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# Noninvasive Monitoring of Adrenocortical Activity in Roe Deer (*Capreolus capreolus*) by Measurement of Fecal Cortisol Metabolites

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A method for measuring glucocorticoids noninvasively in feces of roe deer was established and validated. The enzyme immunoassay (EIA) measures 11,17-dioxoandrostanes (11,17-DOA), a group of cortisol metabolites. Such measurement avoids blood sampling and reflects a dampened pattern of diurnal glucocorticoid secretion, providing an integrated measure of adrenocortical activity. After high-performance liquid chromatography, the presence of at least three different immunoreactive 11,17-DOA in the feces of roe deer was demonstrated. The physiological relevance of these fecal cortisol metabolites to adrenocortical activity was evaluated with an adrenocorticotropic hormone challenge test: cortisol metabolite concentrations exceeded pretreatment levels (31–78 ng/g) up to 13-fold (183–944 ng/g) within 8–23 h. Starting from basal levels between 13 and 71 ng/g, a suppression of adrenocortical activity after dexamethasone administration, indicated by metabolite levels close to the detection limit, was obtained 36–81 h after treatment, whereas unmetabolized dexamethasone was detectable in feces 12 h after its injection. Fecal glucocorticoid metabolite assessment via EIA is therefore of use in the monitoring of adrenocortical activity in roe deer. In a second experiment, capture, veterinary treatment, and transportation of animals were used as experimental stresses. This resulted in a 7.5-fold increase of fecal metabolites (1200 ± 880 ng/g, mean ± SD) compared to baseline concentrations. The administration of a long-acting tranquilizer (LAT), designed to minimize the physiological stress response, 2 days prior to a similar stress event led to a reduced stress response, resulting in only a 4-fold increase of fecal metabolites (650 ± 280 ng/g; mean ± SD). Therefore, LATs should be further investigated for their effectiveness in reducing stress responses in zoo and wild animals, e.g., when translocations are necessary. 

*Key Words:* stress; cortisol; metabolites; feces; noninvasive method; enzyme immunoassay; ACTH challenge; dexamethasone; perphenazine enanthate; long-acting tranquilizer.

## INTRODUCTION

According to current nomenclature, the environmental stimuli that lead to an imbalance of homeostasis are called "stressors," and the corresponding defense reactions of organisms are called "stress responses." The adrenal gland plays a pivotal role in the stress response, which involves the hypothalamic–pituitary–adrenal axis (HPAA) and the sympatho–adrenomedullary system as its two principal components. Stressors stimulate the hypothalamic release of corticotropin-releasing hormone (CRH), inducing the secretion of adrenocorticotropic hormone (ACTH)

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from the pituitary and that of corticosteroids from the adrenal cortex (Ladewig, 1994). During chronic stress conditions the adrenal cortex allows the hypersecretion of glucocorticoids even without the increment of ACTH (Aguilera *et al.*, 1996). Glucocorticoids can improve fitness by energy mobilization (Raynaert *et al.*, 1976) and trigger behavioral changes (Bahr *et al.*, 1998; Korte *et al.*, 1993). It is generally thought that, if severe enough, chronically high levels of glucocorticoids may decrease fitness, for instance, by causing immunosuppression and atrophy of tissues (Munck *et al.*, 1984), and may lead to the suppression of reproductive performance (Liptrap, 1993; Dobson and Smith, 1995; Ferin, 1999). Glucocorticoid secretion can be stimulated pharmacologically after administration of ACTH and depressed after dexamethasone (Dex) injection.

