SHORT COMMUNICATIONS

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Dehorning Does Not Alter the Stress Response in Southern White Rhinoceroses (*Ceratotherium simum simum*) during Transport: A Preliminary Investigation.

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ABSTRACT: Translocation and dehorning are common and important practices for rhinoceros management and conservation. It is not known if dehorning causes a stress response or negatively affects rhinoceroses during transport. Twenty-three subadult wild white rhinoceros bulls were immobilized and translocated >280 km for population management reasons. Ten animals were dehorned at capture, and 13 animals were transported without dehorning. For transport, five dehorned and six nondehorned rhinoceroses were sedated with azaperone (62.38±9.54 μg/kg) and five dehorned and seven nondehorned rhinoceroses with midazolam (64.61±9.28 μg/kg). Blood samples were collected at capture, start of transport, and after 6 h of transport. Measurements included 10 physiologic variables: hematocrit, total serum protein, creatine kinase (CK), aspartate aminotransferase, gamma-glutamyl transferase (GGT), creatinine, urea, cholesterol, β-hydroxybutyrate, and glucose; and four stress-response variables: cortisol, epinephrine, neutrophil-to-lymphocyte ratio, and leukocyte coping capacity. Using a linear mixed model, CK and GGT were higher in dehorned compared with nondehorned rhinoceroses. There were no significant differences in the other variables between the two groups. The likely cause of these differences is that dehorned animals spent more time in the crate before the start of transport than nondehorned rhinoceroses (3:11±0:54 h vs. 1:12±0:56 h, $P<0.001$). These results indicate that dehorning does not negatively alter the white rhinoceros' physiologic and stress responses during translocation, supporting its use for antipoaching measures.

Key words: Dehorning, stress, stress biomarkers, translocation, white rhinoceros.

The number of southern white rhinoceroses (*Ceratotherium simum simum*) is declining, predominantly due to poaching. One intervention used to reduce poaching incidents is dehorning (Convention on International Trade in Endangered Species of Wild Fauna and Flora 2022). Dehorning, or horn trimming, is the controlled removal of a large part of the rhinoceros' horn through a veterinary procedure (Badenhorst et al. 2016). Another intervention used is translocation to safe, well-protected areas (Emslie et al. 2009). Translocation is defined as "the human-mediated movement of living organisms from one area, with release in another" (International Union for Conservation of Nature/Species Survival Commission 2013). Translocation is associated with stress that can negatively affect animal welfare (Dickens et al. 2010).

Although dehorning and translocations are important conservation methods in the fight against poaching, it is unknown whether dehorning negatively affects rhinoceroses during transport. The aim of our study was to assess and compare physiologic and stress responses in sedated dehorned and nondehorned white rhinoceroses during transportation. We hypothesized that there would be no difference between dehorned and nondehorned animals.

This study formed part of a larger research effort investigating physiologic consequences of capture and transport in white rhinoceroses (Pohlin et al. 2020). Ethical approval was granted

by the University of Pretoria Animal Ethics Committee (V067-17) and the South African National Parks Animal Use and Care Committee (009/17).

A total of 23 subadult male southern white rhinoceroses were transported >280 km within the Kruger National Park, South Africa for management reasons. Four, and in one translocation three, rhinoceroses were captured from the wild and translocated at a time. The first two animals were always dehorned and the last two animals were transported without dehorning.

All rhinoceroses were captured by darting from a helicopter into the gluteal muscle using 3-mL plastic darts with a 60-mm uncollared needle (Dan-Inject, Skukuza, South Africa). Eleven rhinoceroses were immobilized with the drug combination etorphine (Captivon, 9.8 mg/mL, Wildlife Pharmaceuticals, Karino, South Africa) and azaperone (azaperone tartrate, 50 mg/mL, Wildlife Pharmaceuticals); 12 animals were immobilized with etorphine and midazolam (Dazonil® 50 mg/mL, Wildlife Pharmaceuticals). Etorphine doses were based on standardized estimated weight categories; approximately 2.5 µg/kg was administered (Pohlin et al. 2020). Azaperone or midazolam was administered at five times the etorphine dose. The time and distance run from darting until the animal became recumbent were recorded. Dehorning was performed immediately after first contact with the immobilized animal. An electrical saw was used to trim the rostral and caudal horns at 100 mm and 25 mm above the skin–horn interface, respectively (Badenhorst et al. 2016). After completion, butorphanol (5 mg/mg etorphine; 50 mg/mL, butorphanol tartrate, Wildlife Pharmaceuticals) was administered intravenously (IV) to partially antagonize the etorphine and enable walking and loading of the rhinoceroses into International Air Transport Association-approved transport crates. The time from when the animal became recumbent until it was up and walking into the transport crate was recorded as the down time.

