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Changes in clinical chemistry analytes in boma-adapted compared to free-ranging wild rhinoceroses (*Ceratotherium simum*) transported by airplane compared to road

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Abbreviations

Abbreviation	*Description*
A	Azaperone
ACTH	Adrenocorticotropic hormone
ALB	Albumin
ALB/GLOB	Albumin/Globulin
ALP	Alkaline phosphatase
AST	Aspartate-aminotransferase
B	Butorphanol
BHB	Beta-hydroxybutyrate
CA	Calcium
CHOL	Cholesterol
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CK	Creatine kinase
CL	Chloride
CORT	Cortisol
COVID (-19)	Coronavirus disease (2019)
CREA	Creatinine
CRH	Corticotropin releasing hormone
FE	Iron
GGT	Gamma-glutamyl transferase
GLDH	Glutamate dehydrogenase
GLOB	Globulin
GLUC	Glucose
HP	Haptoglobin
HPA	Hypothalamic-pituitary-adrenal axis
IATA	International Air Transport Association
i.m.	intramuscular
IUCN	International Union for Conservation of Nature

i.v.	intravenously
K	Kalium
LACT	Lactate
LDH	Lactate dehydrogenase
M	Midazolam
MG	Magnesium
NA	Sodium
NaF tubes	Sodium Fluoride tubes
NEFA	Non-esterified fatty acids
N:L ratio	Neutrophil to lymphocyte ratio
PHOS	Phosphor
SAM	Sympathetic-adrenal-medullary system
TBILI	Total bilirubin
TP	Total protein
TRIG	Triglycerides
TSP	Total serum protein

Zusammenfassung

Nashornumsiedlungen sind eine wichtige Managementmaßnahme für die Erhaltung dieser Tierart, bedeuten allerdings zwangsläufig Risiken für die Tiere. Um die Transportmethoden zu verbessern ist es wichtig, die mit diesem Eingriff verbundenen Pathophysiologien besser zu verstehen.

In dieser Studie wurden Blutproben von insgesamt 16 Nashörnern genommen. Acht dieser Nashörner wurden in freier Wildbahn gefangen und anschließend für sechs Wochen in einem Boma (Einzäunung) gehalten. Am Tag des Transports wurden sie via Distanz-Injektion vom Boden aus mit Etorphin und Azaperon immobilisiert und unter Sedierung für 18,48 ± 0,02 Stunden mittels Flugzeug transportiert. Blutproben wurden von einer Ohrvene der immobilisierten Nashörner bei Erstkontakt, unmittelbar nach erfolgreichem Fang und kurz vor der Freilassung nach dem Transport entnommen.

Die Plasma-Konzentrationen von Creatin-Kinase und Aspartat-Aminotransferase stiegen während des Transports an ($p= 0,002$ und $p= 0,019$), was auf eine muskuläre Anstrengung aufgrund des lang andauernden Balancierens und Stehens hinweist. Leichte Erhöhungen der Natrium- und Gesamtbilirubin-Konzentrationen und Verringerungen der Harnstoff-, Kalium-, Cholesterin-, Magnesium- und Phosphor-Konzentrationen deuten auf metabolische Verschiebungen hin ($p< 0,05$). Biochemische Stressmarker wie Glukose, Laktat und Cortisol veränderten sich im Laufe des Transports nicht, was wahrscheinlich auf die lange Zeitspanne zwischen den beiden Zeitpunkten der Probennahme oder auf die Erschöpfung der Hypothalamus-Hypophysen-Nebennieren-Achse durch die vorübergehende Boma-Haltung zurückzuführen ist.

Die Daten dieser Nashörner wurden zusätzlich mit den Daten von den anderen acht freilebenden Breitmaulnashörnern, die von einem Hubschrauber aus mit der gleichen Medikamentenkombination immobilisiert und unmittelbar über die gleiche Distanz in einem geeigneten Straßenfahrzeug (25,92 Stunden) transportiert wurden, verglichen. Die Konzentrationen der oben genannten Analyten war bei den an die Boma angepassten Nashörnern nach dem Fang niedriger ($p= 0,05$, $p< 0,001$ und $p= 0,003$), unterschieden sich aber bei der Freilassung nicht ($p> 0,05$).

Abstract

Translocations are essential for rhino conservation but pose risks to animal welfare. It is therefore important to better understand the pathophysiology associated with this intervention. Sixteen rhinoceroses were included in this study. Eight of these rhinos were wild-caught, boma-adapted (6 weeks) and immobilised with etorphine plus azaperone i.m. by darting from foot and transported by airplane under tranquilization for 18.48 ± 0.02 hours. Paired blood samples were collected from an auricular vein from the immobilised rhinos at first contact during capture and from the sedated (but awake) rhinos just prior to release after transport. Results were compared using a Wilcoxon-rank-sum test.

Creatine-kinase and aspartate-aminotransferase concentrations increased from capture to release ($p= 0.002$, $p= 0.019$, respectively) indicating skeletal muscle exertion after long periods of balancing and standing. Mild increases in sodium and total-bilirubin concentrations and decreases in urea, cholesterol, potassium, magnesium and phosphorus concentrations suggest metabolic shifts ($p< 0.05$). Serum glucose, lactate and cortisol concentrations, which are indicative of stress and exertion, did not change between capture and release, likely due to the long interval between the two sample time-points or due to exhaustion of the hypothalamic-pituitary–adrenal axis in response to temporary boma-confinement.

Compared to data from eight free-ranging white rhinos captured by remote darting from a helicopter using the same drug-combination, and transported over the same distance via road (25.92 hours) immediately after capture, the concentration of these analytes at capture was lower in the boma-adapted rhinos ($p= 0.05$, $p< 0.001$, $p= 0.003$, respectively) but did not differ at release ($p> 0.05$).

1 Introduction

1.1 The endangered rhino

There are currently five species and eleven subspecies of rhinos, which belong to the genus *Ceratotherium* in the family of the *Rhinocerotidae*. In earlier times, they roamed large parts of North America, Europe, Africa and Southeast Asia. Today they live in very small, protected groups in Africa and Asia, with only about 27000 animals left worldwide (1). All rhino species are threatened with extinction, with the greatest threats being the illegal wildlife trade, climate change and habitat loss as well as human disturbance, geological events, invasive plant and animal species and diseases (2).

The five species consist of the greater one-horned rhino (*Rhinoceros unicornis*), the Javan rhino (*Rhinoceros sondaicus*), the Sumatran rhino (*Dicerorhinus sumatrensis*), the black rhino (*Diceros bicornis*) and the white rhino (*Ceratotherium simum*) (1).

The greater one-horned rhino is found in Nepal and India. Thanks to conservation efforts and strict protection, the number of this rhino species has increased from only twelve animals in 1905 to over 4000 living in the wild today (3, 4). It is classified as vulnerable by the International Union for Conservation of Nature (IUCN) Red List (4).

The Javan rhino is listed as critically endangered. Only about 68 animals still live in the forests of Indonesia (5).

The Sumatran rhino is also classified as critically endangered, with the population continuing to decline (6). Its historical range covered several parts of South Asia, but today it is only found in Indonesia with a population of less than 80 individuals (7, 8).

With a global population of 23,432 rhinos (as of the end of 2021), 22,137 of these live on the African continent, which is home to two rhino species: the black and the white rhino (9).

The black rhino is considered as critically endangered (10). Once the largest rhino population with about 100,000 animals in the 20th century, black rhino numbers reached their low point due to unregulated, illegal hunting in the 1990s with only 2,300 animals left (10,11). Despite the current poaching crisis, black rhino numbers have recovered to approximately 6000 individuals due to conservation efforts such as translocation and range expansion. From 2017 to 2021, black rhino numbers have been increasing by 3 % per year (9).

There are two subspecies of the white rhino: the northern and the southern white rhino. Due to excessive poaching, the northern white rhino (*Ceratotherium simum cottoni*) is classified as

critically endangered. Only two females remain, born at Dvur Kralove Zoo in the Czech Republic and are now kept under permanent protection in Kenya's Ol Pejeta Conservancy. The northern white rhino, which once lived in north-western Uganda, southern Chad, south-western South Sudan, eastern Central African Republic and north-eastern Democratic Republic of Congo, has not been found in the wild since 2007 (12, 13). As the two animals left do not breed anymore, the subspecies conservation depends on new reproduction techniques. Scientists explore the possibility of *in vitro* fertilisation, using oocytes from the two females left and cryopreserved sperm collected from the last five rhino bulls when they were still alive. Southern white rhinos could then be used as surrogate mothers. However, the embryo transfer methods still have to be optimized to create new offspring of the northern white rhino (12, 14).
The southern white rhino (*Ceraototherium simum simum*) has been listed as near threatened on the IUCN red list since 2002. This subspecies forms the largest population of the five rhino species but currently undergoes a decreasing population trend, mainly because of the high poaching pressure (12).
At the end of 2021, 15 942 white rhinos were counted across Africa with records from 2017 to 2021 showing a population decline of 3.1 % per year (9).
Over 99 % of southern white rhinos are distributed in only five African countries, including South Africa, Namibia, Botswana, Zimbabwe and Kenya (9). South Africa hosts 80 % of the total southern white rhino population, followed by Namibia, which is home to 7.7 % of these rhinos and Kenya with 5.5 %. Although Kenya, Uganda and Zambia most likely lie outside of the historical range of the southern white rhino, animals currently live there as a result of translocations and reintroductions (9, 15, 16).
Around one third of the rhinos live under semi-wild or intensive conditions and over 50 % of the rhino population is run by private or communal ownership (9, 17). Although the numbers of privately owned rhinos are rising, the costs for keeping and protecting them from poaching activities are too. This leads to reduced sale prices for live rhinos and subsequently contributes to a decrease of rhino population growth (12,18). The other 50 % of rhinos live in government protected areas, with the largest population in Kruger National Park in South Africa (12).

1.2 Poaching and illegal horn trade

In 1895, southern white rhinos were on the verge of extinction, with only 20–50 individuals left in Kwazulu-Natal, South Africa. This was caused by excessive sports hunting and expansion of agricultural land (15). Conservation measures, including thousands of translocations, made

it possible to increase the number of southern white rhinos by 7.1 % per year from 1992–2010, reaching a number of approximately 20,000 animals, divided into over 400 subpopulations (12, 15, 18, 19). As a result of increased poaching starting from 2007 onwards, the population size is continuously decreasing since then. Especially in Kruger National Park the killing of rhinos for their horn poses a major threat to white rhinos (12).

Poaching peaked in 2015 when 5.3 % of the southern white rhino population in Africa was killed. Since then, poaching incidents have decreased due to conservation efforts and law enforcement actions (9, 12). From 2018 to 2021, 2707 poaching incidents were recorded across the African continent, 90 % of which took place in South Africa. When the Covid-19 pandemic occurred in 2020, poaching activities in Kruger National Park decreased by 79.4 % due to lockdown regulations but increased again in 2021. In 2021, 2.3 % of the rhino population was poached, not including orphaned calves. It is also important to note that not every rhino poached will be found and that a poached rhino might be pregnant or leave an orphaned calf behind (9). In 2022, 124 rhinos were reported to be poached in Kruger National Park and 244 rhinos in KwaZulu-Natal, most of them in provincial parks. Across South Africa, 86 privately owned white rhinos were killed in 2022 (20).

About 95 % of illegally traded horns are believed to originate from poached rhinos (18). The other small percentage is stolen or illegally sold from private stockpiles, museums and exhibitions, or comes from 'pseudo-hunting' (12). It is estimated that between 4,593 and 5,186 rhino horns entered the illegal trade between 2018 and 2020. About half of the rhino horn was seized by the police or at the site of poaching, leaving about 1,724 to 2,768 horns actually entering the market. This would mean that 575 to 923 rhino horns would be traded annually from 2018 to 2020. As 2020 was an exceptional year due to Covid and due to conservation plans and law enforcement actions, the numbers in this period were lower than in previous years (9).

