

CHAPTER 3: CHALLENGES TO ANIMAL WELFARE ASSOCIATED WITH CAPTURE AND LONG ROAD TRANSPORT IN BOMA-ADAPTED BLACK (*DICEROS BICORNIS MINOR*) AND SEMI-CAPTIVE WHITE (*CERATOTHERIUM SIMUM SIMUM*) RHINOCEROS

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This research paper has been adjusted to this thesis format.

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CHAPTER 4: ELECTROLYTE AND ACID-BASE RESPONSES TO CAPTURE AND TRANSPORT IN WILD SOUTHERN WHITE RHINOCEROS BULLS (*CERATOTHERIUM SIMUM SIMUM*) SEDATED WITH EITHER AZAPERONE OR MIDAZOLAM

This chapter has been submitted as a research paper for publication and is currently under review by the Journal of Veterinary Anaesthesia and Analgesia:

Pohlin F, Buss P, Hooijberg EH, Meyer LCR. Midazolam alters acid-base status less than azaperone during the capture and transport of wild southern white rhinoceros (*Ceratotherium simum simum*).

CHAPTER 5: HAEMATOLOGICAL AND IMMUNOLOGICAL RESPONSES TO CAPTURE AND TRANSPORT IN WILD SOUTHERN WHITE RHINOCEROS BULLS (*CERATOTHERIUM SIMUM SIMUM*) SEDATED WITH EITHER AZAPERONE OR MIDAZOLAM

This chapter is in preparation to be submitted as a research paper for publication.

Pohlin F, Hoojiberg EH, Buss P, Huber N, Viljoen FP, Blackhurst D, Meyer LCR. Haematological and immunological responses to capture and transport in wild southern white rhinoceros bulls (*Ceratotherium simum simum*) and their modulation by midazolam compared to azaperone.

oxidative stress could be implemented in post-release monitoring and investigated together with spatial, behavioural, hormonal, and disease measurements to identify animals with chronic stress and increased risk of developing morbidity after translocation. These measurements could be used to further investigate the effectiveness and clinical importance of applied interventions that aim to mitigate translocation stress.

Regardless of the mounting of an acute or chronic stress response, factors like **muscular fatigue, energy imbalance and dehydration** became important with increasing transport time in both black and white rhinoceros, representing stressors themselves.

All animals, including the ones transported for only six hours, experienced a pronounced increase in CK and AST over time indicating that myocyte metabolic dysfunction and skeletal muscle damage occurred ([chapter 3](#) and [4](#)) (260). These findings are also common in transported domestic and other wild animals (95,124,141,220) and have been attributed to tiredness and fatigue associated with a prolonged state of muscle contraction due to standing and might be intensified in rhinoceros because of their heavy weight (68). Intense exercise during the capture, repeated intramuscular administration of the tranquilising drugs, and dehydration likely further contribute to this issue (48,221,239).

In domestic animals, it is believed that increases in muscle enzymes tend to be indicative of knocks and bruises (68). Rhinoceros are generally sedated during transport and the drugs used for sedation, including the benzodiazepines, are known to affect neuromuscular processing related to balance control (304). These effects mean that the risk of losing balance to sudden perturbations during transport, and being knocked and bruised, are probably high in transported rhinoceros.

Interventions that might alleviate muscle injury could be: (1) providing padding to the transport crate, (2) motivating the rhinoceros to stand more comfortably (e.g. providing food might motivate animals to stand more put in order to eat), and, or (3)

using drugs, or doses, that cause less ataxia and allow the animal to have better footing and lay down when required. These interventions need to be systematically investigated in future research.

Another factor that became more important over time was energy metabolism. Glucose concentrations did not differ between “capture” and “after transport” samples in black and white rhinoceros transported over a long time ([chapter 3](#)). In the white rhinoceros bulls, glucose peaked at capture, likely in response to elevated adrenaline concentrations from a fight or flight response, but did not change throughout a six hour transport ([chapter 4](#)). Although glucose concentrations gave no indication that rhinoceros entered a negative energy balance, increasing BHB and NEFA concentrations (white rhinoceros [chapter 3](#) and [4](#)) over time demonstrated that there was mobilisation of lipid stores from the adipose tissue to generate energy (258). The black rhinoceros in [chapter 3](#) only showed an increase in plasma triglyceride concentrations, which could also indicate the mobilisation of lipid stores for energy, or could be the result of oxidative stress. Again, the effect of temporary captivity on these clinical chemistry analytes should be investigated in more detail. To prevent rhinoceros from entering a negative energy balance during transport, further research on nutritional planning (i.e. type and amount of food) prior to, and during translocation, is also necessary.

