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Wildlife capture in Zambia – An evaluation of immobilization techniques and physiological parameters on wild ungulates

Immobilisering av ville ungulater i Zambia – en evaluering av fangstmetoder og fysiologiske parametere

Authors: Eira Moen Attaei Kachouie & Ingeborg Rindalsholt
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Supervisors: Anne Randi Græsli and Mette Helen Bjørge Müller

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Preface

Wildlife medicine is an exciting part of veterinary medicine. However, it is a minor part of our veterinary study program in Norway. By joining FORTECASE's project, we would get the opportunity to experience how the management of wild ungulates is done in Zambia and learn about wildlife medicine and immobilization techniques from professional wildlife veterinarians. This study will also provide us with perspectives on how capture teams in Zambia maintain animal welfare during capture.

As a veterinarian, there are many job opportunities concerning wildlife management and immobilization, particularly within the field of wildlife conservation and disease surveillance programs. In Norway, we do research on and management of wild animals and zoo animals where veterinarians have a crucial role, especially during captures, like handling immobilization drugs, monitoring, treatments and surgery. As a livestock veterinarian, tasks like chemical immobilization of lost and wild heifers during the pasture season can be part of your responsibilities.

Research and knowledge on wildlife and immobilization techniques are crucial for improving animal welfare. We hope that the experiences and data we collected during our study can serve as valuable inspiration for further research.

Summary

Title: Wildlife capture in Zambia – an evaluation of immobilization techniques and physiological parameters on wild ungulates

Authors: Eira Moen Attaei Kachouie & Ingeborg Rindalsholt

Supervisors: Anne Randi Græsli, Department of Forestry and Wildlife Management, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, Koppang, Norway
Mette Helen Bjørge Müller, Associate Professor at Department of Production Animal Clinical Sciences at NMBU.

There is an increasing need for the development of immobilization techniques on wild ungulates. Many African ungulates are kept in national parks, conservation parks and game ranches, and the immobilization of these ungulates are often necessary managing such areas. Conserving wild species carries a significant responsibility for ensuring proper animal welfare. However, knowledge of the capture-induced stress in wild African ungulates is limited. This study aims to evaluate different immobilization techniques and physiological parameters like body temperature, serum cortisol and blood biochemistry values in wild Zambian ungulates. This study consists of both physical capture by boma and chemical capture by darting, and the ungulates were captured for translocation reasons. A total of 15 ungulates were captured including the species impala (*Aepyceros melampus*), sable antelope (*Hippotragus niger*), roan antelope (*Hippotragus equinus*), puku (*Kobus vardonii*) and kudu (*Tragelaphus stresiceros*). Venous blood samples were collected from 12 of the captured ungulates. The samples were analysed for cortisol by DetectX® Cortisol ELISA kit. The selected biochemistry parameters ALT, AST, CK, glucose and creatinine, were analysed by Atellica® CH Analyzer. The blood sample results indicated a correlation between cortisol

levels and body temperature with elevated cortisol levels and elevated body temperature. We found no correlation between body temperature and ambient temperature. The ungulates with the highest CK measurements exhibited significant muscle activity prior to sampling. During the captures of this study, there was one recorded incident of mortality and three incidents of capture-related injuries. There is a critical need for further research to enhance animal welfare during the capture of wild African ungulates, with the goal of minimizing capture-induced morbidity and mortality. The research should include a variety of ungulate species to address their unique requirements.

Abbreviations

Alpha-2 agonist	Alpha-2 adrenoreceptor agonist
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BT	Body temperature
CBG	Corticosteroid binding globulin
CK	Creatine kinase
CM	Capture myopathy
CNS	Central nervous system
CPR	Cardiopulmonary resuscitation
CRT	Capillary refill time
DNPW	Department Of National Parks and Wildlife
GC	Glucocorticoid
GCM	Glucocorticoid metabolite
GMA	Game Management Areas
HPA	Hypothalamic-pituitary-adrenal

HR	Heart rate
IM	Intramuscular injection
IV	Intravenous injection
NA	Norepinephrine
NMDA	N-methyl-D-aspartate
PNS	Peripherial nervous system
RR	Respiratory rate
SC	Subcutaneous injection
SNS	Sympathetic nervous system
UNZA	University of Zambia
ZAWA	Zambian Wildlife Authority

1 Introduction

1.1 Human-wildlife interaction and One Health

Due to the rapid climate change, causing a continuous reduction of natural habitats and resources, human-wildlife and livestock-wildlife interactions are increasing (Narayan & Rana, 2023: As reviewed in Jones et al., 2013.). As well as creating a forced co-existence, human activities such as hunting, road traffic, agriculture, and expansion of livestock farming, have led to increased mortality of wild species and a loss of biodiversity (Narayan & Rana, 2023). Wildlife contact, combined with other factors, such as international travel and trade, are contributing to the risk of development and spreading of diseases between animals and humans, e.g. zoonoses (McMichael, 2004; As reviewed in Leal Filho et al., 2022). This is making the concept of One Health more crucial than ever before (Mackenzie & Jeggo, 2019). Defined by the World Health Organization (WHO), the One Health principles consist of close multisectoral collaboration and global communication toward solving issues involving animal, human, and environmental health (WHO, n.d.). However, despite the One Health approach securing global health, wildlife health surveillance is often neglected when it comes to global and national prioritization, and the risk of pathogen spillover events is evident (Machalaba et al., 2021; As reviewed in Ryser-Degiorgis et al., 2015). Pathogen spillover events occur when pathogens move from domesticated livestock to wild species, for example by using shared grazing areas and water sources. The events contribute to the formation of wild reservoirs within the wildlife population. Such reservoirs can later lead to spillback events, returning the pathogen back to the livestock population (Ryser- Degiorgis et al., 2015). The risk of spillover events with zoonotic pathogens is rising with the increasing human-wildlife contact and public interest in wildlife (As reviewed in Ryser-Degiorgis, 2013). The spillover of bovine tuberculosis from livestock, creating a wildlife reservoir, is an

example of such transmission of zoonotic pathogens (Ryser-Degiorgis et al., 2015). In sub-Saharan countries, such as Zambia, the risk of bovine tuberculosis transmission is at a level where community campaigns are recommended (Monde et al., 2023).

Population growth and the subsequent intensification of industrial agriculture are demanding larger quantities of land areas used for human activities, resulting in the loss of important habitats, and contributing to the increasing human-wildlife interaction (Jones et al., 2013; Narayan & Rana, 2023). Imposing a human co-existence on wildlife is not only problematic considering the One Health principles and future pandemic prevention. Co-existence is also causing a growing ethical responsibility for the preservation of Earth's ecosystems and biodiversity. To develop sufficient pandemic prevention plans rooted in the One Health concept, minimize human-wildlife conflicts, and simultaneously secure wildlife conservation work, information on how to properly manage the afflicted wildlife is crucial. Increased wildlife health monitoring and wildlife-focused research are therefore needed (Machalaba et al., 2021).

1.2 Human-wildlife conflicts and African wildlife

African wildlife is known for its variety of species and their distinctiveness. This gives this wildlife a broad spectrum of positive values, ranging from socio-cultural aspects to nutritional values, as well as having a huge economic potential (Chardonnet et al., 2002). Activities, such as wildlife hunting, wildlife trade, and eco-tourism have contributed to the rise of the game ranching industry, increasing human-wildlife contact (Carruthers, 2008).

Close contact with wild species is lamentably also causing issues affecting both the wild species and humans. The term human-wildlife conflict (HWC), contrary to the neutral expression of human-wildlife interaction, refers to the conflicts that appear with increasing contact (IUCN, 2021). The cause of HWCs varies with the specific species involved, as well

as geographic location, but the main contributing factor is usually competition for land areas and resources (Lamarque et al., 2009). In addition, HWCs do not only arise from actual threats or after a damaging event. Conflicts also exist because of the perceived threats against human interests and the fear of potential problems arising. This fear is often driven by prejudices against the appearance or behaviour of the specific species of wildlife being in close contact with humans (IUCN, 2021). As a result, HWCs are changing our tolerance for wild species, causing certain species to be viewed as pests without having done any actual damage (Gross et al., 2021). The retaliatory killing of African elephants *(Loxodonta africana*) is an example of HWCs due to crop damage (Nyirenda et al., 2015). Large carnivores such as African lions (*Phantera leo*) are killed due to livestock losses (Sebsibe, 2022). Lions are also being killed in instances where the livestock damage was caused by other carnivore species such as hyena (*Crocuta crocuta)*, an example where the killing often is driven by retaliation or by fear (Sebsibe, 2022; Felix et al., 2022). Lack of government regulation of HWC, regardless of whether the conflict is caused by large herbivores, carnivores, or humans, is providing for illegal wildlife trade and poaching (Kuiper et al., 2023; Sebsibe, 2022). Although there is little available research, HWC and illicit activities considering wildlife are most likely connected (Moreto, 2019). HWCs are not only occurring in African countries. The conflicts are a worldwide issue, and in many cases resulting in serious injuries and mortalities on both humans and animals (SSC: Human-Wildlife Conflict & Coexistence Specialist Group, 2024; Gross et al., 2021; Lamarque et al., 2009).

Many HWCs are now escalating due to the rapidly changing environment. Disagreements on how to mitigate these impacts, in a way that safeguards the interests of all involved, have led to inter-human conflicts as well (SSC: Human-Wildlife Conflict & Coexistence Specialist Group, 2024) The management of the endangered grey wolf *(Canis lupis)* in European countries like Norway, is an example of HWC causing political divisions (Strand et al., 2019;

Skogen & Krange, 2020). Inter-human conflicts, involving different interests, cultural aspects, moral viewpoints, political views, and legislative solutions, are making HWCs harder to manage (SSC: Human-Wildlife Conflict & Coexistence Specialist Group, 2024). In summary, HWCs are causing problems for humans, livestock, and wildlife, as well as having negative impacts on food security, the economy, and global conservation work (Gross et al., 2021; Lamarque et al., 2009). When drawing up future wildlife conservation programs, management of potential HWCs should be included, as a successful conservation often results in increased wildlife populations elevating the risk of conflicts (IUCN, 2021).

1.3 Wildlife management and immobilization

With increasing HWCs, the need to properly manage wild species is rising. Capture and translocation of wild animals is one of many methods for reducing conflicts occurring in African countries (Lamarque et al., 2009). Another method for reducing the HWCs is the establishment of national parks, conservation parks and game ranches, however it is important to mention that uncoordinated planning of such areas can escalate the conflicts instead of mitigating them (Lamarque et al., 2009). A successful operation of such areas is dependent on having different methods of translocation, capture, and immobilization suitable for the various species being kept there (Carruthers, 2008). Proper immobilization techniques are also required for numerous research-related projects, especially when working with future disease prevention plans rooted in the One Health principles as mentioned (Zeiler & Meyer, 2017a; Machalaba et al., 2021). In addition, wildlife husbandry and wildlife medicine are growing subjects in the veterinary field, contributing to the development of wildlife immobilization as a new field of study (Zeiler & Meyer, 2017a). Managing populations of wild African ungulates in game ranches is an example of such wildlife husbandry (Carruthers, 2008). When gathering large groups of wild ungulates in limited areas, humans take on a responsibility to safeguard the animal welfare, while at the same time preventing the spread of infectious

diseases and injuries. As several wild ungulate species have great economic value, and any handling and translocation can cause fatal consequences by stress or physical traumas, correct handling is a necessary (As reviewed by Ryser-Degiorgis et al., 2015 and Breed et al., 2019). Because of this, multiple studies evaluating the different immobilization techniques used on wild ungulates, have now been published (Zeiler et al., 2015; Zeiler & Meyer, 2017a; Zeiler & Meyer, 2017b; Jacques et al., 2009; Meyer et al., 2008a; Meyer et al., 2008b; Meyer et al., 2010; Thompson et al., 2020; Arnemo et al., 2006).

1.4 Zambia

Zambia is a landlocked Sub-Saharan country, located in Southern Africa, illustrated in Figure 1. The country is divided into ten provinces: Central, Copperbelt, Eastern, Luapula, Muchinga, Northern, North-Western, Southern, Western, together with the capital Lusaka (The World Factbook, 2024). Zambia has an estimated population of 20,569,737 people. The land area consists of 752,610 km^2, being mostly savannas and grasslands (FN- sambandet, 2021). Zambia has reserved approximately 30% of its land areas for conserving and managing wildlife, with 20 National Parks and 35 Game Management Areas (GMAs) (Ministry of Tourism, n.d.). The GMAs are functioning as a buffer zone between National parks and adjacent land, working as interface areas between livestock and wildlife, preventing human-wildlife conflicts. The reserved land areas are administered by The Department of National Parks and Wildlife (DNPW) governed by The Ministry of Tourism and Arts (Ministry of Tourism, n.d.). In addition to the national parks and GMAs, wildlife is also managed through the wildlife ranching industry (Lindsey et al., 2013).

***Figure 1**: Map over Zambia, the Central Province is marked. Map over Africa, Zambia is marked. Figures made using www.mapchart.net*

1.4.1 Wildlife ranching in Zambia - Ungulates

Wildlife or Game ranching in Zambia consists of extensive or fenced privately owned ranches that are governed by the Zambia Wildlife Authority (ZAWA). The Zambian wildlife ranching industry has expanded considerably since the late 1990s (Lindsey et al., 2013). Combined with the protected area network (National Parks and GMAs), the ranching areas have a huge potential to contribute to conservation work (Lindsey et al., 2013). Zambia has marginal land suitable for agriculture and livestock, and wildlife ranching is therefore providing an important alternative for utilizing these areas to the fullest (Lindsey et al., 2013). Livestock farming and wildlife ranching are often combined, increasing the farmer's income. Combining these activities leads to the best possible utilization of the land areas available, as the wild species manage to utilize the natural vegetation and resources better than the domesticated species (Lindsey et al., 2013).

The terms wildlife and game are generally indistinguishable, however, when put in context with ranching, the term game usually refers to ungulate species (Carruthers, 2008). Regarding classification, the term ungulate has no taxonomic significance and is defined as a mammal with hooves (Huffman, 2023; Estes, 2024). Ungulates consist of a diverse group of herbivores, divided into the two taxonomic orders: Artiodactyla (even-toed animals) and Perissodactyla (odd-toed animals) (Huffman, 2023). Wild ungulates represent the majority of animals being kept on Zambian wildlife ranches, with impala (*Aepycers melamous*), kudu (*Tragelaphus strepsiceros*) and bushbuck (*Tragelaphis scriptu*s) being some of the most common species (Lindsey et al., 2013). Wildlife ranches contribute to maintaining the ungulate biodiversity in Zambia, and wild ungulates on ranches comprise 16.8% of the total populations occurring in the protected area networks and ranches combined (Lindsey et al., 2013). Eland antelope *(Taurotargus oryx*) and roan antelope *(Hippotargus equinus)* are examples of other ungulate species where wildlife ranches contain significant portions of the national population (Lindsey et al., 2013). Roan antelope and eland antelope are often referred to as antelopes (Estes, 2024). Similar to the term ungulate, antelope is not in the taxonomic system (Estes, 2024). Species referred to as antelopes are taxonomically placed in the Bovidae family, under the order of Artiodactyls, without being viewed as cattle, sheep, or goats (Huffman, 2023). Africa, having 71 species of antelopes is viewed as the continent of antelopes (Estes, 2024). Like other species in the Bovidae family, antelopes are ruminants (Estes, 2024).

The Zambian wildlife ranching industry, containing large numbers of wild ungulates, is in addition to the conservation work also contributing to the country`s food security through game meat (Lindsey et al., 2013) Moreover, the industry is providing numerous positions of employment including sectors in ecotourism, with activities such as hunting, safari, wildlife sale and auctions (Lindsey et al., 2013; Carruthers, 2008). As the industry is expanding, the

need for new positions of employment also follows, and the number of wildlife specialists in southern Africa is increasing (Lindsey et al., 2013; Carruthers, 2008). A growing wildlife industry leads to additional knowledge on wildlife husbandry, and the subject of animal welfare must be included in the expansion. It is therefore essential that wildlife veterinarians and other specialists within the field (e.g. ecologists and conservation experts) follow the expansion closely (As reviewed in Ryser-Degiorgis, 2013).

1.5 Methods for immobilization

When handling wild ungulates, adequate immobilization is needed. Wild ungulates are captured primarily for marking, medical treatments, research, translocation projects, and population monitoring (West et al, 2014; Clarke et al., 2014). Wild ungulates can be immobilized by either chemical or physical capture (Kaarakainen, 2019). Factors that need to be considered when selecting the capture method are human safety for those handling the animals, safety for the animals, and the animal's welfare including the opportunity to monitor the animals until full recovery from the physical or chemical effects (Sontakke et al., 2017). Unfortunately, immobilization of wild ungulates comes with a high risk of morbidity and mortality due to stress and injuries (Zeiler & Meyer, 2017a).