The poaching of rhinos is driven by the demand for their horns in Asia, particularly Vietnam and China, where the horn is used in traditional Chinese medicine as a status symbol and for decoration (12).

1.3 Law enforcements and conservation measures

African rhinos and their products were first listed in 1977 in Appendix 1 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which prohibits their commercial trading. After rhino populations increased, the southern white rhino is listed

in Appendix 2 since 1994. This allows the export of hunting trophies and translocations of this species to “appropriate and acceptable destinations” (12).

Apart from the CITES restrictions, there are many regional, national and international rhino conservation initiatives that contribute to their protection. There are the South African Development Community Rhino Management Group, the East African Rhino Management Group and the Southern African Rhino and Elephant Security Group, to name a few. The IUCN African Rhino Specialist Group addresses continental rhino conservation (12).

Eleven range state conservation plans were active in Africa during 2018 to 2020 (9). These include research, biological management of populations, promoting sustainable conservation and finance and education for rhino conservation (12, 21). Due to the difficulty to effectively protect rhinos in the wild, other measures, such as dehorning, are common conservation procedures. Between 2018 and 2021 2,217 rhinos were dehorned to make them less attractive to poachers. 391 rhinos were translocated in order to form new populations, to move them to more protected areas and to promote long-term genetic conservation of the rhinos (9, 21). Rhino sanctuaries, where injured and orphaned rhinos receive medical treatment, are raised, rehabilitated and rewilded as well as protected fenced rhino conservation areas further contribute to the protection of the species (9, 12).

1.4 Translocations

1.4.1 Reason for translocating

Currently the world is experiencing its 6th mass extinction due to habitat loss, biological invasions and above all, climate change. Therefore, it is becoming increasingly relevant to implement appropriate conservation plans for endangered species and to continuously adapt and improve them.

Regarding the conservation of rhinos, a common strategy to improve their chance of survival is conservation translocation. It is defined as “the intentional movement and release of a living organism where the primary objective is a conservation benefit” and includes capture, temporary captivity, transport and release (19, 22). The goal is to achieve a benefit for an entire population, ecosystem or species and not only for an individual animal. Consequently, translocations of animals to sites with ecologically better and/or more protected areas can secure a species’ population. Conservation translocations include conservation introductions such as assisted colonisation and ecological replacement, reintroductions and reinforcements.

While the conservation introductions comprise translocations of animals into new areas outside their native range, the reintroduction is about releasing a population into an area within their historical range from which it has disappeared. Reinforcement, on the other hand, involves releasing animals into an existing population of conspecifics in order to improve the viability of the population (22, 23).
As translocations require a lot of time, money, detailed planning, experienced staff and pose risks to human and animal welfare, it is important to reflect on whether they make ecological sense (22).

1.4.2 Translocation phases

Translocations of rhinos can be done in different ways: Wild animals can either be captured in the veld (= field/wild), loaded and directly transported to the destination (= veld capture to veld release), or they can be confined in bomas prior to the transport (= veld capture to boma) and also after the transport (19). A boma is an enclosure built for temporary rhino confinement, which allows the animals to adapt to captive conditions and new diets. Boma-confinement further lowers the risk of rhinos self-traumatising during transport, allows for health checks and a faster capture and loading process on the day of transport (19). The disadvantage of boma confinement of white rhinos is the relative high proportion of rhinos that do not adapt or adapt very poorly, resulting in reduced or no food intake (19).
Capture and transport can be carried out in many different ways and are always adapted to the respective situation. Rhinos can be darted from the ground or from the helicopter and then loaded onto trailers in single or mass crates with individual compartments (19). Common drugs used for capture and transport are mentioned in chapter 1.4.3.
After the transport by road or by airplane, rhinos get released individually either directly into the field or they get released into pre-release bomas. These bomas are commonly used after a prolonged transport and ensure a more controlled release. Furthermore, the stressed animals can be screened for injuries they may have sustained during capture and transport (19, 24).
Pathologies associated with translocations include distress, dehydration, negative energy balance, post-capture anorexia, enterocolitis, muscle exertion and muscle cell damage, which may ultimately lead to the death of the animal (24, 25, 26). Possible side effects of the drugs used for immobilisation and sedation like cardiorespiratory depression or metabolic acidosis also pose risks to animal welfare (26). Rhinos may also self-traumatise or suffer from heat

stress during transport (27, 28). The mortality rate for rhino translocation in South Africa and Namibia is presently estimated to be 5 % (25).
Therefore, it is important to better understand the pathologies associated with translocations and how to minimise their occurrence.

1.4.3 Capture

Capture starts with the immobilisation of the animal by darting from the helicopter, vehicle or on foot. Commonly used drugs for rhino immobilisation are etorphine, an opioid 5 000–10 000 more potent than morphine, in combination with azaperone (28, 29). The induction time after darting is short, with the animal generally being recumbent within a few minutes. Possible side effects of etorphine include respiratory depression, hypoxia, muscle tremors and hypertonicity, hyperthermia, hypertension and inhibition of the intestinal motility (28). To lower the risk of morbidity and mortality, any side effects should be avoided or treated, e.g. by administering oxygen directly into the nostrils to reduce the hypoxaemia and by regulating body temperature with cold water or ice packs to reduce hyperthermia. Azaperone, a butyrophenone derivative, minimises induction time and the hypertension caused by the etorphine through its alpha-1-antagonism, resulting in vasodilation (28). The administration of butorphanol, a mixed opioid agonist-antagonist, reduces muscle hypertonus and tremors which results in altered metabolic oxygen consumption and therewith improved blood oxygenation in etorphine-immobilised rhinos (28).
Besides butorphanol, diprenorphine is often administered to partially reverse the immobilizing effects of the etorphine (28).
For loading into a transport crate, the immobilised and blindfolded animal is first rolled into sternal recumbency in order to help it get up more easily. Then, the partial reversal (e.g. butorphanol) is administered i.v. and the still sedated rhino is pulled up on its legs and slowly “walked” to the transport crate with the help of ropes being placed around the horns and legs. Once in the transport crate, additional partial reversal (e.g. diprenorphine or more butorphanol) is administered to further reduce the immobilising but maintain the sedative effects of the etorphine. Complete antagonism, for example for field release after transportation, can be achieved by injecting naltrexone (28).
For long distance transports, long-acting tranquillizers such as zuclopenthixol acetate are administered prior to the start of transport in order to keep the animals calm during the transport. Additionally, azaperone or midazolam are commonly administered in regular time

intervals as short acting tranquillizers even during short transportations (28).
After successful capture, animals can be placed in bomas (see Chapter 1.4.2) or transported in suitable vehicles or by airplane, following the guidelines for transport of live wild animals and rhinoceroses (19, 22, 30, 31).

1.4.4 Stress response

Wild animals are exposed to a lot of stressors during the described capture techniques such as a noisy helicopter chasing them, the drug effects, human contact, unfamiliar surroundings, loading and handling operations (28, 32). As a survival mechanism, rhinos react with a physiological stress response in which several nervous, endocrine and immune mechanisms are involved and regulated by the sympathetic-adrenal-medullary system (SAM), the hypothalamic-pituitary-adrenal axis (HPA) and the immune system (33). SAM acts as a rapid stress response by triggering increased secretion of epinephrine from the adrenal medulla and norepinephrine from the sympathetic nerves. These catecholamines are agonistic to alpha- and beta-adrenergic receptors, resulting in increased blood pressure, heart rate, cardiac output and bronchiolar dilatation. Other effects are a higher skeletal muscle blood flow, an increased sodium retention in the kidneys, increased glucose (GLUC) concentrations and increase of body temperature (34). The HPA system response, on the other hand, initiates a slower (minutes) but more sustained response than the SAM (seconds) (34). Various stimuli induce the secretion of the corticotropin releasing hormone (CRH) from the hypothalamus, which is followed by an increased adrenocorticotrophin hormone (ACTH) secretion from the pituitary gland. ACTH effectuates the adrenal cortex to release glucocorticoids into blood circulation, which leads to an increased serum cortisol (CORT) concentration (34). Cortisol then affects nearly every organ system, as receptors are located throughout the body (35). Besides its immunosuppressive function, CORT provides energy through causing an increased gluconeogenesis, glycogenolysis and lipolysis (35, 36). It also intensifies the function of catecholamines (37).
Due to the stress-induced immunosuppression, diseases often develop or worsen after translocation (38).

1.4.5 Capture myopathy

Capture myopathy is a stress or exertion induced muscle degeneration (rhabdomyolysis), affecting skeletal as well as cardiac muscles of domestic and wild animals (39). Physical exertion, hours of balancing and standing and limited space during transport, possible trauma and reduced muscle perfusion due to sympathetic effects are among the etiological factors of the disease (39, 40, 41). Injured muscle fibres secrete creatine kinase (CK) and myoglobin and cause a rise in serum lactate (LACT) concentrations (39, 42). Increased LACT leads to an additive metabolic acidosis through lowering the pH, while the increased myoglobin can result in kidney failure (42, 43). This is caused by several mechanisms, including reduced renal blood flow due to vasoconstriction, intraluminal casting and haem protein-induced cytotoxicity (42). Ultimately, capture myopathy can lead to multiple organ failure (44).

Initial clinical signs are shivering, an increased respiratory rate and body temperature, dark red urine and torticollis. In the later course of the disease animals show lameness, stiff extremities, weakness up to high-grade lethargy and gastro-intestinal symptoms like constipation and anorexia (39, 44, 45). The course of disease can be hyper-acute, acute, sub-acute and chronic. Hyper-acute capture myopathy, also known as capture shock syndrome, causes a sudden death of the animal during capture or up to a few hours later (39, 46). Serum biochemistry may show elevated levels of lactate dehydrogenase (LDH), CK and aspartate aminotransferase (AST) (39, 47). Blood gas analysis may illustrate a lowered pH, which may lead to electrolyte imbalances such as hyperkalaemia (43, 46).

Acute capture myopathy or ataxic myoglobinuric syndrome occurs hours or days after capture. Clinical symptoms vary in their severity and reflect the survival chance of the animal. In addition to the prior named elevated blood chemistry analytes, blood urea nitrogen (UREA) can also be increased, due to a reduced kidney function (39, 43, 46). A similar biochemistry profile can be seen in animals with sub-acute capture myopathy, also named ruptured muscle syndrome. It is characterized by the animal showing no clinical signs until one to two days after capture. Later on, released animals present with severe symptoms of muscle injury like torticollis, tetraplegia, hyperflexion and dropped hindquarters. Affected animals mostly die within days to weeks (39, 46, 47).

When animals die within minutes due to a second stressful event after having been captured before, it is referred to chronic capture myopathy, also called delayed per-acute syndrome (43, 48). This course of disease is predicated on a sudden hyperkalaemia, which results in ventricular fibrillation and the consequential death of the animal (48).

As there is currently no treatment for capture myopathy, it is even more important to apply good prevention practices (49, 50), such as the administration of tranquillizers, professional handling of the animal and detailed planning of the capture in order to minimise stress (43).

1.4.6 Biochemical responses to capture, transport and boma confinement

Previous studies have shown increases in total serum protein (TSP), albumin (ALB), sodium (NA), UREA and creatinine (CREA) as a result of dehydration and fluid shifts due to water deprivation during translocation (24, 51–59). Muscle enzymes CK, AST and LDH are often found to be elevated, reflecting the physical and stress-related exertion to which the animals are exposed during capture and transport (57, 58, 60–63).