Finally, black and white rhinoceros transported over a long time (over 19 hours) ([chapter 3](#)) developed dehydration. Long periods of time without access to water (and food) have been identified as a major concern in domestic animal transports (69). Rhinoceros may be exposed to prolonged thirst and dehydration during long transport even if water is offered, as they are usually reluctant to drink during transport from unknown sources (M. Hofmeyr, pers. comm.). It is important to note that there are interactions between the thermal conditions of a journey and the animals’ resistance to the effects of water deprivation (68). In rhinoceros, this resistance appears to be high under natural conditions, because these animals usually

only drink once a day to every second day (29,211). However, if thermal conditions are very hot, rhinoceros are likely to develop dehydration more easily (68,69). Current recommendations state that rhinoceros should be captured when temperatures are lower than 25°C and transported during colder months of the year (24,29). Even if translocations take place during cold and dry seasons, it can still become very hot in the middle of the day, and especially if crates are not adequately ventilated. Therefore, it is important to monitor thermal conditions, especially in the crates during transport, and further investigate the effects of changing thermal conditions on the rhinoceros' resistance to water deprivation. Currently there are no recommended limits for transport-duration, or water deprivation times, for rhinoceros. Importantly, these should be established.

To provide a logical and comprehensive framework for good animal welfare, the Farm Animal Welfare Council of the United Kingdom established the concept of the five freedoms, defining ideal states for an animal's physical and mental condition, which also apply during transport (179). These freedoms include: (1) freedom from hunger and thirst; (2) freedom from discomfort; (3) freedom from pain, injury, or disease; (4) freedom to express normal behaviour; and (5) freedom from fear and distress (73,179). Later, from these five freedoms, five domains of potential animal welfare compromise have been established, namely: nutrition, environment, health, behaviour, and mental state (178). To a certain degree, it appears to be possible to comply with most of these aspects during rhinoceros translocation.

By administering midazolam, instead of azaperone, we attempted to reduce stressors associated with capture and transport and enhance the freedom from fear and distress, or mental state ([chapter 5](#)). However, we were not able to demonstrate a stress reducing effect of this drug at the given dose. Instead, we identified a possible immunosuppressive effect of the benzodiazepine. Further studies are required to find out if this effect is dose-dependent, of prolonged duration, and if it leads to an increase in the rhinoceros' susceptibility to disease after transport. In rats, midazolam reversed

behavioural deficits associated with chronic stress and mitigated stress-induced hyperthermia (191,193). These effects remain to be investigated in transported rhinoceros as the benefit of behavioural coping might be more important than the immunological side effects.

A main limitation of this study was the lack of control animals, which would have helped in 1) better differentiating the effects of capture versus transport, and 2) the effects of tranquiliser-administration during transport. We tried to differentiate the effects of capture from the effects of transport by taking a “start transport (T0)” blood sample. This differentiation worked well with clinical variables that change quickly (e.g. electrolytes), but was only of limited value for slow-changing variables (e.g. N:L ratio). Ideally, a set of control animals should have been immobilised, sampled and then released without any form of transport. Samples would then have had to be collected from these animals after release at the same intervals as for the transported animals without re-immobilising them. Clearly, this would have been extremely difficult and could not be done. Moreover, for ethical reasons, we decided against a non-tranquilised but transported control group, because the risk of injury would have been too high. Nevertheless, we believe that this study allowed us to identify major challenges to animal welfare associated with capture and transport in rhinoceros.

To sum up, rhinoceros experienced respiratory and metabolic (lactic) acidosis during capture and a mild metabolic alkalosis during transport. The mounting of a stress response to capture and transport was associated with characteristic immunological changes. Skeletal muscle fatigue, energy imbalance and dehydration occurred over time. Midazolam reduced the metabolic acidosis during capture, but was associated with immunosuppression.

Based on these results, we recommend the use of midazolam, instead of azaperone, for etorphine-based capture of white rhinoceros. During transport, there appears to be no benefit in using midazolam over azaperone. Further studies investigating

cardiopulmonary, immunological, and behavioural differences between these drugs during rhinoceros translocation are required.

