Journal of Zoo and Wildlife Medicine 33(3): 214–221, 2002

REMOTE ASSESSMENT OF STRESS IN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*) AND BLACK RHINOCEROS (*DICEROS BICORNIS*) BY MEASUREMENT OF ADRENAL STEROIDS IN FECES

John W. Turner, Jr., Ph.D., Peter Tolson, Ph.D., and Noel Hamad, M.S.B.S.

Abstract: This study monitored fecal cortisol and corticosterone levels in 14 black rhinoceroses (*Diceros bicornis*) and in seven white rhinoceroses (*Certotherium simum*) under various conditions of captivity, including translocation. Free cortisol and free corticosterone were measured in methylene chloride extracts of feces, using high-performance liquid chromatography (HPLC). The extraction–assay method was validated for quantitative measurement of these hormones by mass spectroscopy analysis, chemical derivitization, and radiolabel tracking and recovery. Both cortisol and corticosterone were extractable from feces and routinely detectable by HPLC. In three nonstressed, captivity-adapted white rhinoceroses monitored across 21 days of routine activity, fecal cortisol ranged from 2.0 to 7.3 ng/g dry feces and corticosterone from 4.0 to 10.8 ng/g dry feces, with no observable trend. Matched plasma, urine, and fecal samples in these rhinoceroses yielded corticosterone:cortisol ratios of 2.0:1.0, 2.7:1.0, and 2.2:1.0, respectively. Both black rhinoceroses (n = 5) and white rhinoceroses (n = 4) exhibited higher fecal cortisol (6.9- to 10.0-fold) and corticosterone (3.2- to 4.5-fold) levels in association with restraint–translocation than in limited free-roaming conditions. In five black rhinoceroses monitored across 6 wk after release from translocation, fecal levels of both cortisol and corticosterone decreased significantly between week 1 and weeks 4–6. In general, cortisol and corticosterone paralleled each other, with cortisol exhibiting a greater range of response. Measurement of either hormone in feces appears to be reliable for adrenal axis monitoring in the white and the black rhinoceroses.

Key words: *Certotherium simum*, *Diceros bicornis*, rhinoceros, fecal hormones, cortisol, stress.

INTRODUCTION

Both white rhinoceros (*Certotherium simum*) and black rhinoceros (*Diceros bicornis*) are endangered species that do not reproduce well in captivity for reasons that are unclear. Stress, defined as any physical or psychologic stimulus that disrupts the homeostasis of the organism, may contribute to this poor reproductive performance. Stress can cause immunosuppression,[23] disruption of metabolism,[12] and gastrointestinal dysfunction.[17] In primates, prolonged stress can suppress reproduction[8] and can inhibit ovarian function and stimulate spontaneous abortion in females.[31] In stressed males of species as diverse as the human being[24] and the Syrian hamster,[26] testosterone production, which is necessary for spermatogenesis,[1] is decreased.

Plasma cortisol levels have been measured to indicate the level of stress associated with capture and translocation in 18 wildlife species.[18] A variety of adverse effects have been demonstrated on free-ranging black rhinoceroses after captivity and translocation, with severity of effects related to time in transport.[14,15]

From the Department of Physiology and Molecular Medicine, Medical College of Ohio, 3035 Arlington Avenue, Toledo, Ohio 43614-5840, USA (Turner, Hamad); and Conservation and Research, The Toledo Zoo, P.O. Box 140130, Toledo, Ohio 43614, USA (Tolson). Correspondence should be directed to Dr. Turner.

Adrenal glucocorticoid levels in biological sources, such as saliva,[19] cerebral spinal fluid,[6] blood,[27] urine,[3,5] and feces,[16] have been used to assess stress levels. Of these sources only urine and feces can reasonably be expected to be accessed in free-ranging animals. Urine can be extracted from soil,[13] but the yield can be unreliable and the product can be contaminated by soil constituents. Solid feces are produced several times daily in many species, and they can be accessed and monitored without direct animal contact. Techniques for fecal glucocorticoid analysis are established and have been used in various primates[28,34,36] and other mammals.[30] Fecal glucocorticoid levels have adequately predicted plasma levels of corticosterone and cortisol in brown capuchin monkeys (*Cebus apella*).[4] Significant corelations between serum and fecal levels of glucocorticoids have been demonstrated,[21,25,29,32] although fecal monitoring provides a more amortized and blunted picture of response to stress, representing cumulative glucocorticoid secretion over a number of hours.[33] In many species, feces and urine contain free cortisol and corticosterone and their hydrolyzed metabolites, with the majority in hydrolyzed form.[2,7,20] Cortisol metabolites in felids are present only in a polar, nonhydrolyzable form.[9]

In this study we report methods for the extraction of cortisol and corticosterone from rhinoceros feces, and also high-performance liquid chromatog-

raphy (HPLC) analysis and hormone identity verification in these fecal extracts. Matched serum, urine, and fecal hormone levels from rhinoceroses are compared, fecal hormone excretion data across 3 wk in captivity-adapted rhinoceroses are presented, and the effect of restraint and shipping stress on fecal hormone levels are examined.