1.6 Chemical immobilization

Chemical immobilization is a method used for selective capture of one or few individuals, and for larger animals where physical immobilization can be too risky or impractical (Clarke et al., 2014). Chemical immobilization techniques are often favoured over physical during veterinary procedures due to the need for fixation or administration of analgesics. It is an expensive but often a more efficient and safer capture method than physical because the animals are sedated or anesthetized (Clarke et al., 2014). The administration of the drug can be done by remote injection, injection from a close distance or by oral ingestion given in feed

(Kaarakainen, 2019; Kreeger & Arnemo, 2018). Remote injections can be done from a distance by dart guns, crossbows, blow pipes, or pole syringes (Kock & Burroughs, 2021). Immobilization by darting is widely used for managing wild animals during different procedures, like translocations, marking, application of radio transmitters, resources, veterinary treatments, and surgery (Clarke et al., 2014).

1.6.1 Drugs groups and protocols

Chemical immobilization gives a wide spectrum of effects, from gentle suppression of consciousness to general anaesthesia, depending on the type of drug groups used (Kreeger & Arnemo, 2018). General anaesthetics makes the animal unconscious and unaware of its surroundings and a loss of nociceptive perception, and is necessary during surgery procedures (Clarke et al., 2014; Haga et al, 2012). Tranquilizers reduce anxiety without drowsiness, while sedatives have a more profound effect that relieves anxiety and provides drowsiness and depression (Shell., 2015; Kock & Burroughs, 2021). Sedatives and tranquilizers cannot achieve adequate immobilization on their own, and the animal can arouse if it gets sufficiently nociceptive stimulated. These drugs are often used in combination with other drugs during general anaesthesia or during procedures where full unconsciousness is not necessary, like translocation, easy veterinary treatments or marking (Kreeger & Arnemo, 2018). It is common to use a combination of different sedative drugs to induce a state of profound sedation with lower doses and then reduce the chance of undesirable side effects (West et al, 2014; Haga et al, 2012). Capture drugs are often very potent and formulated in relatively concentrated solutions, which allows injection of very small drug volumes mixed in small darts (Kreeger & Arnemo, 2018).

Different combinations of immobilization drugs are more or less suitable for different animal species (Kock & Burroughs, 2021; Kreeger & Arnemo, 2018). Practitioners' experiences with

the use of different drugs, have led to different protocols and handbooks with recommendations for different species. According to Clark et al., 2014, s.578, the ideal anaesthetic drug protocol has: "...a wide safety margin, low mortality rate, rapid and smooth onset of action after intramuscular administration, causes minimal excitement phase, (...), and produce relaxation that facilitates ease of handling of the animal". Kreeger & Arnemo (2018) describes the characteristics of a perfect capture drug being a drug that is potent, fast-acting, has good muscle relaxation and minimal depression of the cardiovascular or respiratory system, is rapidly metabolized, highly water-soluble, has minimal side effects, is safe for pregnant animals, is compatible with other drugs in mixtures and has a low toxicity in humans. They also conclude that the perfect protocol or drug does not exist yet, but those in use have several of these characteristics (Kreeger & Arnemo, 2018; Clarke et al., 2014). The choice of protocols can vary between different capture teams due to their experiences and the purpose of immobilization. It can also depend on the availability of drugs in different countries and the economics (Clarke et al., 2014; Dr. A. C. M. Sitima, 2023, personal communication, 24 June).

Immobilization drugs used for ungulates

In capture protocols for ungulates, a combination of phenothiazines (sedatives/ tranquilizers), alpha-2-adrenoreceptor agonists (sedatives), cyclohexamines (anaesthetics) and opioids (sedatives) are commonly used to get a profound sedation (West et al, 2014). Cyclohexamines can also be given to improve the immobilization once the animal is down (Kreeger & Arnemo, 2018).

Opioids

Opioids are sedatives, and many of them provide satisfactory analgesia (Haga et al, 2012). The mechanism of action is interactions with different opioid receptors in the CNS, PNS and other tissues, which gives a reduction of neuronal excitability (Clarke, et al., 2014). In general, opioids have minimal side effects, but dose-dependent respiratory depression is one of the most important, in addition to bradycardia and motoric excitation (Clarke, et al., 2014; Haga et al, 2012). Opioids commonly used for ungulate immobilization are etorphine, carfentanil and thiafentanil, which are potent opioids that induce a rapid profound sedation. These are often used as the only immobilant, without any additional drugs (Kreeger & Arnemo, 2018; West et al, 2014).

Phenothiazines

Phenothiazines are antipsychotic drugs or tranquilizers, classified as neuroleptics that have antiemetic effects, and give sedation and long-acting tranquilization by sufficient doses, but have minimal to absent analgetic effects (Haga et al, 2012; Shell., 2015). The mechanism of action is blockage of the dopamine receptors in the CNS, and a reduction of psychomotor agitation and aggressiveness (Kreeger & Arnemo, 2018; Haga et al, 2012). Side effects of phenothiazines are vasodilation, hypotension, and hypothermia (Haga et al, 2012). Promazine, perphenazine and acepromazine are commonly used phenothiazines for ungulates (Kreeger & Arnemo, 2018).

Cyclohexamines

Cyclohexamines are anaesthetics that also give analgesia (Haga et al, 2012). They are called dissociative anaesthetics because they induce a dissociative state in human patients and make them feel disconnected from the environment and make them hallucinate (Gitlin et al, 2020). The main mechanism of action is blocking of N-methyl-D-aspartate (NMDA) receptors in the

CNS, which blocks the action of the excitatory glutamate and then prevents central hypersensitivity (Clarke, et al., 2014). In other words, they are NMDA antagonists. Some common side effects of these drugs are excitation of neurons, restlessness and uncontrolled muscle activity. They also cause increased heart rate, stroke volume and arterial blood pressure (Haga et al, 2012). Ketamine and tiletamine are two cyclohexamines commonly used during wildlife immobilization (Kreeger & Arnemo, 2018).

Alpha-2-adrenoreseptor agonists

Alpha-2-adrenoreseptor agonists (hereafter alpha-2 agonists) are classified as a sedative with analgetic and muscle relaxing effects (Haga et al, 2012). The mechanism of action is stimulation of pre- and postsynaptic alpha-2 receptors in the central nervous system (CNS) and the peripheral nervous system (PNS), which activates inhibitory neurons that decrease the release of norepinephrine (NE) (Kreeger & Arnemo, 2018; Sjaastad, et al., 2016). Lack of NE gives decreased alertness and subsequent sedation (Haga et al, 2012). Side effects of alpha-2 agonists are respiratory depression and bradycardia, following an increased risk of hypoxemia (Sjaastad, et al., 2016; Haga et al, 2012). Commonly used alpha-2 agonists for ungulates are xylazine and medetomidine (Kreeger & Arnemo, 2018).

Reversal of immobilization drugs

An antagonist is given to reverse the drugs and to arouse the animals. Naltrexone is a commonly used opioid antagonist, and the work of action is to compete with opioids at the opioid receptors (West et al, 2014). Intravenous (IV) injection gives the most rapid recovery (1-2 minutes), either administrated in the jugular vein or in an ear vein. Intramuscular (IM) injection will give a slower recovery for about 5-10 minutes. The antidote dose is often split between IV, IM and subcutaneous (SC) administration. (Kreeger & Arnemo, 2018). After administration of naltrexone, the animal cannot be re-immobilized in the next 24 hours,

because of the long half-life of naltrexone (Kock & Burroughs, 2021; Pastilha, 2016). Atipamezole is an alpha-2-antagonist, while there are no available antagonists for acepromazine, ketamine and tiletamine (Clarke, et al., 2014).

Calculation of drugs dosages

Calculation of drug dosage can be challenging during chemical immobilization of wild ungulates because there are not possible to do a pre-anaesthetic check to evaluate the health status and weight. Experience and knowledge about the average weight of age classes and sex crucial to avoid over- or underdosing. A handbook with drug doses recommendations for different species, age groups, sizes and sex are therefore recommended to use as a guideline (Dr. A. C. M. Sitima, personal communication, 24 June 2023; Ferreira, 2016).

1.6.2 Darting and induction

When administration of drugs by a dart, the preferable administration site is IM in large muscle masses at the proximal hindlimb, shoulder and neck (see Figure 2) (Kreeger & Arnemo, 2018; Clarke et al., 2014). The target animal can be approached at the ground by foot or by vehicles, or from above in a helicopter. The darting should be done when the animal is standing still at a maximal range of 40-50 meters (Kreeger & Arnemo, 2018).

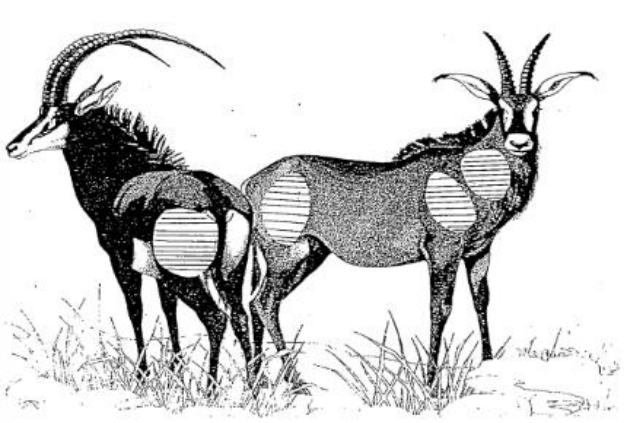

***Figure 2**: Best darting site in ungulates market with stripes (Adapted from Kock & Burroughs, 2012)*

The time interval between darting/injection and the point where the animal is recumbent, is called the induction time. This time is influenced by the sensibility and the type of drug and dosage delivered, the level of anxiety and stress during darting, physiological and physical status and administration site. If the drugs are inadvertently injected SC, the induction time

will be longer and more unpredictable, because the absorption of the drugs to the bloodstream takes longer time (Clarke et al., 2014). Under most circumstances, the darted animal will give signs of being affected within 3-15 minutes after darting IM (Kock & Burroughs, 2021). Some typical signs during the induction period are licking and increased salivation, lowered head, aimless walking, stumbling and falling, before it finally lies down unable to rise. (Kreeger & Arnemo, 2018). The excitement phase is a well described phase of induction where the animal run aimlessly in a "hackney gait". If the drug dose was insufficient, the animal will stay in this phase for a longer time, which increase the risk of injury and hyperthermia, in worst cases exhaustion and death. If the animal stays in the excitement phase for a longer period than excepted, it is necessary to administer some more drugs (Kock & Burroughs, 2021; Pastilha, 2016). The darted animal can walk or run over a long distance. Hence it is important that the capture team has explored the area and terrain in forehand of the capture to minimise the risk of morbidity and mortality of the animal being immobilized (Clarke, et al., 2014).

1.6.3 Handling and care of the animal during immobilization

The first handling of the animal is usually to restrain the head, either by the horns or firmly by the head if the animal does not have horns. The position with the head held up and nose pointing down is important to avoid aspiration of saliva, in addition to protect the handlers from injury from the horns if the animal moves it head. Then assess the state of the animal, both open airways and depth of anaesthesia, in addition to temperature to check for hyperthermia, in case cooling is necessary (Dr. A. C. M. Sitima, 2023, personal communication, 24 June; Kock & Burroughs, 2021). Common ways to assess the dept of anaesthesia is to observe for spontaneous movements, ear twitch (poke inside of ear), jaw tone (spread jaws and feel for resistance), palpebral and cornea reflex (touch eyelashes and cornea, and watch for blinking) (Kock & Burroughs, 2021). To impair view and lower stress it is

usual to blindfold the animal's eyes. It will also protect cornea from direct sunlight, dust and injury. To decrease auditory stimuli, it is also possible to insert cotton in the ears (Clarke, et al., 2014; Vyas., 2017; Kock & Burroughs, 2021). Ruminants should be positioned in sternal recumbency during immobilization with all four limbs tucked under in a natural position to prevent bloating, regurgitation, and aspiration of stomach contents. If the animal has to be positioned on its side for a short period of time, it should be placed in right lateral recumbency (with its left side up) to minimize the pressure on the rumen and in that way avoid regurgitation. The head and neck should still be elevated. Pregnant females should never be rolled over on their back, as this may result in uterus torsion. (Kock & Burroughs, 2021; Kreeger & Arnemo, 2018; Dr. A. C. M. Sitima, 2023, personal communication, 24 June).

1.6.4 Monitoring

It is important to monitor vital signs/parameters during immobilization, including respiratory rate (RR) and depth, cardiac function, body temperature (BT), capillary refill time (CRT) and depth of anaesthesia (Clarke et al., 2014). The first measurement of the RR and HR can be used as a base to evaluate changes that may occur (Kock & Burroughs, 2021). Intranasal oxygen should ideally be given to avoid hypoxia during chemical capture, and a pulse oximeter is valuable equipment that can measure oxygen saturation of haemoglobin (Kock & Burroughs, 2021).

Respiratory system

The respiratory depth and rhythm are as important as respiratory rate. Few deep breaths are better than many shallow breaths, because the respiratory volume must be larger than the dead space, so the oxygen reaches the alveoli and then the blood (Sjaastad et al., 2016). Insufficient circulation of air increases the concentration of carbon dioxide in the bloodstream

(hypercapnia), which causes respiratory acidosis (Kreeger & Arnemo, 2018). Hypercapnia, respiratory acidosis and hypoxia can cause dangerous complications, like seizures, cardiorespiratory collapse, coma and severe organ damage and death (Kock & Burroughs, 2021). Colour of the mucous membranes of the eyes, gums and tongue is an indicator if there is lack of oxygen or not. The membranes should be coloured pink when the blood is appropriate oxygenated and change to become blue if there is a severe lack of oxygen (Clarke, et al., 2014).

Cardiovascular system

HR is often used as a parameter to assess the cardiovascular system. A very fast HR can either be a function of the drugs, physiological response to stress, hyperthermia, hypoxia or shock. A slow heart rate could also be a function of drugs, hypothermia or metabolic disorders (Kreeger & Arnemo, 2018).

Body temperature

Tranquilisers or sedatives may interfere with thermoregulation, so the handlers should be aware of both hypo- and hyperthermia (Kock & Burroughs, 2021). In general, mammals have a rectal temperature range from 37.5-38.8 °C (Kreeger & Arnemo, 2018). If the animal becomes hyperthermic (> 2 °C above normal BT), cool the animal with water and provide shade. Blankets and hot water bottles can be necessary to prevent hypothermia (> 2 °C below normal BT) (Kreeger & Arnemo, 2018). It is important to be aware that many African antelopes are sensible to low ambient temperature, especially during transport in cool weather (Kock & Burroughs, 2021).

Health examination

It is important to check the animal for wounds and injuries, during immobilization. It is practical to start from the nose and work toward the tail and look for blood, swelling,

ectoparasites, hear loss, fractures, diarrhoea, dehydration and to measure a body condition score. A high parasite load can indicate an animal in a poor condition, which can impact the recovery from anaesthesia, as well as long-time survival (Kreeger & Arnemo, 2018). If other conditions like wounds and parasites are detected, treatment can also be necessary (Dr. A. C. M. Sitima, 2023, personal communication, 24 June; Kock & Burroughs, 2021).

1.6.5 Human risks during chemical immobilization

Drug handling

The opioids etorphine, carfentanil and thiafentanil are very potent immobilization drugs. Carfentanil are quoted to 10 000 times more potent than the commonly used human and veterinary preparate morphine (Clarke et al., 2014; West. et al, 2014). Hence, there is an increased risk of intoxication if the practitioner gets exposed to only small volumes of the drug through small skin cuts, needle sticks, in the mouth or by nasal exposure. This can cause nausea, dizziness or profound sedation, respiratory depression, hypotension, and cardiac arrest (Clarke et al., 2014). It is therefore important to handle the drugs with respect, and a good practice is to always wear gloves when the drugs are handled. A human antagonist should be drawn up and ready to administer in case of an incident like self-administration or if the drug comes in contact with the membranes of the eyes, mouth or nose. Naloxone and naltrexone are antagonists to both etorphine and carfentanil (Clarke et al., 2014). If the drug comes in contact with intact skin, wash immediately with large amount of water. It is also recommended that the capture crew know basic first- aid techniques, like cardiopulmonary resuscitation (CPR) (West et al, 2014; Kock & Burroughs, 2021).

Handling of the darted animal

Handling the restrained animal is often one of the most critical stages during the immobilization process where both personnel and the animal can be harmed. Ungulates can

have spontaneous movements like kicks during anaesthesia, and some of them have horns that can harm the personnel (West et al, 2014; Dr. A. C. M. Sitima, 2023, personal communication, 24 June). Sometimes it can be necessary to hobble the legs of ungulates to prevent spontaneous kicks (Kreeger & Arnemo, 2018).

