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ORIGINAL PAPER

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Establishing baseline values of parameters potentially indicative of chronic stress in red deer (*Cervus elaphus*) from different habitats in western Germany

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Abstract The use of habitats by wild animals is commonly assumed to be decreasing due to human activities, such as tourism or the installation and use of wind-energy plants. These anthropogenic interferences may subject animals to chronic stress. To be able to objectively characterise the effects on animal populations or on individual animals, the collection of data that might be suitable to monitor such chronic stress is required. In this study of hunted red deer, we report data that are related to adrenal activity and are not affected by the acute stress induced by hunting. Adrenal glands and samples from ileal digesta were collected from 75 hunted deer from seven different habitats in the German Rhineland. The adrenal glands were evaluated histomorphometrically; in the digesta, the concentration of cortisol metabolites, i.e. of 11,17-dioxoandrostanes (11,17-DOA), was measured. Digesta were also examined for parasites. Animals were grouped according to age, sex, habitat, and hunting method. Animals were infected with gastrointestinal helminths and lungworms; examination for liver flukes was negative. Significant differences were not established among the different groups for any of the recorded parameters. For sex, a tendency ($P = 0.11$) towards higher DOA levels was observed in female deer when compared to male deer. The variability of the parameters together with the lack of identifiable influences of hunting indicates that chronic stress might indeed have been a relevant factor. However, none of the parameters analysed can presently be validly used to evaluate habitat quality for red deer since physiological stressors cannot be differentiated.

Keywords Adrenal gland · Glucocorticoids · Faeces · Digesta · Parasites · Red deer

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Introduction

Animals have evolved mechanisms to cope with the short-term stressors affecting their lives. Survival is important and the cost of stress is less important to the animal. Only if the resources needed for a stress response are shifted away from other biological functions will these functions be impaired. Chronic stress is known to result in disturbances of behaviour, reproduction, immune defense and other pathological alterations (Munck et al. 1984; Moberg 2000). Because of its effects on reproduction and mortality, stress has been hypothesised to be of major importance in the regulation of natural populations (Harper and Austad 2000). In order to be able to evaluate the habitat of wildlife species for potential stressors that may exert chronically stressful effects on the respective population, it is necessary to be able to determine chronic stress in the animals concerned (Bubenik and Bubenik 1965).

During stressful events, the adrenal glands secrete stress hormones. When monitoring the blood concentrations of cortisol, the main glucocorticoid secreted from the adrenal cortex, natural circadian and seasonal rhythms have to be taken into account, and therefore this approach is limited to laboratory set-ups. Although some of these problems can be mitigated, e.g. by measuring cortisol levels in saliva (Kirschbaum and Hellhammer 2000), any attempt to collect blood or other body fluids from wild free-ranging animals induces an acute stress response, and such measurements will thus not allow for the evaluation of chronic stress.

Assessment of faecal glucocorticoid metabolites has been proposed as a non-invasive method applicable also to wild animals (Palme et al. 1999; Harper and Austad 2000). The concentrations of 11,17-dioxoandrostanes (11,17-DOA) in faeces have been demonstrated to correlate with blood cortisol levels with a delay of about 10 h in ruminants (Palme et al. 1999). Faecal samples can also be easily collected from free-ranging animals, but assignment of the samples to individual animals is hardly possible and in hunted animals droppings may not be collected from the rectum since defaecation is a concomitant phenomenon of acute stress (Vessier and Le Neidre 1992). Moreover, the faecal concentrations of the cortisol metabolites are dependent on microbial fermentation, i.e. once the faeces are deposited, concentrations will change. In cattle, it has been demonstrated that 11,17-DOA concentrations will more than double within 4 h of storage at room temperature (Möstl et al. 1999). Data from faecal glucocorticoid measurements in wild animals have been published, but most studies were performed on animals from enclosures in which the time between defaecation and sample collection could be limited to consistently short intervals (Harper and Austad 2000; Teskey-Gerstl et al. 2000).

Other approaches to characterise chronic stress in wild animals are likely to be applicable also in animals that have been shot, e.g. evaluation of the adrenal glands for signs of hypertrophy (for review see Ladewig 1994).

We aimed to record parameters that might be useful as indicators of chronic stress in different populations of free-ranging red deer in order to collect data on variability and to identify factors of influence such as age and sex (Benert 1981). Considering the limitations outlined before, we collected the adrenal glands from hunted animals and also measured 11,17-DOA in samples obtained from the small intestine, i.e. in ileal digesta. Adrenal glands were weighed, measured and evaluated histologically.

