Evaluation of biochemical data collected from chemically immobilised semi-captive Southern White Rhinoceros (*Ceratotherium simum simum*) in the Eastern Cape, South Africa

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Photograph by Gemma Crowley: blood sample being taken from an chemically immobilised adult male southern white rhinoceros

Scientific Paper submitted in part fulfilment of the requirements for the degree of Master of Science in Wild Animal Biology, University of London, 2016-17

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Declaration: The data for this project was collected by Gemma Crowley BSc (Hons) RVN, Dr William Fowlds BVSc, Dr Emily Baxter BVetMed MRCVS and Sister Candice Momberg Dip. VetNurse. The data was collated, analysed and written up solely by Gemma Crowley BSc (Hons) RVN.

Word count: 5,000

Abstract

Currently there is a lack of data with regards to the normal biochemistry values and the effects of the duration of chemical immobilisation on biochemical values of blood in semi-captive southern white rhinoceros (*Ceratotherium simum simum*) in the Eastern Cape. Biochemistry plays a pivotal role in veterinary medicine diagnostics and is being used increasingly in wildlife, as well as in domestic species. Unfortunately in wildlife, particularly free-living and semi-captive species, there are very few normal blood chemical values available, and therefore interpretation of results and identification of abnormal results is difficult.

Biochemical panels were analysed on 19 individual semi-captive southern white rhinoceros from the Eastern Cape. These animals were chemically immobilised between May 2017 and July 2017 for existing rhino management and veterinary procedures. The rhino were immobilised using Etorphine (M99®), Azaperone (Stresnil®) and Hyaluronidase (Hyalase®). Blood samples were collected from the auricular vein using an 18G vacutainer needle and shoulder directly into lithium heparin and serum blood tubes (BD Vacutainer; Becton and Dickinson, Plymouth, UK). One sample was taken as soon as possible after chemical immobilisation and a second sample was taken shortly before reversal with Naltrexone (Trexonil®). Heparinised plasma samples were run in the field using a bench-top chemistry analyser, the Abaxis Vetscan VS2 (Abaxis Diagnostics, California, USA); serum samples were stored in cooler boxes containing ice packs until they could be centrifuged and analysed using the Abaxis Vetscan VS2 in the laboratory within five hours.

The objectives of this study were to determine whether the duration of chemical immobilisation has a direct effect on the biochemical values of blood taken from semi-captive southern white rhinoceros in the Eastern Cape; whilst also establishing reference intervals of biochemical values in order to ascertain a baseline of what is expected in healthy southern white rhinoceros for comparative purposes in clinical cases and traumatised rhino.

Significant differences between the first and second samples were seen in alb, ALP, AST, Ca, glob, Mg and TP; suggesting that duration of chemical immobilisation does have a significant impact on biochemical intervals in southern white rhinoceros. Biochemical reference intervals were established using the first blood samples collected from the southern white rhinoceros in this study (n=19). The paucity of research on biochemical values in semi-captive southern white rhino warrants the undertaking of this study and reinforces the importance of collating existing data to establish a fully functional set of biochemical intervals for this species. Furthermore, this study allows for wildlife veterinarians treating future semi-captive white rhinoceros to determine whether individuals are suitable for prolonged chemical immobilisation.

Key words: *Ceratotherium simum simum*, southern white rhinoceros, biochemistry, chemical immobilisation, heparinized plasma, serum.

Scientific Paper submitted in part fulfilment of the requirements for the degree of Master of Science in Wild Animal Biology/Health), University of London, 2016-17

1.0 Introduction

1.1 Ecology and threats to the species

Southern white rhinoceros (*Ceratotherium simum simum*) are near threatened grazing rhinoceros that inhabit the subtropical grasslands, savannas and shrublands of sub-Saharan Africa (Cromsigt and Te Beest, 2014; Emslie, 2012; Waldram et al., 2008). There is currently estimated to be between 19,682 and 21,077 wild southern white rhino remaining (Emslie, 2012). However, the rapid escalation of poaching for the international rhino horn trade in Africa, particularly South Africa, greatly threatens the survival of white rhino populations with a predicted loss of well in excess of 30% over three generations (Emslie, 2012).

The majority of the remaining white rhino in South Africa are now concentrated in fenced reserves, sanctuaries, conservancies, rhino conservation areas and intensive protection zones where anti-poaching and law enforcement effort can be concentrated at effective levels (Emslie, 2012). Thus wildlife conservationists claim that there are no truly wild white rhino in South Africa, and therefore white rhinos in South Africa are referred to as semi-captive.

The rhino inhabiting protected areas often require veterinary intervention or conservation management procedures such as translocation, fitting trackers or de-horning, and consequently the chemical immobilisation of rhino is required (Kock et al., 1995). It is anticipated that the pressure of poaching will force rhino into small to medium sized reserves (i.e. 1000 to 30,000 hectares) in the future to improve security efficacy, and will hence result in more management and veterinary procedures than what is currently considered necessary (W Fowlds 2017, personal communication, 28 May).

1.2 Chemical Immobilisation

Rhinoceros have been chemically immobilised as part of conservation management plans since the 1960's, however chemical immobilisation is not without risk and consequently it is vital to fully understand the effects of immobilisation on individuals to help improve anaesthetic protocols and establish a safe period of immobilisation for future veterinary procedures (Kock et al., 1995). Chemical immobilisation can place rhinos at risk of physiological detriment due to the stress of the capture and drug related side effects (Kock et al., 1995; Wegner et al., 2007). One of the most common reasons for chemical immobilisation is for conservation translocation; the IUCN Rhino Specialist Group estimates a 5% mortality rate for translocated rhinos in South Africa and Namibia (Emslie, 2012). Morbidity and mortality is often caused post translocation due to the fact that the individual may have suffered from an underlying disease or condition that may have been amplified by chemical immobilisation; such conditions could have been detected previous to translocation by taking biochemical and haematological samples (Emslie et al., 2012; Kock et al., 1990).

The effect of chemical immobilisation on various biochemical parameters in wild animals has been reported in a number of species (Kock et al., 1987a; Kock et al., 1987b; Kock et al., 1993; Marco and

Lavin, 1999; Wessen et al., 1979). The relationship between anaesthesia and biochemical values has also been analysed in a number of domestic species (Gil et al., 2004; Bougherara and Bouaziz, 2014). However, there are currently no known studies that investigate the effect of duration of chemical immobilisation on biochemical parameters in semi-captive southern white rhino, potentially due to difficulty collecting samples.

A study by Kock et al. (1990b) investigating baseline biological data from black rhinoceroses suggests that those individuals that have undergone prolonged periods of immobilisation may have elevated levels of aspartate transaminase, blood urea nitrogen and globulin, and decreased levels of phosphorus, magnesium, albumin, calcium and creatine kinase. In addition, significant differences in glucose levels related to capture stress were observed in black rhino following capture (n=64) and translocation (Kock et al., 1990a). Another study found increased protein concentration following transportation of black rhino (n=43) and neutrophilia and anaemia in rhino transported over longer distances (Kock et al., 1999).

1.3 Clinical biochemistry

In order to establish the effect of duration of chemical immobilisation on individuals, biological samples can be taken to observe the change in biochemical parameters that occur in the body. Biochemistry plays a pivotal diagnostic role in veterinary medicine and is being used recurrently in wildlife, as well as domestic species (Borjesson et al., 2000; García et al., 2010; Paul-Murphy et al., 1994; Nordøy and Thoresen, 2002). Unfortunately, very few reference blood chemical values are available, particularly for free-living wild species due to the difficulty collecting samples, and therefore interpretation of results and identifying abnormal results is difficult (Mathebula et al., 2012).

Knowledge of biochemical parameters of free-ranging and semi-captive wildlife is important for assessing and managing populations. Biochemical samples collected from free-living and semi-captive wildlife are valuable resources for disease surveillance, establishing reference intervals and creating a biobank as a source for future genetic and serologic studies (Mathebula et al., 2012). Blood parameters used to diagnose diseases amongst wildlife can be used to assess the physiological status of a population. Unfortunately most clinical biochemistry information has been collected from captive wildlife and therefore further research into biochemical values for wild animals is warranted (Jones et al., 2014; Marco et al., 2000; Okotie-Eboh et al., 1992; Pospíšil et al., 2014).

There are currently few existing reference intervals for free-living or semi-captive wild animals, despite being a vital diagnostic tool for both individual and population health. Evidently it is extremely difficult to collect sufficient biochemical samples from free-living or semi-captive wild animals in order to establish a large enough bank of samples to create reference intervals, particularly in the case of endangered or elusive animals. From the literature it is clear that there is no recent, fully established set of reference intervals for free-living or semi-captive white rhinos, only baseline biochemical data from (n=181) white rhino in the Kruger National Park (Mathebula et al., 2012), baseline data from semi-captive rhino (n=16) in a safari park in Virgina (Seal et al., 1976) and a fully established set of

reference intervals for captive white rhinos in British and Irish Association of Zoos and Aquariums (BIAZA) zoos (Teare, 2013).

References to biochemical values are important to be able to distinguish between clinically healthy and clinically ill animals. For reference intervals to be reliable, it is important to have access to a range of ages and different sexes within species to prevent misdiagnosis (García et al., 2010; Kaneko et al., 2008; Nordøy and Thoresen, 2002).

The relationship between age, sex and biochemical values has been analysed in a number of wild animals, particularly non-human primates, but also marine mammals, artiodactyls and equids (Elkhair, 2016; Kiran et al., 2012; Miknienė et al., 2013; Norman et al., 2013; Shukan et al., 2012; Wu et al., 2014; Xi et al., 2013). From these studies it is evident that age and sex have a statistically significant impact on biochemical values. In non-human primate's age and sex had a statistically significant impact on AST, ALT, ALP and GGT (Wu et al., 2014; Xi et al., 2013).

Establishing a reliable set of reference intervals and the effect of the duration of chemical immobilisation could allow for early detection of medical concerns in white rhino that may not be suitable candidates for repeated or extended periods of immobilisation, such as for translocation. Values from individual animals can be compared with the distribution of values from a reference population to identify a physiological change or disease and establish a course of treatment and management for that animal (Geffré et al., 2009). Greater understanding of factors impacting successful chemical immobilisation of rhinos will enhance the management and conservation efforts crucial to saving this species (Mathebula et al., 2012).

2.0 Materials and methods

2.1 Data Collection

Blood samples were collected from 19 chemically immobilised semi-captive southern white rhinoceros undergoing existing veterinary and rhino management procedures in game reserves, sanctuaries, conservancies, rhino conservation areas and intensive protection zones in the Eastern Cape, South Africa. Only clinically healthy rhino were sampled for this study, health status was determined according to physical examination, body condition score, and previous medical history. The 19 rhinos were split into age and sex associations; male (n=6), female (n=13), adults (over 7 years) (n=8), sub-adults (3-7 years) (n=7) and juveniles (<3 years) (n=4). The samples were collected from the auricular vein of the left ear as soon as safely possible after immobilisation and just before reversal. The rhino were immobilised using the protocol in Table 1 (Radcliffe et al., 2000; Portas, 2004).

Age/sex	Primary drug	Additional drugs	Narcotic antidote	Tranquillizer/sedative	Drug delivery system
Adult bull and cow	4mg Etorphine (M99®)	2000IU Hyaluronidase (Hyalase®) 20mg Butorphanol®	40mg Naltrexone (Trexonil®)	40mg Azaperone (Stresnil®)	Pneu-dart® (from helicopter)
Sub-adult bull and cow	2mg Etorphine (M99®)	2000IU Hyaluronidase (Hyalase®) 20mg Butorphanol®	20mg Naltrexone (Trexonil®)	20mg Azaperone (Stresnil®)	Pneu-dart® (from helicopter)

Table 1: Protocol used for darting white rhino, including equipment and chemical restraint agents

Anaesthesia was maintained by veterinary professionals to include veterinary surgeons and veterinary nurses. Physiological parameters including temperature, respiration, pulse rate, blood pressure, mucous membrane colour, capillary refill time, and blood oxygen levels were monitored and recorded throughout the procedure using an anaesthetic monitoring sheet (Appendix II). General health from physical appearance, clinical history and body condition score (Appendix III), sex, age (as an estimate), and reason for veterinary intervention were also recorded for reference to coincide with the biochemical panels.

