

Noninvasive Fecal Monitoring of Glucocorticoids in Spotted Hyenas, *Crocuta crocuta*

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The aim of this study was to validate a method for measuring glucocorticoids noninvasively in feces of spotted hyenas (*Crocuta crocuta*). Three established enzyme immunoassays (EIA) for cortisol, corticosterone, and 11-oxoetiocholanolone were tested, but proved unsatisfactory. A new EIA using another corticosterone antibody was established and was used for all subsequent analyses; this EIA was validated by demonstrating parallelism between serial dilutions of spotted hyena fecal extracts and dilutions of standard corticosterone and by the recovery of corticosterone added to fecal extracts. High-performance liquid chromatography (HPLC) fractions analyzed by EIA showed various immunoreactive substances with polarities of unconjugated steroids. The physiological relevance of fecal glucocorticoid metabolites was further validated by demonstrating that (1) injection of exogenous ACTH to four males and two females led to a significant increase in fecal glucocorticoid metabolites within 24–50 h, (2) the translocation of a male spotted hyena to a new enclosure resulted in a fivefold increase compared to baseline concentrations, and (3) agonistic social interactions and physical conflict resulted in large increases of fecal glucocorticoid metabolites in both protagonists. Fecal steroid assessment is therefore of use in monitoring adrenal activity in spotted hyenas. © 1999 Academic Press

Key Words: feces; cortisol; corticosterone; stress; noninvasive method; enzyme immunoassay; ACTH challenge; translocation; aggression

Although there is no standard definition and no single physiological measure of “stress” (see Hofer and East, 1998), adverse stimuli are known to activate the hypothalamus–pituitary–adrenocortical axis (HPA axis), which results in the release of glucocorticoids. Plasma glucocorticoid levels are considered good indices of stress in many vertebrates (fish: Bonga, 1997; reptiles: Greenberg and Crews, 1990; Knapp and Moore, 1997; birds: Wingfield *et al.*, 1995; Silverin, 1997; mammals: Bradley *et al.*, 1980; Sapolsky, 1985; Alexander and Irvine, 1998) and can have opposing consequences upon health. They can improve fitness, for instance, by mobilizing energy (e.g., Lee and Cockburn, 1985) or by triggering physiological and behavioral changes during the “emergency life history stage” (Wingfield *et al.*, 1998), and chronically high levels may decrease fitness, for instance, by causing immunosuppression and atrophy of tissues (Munck *et al.*, 1984; Lee and Cockburn, 1985; Sapolsky, 1987). Because of these potential effects on physiological status, the measurement of glucocorticoids can be valuable in studies of evolutionary ecology, conservation biology, and animal welfare.

Spotted hyenas (*Crocuta crocuta*) live in large, highly structured, female-dominated groups (or clans) with separate male and female dominance hierarchies (Kruuk, 1972; Frank, 1986; Hofer and East, 1993). Social stress within a clan may be sufficient to decrease the reproductive success or survival of certain individuals. It would thus be useful to examine whether individual

life-history parameters, such as rank and reproductive success, are associated with particular glucocorticoid profiles. Holekamp and Smale (1998) showed that plasma cortisol concentrations in immigrant male spotted hyenas depend on social rank and clan tenure. Because spotted hyenas may become increasingly dependent on conservation efforts (Hofer and Mills, 1998), monitoring stress hormones may also become a useful tool for identifying situations likely to cause stress. This information would be of value both for the conservation management of wild spotted hyena populations and for the welfare of animals in captivity.

Traditionally, glucocorticoids have been measured in blood plasma; however, capture and blood sampling procedures are usually stressful (Sapolsky, 1982; Wingfield *et al.*, 1992). Furthermore, as plasma glucocorticoids can vary circadianly and have pulsatile secretory patterns (e.g., Fulkerson and Tang, 1979; Thun *et al.*, 1981; Monfort *et al.*, 1993), a blood sample represents plasma glucocorticoid levels within a narrow time frame. Thus, noninvasive methods, such as measuring glucocorticoids in feces, are desirable because animals need not be captured, several samples can be obtained from individuals, and fecal glucocorticoid metabolites represent pooled fractions of plasma glucocorticoids, providing an integrated measure of adrenal status. Although Sousa and Ziegler (1998) found diurnal fluctuations in fecal glucocorticoid levels in common marmosets, it is likely that fluctuations due to secretory patterns are attenuated in feces.