Materials and methods

Samples were obtained from a total of 75 individuals. Animals were either shot during silent beat hunting ($n = 64$) or during still hunting ($n = 8$) in different parts of western Germany. Three animals were shot in an enclosure. The following age groups were included: 23 calves (about 5 months old, 10 males, 13 females), 15 young deer (about 1.5 years of age; 3 spike stags, 12 hearsts) and 37 deer older than 18 months (17 stags, 20 hinds). Most of the animals were from the hunting grounds of the Prince of Wied in Neuwied ($n = 57$); the remaining animals were from habitats in the Westerwald ($n = 5$), in Bad Driburg ($n = 2$), Gemünd ($n = 6$), Vogelsang ($n = 2$), and from an enclosure in Warstein ($n = 3$).

Shot animals were either first transferred to a dissecting place or were immediately eviscerated where shot. The age of the animals was estimated based on body size, dental status and, where applicable, on antler growth. In case of the animals sampled at Neuwied, the dressed body weights were recorded. During evisceration, both adrenal glands were collected if found intact. For histological assessments, the adrenal glands were fixed in 4% acid-free formaldehyde (Roti-Histofix, Carl Roth, Karlsruhe, Germany), dehydrated and embedded in paraffin (Roti-Plast, Carl Roth). Histological sections of 5–6 μm thickness were cut on a microtome (Leitz, Wetzlar, Germany) and stained with haematoxylin and eosin. Gross appearance was microscopically evaluated, the areas of the cortical and medullar portions were determined (pixel units) using an image-analysis program (Leica), and the ratio of cortex to medulla was calculated.

Ileal digesta were collected, aliquoted and either immediately frozen and stored at −20°C until analysed for 11,17-DOA (Möstl et al.1999) or were tested for the presence of coccidia oocysts and helminth eggs using a faecal flotation technique together with MacMaster helminth egg-counting slides to estimate the parasite burden. In addition, presence of liver flukes and lung worms was investigated in the samples using the sedimentation technique and the Baermann technique respectively. For technical reasons, parasitological examination was done on only 41 of the 75 animals under investigation.

Data were first subjected to descriptive statistical analysis, i.e. the distributions were assessed and depicted as box-plot diagrams where appropriate. For comparisons of two or more groups, the non-parametric Kruskal-Wallis test was applied. Relationships between different parameters were evaluated by calculating the coefficients of correlation (normally distributed data: Pearsons's correlation coefficient; nonparametric approach: Spearman's rank correlation coefficient).

Results

Age, sex, provenance and mode of hunting had no effect ($P > 0.05$) on the adrenal cortex-to-medulla ratio in either the left or the right gland. As shown in a representative example (Fig. 1) for different ages and sexes, the cortex comprised about 80% of the cross-sectional area. In cases where both adrenals of an individual could be obtained and histologically examined, values obtained for the entire cross-sectional area from both the right and the left gland were related ($n = 13$, $r = 0.58$, $P < 0.05$). An almost significant relationship existed between the size of the right adrenal gland, i.e. its cross-sectional area, and dressed body weight in the animals for which both parameters could be recorded ($n = 27$, $r = 0.52$, $P = 0.06$).

The concentration of 11,17-DOA measured in ileal digesta showed high individual variation, with values ranging from 9.7–148 ng/g wet wt. digesta. When concentrations in digesta of male and female deer were compared (Fig. 2a), there was a tendency ($P = 0.113$) towards higher values in females. Concentrations were

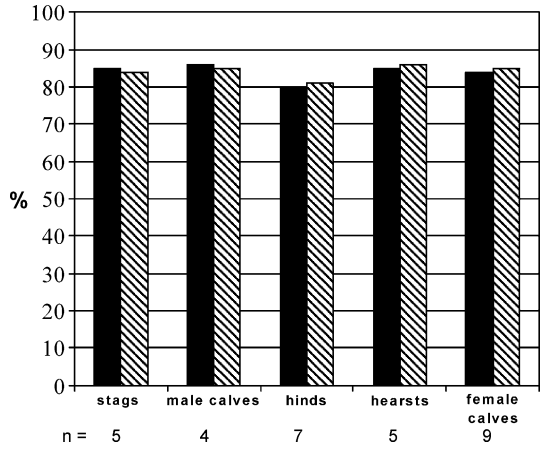

Fig. 1 Proportions of the adrenal cortex (means) in the cross-sectional area from the middle part of the right (*solid bars*) and the left adrenal gland (*shaded bars*) from deer of different ages and sex. Data were obtained from 44 animals for the right gland and from 31 for the left gland. Data for the three spike stags are not shown since only the left gland could be obtained from one of these animals. There was no statistical difference ($P > 0.05$) between the groups