Transport started once the three to four animals of the respective translocation had been captured and loaded. Diprenorphine (3 mg/mg etorphine; Activon, 12 mg/mL, Wildlife Pharmaceuticals) was administered and, during transport, azaperone or midazolam was readministered intramuscularly every 2 h at 25 times the etorphine dose, respectively. Details regarding the capture, transport, and blood sample collection are available in Pohlin et al. (2020). Overall, 5/11 animals tranquilized with azaperone were dehorned and 6/11 were not; 5/12 animals tranquilized with midazolam were dehorned and 7/12 not. At the destination, after 6 h of transport, 80 mg of naltrexone (Trexonil, 50 mg/mL, Wildlife Pharmaceuticals) was administered IV and the rhinoceroses released back into the wild. The time each animal spent in the transport crate was recorded as translocation time.

We used blood samples collected at capture, start of transport (t0), and after 6 h of transport (t6). These were collected from an auricular IV catheter directly into EDTA, lithium heparin, and serum tubes (BD Vacutainer, Becton Dickinson, Oxford, UK). Plasma for epinephrine measurement was harvested from EDTA tubes as soon as possible and snap-frozen in liquid nitrogen. Lithium heparinized whole blood was used to measure the leukocyte coping capacity (LCC) within 10 min after collection. Serum tubes were stored in a cooler box with ice and centrifuged within 12 h. Serum was aliquoted and stored at −80 C until further analysis. We analyzed 10 physiologic and four stress-response variables. Packed cell volume was measured using the manual method (Steyrer et al. 2022). Serum biochemistry variables were analyzed using an automated analyzer (Cobas Integra 400 Plus, Roche Diagnostics Ltd., Rotkreuz, Switzerland) using commercially available kits and included total serum protein (TSP), creatine kinase (CK), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), creatinine (Crea), urea, cholesterol (Chol), and β-hydroxybutyrate (BHB). Using EDTA blood samples, glucose (Gluc) was analyzed immediately using a portable point-of-care blood gas analyzer with test cards (Kyron Laboratories, Johannesburg, South Africa). Stress-response variables included serum cortisol and plasma epinephrine concentrations, neutrophil-to-lymphocyte ratio (N:L ratio), and LCC, and were analyzed as described (Pohlin et al. 2020).

Statistical tests were performed using RStudio 1.2.5033 (R Foundation for Statistical Computing, Vienna, Austria). Data were assessed for normality by calculating descriptive statistics and plotting histograms. Mean and SD were calculated for each analyte per sample time point and group and interval plots were generated for descriptive purposes. Changes over time and between groups for variables of interest were assessed using a linear mixed model (lme4-package), with time, drug (azaperone vs. midazolam), and dehorned (yes vs. no) as fixed factors, and rhinoceros as random factor. Because of the small sample size, nonparametric analyses were used to compare the difference in time to recumbency, distance run after darting, down time, and translocation time between dehorned and nondehorned rhinoceroses using a Wilcoxon-test. A P-value <0.05 was considered significant for all tests.

A descriptive analysis of results is provided in Table 1. Model-based analyses, including coefficient estimates and corresponding standard errors and significance levels, are presented in Table 2.

There was no difference in time to recumbency ($P=0.67$) or the distance run after darting ($P=0.78$) between dehorned and nondehorned animals. However, the down time (33±7 min vs. 27±5 min; $P=0.03$) and translocation time (10:54±1:12 h vs. 8:48±1 h; $P<0.001$) was longer in dehorned than in nondehorned rhinoceroses. Dehorned animals were captured first, resulting in a longer waiting period from capture to the start of transport (3:11±0:54 h vs. 1:12±0:56 h).

Only CK and GGT were higher in dehorned compared with nondehorned rhinoceroses ($P<0.05$, Table 2). There was no significant difference in any of the other physiologic variables between animals that were dehorned or not. However, we found a strong and significant effect of time across all physiologic variables. Packed cell volume, TSP, Crea, Chol, and Gluc decreased and CK, AST, urea, and BHB increased from capture to t6 ($P<0.05$, Table 2), regardless of the dehorning status of the animals. Gamma-glutamyl transferase mildly increased from capture to t0 and returned to capture levels thereafter. Drug effect was not associated with differences in any of the physiologic variables.

We detected no differences in serum cortisol, plasma epinephrine, N:L ratio, and LCC between dehorned and nondehorned rhinoceroses. The effects of drug and time have been reported elsewhere (Pohlin et al. 2020). Briefly, serum cortisol concentrations increased from capture to t0 and decreased thereafter; N:L ratio increased from t0 to t6, whereas plasma epinephrine concentrations were above the laboratory detection limit (5 nmol/L) only at capture. Leukocyte coping capacity did not change significantly over time. The drugs used were not associated with changes in these stress variables (Table 2).