An intravenous cannula was placed in the auricular vein in the right ear of each rhino to ensure constant intravenous (I/V) access and to allow for administration of I/V drugs. Diprenorphine (M5050®) was administered to correct any respiratory depression when required. Butorphanol was administered routinely once the rhino was immobilised to allow for partial reversal. Administration of Ketamine (Ketaset®) was given at the veterinary professional's digression to maintain anaesthesia if required. Intravenous fluid therapy (IVFT) was only used in those rhino that underwent extended immobilisation times, over 90 minutes (n=1).

All rhinos were treated with prophylactics routinely, including dart wound treatment (Rilexine®), multivitamins (MultiVit®) and antibiotics (Lentrax®). Rhinos were fully reversed with Naltrexone (Trexonil®), given intramuscularly (I/M) to prevent renarcotisation. Each rhino was monitored by the veterinary professionals present post procedure until deemed fully reversed and recovered, and were continuously monitored for the remainder of the day by rangers or anti-poaching units.

Blood samples were collected from the auricular vein in the left ear using an 18G vacutainer needle and shoulder directly into lithium heparin and serum blood tubes (BD Vacutainer; Becton and Dickinson, Plymouth, UK). Where possible heparin samples were taken and run in the field but when circumstances prevented use of the Abaxis Vetscan VS2 in the field, serum samples were taken and

stored in cooler boxes containing ice packs until they could be centrifuged and analysed using the Abaxis Vetscan VS2 in the laboratory within five hours. All samples were collected by the author where possible, where not possible samples were collected by another veterinary professional according to the standard operating procedure (SOP) (Appendix I). All vacutainers were labelled with the date, patient ID, microchip number and location, and the samples were labelled with the time and either one or two to represent at what point in the anaesthesia the sample was taken.

Of the 38 samples taken from the white rhino sampled (n=19), 24 samples were stored in serum tubes and cooler boxes until they could be centrifuged and analysed in the laboratory, and 14 samples were stored in lithium heprin tubes and analysed directly in the field.

The samples were analysed using a benchtop biochemistry analyser, Abaxis Vetscan VS2 (Abaxis Diagnostics, California, USA), using the Large Animal Rotor, as recommended (M Miller 2017, personal communication, 28 May). All analyses were carried out by the author. The biochemical panel included the following eleven analytes: Albumin (alb), alkaline phosphatase *(*ALP), aspartate transaminase (AST), urea nitrogen (UN), calcium (Ca), creatine kinase (CK), gamma-glutamyltransferase (GGT), globulins (glob), magnesium (Mg), phosphorous (phos), and total protein (TP). As previous to this study there were no recorded biochemical values for semi-captive southern white rhino in South Africa, Abaxis (Abaxis Diagnostics, California, USA) uploaded normal biochemical parameters from the domestic horse (*Equus domesticus*) to produce intervals on the Abaxis machine (Barrelet and Ricketts, 2002; Driessen et al., 2002; Kaneko et al., 2008; Steffey et al., 1993; Watson et al., 2002). The results from the Abaxis Vetscan VS2 were then inserted into a Microsoft Excel™ spreadsheet along with the total time of immobilisation, physiological parameters (temperature, respiration, and pulse), sex, age (as an estimate), and reason for veterinary intervention.

2.2 Quality Assurance

To provide quality assurance a SOP (Appendix I) was created to ensure that sample collection, sample handling, and sample processing was performed in a standardised manner (Flatland et al., 2010). Considerations were given to any potential adverse effects to samples caused by preanalytical factors. Rejection criteria were established and all samples were checked for potential endogenous interference, such as hemolysis, icterus or lipemia, and for any exogenous interference, such as administration of additional drugs or intravenous fluids, previous to analysis. Any samples that were deemed to have undergone any form of endogenous interference were discarded (Dimeski, 2008). Samples were analysed using methods monitored with strict quality control procedures (Flatland et al., 2010). Abaxis (Abaxis Diagnostics, California, USA) were consulted to ensure optimum operation of the Abaxis Vetscan VS2 machine.

2.3 Data Analysis

The 38 data sets collected were inserted into a Microsoft Excel™ spreadsheet and then exported to SPSS 23.0 statistical software package. To establish whether significant differences existed between

the first and the second blood samples taken, a paired t-test was used to decipher whether duration of immobilisation has a significant effect on biochemical parameters. The Kolmogorov-Smirnov goodness-of-fit hypothesis test was performed to determine a normal distribution (Horn et al., 2001; Seigel, 1988).

To determine the effect of other covariates such as age and sex on the biochemical parameters obtained, SPSS 23.0 was used to construct individual general linear. The difference between the first blood sample and the second were calculated (a-b) and then the difference was divided by the second result ((a-b)/b) to obtain the percentage difference.

Reference intervals for southern white rhino were determined according to guidelines set by the ASVCP and CLSI (Friedrichs et al., 2012; Horowitz et al., 2008). The mean, mode and median were calculated using the data from the first sample taken. SPSS 23.0 was used to construct frequency tables and histograms, as recommended for lower sample sizes (<120) (Friedrichs et al., 2012; Horowitz et al., 2008). The data was inspected visually to identify the type of distribution and identify any outliers (Chua et al., 1978; Friedrichs et al., 2012). Outliers were detected using Tukey and Dixon methods, any outliners identified were omitted from the reference intervals. To determine normal or Gaussian distribution of data, the Kolmogorov-Smirnov goodness-of-fit hypothesis test was performed (Horn et al., 2001). The significance level (P) was set at ≤0.05. For the parameters that followed Gaussian distribution, the 95% confidence interval (mean ± 1.96SD) was used to establish the reference intervals of the rhinoceroses. Due to the limited sample size, separate age and sex parameters were not distinguished.

3.0 Results

All the raw biochemical and demographic data collected for use in this study is listed in Table 2. The results from the paired t-test to determine whether duration of immobilisation had a significant effect on biochemical parameters are listed in Table 3. A frequency table displaying mean, mode and median of the biochemical values of semi-captive southern white rhinoceros (n=19) are listed in Table 4**.** The reference ranges derived from the first blood sample taken from semi-captive rhinoceroses (n=19) are displayed in Table 5. The general linear model to show univariate analysis of variance of the percentage difference between first and second blood samples against immobilisation time, age and sex is listed in Table 6.

Several statistically significant differences between the first and second blood samples have been found. Significant differences between the first and second samples were seen in alb (Figure 2a), ALP (Figure 2b), AST (Figure 2c), Ca (Figure 2e), glob (Figure 2h), Mg (Figure 2i) and TP (Figure 2k). The southern white rhinoceros were found to have an average decrease in alb (Figure 2a), ALP (Figure 2b), AST (Figure 2c), Ca (Figure 2e), glob (Figure 2h), Mg (Figure 2i) and TP (Figure 2k). There were no significant differences seen between the first and second samples in UN (Figure 2d),

CK (Figure 2f), GGT (Figure 2g) and phos (Figure 2j). Despite the lack of significance, UN, CK, GGT and phos also showed an average decrease in biochemical value as duration of immobilisation increased.

The effect of sex and age was investigated (Table 6) using a general linear model. Age did not appear to have a significant effect on the difference in biochemical values for any of the biochemical values measured. Sex only appeared to have a significant effect on ALP (0.005).

Biochemical reference intervals were established using the first blood samples collected from the white rhino in this study (n=19) (Table 5).

4.0 Discussion

In all semi-captive southern white rhino chemically immobilised using M99®, Stresnil® and Hyalase®, the duration of chemical immobilisation had a statistically significant effect on several of the biochemical values investigated, as expected. However, some of the values that were expected to have the most significant change due to results from previous studies, UN, CK and phos, in fact had no significant change; and the value that had the most notable change in this study, ALP, had no significant change throughout immobilisation in previous studies (Kock et al., 1990a; Kock et al., 1990b). This may have been due to difference in sub-species or perhaps due to sample size or the different methods of blood biochemical analysis used. Further to this, previous studies suggested that long periods of chemical immobilisation will result in elevated levels of AST, UN, glob and decreased levels of phos, Mg, alb, Ca and CK (Kock et al., 1990a; Kock et al., 1990b). In this study AST levels decreased significantly, UN levels decreased marginally but not significantly, and glob levels decreased significantly, contradictory to previous studies. However, levels of alb, Ca and Mg decreased significantly and levels of CK and phos decreased marginally (Kock et al., 1990a; Kock et al., 1990b).

Overall, all of the analytes appeared to undergo an average decrease from the initial first sample to the second sample taken. The results from the majority of rhino sampled appeared to fluctuate randomly with a mild visible decrease as immobilisation time increased, with the exception of one rhino that underwent a notably longer immobilisation period and received IVFT, which may have had a diluting effect. This may have skewed the overall average causing the appearance of a distinct decrease in values, when actually the distribution was random for the majority of results.

An average decrease in CK was expected, as this has been previously reported in black rhinoceros (Kock et al., 1990a). High levels of CK are expected at the first blood sampling as high CK is indicative of active or recent muscle damage which is often as a result of extra-pyramidal side effects caused by chemical immobilisation, such as muscle tremors (De Lange, 2016). Evidently as the immobilisation stabilises and the muscle tremors decrease due to the administration of a partial

reversal such as Butorphanol, CK is likely to decrease, as shown in this study (Kock et al., 1990a; Morkel, 1989). In a previous study on black rhinoceros, AST appeared to mimic the reaction of CK (Kock et al., 1990a), however Kaneko (1980) suggests that in domestic horses an increase in AST levels was in fact followed by a decrease in CK, in this study an initial high level of AST was observed, followed by a decrease in CK.

Significant alterations in serum proteins are not often observed as a result of short periods of immobilisation, and are more commonly as a result of nutritional influences (Kaneko, 1980). In this study however statistically significant differences were seen between the first alb, TP and glob samples and the second samples taken. Significant differences in protein levels may be indicative of adrenal activity and nitrogen loss caused by capture stress (Kock et al., 1990a).

Clinically significant differences between initial UN samples and later samples are commonly observed in rhino that have suffered from chronic renal damage, thus significant changes were not expected to be seen in this study due to the elimination of clinically ill rhino (Kock et al., 1990a; Kock et al 1990b).

The most significantly changed biochemical interval as a result of increased immobilisation time was ALP. ALP often fluctuates as a result of liver damage, in rhino this may be due to consumption of toxic plants or due to repeated chemical immobilisation (Fernandez and Kidney, 2007). Few previous studies examining the result of capture or chemical immobilisation have investigated changes in ALP, and thus limited comparisons can be made (Kock et al., 1987a; Kock et al., 1987b; Kock et al., 1990a; Kock et al., 1993; Marco and Lavin, 1999; Wessen et al., 1979). Changes in ALP are often associated with alterations in GGT as both can be used to detect liver damage; in addition elevations in GGT can be used to detect intestinal disease domestic horses (Gardner et al., 2005; Underwood et al., 2010). However, in this study changes in GGT were not statistically significant.

The Ca values obtained in this study appeared to be similar to those values reported from semi-captive and captive white rhino populations (Seal et al., 1976; Teare, 2013). Fluctuations in Ca as a result of extended chemical immobilisation time has yet to be reported in rhino, however in domestic horses decreases in calcium were observed in healthy horses during and post anaesthesia which was suggested to be due to an increase in phos levels as a result of alteration in renal function (Edner et al., 2007; Watson et al., 2002). In this study the phos levels decreased, however the results were not statistically significant. Blood values of another mineral, Mg, changed significantly from the initial sample and the second sample. Changes in Mg are often associated with changes in dietary intake or lactation, but when an animal is chemically immobilised Mg and losses in other minerals such as Ca and phos are often seen due to fluid loss (O'kelley, and Fontenot, 1969; Wagner, 2008).

Other covariates including age and sex were investigated to reveal that both had little to no significant effect on biochemical values during chemical immobilisation. Only ALP appeared to be significantly affected by sex, this may be due to the fact that the majority of rhino sampled were female (n=13) and

thus resulted in a bigger variation in ages compared to the males (n=6). ALP has been reported as notably higher in older animals as a result of damage to liver, bones or gall bladder and thus due to the higher number of female adults (n=6), this has a corresponding effect on the resultant biochemical values of the female rhinos in this study (Fernandez and Kidney, 2007). In contrast, ALP values have been reported as significantly higher in sub-adult and juvenile black rhinoceros and white rhinoceros when compared to adults, as well as in colts and fillies when compared to adult domestic horses (Mathebula et al., 2012; Muñoz et al., 2012). This may also correlate with the results from this study due to the higher number of sub-adults and juveniles (n=6) being female, compared with just four males being classed as sub-adult or juvenile.