Due to the activation of the SAM system and the resulting increased CORT secretion, elevated CORT after capture has been recorded in white and black rhinos (59, 62). Glucose concentrations in black rhinos were reported to be significantly increased after translocation (62). Hypoglycaemia, however, can occur because of prolonged transport, fasting and exhaustion and has been documented in domestic animals (40). As reported in transported white rhinos and dromedary camels, non-esterified fatty acids (NEFA) can be mobilised in order to generate energy and consequently lead to an increase of this blood biochemistry analyte (24, 64). Ketone bodies like beta-hydroxybutyrate (BHB) are also synthesized in reaction to a negative energy balance and are commonly increased in transported domestic animals (65, 66, 67) and similar findings were documented in white rhinos after transport (24). Triglyceride levels (TRIG) also showed significant changes after transport in white rhino bulls (59). Pohlin et al. (2020) documented increased TRIG in white rhinos after capture, indicating lipid peroxidation in the course of an acute phase reaction (59). After the transport, TRIG were found to be decreased again (59). Kock et al. (1990) further recorded increased Bilirubin concentrations (TBILI) and decreased cholesterol (CHOL), calcium (CA), magnesium (MG) and phosphorus (PHOS) concentrations in black rhinos after capture and transport (59). Other studies by Leiberich et al. (2022) and Pohlin et al. (2020) investigating the welfare of rhinos during transport also illustrated a decrease of potassium (K) and an increase in chloride (CL) along with the latter named changes (24, 68).

Glutamate dehydrogenase (GLDH) was found to be decreased after transport of white rhinos, while other liver enzymes did not change significantly (24, 68).

Furthermore, other studies showed positive acute phase reactants like haptoglobin (HP) to be increased in water buffalo calves after transport (69). However, in white rhino bulls HP

concentrations decreased from capture to the start of transport (59). This finding likely represents relative changes resulting from fluid shifts associated with haemoconcentration at capture (59). Serum iron (FE), a negative acute phase reactant, was documented to decrease in white rhinos over the time of transport (59, 24).

Kock et al. (1990) investigated changes in biochemical analytes related to capture, transport and boma-confinement in black rhinos. Results showed increased CORT from capture to the end of transport but decreased serum CORT after boma confinement, indicating a recovery from the experienced stress at capture (62). The blood biochemistry of these rhinos also illustrated a decrease in CK, LDH and GLUC linked to an extended boma period (62). Due to chronic stress related to trauma and diet changes, ALB declined while globulin (GLOB) concentrations increased, resulting in a decreased ALB/GLOB ratio. Besides increased UREA, further findings were increased AST concentrations and decreased CREA and K as a result of high dietary intake due to food supplementation during boma confinement (62).

A study by Miller et al. (2022) investigated the effects of boma confinement on white rhinos and documented increased ALB, UREA and total protein (TP), most probably due to decreased water intake and food supplementation (70). Further findings in well-adapted rhinos were a decrease in the liver enzymes alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT), a decline of MG and increased CK and AST (70). Although statistical analyses showed significant changes in these analytes from capture to release, most changes remained within reference intervals or showed a trend of returning back to normal (70, 71, 72).

It is important to note that factors like stress, water and food intake, used drugs for immobilisation and sedation as well as environmental conditions and the animals' welfare affect the blood biochemistry (26).

1.5 Study objectives and hypotheses

The aim of this study was to assess and compare two different capture (boma vs. veld) and transportation (road vs. airplane) methods in white rhinos by evaluating changes in clinical chemistry analytes.

The following hypotheses were investigated:

1. Metabolic shifts, muscle enzyme concentrations and stress associated serum analytes will increase from capture to release in boma-caught rhinos transported by airplane.

2. Metabolic shifts, muscle enzyme concentrations and stress associated serum analytes will be higher in rhinos caught in the veld (wild) compared to rhinos darted in the bomas (temporary confinement) at the respective time of capture.
3. Metabolic shifts, muscle enzyme concentrations and stress associated serum analytes will be higher in road transported compared to air transported rhinos.

2 Material and methods

The translocations were serving conservation purposes and followed the guidelines for the transport of live wild animals and rhinoceroses (19, 22). The rhino crates used were International Air Transport Association (IATA) compliant (30). The opportunistic sample collection was approved by the University of Pretoria Animal Ethics Committee (V067-17).
An environmental logger (Kestrel DROP D2AG Livestock Heat Stress Monitor, KestrelMeters, Parker, CO, USA) recorded the environmental temperature, relative humidity and the heat stress index at 30 min. intervals inside the transport crates. Water was not provided to the animals, as past experience has shown that rhinos do not drink during transport and affixed water containers are known to cause injury (31). At the heat of the day, rhinos were doused with water during stops and small amounts of alfalfa hay were offered to some of the animals.

2.1 Transport by road ("veld" to "veld")

A total of 32 southern white rhinos were transported by road (fig. 1–6) from a 340 ha game farm in the Free State in South Africa to Botswana in 2017 as described in Pohlin et al. (24). For reasons of comparison, eight of the road transported animals of similar age and size like the air transported rhinos were included in this study, consisting of two adults, one calf and five subadults (four males and four females).

Figure 1: route of road transport (1)

The animals were captured from the veld by darting from the helicopter using 2.0 ml darts (Pneu-dart, Inc.®, Williamsport, Pennsylvania, USA) with 63.5 mm barbed needles. Etorphine (4.5–5 mg/adult, 3–3.5 mg/subadult, 0.5 mg/calf, i.m., Captivon®, 9.8 mg/ml, Wildlife Pharmaceuticals, South Africa) and azaperone (30–40 mg/adult, 20–30 mg/subadult, i.m., Azaperone tartrate, 50 mg/ml, Wildlife Pharmaceuticals, South Africa) plus hyaluronidase (5000 IU/adult, i.m., Hyalase®, Kyron Laboratories, Johannesburg, South Africa) were used for the immobilisation of these rhinos. As soon as the animals were immobilised, blood samples were taken from an auricular vein on first contact with the animal (24).

One subadult rhino (Rhino ID= 27) additionally received vitamin C (5 g/rhino, i.m, Ascorbic acid, 500 mg/5 ml, Fresenius Kabi, Bloemfontein, South Africa) to support the animals' antioxidant defences (73).

Diprenorphine (0.2–0.8 mg/adult and 0–0.1 mg/juvenile, i.v., M5050®, 12 mg/ml, Novartis, Midrand, South Africa) was administered to all rhinos within ten minutes of darting in order to partially reverse the immobilising effects of the etorphine and load the rhinos into the transport crates. Each rhino was loaded into its own separate crate. To further reverse any immobilising effects of the etorphine, the loaded rhinos received another 2.5–15 mg of diprenorphine i.v. Furthermore, zuclopenthixol acetate (100–250 mg/adult, 10–50 mg/juvenile, i.m., Clopixol-Acuphase ®, 50 mg/ml, H. Lundbeck Pty. Ldt, Randburg, South Africa), a long-acting tranquillizer, was given to calm the animals during transport. The recording of the transport time started once all rhinos were loaded. The loading process took approximately 3 h 28 min (24).

The truck stopped every 2–4 hours in order to give top-up doses of azaperone and/or midazolam (10–20 mg/adult, 2.5–10 mg/juvenile, i.m., Dazonil®, 50 mg/ml, Wildlife Pharmaceuticals, South Africa) when needed. All rhinos received at least one additional dose of azaperone (100–120 mg/adult, 20 mg/juvenile, i.m.), in the calf combined with midazolam (10 mg/juvenile, i.m.) (table 1).

Rhino 20 and 21 frog-legged and showed a dog-sitting position, respectively, after over 24 hours of transport and were administered corticosteroids i.m.

At the release site, all rhinos were re-immobilised by administering etorphine (3.5–6 mg/adult, 0.5–2.5 mg/juvenile, i.m.) and azaperone (20–40 mg/adult, i.m.) or etorphine (3.5–6 mg/adult, 0.5–2.5 mg/juvenile, i.m.) in combination with midazolam (5 mg, i.m.) in juveniles. Six of the rhinos additionally received butorphanol (10–20 mg/adult 5 mg/juvenile, i.v., Butorphanol tartrate, 50 mg/ml, Wildlife Pharmaceuticals) in order to mitigate the hypoxaemia associated with muscle tremors (74). The animals were restrained with ropes during their release from the transport crates until recumbent. Blood samples were then collected from an auricular vein and GPS-tracking devices mounted. Afterwards, naltrexone (70–90 mg/adult, 20 mg/calf, i.v.,Trexonil®, 50 mg/ml, Wildlife Pharmaceuticals) was given at 20 times the etorphine dose in mg to fully reverse immobilisation and release the rhinos into the wild.

Table 1: Identifications of rhinos and drugs administered at road transport. A= Azaperone. M= Midazolam. B= Butorphanol.

Rhino ID		**19**	**20**	**21**	**24**	**26**	**27**	**28**	**33**
Sex		♀	♂	♀	♀	♀	♂	♂	♂
Age		Sub-adult	Calf	Adult	Sub-adult	Sub-adult	Sub-adult	Sub-adult	Adult
Number of sedation top-ups		1	3	5	2	2	2	2	1
	A (in mg)	120		100 120	120	100		120	120
	M (in mg)		2.5 10	10 20	10	10	10	10	
	A + M (in mg)		20 + 10	120 + 20			120 + 10		
B (in mg) at release			5	20	15	20	20	10	
Re-immobilisation at release		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Fig. 2: Rhino being walked to the transport vehicle after it was darted from the helicopter (2)

Fig. 3: Rhinos in transport crates prior to the long road transport (3)

Fig. 4: Loading rhino crates onto trucks (4)

Figure 5: Rhinos inside their crates transported on trucks by road (5)

Figure 6: Rhino release after transport (6)

2.2 Transport by air (“boma” to “veld”)

All rhinos were captured from the wild and confined in bomas for six weeks prior to the transport. Eight rhinos, consisting of three adults, their calves and two subadults (five females and three males), were subsequently re-captured from the boma and transported by air from South Africa to Botswana. The rhinos were divided into two groups of four rhinos each and transported on two different nights in October 2017.

The rhinos were darted from foot within the boma and immobilised with etorphine (3–3.5 mg/adult, 2–2.5 mg/juvenile, i.m., Captivon®, 9.8 mg/ml, Wildlife Pharmaceuticals, South Africa) in combination with azaperone (40 mg/adult, 20 mg/juvenile, i.m., Azaperone tartrate, 50 mg/ml, Wildlife Pharmaceuticals, South Africa) using 1.5 ml plastic darts (DAN-INJECT, International S.A., Skukuza, South Africa) with 60 mm uncollared needles, propelled by compressed air.

Three rhinos (Rhino IDs= 34, 36 and 37) received an additional 5–10 mg of midazolam (i.m., Dazonil®, 50 mg/ml, Wildlife Pharmaceuticals, South Africa) .

Immediately after recumbency, a blood sample was collected from an auricular vein. Diprenorphine (0.4 mg/adult, 0.3 mg/ juvenile; iv., M5050, 12 mg/mL, Novartis, Midrand, South Africa) was administered in order to load the rhinos into their individual transport crates. After loading, adult animals received another 4.8–9.6 mg of diprenorphine i.v. to complete the etorphine reversal. At the start of the transport, the long-acting tranquillizer zuclopenthixol acetate (175–200 mg/adult, 75–100 mg/juvenile, i.m., Clopixol-Acuphase ®, 50 mg/ml, H. Lundbeck Pty. Ldt, Randburg, South Africa) was given to all animals. Additionally, one subadult female rhino (Rhino ID= 38) received Vitamin C (5 g/rhino, i.m, Ascorbic acid, 500 mg/5 ml, Fresenius Kabi, Bloemfontein, South Africa) and two adult female rhinos (Rhino IDs= 36 and 40) were administered Vit E plus Selenium (500 mg Vit E/rhino, 50 mg Selenium/rhino, i.m., Vitamin E acetate 17 mg/ml, sodium selenite 1.67 mg/ml, Kyron, Midrand, South Africa).