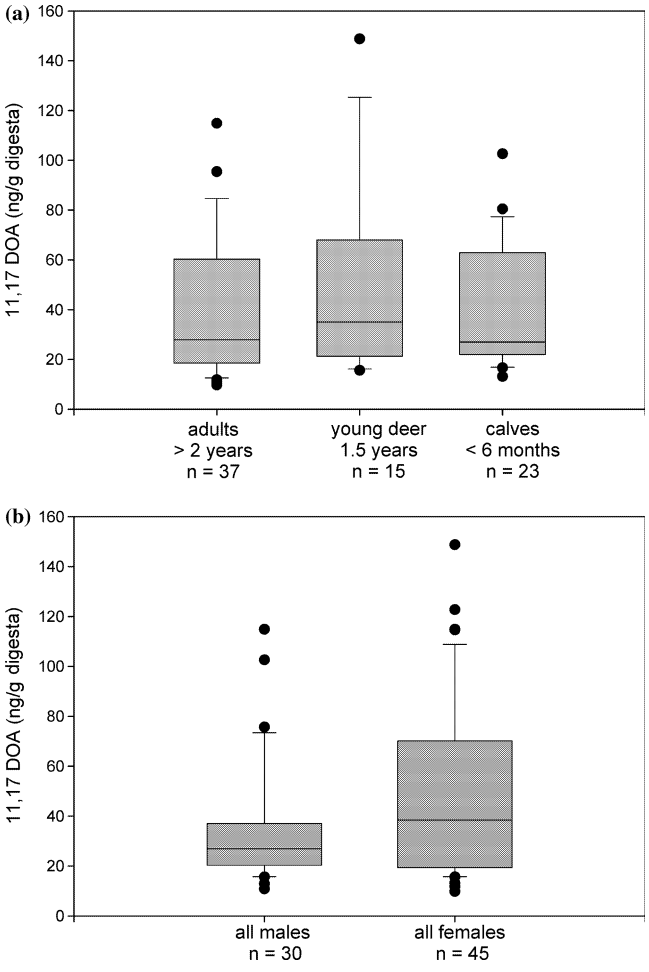

Fig. 2 Box plot showing cortisol metabolite concentrations (*11, 17-DOA* 11,17-dioxoandrostanes) in ileal digesta collected from 65 deer of different ages (**a**) or sexes (**b**). The *horizontal solid line within the box* indicates the median, the *boundaries of the box* indicate the upper and the lower quartile (25th and 75th percentiles respectively); the *lines above and below the box* indicate the 95th and the 5th percentiles respectively. Outliers are depicted as *closed circles*. **a** For the comparison of age groups, no differences were detectable ($P > 0.05$) between the 11,17-DOA concentrations in samples from male or female deer (including all age groups). **b** A tendency ($P = 0.113$) to higher concentrations was observed in females versus males

unrelated to age (Fig. 2b). When comparing the two modes of hunting, the concentrations were more variable in digesta from deer hunted by silent beat than in animals shot in still hunts, but the difference was not statistically significant. Provenance was not significantly related to 11,17-DOA concentrations.

A positive relationship between cross-sectional area of the right and left adrenal gland and 11,17-DOA concentrations in the digesta was observed: $r = 0.48$, $n = 39$ and $r = 0.56$, $n = 28$ respectively ($P < 0.05$).

Parasitological examination showed no flukes in the animals examined. In contrast, helminth eggs (mainly gastrointestinal nematodes) were observed in all samples; lung worm infection was found in most of the animals, with prevalences of 73% for *Dictyocaulus* larvae and 63% for *Varestrongylus* larvae. Coccidiae were sporadically observed, i.e. in 7 out of the 44 animals investigated. There was no obvious relationship between parasitic burdens and the 11,17-DOA concentration.

Discussion

The present study provides ranges of values for parameters potentially indicative of chronic stress in red deer of both sexes and different age classes from habitats exposed to varying levels of human impact.

