Comparing the effects of tranquilisation with long-acting neuroleptics on blue wildebeest (*Connochaetes taurinus*) behaviour and physiology

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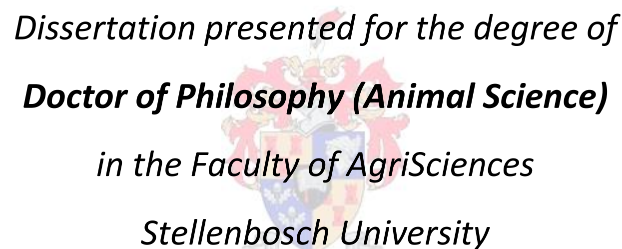

Dissertation presented for the degree of

Doctor of Philosophy (Animal Science)

in the Faculty of AgriSciences

Stellenbosch University

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December 2015

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualifications.

This dissertation includes two original papers published in peer-reviewed journals and four unpublished papers. The development and writing of the papers were the principle responsibility of myself and for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contribution of co-authors.

Date: August 2015

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SUMMARY

In South Africa, large numbers of game animals are translocated annually. These animals are subjected to a great amount of stress and the use of long-acting neuroleptics (LANs) has become a common practice to minimize animal stress. Long-acting neuroleptics suppress behavioural responses without affecting spinal and other reflexes, and can be administered in such a manner that a single dose results in a therapeutically effective tissue concentration for anywhere between three to seven days.

The mammalian stress response consists of a variety of physiological responses, and the study aimed to quantify a number of these responses in blue wildebeest (*Connochaetes taurinus*). This was done in order to compare the effects of a commonly used LAN, Acuphase® (zuclopenthixol acetate in vegetable oil), with a newly developed LAN, Acunil® (zuclopenthixol acetate in a low-release polymer), in minimizing the stress response of blue wildebeest in captivity. A human biotelemetry belt, Equivital™ EQ02, was modified to fit this species, and the results from a validation study indicated that the belt accurately measured heart and respiration rate, respectively, in blue wildebeest. The belt also measured motion accurately, and this made the monitoring of conscious animals prior to and after being treated with a LAN, possible. A faecal glucocorticoid metabolite (FGM) assay was also validated for use in blue wildebeest.

Three sets of trials were performed in which animals received one of three treatments; Acuphase®, Acunil® or a placebo in order to evaluate the effect of each. Animals were monitored for 12 hours before and 12 hours after treatment. The results showed that although both Acuphase® and Acunil® resulted in a decrease in vigilant behaviour and an increase in resting behaviour, similar results were observed when animals received a placebo. Animals treated with Acunil®, however, exhibited a decrease in explorative behaviour as well as an increase in the time they spent eating. Heart rate was unaffected by any of the three treatments, and this lack of effect by either of the LANs may potentially be due to reflex tachycardia in response to hypotension. Respiration rate was lowered by both LANs, specifically during certain behaviours, with this effect being absent in placebo-treated animals. In addition, the motion of the animals indicated that LAN-treated animals had a lowered flight response to a person entering the enclosure.

Endocrine parameters measured in the blood and faeces of the animals before and after treatment revealed a minimal effect. Neither the acute nor the chronic stress response

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appeared to be significantly reduced by treatment with Acuphase® or Acunil®. In addition, immune function (as quantified by white blood cell count and neutrophil response) revealed that the chronic stress of captivity lowered the immune response of the animals. This decrease in immunocompetence, however, could not be ascribed to any of the LAN treatments.

In conclusion, the most pronounced effects observed with the administration of both LANs included a decrease in respiration rate, and responsiveness of the animals. Long term studies on the effect of LAN administration on immune function and endocrine responses may yield more conclusive results regarding the stress responses of wild animals in captivity.

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OPSOMMING

In Suid-Afrika word 'n groot aantal wild jaarliks verskuif. Hierdie diere is onderhewig aan 'n groot hoeveelheid stres en die gebruik van langwerkende neuroleptika (LWN) is 'n algemene praktyk om stres te verminder. Langwerkende neuroleptika onderdruk gedragsresponse sonder om spinale en ander reflekse te beïnvloed en kan op so 'n wyse toegedien word dat 'n enkel dosis toediening 'n terapeuties effektiewe weefsel-konsentrasie vir drie tot sewe dae kan handhaaf.