After 4 hours of road transport, the animals were cross-loaded into an airplane 3–4 hours after the arrival at Durban airport, South Africa (fig. 7a–c). Flight time was recorded to be 2.45 hours in both groups. After the arrival at Maun airport, Botswana, the rest of the transport was continued by road, after cross-loading the rhinos on transport trucks.

Every 1–4 hours, the rhinos were checked and top-up doses of sedatives administered to restless animals. Each rhino received at least two further doses of Azaperone (25–120 mg/adult, 60–100 mg/juvenile, i.m.) or Midazolam (15–50 mg/adult, 10–30/juvenile, i.m.) (table

2). Two of the adult female rhinos (Rhino IDs= 34 and 36) needed seven top-up doses of either azaperone or midazolam until the arrival at the release site.

At the release site, blood samples were taken from an auricular vein right before release from the sedated rhino standing in the crate. Rhinos were not re-immobilised as tracking devices were already mounted during capture. As the animals were not re-immobilised, it was not possible to take blood from each rhino and could therefore only be obtained from five of the eight rhinos. The three adult females and their calves were then released into a boma for one night to allow for mother-calf bonding after transport, and subsequently released into the wild. The two subadults were immediately released into the wild.

Table 2: Identifications of rhinos and drugs administered at air transport. A= Azaperone. M= Midazolam. B= Butorphanol.

Rhino ID	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**
Sex	♀	♂	♀	♂	♀	♂	♀	♀
Age	Adult	Calf	Adult	Calf	Sub-adult	Sub-adult	Adult	Calf
Number of sedation top-ups	7	4	7	4	1	2	2	2
A (in mg)	100	80	100	100				
	25		25	100				
	120		120					
M (in mg)	20	20	20	30				
	25	25	25					
	50		50					
A + M (in mg)	120 + 25	100 + 20	120 + 25	100 + 25	80 + 15	80 + 15	80 + 15	60 + 10
						60 + 15	80 + 15	60 + 10
B (in mg) at release	No	No	No	No	No	No	No	No
Re-immobilisation at release	No	No	No	No	No	No	No	No

Figure 7a

Figure 7b

Figure 7c

Figure 7: (**a**) Preparations for rhino transport by airplane and (**b,c**) loading processes at the airport

2.3 Blood sample analysis

The blood samples were collected into serum and NaF tubes (BD Vacutainer, Becton and Dickinson, Plymouth, UK) and subsequently stored in a cool box filled with ice packs. The tubes were centrifuged within 24 hours. Collected plasma and serum were kept at -20°C for one month and then transferred to a -80°C freezer until analysis in the clinical pathology laboratory of the Onderstepoort Veterinary Academic Hospital (Pretoria, South Africa).

The Cobas Integra 400 Plus automated biochemistry analyser (Roche Diagnostics Ldt., Rotkreuz, Switzerland) was used for the serum clinical chemistry and spectrophotometric plasma analyses. Measured serum analytes included: TSP, ALB, GLOB, ALP, UREA, CREA, NA, K, CL, AST, CHOL, MG, PHOS, TRIG, GGT, GLDH, TBILI, CA, CK, and FE. Measured plasma analytes included GLUC and LACT. Serum CORT was measured using a chemiluminescent immunoassay (Immulite/Immulite 1000 Cortisol ®, Siemens Healthcare, Erlangen, Germany). Furthermore, HP was determined with the same analyser (Cobas Integra 400 Plus) through the binding method using a commercial kit (PHASE Haptolglobin Assay, Tridelta Development Limited, Kildare, Ireland). Serum BHB and NEFA levels were measured with BHB and NEFA kits (Randox Labaratories, Crumlin, Antrim, UK), through kinetic, enzymatic and colorimetric methods.

2.4 Statistical analyses

Statistical analysis was conducted using R 3.6.1 for Windows (The R Foundation, Vienna, Austria). Descriptive tables, scatter plots, mean and standard deviations for each analyte were generated. Non-parametric analyses were used to compare the concentrations of the analytes by using a Wilcoxon-rank-sum test. Changes in blood biochemistry analytes were evaluated and compared between:

- Prior to (“capture”) and after transport (“release”) by airplane
- Capture in the veld and capture in the boma
- After transport by road and after transport by airplane

A p-value < 0.05 was considered significant.

3 Results

3.1 Sample size

All animals survived capture and transport. A total of 29 blood samples were included in this study: Eight blood samples collected at capture in the boma and eight at capture in the veld, another eight at the release site after road transport and five after air transport. However, GLUC, LACT, FE, NEFA, BHB and HP concentrations could not be measured in one of the samples collected post air transport (Rhino ID= 36). Therefore, only four measurements of these analytes were included in the statistical analysis comparing biochemical changes between capture and release of air transported rhinos and between release samples of road and air transported rhinos. The reason there were fewer blood samples collected from air transported rhinos after release compared to capture was that these animals were not re-immobilised after transport and it was hence more difficult to obtain a blood sample from the rhino standing in the crate resulting in less successful sampling at this time point.

3.2 Blood biochemistry at capture and release after air transport

Except for recurring restless behaviour in five rhinos, no further complications occurred during capture in the boma, air transport and release. The mean transportation time by air was 18.48 ± 0.02 hours.

Air transport influenced several clinical chemistry analytes significantly. The mean concentration of each analyte and their standard deviation are shown in table 3. Plasma UREA decreased from capture to release, so did K, CHOL, MG and PHOS. NA, TBILI and NEFA increased (table 3).

Muscle enzymes AST and CK increased over the time of air transport. A more detailed presentation of these analytes and NA and NEFA are shown in fig. 8a–j.

Mean serum CORT and GLUC concentrations did not change significantly from capture to release ($p > 0.05$).

Table 3: Means, standard deviations and p-value of blood biochemistry at capture and release after air transport

Clinical chemistry analyte	Mean concentration and standard deviation at capture (n=8)	Mean concentration and standard deviation at release (n=5)	p-value (Wilcoxon rank sum test)
TSP (g/L)	87.64 ± 6.12	88.12 ± 6.66	0.833
ALB (g/L)	28.63 ± 2.45	30.16 ± 1.41	0.171
GLOB (g/L)	58.84 ± 5.68	57.9 ± 7.61	0.558
ALP (U/L)	70.31 ± 28.38	55.14 ± 22.91	0.524
UREA (mmol/L)	**7.56 ± 0.53**	**6.67 ± 0.87**	**0.045**
CREA (µmol/L)	96 ± 21.16	100.6 ± 13.94	0.622
NA (mmol/L)	**128.56 ± 2.39**	**132.12 ± 0.38**	**0.008**
K (mmol/L)	**5.55 ± 0.56**	**3.41 ± 0.31**	**0.002**
CL (mmol/L)	88.44 ± 1.74	88.48 ± 1.29	1
AST (U/L)	**69.26 ± 17.29**	**122 ± 48.06**	**0.019**
CHOL (mmol/L)	**1.63 ± 0.37**	**1.12 ± 0.18**	**0.019**
MG (mmol/L)	**1.02 ± 0.05**	**0.7 ± 0.09**	**0.004**
PHOS (mmol/L)	**1.54 ± 0.2**	**0.68 ± 0.29**	**0.002**
TRIG (mmol/L)	0.32 ± 0.06	0.34 ± 0.12	0.883
GGT (U/L)	13.04 ± 3.6	14.5 ± 2.67	0.354
GLDH (U/L)	4.26 ± 2.94	3.66 ± 1.56	0.943
TBILI (µmol/L)	**2.19 ± 0.41**	**3.7 ± 0.76**	**0.006**
CA (mmol/L)	2.88 ± 0.03	2.87 ± 0.18	0.607
CK (U/L)	**190.49 ± 87.37**	**6309.76 ± 6065.84**	**0.002**
GLUC (mmol/L)	6.29 ± 1.22	7.13 ± 1.19*	0.368
LACT (mmol/L)	4.13 ± 3.39	5.35 ± 1.09*	0.283
CORT (nmol/L)	43.86 ± 15.05	67.66 ± 27.39	0.222
FE (µmol/L)	21.79 ± 1.71	19.27 ± 1.99*	0.106
NEFA (mmol/L)	**0.13 ± 0.09**	**0.6 ± 0.29***	**0.013**
BHB (mmol/L)	0.29 ± 0.04	0.23 ± 0.06*	0.123
HP (g/L)	0.98 ± 0.93	1.12 ± 0.85*	0.933