MATERIALS AND METHODS

Animal subjects

This study was conducted between August 1991 and June 1996. Fecal samples from 21 different rhinoceroses were studied, including seven (three males, four females) white rhinoceroses and 14 (seven males, seven females) black rhinoceroses. Care and maintenance routines varied across facilities, but diets were similar, consisting of grass hay and standard herbivore pellets (e.g., HMS Zoo Diets, Bluffton, Indiana 46714, USA), with water available ad lib. Eight of the animals (four white rhinoceroses, four black rhinoceroses) were kept in semi–free-roaming enclosures with outdoor–indoor facilities at the Toledo, Cincinnati, Ft. Worth, and Dallas Zoos. Fifteen (three white rhinoceroses, 12 black rhinoceroses) were free-roaming in the Yulee White Oak (Florida) and Glen Rose Fossil Rim (Texas) Wildlife Preserves.

Sample collection

Fecal samples were collected from five black rhinoceroses (two males, three females) immediately upon their arrival in the United States after being crated and translocated from Zimbabwe (they were free-roaming in the White Oak and Fossil Rim Wildlife Preserves). Fecal sample collections continued periodically from these translocated animals for up to 6 wk after their arrival. Fecal samples were collected from four white rhinoceroses, which were crated and translocated within the United States, with continued periodic sample collection in two of these animals for up to 4 wk. In addition, one male and two female white rhinoceroses which were captive for >7 yr at the Toledo Zoo (TZ) served as the source of samples for methodology development, temporal monitoring of unstressed animals, and serum–urine–fecal comparisons.

On five separate days between 16:00 PM and 18:00 PM, 10 ml of blood was obtained from one male and one female rhinoceros, which had been trained to permit ear venipuncture. The blood-sampling events were uneventful and appeared unstressful to either subject. The blood was centrifuged, and plasma was recovered and frozen until assayed. Overnight fecal and urine samples were collected after blood sampling. Each TZ rhinoceros was routinely housed separately at night, enabling identification of urine and fecal samples with a specific individual. Daytime excrement was removed and each floor was scrubbed and rinsed before placement of animals for overnight collections. Each animal routinely defecated in a perimeter or a corner location in the enclosure and urinated onto the sloping floor, which contained a central drain. A container with a filter was placed in the drain to collect up to 1 L of urine. Two 5-ml aliquots of filtered, clear urine in each sample were extracted and analyzed for hormones.

No samples were available from wild-caught animals before crating and translocation. Thus, black rhinoceroses and white rhinoceroses, which were unstressed and adapted to residence at White Oak and Fossil Rim facilities served as unstressed comparators to the translocated animals. Caretakers at various facilities collected overnight fecal samples before 08:00 AM and daytime samples when they were observed. Approximately 200 g of fecal sample per animal was collected and kept frozen at −20°C in plastic bags until it was sent to our laboratory, where it was thawed and processed.

Fecal steroid extraction

At the time this study was performed, radioimmunoassay techniques to analyze fecal glucocorticoids were not well developed. Thus, we used methods for fecal sample preparation and for organic solvent extraction that were compatible with the analysis of free steroids by HPLC. To minimize contamination by straw and other fibrous material, 50 g of each thawed, moist fecal sample was compressed in a tubular, manual-screw steel press to yield 18 ml of fecal fluid. The press was manufactured in this laboratory using a 50-cm vertical screw with a pitch of 1.3 mm/rotation. The relationship between fecal fluid volume and fecal dry weight was established by determining the weight of each fecal sample presqueezed wet, postsqueezed wet, and dried. Sample water content was 70.9–72.1%, and variation appeared to be random. The fecal fluid was stored at −40°C until extracted. Four milliliters of fecal fluid was placed into a 20-ml polypropylene scintillation vial with 12 ml of HPLC-grade methylene chloride (Aldrich Chemical Co., Milwaukee, Wisconsin 53201, USA). The vial was capped, shaken for 1 hr, inverted, and rapidly frozen to solidify the aqueous portion. The liquid methylene chloride layer was poured into a clean vial, and 2 ml of 0.1 N NaOH was added. After 2 min of centrifugation, the vial was inverted, rapidly frozen, and the methylene chloride layer was poured into a clean vial. This step was repeated

using 18 MΩ water, and the methylene chloride layer was centrifuged at 5,000 *g* for 20 min. The supernatant was poured into a clean vial, dried in a mild air stream, and stored at −40°C until analyzed by HPLC (Dionex Corp., Sunnyvale, California 94088-3603, USA). The above method was also used for serum and urine steroid extraction. Sample volumes were established on the basis of the volume needed to provide measurable hormones in the unstressed condition.