Few comparisons can be concluded between the results of this study and previous similar studies as these studies have all been conducted on other species, and thus similar changes cannot be expected (Kock et al., 1987a; Kock et al., 1987b; Kock et al., 1990a; Kock et al., 1993; Marco and Lavin, 1999; Wessen et al., 1979). Comparisons can be made between this study and previous biochemistry studies on white rhino in relation to the effect of chemical agents used to immobilise southern white rhino. Previous studies suggest that white rhino immobilised with M99® may show marked physiological side effects, such as acidosis, hypercapnia, hypertension, hypoxia, acidosis, and tachycardia, which could potentially interfere with biochemistry results (Bush et al., 2004; Emslie et al., 2009). In order to correct hypoxia the administration of respiratory stimulants such a M5050® and Butrophanol is recommended, such stimulants could affect the resultant biochemical intervals (Bush et al., 2004). Similarly those individuals immobilised with Stresnil® may show extra-pyramidal side effects, such as a ‘high stepping gate’, which could potentially interfere with results (Meltzer et al., 2012; Morkel, 1989). Extra-pyramidal side effects can cause a display of dystonia, resulting in muscle tremors, commonly seen in immobilised rhino; such muscle tremors can cause muscle damage, which may result in increased levels of CK (De Lange, 2016).

Comparing the biochemical reference intervals created in this study (Table 5) with prior reference intervals, a number of differences can be identified. Direct comparisons cannot be made as different methods and units will have been used, however similarities can be seen between the results of this study and the baseline data collated by Seal et al. (1976). The biochemical values investigated included similar values as investigated in this study, alb, ALP, UN, Ca and TP. Despite being analysed with different assays, the same units were used and thus some comparisons can be made, such as the reference range for alb (2.1-3.1g/Gl), ALP (46-154U/L), UN (9-17mg/dL) and Ca (10.5-13.4mg/dL).

The study population included 19 rhinos; this was a limiting factor due to the short data collection period. In a study by Kock et al. (1987) it is suggested that reports with a small sample size are limited due to a lack of variation in sex and age, the types of analyses used, and the lack of detailed evaluations of interactions among parameters measured. Despite the recommendation to sample a larger number of animals (n=>120), the necessary number of animals was not realistic, particularly in white rhino, a near threatened species (Emslie et al., 2012; Friedrichs et al., 2012). All rhinos sampled

were deemed healthy, according to the attending veterinarian and any unhealthy rhino were discarded from the study to prevent underlying illness affecting the biochemical values, evidently reducing the sample size. Despite the small sample size, the samples collected were all from semi-captive rhino free roaming in reserves in the Eastern Cape, South Africa, creating a database of biochemical values for semi-captive white rhino for use by local wildlife veterinarians. Moreover, the values obtained in this study are remarkably similar to those collected by Seal et al. (1976) from semi-captive white rhinoceros, as discussed. Such comparisons suggest that the methods used in this study provide a reliable measurement of semi-captive southern white rhinoceros in the Eastern Cape. As previously discussed, according to wildlife conservationists there are no truly wild white rhino in South Africa and thus analysing samples from, and creating a biochemical database for, semi-captive white rhino allows for a true interpretation of the white rhino inhabiting reserves in the Eastern Cape that undergo rhino management procedures which require veterinary intervention (Mathebula et al., 2012).

Due to the nature of the habitat in which many of the rhino management and veterinary procedures took place, and the nature of some of the procedures themselves, it was not possible to run all of the blood samples in the field using the Abaxis Vetscan VS2. Thus 24 of the 38 samples collected were stored in cooler boxes and run using the Abaxis Vetscan VS2 in the more stable environment of the laboratory. This was a limiting factor as there is the potential for serum samples and lithium heparin samples to procedure different results. However, a number of the procedures allowed for the collection of both one lithium heparin and one serum sample at the initial sampling and the second sampling. This legitimised a comparison to be made to determine whether the results differed between the two sampling and analysis techniques. No significant difference was seen between the two methods of sampling despite previous reports of significant differences being observed between serum and heparinised samples (Mathebula et al., 2012).

The Abaxis Vetscan VS2 machine was used to analyse blood samples, using the large animal rotor. Each sample was analysed using this machine without exception, allowing for direct comparisons between individual samples and samples from different animals. As a benchtop in-clinic machine there is perhaps some discrepancy as to whether the quality control is as efficient as sending the samples to a reference laboratory (Rishniw et al., 2012), however as a benchtop machine this allowed for samples to be run in the field immediately after sampling (Mathebula et al., 2012). Use of this machine and other bench top machines such as the IDEXX VetTest have been reported for use in white rhino, with suitable success (Hooijberg et al., 2017; Mathebula et al., 2012).

5.0 Conclusion

When white rhino are chemically immobilised, changes occur in the biochemical content of their blood. Changes in biochemistry appear to increase as the duration of immobilisation increases, with some exceptions. The biochemical value most affected by increased duration of chemical

immobilisation was ALP. The effect of the duration of chemical immobilisation on the biochemical values obtained is highlighted by the fluctuation of biochemical values as immobilisation time increases. It appears that increased immobilisation times result in an increased difference between the biochemical values inferred from the first sample and the values inferred from the second sample. However due to the small sample size, and the small number of extended immobilisation times further research may be warranted.

By providing evidence that the duration of chemical immobilisation does have a statistically significant effect on biochemical values, with the exception of UN, CK, GGT and phos, the research undertaken for this study is justified. The results are of significant value as they prove that current methods of immobilisation and their duration need to be adjusted to have minimal impact on rhino; therefore warranting that blood samples should be taken before extended immobilisation to assess the rhino's current health status and how this will be affected by the chemical immobilisation. This protocol will also decipher whether additional measures or intensive anaesthetic monitoring is required for compromised rhino with biochemical values outside the reference intervals prior to proceeding with an extended immobilisation period, thus reducing the risk of usually unforeseeable anaesthetic related death.

Furthermore, this report provides biochemical reference intervals for semi-captive southern white rhino that can be used by wildlife veterinarians across the Eastern Cape. In order for comparison of these results with previously published data, a valid direct assessment is only possible where the ranges are calculated using the same methods.

Further research and collation of biochemical values of southern white rhinoceros is still taking place. This will allow for further samples to be added to current sample size (n=19) to help create a larger, more reliable set of reference intervals that could be used across South Africa as oppose to just the Eastern Cape area. Moreover, with a larger sample size covariates such as sex and age can be analysed to create specific reference intervals for age and sex, which can in turn allow for a more accurate diagnosis.

6.0 Acknowledgments

The author recognises and thanks Ikhala Veterinary Wildlife Service, with special thanks to Dr William Fowlds, Sister Candice Monberg, and Dr Emily Baxter for their support and guidance in the field, and for allowing the use of the Abaxis Vetscan VS2 machine. Thanks to Dr Balazs Sladovits and Yu-Mei Chang for their advice, support and guidance throughout the project. Finally, thanks to Vetwork UK, The Royal Veterinary College and The Zoological Society of London for their generous funding, without which this project would not have been possible.

7.0 References

Barrelet, A. and Ricketts, S., 2002. Haematology and blood biochemistry in the horse: a guide to interpretation. *In Practice*, *24*(6), pp.318-327.

Biggs, D., Courchamp, F., Martin, R. and Possingham, H.P., 2013. Legal trade of Africa's rhino horns. *Science*, *339*(6123),pp.1038-1039.

Borjesson, D.L., Christopher, M.M. and Boyce, W.M., 2000. Biochemical and hematologic reference intervals for free-ranging desert bighorn sheep. *Journal of Wildlife Diseases*, *36*(2), pp.294-300.

Bougherara, H. and Bouaziz, O. 2014. Effects of the anaesthetic / tranquillizer treatments (Ketamine, Ketamine+Acepromazine, Zoletil) on selected plasma biochemical parameters in laboratory rats. *Central European Journal of Experimental Biology*, 3 (2):1-5.

Bush, M., Raath, J.P., Grobler, D. and Klein, L., 2004. Severe hypoxaemia in field-anaesthetised white rhinoceros (Ceratotherium simum) and effects of using tracheal insufflation of oxygen. *Journal of the South African Veterinary Association*, *75*(2), pp.79-84.

Chua, K.S., Jacob, E., Lam, C.W.K. & Koon, T.I. 1978. Reference Ranges of 17 Serum Biochemical Constituents in a Singapore Population. Singapore Medical Journal, 205-217

Cromsigt, J. P. G. M.; Te Beest, M. 2014. "Restoration of a megaherbivore: Landscape-level impacts of white rhinoceros in Kruger National Park, South Africa". *Journal of Ecology*. 102 (3), pp. 566–575.

De Lange, S.S., 2016. *Tremors in white rhinoceros (Ceratotherium simum) during chemical immobilization* (Doctoral dissertation, University of Pretoria).

Dimeski, G., 2008. Interference testing. *The Clinical Biochemist Reviews*, *29*(Suppl 1), p.S43.

Driessen, B., Zarucco, L., Steffey, E.P., McCullough, C., Del Piero, F., Melton, L., Puschner, B. and Stover, S.M., 2002. Serum fluoride concentrations, biochemical and histopathological changes associated with prolonged sevoflurane anaesthesia in horses. *Journal of Veterinary Medicine Series A*, *49*(7), pp.337-347.

Edner, A.H., Nyman, G.C. and Essén-Gustavsson, B., 2007. Metabolism before, during and after anaesthesia in colic and healthy horses. *Acta Veterinaria Scandinavica*, *49*(1), p.34.

Elkhair, N.M., 2016. Influence of age and sex on certain serum biochemical parameters of dromedary camels. *Nova Journal of Medical and Biological Sciences*, *5*(3).

Emslie, R.H., Amin, R. and Kock, R. eds., 2009. *Guidelines for the in situ re-introduction and translocation of African and Asian rhinoceros* (No. 39). IUCN.

Emslie, R. H., Milledge, S., Brooks, M., Strien, N. J., van and Dublin, H. 2007. African and Asian Rhinoceroses – Status, Conservation and Trade. A report from the IUCN Species Survival Commission (SSC) African and Asian Rhino Specialist Groups and TRAFFIC to the CITES Secretariat pursuant to Decisions 13.23-25 taken at the 13th meeting of the Conference of the Parties, and further deliberations at the 53rd and 54th meetings of the Standing Committee.

Emslie, R. 2012. *Ceratotherium simum*. The IUCN Red List of Threatened Species 2012: e.T4185A16980466. http://dx.doi.org/10.2305/IUCN.UK.2012.RLTS.T4185A16980466.en. Downloade d on 29 January 2017

Estes, R. 1991. *The Behavior Guide to African Mammals: Including Hoofed Mammals, Carnivores, Primates*. University of California Press. pp. 323

Fernandez, N.J. and Kidney, B.A., 2007. Alkaline phosphatase: beyond the liver. *Veterinary Clinical Pathology*, *36*(3), pp.223-233.

Flatland, B., Freeman, K.P., Friedrichs, K.R., Vap, L.M., Getzy, K.M., Evans, E.W. and Harr, K.E., 2010. ASVCP quality assurance guidelines: control of general analytical factors in veterinary laboratories. *Veterinary clinical pathology*, *39*(3), pp.264-277.

Friedrichs, K.R., Harr, K.E., Freeman, K.P., Szladovits, B., Walton, R.M., Barnhart, K.F. and Blanco-Chavez, J., 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology*, *41*(4), pp.441-453.

García, I., Napp, S., Zorrilla, I., Vargas, A., Pastor, J., Muñoz, Á. and Martínez, F., 2010. Determination of serum biochemical reference intervals for the Iberian lynx (*Lynx pardinus*). *The Veterinary Journal*, *183*(2), pp.201-204.

Gardner, R.B., Nydam, D.V., Mohammed, H.O., Ducharme, N.G. and Divers, T.J., 2005. Serum gamma glutamyl transferase activity in horses with right or left dorsal displacements of the large colon. *Journal of veterinary internal medicine*, *19*(5), pp.761-764.

Geffré, A., Friedrichs, K., Harr, K., Concordet, D., Trumel, C. and Braun, J.P., 2009. Reference values: a review. *Veterinary clinical pathology*, *38*(3), pp.288-298.

Gil, A.G., Silván, G., Illera, M. and Illera, J.C., 2004. The effects of anesthesia on the clinical chemistry of New Zealand White rabbits. *Journal of the American Association for Laboratory Animal Science*, *43*(3), pp.25-29.