Concentrations of plasma cortisol have been widely used to evaluate stress responses in feral species, e.g., due to capture and translocation (Morton *et al.*, 1995), anthropogenic disturbances of their environment (Zoldag *et al.*, 1983), or psychogenic stress (Haemisch, 1990). However, capture of animals to obtain blood samples by venipuncture causes stress and thus increased peripheral glucocorticoid concentrations within minutes (Ladewig, 1994; De Villiers *et al.*, 1997). Plasma glucocorticoid measurements are also influenced by pulsatile secretion and by circadian rhythmicity of cortisol secretion (van Cauter *et al.*, 1996). Glucocorticoids are extensively metabolized by the liver. In sheep about 28% of infused [$^{14}$C]cortisol was excreted via feces, mainly (about 95%) as unconjugated metabolites, with a delay time of fecal peak radioactivity of about 12 h (Palme *et al.*, 1996). Although considerable interspecies differences exist in the amounts of steroid metabolites excreted via feces or urine (Palme *et al.*, 1996) and the metabolites formed (Palme and Möstl, 1997; Möstl *et al.*, 1999; Teskey-Gerstl *et al.*, 2000), alternative noninvasive methods for the determination of fecal cortisol metabolites utilizing a newly established 11-oxoetiocholanolone enzyme immunoassay (EIA) have been established in a variety of species (review: Wasser *et al.*, 2000; primates: Bahr *et al.*, 2000; Wallner *et al.*, 1999; okapi: Schwarzenberger *et al.*, 1998; ruminants: Palme and Möstl, 1997; Palme *et al.*, 1999, 2000; horses and pigs: Möstl *et al.*, 1999; hares: Teskey-Gerstl *et al.*, 2000; deer, rhinoceroses, cats, dogs, and guinea pigs: Palme *et al.*, 1998; spotted hyenas: Goymann *et al.*, 1999). This EIA measures 11,17-dioxoandrostanes (11,17-DOA), a group of cortisol metabolites. Such measurement avoids blood sampling and reflects a dampened pattern of diurnal glucocorticoid secretion, providing an integrated measure of adrenal activity (Palme *et al.*, 1999). 11,17-DOA measurements were successfully used to monitor transportation stress in cattle (Palme *et al.*, 2000) and thus seemed promising for assessing adrenocortical activity in roe deer. Due to the increasing demand for methods to quantify adrenal activity in zoo and wild animals in response to stress (e.g., loading, transportation), after pharmacological and management precautions to minimize stress, and the necessity to investigate stress impacts on animal welfare, the roe deer was used as a potential model for related ungulate species.

The objectives of this study were to: (1) validate an enzyme immunoassay to quantify glucocorticoid metabolites in roe deer, (2) assess the potential of this assay to monitor changes in cortisol secretion after an ACTH challenge to stimulate and a Dex administration to suppress adrenocortical activity, (3) study the course of fecal Dex excretion, and (4) test the effect of perphenazine enanthate, a long-acting tranquilizer (LAT), on cortisol secretion and thus on fecal cortisol metabolites in a defined stress situation (loading and transport).

## MATERIALS AND METHODS

### *Experimental Design*

Five hand-reared, castrated male roe deer (6–8 months old, 18–24 kg, housed in a grass enclosure of about 600 m$^2$) were treated with Dex (Voren; 1 mg dexamethasone-21-isonicotinat, i.m.; Boehringer, Ingelheim, Germany) in December 1997 followed by a second treatment with ACTH (Synacthen; 0.25 mg; Ciba-Geigy, Wehr, Germany; i.m. by teleinjection with a blow pipe) 3 months later (March 1998) to suppress or stimulate adrenocortical activity, respectively. Samples were collected for 7 (Dex) and 5 (ACTH) days from all defecations starting on the day before treatment.

In February 1999 four of these roe deer were exposed to a defined and reproducible stress situation

twice, without tranquillizing drugs and 48 h after the administration of perphenazine enanthate (Decentan Depot; Merck KgaA, Darmstadt, Germany). The animals weighed between 23 and 28 kg, and each received a dose of 15 mg (i.e., 0.15 ml) of perphenazine. The stress began at 9:00 a.m. and comprised 15 min of moving the animals out of their enclosure into the restraint-chute area, individual manual restraint, and two injections that were part of the general prophylactic program of the station. Thereafter the animals were loaded into transportation crates (two animals per crate) which were fixed onto a pickup truck (20 min), followed by a 2-h drive with two interruptions of 15 min. Ten minutes after rearrival at the station, the animals were set free into their enclosure. Samples were collected for 8 (ACTH), 5 (Dex), and 3 (LAT) days from all defecations starting on the day before treatment. Samples were collected from individual animals immediately after defecation, maintained on ice in small freezing bags for less than 2 h, and finally stored at −20° until analyzed.

### *Determination of Fecal Cortisol Metabolites*

Fecal samples (0.5 g) were extracted for 30 min with nine volumes of 90% methanol. After centrifugation (15 min at 1200*g*) the supernatant was transferred into a new tube and diluted 1:1 with water, and aliquot portions of 10 $\mu$l were subjected to the two enzyme immunoassays for 11,17-DOA and Dex. All hormone measurements were carried out in duplicate with microtiter plate enzyme immunoassay procedures and the results are expressed as nanograms / gram of fecal wet weight.

The samples were analyzed for cortisol metabolites with an 11,17-DOA EIA according to Palme and Möstl (1997). Previous measurements with a cortisol or a corticosterone EIA did not reveal significant amounts of cortisol metabolites.

Serial dilutions of roe deer fecal extracts yielded displacement curves parallel to those of the standard 11-oxoetiocholanolone. The intra- and interassay coefficients of variation for two biological samples were 10.4% ($n$ = 12) / 7.2% ($n$ = 11) and 18.1% ($n$ = 7) / 11.9% ($n$ = 8), respectively. The detection limit of the assay was 0.2 pg / well.