*n=4

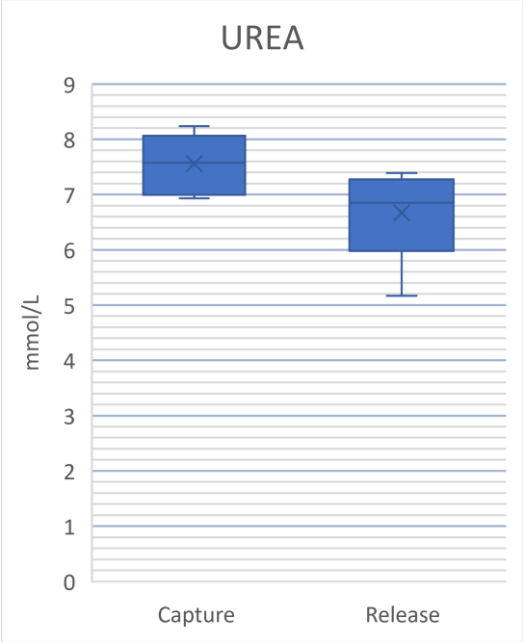


Figure 8a

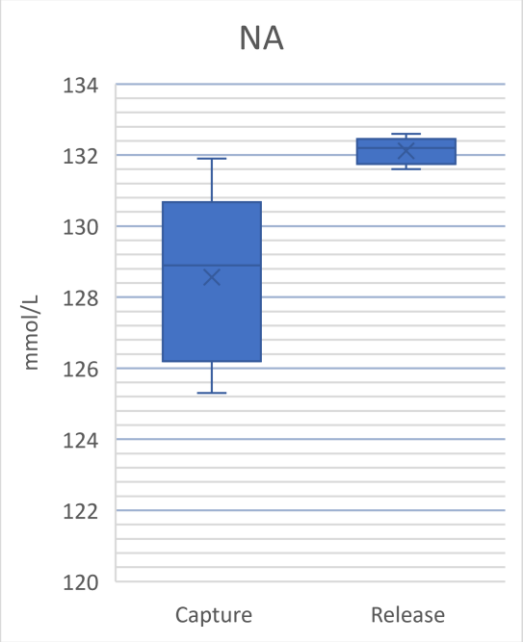


Figure 8b

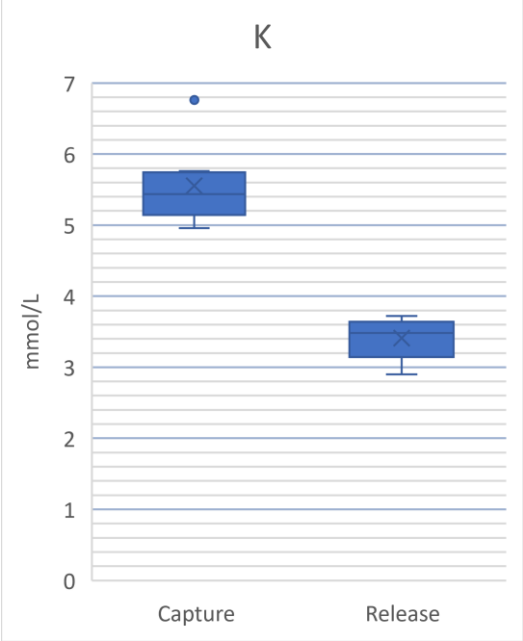


Figure 8c

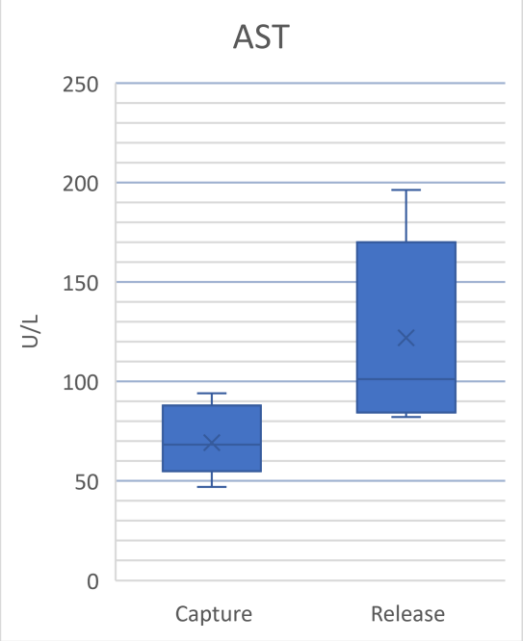


Figure 8d

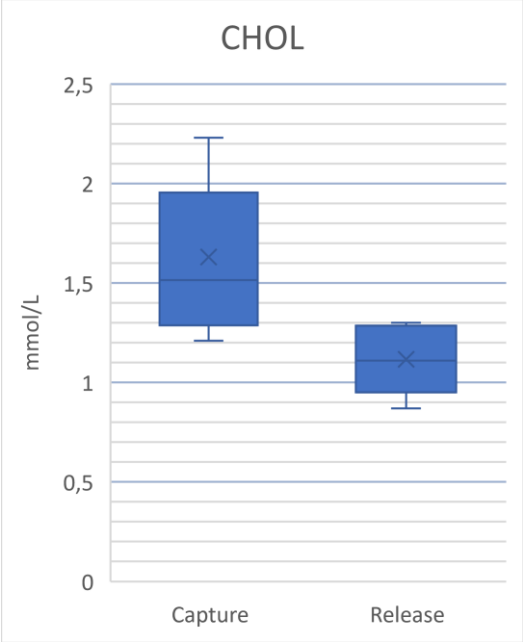


Figure 8e

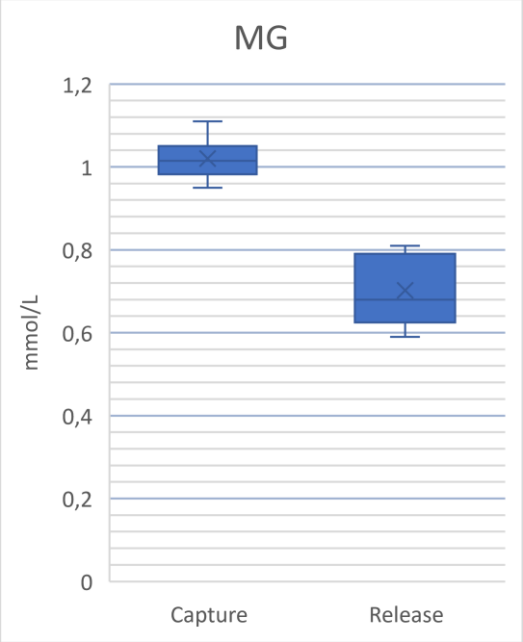


Figure 8f

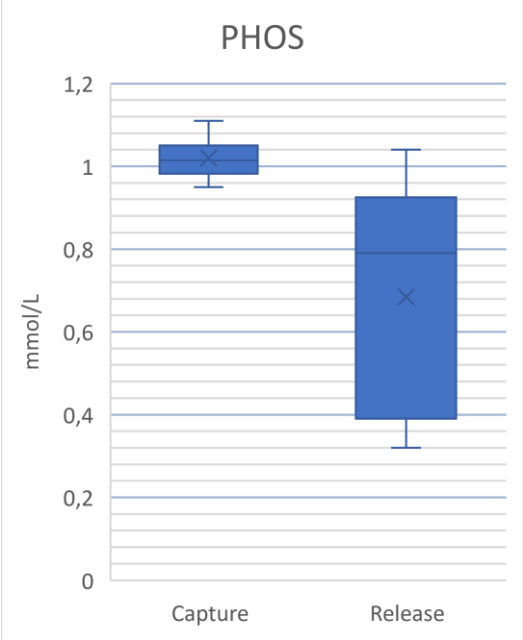


Figure 8g

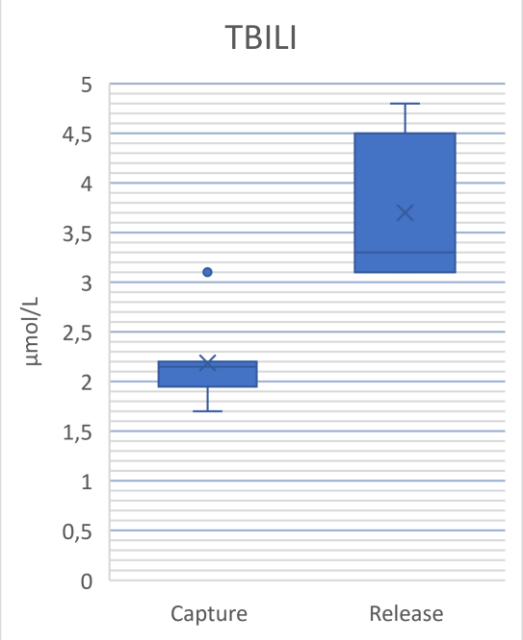
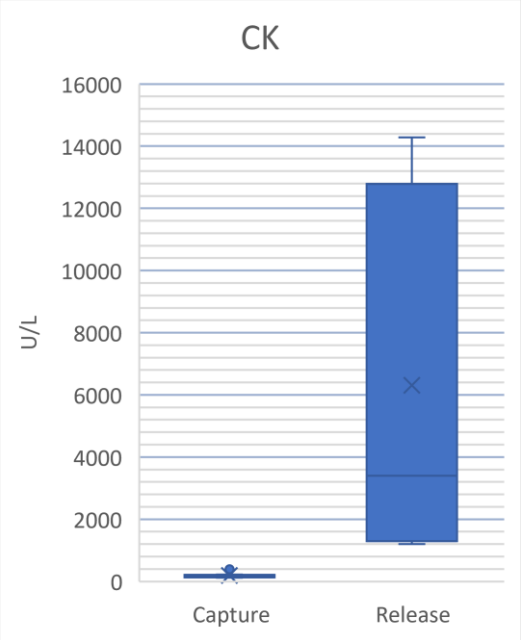


Figure 8h



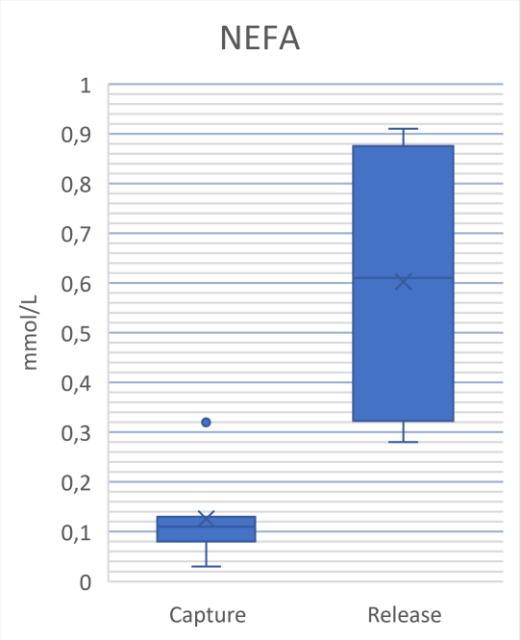


Figure 8i **Figure 8j**

Figure 8: Boxplots of the plasma concentrations of (**a**) UREA, (**b**) NA, (**c**) K, (**d**) AST, (**e**) CHOL, (**f**) MG, (**g**) PHOS, (**h**) TBILI, (**i**) CK and (**j**) NEFA after capture of the boma-adapted rhinos and release after air transport; x=mean

3.3 Blood biochemistry after boma-capture and capture in the veld

No complications occurred during the captures. Mean concentrations and standard deviations of the clinical chemistry analytes are shown in table 4. Significant differences between the two groups were, among others, determined in UREA and BHB, which reached higher concentrations in boma-captured rhinos. The following clinical chemistry analytes were significantly higher in veld-captured rhinos: CREA, CHOL, TRIG, GLUC, LACT and CORT. The acute phase reactant HP also showed higher concentrations in rhinos that were captured in the veld (table 4). Figures 9a–i show the comparison of CORT, GLUC, LACT, CREA and HP values in boma- and veld-captured rhinos.

No significant differences were detected in the muscle enzymes CK and AST.

Table 4: Means, standard deviation and p-value of blood biochemistry at boma-capture and veld-capture

Clinical chemistry analyte	Mean concentration and standard deviation in boma-captured rhinos (n=8)	Mean concentration and standard deviation in veld-captured rhinos (n=8)	p-value (Wilcoxon rank sum test)
TSP (g/L)	87.64 ± 6.12	85.4 ± 7.29	0.505
ALB (g/L)	28.63 ± 2.45	30.87 ± 2.99	0.13
GLOB (g/L)	58.84 ± 5.68	54.54 ± 6.93	0.442
ALP (U/L)	70.31 ± 28.38	97.3 ± 40.19	0.442
UREA (mmol/L)	**7.56 ± 0.53**	**3.5 ± 0.95**	**<0.001**
CREA (µmol/L)	**96 ± 21.16**	**135.5 ± 24.92**	**0.007**
NA (mmol/L)	128.56 ± 2.39	131 ± 2.61	0.093
K (mmol/L)	5.55 ± 0.56	4.91 ± 0.64	0.083
CL (mmol/L)	88.44 ± 1.74	88.42 ± 1.81	0.916
AST (U/L)	69.26 ± 17.29	67.51 ± 16.98	1
CHOL (mmol/L)	**1.63 ± 0.37**	**2.73 ± 1.47**	**0.005**
MG (mmol/L)	1.02 ± 0.05	1.12 ± 0.11	0.058
PHOS (mmol/L)	1.54 ± 0.2	1.51 ± 0.32	0.713
TRIG (mmol/L)	**0.32 ± 0.06**	**0.54 ± 0.15**	**0.004**
GGT (U/L)	13.04 ± 3.6	13 ± 4.25	0.959
GLDH (U/L)	4.26 ± 2.94	4.85 ± 2.39	0.4
TBILI (µmol/L)	2.19 ± 0.41	1.8 ± 0.44	0.29
CA (mmol/L)	2.88 ± 0.03	3 ± 0.24	0.188
CK (U/L)	190.49 ± 87.37	203.21 ± 64.65	0.382
GLUC (mmol/L)	**6.29 ± 1.22**	**7.68 ± 1.12**	**0.05**
LACT (mmol/L)	**4.13 ± 3.39**	**17.05 ± 3.95**	**<0.001**
CORT (nmol/L)	**43.86 ± 15.05**	**75.85 ± 15.87**	**0.003**
FE (µmol/L)	21.79 ± 1.71	22.15 ± 1.73	0.528
NEFA (mmol/L)	0.13 ± 0.09	0.08 ± 0.04	0.167
BHB (mmol/L)	**0.29 ± 0.04**	**0.2 ± 0.05**	**0.004**
HP (g/L)	**0.98 ± 0.93**	**2.13 ± 0.88**	**0.021**