Hooijberg, E.H., Steenkamp, G., Preez, J.P. and Goddard, A., 2017. Analytic and quality control validation and assessment of field performance of a point-of-care chemistry analyzer for use in the White rhinoceros. *Veterinary Clinical Pathology*, *46*(1), pp.100-110.

Horn, P.S., Feng, L., Li, Y. and Pesce, A.J., 2001. Effect of outliers and nonhealthy individuals on reference interval estimation. *Clinical Chemistry*, *47*(12), pp.2137-2145.

Horowitz G, Altaie S, Boyd J, Ceriotti F, Garg U, Horn P, Pesce A, Sine H, Zakowski J. 2008. Clinical and Laboratory Standards Institute: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. 3rd ed. Wayne, PA: CLSI.

Jones, M., Arheart, K. and Cray, C. 2014. Reference intervals, longitudinal analyses, and index of individuality of commonly measured variables in captive bald eagles (*Haliaeetus leucocephalus*). *Journal of Avian Medicine and Surgery*, 28(2):118-126.

Kiran, S., Bhutta, A.M., Khan, B.A., Durrani, S., Ali, M. and Iqbal, F., 2012. Effect of age and gender on some blood biochemical parameters of apparently healthy small ruminants from Southern Punjab in Pakistan. *Asian Pacific journal of tropical biomedicine*, *2*(4), pp.304-306.

Kaneko, J. J. 1980. Clinical Biochemistry of Domestic Animals, 3rd ed. Academic Press, London, U.K.

Kock, M.D., Clark, R.K., Franti, C.E., Jessup, D.A. and Wehausen, J.D., 1987a. Effects of capture on biological parameters in free-ranging bighorn sheep (*Ovis canadensis*): evaluation of normal, stressed and mortality outcomes and documentation of postcapture survival. *Journal of Wildlife Diseases*, *23*(4), pp.652-662.

Kock, M.D., Jessup, D.A., Clark, R.K. and Franti, C.E., 1987b. Effects of capture on biological parameters in free-ranging bighorn sheep (*Ovis canadensis*): evaluation of drop-net, drive-net, chemical immobilization and the net-gun. *Journal of Wildlife Diseases*, *23*(4), pp.641-651.

Kock, M.D., Martin, R.B. and Kock, N., 1993. Chemical immobilization of free-ranging African elephants (*Loxodonta africana*) in Zimbabwe, using etorphine (M99) mixed with hyaluronidase, and evaluation of biological data collected soon after immobilization. *Journal of Zoo and Wildlife Medicine*, pp.1-10.

Kock, M.D., Morkel, P., Atkinson, M. and Foggin, C., 1995. Chemical immobilization of free-ranging white rhinoceros (*Ceratotherium simum simum*) in Hwange and Matobo National Parks, Zimbabwe,

using combinations of etorphine (M99), fentanyl, xylazine, and detomidine. *Journal of Zoo and Wildlife Medicine*, pp.207-219.

Kock, M.D., du Toit, R., Kock, N., Morton, D., Foggin, C. and Paul, B., 1990a. Effects of capture and translocation on biological parameters in free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. *Journal of Zoo and Wildlife Medicine*, pp.414-424.

Kock, M.D., du Toit, R., Morton, D., Kock, N. and Paul, B., 1990b. Baseline biological data collected from chemically immobilized free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. *Journal of Zoo and Wildlife Medicine*, pp.283-291.

Kock, R.A., Mihok, S., Wambua, J., Mwanzia, J., Saigawa, K. 1999. Effects of translocation on hematological parameters of free-ranging black rhinoceros (*Diceros bicornis michaeli*) in Kenya. *Journal of Zoo and Wildlife Medicine*. 30 (3) 389-396.

Mathebula, N., Miller, M., Buss, P., Joubert, J., Martin, L., Kruger, M., Hofmeyr, M. and Olea-Popelka, F., 2012. Biochemical values in free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa. *Journal of Zoo and Wildlife Medicine*, *43*(3), pp.530-538.

Marco, I. and Lavin, S., 1999. Effect of the method of capture on the haematology and blood chemistry of red deer (*Cervus elaphus*). *Research in Veterinary Science*, *66*(2), pp.81-84

Marco, I., Martinez, F., Pastor, J. and Lavin, S., 2000. Hematologic and serum chemistry values of the captive European wildcat. *Journal of wildlife diseases*, *36*(3), pp.445-449.

Meltzer, D., Burroughs, R. and Morkel, P., 2012. Chemical and physical restraint of wild animals. *Greyton, South Africa: International Wildlife Veterinary Services*.

Miknienė, Z., Maslauskas, K., Kerzienė, S., Kučinskienė, J. and Kučinskas, A., 2013. The effect of age and gender on blood haematological and serum biochemical parameters in Žemaitukai horses. *Veterinarija ir zootechnika*, *65*(87).

Morkel, P., 1989. Drugs and dosages for capture and treatment of black rhinoceros Diceros bicomis in Namibia. *Koedoe*, *32*(2), pp.65-68.

Muñoz, A., Riber, C., Trigo, P. and Castejón, F., 2012. Age-and gender-related variations in hematology, clinical biochemistry, and hormones in Spanish fillies and colts. *Research in veterinary science*, *93*(2), pp.943-949.

Nordøy, E.S. and Thoresen, S.I., 2002. Reference Values for Serum Biochemical Parameters in Free-Ranging Harp Seals. *Veterinary clinical pathology*, *31*(3), pp.98-105.

Norman, S.A., Beckett, L.A., Miller, W.A., Leger, J.S. and Hobbs, R.C., 2013. Variation in hematologic and serum biochemical values of belugas (Delphinapterus leucas) under managed care. *Journal of Zoo and Wildlife Medicine*, *44*(2), pp.376-388.

O'halloran, J., Duggan, P.F. and Myers, A.A., 1988. Biochemical and haematological values for mute swans (*Cygnus olor*): effects of acute lead poisoning. *Avian Pathology*, *17*(3), pp.667-678.

Okotie-Eboh, G., Bailey, C.A., Hicks, K.D. and Kubena, L.F., 1992. Reference serum biochemical values for emus and ostriches. *American journal of veterinary research*, *53*(10), pp.1765-1768.

O'kelley, R.E. and Fontenot, J.P., 1969. Effects of feeding different magnesium levels to drylot-fed lactating beef cows. *Journal of animal science*, *29*(6), pp.959-966.

Paul-Murphy, J., Work, T., Hunter, D., McFie, E. and Fjelline, D., 1994. Serologic survey and serum biochemical reference ranges of the free-ranging mountain lion (*Felis concolor*) in California. *Journal of wildlife diseases*, *30*(2), pp.205-215.

Pospíšil, J., Špála, P., Váhala, J. and Kaše, F., 2014. Biochemical Values in the Blood Serum of Zebras Kept in the East-Bohemian Zoological Garden at Dvůr Králové nad Labem. *Acta Veterinaria Brno*, *56*(1-2), pp.181-193.

Portas, T.J., 2004. A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species. *Australian veterinary journal*, *82*(9), pp.542-549.

Radcliffe, R.W., Ferrell, S.T. and Childs, S.E., 2000. Butorphanol and azaperone as a safe alternative for repeated chemical restraint in captive white rhinoceros (Ceratotherium simum). *Journal of Zoo and Wildlife Medicine*, *31*(2), pp.196-200.

Rishniw, M., Pion, P.D. and Maher, T., 2012. The quality of veterinary in-clinic and reference laboratory biochemical testing. *Veterinary clinical pathology*, *41*(1), pp.92-109.

Seal, U.S., Barton, R., Mather, L. and Gray, C.W., 1976. Baseline laboratory data for the white rhinoceros (*Ceratotherium simum simum*). *The Journal of Zoo Animal Medicine*, *7*(1), pp.11-16.

Seigel, A. F. 1988. Statistics and data analysis - an introduction. Wiley, New York.

Shukan, E. T., Boe, C. Y., Hasenfus, A. V., Pieper, B. A., & Snowdon, C. T. (2012). Normal Hematologic and Serum Biochemical Values of Cotton-Top Tamarins (*Saguinus oedipus*). *Journal of the American Association for Laboratory Animal Science : JAALAS*, *51*(2), 150–154.

Steffey, E.P., Giri, S.N., Dunlop, C.I., Cullen, L.K., Hodgson, D.S. and Willits, N., 1993. Biochemical and haematological changes following prolonged halothane anaesthesia in horses. *Research in Veterinary science*, *55*(3), pp.338-345.

Teare, J.A. 2013. "Ceratotherium_simum_No_selection_by_gender__All_a ges_combined_Standard_International_Units__2013_CD ml" *in* Species360 Physiological Reference Intervals for Captive Wildlife: A CD-ROM Resource., Species360 , Bloomington, MN.

Underwood, C., Southwood, L.L., Walton, R.M. and Johnson, A.L., 2010. Hepatic and metabolic changes in surgical colic patients: a pilot study. *Journal of veterinary emergency and critical care*, *20*(6), pp.578-586.

Ververs, C., Van Zijl Langhout, M., Govaere, J. and Van Soom, A. 2015. Features of reproduction and assisted reproduction in the white (Ceratotherium simum) and black (Diceros bicornis) rhinoceros. Vlaams Diergeneeskundig Tijdschrift 84: 175-187.

Wagner, A.E., 2008. Complications in equine anesthesia. *Veterinary Clinics of North America: Equine Practice*, *24*(3), pp.735-752.

Waldram, M.S., Bond, W.J. and Stock, W.D., 2008. Ecological engineering by a mega-grazer: white rhino impacts on a South African savanna. *Ecosystems*, *11*(1), pp.101-112.

Watson, Z.E., Steffey, E.P., VanHoogmoed, L.M. and Snyder, J.R., 2002. Effect of general anesthesia and minor surgical trauma on urine and serum measurements in horses. *American journal of veterinary research*, *63*(7), pp.1061-1065.

Wenger, S., Boardman, W., Buss, P., Govender, D. and Foggin, C., 2007. The cardiopulmonary effects of etorphine, azaperone, detomidine, and butorphanol in field-anesthetized white rhinoceroses (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine*, *38*(3), pp.380-387.

Wu, D., Yi, Y., Sun, F., Zhou, L., Yang, F., Wang, H.,Yue, F. (2014). Effects of Age and Sex on the Hematology and Blood Chemistry of Tibetan Macaques (*Macaca thibetana*). *Journal of the American Association for Laboratory Animal Science : JAALAS*, *53*(1), 12–17.

Xie L, Xu F, Liu S, Ji Y, Zhou Q, Wu Q, et al. (2013) Age- and Sex-Based Hematological and Biochemical Parameters for *Macaca fascicularis*. PLoS ONE 8(6): e64892. https://doi.org/10.1371/journal.pone.0064892.

8.0 Tables

Table 1: Protocol used for darting white rhino, including equipment and chemical restraint agents

Age/sex	Primary drug	Additional drugs	Narcotic antidote	Tranquillizer/sedative	Drug delivery system
Adult bull and cow	4mg Etorphine (M99®)	2000IU Hyaluronidase (Hyalase®) 20mg Butorphanol®	40mg Naltrexone (Trexonil®)	40mg Azaperone (Stresnil®)	Pneu-dart® (from helicopter)
Sub-adult bull and cow	2mg Etorphine (M99®)	2000IU Hyaluronidase (Hyalase®) 20mg Butorphanol®	20mg Naltrexone (Trexonil®)	20mg Azaperone (Stresnil®)	Pneu-dart® (from helicopter)

Table 2: Biochemical analysis of blood taken from semi-captive Southern white rhino in the Eastern Cape. Samples analysed using the Abaxis Vetscan VS2.