Despite these advantages, immunoassays to measure fecal glucocorticoid metabolites are currently described for only a very limited number of mammals (Miller *et al.*, 1991: bighorn sheep; Graham and Brown, 1996, 1997: several felids; Palme and Möstl, 1997: domestic sheep; Whitten *et al.*, 1997: chimpanzee; Jurke *et al.*, 1998: cheetah; Monfort *et al.*, 1998: African wild dog; Sousa and Ziegler, 1998: common marmoset). The present study is part of a larger project examining hormones of free-ranging spotted hyenas in the Serengeti ecosystem. A noninvasive approach to measuring stress in those spotted hyenas is desirable and the objectives of this study were (1) to validate an enzyme immunoassay (EIA) to quantify glucocorticoid metabolites in spotted hyena feces and (2) to assess the potential of this technique to monitor changes in adrenal activity of this species.

METHODS

For the biological validation, the response in terms of the elevation of glucocorticoid metabolites in feces, an adrenocorticotrophic hormone (ACTH) challenge was conducted. Then, two situations that were likely to result in increased stress and glucocorticoid release were examined. In the first situation, an animal was translocated from one zoo to another. The second situation involved social tension between a male and a female spotted hyena.

Animals

Fecal samples were collected from four adult male and two adult female spotted hyenas living in enclosures at four different zoos in Germany and the Czech Republic. Two sibling male–female pairs were housed together; the other two males were kept on their own. When two animals were housed together, rye grains were added to the daily meat rations of one of the animals. As hyenas do not digest rye grains, feces with grains were allied to the individual fed with grains, whereas feces containing no grains were allied to the other individual.

ACTH-Challenge Experiment

Three males and one female were each dart-injected im with 50 IU of a synthetic ACTH preparation (Synacthen Depot 0.5 mg, Ciba-Geigy, Wehr, Germany); one male and one female were each dart-injected im with 200 IU of another ACTH preparation (Acthar Gel, Rhone-Poulence Rorer Pharmaceuticals, Collegeville, PA). All injections were done between 0845 and 1100 h.

Fecal samples were collected 2–3 days preceding ACTH administration and for 5–7 days after the treatment. Enclosures were checked for feces every 1–2 h between 0700 h in the morning and 2000 h in the evening.

Translocation

In summer 1997, a spotted hyena from Münster Zoo was translocated to Munich Zoo. Fecal samples were collected 3 days prior to and for 10 days after trans-

port. Feces from this animal were collected once or twice per day by the animal keepers.

Social Tension

Three days after the start of fecal sampling of a male and female pair that were housed together, the female began to utter low calls (Mills, 1990). These sounds are produced during aggressive interactions (Mills, 1990). The male gave the female a wide berth and stopped feeding. The female continued low calling throughout day 3. On the morning of day 4, the sand in the enclosure was pawed and both animals were excited. The male was interested in the urine of the female and showed flehmen behavior. The female repeatedly chased the male. Both animals repeatedly uttered low calls, pawed the ground, and finally fought with each other for 1.5 h, until they were separated by animal keepers. The ears, forelegs, and flanks of both animals were scratched and bitten. After separation on day 4, both animals were restless but on day 5 they spent most of their time resting in the sun. According to information from the animal keepers these two animals had not previously fought with each other.

All fecal samples were collected 3 days preceding the onset of aggressions, and sampling continued until 5 days after aggressions had ended. Enclosures were checked for feces every 1–2 h between 0700 h in the morning and 2000 h in the evening.