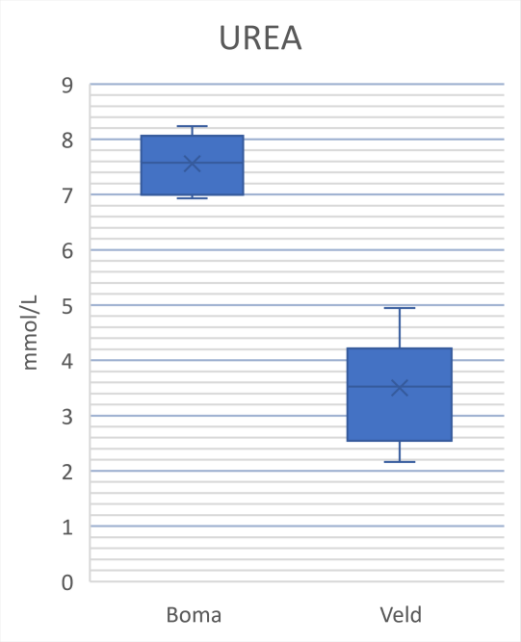


Figure 9a

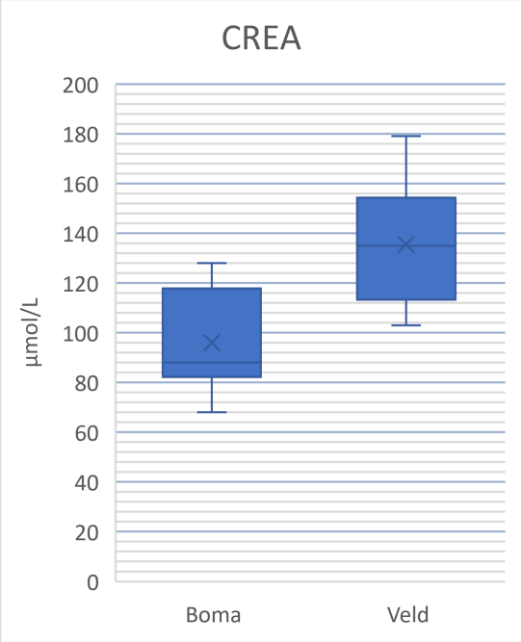
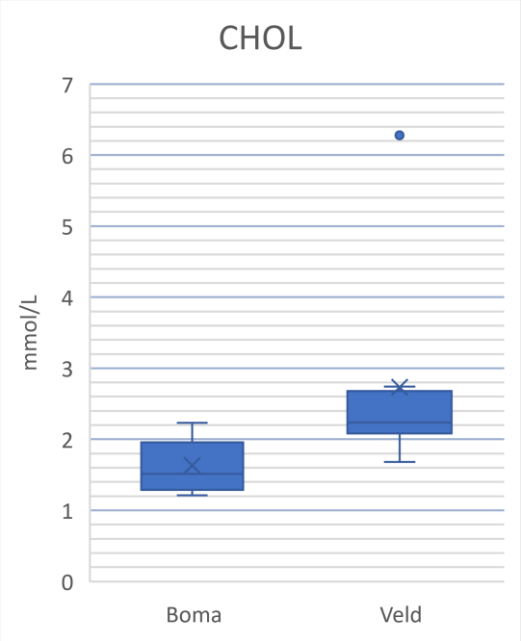


Figure 9b



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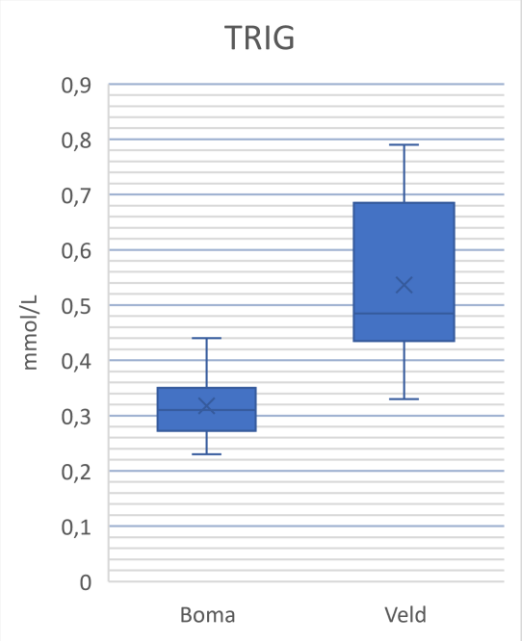

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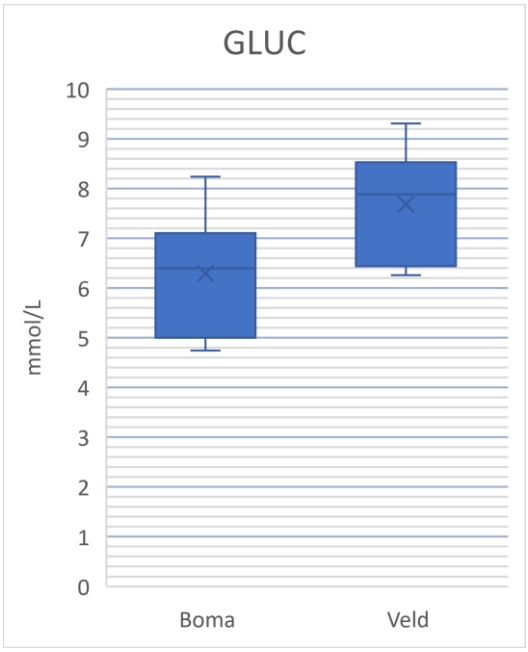

Figure 9e

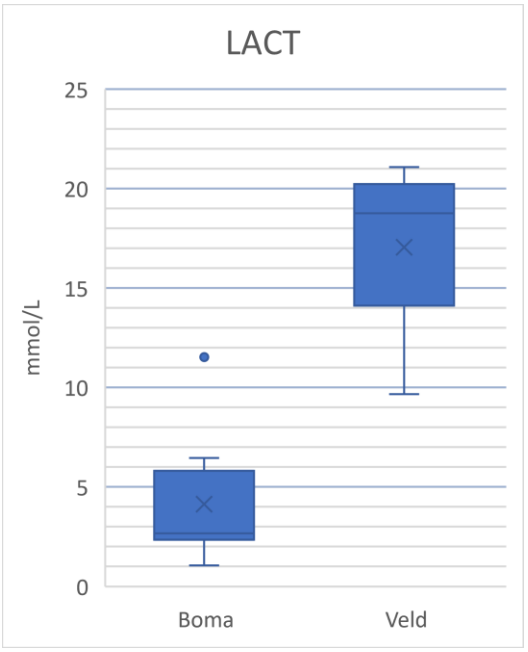

Figure 9f

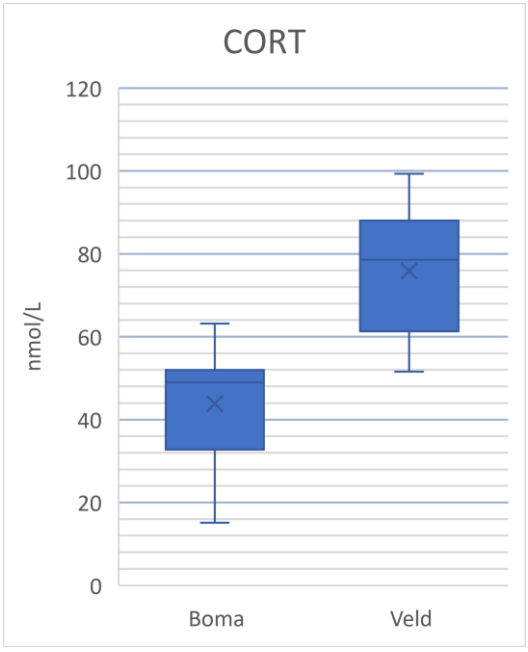

Figure 9g

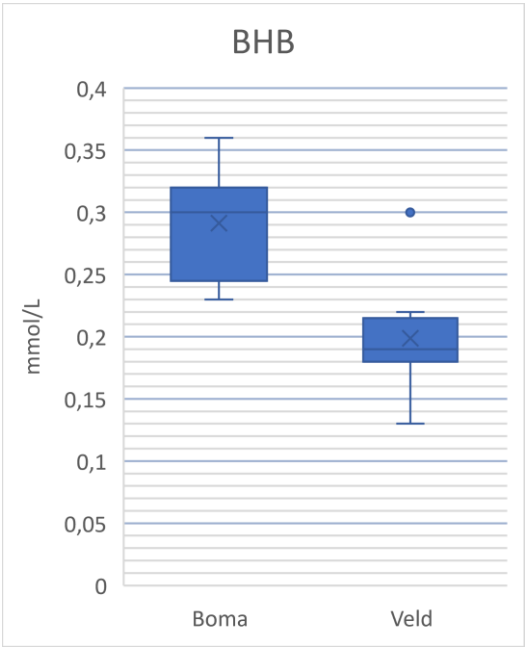

Figure 9h

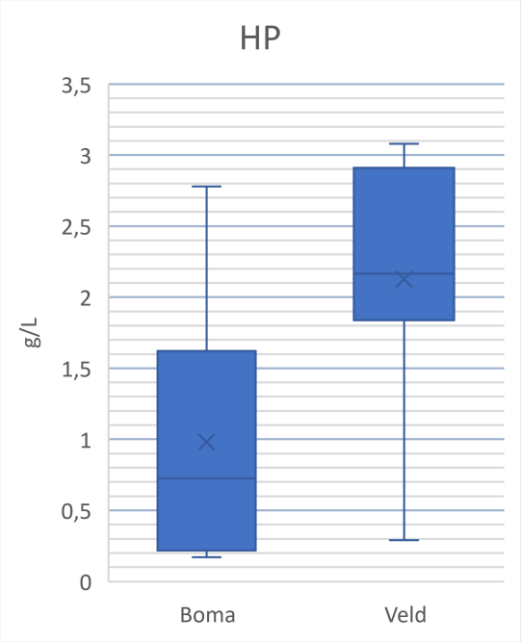

Figure 9i

Figure 9: Boxplots of the plasma concentrations of (**a**) UREA, (**b**) CREA, (**c**) CHOL, (**d**) TRIG, (**e**) GLUC, (**f**) LACT, (**g**) CORT, (**h**) BHB and (**i**) HP at boma-capture and veld-capture; x= mean

3.4 Blood biochemistry after road and air transport

During road transport, the male calf (Rhino ID= 20) struggled to balance during transport and was not able to get up. The female adult rhino (Rhino ID= 21) showed aroused behaviour and dog-sitting during transport. Except for excoriations in three of the rhinos and nervous behaviour of a male subadult (Rhino ID= 28), no further complications occurred. The time from loading to release of the animals was 30.43 ± 1.05 h, with 25.92 h being the actual transport time.

Comparing the two transportation methods, there were significant differences in several clinical chemistry analytes (table 5). A higher mean value of UREA was found in rhinos transported by airplane (p= 0.011), whereas the electrolytes NA and CL showed higher mean concentrations in road transported rhinos. CREA, ALP, CHOL and TBILI also reached higher mean levels after road transport compared to air transport (table 5). The different concentrations of UREA, CREA and ALP are visualised in fig. 10a–g.

There were no significant differences detected in stress-associated analytes like serum CORT and GLUC or muscle enzymes CK and AST.