ID	AGE	SEX	ALB[1]	ALP[1]	AST[1]	BUN[1]	CA[1]	CK[1]	GGT[1]	GLOB[1]	MG[1]	PHOS[1]	TP[1]	TIME	ALB[2]	ALP[2]	AST[2]	BUN[2]	CA[2]	CK[2]	GGT[2]	GLOB[2]	MG[2]	PHOS[2]	TP[2]
1	2.5	M	3.5	110	75	17	12.3	340	9	7.1	2.8	1.50	10.5	25	3.1	90	80	19	12.6	365	13	6.9	2.5	1.6	10
2	4.5	M	3.7	115	84	15	12	320	5	8	3.2	1.70	11.9	16	3.4	63	87	20	12.7	352	8	7.8	2.9	1.74	11.2
3	4.5	F	2.7	120	87	16	13	321	15	6.7	3.1	1.43	9.3	67	2.6	114	76	17	12.6	334	19	6	2.7	1.44	8.6
4	4	F	2.8	102	69	12	12.4	520	14	6.1	2.6	1.49	8.9	50	2.8	103	64	13	12.1	511	12	6	2.4	1.6	8.8
5	5	M	2.7	103	89	11	12.5	298	12	7.3	3.1	0.91	10	37	2.5	88	73	12	12	264	10	6.6	2.8	0.87	9.2
6	1	F	3.1	110	88	17	13.9	240	13	5.6	3	1.75	8.8	35	3	102	80	17	12.9	236	12	4.9	2.9	1.64	7.8
7	7	F	2.9	57	90	18	13.1	221	14	8.3	2.7	1.23	11.3	38	2.7	36	78	19	11.4	182	12	7.4	2.4	1.13	10
8	12.5	F	2.7	40	83	9	12.9	241	9	7.2	2.7	1.14	9.9	47	2.6	43	77	9	12.3	257	6	6.6	2.6	1.29	9.2
9	20	M	2.5	45	66	11	12.4	197	8	8.1	3.6	1.08	10.6	37	2.4	23	58	12	11.6	173	5	7.2	3.3	0.96	9.5
10	17	M	2.6	54	66	17	11.8	191	9	7.5	2.8	0.89	10.1	10	2.4	43	65	17	11.7	197	9	7.5	2.8	0.89	10.1
11	3	F	2.1	113	120	11	12.5	356	10.2	8.1	2.9	1.59	11	40	2	112	114	10	11.8	358	10	8	2.8	1.6	9.2
12	2	F	1.9	133	76	12	11.9	441	18	7.3	2.6	1.86	9.2	41	1.7	130	70	12	11.8	435	17	7	2.5	1.5	9
13	14	F	2	49	76	13	12.3	383	19	9.2	2.5	1.22	11.2	60	1.8	41	74	10	12	360	16	9	2.3	1.14	10.9
14	3	F	2.8	43	123	21	13.7	1225	11	8	3	0.88	10.8	140	2.5	18	102	20	11	1154	5	5.7	2.7	0.85	8.2
15	7	F	2.6	54	58	13	12.4	260	9	7.4	2.8	1.27	10	24	2.7	29	53	13	11.8	215	10	6.9	2.6	1.28	9.7
16	2.5	M	3.1	33	73	21	14	333	8	7.3	2.5	1.23	10.4	54	2.9	22	69	21	12.6	324	7	6.3	2.3	1.22	9.2
17	3	F	2.5	41	177	27	11.8	2088	8	6.3	2.6	0.42	8.8	48	2.3	34	161	26	11	1873	5	5.6	2.3	0.36	8
18	9	F	3	91	52	8	12.8	361	10	6.2	3.4	1.19	9.2	48	2.8	86	51	8	12.2	380	9	5.7	3.1	0.98	8.5
19	10	F	2.5	72	86	8	11.6	988	12	6.2	2	1.35	8.7	73	2.6	60	83	8	11.3	949	12	5.8	2	1.43	8.4

Legend: Identification (ID), first sample ([1]), second sample ([2]), albumin (ALB), alkaline phosphatase (ALP), aspartate transaminase (AST), urea nitrogen (BUN), calcium (CA), creatine kinase *(*CK), gamma-glutamyltransferase (GGT), globulins (GLOB), magnesium (MG), phosphorous (PHOS), and total protein (TP).

Table 3: Paired t-test using averages to determine whether duration of immobilisation has a significant effect on biochemical parameters

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	ALB - ALB	0.1526	0.1264	0.0290	0.0917	0.2135	5.265	18	0.000
Pair 2	ALP - ALP	10.3368	17.7094	4.0628	1.8012	18.8725	2.544	18	0.020
Pair 3	AST - AST	6.474	6.552	1.503	3.316	9.632	4.307	18	0.000
Pair 4	BUN - BUN	-0.316	1.565	0.359	-1.070	0.439	-.879	18	0.391
Pair 5	CA - CA	0.6263	0.7385	0.1694	0.2704	0.9823	3.697	18	0.002
Pair 6	CK - CK	21.316	54.083	12.408	-4.752	47.383	1.718	18	0.103
Pair 7	GGT - GGT	0.8526	2.5411	0.5830	-0.3721	2.0774	1.463	18	0.161
Pair 8	GLOB - GLOB	0.5789	0.5138	0.1179	0.3313	0.8266	4.912	18	0.000
Pair 9	MG - MG	0.2105	0.1150	0.0264	0.1551	0.2659	7.982	18	0.000
Pair 10	PHOS - PHOS	0.03211	0.11928	0.02737	-0.02539	0.08960	1.173	18	0.256
Pair 11	TP - TP	0.7947	0.6346	0.1456	0.4889	1.1006	5.459	18	0.000

Table 4: Frequency table of biochemical values of semi-captive southern white rhinoceros (n=19)

Frequencies

	ALB	ALP	AST	BUN	CA	CK	GGT	GLOB	MG	PHOS	TP
Mean	2.721	75.442	86.21	14.58	12.595	490.74	11.221	7.258	2.837	1.2700	10.032
Median	2.700	72.000	83.00	13.00	12.400	333.00	10.200	7.300	2.800	1.2300	10.000
Mode	2.5[a]	110.0	66[a]	11[a]	12.4	191[a]	9.0	7.3	2.6[a]	1.23	8.8[a]

a. Multiple modes exist. The smallest value is shown

Table 5: Reference intervals for biochemical values of semi-captive southern white rhinoceros (n=19)

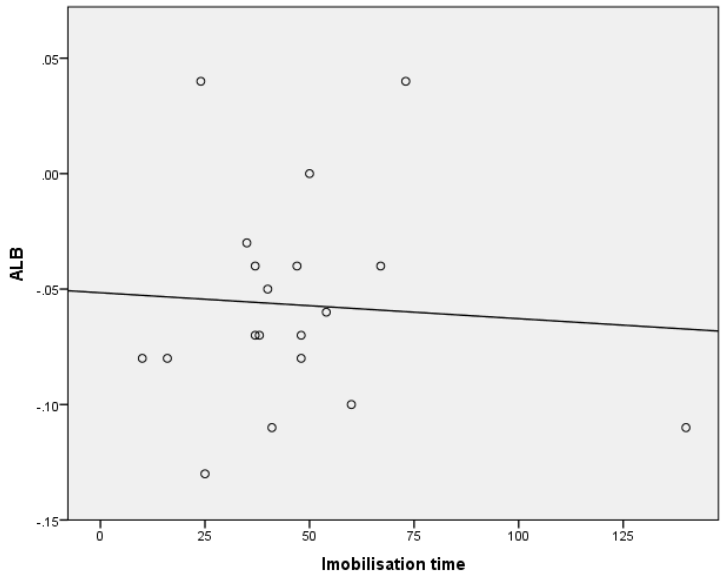
Parameters	Mean	Median	Reference Interval	Units
Albumin	2.7	2.7	2.5 - 3.1	g/dL
Alkaline phosphatase	75.4	72.0	45.0 - 120.0	U/L
Aspartate transaminase	86.2	83.0	79.0 - 123.0	U/L
Urea nitrogen	14.5	13.0	11.0 - 21.0	mg/dL
Calcium	12.5	12.4	12.0 - 13.9	mg/dL
Creatine kinase	490.7	333.0	241.0 - 441.0	U/L
Gamma-glutamyltransferase	11.2	10.2	9.0 - 18.0	U/L
Globulins	7.2	7.3	6.3 - 8.3	g/dL
Magnesium	2.8	2.8	2.6 - 3.4	mg/dL
Phosphorous	1.2	1.2	1.1 - 1.8	mmol/L
Total protein	10.0	10.0	9.2 - 11.3	g/dL

Table 6: Collation of individual general linear models to show the relationships between each biochemical value and the duration of chemical immobilisation, sex and age.

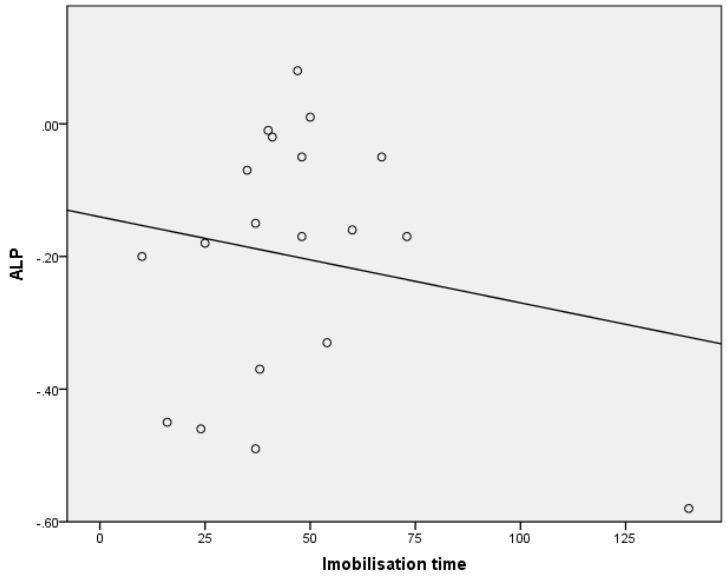
Dependent variable	Significance of sex	Significance of age	Significance of immobilisation time
ALB	0.120	0.432	0.413
ALP	0.005	0.663	0.099
AST	0.663	0.842	0.129
BUN	0.039	0.166	0.334
CA	0.743	0.974	0.011
CK	0.566	0.562	0.800
GGT	0.462	0.100	0.035
GLOB	0.209	0.439	0.000
MG	0.423	0.247	0.371
PHOS	0.637	0.876	0.883
TP	0.340	0.253	0.006

9.0 Figures

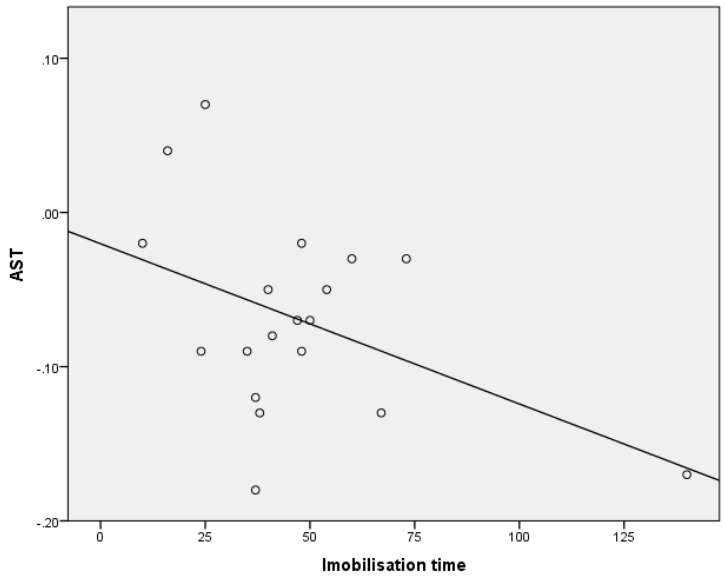
Figure 1: Scatter plots a-k: Scatter plots to display effect of duration of immobilisation on biochemical values using percentage difference.



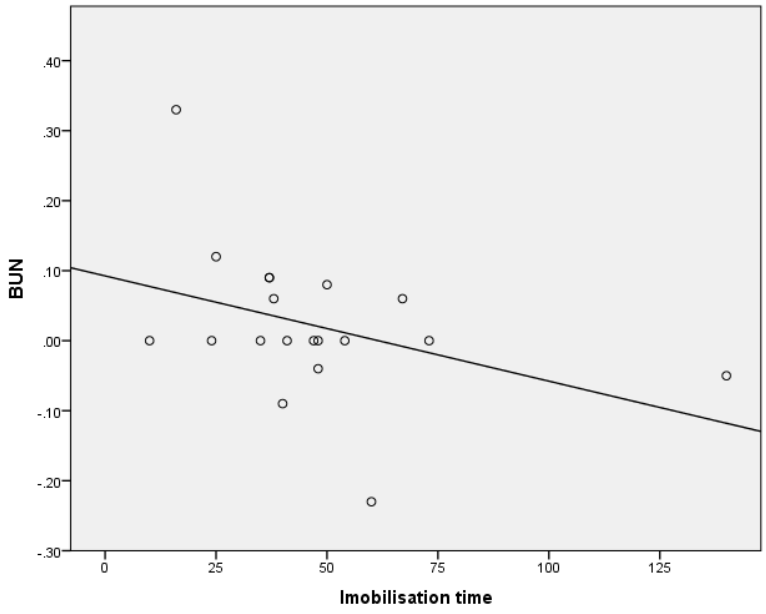
Scatter plot a: Relationship between albumin and duration of immobilisation (average percentage difference between first and second sample = -0.05). Displays overall decrease in albumin value as duration of immobilisation .