Dex was determined with an EIA originally developed for the detection of Dex in equine blood (Friedrich *et al.*, 1992). The used antibody was raised against Dex-21-HS-BSA and cross-reacts with various synthetic glucocorticoids (Dex 100%; flumethasone 103%; betamethasone 45%; prednisolone 17%; triamcinolone 0.2%), whereas natural steroids (cortisol, cortisone, progesterone, pregnenolone, testosterone) had negligible cross-reactivities below 0.4% (Meyer and Dürsch, 1996). Prednisolon-21-HS-horseradish peroxidase was used as enzyme conjugate. The Dex calibration standards were prepared by dilution with assay buffer and ranged from 100 to 0.1 pg / well.

Serial dilutions of fecal extracts yielded displacement curves parallel to those of the standard Dex. The intra- and interassay coefficients of variation for two pool samples were 6.7% / 8.7% (number of assays = 6) and 4.9% / 14.9% (number of assays = 8), respectively. The detection limit of the assay was 0.2 pg / well.

### *High-Performance Liquid Chromatography*

For separation and characterization of cortisol metabolites, methanol extracts of fecal samples were purified on a solid-phase extraction cartridge (Sep-Pak C-18; Waters Associates, Milford, MA). Unconjugated metabolites were eluted with dichloromethane, and high-performance liquid chromatography (reverse-phase) separation of these metabolites was performed on a 150 × 3.9-mm Novapak C18 column (Millipore GmbH, Eschborn, Germany), as described by Teskey-Gerstl *et al.* (2000), with a methanol / water gradient. Following separation, immunoreactivity of each fraction was determined with the 11,17-DOA EIA. In addition, fecal extracts were tested for immunoreactive metabolites in a cortisol and corticosterone EIA (Palme and Möstl, 1997). To calibrate the HPLC column, the elution positions of authentic cortisol (fraction 19), corticosterone (fraction 30), and progesterone (fraction 54) had been previously determined in a separate HPLC run (Fig. 1a).

For separation and demonstration of Dex and possible metabolites, 500 $\mu$l of fecal extracts from pre- and posttreatment samples were loaded onto a Ultrasep ES100 / RP-18 / 6-$\mu$m (Sepserv, Berlin) HPLC column (4 × 250 mm). Steroids were separated by reverse-phase chromatography with a methanol:buffer (20 mM Tris, pH 7.2) mixture (70:30) at a flow rate of 1 ml / min. Fractions were collected at 1-min intervals and diluted with one volume of water before 20 $\mu$l of

the fractions were pipetted into microtiter plate wells. The elution position of Dex on this column had been determined in a separate HPLC run after its injection (Fig. 1b).

### *Analysis of Data*

Basal levels of glucocorticoid metabolites in feces were defined as the mean of the concentrations of all samples collected in the pretreatment period. To investigate the effect of a LAT, all values were allocated into time frames of 6 h and mean concentrations ± SD were calculated for each interval. Statistical analysis was performed by repeated-measurements ANOVA. In addition, the individual maxima of 11,17-DOA concentrations (highest 11,17-DOA concentrations measured after ACTH (or stress) in an animal; 5 to 30 times higher than the basal levels) were compared by the Student *t* test.

## RESULTS

### *HPLC Analysis*

Measurement of 11,17-dioxoandrostanes in the HPLC fractions of a representative fecal extract after ACTH stimulation is shown in Fig. 1a. The analyses revealed large amounts of three different 11,17-DOA (0.5, 3.1, and 4.1 ng/fraction) in fractions 22, 28, and 36, respectively. 11,17-DOA are a group of cortisol metabolites derived from side-chain cleavage. They are 5$\alpha$/5$\beta$-androstanes (ring A reduced), possessing two oxo groups at positions C11 and C17. They are characterized by their cross-reactivities in the 11-oxoetiocholanolone EIA (5$\beta$-androstane-3$\alpha$-ol-11,17-dione). The antibody was developed against an immunogen, which was an 11-oxoetiocholanolone coupled at position C3 to bovine serum albumin, and therefore does not distinguish metabolites, which have the two oxo groups but different groups at C3 (3$\alpha$-ol; 3$\beta$-ol or 3-one). The metabolite eluting around fraction 36 is 11-oxoetiocholanolone (checked by separate injection into the HPLC system). The other substance (fraction 28) elutes around the standard 5$\beta$-androstane-3,11,17-trione and the substance around fraction 22 might be a 5$\beta$-androstane-3$\beta$-ol-11,17-dione. Due to lack of such an exotic steroid standard this could not be confirmed. However, they are well characterized by their immunoreactivity in the 11-oxoetiocholanolone EIA (see above). In contrast to the 11,17-DOA EIA, a specific cortisol EIA detected minor quantities (below 0.05 ng/fraction) of immunoreactive metabolites (fractions 10 and 16) and probably authentic cortisol in fraction 19, which can be inferred from the elution position, which is similar to that of the cortisol standard. The measurement of corticosterone did not reveal detectable amounts in the fractions (not shown).