Capture and transport of dehorned and nondehorned white rhinoceroses induced changes in serum enzymes and metabolites. Creatine kinase and GGT activities were higher in dehorned than in nondehorned rhinoceroses. The likely cause of these differences is the longer down time and time dehorned animals spent in the transport crates; this represents a major limitation of this study and may have influenced results. Journey duration is known to affect the activities of serum CK and other variables (Grigor et al. 1998). Elevated serum CK activities have been reported for transported rhinoceroses, indicating myocyte damage and fatigue that increases over time (Kock et al. 1990). Changes in GGT activity may indicate biliary hyperplasia, cholestasis, or oxidative stress (Xing et al. 2022). These rhinoceroses are known to have developed oxidative stress (Pohlin et al. 2020). However, whether this is the reason for the observed changes in GGT and why there was a difference in this variable between the two groups remains unclear.

Rhinoceroses mounted a stress response to translocation characterized by high serum cortisol, plasma epinephrine, and N:L ratios (Pohlin et al. 2020), which did not differ between dehorned and nondehorned rhinoceroses. Badenhorst et al. (2016) observed a peak in fecal corticosteroid metabolite (FCM) concentrations immediately after immobilization for dehorning, indicating that rhinoceroses had experienced an acute endocrine stress response to dehorning. However, the lack of a control group did not allow the authors to distinguish between the effect of immobilization and dehorning. Our data suggest that the stress response is caused by immobilization and

TABLE 1. Mean±SD of the physiological variables: packed cell volume (PCV), total serum protein (TSP), creatine kinase (CK), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), creatinine (Crea), urea, cholesterol (Chol), β-hydroxybutyrate (BHB), glucose (Glu); and the stress-response variables: serum cortisol, plasma epinephrine concentrations, neutrophil-to-lymphocyte ratio (N:L ratio), and leukocyte coping capacity (LCC) at capture, the start of transport (t0), and at 6 h of transport (t6) for dehorned and nondehorned southern white rhinoceroses (*Ceratotherium simum simum*) tranquilized with either azaperone or midazolam.

	Dehorned (n=10)			Nondehorned (n=13)		
Sample point	Capture	Start transport	Transport 6 h	Capture	Start transport	Transport 6 h
PCV (%)	45±2	38±5	33±3	46±3	36±4	36±2
TSP (g/L)	88±8	83±9	83±10	89±2	78±4	81±3
CK (U/L)	288±205	810±263	2305±1865	205±51	357±123	1,389±767
AST (U/L)	49±14	57±16	79±37	56±8	52±9	72±16
GGT (U/L)	19±8	20±8	18±7	14±3	14±5	14±5
Crea (μmol/L)	142± 23	139±24	138±27	152±28	152±27	148±27
Urea (mmol/L)	3.2±0.3	3.4±0.4	3.7±0.3	3.3±0.5	3.5±0.5	3.8±0.5
Chol (mmol/L)	2.86±0.38	2.75± 0.42	2.47±0.49	2.67±0.63	2.36±0.57	2.30±0.60
BHB (mmol/L)	0.18±0.08	0.37± 0.13	0.45±0.20	0.17±0.03	0.23±0.08	0.56±0.23
Glu (mmol/L)	9.8±3.4	6.0±2.4	8.1±1.2	9.0±2.3	7.6±2.2	7.6±1.6
Cort (nmol/L)	48.5±23.2	128.4±33.5	60.6±22.7	50.4±15.5	105.2±46.5	97.3±53.1
Epinephrine (nmol/L)	11.58±7.90	bdl[a]	bdl	8.77±6.00	bdl	bdl
Epinephrine (ng/mL)	2.12±1.45	bdl	bdl	1.61±1.10	bdl	bdl
N:L ratio	1.64±0.92	3.78±2.28	8.43±3.20	1.50±0.62	3.10 ±2.26	9.83±9.97
LCC	2,785±1,686	3,820±1,571	4,484±2,992	3,297±1,765	4,989±4,173	4,621±3,082

[a] bdl = below detection limit (5 nmol/L).

TABLE 2. Coefficient estimates (standard errors) for drug (azaperone vs. midazolam), dehorning (no vs. yes), and sample time for packed cell volume (PCV), total serum protein (TSP), creatine kinase (CK), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), creatinine (Crea), urea, cholesterol (Chol), β-hydroxybutyrate (BHB), glucose (Glu), serum cortisol, plasma epinephrine concentrations, neutrophil-to-lymphocyte ratio (N:L ratio), and leukocyte coping capacity (LCC) in southern white rhinoceroses (*Ceratotherium simum simum*). Level of significance: $P<0.05$. Reference category: drug azaperone, nondehorned, sample time capture.