Processing of Fecal Samples

Immediately after collection, feces were chopped up and mixed thoroughly, and 15–20 g were taken and stored at -10 to -20° in the respective zoo (for up to 12 days), transported at -10 to -20° , and stored at -70° after transfer to the laboratory. A subsample of 5.33 ± 1.34 g was freeze-dried (72–96 h) with a Christ Alpha I-5 lyophilisator and pulverized with a mortar. A proportion of the resulting powder (0.500 ± 0.001 g) was weighed with a Sartorius Research R 160 P balance and extracted with 4 ml methanol and 0.5 ml double-distilled water (ddH₂O). After vortexing for 30 min, the sample was centrifuged (2500g, 10 min) and the supernatant transferred to a new tube. This methanol extract was used in three enzyme immunoassays (EIA) for cortisol, corticosterone, and 11-oxoetiocholanolone

(described below). Recovery of added ³H-labeled corticosterone was $83.6 \pm 1.0\%$ (mean \pm SEM, $n = 12$).

Serial dilutions of methanol-extracted samples did not show parallelism to hormone standards in the ICN-corticosterone EIA (described below), indicating matrix interferences. Thus, a further extraction step was included to remove substances interfering with the antibody reaction; 5 ml diethyl ether (DEE) and 500 μ l NaHCO₃ were added to 1 ml of the methanol extract, vortexed, and a further 4 ml ddH₂O was added. Then the vial was plugged, turned round four times, and freeze-decanted into a new vial. Finally, the DEE was evaporated at 40° under a stream of nitrogen and the sample resuspended in assay buffer. Mean recovery of ³H-radiolabeled corticosterone was $62.1 \pm 1.0\%$ (mean \pm SEM, $n = 22$). Extracted samples were stored at -40° until assayed.

Enzyme Immunoassays

To compare the efficiency of four different assays for detecting changes in glucocorticoid excretion in response to an ACTH challenge, samples from one female spotted hyena were analyzed using each antibody. Methanol-extracted samples were analyzed with three EIA systems (cortisol, corticosterone, and 11-oxoetiocholanolone; for assay and antibody characteristics, see Palme and Möstl, 1997). An assay for 11-oxoetiocholanolone might be unusual to measure adrenocortical activity, but this assay has been shown to provide powerful results in ungulates (Palme and Möstl, 1997). Briefly, 50 μ l of each sample was pipetted into microtiter plate wells (coated with sheep-anti-rabbit IgG) and 100 μ l antibody and enzyme solution was added. After overnight incubation in the cold room (4°), plates were washed four times with ddH₂O/Tween 20 (1:500000), and 250 μ l enzyme solution (streptavidin-POD conjugated, 500 U, 1:30000; Boehringer, Mannheim, Germany) was pipetted into each well and incubated for 45 min at 4° . Then plates were washed again and 250 μ l tetramethylbenzidine/H₂O₂ substrate solution was added. After incubation (45 min, 4°), the reaction was stopped with 50 μ l H₂SO₄ (3 M) and absorbance measured at 450 nm/630 nm, using a DIAS reader (Dynatech, Guernsey, Great Britain). Standard curves and sample concentrations were calculated with Immunofit 3.0 (Beckman Inc., Fullerton, CA).

To allow further comparisons, DEE-extracted samples were also analyzed in the corticosterone assay. An EIA using another corticosterone antibody purchased from ICN Biomedicals (Costa Mesa, CA) was also set up. As serial dilutions of methanol-extracted samples did not show parallelism to standard corticosterone (see extraction procedures), DEE-extracted samples were used in this assay. To avoid confusion with the other corticosterone antibody tested, this antibody is referred to as ICN-corticosterone antibody. It is reported to cross-react with corticosterone (100%), desoxycorticosterone (0.34%), testosterone (0.10%), cortisol (0.05%), aldosterone (0.03%), progesterone (0.02%), and less than 0.01% for 12 other steroids tested. A previously described EIA system (Möstl *et al.*, 1987; Palme and Möstl, 1987) was modified to allow use of the ICN-corticosterone antibody. Fifty microliters of DEE-extracted samples and an antibody dilution of 1:21000 were used. Biocytinylated corticosterone, serving as label (dilution 1:512000), was synthesized (Palme and Möstl, 1993), using biocytin instead of biotinyl-diaminodioxooctan for coupling. Assay procedures were the same as described for the other assays.