HPLC analysis

For HPLC analysis, dried samples were removed from the freezer and reconstituted in 500 μl of 100% acetonitrile (Aldrich Chemical Company, Milwaukee, Wisconsin 53201, USA). The sample was vortexed for 45 sec, mixed with 1.0 ml of 10% acetonitrile and again vortexed for 45 sec. The sample was then passed though a 0.02-μm syringe filter (Pall Gelman Laboratory, Ann Arbor, Michigan 48103, USA), and a 250-μl aliquot of the sample was injected into the HPLC. Cortisol and corticosterone in reference standards (0.1 μg/ml) and in the above extracts were quantified using an ultraviolet–visible (uv–vis) absorbance detector at a wavelength of 240 nm. The lower limit of detection was 6.3 ng/250 μl injection volume for both standards. The initial eluent condition was 10% acetonitrile and 90% water, with a gradient change over 45 min to yield 90% acetonitrile and 10% water. The flow rate was 1 ml/min. The retention time for cortisol was 16.6 ± 0.2 min and that for corticosterone was 20.1 ± 0.3 min. In reconstituted fecal extracts the nearest detectable peak on either side of the cortisol peak eluted at 15.6 and 17.3 min. On either side of the corticosterone peak the nearest peak was 17.6 and 21.4 min. Water was passed through the system between sample runs to clean the column and minimize variability. There was 6.0% variability in hormone concentration and 0.1% variability in retention time between runs for eight aliquots of the same extract.

Verification of hormone identity

Because fecal extracts contain numerous compounds it was necessary to verify that the HPLC peaks assigned as cortisol and corticosterone contained only these hormones. We determined these identities by derivitization, gas chromatography–mass spectroscopy, and radioactive tracer. Derivitization was performed on cortisol and corticosterone standards and on pooled and concentrated eluent peaks of presumptive cortisol and corticosterone obtained from fecal extracts. The derivatizing agent, methoxyamine-HCl (MOX) in pyridine (Pierce Chemical Corp., Rockford, Illinois 61105, USA), was added to each standard (cortisol, corticosterone) and to the presumptive hormone eluent peak from the fecal extract. The derivitization consisted of conversion of the carbonyl group in the steroid to a methoxime group at positions 3 and 20. This resulted in a characteristic shift in HPLC elution time. After a 15-hr derivatizing incubation[11] each product was extracted and injected into the HPLC. Elution times for derivatized standards were 28.2 min for cortisol and 34.1 min for corticosterone. Spiked, derivatized fecal extract eluents showed peaks at these same times.

Eluent peaks for cortisol and corticosterone standards and presumptive cortisol and corticosterone peaks from fecal extracts were collected and sent to J. Gano (Univ. of Toledo, Toledo, Ohio 43606, USA) for mass spectroscopy analysis. It was necessary to pool peaks from five separate fecal extracts (cortisol and corticosterone pools kept separate) to obtain sufficient material for spectroscopic analysis. The spectroscopic cracking patterns among samples revealed that each presumptive hormone peak from fecal-extract eluents was the hormone expected and that these peaks were not contaminated by other compounds. Final assessment of hormone identity in HPLC eluents was made by spiking fecal extracts with tritiated cortisol or tritiated corticosterone and determining the radioactivity in HPLC eluents from these fecal extracts by liquid scintillation. Only eluent peaks at the retention time for corticosterone or for cortisol showed significant radioactivity and the recovery of the added label was 87.2% and 88.1%, respectively. No assessment of significant HPLC eluent peaks other than those of cortisol and corticosterone was made in this study.