Risks during reversal

Arousing from anaesthesia to a fully awake state can be very stressful for the animal (West et al, 2014). All personnel not needed for the recovery should be watching and observing the recovery from a safe distance, and not in front of the animal. One or a few persons, often the veterinarian that gives the antidote, can remain with the animal until the first sign of recovery (Dr. A. C. M. Sitima, 2023, personal communication, 24 June).

1.7 Physical immobilization

Physical capture techniques are effective during mass captures, but some methods are also designed for individual capture (West et al, 2014). There are different methods, including capture bomas, lasso ropes, nets, net guns, drop-nets, and capture traps (Kock & Burroughs, 2021). In our study capture bomas was the physical capture method used and will therefore be described further.

1.7.1 Capture bomas

The boma is a widely used method of mass capture and can be used to capture most species. A herd can be captured as a single entity with minimal human-animal contact in a short amount of time (Kock & Burroughs, 2021). The boma is made of plastic or nets that are formed to make a large funnel (As reviewed in Laubscher et al., 2015). The plastic walls are commonly strung between trees by wires. The animals are driven into boma either by a helicopter, a vehicle or by foot. The walls of the boma are barriers that make the animals run further down the funnel which becomes more and more narrow (Kock & Burroughs, 2021). A

series of curtains are manually drawn behind the animals once they enter strategic places to prevent the captured animals to turn around and escape. This is done by people in the capture crew that are hiding inside the boma. Good coordination and effective communication within the capture crew are important to make sure that all the target animals have entered the boma before closing it off (Dr. A. C. M. Sitima, 2023, personal communication, 24 June). At the end of the boma there is a crush system and a loading ramp that leads into a transport truck. The boma is often camouflaged by trees and bushes. To avoid the animals getting the scent of the boma, the gate should face into the wind (Kock & Burroughs, 2021). The entrance of the boma should be wide enough for the animals to enter, appropriately about 100-120 meters long (As reviewed in Laubscher et al., 2015). Some ungulates, like kudus, can easily jump over a wall of 2 meters, so the height of the boma walls should not be less than 3-4 meters high (Kock & Burroughs, 2021). Although boma capture is an effective capture method, setting up the boma itself can be very time-consuming (Dr. A. C. M. Sitima, 2023, personal communication, 24 June).

1.8 Adverse health effects

Capture of free-ranging animals require basic knowledge of anatomy, physiology and behaviour of the animals that are captured and challenging field conditions (Clarke et al., 2014). Captures are expensive, due to the use of qualified personnel, equipment and medicaments, and the costs increases if there are animal losses caused by injuries, capture myopathy, abortion due to stress and incorrect use of immobilization drugs (Sontakke et al., 2017). Therefore, it is important to minimize the stressors by using the right type and dosage of drugs that give a rapid induction and recovery, in addition to knowledge about the species immobilized and the capture area (Kreeger & Arnemo, 2018).

1.8.1 Stress mediators influence on physiological parameters

Immobilizations are stressful procedures that lead to an acute stress response that alters the animal's homeostasis (Kreeger & Arnemo, 2018). Potential triggers of stress during a capture are anxiety, fear, exhaustion, pain and anaesthetic drugs. Stress is a way the body prepares itself to cope with a threatening situation, by increasing the muscle activity, body temperature and oxygen demand (Clarke, et al., 2014). These stress responses are mediated by the hypothalamic-pituitary-adrenal (HPA) axis (Figure 3) and the sympathetic nervous system (SNS). The major mediators are the catecholamines (e.g. adrenalin and noradrenaline) that are released by the adrenal medulla and sympathetic nerves in the first phase of a stress response, also known as the fight- or flight response (Sjaastad, et al., 2016). The catecholamines will cause an increase in heart rate, cardiac output, blood pressure and mobilization of energy resources. Furthermore, the blood flow increases to the muscle, blood glucose levels are elevated due to increased gluconeogenesis in the liver and muscles, as well as conversion of lactic acid into glucose in the liver. The ventilation increases and mental activity and alertness becomes sharpened up. All these changes of the animal's physiology are essential for an effective escape. Catecholamines rise rapidly and the effects are almost immediate, but are not sustained, because they are short-lived and quickly metabolised (Clarke, et al., 2014). Further, the HPA-axis will be activated and cause release of cortisol and other glucocorticoids (GCs) from the adrenal cortex, which increases the mobilisation of energy from protein and fat even more. Cortisol also alters cellular functions, affects the homeostasis in the body and suppresses the immune system. In contrast to catecholamines, the concentrations of glucocorticoids take time to rise, approximately 15-30

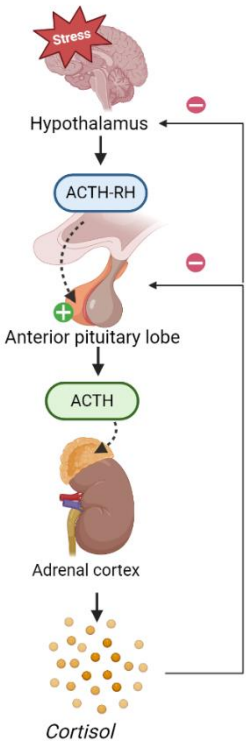

***Figure 3**: Illustration of the positive and negative feedback reactions in the HPA-axis. Figure made using biorender.com*

minutes after the initial stimulus (Kock & Burroughs, 2021). The concentration may remain elevated until the stressor is reduced or removed, but the metabolic and the other effects can sustain even after the concentration is decreased in the blood (Kock & Burroughs, 2021). Persistent exposure to stressors, like transporting, translocations or housing in bomas, causes prolonged release of glucocorticoids and low to moderate release of catecholamines (Kreeger & Arnemo, 2018). If the stress last for hours, days or even years, it is defined as chronic stress (Kreeger & Arnemo, 2018). The prolonged catabolism and immune system suppression make the animal lose weight and more vulnerable to develop diseases, increased chances of developing gastric ulcers and cellular degeneration, as well as reproduction problems like abortion and infertility. They can also get behavioural changes, like depression, anorexia, a depressed flight- or fight response and abnormal aggression (Kock & Burroughs, 2021).

1.8.2 Capture myopathy

Capture myopathy (CM) is a clinical syndrome characterized by pain, muscular stiffness, incoordination, ataxia, oliguria, paresis, paralysis, metabolic acidosis, depression and potentially death. Myopathy refers to diseases that attack muscle fibers (Clarke, et al., 2014). It is probably the most important cause of death and sickness following the capture of ungulates (Kock & Burroughs, 2021). Predisposing factors to CM are a combination of fear, extreme stress and excessive muscular activity, as well as high environmental temperatures, underlying diseases, deficiencies in vitamin E/selenium, dehydration and malnutrition (West et al, 2014). Some capture drugs, such as opioids, can contribute to CM by promoting excitement, muscle rigidity, hypoventilation, catecholamine release and hyperthermia (Clarke, et al., 2014). Animals that survive the acute stage of CM, may die up to days or months after the episode without any signs, as a result of severe tissue damage (Kock & Burroughs, 2021). The tissue damage is a result of altered blood flow to the tissues and decreased delivery of oxygen and nutrients, increased circulating lactic acid (which cause the metabolic acidosis)

and inadequate removal of cellular waste from the cells (Clarke, et al., 2014). CM is a type of exertional rhabdomyolysis, which means breakdown of both skeletal and cardiac muscle. This leads to muscle necrosis and leakage of intracellular muscle contents, such as creatinine kinase (CK), aspartate aminotransferase (AST) and myoglobin into the bloodstream (West et al, 2014). Increased levels of circulating myoglobin can lead to tubular necrosis of the kidneys and renal failure, as well as myoglobinuria (Clarke, et al., 2014). CM are classified into four categories that are useful to predict the outcome of the case, by characteristics, clinical signs and pathology. These are hyperacute or capture shock syndrome, ataxic myoglobinuric syndrome, ruptured muscle syndrome and delayed-peracute syndrome (West et al, 2014; Clarke, et al., 2014). Treatment of CM generally has a low success rate and is logistically difficult in wild animals in field situations. Eventually, treatments recommended in the early phase of CM are fluid administration, analgesics, muscle relaxants, dantrolene (prevent malignant hyperthermia), vitamins (vitamin E/Selenium and vitamin B), oxygen therapy and sodium bicarbonate to combat acidaemia (Clarke, et al., 2014). Prevention is often much more efficient than treating CM and other capture injuries (West et al, 2014). The occurrence of CM can be an indicator of how well animal welfare was considered during capture and translocation (Breed et al, 2019).

1.9 Wildlife and animal welfare

Animal welfare is a complex issue that refers to the feelings and physical well-being of an animal in relation to the conditions it lives and dies in (World Organization for Animal Health, 2024). “The five freedoms”, developed in 1965, describes how the society expect the conditions animals should experience under human care: 1. Freedom from hunger, malnutrition and thirst, 2. Freedom from fear and distress, 3. Freedom from heat stress or physical discomfort, 4. Freedom from pain, injury and disease, 5. Freedom to express normal patterns of behaviour (World Organization for Animal Health, 2023). These freedoms are

used as a framework for further development of animal welfare (World Organization for Animal Health, 2024). Wildlife is safeguarded by various research ethics committees. Prior to any human activities with vertebrate animals, such as immobilization and research, the committees will review all types of research proposals (UNZAREC, 2018). This ensures that ethical assessment and approval take place before research. By following this process, researchers can balance the need for research with considerations of animal welfare and ethics (NENT, 2019).

1.9.1 Improvement of animal welfare during wildlife immobilization

Elimination of stress during capture and immobilization is a key priority to ensure improved animal welfare (Kreeger & Arnemo, 2018). First, careful planning of the capture procedure is crucial. It is important to know the species that are going to be captured and be aware of different species-specific sensitivities, due to drugs and handling (Clarke, et al., 2014). The chase and induction time is a critical phase of immobilization, and it is important to give breaks if the animals are chased over a long distance or time. The drugs in the anaesthetic protocol should give a rapid induction and recovery and have minimal adverse physiological side effects. Eventually, side effects, like depressed ventilation, should be prevented by oxygen supplementation (Kock & Burroughs, 2021). Handling of the animals should be done as quickly as possible and performed by experienced personnel. Eventually, transportation should be as short as possible. Very young, old or heavily pregnant animals, and animals that are in poor condition should not be captured (West et al, 2014). To avoid hyperthermia immobilization should be done at a time of the year and a time of the day when it poses the least hazard to the species. In Zambia, the best time to anaesthetize ungulates are in early spring, fall or in the winter, and in the early morning or evening when the temperature is coolest. The capture teams should have cool water available in case cooling the animal is necessary (Clarke, et al., 2014). Minimising of possible stressors by limiting visual

stimulation, noise, touch and foreign smells, are favourable (Kock & Burroughs, 2021). Capture teams should take all these preventive factors into account to make a more efficient capture that improves the animal welfare and the profitability of the catch by keeping the animals healthy (Kreeger & Arnemo, 2018).

1.9.2 Evaluation of stress in wild ungulates

Evaluating the effect of stressors in animals is challenging, as the development of stress is complex and often multifactorial (As reviewed by Sheriff et al., 2011 and Dantzer et al., 2014). The use of glucocorticoids, either from invasive (blood; plasma or serum) or non-invasive (saliva, faeces, urine, milk, hair or feathers) sample methods, has become a standard in animal welfare studies focusing on stress (Ralph & Tilbrook, 2016; Cook, 2012; Sheriff et al., 2011). Other supplementary measures consist of biochemical blood parameters (e.g. glucose, free fatty acids) and changes in physical parameters (e.g. heart rate, respiratory rate, body temperature) (Dantzer et al., 2014; Cabanac & Guillemette, 2001). Securing such samples from wild species requires a successful live capture, immobilization and in some cases deep anaesthesia of the animal (Dantzer et al., 2014). These methods of management, leads to close human-animal contact and are subsequently influencing the stress response of the targeted animal. Figure 4 illustrates different methods used to assess stress in wild ungulates.

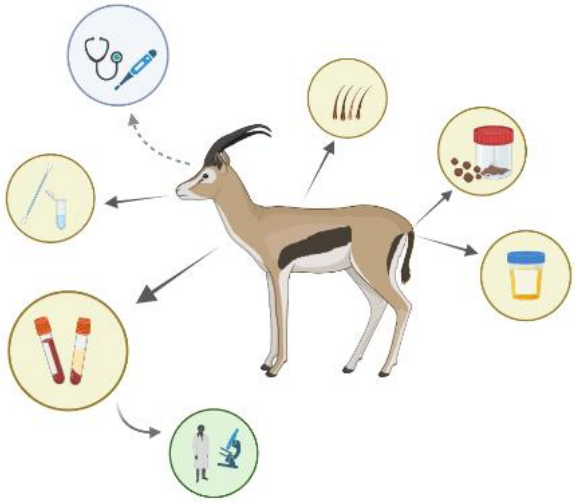

***Figure 4**: Illustration of the different methods for measuring stress responses on wild ungulates, including evaluation of physical parameters, invasive and non-invasive glucocorticoid sampling methods, and biochemistry blood parameters. Figure mate using www.biorender.com.*

Containing wild species of ungulates under human care, either by zoological collections or in game ranches, has led to the need to define species-specific physiological reference values (Zeiler & Meyer, 2017a). Other motives for wildlife interference, like wildlife conservation, HWC prevention projects and veterinary care, are contributing to this need (Sleeman & Widdowson, 1993; Lamarque et al., 2009). Extrapolation of reference values from domesticated ungulates, such as the goat (*Capra hircus*) or sheep (*Ovis aries*), can in many instances cause problems interpreting the reliability. This is particularly relevant in instances where the animal is immobilized with drugs, such as opioids, as different species of ungulates react differently under the influence of immobilizing agents (Pfitzer et al., 2020). Sex and age are also important to consider when interpreting stress measuring samples (As reviewed in Dantzer et al., 2014). When evaluating stress responses, it is favourable to compare the acquired measurements with normal reference values of the targeted species (Pfitzer et al., 2020). Unfortunately, collecting representative data of normal reference values from wild species is challenging, as variations in capture and management protocols can cause different outcomes. This makes the identification of baseline GCs and normal biochemical values problematic (As reviewed in Dantzer et al., 2014). Moreover, stress-related studies on wild

ungulates should also consider the long-term effects of capture, as human interference can influence the animal`s ability to adapt post-release. It is therefore important to find out whether the capture, immobilization or translocation cause changes in the animal`s behaviour (e.g. movement patterns, social status, reproduction and survival) (Jung et al., 2019; Trondrud et al., 2022; Trondrud et al., 2023).

1.9.3 Glucocorticoids as a stress indicator

Glucocorticoid (GC) levels are the most used biomarkers to evaluate physiological stress (As reviewed in Dantzer et al., 2014; Ralph & Tilbrook, 2016; Cook, 2012). Cortisol, cortisone and corticosterone are the major GC responsible for the stress response (Botía et al., 2023). Most mammals have cortisol as their dominant glucocorticoid, whereas other species are corticosterone-dominant (e.g. birds, amphibians and reptiles) (Botía et al., 2023; As reviewed in Sheriff et al., 2011). However, some exceptions to this rule have been observed in species of rodents (Boyle et al., 2021; Sheriff et al., 2011). As ungulates belong in the mammal taxa, the focus in this study is on cortisol.

GCs released from the adrenal cortex are found in the circulatory system either as bound or in free form (As reviewed in Cook, 2012). Most of the circulating cortisol (80%) is bound with a high affinity to corticosteroid-binding globulins (CBGs), whereas the rest is bound to albumin (10%) or exists as “free” un-bound hormone in the circulation (10%) (As reviewed in Bryen, 1980). The free form of GCs is considered as the biologically active portion (Cook, 2012). When the concentration of total GCs in plasma increases, as a response to stress, the increase is mainly due to an increase in the free and active percentage (Cook, 2012). The total glucocorticoid concentration in blood is not automatically the same as the biologically active concentrations. This is something to be aware of before evaluating and comparing the results from different sampling methods (Cook, 2012).