To demonstrate intact Dex or possible metabolites, a separate HPLC column was calibrated with Dex (Fig. 1b, fraction 9). When the HPLC fractions of fecal extracts were analyzed with a specific Dex EIA, unmetabolized Dex was confirmed as the predominant fecal steroid at both 17 and 41 h after its injection. In addition, small amounts of immunoreactivity coeluted close to the solvent front (fraction 10); these were probably conjugated steroids.

### *ACTH Challenge*

The administration of ACTH increased fecal glucocorticoid metabolites. There was considerable interanimal variability in the means of all samples obtained during the 24-h pretreatment phase (basal), in the peak values, and in the time after which peak levels were reached and returned to basal levels (Table 1). Two individual fecal profiles are shown in Figs. 2a and 3a. Following the ACTH injection, fecal metabolite concentrations peaked after 6.1–12.4 h (four animals, mean 9.1 h) and 22.6 h (one animal) and returned to below basal levels within 27.5 to 31 h after treatment. Maximal concentrations of fecal cortisol metabolites ranged from 183 to 944 ng/g feces (mean 459 ng/g), which reflected a 4- to 12.5-fold increase over the basal levels.

### *Dexamethasone Administration*

The effect of the Dex treatment is shown for the same individuals in Figs. 2b and 3b. Again, there was considerable interanimal variability in the pretreatment levels (Table 2), and in one animal (Fig. 3b) a distinct increase of fecal 11,17-DOA occurred 14.6 h after Dex administration. In each animal Dex injection