For adrenal-gland size and composition, the only significant relationship observed was between body weight and adrenal cross-sectional area. However, this relationship was not very strong and thus body weight is not a factor that can easily be used to correct for adrenal-gland size in studies on chronic stress exposure in wild animals. There are various factors that might induce allometric growth of the adrenals in relation to body growth. During ontogenesis, growth of the adrenal glands may be positively or negatively allometric, i.e. exhibit accelerated or reduced growth rates compared to body growth. This has been demonstrated for development in human fetuses (de Aragao and Mandarim-de-Lacerda 1990). Differing genetic background and metabolic status may also affect adrenal size as indicated by the studies of Wise et al. (1993), in which increased adrenal weights were reported for pigs selected for high serum cholesterol concentrations. Differences in adrenal morphological characteristics attributable to sex were not found in the springbok (*Antidorcas marsupialis*) (Rijswijk and Vorster 1995) or for the Chinese water deer (*Hydropotes inermis)* (Hastings et al. 1992).

The concentrations of 11,17-DOA measured in ileal digesta of the red deer were within the range of basal values reported for faeces from white-tailed deer (*Odocoileus virginianus*: about 50–150 ng/g faeces; Washburn and Millspaugh 2002) and for roe deer (*Capreolus capreolus*; 13–78 ng/g faeces; Dehnhard et al. 2001). For dairy cows, faecal 11,17-DOA concentrations ranging from 25–295 nmol/kg, i.e. 8–90 ng/g faeces were reported (Hopster et al. 2002).

Besides sex, for which a tendency towards higher values in digesta from females was observed in this study, none of the factors recorded for the individual animals showed an apparent relationship with 11,17-DOA concentrations. The tendency towards higher values in females corresponds with the more pronounced avoidance behaviour of female red deer. From winter to early summer enemy-recognition distance and flight distance of groups of hinds and calves exceeded those of stag groups (Petrak 1996). Sex differences in faecal 11,17-DOA values have not been reported for wild ruminant species, but there is one report suggesting that sex does not affect cortisol metabolite levels in anonymous faecal samples from red deer (Huber et al. 2003). However, Kock et al. (1987) suggested from their study in bighorn sheep (*Ovis canadensis*) that the difference in blood cortisol levels between animal groups captured with different methods might be at least partially explained by the distribution of sexes within the groups.

The individual variability observed in the present study for 11,17 DOA values might be hypothetically attributed mainly to factors that could not be monitored. Numerous studies have clearly demonstrated that adrenal activity and, in consequence, faecal glucocorticoids increase after exposure of the animals to various stressors or after challenging them with ACTH (adrenocorticotropic hormone) injections, for example (Palme et al. 1999; Dehnhard et al. 2001). Therefore the observed variation might indeed reflect varying levels of stress-inducing social-rank or habitat conditions. It should be noted that this is the first investigation using ileal digesta; it is not yet known whether similar time relations can be assumed as between elevated blood cortisol concentrations and faecal concentrations. Concentrations in ileal digesta might rise earlier and therefore hunting-related effects might not be able to be excluded. However, this cannot be verified on the basis of our data and thus none of the parameters evaluated by us can presently be used to characterise chronic stress in wild deer. The same might be true for other parameters that could be considered in this context, in particular for cortisol determinations in hair. Different glucocorticoids can be measured in human hair (Crimele et al. 1999), and cortisol could indeed be quantified in a hair sample obtained of a red deer from our study (W. Schänzer, Institute for Biochemistry, Deutsche Sporthochschule, Cologne, Germany; personal communication). Nevertheless the problem of individual variation will most probably remain.

Parasitic infections have been related to adrenal activity. Most of the reports demonstrated elevated adrenal activity in animals inoculated with various parasites (e.g. Fleming 1997, 1998), but when considering the time course after infection, cortisol levels varied and may even have decreased (Morales-Montor et al. 2001). The spectrum of the parasites identified in the present study corresponds well with another study in which the parasite fauna of deer from the same habitats or from nearby areas of North Rhine-Westphalia were described in detail (Rehbein et al. 2002). Although occurrence of parasites or their eggs in faecal samples confirms parasitism, a negative faecal exam cannot be taken as clear-cut evidence of the absence of parasites. Moreover, the intensity of parasitic infection cannot validly be derived from faecal egg counts. We therefore refrained from classifying the samples according to this criterion and thus from comparing adrenal-gland size or 11,17-DOA concentrations between such classes.

In conclusion, none of the parameters recorded in the present study can validly be used to mirror chronic stress in wild, free-ranging deer. Whether a study with larger samples would allow more detailed and conclusive statements remains to be clarified. At present the reasons underlying the observed variability cannot be attributed to any of the factors known to increase adrenal activity.

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