By soogdiere bestaan die stres respons uit verskillende fisiologiese reaksies en hierdie studie het beoog om 'n aantal van hierdie reaksies in blouwildebeeste (*Connochætes taurinus*) te kwantifiseer. Dit is gedoen om die gevolge van 'n algemeen gebruikte LWN, Acuphase® (zuklopentiksol-asetaat in groente-olie) te vergelyk met 'n nuut ontwikkelde LWN, Acunil® (zuklopentiksol-asetaat in 'n stadig-vrystellende polimeer) om die voorkoms van stres by blouwildebeeste in aanhouding te verlaag. 'n Biotelemetrie gordel, Equivital™ EQ02, ontwikkel vir menslike gebruik, is aangepas om hierdie spesie te pas. Die resultate van 'n valideringstudie het aangedui dat die gordel hart- en respirasietempo's presies in blouwildebeeste kon meet. Beweging kon ook presies deur die gordel gemeet word, wat die monitering van bewuste diere voor en nadat hulle met 'n LWN behandel is, moontlik gemaak het. 'n Toets vir fekale glukokortikoïed metaboliete (FGM) is ook gevalideer vir gebruik by blouwildebeeste.

Drie stelle proewe is uitgevoer waarin diere een van drie behandelings ontvang het: Acuphase®, Acunil® of 'n plasebo. Diere is vir 12 uur voor en 12 uur ná behandeling gemoniteer. Die resultate het getoon dat, alhoewel beide Acuphase® en Acunil® tot 'n afname in waaksame gedrag en 'n toename in rusgedrag gelei het, dat soortgelyke resultate ook waargeneem is wanneer diere 'n plasebo ontvang het. Maar die diere wat met Acunil® behandel is, het 'n afname in ondersoekende gedrag en 'n toename in die tyd wat hulle aan vreet bestee het, getoon. Hartklop was nie deur enige van die drie behandelings beïnvloed nie, alhoewel die gebrek aan 'n invloed van albei LWN's moontlik aan reflekstagikardie in reaksie op hipotensie, toegeskryf kan word. Asemhalingstempo is deur beide LWN's verlaag met sekere soorte gedrag - dié effek is nie by plasebo-behandelde diere waargeneem nie. Daarbenewens het die beweging van die diere ook aangedui dat LWN-behandelde diere 'n minder prominente vlugreaksie getoon het wanneer 'n persoon in die boma ingestap het.

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Endokriene parameters gemeet in die bloed en mis van die diere voor en ná behandeling het ŉ minimale effek van die neuroleptika getoon. Nie die akute of die chroniese stresreaksie is aansienlik deur behandeling met Acuphase® of Acunil® geïnhibeer nie. Immuunfunksie (gekwantifiseer d.m.v. witbloedseltellings en neutrofielreaksies) is deur die chroniese stres van aanhouding beïnvloed, soos waargeneem in ŉ verlaagde immuunrespons. Die kompromie van immuunrespons is nie beduidend deur LWN behandeling beïnvloed nie.

In samevatting – die mees beduidende invloed van beide LWN's is waargeneem as ŉ afname in onderskeidleik die asemhalingstempo en die vlugreaksie van die diere. Langertermynstudies oor die uitwerking van hierdie LWN's op immuunfunksie en endokriene reaksies op stres kan moontlik meer insig gee in hoe diere op stres a.g.v. aanhouding reageer.

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ACKNOWLEDGEMENTS

In memory of my late husband, Mr Hayden Docking.

The life given to us by nature is short, but the memory of a life well spent is eternal
– Cicero

Your outlook upon life, your estimate of yourself, your estimate of your value, are largely coloured by your environment. Your whole career will be modified, shaped, moulded by your surroundings, by the character of the people with whom you come in contact every day – Dr Orison Swett Marden

Only once I had completed this journey, did I realise the significance of these words. There are many people to whom I would like to express my deepest gratitude for shaping me into the person and researcher I am today.

First and foremost, Prof. Louw Hoffman, for always echoing the words *carpe diem* in my mind. Without your constant encouragement and support (and often relentlessness), I would never have dreamt of completing my PhD. You truly exemplify what a great mentor should be, and I am lucky that I may also now call you my friend.

Secondly, to Dr Cobus Raath and his wife Linda, for taking me on board and giving me this once-in-a-lifetime opportunity. There are no words to convey the gratitude I have for your constant support and for welcoming me into your lives and your home. You always made sure no obstacle was insurmountable and your mentorship and encouragement shaped so much of the person I am today.

To Dr Neville Pitts, for all the supervision, guidance and long (very long) days in the lab. I have learnt so much from you and I am incredibly grateful that you agreed to be part of this project.

To Dr Martin Kidd, for all the statistical analyses. Thank you for your patience and hard work, especially with such a daunting amount of data.

