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Level of fecal glucocorticoid metabolites in cervids as a key to assessing their welfare

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Summary

Cervids are a group of animals that are particularly sensitive to negative stimuli from the environment. Undoubtedly, hunting is one of the factors that negatively affect the welfare of game animals. The aim of the study was to analyze the fecal glucocorticoid metabolites (FGM) of cervids in order to assess the level of welfare of red deer, European roe deer, fallow deer, and Eurasian elk during the non-hunting period. The average concentration of glucocorticosteroid metabolites in the feces of all cervids was 9.849 ng/g. The values ranged from 11.311 ng/g (*Capreolus capreolus*) to 8.536 ng/g (*Dama dama*). It was shown that the welfare of cervids in the study area was maintained during the non-hunting period. This research may be an introduction to further analyses and may contribute to discussion on the desirability of including the assessment of animal welfare in the management of wild animal populations in Poland.

Keywords: Cervidae, stress, hunting, fecal glucocorticoid metabolite

Cervidae are the most numerous family of game animals in Poland. In 2024, their population was estimated at 288,000 for red deer (*Cervus elaphus*), 890,300 for roe deer (*Capreolus capreolus)*, 36,113 for fallow deer (*Dama dama*), and 40,773 for Eurasian elk (*Alces alces*) (1). There has been a continuous increase in the number of cervids, which results in an increase in the harvest of these species. Members of the Cervidae family, except for elk, are therefore the most frequently harvested species of game animals in Poland. Hunting of elk has been subject to a moratorium since 2001 (year-round protection, Journal of Laws 2001 No 43, item 488). According to estimates by the Central Statistical Office, 107.062 red deer, 19.151 roe deer and 10.900 fallow deer were hunted in Poland in the 2023/2024 hunting season (as of March 10, 2024). It is worth emphasizing that this group of animals is highly sensitive, especially to negative stimuli of external origin (2, 20, 54, 66).

The stress response in animals is an important mechanism of adaptation to the environment, the primary purpose of which is survival (45). The physiological response of the body to a strong stimulus is the release of glucocorticosteroid hormones, the secretion of which is regulated by the hypothalamic-pituitary-adrenal axis (HPA hypothalamic-pituitary-adrenal) (17, 42, 45). Cortisol is considered a reliable indicator of the intensity of the HPA axis reaction (33, 58), which triggers the release of energy necessary for the body to take action in response to a stimulus (49). Determining the level of fecal glucocorticoid metabolites (FGM) in animal is a non-invasive method of assessing their welfare, since samples are collected in their habitat without exposing them to stressful stimuli. Such stimuli could not only affect the reading and reliability of the result (31, 48), but also have a negative impact on the health and welfare of cervids (8). The level of cortisol metabolite concentration in feces is strongly correlated with the levels of blood plasma parameters (61). Collection of feces is therefore a non-invasive method of obtaining samples during field studies, which is a convenient alternative to collection of blood samples (60). Methods that do not affect the functioning of animals during sample collection and measurement of the intensity of the stress response are widely used in many fields because of the possibility of repeatability of the test (9, 49, 50, 59, 68). It is estimated that the time from the stressor to the appearance of the glucocorticosteroid

signature in feces is from 6 to 24 hours, depending on the species (49, 60). Moreover, the concentrations of FGM accurately reflect the intensity of the stress reaction, because in the case of feces, we do not measure a pulsatile release of these hormones as we do when they are measured in blood or plasma (59). However, it is necessary to collect fresh feces because FGM are unstable, and their concentration decreases over time (16). Additionally, FGM are less dependent on fluctuations or pulsatile secretion of other hormones (4). It is believed that measuring FGM is one of more accurate methods of determining the endocrine profile of the animal (4, 25, 65).

Frequent and intense negative environmental stimuli can lead to various adverse effects (32, 37), thus affecting the condition of wild animal populations. First of all, increased cortisol levels are associated with reduced individual immunity (27), which in turn translates into weaker resistance to diseases (15). High levels of glucocorticosteroids are also highly correlated with behavioral and physiological disorders (34, 69). An increase in the level of glucocorticosteroid hormones disrupts, among others, the antler shedding cycle in red deer (3, 5) and affects the growth of antlers (18, 28). An adverse effect of cortisol on the body weight of animals, which is one of the determinants of reproductive success, has also been noted (56). As reported by Kotlarczyk (43), stress in hinds has negative effects on the regulation of the estrous cycle and ultimately on maintaining pregnancy.

Activities of hunters, such as taking game inventory, forest maintenance, protection of agricultural crops, or protecting game (e.g. by laying out salt licks or feeding), require their constant presence in hunting grounds. The presence of humans in refuges of animals is one of the factors that may disturb animal welfare. Hunting, however, may be the strongest stressor that negatively affects the welfare of game animals (17, 19). Hunting periods for individual species of deer in Poland are seasonal and closely associated with the intensification of human activities in their habitats. As a consequence, hunting can have a negative impact on the welfare of deer (67). Identifying potential stressors and analyzing their impact on the welfare of game animals can be important in managing their populations (11, 31, 40, 48). The analysis conducted in this study was aimed at determining the level of glucocorticosteroid metabolites in the faeces of cervids in order to assess their welfare level during the non-hunting period.