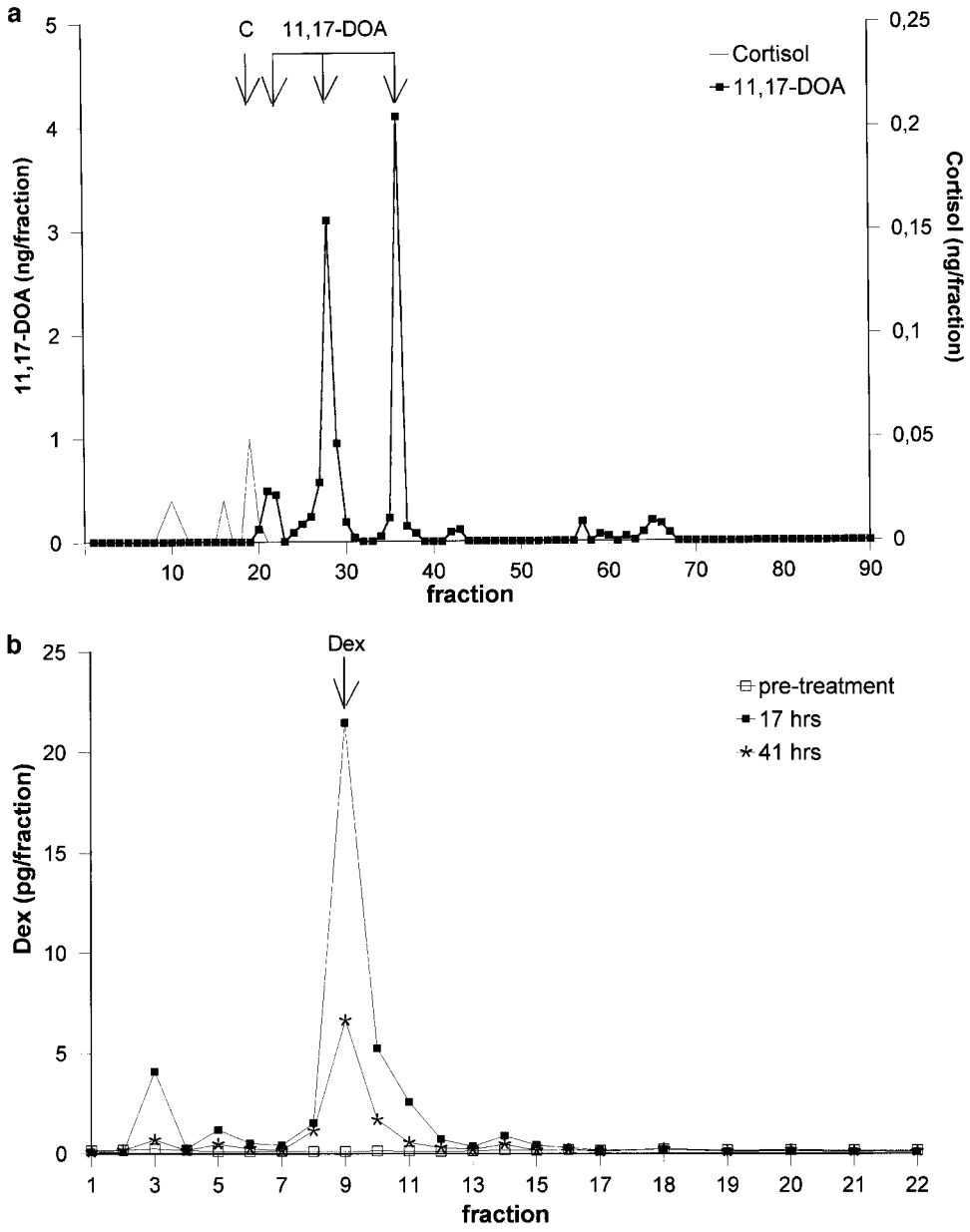


FIG. 1. HPLC-profile of 11,17-dioxoandrostanes (11,17-DOA) and cortisol immunoreactive substances in feces from one roe deer (a) and HPLC profiles of fecal dexamethasone in fecal samples from another roe deer (b) obtained before and 17 and 41 h after dexamethasone application. Arrows indicate elution of cortisol (C), dexamethasone (Dex), and 11,17-DOA.

decreased 11,17-DOA excretion below basal levels after 14.7–32.4 h, and metabolite levels close to the detection limit (1.5 ng / g) were obtained within 24 to 36 h and remained low up to 120 h postinjection (Table 2). The individual profiles of Dex excretion are shown in Figs. 2c and 3c. Maximum concentrations of unmetabolized fecal Dex (88.5–250.8 ng / g) were reached 18.2–33.2 h after injection (mean 22.5 h).

### *Effects of a Long-Acting Tranquilizer on Adrenocortical Function*

Without previous LAT (perphenazine enanthate) administration, the animals seemed very nervous, exhausted, and agitated. All four animals were trembling during the first hour after transport. When the distance between the observer and the animals was

TABLE 1
ACTH Challenge: Individual Basal and Peak Values (ng/g Feces and % Increase) of Fecal 11,17-DOA Concentrations and Time after Which Fecal Peak Levels Were Reached

| | | ACTH Challenge | | |
|---|---|---|---|---|
| Animal | Basal level (mean ± SD) | Maximum (ng/g) | % Increase | Hours after treatment |
| 1 | 51.7 ± 23.9 | 302.0 | 584 | 9.10 |
| 2 | 69.9 ± 23.0 | 944.0 | 1350 | 12.4 |
| 3 | 77.8 ± 30.3 | 392.0 | 504 | 6.10 |
| 4 | 31.3 ± 17.1 | 182.7 | 584 | 22.6 |
| 5 | 73.9 ± 28.9 | 472.0 | 639 | 8.70 |

reduced below 5–7 m the animals escaped (this reflects the flight distance). No animal ruminated during the first 4 h. After LAT administration the reaction of the animals to the catching procedure seemed identical. After the transport, however, the animals did not tremble and seemed relaxed. The animals layed down at once and started ruminating within the first 10 min after transport. The flight distance was reduced to 1–2 m. Defecation did not occur as frequently as during the first trial.

Without LAT administration, fecal DOA values increased from basal levels (range 78 to 246 ng/g) to maximal values of 1000 to 3115 ng/g (1170–3070%) within 14 to 20 h after the start of the loading and transportation stress. The administration of LAT 2 days before a similar stress event resulted in a dampened increase from basal levels (range 76 to 340 ng/g) to 697 to 1225 ng/g (323–1429%) within 10 to 21 h after initiation of stress. As the times and frequencies of defecation differed, all values were allocated into time frames of 6 h (Fig. 4). The fecal metabolite concentrations after LAT administration were not significantly different from those of the control when the concentrations were compared by repeated-measurements ANOVA ($P = 0.19$). However, when the individual maxima of 11,17-DOA concentrations were compared by the Student $t$ test, significantly lower values were obtained after LAT application ($P = 0.027$).