Serial dilutions (1:5 up to 1:80) of spotted hyena fecal DEE extracts yielded displacement curves parallel to standard corticosterone. ICN-corticosterone assay accuracy was $97.6 \pm 4.5\%$ (mean \pm SEM, $n = 32$) and sensitivity was 120 pg/ml. Intra- and interassay coefficients of variation were 7.7 and 8.3% for a high pool and 5.3 and 12.5% for a low pool ($n = 8$). Samples were assayed in duplicate and concentrations are expressed as nanograms/gram of fecal dry matter.

High-Performance Liquid Chromatography

The number and relative proportions of immunoreactive glucocorticoid metabolites in hyena fecal extracts were determined via HPLC. Methanol-extracted samples (1 ml) of one male and one female were diluted with ddH₂O (10 ml) and passed through C-18 matrix columns (Waters, Milford, MA). Columns were then washed with ddH₂O (2×5 ml) and eluted with methanol (4 ml). The methanol was evaporated to dryness under a stream of nitrogen, and the sample was resuspended in 50% methanol and separated on a Novapak C-18 column using a linear gradient of 50–75% methanol (flow rate 1 ml/min; run time 40 min). Ninety-five 330- μ l fractions were collected, dried

under a stream of nitrogen, and reconstituted in assay buffer, and immunoreactive glucocorticoid metabolites were quantified with the ICN-corticosterone EIA as previously described. Elution of [³H] corticosterone was measured by liquid scintillation counting and elution profiles of 17 α ,20 α -dihydroxyprogesterone, 20 α -dihydroxyprogesterone, and cortisol were determined by measuring absorbance at 254 nm.

Statistics

The Friedman test with the post hoc multiple comparisons was calculated according to Conover (1980). All *P* values are two-tailed. Increases in glucocorticoid metabolite excretion after translocation and social tension were considered significant if they exceeded mean baseline concentration plus three standard deviations.

RESULTS

Comparison of Different Antibodies

The assays for cortisol, corticosterone and ICN-corticosterone measured differing baselines with low fluctuations of fecal glucocorticoid metabolites and showed a clear response to an ACTH-induced increase in adrenal activity (Figs. 1a,1c,1d). In contrast, baseline data of the 11-oxoetiocholanolone assay fluctuated and the assay indicated the weakest response to the ACTH challenge (Fig. 1b). Although the cortisol assay (Fig. 1a) showed a clear response to the ACTH challenge, baseline values of this assay were close to or below the detection limit. The corticosterone assay showed a clear response to ACTH, also when extracted with DEE for a direct comparison with the ICN-corticosterone assay (Fig. 1c, open circles). The ICN-corticosterone assay revealed the highest response to ACTH. It thus appeared to be the most promising for further investigations and was used for all subsequent analyses.

HPLC Analysis

HPLC fractions analyzed with the ICN-corticosterone antibody showed various immunoreactive sub-

stances with the polarity of unconjugated steroids, in both a male and a female spotted hyena (Fig. 2). These substances showed a chromatographic mobility of unconjugated steroids, mainly between cortisol and 17 α ,20 α -dihydroxyprogesterone. The first immunoreactive peak (fraction 5) had the polarity of conjugated steroids. Cortisol itself was below the detection limit and corticosterone comprised only a minor fraction of total immunoreactivity.

ACTH Challenge

Administration of ACTH stimulated the production of glucocorticoids, which resulted in an increase of fecal glucocorticoid metabolites (Fig. 3). The ACTH-