Scatter plot b: Relationship between alkaline phosphatase and duration of immobilisation (average percentage difference between first and second sample = -0.04). Displays overall decrease in alkaline phosphatase value as duration of immobilisation increases.



Scatter plot c: Relationship between aspartate transaminase and duration of immobilisation (average percentage difference between first and second sample = -0.07). Displays overall decrease in aspartate transaminase value as duration of immobilisation increases.



Scatter plot d: Relationship between urea nitrogen and duration of immobilisation (average percentage difference between first and second sample = 0.02). Displays overall decrease in urea nitrogen value as duration of immobilisation increases.

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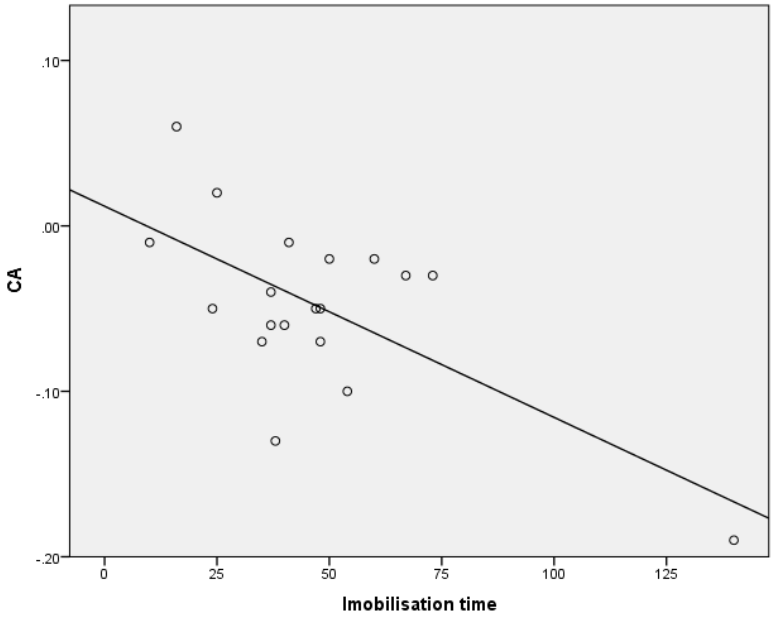
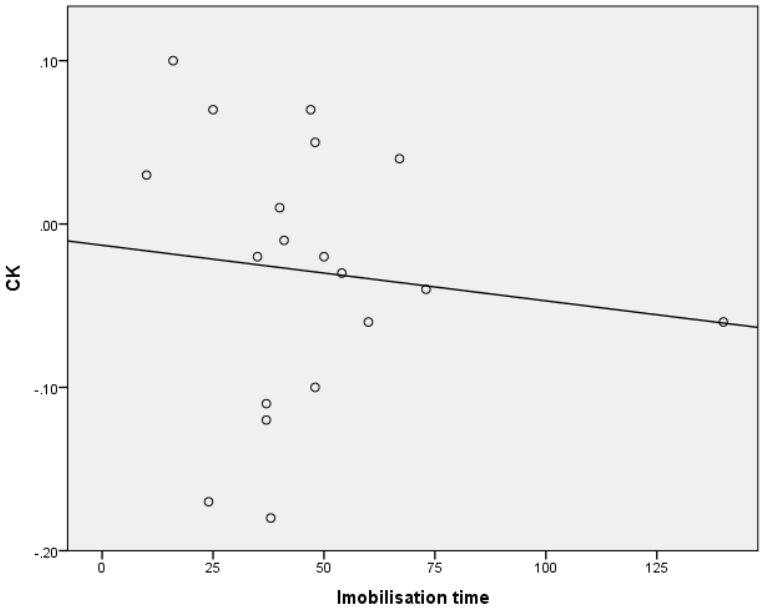
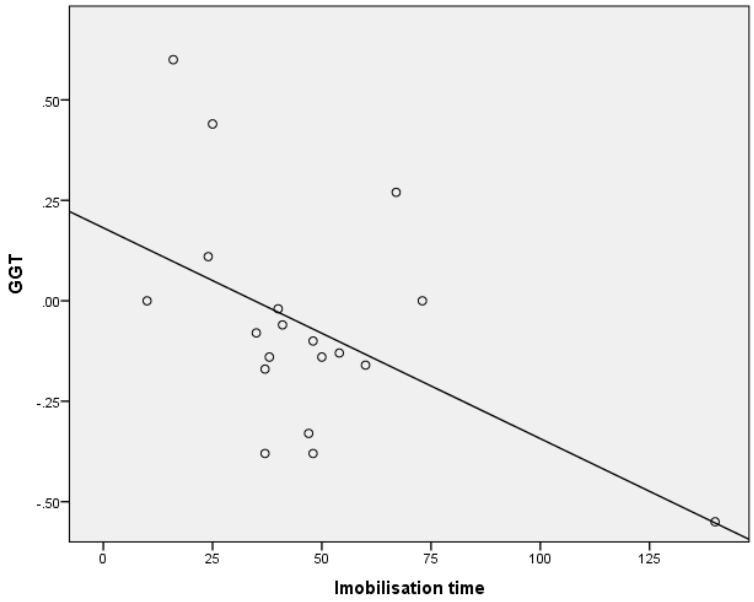


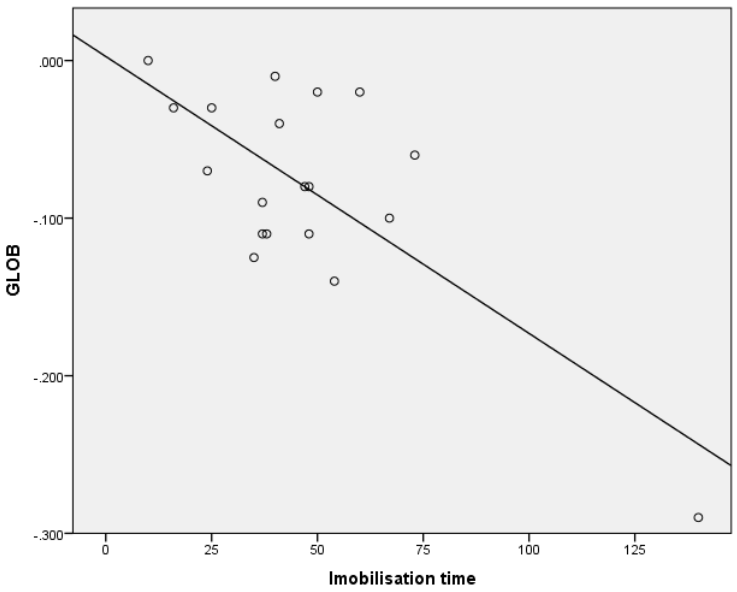
Scatter plot e: Relationship between calcium and duration of immobilisation (average percentage difference between first and second sample = -0.05). Displays overall decrease in calcium value as duration of immobilisation increases.



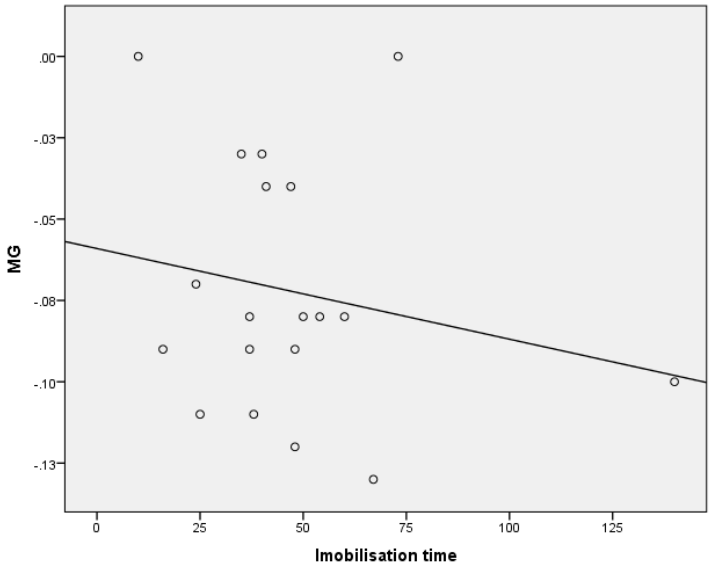
Scatter plot f: Relationship between creatinine kinase and duration of immobilisation (average percentage difference between first and second sample = -0.03). Displays overall decrease in creatinine kinase value as duration of immobilisation increases.



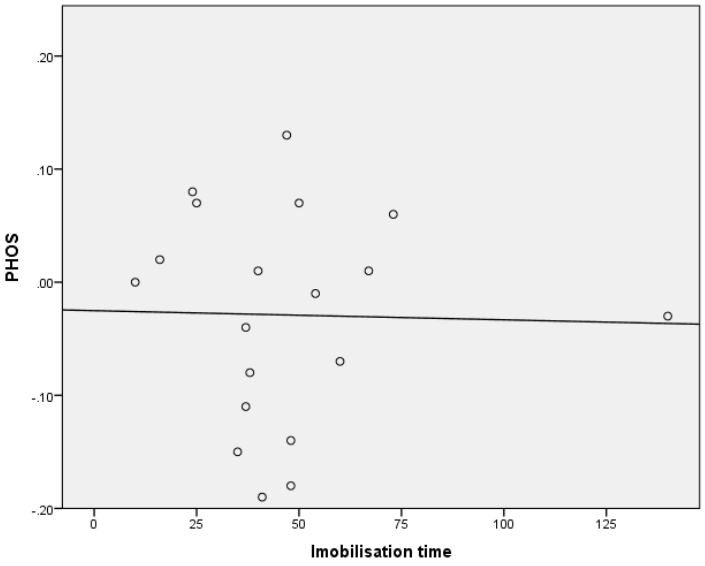
Scatter plot g: Relationship between gamma-glutamyltransferase and duration of immobilisation (average percentage difference between first and second sample = -0.07). Displays overall decrease in gamma-glutamyltransferase value as duration of immobilisation increases.



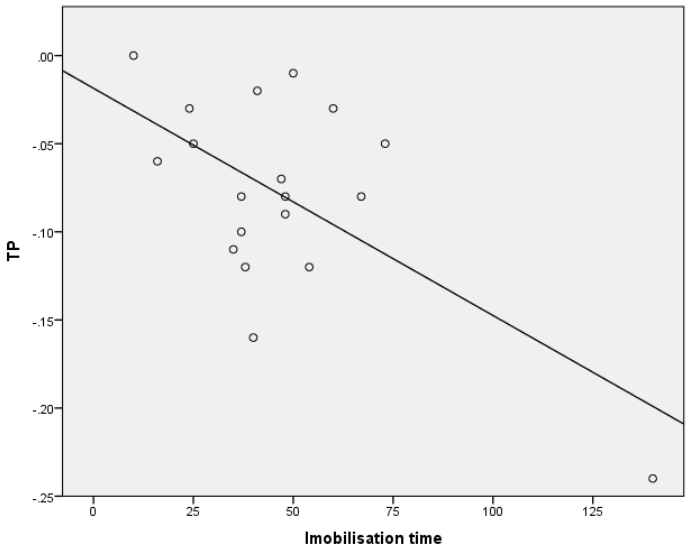
Scatter plot h: Relationship between globulins and duration of immobilisation (average percentage difference between first and second sample = -0.07). Displays overall decrease in globulins value as duration of immobilisation increases.



Scatter plot i: Relationship between magnesium and duration of immobilisation (average percentage difference between first and second sample = -0.07). Displays overall decrease in magnesium value as duration of immobilisation increases.