To improve animal welfare during long transports, future research needs to investigate other interventions that might enhance one, or more, of the “five freedoms”, or “domains” during rhinoceros translocation. Offering fluids and, or, nutritional supplements could increase the freedom of hunger and thirst during rhinoceros transport (nutritional domain). Padding of the transport crates, and better monitoring and regulating of environmental conditions within them, could increase the freedom of discomfort (environmental domain) and the use of midazolam could improve behavioural coping during transport (behavioural domain) and therefore be advised despite its immunological effects. Animal health is an important part of animal welfare and improving animal welfare during transport will increase the freedom of disease during, and after, rhinoceros transport (health domain) (72).

The ultimate goal of this thesis was to help improve the outcome of rhinoceros translocation and contribute towards conservation of the species. The information gained from this thesis, importantly, has paved the way for further studies that can now be aimed at reducing the stressors, and their consequences, that are induced by capture and transport in rhinoceros, thereby improving animal welfare and the success of rhinoceros conservation translocations.

CHAPTER 7: FUTURE RESEARCH DIRECTIONS

The results of this thesis, in particular the work done on the physiological effects of long road transport in black and white rhinoceros and the modulation of these effects by using of midazolam instead of azaperone, point the way for several new studies.

New avenues of investigation could include:

1. Establishment of limits for transport-duration, or water and food deprivation times, in black and white rhinoceros.
2. Exploration of the value of temporary confinement as part of rhinoceros translocation.
3. Exploration of immunological responses to chronic stress by investigating characteristic changes in leukocyte subpopulation numbers and function and inflammatory cytokine concentrations.
4. Investigation of nutritional feasibility and planning (e.g. type and amount of food, potential side-effects) for rhinoceros during transport.
5. Investigation of the effects of changing thermal conditions on the rhinoceros' resistance to water deprivation.
6. Identification of effective methods of fluid administration (e.g. route of administration, type of fluid, and possible parenteral feeding) during long transport in rhinoceros
7. Systematic investigation of midazolam use in black rhinoceros.
8. Further investigation of cardiopulmonary effects when midazolam is used instead of azaperone for the capture of white rhinoceros to determine arterial blood pressure and arterial blood gases.
9. Further investigation of immunological effects associated with the use of midazolam in white rhinoceros to further characterise white cell function and the susceptibility to disease after transport.

10. Exploration of possible behavioural benefits of using midazolam, instead of azaperone, for the transport of white rhinoceros
11. Post-release monitoring should be included in future studies to better evaluate the clinical relevance of measured physiological responses to capture and transport in black and white rhinoceros and their modulation by implemented interventions.

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APPENDICES

PUBLICATIONS

- 1) **Pohlin F**, Hooijberg EH, Meyer LCR. A review on the effects of transport on animal welfare in wild mammalian species. Review article; submitted to the Journal of Zoo and Wildlife Medicine (in review).
- 2) **Pohlin F**, Hofmeyr M, Hooijberg EH, Blackhurst D, Reuben M, Cooper D, Meyer LCR. Challenges to animal welfare associated with capture and long road transport in boma-adapted black (*Diceros bicornis minor*) and semi-captive white (*Ceratotherium simum simum*) rhinoceroses. J Wildlife Dis 2020; 56(2):000-000 (in press).
- 3) **Pohlin F**, Buss P, Hooijberg EH, Meyer LCR. Midazolam alters acid-base status less than azaperone during the capture and transport of wild Southern white rhinoceros (*Ceratotherium simum simum*). Research article; submitted to the Journal of Veterinary Anesthesia and Analgesia (in review).
- 4) **Pohlin F**, Hooijberg EH, Buss P, Huber N, Viljoen FP, Blackhurst D, Meyer LCR. Haematological and immunological responses to capture and transport stress in wild white rhinoceros bulls (*Ceratotherium simum simum*) and their modulation by midazolam compared to azaperone. Research article; in preparation.