Statistical analysis

All data are presented as mean value ($\bar{x}$) ± standard error (SE) in units of nanograms of hormone per gram of dry feces, except for urine and serum data (micrograms per deciliter of fluid). In cases where two groups or conditions were compared, Student's *t*-test was used.[35] Possible temporal changes were assessed by Repeated Measures ANOVA and paired *t*-test.[35]

RESULTS

Both cortisol and corticosterone were readily extracted from rhinoceros feces and were routinely detected by HPLC in these extracts (Fig. 1). Baseline cortisol and corticosterone values for matched serum, urine, and fecal samples are presented for TZ white rhinoceroses in Table 1. Matched samples

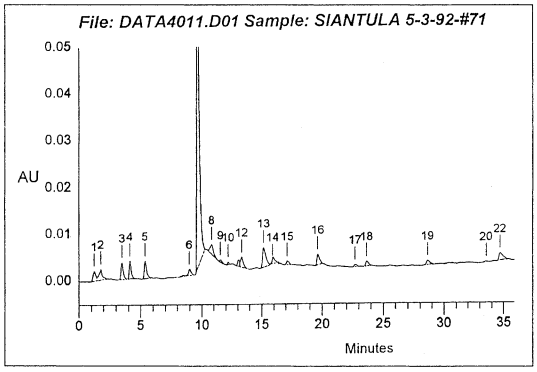

Figure 1. Chromatogram of a methylene chloride–extracted, unspiked fecal sample from an unstressed black rhinoceros. Cortisol is peak #14, and corticosterone is peak #16. AU is absorbance units from UV detector. Identification numbers for peaks 11 and 21 were omitted to reduce congestion. These peaks were not germane to the study.

were not available for black rhinoceroses. In TZ white rhinoceroses the corticosterone:cortisol ratios were 2.0:1.0, 2.7:1.0, and 2.2:1.0 for serum, urine, and feces, respectively. In the TZ white rhinoceroses, defecation frequency varied across days and individuals, with a range of two–four events per 24 hr. No discernible diurnal pattern was noted in samples slotted into three separate daily periods of collection (Table 2).

Many samples were overnight samples, with a maximum possible span of 12 hr from deposit to collection. Because biodegradation was possible across time after samples were deposited, we determined hormone levels in aliquots of the same fecal ball kept at 27°C for 0, 8, and 16 hr after defecation. Cortisol and corticosterone levels as a percentage of the freshest (0 time) aliquot averaged, respectively, 103 ± 3% and 96 ± 4% at 8 hr, and 94 ± 5% and 92 ± 4% at 16 hr in four samples tested.

Baseline values of fecal cortisol and corticosterone were determined in the three captive, unstressed TZ white rhinoceroses over 21 days of routine activities (Fig. 2). Average cortisol values were 2.0–7.3 ng/g dry feces during this period, and average corticosterone levels were 4.0–10.8 ng/g dry feces. The two hormones showed a similar overall pattern, but no trends were observed for either hormone.

Black rhinoceroses and white rhinoceroses exhibited significantly higher fecal levels of cortisol and corticosterone in association with restraint–translocation than in limited free-roaming (adapted to wildlife-preserve captivity) conditions. These levels of cortisol and corticosterone were 6.9-fold and 3.2-fold higher in white rhinoceroses, and 10.0-fold and 4.5-fold higher in black rhinoceroses, respectively. In black rhinoceroses, both cortisol and corticosterone showed a similar pattern during the first 6 wk after arrival at their destination. But levels of cortisol and corticosterone at arrival were 30.1 ± 4.2 ng/g dry feces and 24.2 ± 2.6 ng/g dry feces and thereafter showed a decreasing pattern with values of 8.3 ± 2.4 ng/g dry feces and 9.6 ± 2.2 ng/g dry feces, respectively, after 43 days. For both hormones the average level for the first three collections (week 1) was significantly greater ($P \leq$

Table 2. Fecal cortisol and corticosterone levels in white rhinoceros over 24 hr.

Sampling period	No. of samples[b]	Hormone concentration[a] (ng/g dry feces ± SE)	
		Cortisol	Corticosterone
7 AM–1 PM	6	3.9 ± 1.3	7.6 ± 1.5
1 PM–7 PM	5	5.4 ± 1.1	8.4 ± 1.9
7 PM–7 AM	6	3.6 ± 0.8	6.1 ± 2.3

[a] Values among sampling periods were not different ($P > 0.05$, repeated measures ANOVA).

[b] A sample of every fecal produced across two 24-hr periods was collected from each of the three animals. One animal did not produce a 1 PM–7 PM sample on one day.

Table 1. Glucocorticoid values in matched samples of serum, urine, and feces in captivity-adapted unstressed white rhinoceros.

Hormone	Rhinoceros[a] *n*	Sample[b] *n*	Serum (μg/dl ± SE)	Urine[c] (μg/dl ± SE)	Feces (ng/g dry feces ± SE)
Cortisol	2	5	0.51 ± 0.24	2.5 ± 0.51	3.40 ± 0.44
Corticosterone	2	5	1.01 ± 0.42	6.8 ± 3.2	7.56 ± 0.93

[a] Both animals (one male, one female) had been trained to permit ear venipuncture, were tractable, in captivity >7 yr, and experienced no unusual events during the collection periods.