## DISCUSSION

In recent years, as a result of the increased concern for the conservation of wildlife, numerous studies have been conducted to measure stress responses in a variety of species. Capture of animals to obtain blood samples for cortisol measurements, however, causes stress and virtually none of the results obtained in this manner represent true resting baseline cortisol levels. However, noninvasive techniques for measuring fecal glucocorticoid metabolites have recently become available for use with domestic livestock (Palme and Möstl, 1997; Palme *et al.*, 1999, 2000) and wildlife (Goymann *et al.*, 1999; Bahr *et al.*, 1999; Schwarzenberger *et al.*, 1998; Graham and Brown, 1996, 1997). Before assays to measure fecal glucocorticoid metabolites can be applied to a particular species, they must be vali-

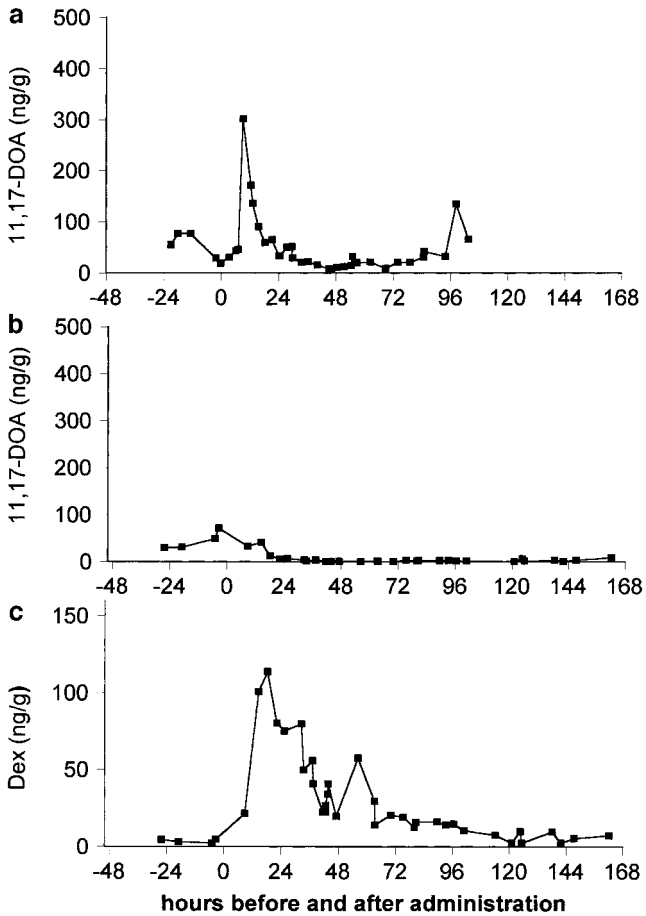


FIG. 2. Stimulation and suppression of adrenal activity after application of ACTH (a) and dexamethasone (b), respectively. Samples were collected for 5 (ACTH) and 7 (Dex) days from all defecations starting on the day before treatment. The samples were analyzed for cortisol metabolites (11,17-DOA). In addition, fecal Dex (c) was measured in the same individual. (0, time of administration; note different y axis scales).

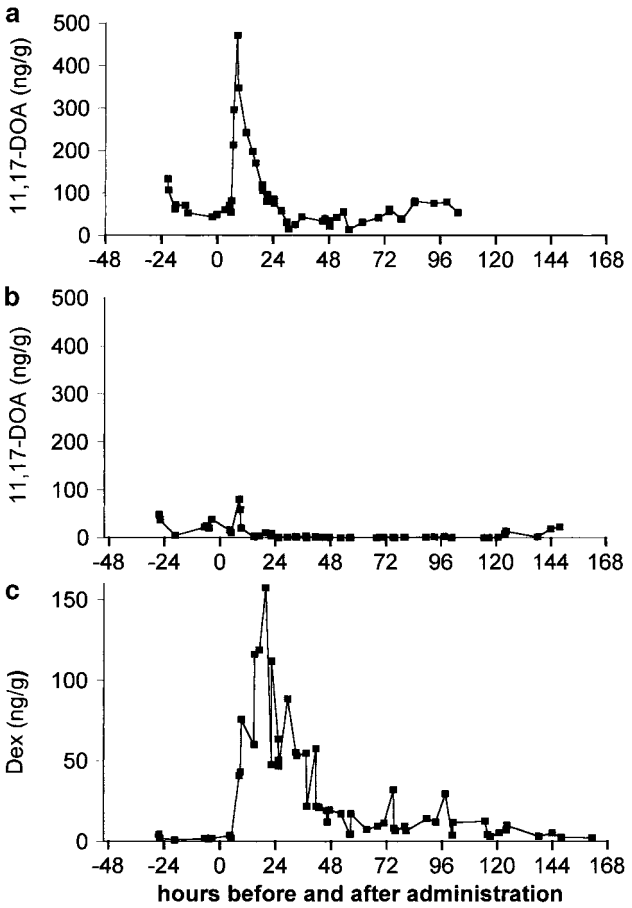


FIG. 3. Fecal 11,17 DOA concentrations during an ACTH challenge (a) and a dexamathasone application (b) with the respective concentrations of fecal Dex (c) in a second individual.