CHALLENGES TO ANIMAL WELFARE ASSOCIATED WITH CAPTURE AND LONG ROAD TRANSPORT IN BOMA-ADAPTED BLACK (*DICEROS BICORNIS*) AND SEMI-CAPTIVE WHITE (*CERATOTHERIUM SIMUM SIMUM*) RHINOCEROSES

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ABSTRACT: Capture and transport are part of translocation and expose animals to a variety of stressors that can lead to morbidity and mortality. We aimed to establish a better understanding of the physiologic responses to capture and transport in black (*Diceros bicornis*) and white (*Ceratotherium simum simum*) rhinoceroses in Southern Africa. Fourteen adult black rhinoceroses were transported 600 km by vehicle and 32 white rhinoceroses (24 adults and 8 juveniles) were transported 1,300 km by vehicle. The black rhinoceroses had been wild-caught and boma-adapted over 6 wk prior to the translocation and were only sedated to allow for loading into the transport crates. The white rhinoceroses originated from a game farm and were chemically immobilized from a helicopter and then loaded. Paired blood samples were collected from animals at loading (capture) and after transport and evaluated for changes in clinical chemistry analytes, acute phase reactants, and oxidative stress biomarkers. The Wilcoxon rank sum test was used to compare changes in measured analytes from capture and after transport. All rhinoceroses survived capture and transport. Rhinoceroses experienced total body water loss, mobilization of energy reserves, and muscular damage. Alterations in acute phase reactants suggested that animals mounted a stress response. Oxidative stress was observed in black rhinoceroses. We identified the following challenges to animal welfare during transport: hydration status, energy balance, skeletal muscle fatigue, and stress-induced immunomodulation. Measures to mitigate these challenges, such as administration of fluids, need to be included in the planning of future translocations.

Key words: Energy balance, fatigue, hydration, rhinoceros, stress, translocation, transport.

INTRODUCTION

The Southern-central black rhinoceros (*Diceros bicornis minor*) is listed as critically endangered, and the Southern white rhinoceros (*Ceratotherium simum simum*) as near threatened, by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Emslie 2011, 2012). The main reasons for these assessments are the continued and increased poaching threat and the increasing illegal demand for rhinoceros horn associated with the increased involve-


ment of organized international criminal syndicates in rhinoceros poaching (Emslie et al. 2016). Translocation for population reintroduction or reinforcement, or metapopulation management, represents an essential tool for the management of these species and is an integral part of national and international rhinoceros conservation plans (Knight 2017). Translocation involves capture, temporary captivity, transport, and release into a novel environment, exposing the animals to a variety of stressors such as prolonged periods of

CONGRESS ORAL PRESENTATIONS RELATED TO THIS THESIS

- 1) **Pohlin F**, O'Dell JH, Hooijberg EH, Cooper D, Leeming R, Flamand J, Meuffels J, Meyer LCR. Effects of transport on clinical chemistry analytes in black rhinoceros (*Diceros bicornis*) translocated in South Africa. Wildlife Group of the South African Veterinary Association (SAVA) Annual Congress, Muldersdrift, South Africa, March 2018.
- 2) **Pohlin F**, Hofmeyr M, Reuben M, Hooijberg EH, Meyer LCR. Effects of capture and transport on clinical chemistry analytes in white rhinoceros (*Ceratotherium simum*) translocated for over 30 hours. Joint AAZV/ EAZWV/ Leibniz-IZW Zoo and Wildlife Health Conference, Prague, Czech Republic, October 2018. Murray Fowler International Conference awardee.
- 3) **Pohlin F**, Buss P, Hooijberg EH, Meyer LCR. Using haematological measurands to assess translocation-stress in white rhinoceros (*Ceratotherium simum*) sedated with either azaperone or midazolam. Wildlife Group of the SAVA Annual Congress, Muldersdrift, South Africa, March 2019.

CONGRESS POSTER PRESENTATIONS RELATED TO THIS THESIS

- 1) **Pohlin F, Hofmeyr M, Reuben M, Hooijberg EH, O'Dell JH, Cooper D, Meyer LCR.** Effects of capture and transport on clinical chemistry analytes in black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. Faculty day, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort, South Africa, August 2018. Winner of the best poster award.



Effects of capture and transport on clinical chemistry analytes in black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses

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Introduction

Translocation is a strategy used for the management and conservation of black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros populations in Southern Africa.¹ Capture and transport are part of translocation and expose the animals to a variety of stresses that might ultimately lead to translocation failure.² The aim of this study was to establish a more comprehensive understanding of the pathophysiology and "stress" associated with capture and transport of African rhinoceroses.