	Dependent variable													
	PCV (%)	TSP (g/L)	CK (U/L)	AST (U/L)	GGT (U/L)	Crea (μmol/L)	Urea (mmol/L)	Chol (mmol/L)	BHB (mmol/L)	Glu (mmol/L)	Cort (nmol/L)	Epinephrine (ng/mL)	N:L ratio	LCC
Drug midazolam	0.27	−2.72	−109.44	−4.58	−2.27	5.73	0.00	−0.04	0.02	1.04	13.60	−0.11	0.34	172.36
	(1.05)	(2.46)	(215.04)	(6.76)	(2.46)	(11.05)	(0.18)	(0.22)	(0.04)	(0.57)	(11.02)	(0.64)	(1.28)	(716.66)
Dehorned yes	−0.54	1.79	**475.34**[a]	1.24	**5.09**[a]	−10.03	−0.10	0.25	0.02	−0.06	−4.62	0.21	−0.18	−599.51
	(1.06)	(2.48)	(216.49)	(6.81)	(2.48)	(11.13)	(0.18)	(0.22)	(0.04)	(0.58)	(11.11)	(0.64)	(1.29)	(722.15)
Sample time	−8.83*	−8.46[a]	312.73	0.83	0.88[a]	−1.17	0.12[a]	−0.22[a]	0.12[a]	−2.45[a]	65.68[a]	−1.37[a]	1.83	1,406.74
Start transport	(0.81)	(0.91)	(225.07)	(3.30)	(0.45)	(1.61)	(0.05)	(0.05)	(0.04)	(0.64)	(9.69)	(0.52)	(1.30)	(788.60)
Sample time	−10.96[a]	−6.65[a]	1,553.07[a]	21.52[a]	−0.13	−4.74[a]	0.46[a]	−0.38[a]	0.33[a]	−1.56[a]	31.74[a]	−1.76[a]	7.66[a]	1,487.21
Transport 6 h	(0.81)	(0.92)	(227.91)	(3.35)	(0.45)	(1.64)	(0.05)	(0.05)	(0.05)	(0.64)	(9.69)	(0.52)	(1.30)	(788.60)
Constant	45.75[a]	89.24[a]	91.43	55.18[a]	14.98[a]	148.81[a]	3.34[a]	2.66[a]	0.16[a]	8.83[a]	44.50[a]	2.61[a]	1.46	3,244.83[a]
	(1.01)	(2.17)	(224.34)	(6.07)	(2.12)	(9.49)	(0.15)	(0.19)	(0.04)	(0.61)	(10.96)	(0.56)	(1.33)	(763.29)
Observations	69	68	68	68	68	68	68	68	68	69	69	20	69	69
Log likelihood	−172.01	−191.37	−518.027	−267.205	−160.94	−246.26	−11.31	−14.77	22.80	−149.94	−328.45	−26.25	−196.93	−605.51
Akaike information criterion	358.02	396.74	1,050.05	548.410	335.88	506.52	36.63	43.55	−31.60	313.88	670.90	66.50	407.85	1,225.03

[a]$P<0.05$.

translocation, not by dehorning itself, since we found no differences between dehorned and nondehorned rhinoceroses. Similarly, Penny et al. (2020) measured FCM in dehorned rhinoceroses and found no long-lasting stress responses to this procedure. However, because of variable time-sample intervals, the authors might have missed peak FCM concentrations in that study. To appropriately capture and interpret a stress response, it is important to consider that various techniques integrate the effects of stress on different timescales. Short-term responses to acute stressors can be assessed by measuring plasma epinephrine concentrations (seconds to minutes). Serum cortisol concentrations change within minutes, whereas N:L ratio and LCC are characterized by a longer response latency (minutes to hours; Gormally and Romero 2020). Integrating a combination of these different stress response variables in our rhinoceroses enabled a multimodal assessment of the stress response to translocation and dehorning on different timescales, minimizing the likelihood of missing peak stress responses. Unfortunately, because of the wild nature of the rhinoceroses, no blood samples could be collected before immobilization from conscious, nonstressed animals as individual baseline values.

Our findings support the use of dehorning as an effective antipoaching tool in translocation programs. However, new research in black rhinoceroses (*Diceros bicornis*) indicates that dehorning may have demographic and behavioral consequences over the long term (Duthé et al. 2023). Whether these findings are applicable to white rhinoceroses requires further investigation, but dehorning may have other unexplored clinical effects.

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