dated. Antibodies against plasma glucocorticoids coupled to bovine serum albumin do not necessarily bind to the relevant fecal metabolites, and there is no single major metabolite but rather several different metabolites (Palme and Möstl, 1997; Möstl *et al.*, 1999; Teskey-Gerstl *et al.*, 2000; Goymann *et al.*, 1999). In addition, use of different glucocorticoid antibodies resulted in the detection of different patterns of fecal metabolites after ACTH challenge in hyenas (Goymann *et al.*, 1999).

The aim of this study was to investigate whether measurement of fecal glucocorticoid metabolites is a suitable technique for monitoring adrenocortical activity in the roe deer. The results obtained from HPLC followed by EIA showed that the 11,17-DOA antibody detected three different fecal 11,17-dioxoandrostanes. Several lines of evidence suggest that such measurement clearly reflects adrenal activity: (1) an ACTH challenge led to individual increases in fecal 11,17-DOA levels exceeding pretreatment levels by up to 16-fold; (2) Dex suppressed adrenocortical activity, and glucocorticoid metabolite levels close to the detection limit were measured within 24 to 36 h after its injection; and (3) stressful situations (loading, transport) led to distinct increases in fecal glucocorticoid metabolites. Therefore, the measurements of fecal DOA is an appropriate technique for monitoring adrenocortical activity in roe deer. Compared to the 11,17-DOA EIA, a cortisol or a corticosterone EIA confirmed only minor or zero quantities of the relevant metabolites and seemed unsuitable for measurements in roe deer. It can be assumed that in roe deer nearly all cortisol is metabolized. This corresponds with radiometabolic studies in sheep in which authentic radiolabeled cortisol was undetectable in fecal samples (Palme and Möstl, 1997) and the measurement of 11,17-DOA is the only reliable technique for stress monitoring in sheep. In contrast to cortisol, Dex was found to be almost unmetabolized in fecal samples. This confirms the higher biopotency of Dex, which is protected against metabolism due to several modifications in the pregnen skeleton. The presence of unmetabolized Dex in feces up to 48 h after injection might offer an analytical technique to detect the synthetic glucocorticoid in species, e.g., horses or calves, in which an illegal administration of Dex is suspected (Friedrich *et al.*, 1992).

TABLE 2

Dexamethasone Suppression: Individual Basal 11,17-DOA Concentrations, Time after Which a Decrease Below Basal Levels Was Measurable, Maximal Concentrations of Fecal Dex, and Time after Which They Were Measured

| | 11,17-DOA | | Fecal dexamethasone | |
|---|---|---|---|---|
| Animal | Basal level (mean ± SD) | Decrease below Basal − SD (h) | Maximum (ng/g) | Hours after treatment |
| 1 | 45.5 ± 16.8 | 18.2 | 113.7 | 18.2 |
| 2 | 12.9 ± 3.70 | 32.4 | 88.50 | 21.7 |
| 3 | 23.7 ± 6.70 | 25.6 | 191.4 | 33.2 |
| 4 | 70.8 ± 33.9 | 29.4 | 250.8 | 19.6 |
| 5 | 27.9 ± 13.5 | 14.7 | 157.4 | 19.7 |

*Note.* The decrease was defined as a decrease below basal levels − SD followed by concentrations below that value in at least three samples.