Methods

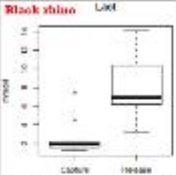
<h5>Black rhinoceros</h5> <ul style="list-style-type: none"> ■ Captured in bomas ■ 14 rhinos - all adults ■ 1 female - 1 male ■ transported over 800 km (18-23 hours) ■ Sedated during transport ■ Released into the wild 	<h5>White rhinoceros</h5> <ul style="list-style-type: none"> ■ Captured in the wild ■ 32 rhinos - 24 adults, 8 calves ■ 18 females - 8 males ■ transported over 1,800 km (30.5-40 hours) ■ Sedated during transport ■ Released into the wild
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Paired blood samples were collected from the animal's auricular vein at capture and release and changes in selected clinical chemistry analytes were compared using the Wilcoxon rank sum test.

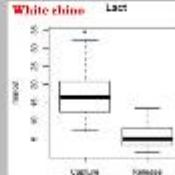
Results

Clinical chemistry analyte	Black rhino	White rhino	Clinical chemistry analyte	Black rhino	White rhino
Albumin	↑	n.s.	Koagulation	↓	↓
Alkaline phosphatase	n.s.	n.s.	Prothrombin	↓	↓
Aspartate aminotransferase	↑	↑	Serum amyloid A	↑	↑
β-hydroxybutyrate	n.s.	↑	Sodium	↑	↑
Chloride	↑	↑	Total bilirubin	↑	↑
Cholesterol	↓	n.s.	Total serum protein	↑	n.s.
Cortisol	n.s.	n.s.	Total calcium	↑	↓
Creatinine	n.s.	↑	Urea	n.s.	↑
Creatine kinase	↑	↑			
γ-glutamyl transferase	n.s.	n.s.			
Glucocorticoid	n.s.	n.s.			
Glucose	n.s.	n.s.			
Haptoglobin	n.s.	n.s.			
Iron	↓	↓			
Lactate	↑	↓			

n.s., non significant
↑ increase
↓ decrease



Black rhino Lact



White rhino Lact

The difference in serum lactate concentrations between capture and release are more likely reflecting the different capture techniques used in the black (bomas) vs. white (wild) rhinoceroses.
Note: variability was greater between species, life-history stage, gender and individuals in all clinical chemistry analytes.

Conclusion

Transport causes changes in clinical chemistry analytes in black and white rhinoceroses. Similar changes have been reported in transported farm-animals and attributed to³

- Dehydration and deprivation of food and water
- Carabolic & anaerobic shifts in carbohydrate and lipid metabolism
- Muscle damage due to handling, vigorous exercise, or myopathy
- Acute phase response
- "Stress". No significant increase in cortisol might not necessarily mean no stress. It might reflect:
 - Efficacy of the sedative drugs
 - Habituation to transport "stress"
 - "Exhaustion" of the hypothalamic-pituitary-adrenal axis

DIFFERENCES between


- Species
- Capture & management techniques
- Age, gender & life-history stage and their implications to animal welfare need to be investigated further!

Acknowledgements

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2) Pohlin F, Buss P, Hooijberg EH, Meyer LCR. Stress haemoconcentration during the capture and transport of free-ranging white rhinoceros (*Ceratotherium simum*) sedated with either azaperone or midazolam. Joint Leibniz-IZW/ EAZWV/ ECZM Zoo- and Wildlife Health Conference, Kolmården, Sweden, June 2019.

Stress haemoconcentration during the capture and transport of free-ranging white rhinoceroses (*Ceratotherium simum*) sedated with either azaperone or midazolam

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Introduction

Translocation represents an essential practice used in the management of white rhinoceroses.¹ Capture and transport are part of translocation, and are associated with stress which could ultimately lead to translocation failure.² Haemoconcentration indicates an increased ratio of red blood cells and large molecules (>69 kDa) to the plasma volume and has been associated with acute stress in laboratory animals.³ Here, we measured the response of common indicators of haemoconcentration to capture and transport in free-ranging white rhinoceroses sedated with either azaperone or midazolam.

Results

■ Azaperone
■ Midazolam

Mean ± SEM
 * Significant difference from T1 in the other sample point (p < 0.05)
 † Not found to be significantly different from control (p > 0.05)

Methods
Study animals: 83 free-ranging sub-adult white rhinoceros bulls
2 groups:
 ■ Azaperone (n=11, ZapsolX, Wildlife Pharm., 50 mg/mL)
 ■ Midazolam (n=18, DaocidX, Wildlife Pharm., 50 mg/mL)
Capture: from helicopters
 ■ Etorphine (3-4 mg, i.m.; Captivetop, Wildlife Pharm., 0.8 mg/mL)
 ■ Azaperone/midazolam (8 x strobilium dose, mg, i.m.)
Loading:
 ■ Butorphanol (18-28 mg, i.v.; Wildlife Pharm., 80 mg/mL)
 ■ Diprenorphine (0-18 mg, i.v.; Actwan, Wildlife Pharm., 12 mg/mL)
Transport:
 ■ Azaperone/midazolam (25 x etorphine dose, mg, i.m.), every 3 hours
Serial blood samples were collected from an antecubital intravenous catheter at (T1) capture; (T2) start of transport; and (T3) after six hours of transport. Changes in measured variables over time and between groups were compared using general mixed effects models.