Serum cortisol

Collecting blood samples for measuring serum cortisol concentrations is perceived as an invasive sampling method, as it requires arterial or venous puncture (As reviewed in Sheriff et al., 2011 and Cook, 2012). When collecting blood from wild species, success depends on having adequate fixation and immobilization of the animal (Sheriff et al., 2011). Blood sampling from large species of wild ungulates is dependent on chemical immobilization methods, often contributing to a longer capture time (Dr. A. C. M. Sitima, 2023, personal communication, 24 June). Depending on experience, equipment and the chosen capture method, variations in the timeframe of capture-start to sampling, are difficult to prevent (As reviewed in Dantzer et al., 2014). Such variations make the comparison of results from different study individuals more difficult. However, compared to the other sampling methods, serum samples make it possible to measure the direct adrenal production of glucocorticoids, instead of the metabolised by-products of glucocorticoids (GCM) obtained from non-invasive GC sampling (e.g. faeces, urine, milk, hair, or feather samples) (Sheriff et al., 2011). This makes serum samples relevant when the main interest is to evaluate the acute stress response in wildlife and the method can therefore be classified as an instantaneous measure of stress (As reviewed in Dantzer et al., 2014).

1.9.4 Biochemical blood markers

Collecting blood samples from wildlife for serum cortisol measurements, are simultaneously providing an opportunity to measure other components of the blood (e.g. biochemistry, haematology, reproductive hormones, etc.). This makes it possible to get multiple results from one single sample, and the glucocorticoid concentrations can be evaluated against the overall condition of the captured animal (As reviewed in Sheriff et al., 2011). Biochemical blood markers (e.g. glucose, AST, ALT, CK and creatinine) can be used to assess animal homeostasis during capture of wild ungulates (Marco et al., 1997). Biochemical parameters

can also be used as an indicator of chronic stress, as the measurements give an impression of the general health of the captured animal (As reviewed in Dantzer et al., 2014). Studies focusing on capture-related stress and biochemical blood markers in wild ungulates have therefore been published (Marco et al., 1997; Cheney & Hattingh, 1988; Harms et al., 2012; Pfitzer et al., 2020). This makes biochemistry relevant when assessing the stress-impact of different immobilization methods.

ALT (alanine aminotransferase) and AST (aspartate aminotransferase) can both be used to evaluate the liver function, as elevated blood concentrations indicate damage to the cells containing these enzymes (Ferrier, 2014). Conditions like viral hepatitis, neoplasia, toxic injury, physical trauma or prolonged circulatory collapse can cause significantly high measurements (Ferrier, 2014; NMBU, 2024). As ALT is found in small concentrations in the liver, especially in ruminants, it has poor diagnostic value in species of ungulates (Marco et al., 1997; NMBU, 2024). Contrary to ALT, hepatic cells contain large amounts of AST, making AST a sensitive parameter on liver damage (NMBU, 2024). However, as AST is present in multiple cell types such as cardiac and skeletal muscle, interpretation of elevated AST levels should be done together with other muscle-specific blood parameters (e.g. creatine kinase) as the AST levels can be elevated due to muscle trauma, injury or disease (Ferrier, 2014; NMBU, 2024). Elevated concentrations of creatine kinase (CK) in serum can be used diagnostically as an indication of both skeletal and heart muscle damage (Ferrier, 2014). Common causes for elevated serum CK concentrations are overexertion of skeletal muscles, IM injections, capture myopathy or damage caused by pressure due to prolonged lying (NMBU, 2024; Breed et al., 2019). In instances of muscle inflammation, the serum concentrations of CK increase faster than AST. However, the AST concentrations are elevated for a longer time-period than the CK measurements (NMBU, 2014). As muscle enzymes concentrations (AST and CK) reflect alterations caused by capture and handling of

wild ungulates, they become important parameters when evaluating the impact of both physical and chemical immobilization (Marco et al., 1997; Harms et al., 2012; Breed et al., 2019). Moreover, creatinine measurements can be used to evaluate renal function (NMBU, 2024). Normally, creatinine is rapidly eliminated from the circulation by glomerular filtration. Any rise in serum creatinine levels can therefore be used diagnostically as an indicator of renal disease or reduced perfusion of the kidneys (Ferrier, 2014). In some ungulates, increased serum creatinine due to muscular activity and vasoconstriction in the kidneys caused by the catecholamines released in the capture induced stress response, has been described (Breed et al., 2019; Marco et al., 1997). Hyperglycaemia is another result from the catecholamine and glucocorticoid activation during capture, as elevated serum glucose concentrations in captured wild ungulates has been documented (Kock et al., 1987; Marco et al., 1997; Gerlach et al., 2017; Pfizer et al., 2020). Glucose concentrations should therefore also be included in the biochemistry profile.

2 Aims

The main aim of this study was to evaluate different immobilization techniques used for capturing ungulates in Zambia and assess physiological parameters related to the immobilization with a focus on capture-induced stress.

Objectives:

1. Preform different immobilization techniques in the field in Zambia on at least two different species, and 10-15 animals per species.
2. Evaluate the cortisol levels shortly after immobilization by analysing total cortisol levels in serum.
3. Analyse serum biochemistry parameters and body temperature shortly after immobilization.
4. Assess injuries and mortality related to the capture.

3 Material and methods

3.1 Study design

This study is an explorative study investigating the physiological responses to different immobilization strategies used on ungulates in Zambia.

3.1.1 Study area and selection of animals

The study area consists of three privately owned game ranches in the Central Province of Zambia; Noah's Ark Conservancy in Mkushi on the 25th of June (location A), Kabwe on the 7th of July (location B) and Protea Game Reserve Chisamba on the 13th of July (location C). See Figure 5 for the locations in the study area.

The study population consisted of five different species of wild ungulates of both sexes. The ungulate species were impala (*Aepyceros melampus)*, puku (*Kobus vardoni*), roan antelope (*Hippotragus equinus),* sabel antelope (*Hippotragus niger)* and kudu (*Tragelaphus strepsiceros*). The animals were not captured for this study only, as our project participated in captures where the main goal was to capture and immobilize animals for translocation and sale. Therefore, the species and number of animals were chosen based on available capture assignments from the 20th of June until the 31st of July 2023.

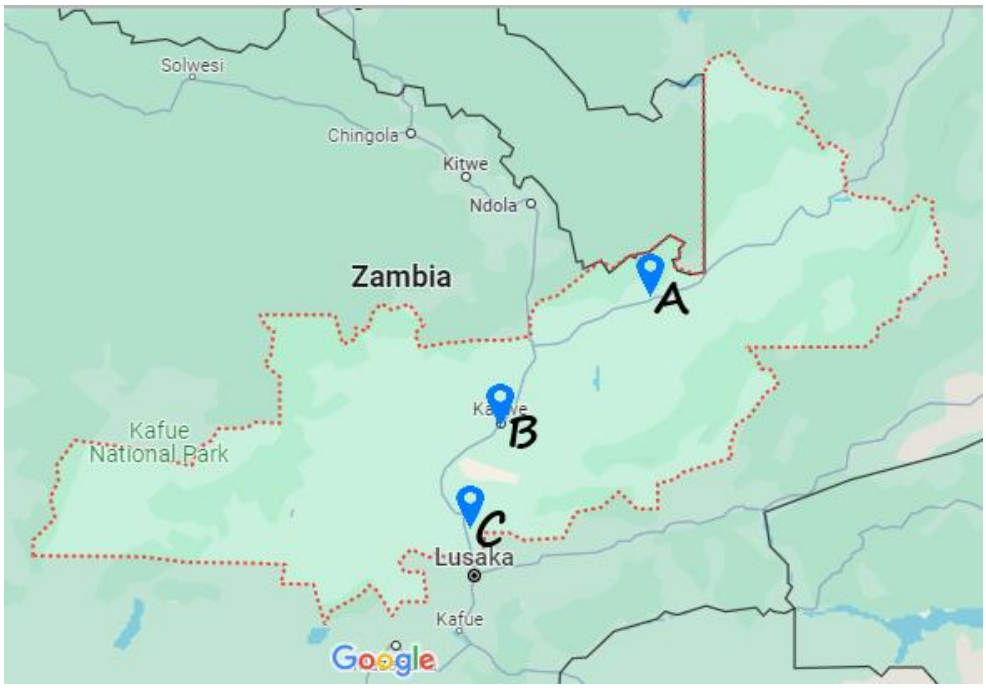

Figure 5: *Map showing the different locations of capture and sampling in this study. The Central Province of Zambia is outlined in red.* ***A****: Noah`s Ark Concervancy, Mkushi.* ***B****: Kabwe.* ***C****: Protea Game Reserve, Chisamba. The figure is made using Google Maps (2024).*

3.2 Immobilization techniques

3.2.1 Physical immobilization

Our study participated in a physical capture of impala at Noah`s Ark conservancy in Mkushi (Location A). At this location, the immobilization technique consisted of using a boma (Figure 6) to perform a mass capture of impalas. Other species of ungulates that followed, such as puku (*Kobus vardonii)*, were also included. The boma was in an area with varied vegetation, and it was carefully camouflaged by trees and bushes. The capture was initiated in the afternoon. A group of experienced capturers located the herd, and chased them by foot and by vehicle, towards the funnel opening of the boma. As soon as the selected animals were on the inside, the enclosure was closed manually by men dragging the tarpaulin curtains shut. After the chase and capture, the animals were given 12-24 hours to rest and to acclimatize to the boma environment. The sampled animals in our study stayed in the boma overnight,

before being loaded on the transportation vehicle. The sampling itself took place after the loading procedure, and the selected animals were lifted down from the ramp manually by a group of two to three men.

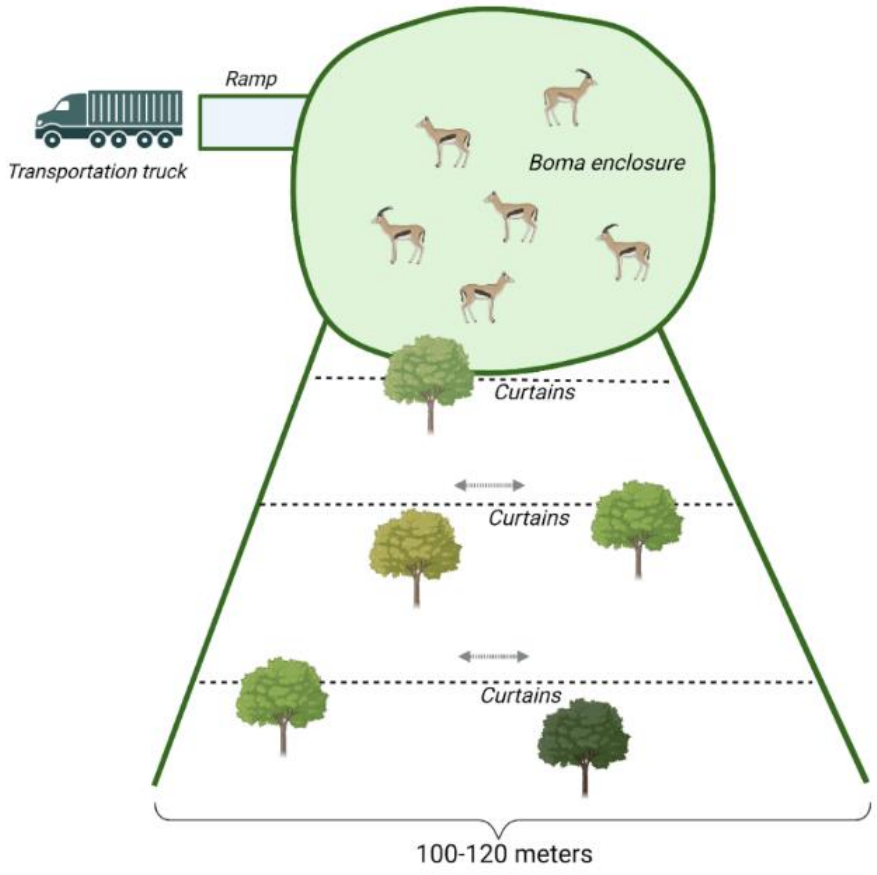

Figure 6: *An illustration of the capture boma used in our study. Figure made by using www.biorender.com.*

3.2.2 Chemical immobilization

***Table 1**: Drug protocols for chemical captures in Kabwe and Chisamba.*

Location	Species	Sex	Type of drugs administered and dose (mg)	Comments
Kabwe (B)	Roan (B004)	F	Etorphine [1] Ketamine[1] Naltrexone[1]	
Kabwe (B)	Roan (B005)	F	Etorphine [1] Ketamine[1] Naltrexone[1]	
Kabwe (B)	Roan (B006)	F	Etorphine [1] Ketamine[1] Naltrexone[1]	
Kabwe (B)	Roan (B007)	F	Etorphine [1] Ketamine[1] Naltrexone[1]	
Kabwe (B)	Roan (B008)	M	Etorphine [1] Ketamine[1] Naltrexone	Given additional dosages with ketamine three times after darting
Kabwe (B)	Sable (B009)	M	Etorphine [1] Ketamine[1] Naltrexone[1]	
Chisamba (C)	Impala (C010)	M	Etorphine: 3 mg Naltrexone: 30 mg	
Chisamba (C)	Sable (C011)	F	Etorphine[1] Perphenazine: 50 mg Naltrexone[1]	
Chisamba (C)	Sable (C012)	M	Etorphine[1] Perphenazine: 50 mg Naltrexone[1]	
Chisamba (C)	Impala (C013)	F	Etorphine: 2 mg Naltrexone: 20 mg	
Chisamba (C)	Impala (C014)	F	Etorphine: 2 mg Naltrexone: 20 mg	
Chisamba (C)	Kudu (C015)	F	Etorphine: 8 mg Perphenazine: 60 mg Naltrexone: 80 mg	

***Sex**: **M**: Male, **F**: Female. [1] Unknown dosage.*

Drugs and equipment

All the chemically immobilized ungulates were darted from the truck with a Model 389 Pneu-Dart cartridge fired projector (Pneu-Dart, Inc. Williamsport, Pennsylvania, USA), using red and green 22 calibre cartridge and Pneu-Dart darts (Type C, 1.5 cc, long wire barbed tri-port cannula needle, Pneu-Dart, Inc. Williamsport, Pennsylvania, USA) (Figure 7, middle and right side). To secure a proper administration of drugs, the size of the needle was selected according to the size of the animals. The darts were always located in the muscles of the gluteal or scapular area.

In Kabwe (location B) ungulates were immobilized using darts loaded with a drug combination of ketamine and etorphine hydrochloride M98 (Captivon 98®, etorphine 9.8 mg/mL, Wildlife Pharmaceuticals (Pty) Ltd., White River, South Africa).) In Protea Game Reserve Chisamba (location C) the larger ungulates (sable and kudu) were immobilized with perphenazine enanthate (Trilafon LA ® 108.2 mg/mL, Kyron Laboratories (Pty) Ltd., South Africa) combined with etorphine hydrochloride M99 (M99®, etorphine 9.9 mg/mL, Novartis Animal Health, Basel, Switzerland), while impalas only were sedated with etorphine. Sterile water was added to fill the darts. If needed, additional dosages of ketamine were given to the larger ungulates IM by hand injection. Naltrexone hydrochloride (Trexonil® 50 mg/mL, Wildlife Pharmaceuticals (Pty) Ltd., South Africa) was given as the opioid reversing agent (Figure 7, left side). It was administered IV by the auricular veins and SC in the neck area once the animals were on the transportation vehicle. The drug doses (mg/ungulate) on each drug used during our study were not clearly established, but some of them are shown in Table 1.

Figure 7: *Photos from location B. Left photo: Trexonil ® 50 mg/mL used as a reversal. Middle photo: A Pneu-Dart. Right photo: Eira Moen Attaei Kachouie testing the dart rifle. Photos by: Eira Moen Attaei Kachouie, Ingeborg Rindalsholt and Elise Lismoen.*

Handling of the immobilized ungulates

Darted animals were approached by experienced members of the capture crew after the first sign of effect. A team, consisting of two to six men depending on the size of the animal, was responsible for physically immobilizing the animals during sampling and transportation. The ungulates were placed in sternal recumbency with their head held up and nose down. Their eyes were blindfolded with a blanket. Impalas were sometimes held in a lateral recumbency with left side up with their head and neck held down at the ground. Two men were always responsible for fixating the horns on the largest ungulates, both to avoid damage and to keep the head and neck steady for sampling. The animals were blindfolded, and the dart was removed in a rotating motion to release the barbed wire from the cannula.

Pick-up trucks were used to move the ungulates from the area of capture to the vehicles used for further transportation. The larger ungulate species (sable antelope, roan antelope and kudu) were transferred from the ground and onto a tarpaulin sheet reinforced with fabric straps. By using strap handles along the tarpaulin edge, the antelopes were lifted from the

ground to the pick-up truck bed, and thereafter from the pick-up truck and over to the transportation vehicle. The lifting of the ungulates was performed by a group of 10-12 men. The impalas were lifted and handled by the same method described in the paragraph on physical immobilization (3.2.1). After sampling, the captured antelopes were loaded onto the transportation vehicle. The majority of the captured antelopes were held in individual compartments of the transportation vehicle. Animals held in a shared compartment, were grouped according to size, sex and species. This was done to prevent conflicts.