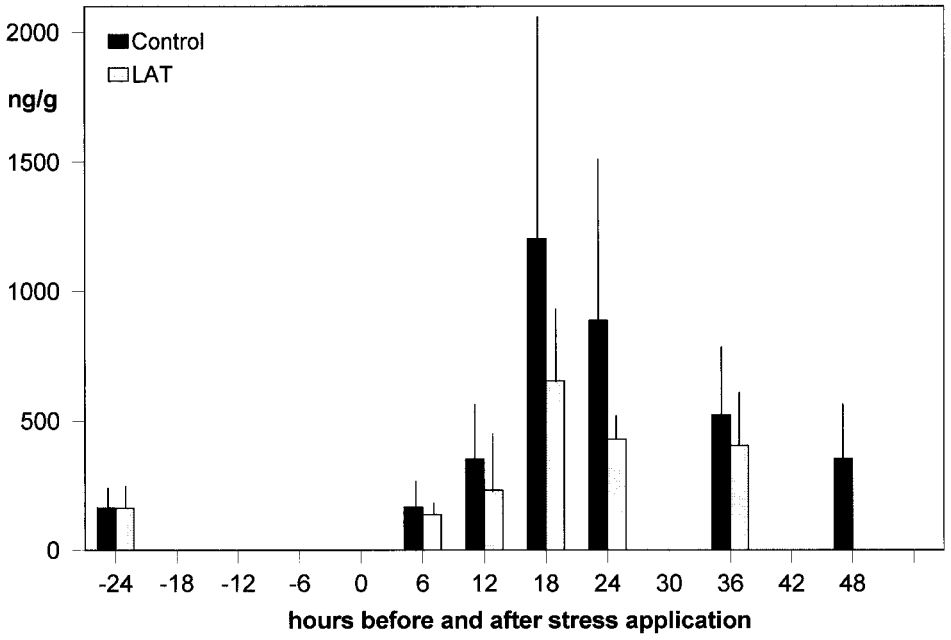


FIG. 4. Fecal 11,17 DOA concentrations (mean ± SD) of four roe deer after a standardized stress exposure without (control) and with pretreatment with a long-acting tranquilizer (LAT). As the times and frequencies of defecation differed, all values were allocated into time frames of 6 h.

The injection of exogenous ACTH led to individual peaks of fecal 11,17-DOA in all individuals after 6.1 to 22.6 (mean 11.8) h. Based on an immediate adrenocortical response to exogenous ACTH, with peak levels in blood plasma being reached within 30 min (Ladewig, 1994), this mean value mainly reflected the delay of fecal glucocorticoid metabolite secretion, which roughly corresponds to the food transit time from the duodenum to the rectum and is comparable to data from sheep (11.7 h; Palme *et al.*, 1996).

When adrenocortical activity was suppressed by Dex injection in individual roe deer, a decreased 11,17-DOA concentration, close to the detection limit, was obtained within 24 to 36 h and adrenocortical activity remained suppressed for at least 120 h after injection. Even if Dex was already detectable in feces after 5.9 h, maximal fecal concentrations of unmetabolized Dex were reached approximately 22.5 h postinjection, which fits with the arrival at the lowest 11,17-DOA concentrations. The temporal discrepancy between the ACTH-dependent fecal 11,17-DOA peak level (9.1 h) and the fecal Dex level (22.5 h) of approximately 13 h, however, might reflect the delay from the i.m. injection to the absorption of Dex into the circulation to its biological action.

A stress event in roe deer resulted in a distinct increase in fecal cortisol metabolite concentrations 12 h after the onset of the stressful situation, which seemed to be higher when compared with the ACTH challenge 1 year before. After the stress event the animals were nervous and exhausted and no ruminant activity occurred during the first 4 h. After pretreatment with LAT and a similar stress the animals seemed more relaxed and started ruminating within the first 10 min. These behavioral observations were supported by the analytical data, which demonstrated that a less-pronounced increase in fecal metabolites occurred 12 h after the onset of the stress event. Compared to the control experiment, this difference did not reach statistical significance, which was probably due to the limited number of animals investigated. Nevertheless, these results might recommend the use of a LAT and they support results from Ebedes and Raath (1999), in which plasma cortisol levels were reduced from 9.26 to 1.78 ng / ml (81%) after perphenazine enanthate administration to nyalas (*Tragelaphus angasi*). LATs have been used in the capture and relocation of free-ranging and captive South African wildlife since the 1980s (Ebedes and Raath, 1999). The mortality rates at capture of notoriously nervous ani-

mals, such as nyala or reedbuck (*Redunca arundinum*), could be dramatically reduced by the use of LATs (Ebedes and Raath, 1999). LATs have been used also in zoos in Europe (Winterer, 1997), and their application has been interpreted as an obligatory contribution to animal welfare (Wiesner, 1998). Based on fecal glucocorticoid metabolite measurements, the present results are less clear. The fecal metabolite measurements revealed a reduction of glucocorticoid secretion of only approximately 46%. This might be due to species differences in the effectiveness of the drug, the dosage, the intensity of the stress, and the analytical procedure. Whereas the measurements in the nyala reflect one-point measurements in blood plasma immediately after stress application, the present analysis of fecal metabolites provided an integrated measure of adrenal activity (Palme *et al.*, 1999) over several hours. Based on the data from the nyala, a cautious interpretation of the behavioral observations, and the analytical data, drug tranquilization by teleinjection with a blow pipe (to avoid physical restraint stress) should be integrated into capture and transport protocols for wild animals and should improve animal welfare.

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