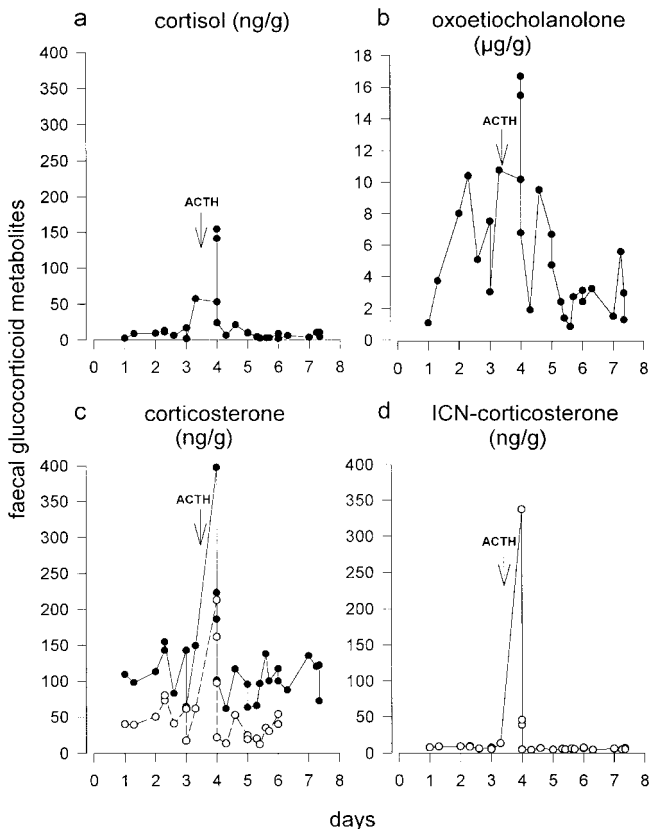


FIG. 1. Change in fecal glucocorticoid metabolites during an ACTH challenge experiment (50 IU ACTH) in female spotted hyena 5, measured with four different antibodies against (a) cortisol, (b) 11-oxoetiocholanolone, (c) corticosterone, and (d) ICN-corticosterone. Arrows indicate time of ACTH injection. Closed circles represent methanol-extracted samples, open circles represent DEE-extracted samples. (Note different y axis scale for 11-oxoetiocholanolone.)

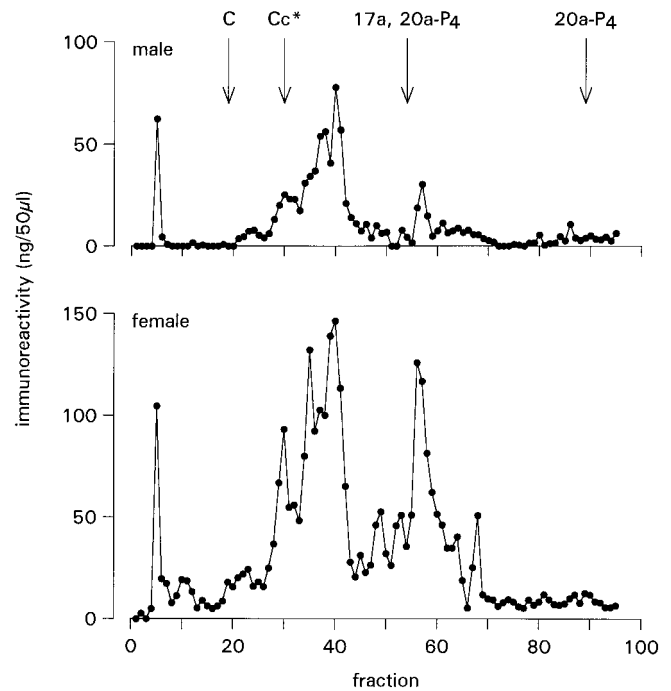


FIG. 2. HPLC profiles of ICN-corticosterone immunoreactive substances in feces of a male (no. 4) and female (no. 6) spotted hyena. Arrows indicate elution of cortisol (C), radiolabeled corticosterone (Cc*), 17 α ,20 α -dihydroxyprogesterone (17a,20a-P₄), and 20 α -dihydroxyprogesterone (20a-P₄).

induced peak in fecal glucocorticoid metabolites occurred 26 ± 5 h after the ACTH injection (Fig. 3 and Table 1). Mean fecal glucocorticoid concentrations in six spotted hyenas differed significantly between the three periods before (days -1 and 0), during (days 1 and 2), and after (days 3 and 4) the ACTH challenge (Friedman test, $\chi^2 = 6.333$, $n = 6$, $df = 2$, $P = 0.0421$, Table 1). Mean levels on peak excretion days 1 and 2 were significantly higher than those of the control periods before (days -1 and 0, post hoc multiple comparisons: $P < 0.05$, Table 1) and after the treatment (days 3 and 4, post hoc multiple comparisons: $P < 0.01$, Table 1).

Translocation

Fecal glucocorticoid levels increased after translocation of a male spotted hyena to a new enclosure. Three days after transport (day 7) levels reached their maximum and were significantly higher than initial baseline levels (Fig. 4). By day 9, fecal glucocorticoid metabolites had returned to the baseline.