T1 -
 Effect of catecholamine-release (etorphine/stress)
 ■ Splenic contraction: acylcysteine-release
 ■ Hypertension: increased hydrostatic pressure leads to fluid shifts from the vessels into the extravascular space¹
T1* Azaperone: α-1-adrenergic-blocking effect becomes apparent as capture-stress wears off
 ■ Splenic outpouring of acylcysteine¹

T1 -
 Effect of catecholamine-release (etorphine/stress)
 ■ Splenic contraction: release of acylcysteine that may be more aneocytic than circulating red blood cells

T1 -
 Effect of catecholamine-release (etorphine/stress)
 ■ Hypertension: plasma passes into interstitial spaces, plasma proteins are unable to passively pass through capillary pores¹
T1-T3 Midazolam: immunosuppressive? less stress?⁴
 Albumin²
 ■ Decrease during acute phase reaction/stress
 ■ Fluid shifts: acid-base balance
 Globulin²
 ■ Increase during acute phase reaction/stress

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Acknowledgements

We thank EAZWV for providing the clinical pathology laboratory at the Onderstepoort Veterinary Academic Hospital, and all members and volunteers for field and lab assistance.

Conclusion

Capture and transport caused changes in blood haematological measurements in white rhinoceros bulls.

- Capture was associated with acute stress
- Recovery from the acute capture-stress occurred by the start of transport
- With transport-duration other effects became more prominent
- Immunological effects of stress
- Effect of the drugs used
 - Midazolam (anxiolytic) appeared to influence the stress-induced immune-response
 - α-1-adrenergic-blocking effect of azaperone on the splanchnic

Better understanding the clinical relevance of the rhinoceroses' response to the sedative drugs administered during transport is critical as it may play a role in the development of disease and translocation failure.

3) **Pohlin F, Buss P, Hooijberg EH, Meyer LCR.** The effect of capture and transport on serum cortisol and total thyroxine concentrations in white rhinoceros (*Ceratotherium simum simum*) sedated with either azaperone or midazolam. Conference of the International Society of Wildlife Endocrinology (ISWE), Skukuza, Kruger National Park, South Africa, October 2019. Cayman Chemical travel grant awardee. Winner of the best poster award.

The effect of capture and transport on serum cortisol and total thyroxine concentrations in white rhinoceroses (*Ceratotherium simum simum*) sedated with either azaperone or midazolam

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³ Conservation Ecology, 2010 Rhodes, Wildlife Research Centre, University of Pretoria, Onderstepoort, South Africa

INTRODUCTION

Translocation is an effective tool for rhinoceros conservation.
Capture and transport are part of translocation and expose the animals to stressors.
Stress affects thyroid gland function and thus energy, bone mass and reproductive performance.
Midazolam has strong anxiolytic properties and might be better in reducing stress than azaperone, which is more commonly used in rhinoceros translocation.⁴

Study aims:

- To assess the effects of capture and 6-hour road-transport on serum cortisol and total thyroxine concentrations in free-ranging white rhinoceros bulls.
- To determine whether these effects can be reduced by using midazolam instead of azaperone.

MATERIALS AND METHODS

Study animals: 23 wild sub-adult white rhinoceros bulls.

Two groups:

- Azaperone (n=11, Zapan[®] Wildlife Pharmaceuticals, 50 mg/mL)
- Midazolam (n=12, Dormin[®] Wildlife Pharmaceuticals, 50 mg/mL)

Captives: 100% halothane

- Etorphine (3-4 mg, Linc; Captivet[®] Wildlife Pharmaceuticals, 9.8 mg/mL)
- Azaperone or midazolam (5x etorphine dose, mg, Linc)

Transport:

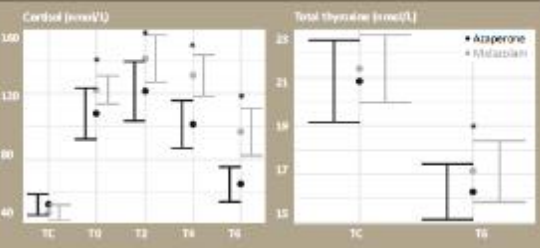
- Azaperone or midazolam (25 x etorphine dose, mg, Linc), every 2 hours

Serial blood samples:

- Collected from an aural artery: intravenous catheter
- Time points: capture (TC), start of transport (T0), and at two (T2), four (T4) and six (T6) hours of transport
- Measurements: serum cortisol (TC to T6) and total thyroxine (TC and T6)
- Analytical method: Siemens reagents 1000
- Statistical analysis: general mixed effects models

RESULTS


- Cortisol concentrations increased, peaked at two hours of transport, and decreased thereafter.
- Total thyroxine decreased from capture to after transport.
- No significant differences between the two groups.
- Midazolam sedated rhinoceroses tended to have higher cortisol concentrations than azaperone sedated rhinoceroses.



* Significant difference from TC over time (P<0.05)

CONCLUSION

- Rhinoceroses mounted a stress response to capture and transport.
- The decrease in total thyroxine concentrations was unexpected.
 - Transport of stressed animals results in total thyroxine loss?⁵
- Possible causes for the decrease in total thyroxine in our rhinoceroses could be:
 - Hypermetabolic state during capture.⁶
 - Food restriction and hypometabolism during transport.
 - Chronic stress.⁴
- Midazolam-use did NOT decrease the magnitude of stress response.
 - Dose too low?⁷
 - Direct effect on adrenal glands via peripheral benzodiazepine receptors?⁴
- Further research:
 - Serial measurement of total thyroxine and other thyroid hormones.
 - Investigation of midazolam use at different dosages.
 - Include post-release monitoring including reproductive success!



References:

1. AMEY SM, SMITH A, JONES S, et al. (2010) Individual variation in the stress response to collection events in a large African ungulate. *Journal of Animal Ecology*, **79**, 1185-1192.
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Acknowledgements: We thank Cayman Chemical for the financial support for the travel grant. We also thank the staff of the Kruger National Park for their assistance during the fieldwork.






OTHER PRESENTATIONS OR INITIATIVES RELATED TO THIS THESIS

- 1) Online presentation on “translocation stress in wildlife” at the joint Southern Africa Wildlife Disease Association Student Chapter (SAWDASC)/ Zoo and Wildlife Medicine Study Group (ZWMSG) online journal club, 2017 (repeated 2018).
- 2) Oral presentation on “translocating wildlife” at the Conservation of Exotics, Zoo- and Wildlife Symposium, Ghent University, Merelbeke, Belgium, 2017.
- 3) “Run rhino run” flash tattoo (small tattoos for a good cause), raising public awareness for rhinoceros poaching and funds for this project by [DEFF INK](#) tattoo, Berlin, Germany 2018.



[KOBEN](#) 2018

- 4) Oral presentation on “rhinoceros translocation” at the rhinoceros conservation fundraiser „Studierende gegen Wilderei - Ein Abend für den Nashornschutz“, Vetmeduni Vienna, Austria, 2018.
- 5) Oral presentation on “physiological responses to capture and transport in black and white rhinoceros” at the Wildlife Disease and Conservation Evening of the SAWDASC at the University of Cape Town, Cape Town, South Africa, 2019.
- 6) Presentation evening to the general public on “illegal wildlife trafficking and rhinoceros poaching” at UFO, Bruneck, Italy, 2019.
- 7) Interactive children workshop on “illegal wildlife trafficking and rhinoceros poaching” at the Paul-Troger middle school (grade 6 to 8), Welsberg-Taisten, Italy, 2020.

Congratulations to [Neil Aldridge](#) for winning the 1st price in the World Press 2018 Photo Contest, category: environment, singles. This photo shows a young southern white rhinoceros, immobilised and blindfolded, which is about to be released into the wild in the Okavango Delta, Botswana, after being captured and transported from South Africa for protection from poachers ([chapter 3](#)).