[b] Each sampling period consisted of afternoon blood collection followed by overnight collection of urine and feces.

[c] Reported on volume basis because creatinine values across these samples varied <10%.

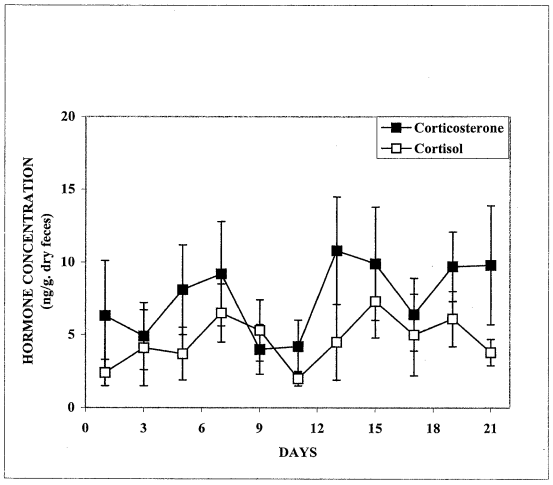

Figure 2. Baseline values of fecal cortisol and corticosterone in three, captivity-adapted white rhinoceroses across time under routine, unstressed conditions.

0.01, paired *t*-test) than the average level for the last three collections (weeks 4–6).

Samples were also collected from captivity-adapted white rhinoceroses and black rhinoceroses in several different facilities. In these samples the average levels (nanograms per gram of dry feces) of both glucocorticoids for black rhinoceroses and for white rhinoceroses were lower (<0.01, *t*-test) than those in samples obtained at arrival from the translocated rhinoceroses of both species (Table 3).

In two white rhinoceroses for which we obtained samples during the 4-wk after translocation, cortisol and corticosterone showed a pattern similar to that for black rhinoceroses with average values of 34.5 and 25.6 ng/g dry feces, respectively, upon arrival and 12.2 and 14.1 ng/g dry feces, respectively, after 4 wk. Relative to these same captivity-adapted black rhinoceroses, samples collected from the translocated black rhinoceroses 4–6 wk after arrival showed average cortisol and corticosterone values that were 2.8- and 1.8-fold greater, respectively.

DISCUSSION

This is the first study of rhinoceros corticosterone and the first comparison of coincident cortisol and corticosterone levels and their response to stress in rhinoceros. At the time this study was initiated, reliable immunoassay methods for fecal glucocorticoids were not available. Such assays faced the difficulty of possible cross-reactivity with a variety of glucocorticoid metabolites present in feces, which could confound the interpretation of experiments. More recently, sensitive and reliable fecal glucocorticoid immunoassays have become available for stress assessment in a variety of species,[30] and such assays have practical application for this purpose. Although the HPLC method avoided the historical immunoassay problems associated with fecal analysis, it was inefficient. Nonetheless, HPLC was used throughout the present study for continuity and because it has some desirable characteristics for examining steroid-related physiology in fecal samples. It permits simultaneous measurement of multiple hormones in one pass through the column, which enables, for example, assessment of multiple stress-related and reproductive hormones concurrently in a single extract for purposes of examining possible relationships between stress and reproduction. It is also useful in experiments with a small number of subjects or samples and for ongoing monitoring of individuals in which regular data feedback is desired for assessment or decision making.

Evaluation of our HPLC methodology revealed fecal cortisol and corticosterone measurement by this means to be accurate and reproducible. Serum and fecal levels in the white rhinoceros were well correlated for both hormones, indicating that fecal hormone levels were a fair measure of adrenocor-

Table 3. Effect of restraint/translocation stress on fecal cortisol and corticosterone levels in white rhinoceros and black rhinoceros.

Species/condition[b]	No. of animals	Hormone concentration[a] (ng/g dry feces ± SE)	
		Cortisol	Corticosterone
White rhinoceros/unstressed	6	4.1 ± 0.6	8.6 ± 1.0
White rhinoceros/stressed	4	28.3 ± 3.4	27.2 ± 2.8
Black rhinoceros/unstressed	6	3.0 ± 0.9	5.4 ± 1.7
Black rhinoceros/stressed	5	30.1 ± 4.2	24.2 ± 2.6

[a] Stressed different ($P < 0.01$, *t*-test) from respective unstressed in all cases.