Material and methods

Characteristics of the study area. The Lubartów Forest District (LFD) is located in central and eastern Poland (51° 27' N, 22° 29' E). Poland has a temperate, warm transitional climate. The forests of the LFT are located mostly in the IV Mazovian-Podlasie Region, the 5th district of the Podlasie Lowland, and the Siedlce Upland. The terrain is flat and not very diverse. The annual rainfall is 552 mm, and the average annual temperature is +7.7°C. The LFD is characterized by a large diversity of habitats and soil fertility. It is estimated that forests cover 24.9% of the region, with 49% of the forested area occupied by coniferous tree species and 38% by mixed forests with a predominance of deciduous species (https://www.lublin.lasy.gov.pl/). The density of cervids in the LFD amounts to 16.03 per 1000 ha of forest area for red deer, 2.08 per 1000 ha of total area for roe deer, and 10.15 per 1000 ha of forest and marsh area for elk. It should be mentioned that Eurasian elk, as a game species, is subject to a year-round moratorium in accordance with the Regulation of the Minister of the Environment of April 10, 2001 (https://isap.sejm.gov.pl/). This study was not subject to evaluation and approval by the local Ethics Committee because the biological material was obtained in a completely non-invasive manner.

Biological sample material. Faecal samples from cervids were collected in the LFD. Twenty-seven samples were obtained between March 15 and March 30, 2024. This period was selected because, in the Lublin province, cervids are not hunted from March 1 until the start of the roe deer season, i.e. May 11. Qualified employees of the LFD collected fresh faeces immediately after defecation of cervids spotted in forest complexes. After having been spotted, a cervid was followed for approximately 70-80 meters until it defecated. Then, the faeces were collected using disposable gloves directly into a string bag. To avoid taking a faecal sample from the same individual, they were collected in different locations within the LFD. All samples were therefore considered independent and unique. Samples were collected in the early morning hours from 5:00 to 9:00. This avoided diurnal fluctuations in cortisol and their potential impact on the results of the final analysis (31, 38, 44, 55). After collection, fecal samples were cooled to 4°C. Within 60 minutes, feces were frozen at –20°C. The procedure for collecting feces in the field was carried out in accordance with the guidelines of the American Society of Mammalogists (62). Then, the frozen samples were placed in a drying oven (at 57°C for 72 hours). Completely dry samples were ground to obtain a homogeneous powder (31, 47, 63). Metabolites were extracted by mixing 0.5 g of the sample with 5 ml of methanol and stirring for 30 s (30). The mixture was left in the mixer for 14 h and then stirred again for 10 s. Samples were centrifuged for 20 min. The supernatant was separated and stored at –20°C until samples were analyzed. Glucocorticosteroid concentrations were measured using the R4866 antibody (CJ Munro, University of California, Davis, CA, USA).

Descriptive statistics were used to summarize the set of values obtained. Statistical calculations were estimated at the significance level of $\alpha = 0.005$ using the statistical package Statistica 13.3.

Results and discussion

During the study, twenty-seven fecal samples (Tab. 1) were collected from four different species of cervids. The lowest recorded value of FGM was 6.517 ng/mg (in a sample from a fallow deer) and the highest was 13.467 ng/mg (in a sample from a European roe deer) (Tab. 1).

Tab. 1. Concentration of FGM in samples collected from the four cervid species

Species	Red deer	Fallow deer	Roe deer	Eurasian elk
FCM [ng/g]	9.428	9.027	12.484	10.355
	12.506	8.430	10.296	9.692
	10.871	6.517	9.881	8.881
	9.681	10.947	12.293	7.935
	9.854	7.230	9.925	7.598
	11.026	9.063	11.268	9.762
	10.243	–	10.873	–
	–	–	13.467	–
Number of animals	7	6	8	6
Total	27			

Tab. 2. Descriptive statistics for the parameters analyzed in the four cervid species

Species	Number of animals	Mean FGM [ng/g]	Standard deviation	Minimum	Maximum	Standard error	Coefficient of variation
Red deer	7	10.515	1.059	9.428	12.506	0.400	0.100
Fallow deer	6	8.535	1.557	6.517	10.947	0.635	0.182
Roe deer	8	11.310	1.319	9.881	13.467	0.466	0.116
Eurasian elk	6	9.037	1.095	7.598	10.355	0.447	0.121
Mean FGM	9.849						

The mean concentration of FGM in all samples was 9.849 ng/g. The highest mean value was determined for the European roe deer (11.311 ng/g) and the lowest for the fallow deer (8.536 ng/g). The range of individual values was largest for fallow deer and smallest for Eurasian elk (Tab. 2).