Table 5: Means, standard deviations and p-value of blood chemistry at release after road and air transport

Clinical chemistry analyte	Mean concentration and standard deviation after road transport (n=8)	Mean concentration and standard deviation after air transport (n=5)	p-value (Wilcoxon rank sum test)
TSP (g/L)	86.59 ± 8.68	88.12 ± 6.66	0.833
ALB (g/L)	32 ± 2.5	30.16 ± 1.41	0.171
GLOB (g/L)	54.59 ± 8.14	57.9 ± 7.61	0.724
ALP (U/L)	**95.53 ± 33.74**	**55.14 ± 22.91**	**0.045**
UREA (mmol/L)	**4.59 ± 1.34**	**6.67 ± 0.87**	**0.011**
CREA (µmol/L)	**160.25 ± 32.76**	**100.6 ± 13.94**	**0.003**
NA (mmol/L)	**137.35 ± 2.13**	**132.12 ± 0.38**	**0.004**
K (mmol/L)	4.15 ± 0.94	3.41 ± 0.31	0.107
CL (mmol/L)	**95.19 ± 1.61**	**88.48 ± 1.29**	**0.002**
AST (U/L)	185.12 ± 105.91	122 ± 48.06	0.284
CHOL (mmol/L)	**2.31 ± 1.05**	**1.12 ± 0.18**	**0.008**
MG (mmol/L)	0.82 ± 0.17	0.7 ± 0.09	0.106
PHOS (mmol/L)	0.94 ± 0.52	0.68 ± 0.29	0.354
TRIG (mmol/L)	0.45 ± 0.08	0.34 ± 0.12	0.093
GGT (U/L)	12.66 ± 3.05	14.5 ± 2.67	0.305
GLDH (U/L)	2.81 ± 0.95	3.66 ± 1.56	0.304
TBILI (µmol/L)	**5.55 ± 2.28**	**3.7 ± 0.76**	**0.034**
CA (mmol/L)	2.92 ± 0.2	2.87 ± 0.18	0.769
CK (U/L)	7013.36 ± 7519.25	6309.76 ± 6065.84	0.833
GLUC (mmol/L)	8.65 ± 1.33	7.13 ± 1.19*	0.109
LACT (mmol/L)	6.53 ± 3.34	5.35 ± 1.09*	0.808
CORT (nmol/L)	71.1 ± 31.6	67.66 ± 27.39	0.833
FE (µmol/L)	17.54 ± 4.37	19.27 ± 1.99*	0.61
NEFA (mmol/L)	0.56 ± 0.26	0.6 ± 0.29*	0.808
BHB (mmol/L)	0.32 ± 0.1	0.23 ± 0.06*	0.126
HP (g/L)	2.39 ± 1.21	1.12 ± 0.85*	0.073

*n=4

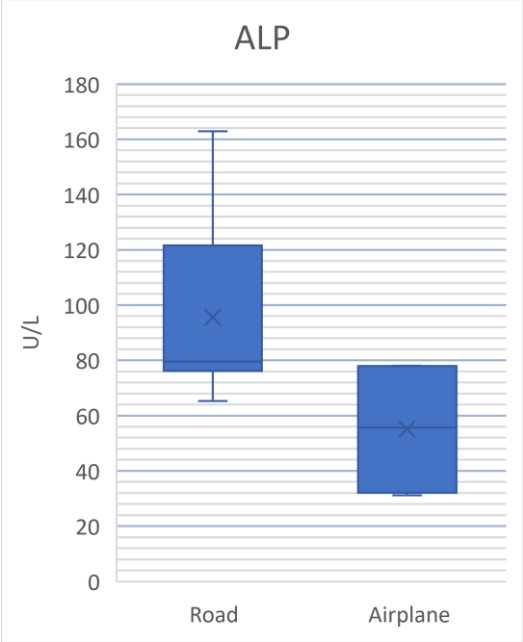

Figure 10a

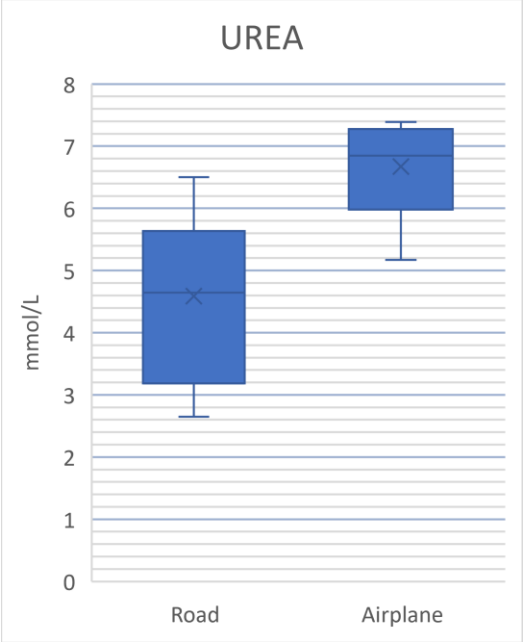

Figure 10b

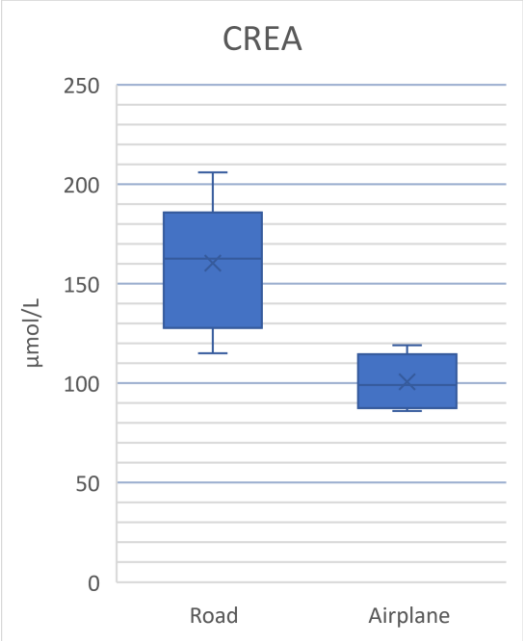

Figure 10c

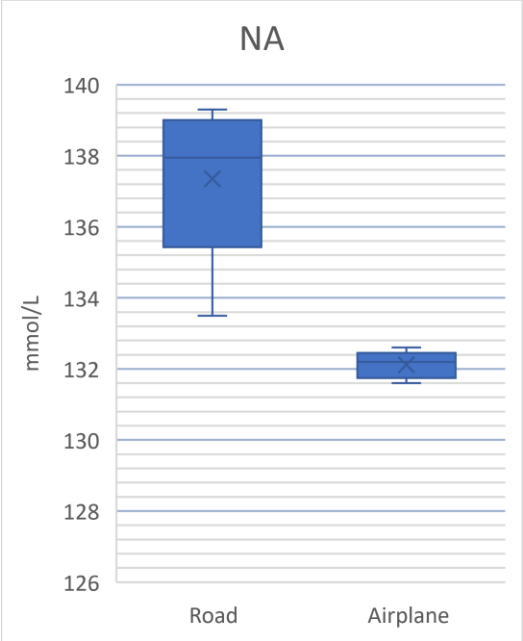

Figure 10d

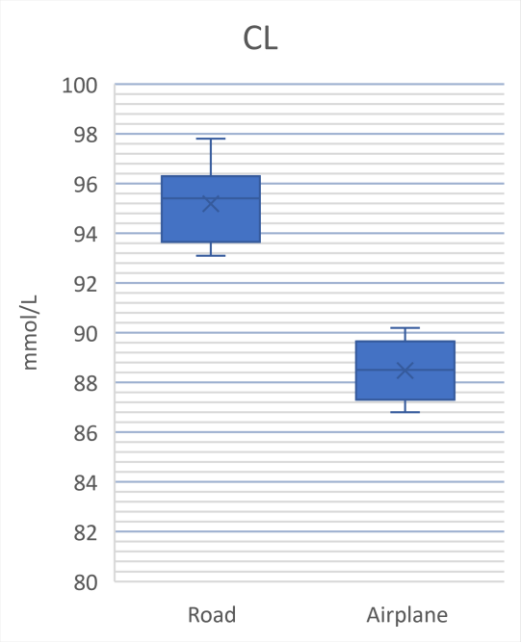

Figure 10e

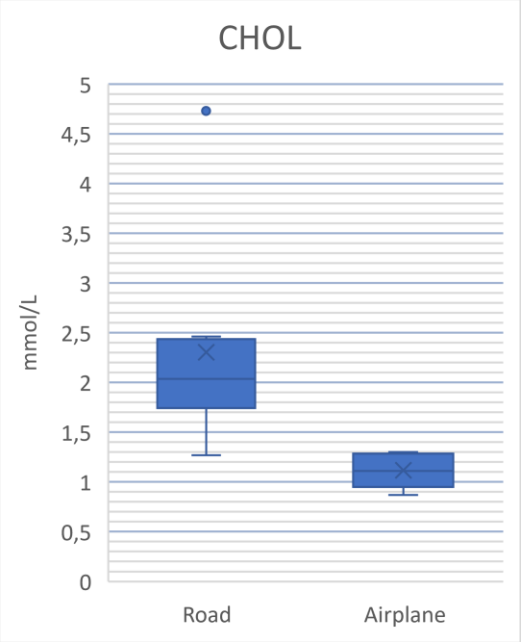

Figure 10f

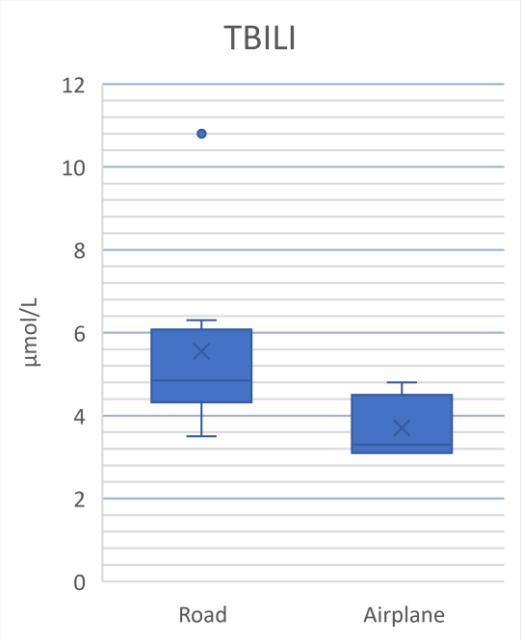

Figure 10g

Figure 10: Boxplots of the plasma concentrations of (**a**) ALP, (**b**) UREA, (**c**) CREA, (**d**) NA, (**e**) CL, (**f**) CHOL and (**g**) TBILI at release after road and air transport; x= mean

4 Discussion

4.1 Changes of the blood biochemistry from capture to release

Several clinical chemistry analytes which reflect the body's energy balance changed significantly from capture to release. These include UREA, K, PHOS, MG, NEFA and TBILI. Although the clinical biochemistry changed over the time of transport, most of these analytes remained within previously reported reference intervals (71, 75).

Magnesium, PHOS and K decreased, most likely due to food deprivation during transport. This finding has already been documented in previous studies as a common finding in transported animals (24, 62, 68, 76, 77). Increases in TBILI are an indication of a disturbed lipid metabolism in relation to decreased food intake (76, 77, 78). In addition, the stress-induced increase in CORT and catecholamines stimulates serum lipase, which leads to a higher concentration of free fatty acids that accumulate in the liver (79, 80). The mobilisation of lipid stores in response to a negative energy balance and an activation of the HPA axis further results in the elevated concentrations of NEFAs (81). A mild decrease of CHOL showed from capture to release, likely because of the changes in the lipid metabolism and the reduced reverse cholesterol transport due to an acute phase reaction (82, 83, 84). Decreases of CHOL may also occur due to the altering effects of CORT on the CHOL metabolism (85). The measured acute phase reactants HP and FE did not significantly differ at capture and release, which is in contrast to other studies that included a greater sample size. Hence, the smaller sample size used for the statistical analysis of these analytes could be the reason we did not detect this change in our rhinos. However, HP concentrations take approximately 24–48 hours to peak (86). Because the air transport took less long, we were not able to detect this change.