[b] "Unstressed" samples were collected from unrestrained animals that were well adapted to their captive environment. "Stressed" samples were obtained from animals that had been restrained, crated, and shipped, and samples were collected within 24 hr of arrival.

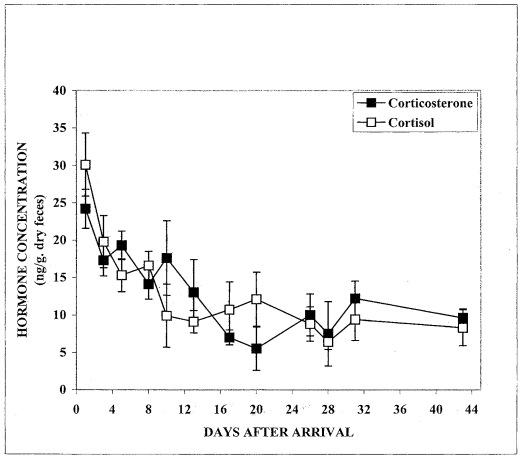

Figure 3. Fecal cortisol and corticosterone levels in black rhinoceroses associated with adaptation to a novel environment after aerial translocation from Zimbabwe to the United States. For each point, $n = 5$, except $n = 4$ on days 8, 9, and 43, and $n = 3$ on day 20.

ticol activity. Black rhinoceros data were insufficient to establish this relationship, but coincident serum and fecal samples obtained on two occasions from two black rhinoceroses yielded ratios for both cortisol and corticosterone, which were similar to those observed in the white rhinoceroses.

In matched serum, urine and fecal samples from white rhinoceroses, the corticosterone:cortisol ratio was consistently 2.0 or greater. Although fecal corticosterone levels exceeded fecal cortisol levels in the unstressed condition in both white rhinoceroses and black rhinoceroses, the stressed condition was associated with greater cortisol than corticosterone levels in both species (Table 3).

There were no consistent differences in temporal patterns between cortisol and corticosterone either in white rhinoceroses during an unstressed baseline (Fig. 2) or in black rhinoceroses during an adaptation to a novel environment (Fig. 3). Nonetheless, overall results suggest that measurement of levels of either hormone alone may be satisfactory for quantification of rhinoceros stress. In comparing the highest (arrival) vs. lowest (adapted) levels for each hormone, it appears that the cortisol response may be more labile than the corticosterone response.

Diurnal fluctuations of both serum cortisol and corticosterone levels have been demonstrated for numerous species.[1] Our failure to observe such a pattern is not, however, surprising because a given fecal sample contained glucocorticoids accumulated across a number of hours and within- or between-animal variation could mask small shifts.

In both white rhinoceroses and black rhinoceroses the levels of both cortisol and corticosterone associated with restraint–shipping were several-fold higher than levels in the same animals after 6 wk of adaptation to a new environment to free-roaming on a preserve and in previously adapted, resident animals in similar environments. These results support the intuitive perspective that restraint–crating–translocation is highly stressful. Fecal glucocorticoid response to exogenous ACTH, commonly used as a measure of maximal adrenocorticol responsiveness,[30] was not determined in this study. But physical restraint and handling are highly stressful for other species.[10,22] Thus, the cortisol–corticosterone levels in the crated and shipped rhinoceroses in the present study are likely to represent the upper end of the physiologic response.

The 6.9- to 10.0-fold and statistically significant difference between the highest (restraint) and lowest (captive, adapted) fecal levels of cortisol, for example, demonstrate that intrinsic variability did not mask the ability to detect a response, an issue often raised with fecal monitoring. But it remains to be determined whether more moderate stress differences, such as those that may be caused by different captive environments or specific long-term social interactions, will be detectable. Considering the small number of rhinoceroses usually available at a given facility, it is probable that the best opportunity to observe such differences will be in a large number of samples taken over many weeks or months, i.e., within-animal, repeated-measure design.

Regarding black rhinoceros, which were translocated, average levels of both hormones decreased over the first 14 days after arrival at the new site, but between 18 and 43 days the levels did not decrease further. Interestingly, average levels at 43 days in these black rhinoceroses (Fig. 3) remained greater than the average levels in adapted black rhinoceroses, which had been at their facility for over 1 yr (Table 3). Direct comparisons are prohibited here because all animals were not at the same facility, and sample collections were not on the same days. But these data suggest that the recent arrivals were not completely adapted after 6 wk.