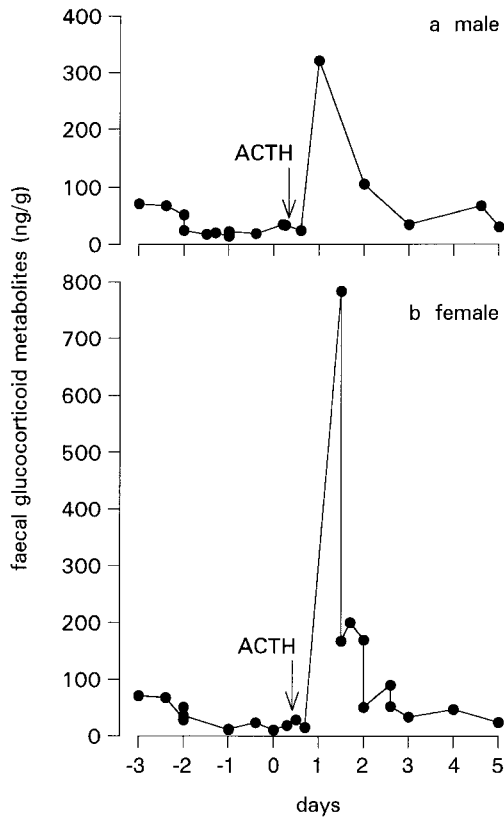


FIG. 3. Faecal glucocorticoid metabolites during an ACTH challenge experiment with 200 IU ACTH in (a) male 4 and (b) female 6.

Social Tension

Social tension and fighting between a male and female spotted hyena led to a significant increase in faecal glucocorticoid metabolites in both animals (Fig. 5). The increase occurred within 24 h after onset of the

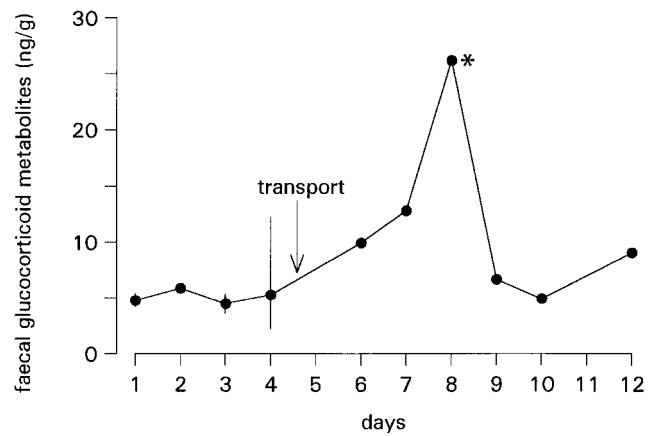


FIG. 4. Faecal glucocorticoid metabolites of male no. 1 before, during, and after translocation (data represent either single samples or daily means; error bars indicate daily concentration range level labeled with * exceeds mean + 3SD).

social tension. The patterns of glucocorticoid clearance were quite different, but levels of both animals returned to baseline after day 6, the second day after aggressions had ceased.

DISCUSSION

Methods of measuring faecal glucocorticoid metabolites have only recently become available (feces: Miller *et al.*, 1991; Graham and Brown, 1996, 1997; Palme and Möstl, 1997; Whitten *et al.*, 1997; Jurke *et al.*, 1998; Monfort *et al.*, 1998; Sousa and Ziegler, 1998; bird

TABLE 1

Mean Faecal Glucocorticoid (GC) Levels before (Days -1\0), during (Days 1\2), and after (Days 3\4) an ACTH Challenge, as Well as ACTH-Induced GC Peak Concentrations