3.2.3 Clinical observations and monitoring of variables during capture

All clinical observations and variables were registered on a capture form (available in Appendix 1). The body condition and age of the animals were estimated by the responsible veterinarian at the capture site. The immobilization degree was evaluated based on jaw tone (relaxed or tense), palpebral reflex (absent or present), and the degree of voluntary movements. Any signs of pre-capture, post-capture injuries or other signs of disease was recorded.

The monitoring during both physical and chemical immobilization was performed in the same way as soon as possible, somewhere between 1-30 minutes after the animals were immobilized. Physical parameters that were monitored; Heart rate (HR) (measured by auscultation with a stethoscope) (Figure 8), respiratory rate (RR) (registered by visual inspection of thoracic movements, any abnormal respiratory patterns were noted as a comment) and temperature (measured as rectal temperature using a digital thermometer).

Specific points of time were registered during the capture to calculate relevant time intervals including the start of the chase, hit time of the dart, the first sign of the effect from the immobilising agents, time of successful immobilisation/ capture, sampling time, the time of antagonist administration and the time for release/loading onto transportation vehicle.

Environmental parameters such as weather conditions, ambient temperature and barometric pressure were recorded before the start of capture. The weather condition was described subjectively based on cloud cover and the degree of wind. Ambient temperature and barometric pressure were recorded by using an i-STAT® 1 Analyzer (Abbott Laboratories, Illinois, United States).

Figure 8: *Ymke Masurel monitoring the physically captured male impala (A002) in Mkushi. Photo by Thea Kleiven.*

3.3 Sampling

Venous blood samples were collected from both physically and chemically captured ungulates. The sampling was done as soon as possible, somewhere between 1-30 minutes after the animals were immobilized. During the capture, additional samples like saliva, deep nasal swabs, faecal and hair samples were taken for other research purposes. The additional samples will not be discussed further in this study.

3.3.1 Blood sampling

The venous blood samples were taken from the jugular vein using a vacutainer system (BD Vacutainer®, BD Diagnostics, Preanalytical Systems, Franklin Lakes, NJ, USA). For small ungulate species like impalas and pukus, the 22 x $1^{1/2}$ –gauge vacutainer needle was used. For larger antelopes like sables, roans and kudus, the 21 x $1^{1/2}$-gauge vacutainer needle was used. Blood was sampled 1-5 minutes after they were immobilized either chemically or physically. The blood for biochemistry was collected in 4 mL CAT serum clot activator tubes (Vacuette®, Greiner Bio-One International AG, Kremsmünster, Austria). The blood samples were labeled with the animal`s ID number and placed in a holder stand at ambient temperature for 20-60 minutes to ensure the start of the clotting process. Afterwards, the samples were stored in a cooling box and transported to the laboratory.

Figure 9: *Ingeborg Rindalsholt collecting venous blood from a sable antelope in Chisamba. Photo by Eira Moen Attaei Kachouie.*

3.4 Storage and shipment of serum samples

The blood samples were brought to the laboratory at UNZA the same day as the sampling. At the laboratory, the blood was centrifuged at 3500 rpm for 3 minutes or 5 minutes to separate the serum (TOMY MX-207 high-speed refrigerated micro centrifuge, Tokyo, Japan). Then

the serum was stored in 2 ml microtubes (Eppendorf® tubes, VWR) at -80 °C. The frozen samples were shipped to Norway and analysed at the laboratory. The samples were shipped to Faculty of Veterinary Medicine, NMBU, on dry ice using DHL shipment services. When arriving the laboratory, the samples were frozen at –80 °C until analyses.

3.5 Analysis of serum samples

3.5.1 Cortisol in serum

The serum cortisol was measured quantitatively with the DetectX® Cortisol ELISA Kit (Arbor Assays, Michigan, USA) and validated following the protocol provided by the manufacturer. A standard curve was made to calculate the results and all standards and samples were run in duplicate using an ELISA-plate with 96 wells. The plate was read in the Agilent BioTek Epoch microplate spectrophotometer (Agilent Technologies, Highland Park Winooski, USA) using a 450 nm wavelength and further analysed using an online data analysis tool for life sciences assays (MyAssays Limited, Brighton, UK, www.myassays.com). Serum cortisol concentrations were expressed in pg/ml, and the DetectX® Cortisol ELISA Kit has a sensitivity of 27.6 pg/mL.

The serum cortisol levels were converted from pg/mL to nmol/L, by the help of the conversion factor in the DetectX® Cortisol ELISA Kit protocol (1 pg/mL cortisol = 0.00276 nmol/L cortisol). See Appendix 2 for the protocol.

3.5.2 Serum biochemistry

The biochemical profile included alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), creatinine kinase (CK), amylase, lipase, total protein, urea, creatinine, bile acids, total bilirubin, cholesterol, triglycerides, free fatty acids (FFA), glucose, inorganic phosphorous, calcium, sodium, potassium, chloride and sodium-potassium ratio. Serum protein electrophoresis yielded two protein fractions: albumin and globulins, and the

albumin-globulin ratio. All clinical chemistry analyses, except albumin analysis, were carried out with an Atellica® CH Analyzer (Siemens Healthcare, Tarrytown, NY, USA). Different reagents from Siemens Healthcare were used for each analyte except FFA which NEFA-HR (2) FUJIFILM (Neuss, Deutschland). Albumin was carried out with SEBIA Capillarys 2.0 (UK) that analyses protein electrophoresis. ALT, AST, CK, glucose and creatinine, shown in Table 3, were selected from the biochemical profile. The full biochemical profile is available in Appendix 5.

3.6 Statistical analyses

An Excel database was created based on the information from each individual capture form used in the field. The database included the individual ID numbers of the captured animals, and the corresponding clinical parameters of each animal. The different time registrations made during the capture process were also included in the database, and relevant time intervals were calculated. The results from the DetectX® Cortisol ELISA Kit and the serum biochemistry samples were registered in the Excel database, and thus connected to the clinical parameters from the field. The database was used to visualize the distribution of data, measures of central tendency (median, average and range) and the degree of variation (standard deviation). Descriptive statistics were applied, with dot plots and bar charts created in Excel to illustrate the results.

3.7 Ethical considerations

Ethical clearance was granted from the University of Zambia health and research ethical committee IRBno.00011000, IORGno: 0009227 and FWA no 00026270.

4 Results

4.1 Locations and study selection

Five impala, five roan antelopes, three sable antelopes, one kudu and one puku were captured in three different locations in the Central Province of Zambia from June 24th to July 13th, 2023. The study selection included five males and ten females. Three of the animals were physically immobilized, while twelve animals were chemically immobilized (Figure 10). See Table 2 for general data collected in the field during immobilization.

4.1.1 Location A – Noah's Ark Conservancy, Mkushi

In Mkushi one male impala and one female puku were sampled. They were captured physically in a boma with approximately 15 other ungulates the day before sampling, in connection with translocation. Since the other impalas in the boma were assumed pregnant and further handling would increase the risk of abortion and death do to stress, no other animals were taken samples of from this location. The blood sample from the puku were not analysed because of low quantities of serum.

4.1.2 Location B – Kabwe

In Kabwe five roan antelopes and one sable antelope were sampled while being chemically immobilized for translocation. A male roan antelope (B008) was not properly sedated to take samples from, resulting in the sample size of five animals from this capture site.

4.1.3 Location C – Protea Game Reserve, Chisamba

In Chisamba we sampled blood from three impalas, two sable antelopes and one kudu during chemical immobilization for translocation.

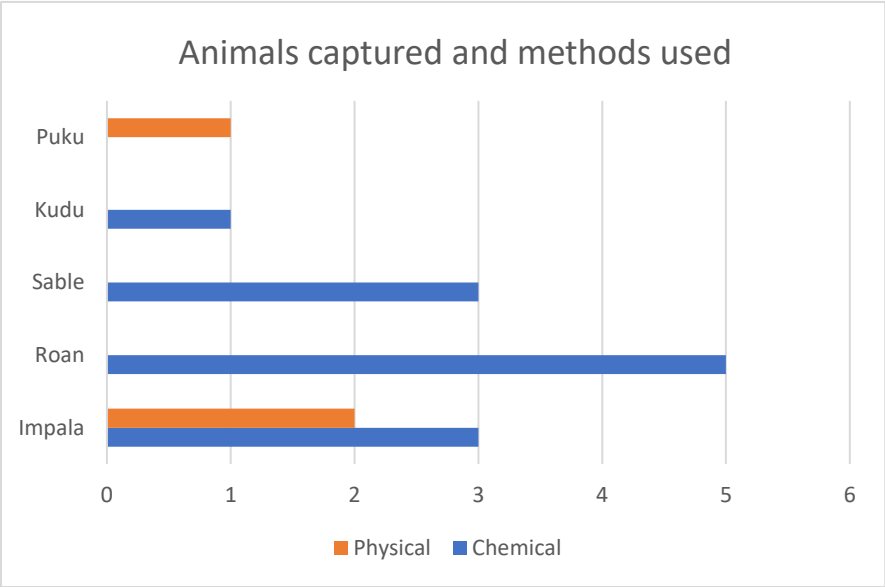

Figure 10: *Illustrative bar chart showing the total number of animals captured and immobilization methods used.*

4.2 Clinical observations

An overview of the captures including the animal ID, species and sex, location of the captures, method of the immobilization, body temperature, respiratory rate (RR) and samples taken are given in Table 2.

Table 2: *Clinical observations.*

Animal_ID	Location	Amb.temp	Method	Species	Sex	Body temp.	RR	Samples
001	A		P	Impala	F			
002	A		P	Impala	M	39.4 °C	76	Blood
003	A		P	Puku	F	39.4 °C	48	Blood
004	B		C	Roan antelope	F		36	Blood
005	B		C	Roan antelope	F	38.2 °C	28	Blood
006	B	23.6 °C	C	Roan antelope	F			Blood
007	B	26.3 °C	C	Roan antelope	F	38.7 °C	28	Blood
008	B		C	Roan antelope	M		32	
009	B		C	Sable antelope	M		48	Blood
010	C	18.7 °C	C	Impala	M	38.5 °C	64	Blood
011	C	21.8 °C	C	Sable antelope	F	37.0 °C		Blood
012	C	21.8 °C	C	Sable antelope	M	37.9 °C		Blood
013	C	22.2 °C	C	Impala	F	41.7 °C		Blood
014	C		C	Impala	F	40.5 °C		Blood
015	C	22.1 °C	C	Kudu	F	38.6 °C	28	Blood

Location: *Location of immobilization. A: Noah's Ark Concervancy, Mkushi. B: Kabwe. C: Protea Game Reserve, Chisamba.* ***Amb.temp****: Ambient temperature at the capture site measured in C°.* ***Method:*** *Immobilization method. P: Physical. C: Chemical.* ***Sex:*** *Sex of immobilized animal. F: Female. M: Male.* ***Body temp:*** *Rectal temperature in °C.* ***RR:*** *Respiratory rate, breaths per minute.* ***Samples****: Collected samples, venous blood samples.*

4.2.1 Body temperature

The body temperature of the two physically immobilized ungulates was 39.4 °C. The body temperature ranged from 37.0°C – 41.7°C (median 38.6 °C, mean 38.9°C) among the chemically immobilized ungulates (n=8).

4.2.3 Respiratory rate

Respiratory rate was registered for nine ungulates. The RR for physically immobilized ungulates (n=2) ranged from 48-76 breaths per minute while chemically immobilized (n=7) ranged from 28-64 breaths per minute (median: 32, mean 38).

4.2.4 Heart rate

The heart rate (HR) was not registered as planned, due to limitations in time during immobilization.

4.2.5 Immobilization degree

Evaluation of the immobilization degree was not performed as intended. All the chemically immobilized ungulates (n=12) had presence of the palpebral reflex, a tense jaw tone and regular voluntary movements. The immobilization degree was therefore performed by a more subjective assessment of the animal`s level of awareness and the degree of physical fixation required. The degree of immobilization was evaluated as low- moderate on all animals captured alive (n=14). However, it was sufficient for collecting venous blood samples on most of the captured ungulates in this study (n=12).

4.2.6 Injuries and mortality

There were four registrations of injuries or mortalities. One impala (A001) was found dead in the boma during the physical capture in Mkushi. Another impala in Mkushi (A002) was bleeding from both nostrils and mouth prior to sampling, due to trauma during loading and

capture in the transport vehicle. A sable antelope (B009) in Kabwe got a large and deep wound (2-3 cm in diameter) near the bone in the gluteal area caused by the dart and was therefore given penicillin IM. An impala (C013) in Chisamba was hit by the dart in the perineum area.

4.3 Time utilization

Time registrations were made during the chemical captures. Two different time intervals were calculated from these registrations: Handling time (the time of capture until the time of antagonist administration) and Total time (the start of chasing until the time of antagonist administration). The mean handling time was 13 minutes (range 2 to 24 min, median 13 min), whereas the mean total time was 28 minutes (range 7 to 62 min, median 23 min). The time intervals are available in Appendix 2.

4.4 Sampling

Venous blood samples were obtained from 13 of 15 immobilized animals. Samples were not obtained from one impala (A001) and one roan antelope (B008). The impala was found dead one day after physical capture by boma, and potential blood samples were evaluated as being non-reliable, as the time of death was unknown. The roan antelope was not immobilized sufficiently, and samples were therefore not taken based on a safety assessment.

4.5 Laboratory results

After serum extraction from the 13 venous blood samples, 12 samples had enough material to be analysed, as the sample from the puku (A003) contained less volume than required for the analyses (< 0.5 mL).

4.5.1 Serum cortisol

Serum cortisol was measured from twelve individuals (physical capture n=1, chemical capture n=11) as duplicated ELISA measurements. Average serum cortisol concentrations for each individual were calculated from the duplicate ELISA measurements and are shown in Figure 11. The concentrations are measured in picogram per millilitre (pg/mL).

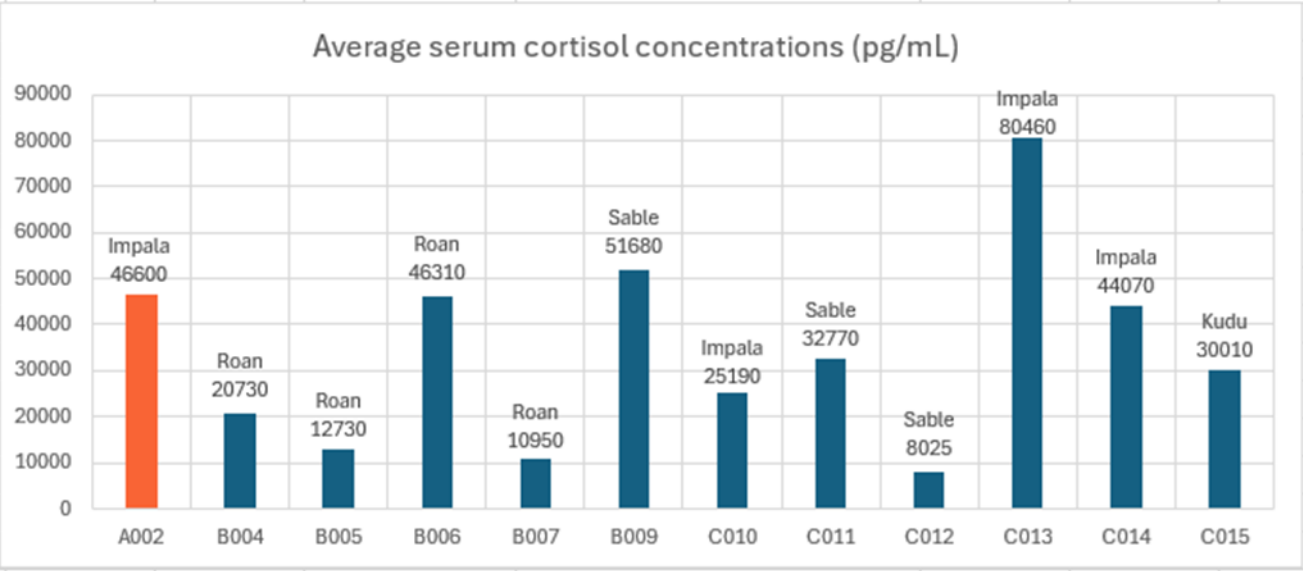

***Figure 11**: Average serum cortisol concentrations. Orange bar: physical immobilization. Blue bars: chemical immobilization. Samples from one impala (A001), one puku (A003) and one roan antelope (B008) were not analysed.*

The measured serum cortisol concentrations showed great individual variation in the study population. The chemically immobilized ungulates (n=11) had a median serum cortisol concentration of 30,010 pg/mL (mean: 32,993 pg/mL, median 30,010 pg/mL), with a range from 8,025-80,460 pg/mL. The individual with highest measured serum cortisol concentration (80,460 pg/mL) was a chemically captured impala (C013). The individual with the lowest serum cortisol concentration (8,025 pg/mL) was a chemically immobilized sable antelope (C012).