Sodium levels were found to be slightly increased at release, indicating changes in the extracellular fluid compartment and relative body water loss likely due to water deprivation during transport (87). This finding correlates with the results of previous translocation studies investigating changes in electrolyte concentrations (24, 51–59). Surprisingly, UREA decreased from capture to release. Urea nitrogen is a product of the amino acid metabolism in the liver (88). Decreased protein intake during transport may have caused this finding, as the animals were fed in a boma prior to capture. Despite the reduced serum concentration of this analyte at release compared to the time of capture, UREA concentrations were above reference intervals at both sample time points (71, 75). The elevation in this analyte may reflect fluid

shifts and body water loss in response to the water deprivation (88). Even in the bomas, prior to translocation, it could have been that rhinos did not drink properly (25).

Muscle enzymes were significantly higher at release compared to the time of capture. Aspartate aminotransferase is a hepatocellular enzyme which is sensitive to liver and muscle damage (89). The increase of this analyte and CK is a common finding in transported animals, due to prolonged balancing and standing as well as stress-related exertion (57, 58, 60–63). The resulting continuous contraction of muscle tissue leads to decreased perfusion and thus to muscle hypoxia (39). An elevation of these muscle enzymes can also reflect the increased fat accumulation in the liver as a consequence of food deprivation (90, 91). In humans, CK is known to respond more rapidly to muscle damage than AST and can rise well above reference intervals, which has also been observed in the rhinos' blood biochemistry at release (92). These findings also correlate with the persistent restless behaviour of the animals during air transport. Leiberich et al. (2022) reported lower levels of AST and CK in rhinos that were fed during transport compared to non-fed rhinos (68). In order to determine whether feeding rhinos can improve muscle enzyme values during air transport, further studies are needed.

Stress-associated clinical chemistry analytes like CORT and GLUC did not increase significantly from capture to release. The small sample size and an exhaustion of the HPA due to the boma-confinement may explain this finding (62). Other causes may be due to the effects of the sedatives and the timing of sampling. Serum cortisol values physiologically fluctuate throughout the day. Warris et al. (1995) and Knowles et al. (1995) reported that CORT in transported cattle and sheep peaked within three hours after start of transport and decreased again within the following nine hours (93, 94). We might have missed peak serum CORT concentrations in our animals due to the long sampling time interval between capture and release and therefore missed the increase of this analyte.

4.2 Comparison of the blood biochemistry of boma-capture with veld-capture

When comparing the two different capture methods, significant differences were found in the acute phase response, the energy balance, stress-associated analytes and the hydration status of the rhino. However, most of the analytes were not outside their reference intervals (71, 75).

Significantly different serum concentrations of acute phase reactants occurred for HP, TRIG and CHOL. Haptoglobin levels were found to be higher in veld-caught rhinos, indicating the initiation of a stronger acute phase response with this capture method (26, 95). It is important to note that it is controversial whether HP plays a role as a major acute phase reactant in rhinos, implying that the increase in concentrations may not be pronounced enough (26, 95). This analyte takes 24 hours to reach peak-plasma concentrations so that only an initial increase can be detected at such an early time point (86).

Triglycerides also reached higher concentrations in veld-captured rhinos. These increases are most likely due to rapid changes in lipid metabolism, caused by acute phase and stress responses associated with the capture (79, 81, 59). In contrast to the latter discussed changes, CHOL concentrations were higher in veld-captured rhinos, whereas they normally decrease during an acute phase reaction, due to reduced reverse cholesterol transport (82, 83, 84). This finding probably reflects differences between the energy balances of veld-caught rhinos living in a semi-wild environment and the wild rhinos being temporarily kept in bomas (96). Moreover, increases in CHOL may also occur due to haemolysis, which could have been caused by the helicopter-chase in the veld-caught rhinos (96).

In order to generate energy, ketone bodies like BHB are synthesized in the liver during the mobilisation of lipid stores (97). This analyte showed slightly higher concentrations in the boma-captured rhinos. This may be a consequence of anorexia and decreased food intake of wild rhinos that are temporarily kept in bomas, which consequently leads to a negative energy state of these animals (25, 98). In line with this finding, the concentrations of UREA exceeded the reference intervals in the boma-captured rhinos indicating a decreased water intake (62, 68, 71, 75, 87).

Serum creatinine, a product of muscle metabolism and marker for a reduced glomerular filtration rate, reached higher concentrations in rhinos caught in the veld (87). This result is not surprising, as veld-captured rhinos ran longer distances during the capture process, leading to a greater degree of body water loss and muscle damage.

Muscle exertion induces anaerobic glycolysis in order to generate more energy (99). The resulting elevated serum LACT reached concentrations more than four times higher in the veld-captured than in the boma-captured rhinos and exceeded former reported reference intervals (100). From these results, it can be concluded that rhinos captured in the veld experience greater muscle exertion than rhinos captured in a boma. This is not surprising as rhinos captured in the veld typically run away from the helicopter, often over long distances, until they

have been darted and immobilised (101). In addition, hypoxaemia and acidaemia caused by etorphine and immobilisation also lead to an increase in LACT concentrations (72, 102).
The measured stress-associated analytes GLUC and CORT showed higher values in veld-captured rhinos. Hyperglycaemia, induced by an activation of the HPA, the SAM and increased pro-inflammatory cytokines often occurs as an adaptive response in stressed animals and has already been reported in transported rhinos (34, 62). This finding correlates with the higher CORT concentrations in the veld-captured rhinos indicating that veld-capture is more stressful than boma-capture.
To more accurately assess whether rhinos captured in the veld experience more stress than rhinos captured in the boma, other parameters such as N:L ratio, plasma adrenaline, oxidative stress biomarkers and leucocyte coping capacity could be included in the analysis (26, 103).

4.3 Comparison of the blood biochemistry at release after road and air transport

The comparison of the two different transport methods showed significant differences in some clinical chemistry analytes. Again, most analytes remained within their reference range (71, 75).
Alkaline phosphatase, an enzyme found in renal tubule cells, bile, bones and the intestinal mucosa, reached higher concentrations in rhinos transported by road (104). The elevated liver enzyme levels could be due to the higher doses of anaesthetics and sedatives administered to the road transported rhinos as they were again immobilised at release, unlike the rhinos transported by airplane. In addition to the increased load on the liver from metabolism and the breakdown of administered drugs, stress and food deprivation may also contribute to an increase in ALP associated with the longer transport time.
Creatinine, NA and CL also showed higher concentrations in the road transported rhinos. While NA and CL were still within their reference ranges in both groups, the serum CREA concentration was observed to be slightly elevated in the road transported rhinos. These differences were most likely due to the longer transport time by road and the associated longer water deprivation, resulting in a greater body water loss in this group of rhinos (87). Hypovolaemia, which can possibly be induced by anaesthetics and sedatives, and muscle injury may further promote the rise in CREA, NA and CL concentrations (28, 105, 106, 107). Increases in these values have been documented in many previously transported animals (24, 51–59).

Urea nitrogen is found to be more concentrated and above the reference range in the rhinos captured in the boma and transported by airplane (71). This elevation is once more reflecting the increased body water loss during transport (88). Since CREA, NA and CL are more sensitive to fluid shifts, the difference in UREA concentrations between the two groups is most likely due to the difference in feed quality and quantity (87, 105, 106, 107).
Cholesterol and TBILI concentrations were higher in road transported rhinos. As described in chapter 4.1. these findings may reflect a changed lipid metabolism in the liver due to food deprivation (76, 77, 78). Higher levels of these analytes in the rhinos transported by road compared to the rhinos transported by airplane are probably due to the longer transport time and thus a longer period of fasting.
No significant differences were detected in stress-associated analytes or muscle enzymes, probably due to the small sample size.

4.4 Limitations

A main limitation to the validity of this study lies in the different anaesthesia protocols. This relates to the comparison between the two different modes of transport, as the rhinos transported by road were immobilised two times, in contrast to those transported by airplane, who were only immobilised at capture. In addition, some of the rhinos transported by road were given butorphanol and two rhinos received corticosteroids during road transport. Corticosteroids, anaesthetics and sedatives used during translocation have previously been shown to affect the biochemistry of the blood, so an accurate comparison of biochemical analytes between the two modes of transport is limited (28, 102, 108, 109).
Furthermore, road transported rhinos were caught by helicopter whereas rhinos transported by air were captured from bomas. The rhinos would have had to be captured in the same way to more accurately assess the sole difference in blood biochemistry between the two transported groups.
Finally, it is important to mention that in order to be able to make a more precise statement about the best translocation method, other parameters, like overall behaviour during transport, the haematology, oxidative stress markers, etc. must also be taken into account.

5 Conclusion

This study has demonstrated the effects of different capture and transport methods on the blood biochemistry of rhinos.

Transport has shown to negatively affect energy balance and lead to mobilisation of lipid stores. The rhinos also suffered relative water loss and muscle exertion, but no increased stress-related chemical analytes were detected between capture and release.

When comparing the two capture methods, boma capture was found to have positive effects on rhino blood biochemistry. Boma-caught rhinos showed a lower degree of dehydration, less muscle exertion and lower levels of stress-related clinical chemistry analytes. However, they showed metabolic shifts due to nutritional imbalances associated with temporary boma confinement.

Rhinos captured in the veld showed a stronger acute-phase response.

In addition, rhinos transported by road have been shown to suffer greater water loss and more effects of food deprivation than rhinos transported by air, probably due to the longer transport time.

Further research under a more controlled setting is needed to determine the most appropriate translocation method and how to mitigate pathologies associated with rhino translocation.

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Figures

1. Photo by Guilbert Gates. Smithsonian Magazine; 2018. Available from; URL: https://www.smithsonianmag.com/science-nature/dangerous-work-relocating-5000-pound-rhinos-180969008/

2. Photo by Jason Florio. Smithsonian Magazine; 2018. Available from; URL: https://www.smithsonianmag.com/science-nature/dangerous-work-relocating-5000-pound-rhinos-180969008/

3. Photo by David Murray. Rhinos Without Borders Gallery 2015–2017.

4. Photo by Jason Florio. Smithsonian Magazine; 2018. Available from; URL: https://www.smithsonianmag.com/science-nature/dangerous-work-relocating-5000-pound-rhinos-180969008/

5. Photo by Jason Florio. Smithsonian Magazine; 2018. Available from; URL: https://www.smithsonianmag.com/science-nature/dangerous-work-relocating-5000-pound-rhinos-180969008/

6. Photo by David Murray. Rhinos Without Borders Gallery 2015–2017.

7 a–c. Photo by David Murray. Rhinos Without Borders Gallery 2015–2017.

Congress poster presentations related to this thesis

(Attended conference together with main external supervisor Dr. Leiberich)

Schönlechner M[1], LCR Meyer[2,3], Hooijberg EH[2,4], Hofmeyr MS[5,6], Cooper D[7], Reuben MMB[8] et al. Changes in clinical chemistry analytes in boma adapted compared to wild white rhinoceroses (*Ceratotherium simum*) transported by airplane compared to road. EAZWV Zoo and Wildlife Health Conference, Valencia, Spain, June 2023.