No.	Sex	ACTH (IU)	Mean (\pm SEM) glucocorticoid concn (ng/g)			GC peak conc. (ng/g)	GC peak delay (h \pm range)
			Days -1\0	Days 1\2	Days 3\4		
1 ^a	m	50	4.2 \pm 0.9 (11)	10.5 \pm 6.5 (7)	1.9 \pm 0.3 (6)	32.1	24 \pm 9
2	m	50	14.9 \pm 1.7 (9)	12.8 \pm 2.3 (11)	7.6 \pm 1.0 (7)	29.5	16 \pm 5
3	m	50	64.8 \pm 11.3 (5)	81.2 \pm 28.3 (4)	80.7 \pm 10.5 (3)	164.1	50 \pm 0
4	m	200	25.1 \pm 3.3 (6)	213.8 \pm 107.9 (2)	51.1 \pm 16.3 (2)	321.7	16 \pm 6
5	f	50	9.0 \pm 0.6 (8)	39.8 \pm 27.3 (12)	5.9 \pm 0.4 (8)	337.2	16 \pm 5
6	f	200	17.3 \pm 2.3 (8)	216.7 \pm 105.0 (6)	35.4 \pm 6.5 (3)	783.7	32 \pm 1

$P < 0.05$

$P < 0.01$

Note. Numbers in parentheses refer to number of faecal samples on which the calculation of means is based.

^a Data are means combined from two ACTH-challenge tests conducted on this individual.

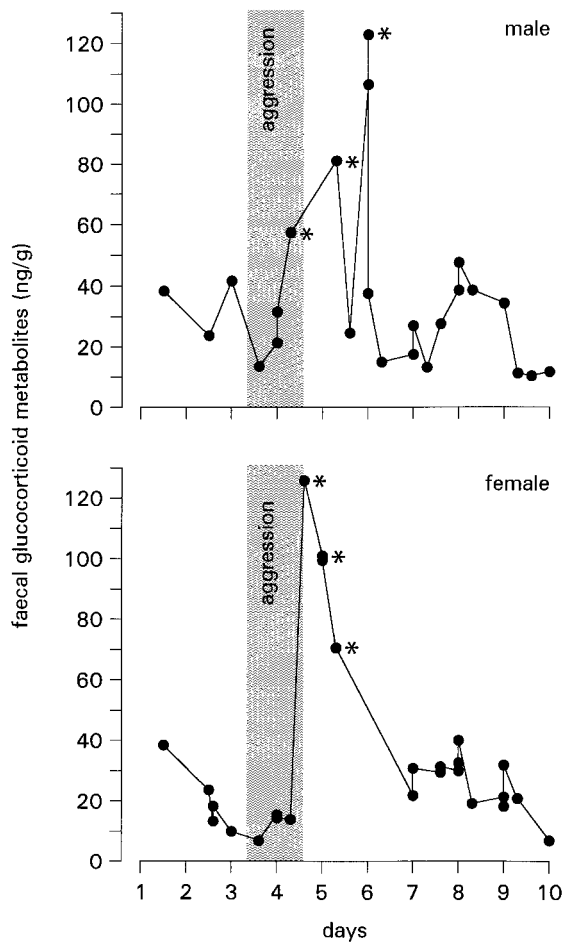


FIG. 5. Faecal glucocorticoid profiles before, during, and after social tension (shaded areas) in male 4 and female 6 (levels labeled with * exceed mean + 3 SD).

droppings: Wasser *et al.*, 1997) and few researchers have applied these noninvasive techniques to free-ranging species (feces: Creel *et al.*, 1997; bird droppings: Kotrschal *et al.*, 1998; Wasser *et al.*, 1997). Before such methods are applied to a particular species, the assays used to measure faecal glucocorticoid metabolites must be validated. Antibodies against plasma glucocorticoids do not necessarily bind to faecal metabolites of these steroids in all species. The 11-oxoeticholanolone assay gives good results in cattle, horse, okapi (*Okapia johnstoni*), roe deer (*Capreolus capreolus*), and sheep (Palme and Möstl, 1997; Dehnhard *et al.*, 1998; Palme *et al.*, 1998; Schwarzenberger *et al.*, 1998), but not in spotted hyenas (this study). As both male and female spotted hyenas are known to have similar