To Jackie Viljoen, for all her dedication and hard work in editing the language in this dissertation. Your expertise, patience and long hours made it all come together.

To my family, especially my parents, Jacques and Esther, and my brother, Leon. I have made you climb this mountain with me like you had no choice in the matter. Lucky for

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me, you all did it with a smile. As always, you guys are my world and without your love and support, I would not be here.

To two of the most significant people in my life, Richard Williams and Katherine Laubser. You have both changed my life in such unique ways and have endured so many of my quirks and 'moments'. Thank you from the bottom of my heart for standing by me throughout this journey.

To all my friends, you know who you are. Thank you for your support these past few years. I truly appreciate your constant motivation and belief in me.

And, finally, thank you to everyone who put so much blood, sweat and tears into this project. Thank you to Louis van Wyk, Bjorn Nel, Dr Derik Venter, Michele and Geran Raath, the team at Wildlifevets.com and all the students and interns who spent many, many long hours collecting data with me over the years. I can say with absolute certainty that without your help, this project would not have been possible. Your constant optimism and enthusiasm made this project a pleasure, and I walk away from it with many fond memories. Again, there are no words to express how deeply grateful I am for the long days and late nights you spent working with me.

For the financial support, I owe my deepest appreciation to the South African National Research Foundation (NRF) and the South African Veterinary Foundation (SAVF). In addition, thank you to the University of Stellenbosch and the University of the Witwatersrand for assistance in this regard.

Lastly, but most importantly, my grateful thanks to Wildlife Pharmaceuticals SA (Pty) Ltd, without whom this project would not have been possible. Thank you for your financial contribution and the supply of many of the drugs used in this study. Thank you also for the use of the veterinary and wildlife facilities and most of all, for providing the initiative for this study.

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NOTES

This dissertation is presented in the format prescribed by the Department of Animal Sciences, Stellenbosch University. The structure is in the form of one or more scientific reviews and research papers, some of which have been published. The dissertation is prefaced by an introduction chapter with the study objectives followed by two literature review chapters. To prevent repetition between research chapters, a separate Materials and Methods chapter has been inserted so that the Materials and Methods section in each research chapter is abbreviated. The research chapters are culminated with a chapter for elaborating a general discussion and conclusions. Language, style and referencing format used are in accordance with the requirements of the African Journal of Wildlife Research. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Results and reviews from this dissertation that have been published in the following journals:

- Laubscher, L.L., Hoffman, L.C., Pitts, N.I., & Raath, J.P. (2015). Validating a Human Biotelemetry System for Use in Captive Blue Wildebeest (*Connochaetes taurinus*). *Zoo Biology,*34, 321–327. doi: 10.1002/zoo.21222.
- Laubscher, L.L., Hoffman, L.C., Pitts, N.I., & Raath, J.P. (2015). Non-chemical techniques used for the capture and relocation of wildlife in South Africa. *African Journal of Wildlife Research*, 45(2), *In Press*.

Results and reviews from this dissertation that are under review in the following journals:

- Laubscher, L.L., Hoffman, L.C., Pitts, N.I., & Raath, J.P. (under review). Behavioural and physiological responses to zuclopenthixol acetate in blue wildebeest (*Connochaetes taurinus*). *African Journal of Wildlife Research.*
- Laubscher, L.L., Hoffman, L.C., Pitts, N.I., & Raath, J.P. (under review). The effect of a slow-release formulation of zuclopenthixol acetate (Acunil®) on captive blue wildebeest (*Connochaetes taurinus*) behavior and physiological responses. *Journal of Zoo and Wildlife Medicine*.

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Articles from this dissertation that have been published in the magazine *Game & Hunt*:

- Laubscher, L.L (July, 2015). Effects of long-acting tranquilizers in blue wildebeest using modern technology – Part I. *Game & Hunt*.
- Laubscher, L.L (August, 2015). Effects of long-acting tranquilizers in blue wildebeest using modern technology – Part II. *Game & Hunt*.

Results from this dissertation that have been presented at the following conferences:

- Laubscher, L.L., Hoffman, L.C., Pitts, N.I., & Raath, J.P. (2012). The effect of tranquilization on the behaviour of blue wildebeest (*Connochaetes taurinus*) in boma captivity. Symposium of the South African Wildlife Management Association, Bela-Bela, South Africa: 16-19 September, 2012. Oral Presentation.
- Laubscher, L.L., Hoffman, L.C., Pitts, N.I., & Raath, J.P. (2014). Changes in behavioural and physiological parameters in Blue Wildebeest (*Connochaetes taurinus*) due to tranquilization with a long-acting neuroleptic. Port Elizabeth, South Africa. 31 August – 3 September, 2014. Poster Presentation.