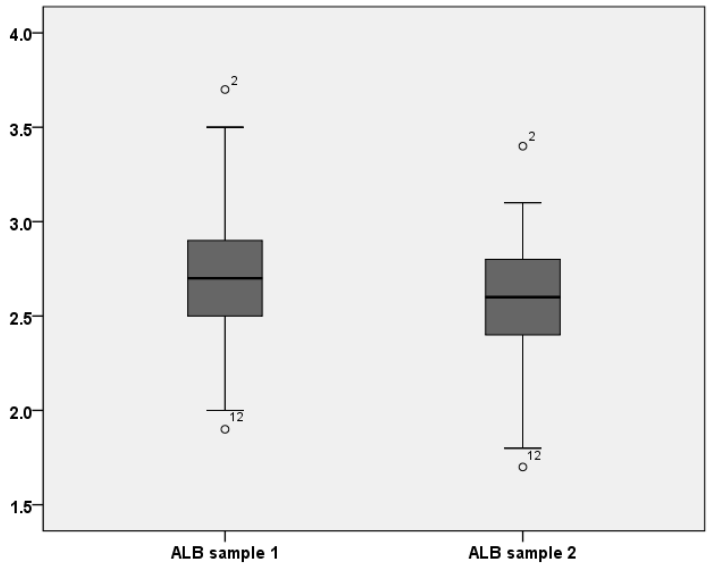


Scatter plot j: Relationship between phosphate and duration of immobilisation (average percentage difference between first and second sample = -0.03). Displays overall decrease in phosphate value as duration of immobilisation increases.

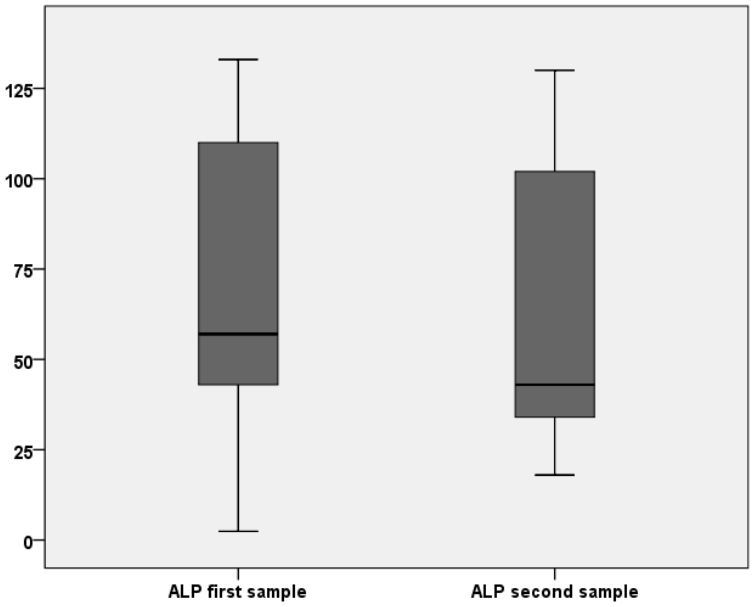


Scatter plot k: Relationship between phosphate and duration of immobilisation (average percentage difference between first and second sample = -0.03). Displays overall decrease in phosphate value as duration of immobilisation increases.

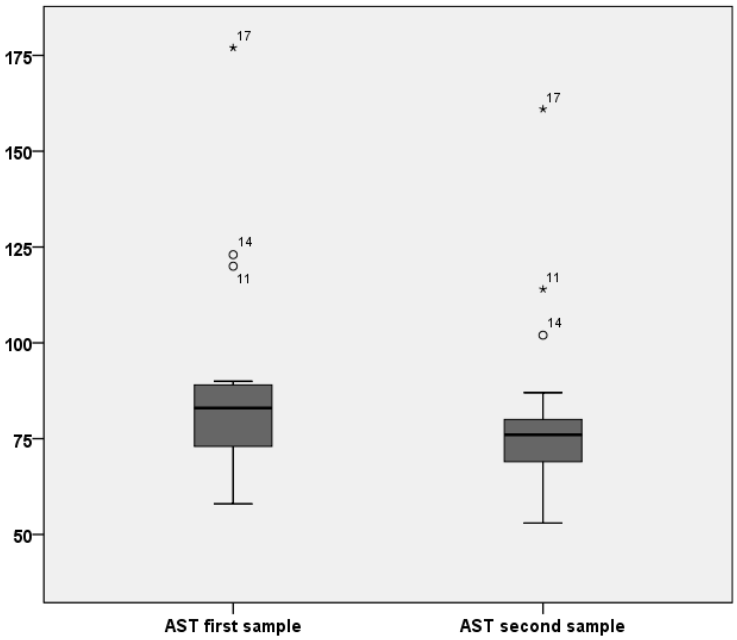
Figure 2: Box plots a-k: Comparison of initial biochemical values from sample one and biochemical values from sample 2 taken from white rhinoceros.



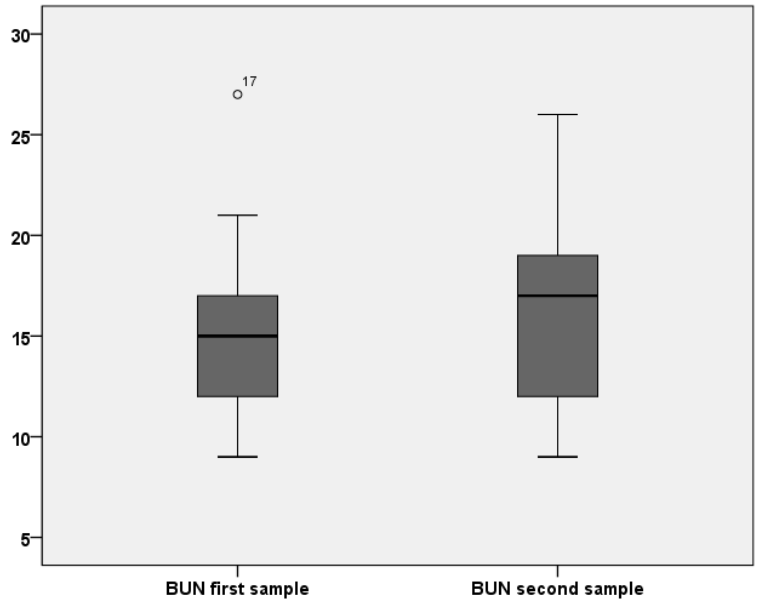
Box plot a: In this study there was a significant difference (P=0.000) between the albumin value obtained from the first sample and the albumin value obtained from the second sample.



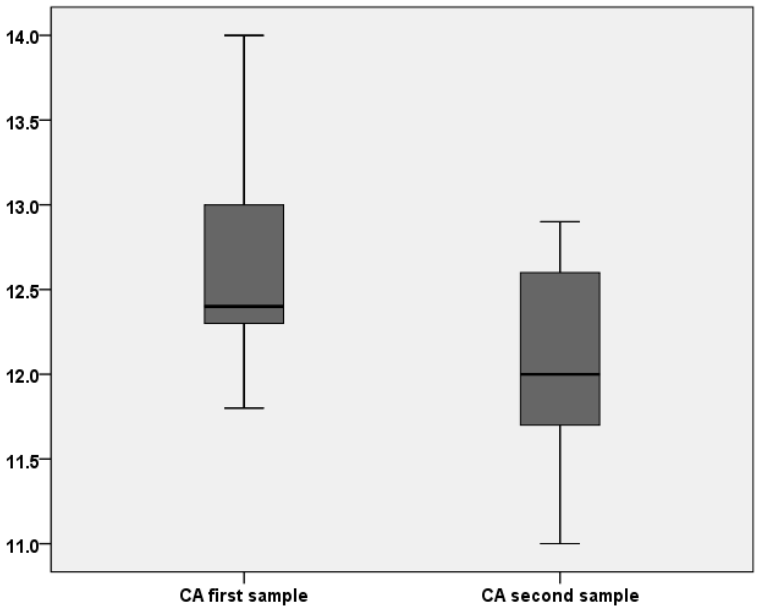
Box plot b: In this study there was a significant difference (P=0.020) between the alkaline phosphatase value obtained from the first sample and the alkaline phosphatase value obtained from the second sample.



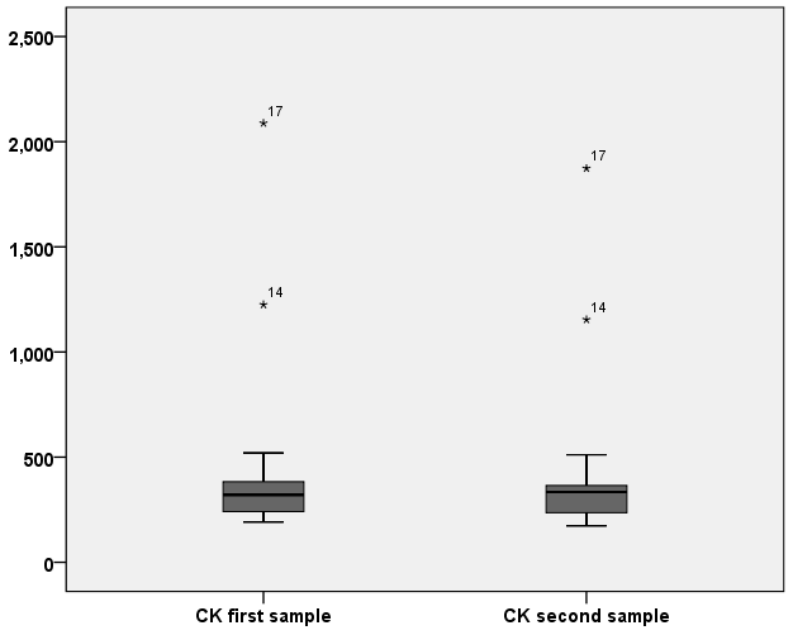
Box plot c: In this study there was a significant difference (P=0.000) between the aspartate transaminase value obtained from the first sample and the aspartate transaminase value obtained from the second sample.



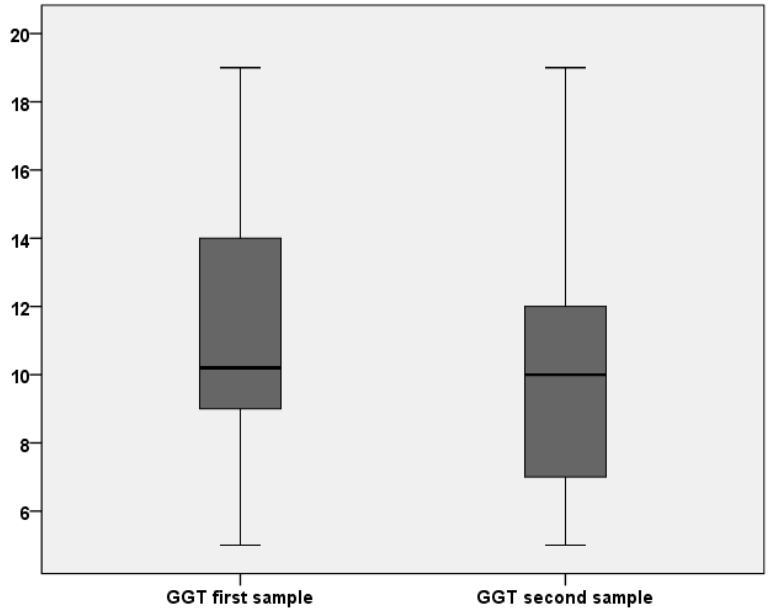
Box plot d: In this study there was not a significant difference (P=0.391) between the urea nitrogen value obtained from the first sample and the urea nitrogen value obtained from the second sample.



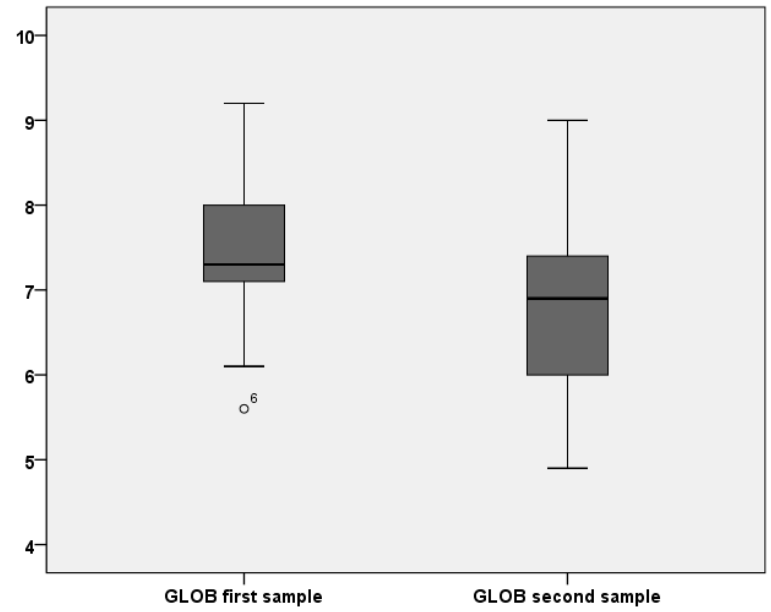
Box plot e: In this study there was a significant difference (P=0.002) between the calcium value obtained from the first sample and the calcium value obtained from the second sample.