ANIMAL ETHICS CERTIFICATES



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	Pharmacological management of stress and its pathophysiological consequences during the transport of the free-ranging white rhinos (<i>Ceratotherium simum</i>)
PROJECT NUMBER	V067-17
RESEARCHER/PRINCIPAL INVESTIGATOR	F Pohlin

STUDENT NUMBER (where applicable)	U_17310441
DISSERTATION/THESIS SUBMITTED FOR	PhD

ANIMAL SPECIES	White rhinos (<i>Ceratotherium simum</i>)	White rhinos (<i>Ceratotherium simum</i>)
NUMBER OF SAMPLES	3-6 (Pilot study)	50 Experiment
Approval period to use animals for research/testing purposes		June 2017- June 2018
SUPERVISOR	Prof. L Meyer	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	26 June 2017
CHAIRMAN: UP Animal Ethics Committee	Signature	

54285-15



UNIVERSITEIT VAN PRETORIA
 UNIVERSITY OF PRETORIA
 YUNIBESITHI YA PRETORIA

Animal Ethics Committee


PROJECT TITLE	Pharmacological management of stress and its pathophysiological consequences during the transport of the free-ranging white rhinos (<i>Ceratotherium simum</i>)
PROJECT NUMBER	V067-17 (Amendment 1)
RESEARCHER/PRINCIPAL INVESTIGATOR	F Pohlin

STUDENT NUMBER (where applicable)	U_17310441
DISSERTATION/THESIS SUBMITTED FOR	PhD

ANIMAL SPECIES	White rhinos (<i>Ceratotherium simum</i>)	
NUMBER OF SAMPLES	32	
Approval period to use animals for research/testing purposes	January 2018 – January 2019	
SUPERVISOR	Prof. L Meyer	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED (* with condition)	Date	7 February 2018
CHAIRMAN: UP Animal Ethics Committee	Signature	

CONDITION

Please submit a detailed progress report of the pilot study as well as an incident report if any occurred

S4285-15



**Faculty of Veterinary Science
Animal Ethics Committee**

5 August 2019

**Approval Certificate
Annual Renewal
(Extension 2)**

AEC Reference No.: V067-17
Title: Pharmacological management of stress and its pathophysiological consequences during the transport of free-ranging rhinoceroses in Southern Africa
Researcher: Mrs F Pohlin
Student's Supervisor: Prof LCR Meyer

Dear Mrs F Pohlin,

The **Annual Renewal** as supported by documents received between 2019-06-06 and 2019-07-29 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2019-07-29.

Please note the following about your ethics approval:

1. The use of species is approved:

Species and Samples	Number
White rhinoceros (<i>Ceratotherium simum</i>)	88

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2020-08-05.
3. Please remember to use your protocol number (V067-17) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Prof. V. Naidoo
CHAIRMAN: UP-Animal Ethics Committee

To develop and manage a system of national parks that represents the biodiversity, landscapes, and associated heritage assets of South Africa for the sustainable use and benefit of all.



ANIMAL USE AND CARE COMMITTEE: APPLICATION FOR APPROVAL

A. PROJECT DETAILS

Project Title:	Pharmacological management of stress and its pathophysiological consequences during the transport of free-ranging white rhinoceros (<i>Ceratotherium simum</i>).		
Researcher	Friederike Pohlin	SANParks Reference No.	009/17

B. SCIENTIFIC REVIEW STATEMENT

(Every application should be supported by a declaration that it has undergone prior scientific review through at least one of the SANParks Research Nodes.)

This research protocol has been reviewed by the Savannah and / or Arid Research Centres SANParks and has been judged to be of national importance, designed in accordance with accepted scientific practices and norms and is in the opinion of the reviewers likely to be successful in achieving its objective.

Name: H Hendricks Designation: Sr GM: CSD Signature:  Date: 30/8/2017
 Ps. As per Research Approval process.

Note: In accordance with the South African National Standard (SANS 10386-2008): "The Care and Use of Animals for Scientific Purposes", an animal is regarded as being "live, sentient non-human vertebrate, including eggs, foetuses and embryos, that is, fish, amphibians, reptiles, birds and mammals, including domestic animals, purpose-bred animals, farm animals, wildlife and higher invertebrates such as advanced members of the Cephalopoda and Decapoda".

This form should be submitted with the SANParks standard Research Project Application, and (where relevant) the following supporting documents: CVs of practitioners in support of competence to handle or treat animals, notices of approval of other ethics committees, diagrams or references illustrating the equipment and/or techniques to be applied.

For Administrative Purposes			
Submission Date	27 th July, 2017	APPROVED	DISAPPROVED
AUCC approval / Disapproval Date	30/8/2017	Signature 	
Reason for Decision	The study fully meets ethical standards		

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