Jachowski et al. (36) showed that the concentration of FGM for *Cervus elaphus* living in an environment exposed to human disturbance was almost twice as high (i.e. 20.0 ng/g) as in the present study. Analyses of red deer living in the Greater Yellowstone Ecosystem revealed that the average level of these metabolites was three times as high (approximately 30 ng/g) (26). On the other hand, in a study aimed at determining the effect of the season on the FGM concentration, Huber et al. (35) obtained values ranging from 0 to approx. 850 ng/g, with a peak in winter months (December and January). Millspaugh et al. (47) found that the concentration of FGM in red deer in winter months amounted to 3.42 ng/g for males and 34.21 ng/g for females. It should be emphasized that these were the lowest values obtained from year-round observations of this species. The values obtained in the present study were also within the range given by Millsqaugh et al. (47).

Konjević et al. (41) in their annual study conducted on breeding fallow deer showed the influence of season on the readings of FGM. The results were highest in winter (from 430-2385 ng/g) and lowest in late summer (95-2071 ng/g). Lower values were determined for wild fallow deer in Mediterranean habitats (Veliki Brijun Island, Croatia). The concentration of FGM was highest in samples collected in November (from 50-2035 ng/g) and lowest in those collected in July (10-195 ng/g) (41). FGM values obtained in all of the above-mentioned analyses were higher than those for fallow deer in the present study (8.535 ng/mg).

In studies conducted on male roe deer kept in a pen, Dehnhard et al. (14) determined the base level of FGM at the level of 31-78 ng/g. FGM concentrations for trapped roe deer ranged from 167 to 4914 ng/g (6) and were much higher than the values obtained in the present study (11.310 ng/g).

The average concentration of FGM for Eurasian elk in the present study was 9.037 ng/g (Tab. 2). This is also a very low value compared to reports from other researchers. Tomeo (64), examining the effects of winter recreation/sporting human activity on stress in elk in Alaska, documented FGM at 77.84 ng/g (lowest reading). Ensminger et al. (23), in their study on the effects of hunting activity on FGM, found values ranging from 10 to approximately 225 ng/g. FGM levels in elk exposed to gray wolf predation ranged widely, peaking at approximately 825 ng/g. The authors explained this by a late gestational increase in plasma steroid binding globulin in females (12).

The studies and analyses we conducted indicate a low exposure to stress in the cervids studied. It should be emphasized, however, that there are currently no other reports on FGM for cervids in the study area. It is therefore impossible to conduct a comparative analysis. On the other hand, the average concentrations were very low compared to those reported by the other researchers cited here. The discrepancies may be due to the climate, different environmental factors (temperature, humidity, etc.), differences between species (35), and direct human intervention, e.g. through capture (14). Previous studies conducted on male roe deer show a wide range of cortisol concentrations in fur, which may indicate that, in the case of cervids, individual variability, including the time of exposure to a given stimulus or individual resistance to stress, may be a decisive factor influencing the secretion of cortisol from the adrenal cortex (18, 39). Disproportions between species will result primarily from differences in the food base, as well as different rates of metabolic processes and rates of excretion of steroid metabolites (38, 46, 48, 59). In addition, important factors influencing the secretion of glucocorticosteroids in free-living

animals are the variability of seasons and prevailing climatic conditions, as well as the seasonality of reproduction (10, 22, 24, 29, 31, 57). Corlatti et al. (10) and Huber et al. (35) showed that during periods of low temperatures with snow cover, the concentrations of FGM for red deer increased. This was directly related to the process of adaptation of the organism to difficult environmental conditions, including limitated resources. It was also proven that the FGM level was significantly higher for red deer stags that gathered larger harems during the rutting period (52, 53). Pavitt et al. (51) also showed that in red deer does, higher levels of FGM were characteristic of lactating females, i.e. those incurring the highest costs in terms of increased energy and metabolic demand (7, 31). In ungulates, pregnancy places a similar burden on the female's body as lactation, and therefore FGM remain at a similar level in both physiological states (21). Therefore, the sex of the animal may influence FGM concentration.

Based on the present research and comparative analysis, we can conclude that despite the absence of hunters in the hunting grounds during sampling and the lack of any hunting pressure, the cervids may have been affected by other negative environmental factors related, for example, to temperature fluctuations, the sex or physiological state of the animals, or their individual reactivity to negative stimuli. These factors may have produced a slight increase in the concentration of FGM. It should be noted here that the analysis of the FGM concentration is a reliable and, above all, non-invasive method of monitoring the welfare of animals exposed to various negative factors, both exogenous and endogenous. The results obtained in this study can be regarded as a preliminary assessment that needs to be complemented by further analyses. They demonstrate the usefulness of the adopted method in studies on free-living animals. Due to climate variability and its impact on fluctuations in the secretion of glucocorticoid hormones, reference values used as the basis for assessing animal welfare must be determined from data collected in specific areas. In the near future, they may be used to precisely determine the level of welfare of Polish game populations, which should be an important tool for management of hunt animals in Poland.

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