g. BUBENIK et al., 1983; HUBER et al., 2003a). Since GC plays a significant role in the regulation of glucose metabolism, it is believed that this change in adrenal activity represents adaptation to a harsh environment and relatively low forage availability. Consequently, an increasing production of GC leads to a shift from anabolic to catabolic metabolism. This shift means that the energy requirements of the organism will be satisfied primarily from its own reserves rather than from feedstuffs. A study performed by PEREIRA et al. (2006) on pampas deer in Brazil showed a similar pattern of glucocorticoid excretion, but in that case the dry season was found to be the main driver of differences between the summer and winter periods. This is of particular importance when planning autumn and winter-time feeding of farmed and otherwise captive fallow deer, especially males that enter the winter after the highly demanding rutting season. In breeders' practice this shift in metabolism means that the main period for supplemental feeding is late summer and autumn.

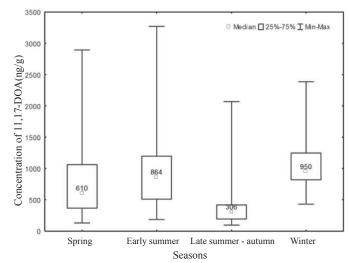


Fig. 1. Concentrations of fGCM (11,17-DOA) in fallow deer (ng/g) during the different seasons of the year

Interestingly, when compared with fGCM levels in fallow deer from the Brijuni Islands (KONJEVIĆ et al., 2011), the values obtained in this study are lower for every period studied. These differences may be explained partly by habituation resulting from the smaller living area and consequently close human-animal contact during daily, routine activities at the Radobojski Orehovec breeding site. In contrast, fallow deer on the Brijuni Islands are capable of avoiding humans by simply moving to other grazing or forested areas, thus retaining wilder, natural conditions. Similar effects of habituation on fGCM values were observed in wild western lowland gorillas (SHUTT et al., 2014). Furthermore, as regular daily activities such as searching for food and water, or avoidance of predators, affect glucocorticoid activity, the less challenging and more predictable captive environment will likely result in lower fGCM values (SMITH et al., 2012).

The observed range between minimal and maximal values may be attributable to high individual variations in adrenal activity, which is further emphasized by herd hierarchy, perception of the environment, physiological status, etc. (GOYMANN, 2012).

The results obtained in this study represent reference fGCM values for captive breeding of fallow deer in inland Croatia. Despite the fact that there is an increasing number of studies that indicate the problematic use of glucocorticoid levels in stress evaluation (VOELLMY et al., 2014), understanding of reference values and their comparison with levels after human-induced disturbances (e.g.: management techniques, handling, transportation, etc.), might be useful to identify fGCM levels that are the

result of potentially stressful events (KONJEVIĆ et al., 2011). Therefore, glucocorticoid excretion, when combined with other parameters, could provide answers regarding the averseness of certain events in the animal's environment (MORMEDE et al., 2007). In conclusion, monitoring fGCM levels is a valuable non-invasive tool that enables long-term longitudinal studies, which can provide insights into levels of stress (HUBER et al., 2003b; THIEL et al., 2008, 2011; VAN METER et al., 2009; KONJEVIĆ et al., 2011; PIROVINO et al., 2011; REHNUS et al., 2014; FORMENTI et al., 2015) and help wildlife managers to adopt adequate management strategies.

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SAŽETAK

Praćenje metabolita glukokortikoida u izmetu (fGCM) različitih vrsta divljih životinja predstavlja značajnu neinvazivnu metodu koja omogućava razumijevanje utjecaja godišnjeg doba, spola, dobi i fiziološkog statusa na organizam životinje. Pored toga omogućava i prepoznavanje možebitnih stresora s ciljem prilagodbe modela gospodarenja/upravljanja i posljedičnog ublažavanja negativnih posljedica stresa. U ovom radu prikazani su rezultati praćenja aktivnosti kore nadbubrežne žlijezde gaterski uzgajanih jelena lopatara na području kontinentalne Hrvatske. Vrijednosti fGCM u izmetu jelena lopatara određivane su imunoenzimnim testom. Dobiveni rezultati potvrdili su sezonski ritam lučenja kortizola pri čemu su najviše koncentracije 11,17-dioksoandrostana (11,17-DOA) zabilježene tijekom zime (950; 430-2385 ng/g, prikazane kao srednja, minimalna i maksimalna vrijednost), a nešto niže tijekom ranog ljeta (864; 186-3271 ng/g) i proljeća (610; 129-2896 ng/g). Značajno niže koncentracije utvrđene su tijekom kasnog ljeta (306; 95-2071 ng/g). Razvidno je da dinamika lučenja fGCM u gaterski uzgajanih jelena lopatara prati istu u slobodno živućih jedinki, ali su vrijednosti niže za svako godišnje doba. Uočene razlike mogu biti posljedica prilagodbe na blizinu čovjeka i rutinske zahvate u uzgoju, kao i manje zahtjevnog i predvidljivog okoliša u zatočeništvu.

Ključne riječi: jelen lopatar, metaboliti kortizola, 11,17-DOA, izmet