CONCLUSIONS

This study, which is the first to measure rhinoceros corticosterone levels and their physiologic response to stress, demonstrates that remotely collected feces can be a reliable resource for monitoring stress-related glucocorticoids (cortisol and corticosterone) in both black and white rhinoceros. Fecal levels of these hormones in the white rhinoceros can reflect average blood and urine levels of

these hormones. Daily variability in these fecal hormones in both species is not so great as to mask response to the stressful condition of restraint–translocation. Furthermore, cortisol and corticosterone levels in black rhinoceros decrease during the several weeks after stress. Fecal glucocorticoid measurement has potential as a noninvasive tool for stress monitoring in rhinoceros.

Acknowledgments: This study was supported by Institute of Museum Services Conservation Grants (IC-00359-90 and IG-10178-91) the Toledo Zoo and the Medical College of Ohio. We thank Dr. E. Blumer, K. S. Burns, J. Roehrs, K. Barton, C. Kern-Buell, A. Postma; G. Favata and R. Gould for assistance; and the personnel at Yulee White Oak Preserve, the Glen Rose Fossil Rim Preserves and at the Cincinnati, Ft. Worth, and Dallas Zoos who collected samples.

LITERATURE CITED

1. Asterita, M. F. 1985. The Physiology of Stress. Human Sciences Press, New York.

2. Bahr, N. I., R. Palme, U. Mohle, J. K. Hodges, and M. Heistermann. 2000. Comparative aspects of the metabolism and excretion of cortisol in three individual nonhuman primates. Gen. Comp. Endocrinol. 117: 427–438.

3. Beerda, B., M. B. H. Schilder, N. S. C. R. M. Janssen, and J. A. Mol. 1996. The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs. Horm. Behav. 30: 272–279.

4. Boinski, S., S. P. Swing, T. S. Gross, and J. K. Davis. 1999. Environmental enrichment of brown capuchins (Cebus apella): behavioral and plasma and fecal cortisol measures of effectiveness. Am. J. Primatol. 48: 49–68.

5. Brown, J. L., C. M. Wemmer, and J. Lehnhard. 1995. Urinary cortisol analysis for monitoring adrenal activity in elephants. Zoo Biol. 14: 533–542.

6. DeRiu, P. L., V. Petruzzi, M. A. Caria, O. Mameli, A. R. Casu, S. Nuvoli, A. Spanu, and G. Madeddu. 1997. Beta-endorphin and cortisol levels in plasma and CSF following acute experimental spinal traumas. Physiol. Behav. 62: 1–5.

7. Docke, F. 1994. Veterinarmedizinische Endokrinologie, 3rd ed. G. Fischer, Jena, Germany.

8. Dunbar, R. I. M. 1989. Reproductive strategies of female gelada baboons. *In:* Rasa, A. E., C. Vogel, and E. Voland (eds.). The Socio-Biology of Sexual and Reproductive Strategies. Univ. Press, Cambridge. Pp. 74–92.

9. Graham, L. H., and J. L. Brown. 1996. Cortisol metabolism in the domestic cat and implications for noninvasive monitoring of adrenocortical function in endangered felids. Zoo Biol. 15: 71–82.

10. Gregory, L. F., T. S. Gross, A. B. Bolten, K. A. Bjorndal, and L. J. Guillette, Jr. 1996. Plasma corticosterone concentrations associated with acute captivity stress in wild Loggerhead sea turtles (*Caretta caretta*). Gen. Comp. Endocrinol. 104: 312–320.

11. Horning, M. G., A. M. Moss, and E. C. Horning. 1968. Formation and gas-liquid chromatographic behavior of isometric steroid ketone methoxime derivatives. Anal. Biochem. 22: 284–294.

12. Jayo, J. M., C. A. Shively, J. R. Kaplan, and S. B. Manuck. 1993. Effects of exercise and stress on body fat distribution in male cynomolgus monkeys. Int. J. Obes. Relat. Metab. Disord. 17: 597–604.

13. Kirkpatrick, J. F., L. Kasman, B. L. Lasley, and J. W. Turner, Jr. 1988. Pregnancy determination in uncaptured feral horses. J. Wildl. Manag. 52: 305–308.

14. Kock, M. D., R. DuTout, D. Morton, K. Kock, and B. Paul. 1990. Baseline biological data collected from chemically immobilized free-ranging black rhinoceros (*Diceros bicornis*) in Zimbabwe. J. Zoo Wildl. Med. 21: 283–291.

15. Kock, R. A., S. R. Mihok, J. Wambua, J. M. Wanzia, and K. Saigawa. 1999. Effects of translocation on hematologic patterns of free-ranging black rhinoceros (*Diceros bicornis michaeli*) in Kenya. J. Zoo Wildl. Med. 30: 389–396.