The species with the highest serum cortisol concentration was impala (n=4) with a mean concentration of 49,907 pg/mL (range: 25,190 pg/mL – 80,460 pg/mL, median: 44,070 pg/mL). The species with the lowest cortisol concentration was roan antelope (n=4) with a

mean concentration of 22,680 pg/mL (range: 10,950 pg/mL – 46,310 pg/mL, median: 16,730 pg/mL). The sable antelopes (n=3) had a mean cortisol concentration of 30,825 pg/mL (range: 8,025 pg/mL – 51,680 pg/mL, median: 32,770 pg/mL).

4.5.2 Body temperature and serum cortisol levels

Of the captured animals in this study from which serum cortisol was measured (n=12), body temperature was registered in nine. Shown by Figure 12, there was a correlation between high serum cortisol concentrations and high body temperature measurements. The species having lowest measured body temperature was the sable antelopes (n=2) with a mean temperature of 37.5 °C. The roan antelopes (n=2) had a mean temperature of 38.5 °C, whereas the impalas (n=4) had a mean temperature of 40.0 °C. The chemically immobilized impalas (n=3) had a mean temperature of 40.2 °C.

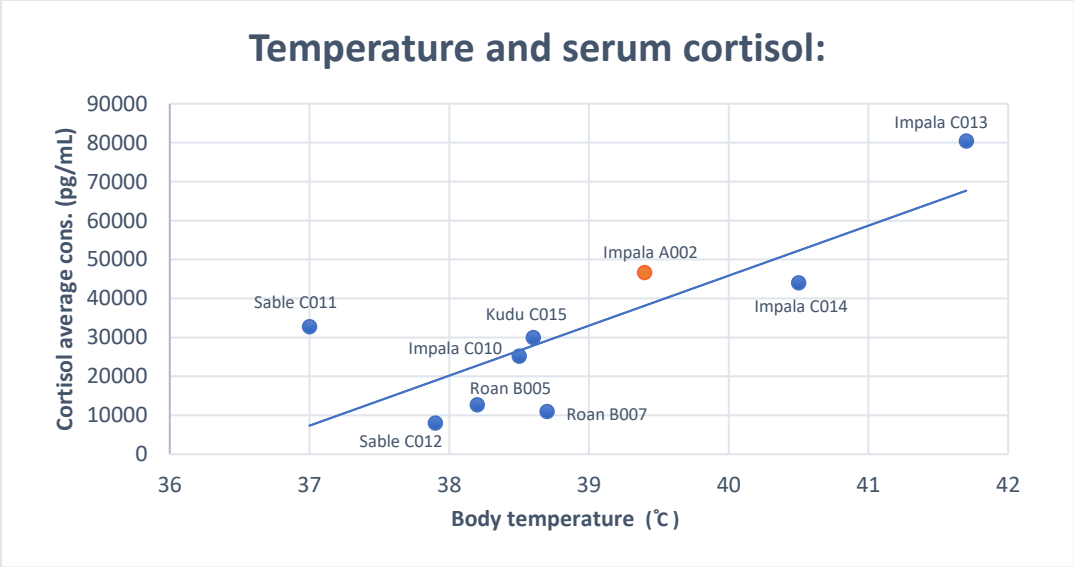

Figure 12: *Temperature and serum cortisol. Orange dot: physical immobilization. Blue dots: chemical immobilization.*

4.5.3 Biochemistry results

The biochemical results of ALT, AST, CK, glucose and creatinine are shown in Table 3. The average concentration of ALT of all the captured ungulates (n=12) was 54 U/L (range: 10 U/L – 110 U/L, median: 48 U/L). The average concentration of AST (n=12) was 168 U/L (range:

32 U/L – 492 U/L, median: 142 U/L). The captured impalas (n=4) had an average AST concentration of 301 U/L (range: 175 U/L – 492 U/L, median: 269 U/L), whereas the roan antelopes (n=4) and sable antelopes (n=3) had average AST concentrations of 118 U/L (range: 74 U/L – 163 U/L, median: 117 U/L) and 63 U/L (range: 32 U/L – 112 U/L, median: 45 U/L), respectively. Of the chemically immobilized impalas (n=3) the average concentration of CK was 845 U/L (range: 396 U/L – 1,092 U/L, median: 1,047 U/L). The roan antelopes (n=3) had large variations in CK levels, ranging from 152 U/L – 2,844 U/L (average: 1,777 U/L, median: 2,335 U/L). The sable antelopes (n=3) had low measurements of CK with an average concentration of 426 U/L (range: 157 U/L – 644 U/L, median: 477 U/L). The kudu (n=1) had the lowest CK concentration, with 128 U/L. Nine of the sampled animals had blood glucose > 4.5 mmol/L, with the average concentration of all the sampled ungulates (n=12) being 8.1 mmol/L (range: 3.5 mmol/L – 16.2 mmol/L, median: 7.2 mmol/L). The creatinine measurements showed little variation between the sampled ungulate species with an average concentration of 156 µmol/L (median: 154 µmol/L). The highest creatinine measurement of 229 µmol/L was collected from a roan antelope (B006), whereas the lowest measurement of <13 µmol/L was from an impala (C010). The roan antelope and impala were both captured chemically. The additional biochemistry values obtained in this study are available in Appendix 5.

Table 3: *Selected biochemistry results.*

Animal_ID	Method	Species	ALT	AST	CK	Glucose	Creatinine
			U/L	U/L	U/L	mmol/L	µmol/L
A002	P	Impala	99	492	5716	6.1	138
B004	C	Roan	40	74	152	6	183
B005	C	Roan	44	102	2335	3.5	160
B006	C	Roan	49	163	2844	16.2	229
B007	C	Roan	72	132		5.1	113
B009	C	Sable	47	112	477	10.5	168
C010	C	Impala	110	218	1047	8.2	<13
C011	C	Sable	32	45	644	4.5	149
C012	C	Sable	40	32	157	4.2	119
C013	C	Impala	55	319	1092	11.8	154
C014	C	Impala	50	175	396	8.6	150
C015	C	Kudu	10	151	128	12.3	157

Method*: Immobilization method.* ***P****: Physical.* ***C****: Chemical.*

5 Discussion

The main aim of this study was to evaluate the different immobilization techniques commonly used for capturing wild antelopes in Zambia and to assess stress-related physiological parameters shortly after immobilization. By participating in capture assignments with different capture teams, we observed variations in both capture techniques and stress-reducing measures.

5.1 Interpretation of results

5.1.1 Serum cortisol concentrations

Serum cortisol concentrations were analysed in 12 ungulates, where one of them was physically immobilized and eleven were chemically immobilized. A chemically captured impala (C013) had the highest serum cortisol levels in our study (222.1 nmol/L). The sampling from this impala was done after administration of the opioid reversal and after it was moved to the transportation vehicle. The chemically captured sable antelope B009 had the second highest cortisol levels (142.6 nmol/L) and was sampled after it was lifted up on a pick-up truck. Both individuals showed elevated signs of stress during fixation and sampling. The high cortisol levels were therefore expected. The only physically captured ungulate, an impala (A002), had the third highest serum cortisol levels (128.6 nmol/L). We expected that this individual would have the highest levels of cortisol, since it had been exposed to stress over a longer time and handled without any sedation during the sampling and clinical examination. However, this was not the case in our study. To elaborate better on the differences between cortisol levels on the different capture methods, we should have had more samples of physically captured individuals to compare with.

The chemically captured sable antelope C012 had the lowest cortisol levels (22.1 nmol/L). This male was captured together with a female sable antelope C011 with higher cortisol levels

(90.4 nmol/L). These ungulates were handled for a shorter time and sampled before being moved to the pick-up truck and administered antagonists. The immobilization degree was considered as moderate, and the cortisol levels were expected to be low. Despite similar handling, the two ungulates had quite different cortisol levels. Cortisol measurements can have been influenced by other factors, like sex and age as reviewed in Dantzer et al., 2014.

Although there is limited information on serum cortisol reference values for African ungulates, some studies about impala have been published (Meyer et al., 2008b; Zeiler & Meyer, 2017a). In a previous study, 15 wild impalas were captured chemically and placed in a confinement (boma) to investigate welfare aspects and the effects of different immobilization and anaesthesia protocols (Zeiler & Meyer, 2017a). Total serum cortisol concentrations were measured immediately after immobilization during weeks 3, 7, 9, 11, and 13 (Zeiler & Meyer, 2017a). The sequence of events leading up to sampling in their study and ours is most similar during the initial sampling in week 3, as our study does not involve repeated captures. Consequently, we are only comparing the cortisol results from week 3 to those in our study. During week 3, the previous study measured average serum cortisol concentrations of 84.4 nmol/L (range: 21.5 nmol/L-188.0 nmol/L) (Zeiler & Meyer, 2017a). When we converted our serum cortisol results from pg/mL to nmol/L, the sampled ungulates in our study had an average serum cortisol concentration of 94.2 nmol/L (range: 22.2 nmol/L – 222.1 nmol/L). Although our study consists of several different species of ungulates, and not only impalas, this comparison indicates that our cortisol measurements in immobilized African ungulates are similar to previous publications.

During personal communication with Dr. A. C. M. Sitima, we were told that impalas in general are one of the most stressed ungulate species, while sable antelopes often are less stressed. This difference was also observed during our fieldwork. Impalas were more

explosive when approached by car, whereas sable antelopes remained undisturbed even at a closer distance. Impalas are known to be a highly stressed species, and their susceptibility to capture myopathy and other related injuries makes them a popular research focus for improving animal welfare during capture (As discussed by Zeiler & Meyer in 2017a, Zeiler in 2018, and Meyer et al. in 2008a).

5.1.2 Serum cortisol and temperature

In our study, we observed that cortisol levels and body temperature tend to increase during a stress response. Figure 12 illustrates a general correlation between serum cortisol and temperature. Specifically, ungulates with elevated body temperature also exhibited higher serum cortisol levels. However, when we examined the ambient temperature during capture (as presented in Table 2), we found that the ungulates with the highest body temperature did not necessarily experience the hottest ambient temperature. Moreover, these individuals were not the ones with the highest degree of muscle activity prior to sampling. The chemically captured impala C013 which had the highest serum cortisol levels, also had the highest body temperature (41.7 °C). However, it is important to consider that our captures were done during wintertime in Zambia, and the ungulates were therefore used to higher ambient temperatures than during these captures.

A study investigating the patterns and mechanisms of capture-induced hyperthermia in habituated and less habituated impalas with implanted miniature thermometric data loggers found that the rapid rise in body temperature is caused predominately by stress (Meyer et al., 2008a). According to this study, activity levels, the effects of drug or environmental conditions does not play quite a certain role in capture-induced hyperthermia (Meyer et al., 2008a).

5.1.3 Biochemistry

Limitations in information on baseline blood chemistry values in the ungulate species sampled in our study make the interpretation of our results challenging. Although cross-species comparison is not recommended, we have chosen to compare our results with species of both domesticated and wild African ungulates, as other suitable references are few. When comparing our measurements with the biochemistry reference values used for domesticated cattle (*Bos taurus*) and sheep (*Ovis aries*) by the central laboratory at NMBU (Appendix 6) we observe that only two individuals (C011 and C015) are within the reference range of ALT for cattle (0 U/L – 38 U/L). However as most of the individuals > 38 U/L have concentrations close to 50 U/L, the increases are not of significant value. Two impalas A002 and C010, had higher measurements of 99 U/L and 110 U/L respectively, thereby standing out from the rest of our study population. However, when comparing our measurements with previous studies on African ungulates, a study done on black faced impala (*Aepyceros melampus petersi*) immobilized by net and given haloperidol IV as a tranquilizer before sampling, the measured serum ALT concentrations was 28.8 U/L – 76 U/L (Karesh et al., 1997). Another study conducted on five different physically immobilized species of duiker (*Cephalophus spp*.) measured average serum ALT concentrations of 39.3 U/L – 91.2 U/L (Karesh et al., 1995). The ALT range obtained in our study (10 U/L – 110 U/L) is close to previous results collected from similar species, and the two higher measurements of ALT becomes less significant. When evaluating our AST measurements, seven of our ungulates had concentrations above the reference range for cattle (0 U/L – 125 U/L). Moreover, two of our ungulates, the physically captured impala (A002) and a chemically captured impala (C013), had serum AST concentrations above the reference range for sheep (0 U/L – 220 U/L). However, when comparing our AST range (32 U/L – 492 U/L) to the measurements of the black faced impalas (26.6 U/L – 430 U/L), the differences are less prominent (Karesh et al., 1997).

Only three of our chemically immobilized ungulates, one roan antelope (B004), one sable antelope (C012) and the kudu (C015) had CK concentrations < 380 U/L, the reference range of CK in cattle and sheep being 0 U/L – 355 U/L and 0 U/L – 380 U/L, respectively. Five of our captured ungulates, three impalas (A002, C010, C013) and two roan antelopes (B005, B006), had CK serum concentrations > 1,040 U/L. As the concentrations are significantly higher than the CK base values of the domesticated species, there is a high probability that our elevated values are due to capture-related reasons. However, serum CK concentrations higher than the ones obtained in this study have been documented in other species of ungulates. For example, serum CK concentrations of 1, 461, 000 U/L was measured in an Arabian oryx (*Oryx leucoryx*) with capture myopathy that had been chemically immobilized for transportation (Vassart et al., 1992). In our study, the impala (A002) that was captured physically in a boma had the highest CK levels (5,416 U/L). The impala had the longest exposure to stress because it was chased into the boma one day and fixated for sampling the next day. The roan antelope B006 had the second highest CK levels (2,844 U/L) and the highest cortisol levels of the roan antelopes (127.8 nmol/L). This ungulate had the longest total time of approximately 60 minutes as it was lost after darting before it was found, captured, transported, and then taken samples of. High CK levels can be a result of capture myopathy, as documented with the Arabian oryx, where extreme stress and excessive muscular activity leads to muscle damage and leakage of CK into the bloodstream (West et al, 2014; Vassart et al., 1992). Because both of our individuals with the highest CK levels (A002, B006) were exposed to a prolonged time of muscle activity during capture, the high measurements of CK in our study can be caused by exhaustion and stress during capture. However, the kudu C015 is an exception, because it was also chased over a long period of time during the induction phase but had the lowest CK levels in our study (128 U/L). To our

knowledge, this is the first study measuring CK levels in kudu, this makes it challenging to determine if the measurements are affected by the capture. In addition, kudus are known to be prone for excessive running during induction with opioids, putting them at risk of developing CM and hyperthermia (Kreeger & Arnemo, 2018).

The previous study on black faced impalas resulted in the range of serum creatinine concentrations of 115 µmol/L – 239 µmol/L (Karesh et al., 1997). Whereas the reference ranges of serum creatinine in cattle (75 µmol/L – 125 µmol/L) and sheep (62 µmol/L – 140 µmol/L), show lower concentrations. In our study, the highest creatinine concentration of 229 µmol/L was from a chemically immobilized roan antelope (B006). This antelope was as mentioned, lost for some time after darting. Although our highest serum creatinine measurement is above the reference values of the domesticated species, it is lower than the highest concentrations measured in the black faced impalas. Only four individuals from our study, the physically captured impala (A002), one roan antelope (B007), one impala (C010), and one sable antelope (C012) had serum creatinine levels below the maximum baseline levels of sheep (< 140 µmol/L) and is thus evaluated as low measurements.

Nine of our captured ungulates had blood glucose levels above the reference range for domesticated cattle (1.8 mmol/L – 4.3 mmol/L), and four samples (B006, B009, C013, C015) were significantly higher with concentrations > 10 mmol/L. Elevated blood glucose was also reported in the captured black faced impala (average: 9.6 mmol/L, range: 7.0 mmol/L – 13.1 mmol/L) and the five different species of duiker (range: 1.4 mmol/L – 16.6 mmol/L) (Karesh et al., 1997; Karesh et al., 1995). The elevated levels of blood glucose in this study are most likely due to the capture-induced stress and immobilization, as similar findings have been recorded in previous immobilizing studies on other ungulate species (Marco et al., 1997;

Harms et al., 2012). However, in a study measuring blood glucose in impala and blesbok (*Damaliscus pygargus phillipsi*) immobilized with etorphine, the captured blesboks had lower concentrations of serum glucose than the impalas (6.4 nmol/L – 7.7 nmol/L), ranging from 5.0 mmol/L – 5.3 mmol/L (Pfitzer et al., 2020). This shows that further research on biochemistry parameters in larger species of wild ungulates is needed, as cross-species comparison is problematic. It should be mentioned that because of variations in the time between sampling and centrifugation of our venous blood samples, the serum could have been in contact with erythrocytes longer than recommended. Prolonged contact with erythrocytes can result in lower glucose concentrations compared to blood centrifuged immediately after sampling, due to continued glycolysis in the erythrocytes (Stockham and Scott, 2008).