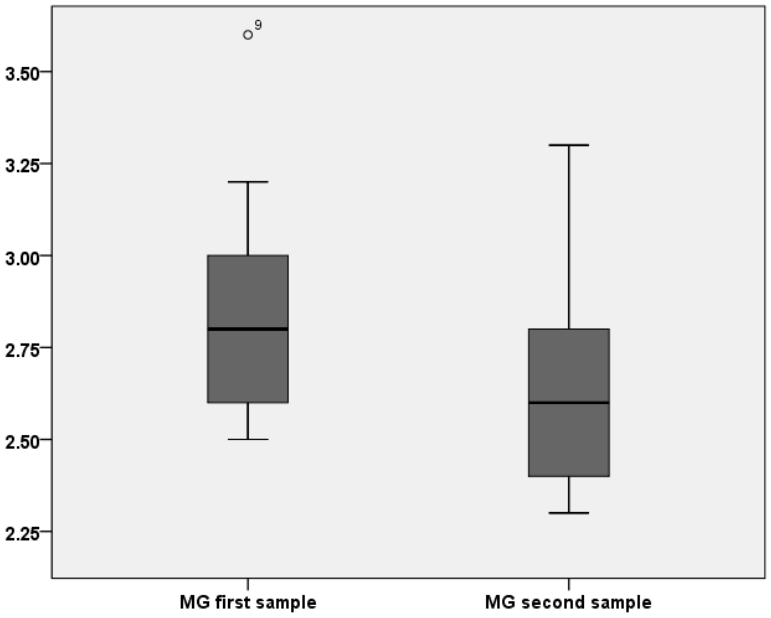
Box plot f: In this study there was not a significant difference (P=0.103) between the creatinine kinase value obtained from the first sample and the creatinine kinase value obtained from the second sample.



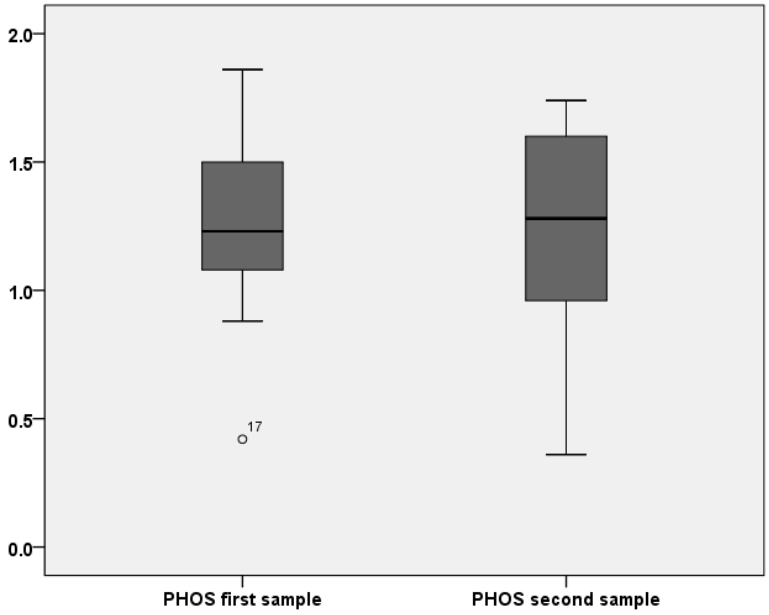
Box plot g: In this study there was not a significant difference (P=0.161) between the gamma-glutamyltransferase value obtained from the first sample and the gamma-glutamyltransferase value obtained from the second sample.



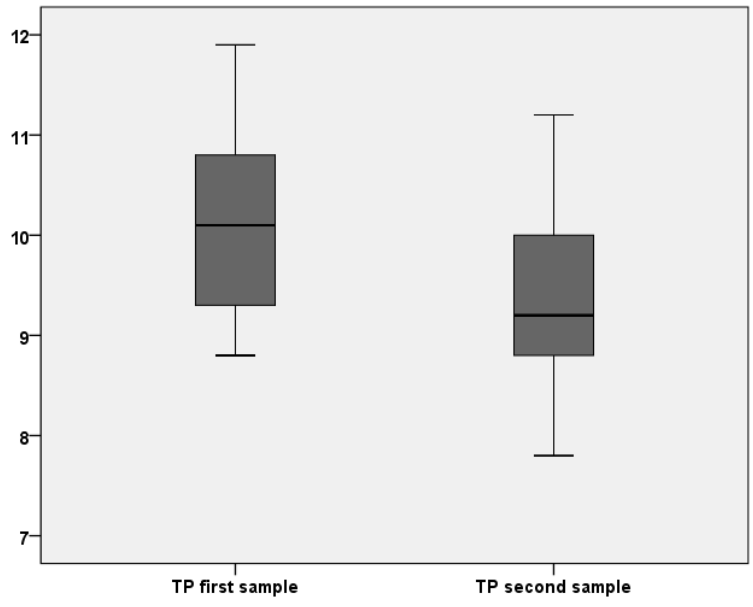
Box plot h: In this study there was a significant difference (P=0.000) between the globulins value obtained from the first sample and the globulins value obtained from the second sample.



Box plot i: In this study there was a significant difference (P=0.000) between the magnesium value obtained from the first sample and the magnesium value obtained from the second sample.



Box plot j: In this study there was not a significant difference (P=0.256) between the phosphate value obtained from the first sample and the phosphate value obtained from the second sample.



Box plot k: In this study there was a significant difference (P=0.000) between the total protein value obtained from the first sample and the total protein value obtained from the second sample.

10.0 Appendices

Appendix I: MSc Wild Animal Biology Research Project Data Collection and Sample Storage SOP

Appendix II: White Rhino (*Ceratotherium simum*) Anaesthetic Monitoring Sheet

Appendix III: White Rhino (*Ceratotherium simum*) Body Condition Score Sheet

Appendix IV: Task division

MSc Wild Animal Biology Research Project
Data Collection and Sample Storage SOP

Gemma Crowley BSc (Hons) RVN

Brief

The aim of this study is to determine how duration of immobilisation effects the biochemical values of blood taken from semi-captive white rhinoceros in the Eastern Cape; whilst also establishing biochemical reference intervals in order to ascertain a baseline of what is expected in heathy white rhino for comparative purposes in clinical cases and traumatised rhino. The biochemical values collected in this study will determine the effects of duration of immobilisation on biological parameters and create a database in which wildlife veterinarians can refer to when treating future white rhinoceros.

Collection Protocol

1. Record initial physiological parameters (temperature, respiration, and pulse) on anaesthetic monitoring sheet
2. Place an intravenous cannula in the right auricular vein of each rhino to ensure constant intravenous (I/V) access
3. Take first blood sample from the left auricular vein and empty syringe into a vacutainer
4. Label vacutainer with SAMPLE 1, the date, time and ID (i.e. 1 for the first rhino, 2 for the second rhino and so on)
5. Place vacutainer in cool box, or if the portable Abaxis Vetscan VS2 is available run the first sample using the large animal rotor and collect data
6. Fill in anaesthetic monitoring sheet. Record general health from physical appearance, body condition score to assist in determining the overall health of each individual (see BCS sheet), sex, age (as an estimate), and reason for veterinary intervention
7. Undertake procedure, continuing to record physiological parameters on anaesthetic monitoring sheet and any drugs administered
8. Just before reversal take second blood sample from the left vena auricularis and empty syringe into a vacutainer
9. Label vacutainer with SAMPLE 2, the date, time and ID (i.e. 1 for the first rhino, 2 for the second rhino and so on)
10. Place vacutainer in cool box, or if the portable Abaxis Vetscan VS2 is available run the second sample using the large animal rotor and collect data

Anaesthesia should be maintained by veterinary professionals *and* physiological parameters should be monitored to include: temperature, respiration, pulse, blood pressure, mucous membrane colour, capillary refill time, and blood oxygen levels, and recorded throughout the procedure using the anaesthetic monitoring sheet provided.

Sample Storage

Once collected the samples should be transferred to vacutainers for storage and should either be analysed straight away using the portable Abaxis Vetscan VS2 using the large animal rotor, or if the Vetscan is not available the vacutainers should be stored in a cool bag containing ice packs at 1-12°C until they can be analysed in the laboratory.

Scientific Paper submitted in part fulfilment of the requirements for the degree of Master of Science in Wild Animal Biology/Health), University of London, 2016-17

White Rhino (*Ceratotherium simum*) Anaesthetic Monitoring Sheet

Date:
Weather:

Location:

Veterinary Surgeon(s):

Veterinary Nurse(s):

Samples collected by:
Procedure/reason for veterinary intervention:

Monitoring sheet completed by:

Patient ID:
Sex:
Overall health:

Age (estimate):
Body condition score: 1 2 3 4 5

Darted by:
Darted from (helicopter or vehicle):
Dart gun used:

Comments:

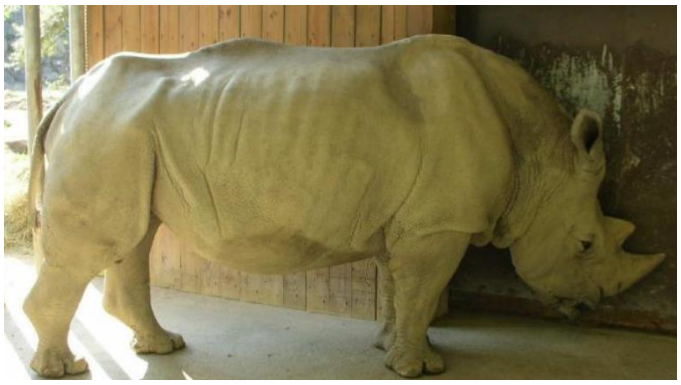
Time Darted	Time Down	Time Reversed	Time Up
Dart site	**I/V Cannula Site**	**Blood Sample Site 1**	**Blood Sample Site 2**
Initial Heart Rate	**Initial Resp. Rate**	**Initial Temp.**	**Initial Blood O_2**

Drug	Dose (mg)	Route	Time

Time	Heart Rate	Pulse Rate	Resp. Rate	Temp.	MM Colour	BP	Blood O_2	Comments

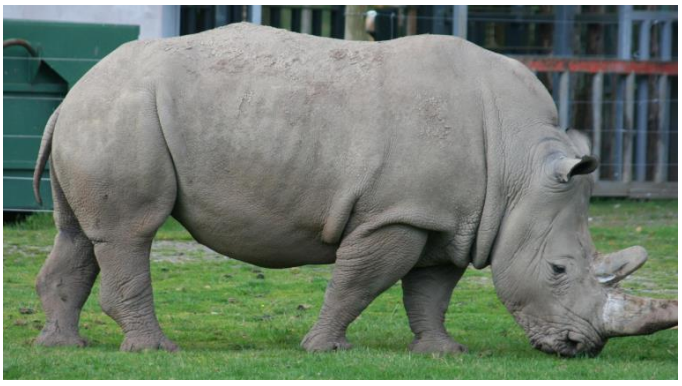
White Rhino (*Ceratotherium simum*) Body Condition Score Sheet

Versteege, L., 2011. Body condition scoring of captive white rhinoceros (*Ceratotherium simum*). Proceedings of the 2011 International Elephant and Rhino Conservation and Research Symposium, Rotterdam 10-14 October 2011: 1123-1137



BCS 1-2

BCS 3



BCS 4

BCS 5

Task Division

Gemma Crowley has been responsible for

- Applying for additional funding to Vetwork UK
- Devising the SOP for collection of fresh blood samples from semi-captive southern white rhinoceros from reserves across the Eastern Cape
- Obtaining fresh blood samples from semi-captive southern white rhinoceros from reserves across the Eastern Cape
- Analysing fresh blood samples using the Abaxis Vetscan VS2 analyser
- Archiving blood samples
- Writing the literature review and grant application
- Organising all obtained data
- The basic statistical analysis of the data
- Writing of the scientific paper

Dr William Fowlds has been responsible for

- Coordination with game reserves and conservancies in the Eastern Cape
- Providing veterinary consumables to allow for blood collection
- Providing the Abaxis Vetscan VS2
- Corrections to the literature review, grant application and scientific paper

Dr Balázs Szladovits has been responsible for

- Corrections to the scientific paper