16. Monfort, S. L., K. L. Mashburn, B. A. Brewer, and S. R. Creel. 1998. Evaluating adrenal activity in African wild dogs (Lycaon pictus) by fecal corticosteroid analysis. J. Zoo Wildl. Med. 29: 129–133.

17. Monnikes, H., B. G. Schmidt, J. Tebbe, C. Bauer, and Y. Tache. 1994. Microinfusion of corticotropin releasing factor into the locus coeroleus/subcoeroleus nuclei stimulates colonic motor function in rats. Brain Res. 644: 101–107.

18. Morton, D. J., E. Anderson, C. M. Foggin, M. D. Kock, and E. P. Tiran. 1995. Plasma cortisol as an indicator of stress due to capture and translocation in wildlife species. Vet. Rec. 136: 60–63.

19. Ohl, F., C. Kirschbaum, and E. Fuchs. 1999. Evaluation of hypothalamo-pituitary-adrenal activity in the tree shrew (Tupaia belangeri) via salivary cortisol measurement. Lab. Anim. 33: 269–274.

20. Palme, R., P. Fischer, H. Schildorfer, and M. N. Isamail. 1997. Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. Anim. Reprod. Sci. 43: 43–63.

21. Palme, R., and E. Mostl. 1997. Measure of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int. J. Mamm. Biol. 62: 192–197.

22. Palme, R., C. Robia, W. Baumgartner, and E. Mostl. 2000. Transport stress in cattle as reflected by an increase in faecal cortisol metabolite concentrations. Vet. Rec. 146: 108–109.

23. Peristein, R. S., M. H. Whitnall, J. S. Abrams, E. H. Mougey, and R. Neta. 1993. Synergistic roles of interleukin-6 and interleukin-1 and tumor necrosis factor in adrenocorticotropin response to bacterial lipopolysaccharide in vitro. Endocrinology 132: 946–952.

24. Singer, F., and B. Zumoff. 1992. Subnormal serum testosterone levels in male internal medicine residents. Steroids 57: 86–89.

25. Stravisky, R. 1994. Socioendocrinology: Noninvasive Techniques for Monitoring Reproductive Function in

Captive and Free-Ranging Primates. Ph.D. Dissertation, Emory Univ., Atlanta, Georgia.

26. Tsuchiya, T., and I. Horii. 1995. Different effects of acute and chronic immobilization stress on plasma testosterone levels in male Syrian hamsters. Psychoneuroendocrinology 20: 95–102.

27. Turner, J. W., Jr., and T. H. Fine. 1991. Restricting environmental stimulation influences levels and variability of plasma cortisol. J. Appl. Physiol. 70: 210–213.

28. Wallner, B., E. Moarl, J. Dittami, and H. Prossinger. 1999. Fecal glucocorticoids document stress in female barbary macaques (Macaca sylvanus). Gen. Comp. Endocrinol. 113: 80–86.

29. Wasser, S. K., K. Bevis, G. King, and E. Hanson. 1997. Noninvasive physiological measures of disturbances in Northern spotted owl. Cons. Biol. 11: 1919–1922.

30. Wasser, S. K., K. E. Hunt, J. L. Brown, K. Cooper, C. M. Crockett, U. Bechert, J. J. Millspaugh, S. Larson, and S. L. Monfort. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. 120: 260–275.

31. Wasser, S. K., and A. K. Starling. 1988. Proximate and ultimate causes of reproductive suppression among female yellow baboons at Mikumi National Park, Tanzania. Am. J. Primatol. 16: 97–121.

32. Whitten, P. L. 1997. Noninvasive methods for the study of stress, reproductive function, and aggression. Am. J. Phys. Anthropol. 24: 239.

33. Whitten, P. L., D. K. Brockman, and R. C. Stavisky. 1998. Recent advances in noninvasive techniques to monitor hormone-behavior interactions. Yearbook Phys. Anthropol. 41: 1–23.

34. Whitten, P. L., and E. Russell. 1996. Information content of sexual swellings and fecal steroids in sooty mangabeys (*Cercocebus torquatus* atys.). Am. J. Primatol. 40: 67–82.

35. Zar, J. H. 1984. Biostatistical Analysis, 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey.

36. Ziegler, T. E., G. Scheffler, D. Wittwer, N. Schultz-Darken, C. T. Snowdon, and D. H. Abbott. 1996. Metabolism of reproductive steroids during the ovarian cycle in two species of callitrichids, Saguinus oedipus and Callithrix jacchus, and estimation of the ovulatory period from fecal steroids. Biol. Reprod. 54: 91–99.

Received for publication 6 September 2001