When interpreting the glucose levels and other biochemical blood values, it is important to consider whether there has been haemolysis in the samples (Harms et al., 2012). Haemolysis was registered in one of our samples from an impala (C010). An overestimation of AST, creatinine and CK and an underestimation of ALT, and glucose has been reported in a study on haemolysis in serum biochemistry of humans (Lippi et al., 2006). However, in a study on bovine blood chemistry parameters, haemolysis had no significant effect (Jacobs et al., 1992). As there are no data on the effect of haemolysis on the serum biochemistry of African ungulates, the presence of haemolysis in this study is difficult to assess. When comparing individual C010, the findings are different than with the registered haemolytic effects on humane serum, as the sample from C010 had the highest ALT concentrations (110 U/L) and lowest creatinine measurements (< 13 µmol/L) in our population, the AST concentration (218 U/L) being close to the average of the other chemically immobilized impalas (301 U/L). Since this individual had significantly lower creatinine concentrations than the other, and as it was the only sample with haemolysis, the creatinine measurements are likely affected by

haemolytic effects. Nevertheless, the effects of haemolysis on wildlife serum biochemistry results should be researched further for a better understanding (Harms et al., 2012). It is important to mention that the analysing methods used in this study have not been validated for African ungulates. The samples have been analysed by using humane biochemical analysing methods adapted for the interpretation of samples from domesticated species of ungulates.

5.2 Extrapolation and interpretation of wildlife samples

Although the focus on research concerning wildlife health and welfare is increasing, there is a lack of knowledge (e.g. behaviour patterns, anatomy, physiology, and blood parameters) on numerous wild species, making future investigations on wildlife challenging (Ryser-Degiorgis, 2013). Laboratory work is often based on values from domesticated species similar to the wild species being investigated, causing a risk of error in result interpretation (Ryser-Degiorgis, 2013). Results should therefore be evaluated with caution when values are extrapolated from domesticated ungulates to wild ungulates (Zeiler & Meyer, 2017a).
This lack of species-specific reference values also applies to wild African ungulates, where the impala is often used to assess the other species (Pfitzer et al., 2020; Zeiler & Meyer, 2017a). Due to their small size and availability, as well as being relatively easy to physically restrain, the impala has been used in numerous capture studies involving both physical and chemical immobilization (Meyer et al., 2008a; Meyer et al., 2008b; Meyer et al., 2010; Zeiler & Meyer, 2017a; Zeiler & Meyer, 2017b; Basson et al., 2021; Buck et al., 2017; Gerlach et al., 2017; Cheney & Hattingh, 1988; Gandini et al., 1989; Hattingh et al., 1988; Mtetwa et al., 2020). However, some of these studies have shown that impalas are naturally more susceptible to stress and the following consequences of capture and sedation (e.g. respiratory depression) resulting in a high risk of morbidity and mortality (Meyer et al, 2008a; Zeiler & Meyer, 2017a; Zeiler & Meyer, 2017b; Pfitzer et al., 2020; Chu et al., 2020; Knottenbelt, 1990). It has been shown that species of African ungulates respond differently to different

immobilizing agents (Pfitzer et al., 2020). Similar to the use of values from domesticated species, extrapolation of immobilization results and clinical parameters from one species of African ungulate to another is therefore not recommended (Pfitzer et al., 2020). However, few immobilization studies aim to establish species-specific ungulate reference values for other African antelopes (Kaufmann, et al., 2015; Hattingh, 1988; Bailey et al., 1995; Drevemo & Karstad, 1974). As an example, no other reports on blood biochemistry values from sable antelope have been found. Studies refining specific reference values require data from many individuals. Due to the high risk of morbidity and mortality when capturing wild ungulates, future research poses ethical questions (Knottenbelt, 1990; Zeiler & Meyer, 2017a). Nevertheless, knowledge on wildlife and species-specific recommendations for immobilization is needed (Ryser-Degiorgis, 2013). Additional data collection, from multiple species of African ungulates, should therefore be done simultaneously with immobilization during routine management procedures (Pfitzer et al., 2020). To estimate baseline values in wild species, the blood sampling and registration of clinical parameters should be performed as fast as possible after the animal has been immobilized (Drevemo & Karstad, 1974). Moreover, it is important that studies without prestigious findings, containing less impressive scientific data (e.g. negative results, baseline values and case reports), also get published (As reviewed in Ryser-Degiorgis, 2013). Such studies should not be neglected as they can contain important findings leading to the development of better reference values, and thereby improving future wildlife research.

5.3 Immobilization drugs impact on physiology

Etorphine was the opioid that was used during our study and was administered in all the chemically captured ungulates, with a combination of either ketamine or perphenazine to the larger ungulates. Sedatives and tranquilizers can reduce the ungulate's perception of stressful stimuli, which can have an impact on the physical parameters measured in our study like body

temperature, RR and cortisol levels. Opioids can also inhibit, slow or increase the endocrine release of cortisol, and the different effects can vary among different species (West et al, 2014; Trondrud et al, 2022). Opioids are also known to give dose-dependent respiratory depression, hypoxia and hyperthermia which make wild ungulates exposed to CM. Since we only participated in captures for translocation, where the opioids were used to induce a rapid sedation to make immobilization possible, and then reversed immediately when the ungulates were restrained and loaded on the transport, we did not have the opportunity to use pulse oximetry to measure oxygen saturation of haemoglobin or supply the ungulates with oxygen. The only way we could evaluate the circulatory and respiratory state, was to count the RR, look at respiratory pattern and measure rectal temperature during a quick clinical examination as we took the samples, before they were transported away. A continuous monitoring of the ungulates was therefore not possible and the evaluation of opioids impact on physiology parameters was difficult. However, our biochemistry results can give an indication of tissue trauma due to hyperthermia or hypoxia, but not if the opioids are the main reason for these alterations.

5.4 Evaluation of immobilization methods

During our stay in Zambia, we gained experience in both physical and chemical immobilization of wild African ungulates. Of all three capture assignments included in this study, only physical capture resulted in mortality (A001). However, it is important to mention that capture-related injuries can have a delayed presentation, as seen with capture myopathy (Kock & Burroughs, 2021). This study did not address the long-term effects and consequences of capture, as monitoring and sampling were only conducted once shortly after immobilization. However, we observed that the capture teams worked effectively to minimize the time spent per animal so that the stress responses and risk of injury or mortality were reduced as much as possible.

Concerning the immobilization methods, physical immobilization by boma proved to be an effective technique when capturing large numbers of animals simultaneously. If samples were not to be taken, capture by boma does not require as close human contact and handling compared to physical capture by net or chemical immobilization. We observed that immobilization of the physically captured impala (A002) was efficient for sampling, as the animals got into a state of freeze while being fixated. However, this sampling method would be difficult to do in the larger ungulate species without any sedation. Since the number of samples from physically immobilized animals in this study is limited, the level of stress experienced during physical immobilization and sampling is challenging to determine. As the impalas captured in the boma were pregnant, the responsible veterinarian did not want to risk any further mortality and abortion caused by stress. Thus, the number of animals captured physically in our study was lower than initially planned. This made it difficult to compare the two immobilization methods used in this study. However, this shows that animal welfare was prioritized before research. Although the capture of pregnant animals is not recommended, the physically immobilized impalas were captured during their peak reproductive period because it coincided with the most optimal weather conditions for capture in Zambia (Dr. A. C. M. Sitima, 2023, personal communication, 24 June). The veterinarian leading the capture assignments is financially responsible if any mortality occurs, this applies to both immobilization methods (Dr. A. C. M. Sitima, 2023, personal communication, 24 June). As species of African ungulates have great economic value, the responsible veterinarian needs to make several difficult decisions to ensure a successful capture.

When capturing ungulates chemically, we experienced that the darted ungulates had a lower degree of sedation than we anticipated beforehand, as additional physical fixation was always needed, and the captured animals seemed conscious. Having little experience with sampling African ungulates, and working under a tight time schedule, we managed to collect venous

blood samples as planned. After working on this project, we consider both immobilization methods used in this study as suitable for sample collection.

5.4.1 Drug protocols and degree of sedation

We experienced that both capture teams were careful when it came to the use of opioids and gave antidots as fast as possible to minimize the risk of side-effect complications and mortality. As the ungulates used in our study were captured for translocation, it was also prioritized that the animals were in a transportable condition.

Sable antelopes, roan antelopes and the kudu were given a combination of etorphine and sedatives/tranquilizers, and a reversal with naltrexone after they were loaded at the transportation vehicle. The impalas were only given etorphine and a reversal with naltrexone soon after they were captured, which means they were not calmed with any sedatives or tranquilizers during the transport to the new area. Impalas are very susceptible to respiratory depression when sedated with opioids, and losses are relatively high when etorphine is used, according to Kock & Burroughs, 2021. The immobilization handbooks by Kreeger & Arnemo (2018) and Kock & Burroughs (2021) recommend thiafentanil with a combination of medetomidine (alfa-2 agonist) as the drugs of choice when it comes to impalas, because it gives shorter induction and fewer side effects than etorphine alone. They also recommend having a dose of butorphanol available to stimulate respiration if necessary. The reason why impalas did not achieve any tranquilizers during our captures remains unclear. However, tranquilizers can also give adverse effects such as respiratory depression and thermoregulation alteration, which may exacerbate when used in combination with opioids (Kreeger & Arnemo, 2018). This could be a good reason why the capture teams decided not to give the impalas tranquilizers prior to translocation. Larger ungulates are also recommended to be immobilized with thiafentanil, but combinations with etorphine and ketamine or perphenazine

as used in this study are also described in the immobilization handbooks (Kreeger & Arnemo, 2018; Kock & Burroughs, 2021). Sedatives/tranquilizers are recommended to use with opioids to avoid excitation upon the induction phase (Kreeger & Arnemo, 2018; Kock & Burroughs, 2021). There is also a greater risk of animal and human injuries if the larger ungulates are not properly calmed.

We experienced that the ungulates in our study had a low to moderate degree of sedation. One male roan antelope (B008) was not sufficiently immobilized to sample, also after receiving three additional dosages of ketamine IM. We do not have the accurate drug dosages that were used for each species, so we cannot comment if the dosages were high or low by comparing them with the drug protocols in the handbooks. However, we know that captures and close contact with humans during handling is quite stressful for wild ungulates. This stress can lead to increased arousal, necessitating higher drug doses to achieve sufficient sedation. However, we must take into consideration that we were participating in capture assignments for translocation and not for research purposes, so a profound sedation was not necessary when the ungulates only should be handled for a short period of time to get them into the transport vehicle. Immobilizing agents, like etorphine, are expensive drugs. This can lead to a sparse usage of the drugs (Dr. A. C. M. Sitima, 2023, personal communication, 24 June). However, lowering the drug dosage can be beneficial when it comes to reducing side effects such as respiratory depression (Clarke et al., 2014).

5.4.2 Injuries and mortality related to the captures

Wildlife captures come with some risks of injuries and mortality and the captures we participated in during our study emphasized that. Two of the injuries were caused by the dart, while traumatic epistaxis was caused during the physically handling at the transport vehicle. Accurate velocity and placement of the dart are critical to avoid injury, like puncturing of

body cavities and other vital structures and bone fractures (Clarke et al., 2014). Especially small ungulates like impalas are exposed to darting injuries due to inappropriate dart placement (Kreeger & Arnemo, 2018). Dart-associated trauma often results from inappropriate use of equipment or inexperienced personnel (Clarke et al., 2014). We had one case of mortality during the physical capture in the boma. The cause of death of this impala was not clear. However, during field necropsy the preliminary diagnosis made by the veterinarian was pneumonia. Capture-related mortality is an important consequence when capturing wild African ungulates, as shown by this study and in previous studies (Zeiler & Meyer, 2017a; Chu et al., 2020).

5.5 Assessment of sampling methods - Glucocorticoids

In this study, glucocorticoids were measured by analysing serum samples. However, as previously mentioned, cortisol can also be measured by using saliva samples or through glucocorticoid metabolites (GCM) in faecal, urine or hair samples. When GCs are used as the primary indicator for stress, it is essential to be aware of the natural physiological fluctuations in the hormone levels. The secretion of GCs depends on multiple factors, such as seasonal and circadian variation, species, age, and sex (Sjaastad et al., 2016; As reviewed in Dantzer et al., 2014). Although the secretion of GCs is fluctuating, an increase in plasma secretion caused by stress will make such variations less prominent (Sjaastad et al., 2016). The concentration of GCs is also dependent on whether the animal has been exposed to episodes of stress prior to the capture (e.g. predators, periods of prolonged starvation, habitat restrictions, parasites, disease, or anthropogenic interference, etc.) (As reviewed in Dantzer et al., 2014). Additionally, in studies where re-capture and re-sampling are a part of the methodology, the possibility for the animal to develop habituation towards human exposure and sampling protocols is present. Habituation causes difficulties when extrapolating the finding to the remaining wild population. This can influence the interpretation of stress measures and is

therefore important to consider when studying wildlife stress responses (Cyr & Romero, 2009). Re-capture and re-sampling did not occur in our study, and the probability that habituation affected our cortisol measurements is low. However, as the pre-capture stress exposure of our captured ungulates is unknown, the possibility that the cortisol measurements were influenced by other factors cannot be ruled out. Nevertheless, the ungulates captured in our study were evaluated to be in good health, and we therefore believe that our serum cortisol concentrations are connected to the capture-induced stress response.

When choosing between the different methods for evaluating stress in wild ungulates, venous blood samples were prioritized due to our previous experience in collecting venous blood from domesticated species of the Bovidae family. We experienced that blood sampling by vacutainer was a reasonable technique for wild ungulates, as it could be performed quickly in the fields, resulting in a shorter handling time. Additionally, the collection of blood samples gave us the opportunity to perform biochemical analyses, giving us more information on the general state of the captured ungulates. However, as venous blood sampling is an invasive method, other GC sampling techniques could have been considered. Depending on the scope and the goals of future studies, it may be advisable to use several methods for stress evaluation to provide opportunities for comparison. Although it should be mentioned that finding correct correlations between blood GC concentrations and GC concentrations from other tissue sampling methods (GCMs), is complicated (Romero & Beattie, 2021). An example of an alternative GC sampling method is measuring cortisol from saliva, which is often seen as a less invasive method, as it only requires oral access to the selected animal. The sampling itself is relatively quick to perform, and in a study where saliva samples for measuring cortisol in sheep, 30 seconds of sampling was considered sufficient (Andanson et al., 2020). Nevertheless, when it comes to wild species, the collecting of saliva will most likely require a degree of immobilization. The method can therefore be seen as somewhat

invasive based on the animal´s size, awareness, and amount of handling. Lastly, it is important to mention that the physiology of glucocorticoids is complex. Using GCs to evaluate stress responses in wild animals cannot be done with complete certainty as the interpretation of the results heavily relies on species- and context-specific conditions (Romero & Beattie, 2021).

5.6 Strengths and limitations of the study

A significant limitation of this study was that few capture assignments were conducted during our stay in Zambia. This caused the number of sampled animals to be significantly lower than planned. The original goal was to obtain samples from at least 50 individuals of two different sized species of ungulates: impala and sable ntelope. As no separate captures were organized for this project, we were dependent on taking part in capture assignments with purposes other than research. The ungulates in our study were immobilized for translocation and sale, and the selection of animals was therefore done by the capture teams, depending on the capture assignments. This led to a variation of species being captured. Subsequently, our study consists of a smaller collection of samples from a greater variety of species, than initially planned. However, a limited sample size of 6-15 is frequently encountered in wildlife research due to logistical challenges and ethical considerations (Pfitzer et al., 2020; Zeiler & Meyer, 2017a).

Of the capture assignments used in this study, most of the sampled animals were chemically immobilized. This made a comparison of the two capture methods and following stress-responses, difficult. Since the ungulates in our study were immobilized for translocation, the capture crews had a tight schedule. We experienced that there was a short amount of time to do clinical examination and sampling. For example, at capture location C, the responsible veterinarian appointed approximately 2 minutes for us to take samples before the animals were loaded onto the transportation vehicles. Regardless of this, we experienced that taking

part in non-research capture assignments, to collect samples and clinical data, is possible. However, this way of co-working is dependent on precise planning beforehand, involving both parties, to ensure that the purpose of both groups is clarified and maintained. We experienced that a protocol combining the goals and tasks of both parties is important, mostly due to the large number of people in the field. Unexpected events need to be limited to ensure minimal variation in the study material. For example, during chemical immobilization, we were not sitting in the same car as the veterinarian who darted the animals, and the hit times were sometimes difficult to estimate accurately.