plasma-androgen concentrations (Licht *et al.*, 1992) and the 11-oxoeticholanolone assay cross reacts with 5 β -androstanes, it might be that the response to ACTH was superimposed by faecal androgen metabolites, measured with this assay. Also, antibodies differ in their ability to detect a species hormonal response to an ACTH challenge. The comparison of four antibodies in the current study demonstrated that the ICN-corticosterone antibody was the most appropriate antibody to measure the hormonal response to ACTH in spotted hyenas, as all values were far above the detection limit and the antibody had the highest level of resolution regarding the ACTH challenge. In contrast, although showing a clear response to ACTH, many values measured by the cortisol antibody were close to or even below detection limit. The resolution of the other corticosterone antibody was far less than the ICN-corticosterone antibody, even when using DEE-extracted samples, to allow a direct comparison with the ICN-corticosterone antibody. The 11-oxoeticholanolone antibody, on the other hand, measured most in terms of absolute concentrations, but had the lowest level of resolution compared with all other antibodies.

The aim of this study was to investigate whether measurement of faecal glucocorticoid metabolites is a suitable tool to monitor adrenocortical activity in spotted hyenas. HPLC analysis confirmed that the ICN-corticosterone EIA used in this study detected one immunoreactive metabolite with the polarity of conjugated steroids and several immunoreactive metabolites with the polarity of unconjugated steroids. The DEE-extraction procedure excluded conjugated steroids and thus only the unconjugated steroids contributed to the values measured with the enzyme immunoassay. Although the exact identity of these metabolites remains unknown, several lines of evidence suggest that they were relevant indicators of adrenal activity: (1) administration of exogenous ACTH led to a significant increase in these faecal metabolites in six different individuals, (2) faecal glucocorticoid levels rose after translocation of a male spotted hyena, and (3) a socially stressful situation, where two spotted hyenas fought each other, led to a substantial increase in faecal glucocorticoid metabolites in both animals. Both translocation and fighting are known to enhance glucocorticoid secretion in mammals (translocation: e.g., Carlstead *et al.*, 1993; fighting: e.g., Alberts *et al.*, 1992;

Sachser *et al.*, 1994) and thus add additional evidence to the ACTH-challenge experiment.

Fecal glucocorticoid metabolites in spotted hyenas increased within 24–50 h after injection of ACTH and the onset of a socially stressful situation. Palme *et al.* (1996) suggested that the delay of fecal glucocorticoid excretion in sheep, ponies, and pigs roughly corresponds with the food transit time from duodenum to rectum in these animals. In spotted hyenas, the fecal remains of rye grains and carrots were found approximately 24 h after consumption (personal observation). Thus, taking gut passage time as an approximate measure of food passage time between duodenum and rectum, the time lag of glucocorticoid excretion in feces appears to correspond with food transit time in spotted hyenas as well.

Because fecal glucocorticoid metabolites increased within 50 h after ACTH challenge, it is puzzling that the peak in fecal glucocorticoid metabolites occurred only on the third day after the translocation (see Fig. 4, day 8). As feces were collected only once per day after the arrival in the new zoo, it might be that feces collected on day 8 were actually “deposited” on day 7. If so, the fecal increase in glucocorticoids would be in the expected time range after the transport.

The ICN-corticosterone antibody used in this study had been previously applied to measure fecal glucocorticoid metabolites in domestic cats (*Felis catus*, Graham and Brown, 1996), and African wild dogs (*Lycaon pictus*, Monfort *et al.*, 1998). In African wild dogs, the antibody binds to a single metabolite of intermediate polarity between cortisol and corticosterone, whereas in domestic cats it binds to several highly polar metabolites, which are probably conjugated steroids. Additionally, the present study showed that the corticosterone antibody reacted with one conjugated and several unconjugated metabolites in spotted hyena feces. Thus, although the identity and proportion of glucocorticoid metabolites differ in these three carnivore species, the antibody proved useful to measure changes in adrenocortical activity in all three carnivores. Because domestic cats, African wild dogs, and spotted hyenas belong to three different families of carnivores, the results of the present study support earlier suggestions that this particular antibody may be useful for measuring fecal metabolites of glucocorticoids in other carnivore species as well (Monfort *et al.*,

1998). Having shown that fecal monitoring of glucocorticoids is a useful tool for measuring adrenal activity in spotted hyenas, our future focus will be on finding endocrine correlates of social and environmental stressors in free-ranging Serengeti spotted hyenas.

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