Nevertheless, we believe that future captures for sampling and research on wild ungulates should be considered as to whether they can be performed with dual purposes, done in combination with already planned capture assignments. Previous studies have shown that ungulates being captured are at a high risk of being exposed to stress-related injuries, sometimes leading to mortality (Zeiler & Meyer, 2017a; Knottenbelt, 1990). Finding a way to research captures already being done by the ranching industry should be considered as a method for minimizing the number of captures done one African ungulates.

6 Conclusion

This study participated in both physical and chemical captures of five different species of African ungulates (impala, puke, sable antelope, roan antelope, and kudu) in three different locations in the Central region of Zambia. This is, to our knowledge, the first study of capture-induced stress on wild Zambian ungulates, where the collection of data is done by participating in already planned capture assignments. We experienced that taking venous blood samples from both physically and chemically immobilized wild ungulates was possible. The blood sample results indicates that there was a correlation between cortisol levels and body temperature, with elevated cortisol levels and elevated body temperature. We found no correlation between high body temperature and high ambient temperature. Moreover, we observed that the ungulates with the highest CK measurements also had a high degree of muscle activity before sampling. During our captures, there was one incident of mortality and three incidents of capture-related injuries. This highlights that immobilizing wild ungulates is not without risks. Further research on the improvement of animal welfare during wildlife captures is needed. This research relies on the collection and publication of data related to species-specific reference values. Increased knowledge in this area can lead to improved handling and conservation of wildlife in the future.

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8 Sammendrag

Tittel: Immobilisering av ville ungulater i Zambia – en evaluering av fangstmetoder og fysiologiske parametere.

Forfattere: Eira Moen Attaei Kachouie & Ingeborg Rindalsholt

Veiledere: Anne Randi Græsli, Veterinær, PhD, Fakultet for anvendt økologi, landbruksfag og bioteknologi, Institutt for skog- og utmarksfag, Høyskolen i Innlandet.

Mette Helen Bjørge Müller, Førsteamanuensis institutt for produksjonsdyrmedisin, NMBU.

Det er et økende behov for utvikling av immobiliseringsteknikker for ville ungulater. Mange afrikanske ungulater av høy verdi holdes i nasjonalparker, verneområder og viltreservater. Immobilisering av ungulatene er ofte nødvendig for å kunne forvalte slike områder. Bevaring av ville arter innebærer et betydelig ansvar for å sikre god dyrevelferd. Det er begrenset med kunnskap om i hvor stor grad immobilisering fører til stress hos ville afrikanske ungulater. Denne studien har som mål å vurdere ulike immobiliseringsteknikker og fysiske parameter, som kroppstemperatur, serumkortisol og biokjemiske verdier hos ville ungulater i Zambia. Studien tar for seg både fysisk fangst med boma og kjemisk fangst ved bruk av bedøvelsespiler. Studiet deltok på immobiliseringsoppdrag, med andre formål enn forskning, da dyrene ble fanget for å transporteres til nye områder. Totalt 15 ungulater ble fanget, inkludert impala (*Aepyceros melampus*), sabelantilope (*Hippotragus niger*), roanantilope (*Hippotragus equinus*), puku (*Kobus vardonii*) og kudu (*Tragelaphus stresiceros*). Venøse blodprøver ble tatt fra 12 av de immobiliserte ungulater. Prøvene ble analysert for serumkortisol ved hjelp av DetectX® Cortisol ELISA-kit. Biokjemiske parameter (ALT, AST, CK, glukose og kreatinin) ble analysert ved hjelp av Atellica® CH Analyzer. Resultatene fra blodprøvene viste en sammenheng mellom forhøyede kortisolnivåer og høy kroppstemperatur, men at

omgivelsestemperatur ikke påvirker kroppstemperatur i stor grad. Ungulater med mest muskelaktivitet før prøvetaking, hadde høyest CK-verdier. Av de 15 dyrene som ble fanget i denne studien, var det ett individ som døde, mens tre som hadde fått fangstrelaterte skader. For å forbedre dyrevelferden ved immobilisering av ville ungulater, slik at tilfeller av fangstrelatert morbiditet og mortalitet reduseres, er det nødvendig med videre forskning. Det er viktig at man vektlegger artsspesifikke forskjeller i denne forskningen, slik at immobiliseringer kan tilrettelegges på best mulig måte.

9 References

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10 Appendices

Appendix 1. Capture form

CAPTURE FORM:

General:

Date: _____ Location: _____

Ambient temperature: _____

Weather conditions: _____

Barometric pressure:_____

Pre-capture observations:

First observed (time):_____

Estimated herd size: _____

Herd behavior/appearance: _____

Animal info:

Animal ID-number: _____ Herd number: _____

Species: _____ Sex: O M O F (O P)

Estimated age: _____ Estimated weight: _____

BCS: _____ Coat quality: _____

PHYSICAL IMMOBILIZATION:

Method used: _____

Start chase	
Captured	

Comments:

CHEMICAL IMMOBILIZATION:

Drug protocol: *(ml/kg)* *(Dosage)*

	mg/ml		
	mg/ml		
	mg/ml		
	mg/ml		
	mg/ml		
	mg/ml		

Dart locations: _____ Dart amount: _____

Induction: *(Time)* *(Refill time)*

Start chase				
HIT TIME				
First sign of effect				
Lays down				
Captured				

Oxygen start:_____Oxygen discontinued: _____

Immobilization degree:

Induction quality:	O smooth	O rough	
Movement:	O head	O neck	O limbs
Muscle condition:	O relaxed	O tense	O tremors
Palpebral reflex:	O absent	O present	

Pre-capture movement:

O Standing still

O Walks

O Runs

O Flight

Positioning:

O Sternal recumbency

O Right side

O Left side

Comments 1:

Clinical monitoring:

Time	HR	RR	Temp	CRT	Mucus m.	Reflexes	SpO2	Comments

Respiratory pattern: O Deep O Superficial *Comments:* _____

Lung auscultation: _____

Spontaneous cough: _____

Nasal discharge: Quantity: _____ Quality: _____

RECOVERY:

Antagonist: *(ml/kg) (Dosage) (Time)*

mg/ml			
mg/ml			
mg/ml			
mg/ml			

Recovery *(Time)*

Head up/first sign	
Stands	
Walks/Runs	
Left behind/finds herd	

Comments 2:

SAMPLES:

Nasal swabs:

O Deep O Left O Right

O Superficial O Left O Right

Venous blood: O Serum O EDTA O Heparin

Sample site: _____

Arterial blood: *(sample taken)* *(sample analyzed)*

1st arterial sample		
2nd arterial sample		

Other samples:

O Saliva

O Feces

Appendix 2. Time utilization

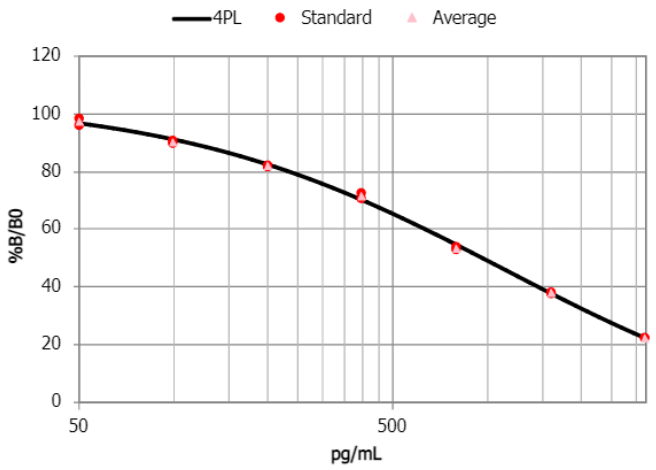
ID- number	**Handling time *(hh:mm)***	**Total time *(hh:mm)***
	(Antagonist- captured)	(Antagonist - start chase)
Roan B005	00:15	00:23
Roan B006		01:02
Roan B007	00:11	
Roan B008	00:24	00:34
Impala C010		00:07
Sable C011	00:13	00:15
Impala C013	00:02	
Average	00:13	00:28
Min	00:02	00:07
Max	00:24	01:02
Median	00:13	00:23

Appendix 3. Cortisol Elisa protocol

DetectX® Cortisol ELISA Kit protocol, Available at:

https://www.arborassays.com/documentation/inserts/K003-H.pdf

Appendix 4. Serum cortisol results



a		-12,6
b		-0,8324
c		1135
d		104,6
MSE		0,8399
R^2		0,9987
SS		5,879
SYX		1,4

Calibrator	Conc.	Wells	Raw	Raw (Average)	SEM	Backfit	Recovery %
Standard1	3200	A1	22,2	22,2	0,0485	3191	99,71
		A2	22,1			3206	100,2
Standard2	1600	B1	38,3	38	0,291	1562	97,6
		B2	37,7			1600	100
Standard3	800	C1	53,7	53,3	0,437	825,9	103,2
		C2	52,8			856,6	107,1
Standard4	400	D1	70,7	71,6	0,922	385,3	96,32
		D2	72,5			350,9	87,72
Standard5	200	E1	81,8	81,7	0,0971	204,9	102,4
		E2	81,7			207,5	103,7
Standard6	100	F1	90,4	89,9	0,485	104,7	104,7
		F2	89,4			114,7	114,7
Standard7	50	G1	95,9	97,1	1,17	54,22	108,4
		G2	98,3			36,21	72,43

Sample	Dilution	Wells	Raw	%	Conc.(pg/ml)	Conc. (Average)(pg/ml)	%CV	SD	SEM	Cortisol in nmol/L
002-A	100	A3	0,711	66,8	52010	46600	16,4	7640	5400	
		A4	0,762		41200					128,616
004-B	100,0000	B3	0,867	81,7	23740	20730	20,6	4260	3010	
		B4	0,913		17710					57,2148
005-B	100,0000	C3	0,995	88,4	8946	12730	42	5350	3780	
		C4	0,923		16510					35,1348
006-B	100,0000	D3	0,758	66,9	41990	46310	13,2	6120	4330	
		D4	0,717		50640					127,8156
007-B	100,0000	E3	0,961	90	12290	10950	17,4	1900	1350	
		E4	0,988		9603					30,222
009-B	100,0000	F3	0,705	64,5	53400	51680	4,69	2430	1720	
		F4	0,72		49970					142,6368
010-C	100,0000	G3	0,847	78,5	26640	25190	8,15	2050	1450	
		G4	0,867		23740					69,5244
011-C	100,0000	H3	0,802	73,8	33870	32770	4,73	1550	1100	
		H4	0,815		31680					90,4452
012-C	100,0000	A5	0,991	93	9319	8025	22,8	1830	1290	
		A6	1,02		6731					22,149
013-C	100,0000	B5	0,612	54,3	78980	80460	2,59	2080	1470	
		B6	0,603		81930					222,0696
014-C	100,0000	C5	0,708	68,2	52700	44070	27,7	12200	8630	
		C6	0,793		35440					121,6332
015-C	100,0000	D5	0,813	75,5	32010	30010	9,42	2830	2000	
		D6	0,838		28010					82,8276
B0		H1	1,01	100	71,57	71,57	-	-	0	
		H2	1,14		> Curve					
NSB		H5	0,047	0	14580	14430	1,47	212	150	
		H6	0,049		14280					

Appendix 5. Biochemistry results

Animal_ID	Immob. method	Species	Albumin	Albumin - elfo	Albumin-raw	Albumin globulin (ratio)	Alkaline phosphatase	ALT	Amylase	AST	CK	Phosphorus inorganic	Free fatty acids	Bile acids	Globulin	Glucose
			g/L	g/L	g/L		U/L	U/L	U/L	U/L	U/L	mmol/L	mmol/L	µmol/L	g/L	mmol/L
A002	P	Impala	28	25,2	28	0,9	50	99	101	492	5716	1,6	0,4	15	30	6,1
B004	C	Roan	40		40	1,2	100	40	25	74	152	2,4	0,4	31	34	6
B005	C	Roan	46	43	46	1,5	80	44	20	102	2335	2,4	0,2	3	31	3,5
B006	C	Roan	39	34,9	39	1,3	108	49	41	163	2844	2,3	0,4	8	31	16,2
B007	C	Roan					79	72	21	132		2,2	0,2			5,1
B009	C	Sable	37	32,9	37	1,1	78	47	22	112	477	1,5	0,5	4	33	10,5
C010	C	Impala	42		42		184	110	107	218	1047	2,4	0,2	1		8,2
C011	C	Sable	37	33,9	37	1,3	84	32	32	45	644	2,5	0	11	29	4,5
C012	C	Sable	37		37	1,1	70	40	24	32	157	2,1	0,1	5	33	4,2
C013	C	Impala	36	31,9	36	1,7	217	55	196	319	1092	2,9	0,3	22	20	11,8
C014	C	Impala	34	31,5	34	1,3	53	50	109	175	396	2,3	0,5	3	26	8,6
C015	C	Kudu	35	32,1	35	0,9	76	10	70	151	128	2,8	0,1	16	41	12,3

Animal_ID	Immob. method	Species	Potassium	Calcium	Chloride	Cholesterol	Creatinine	Lipase	Sodium	Na/K-Ratio	Tot bilirubin	Total protein	Triglycerides	Urea	Comments
			mmol/L	mmol/L	mmol/L	mmol/L	µmol/L	U/L	mmol/L		µmol/L	g/L	mmol/L	mmol/L	
A002	P	Impala	8,6	2,4	111	1	138	18	156	18,1	6	58	0,3	6,4	
B004	C	Roan	6,1	2,7	100	2	183	18	140	23,1	5	74	0,3	4	
B005	C	Roan	6,8	2,8	107	2	160	18	143	21,1	<3,0	77	0,2	5,6	
B006	C	Roan	6	2,4	101	2,1	229	18	143	23,8	8	70	1,4	4,6	
B007	C	Roan	7,5	2,5	100	2,2	113	26	133	17,7	3				
B009	C	Sable	5,3	2,2	105	1	168	22	142	26,9	8	70	0,3	4,8	
C010	C	Impala	6,8	2,4	109	1,8	<13	45	145	21,2	<3,0				Haemolysed
C011	C	Sable	6,8	2,7	104	1,4	149	15	141	20,7	<3,0	66	<0,11	3,2	
C012	C	Sable	6	2,6	104	1,3	119	19	139	23,3	<3,0	70	0,3	3,2	
C013	C	Impala	7,7	2,6	112	1,5	154	19	156	20,3	<3,0	56	1,4	5,9	
C014	C	Impala	5,9	2,3	111	0,9	150	15	152	25,7	<3,0	60	0,9	5,9	
C015	C	Kudu	6,7	2,3	104	1,8	157	22	142	21,3	<3,0	76	0,5	2,8	

Appendix 6. Reference values biochemistry, Central Laboratory NMBU

STORFE			SAU		
STORFE	ALT	0-38			
STORFE	AST	0-125	SAU	AST	0-220
STORFE	b-HBA	0-2.0	SAU	b-HBA	0-1.0
STORFE	CK	0-355	SAU	CK	0-380
STORFE	Fosfor uorg	1.4-2.7	SAU	Fosfor uorg	0.5-2.7
STORFE	Fosfor, uorg	1.4-2.7	SAU	Fosfor, uorg	0.5-2.7
STORFE	GD	0-63	SAU	GD	0-70
STORFE	GGT	0-46	SAU	GGT	0-90
STORFE	Glukose	1.8-4.3	SAU	Glukose	2.7-4.2
STORFE	Hematokrit	0.23-0.31	SAU	Hematokrit	0.31-0.43
STORFE	Hemoglobin	88-125	SAU	Hemoglobin	106-146
STORFE	Kalium	3.8-5.6	SAU		
STORFE	Kalsium	2.2-2.7	SAU	Kalsium	2.2-2.9
STORFE	Kolesterol	1.9-10.8	SAU		
STORFE	Kreatinin	75-125	SAU	Kreatinin	64-140
STORFE	Magnesium	0.7-1.3	SAU	Magnesium	0.9-1.4
STORFE	MCHC	360-400	SAU	MCHC	310-380
STORFE	MCV	40-53	SAU	MCV	21-32
STORFE	PLT	200-600	SAU	PLT	160-560
STORFE	RBC	4.6-6.9	SAU	RBC	10.9-16.3
STORFE	RDW	16-21	SAU	RDW	16-30
STORFE	Tot bilirubin	0-6	SAU	Tot bilirubin	0-4
STORFE	Totalprotein	60-80	SAU	Totalprotein	60-80
STORFE	Urea	1.7-8.4	SAU	Urea	4.7-12.2

Cattle = Storfe, Sheep = sau

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Postboks 5003
NO-1432 Ås
67 23 00 00
www.nmbu.no