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ABBREVIATIONS/ACRONYMS

ACTH = adrenocorticotropic hormone

ALT = alanine aminotransferase

AST = aspartate aminotransferase

AUC = area under the curve

bpm = beats per minute

CBGs = corticosteroid binding globulins

CK = creatine kinase

CL = chemi-luminescent

CM = capture myopathy

CNS = central nervous system

CRH = corticotrophin-releasing hormone

DEAT = Department of Environmental Affairs and Tourism

ECG = electrocardiogram

EDTA = ethylenediaminetetraacetic acid

EIA = enzyme-immunoassay

GCs = glucocorticoids

GDP = gross domestic product

HPA = hypothalamic-pituitary-adrenocortical

HPLC = high-performance liquid chromatography

IM = intramuscular

IUCN = International Union for Conservation of Nature

IM = intramuscular

IV = intravenous

LANs = long-acting neuroleptics

LCC = leukocyte coping capacity

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LDH = lactate dehydrogenase

LSMean = least square mean

N:L = neutrophil to lymphocyte ratio

NADPH = nicotinamide adenine dinucleotide phosphate

NAMC = National Agricultural Marketing Council

PBS = phosphate buffer solution

PCV = packed cell volume

PMA = phorbol 12-myristate 13-acetate

PNI = psychoneuro-immunology

PostT = post-treatment

PreT = pre-treatment

PST = Polar Sports Tester

RBC = red blood cell count

REML = restricted maximum likelihood estimation

RIA = radio-immunoassay

RLU = relative light units

ROS = reactive oxygen species

SABS = South African Bureau of Standards

SAM = sympatho-adrenal medullary axis

SANParks = South African National Parks

SEM = standard error of the mean

SNS = sympathetic nervous system

VCO = voltage control oscillator

VP = vasopressin

WTA = Wildlife Translocation Association

WBC = white blood cell count

μL = microlitre

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CHAPTER 1

General Introduction

1.1 BACKGROUND

Throughout South Africa there is an ever-increasing trend toward game ranching, and the sale and translocation of wildlife species in the country. The translocation of animals generates a significant amount of income, with an estimated contribution of about 16% to the total wildlife industry turnover (National Agricultural Marketing Council [NAMC], 2006). It is estimated that capture and translocation operations generate an annual income of between R750 to R900 million, with an estimated 300 000 animals being translocated annually. No exact records are however, kept so that these amounts may in fact be much higher (Dry, 2010; Dugmore, 2013; NAMC, 2006; Saayman, Van der Merwe, & Rossouw, 2011; Steyn, 2012; Van Hoving, 2011). Concurrently, there has been an increase in concern for the welfare of the animals and the minimisation of financial losses due to stress-related mortalities and injuries (Read, Caulkett, & McCallister, 2000). In South Africa, the most significant losses during wildlife capture and translocation occur as a result of capture myopathy, and according to, more animals died of capture myopathy in the last 30 years in Southern Africa than from any other wildlife disease. Animal injuries and capture myopathy are both stress-related, and it is thus in the interest of all parties involved to minimise the stress experienced by the animals before, during, and after translocation.

The use of long-acting neuroleptics (LANs) has become increasingly popular since they can induce longer-lasting sedation that can reduce animal anxiety during long-distance transportation, assist in acclimatising animals recently captured and introduced into foreign environments, and sedate animals sufficiently to enable them to cope with stressful activities that are associated with game auctions The extended action of LANs is achieved through the esterification of the active compound which is dissolved in vegetable oil, allowing for delayed hydrolysis and slow absorption into the blood (Fick, Matthee, Mitchell, & Fuller, 2006). Long-acting neuroleptics act primarily as antipsychotics by blocking dopamine receptors in the limbic system, and no specific pharmacological antidotes are available for LANs. In humans, LAN efficacy and success depend mainly on individual dosage administered, after titration of the dose to achieve

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the most effective dose regimen (Raath, personal communication, 17 April, 2013). In wildlife, however, individual dose titration is rarely possible, which complicates the establishment and quantification of potential side effects, which is likely to be higher in animals than in humans (Raath, personal communication, 17 April, 2013; Read, 2002). Side effects may include, amongst others, allotriophagia (chewing), catatonia and torticollis (abnormal head or neck positioning), with treatment of these side effects generally symptomatic in nature (Kock & Burroughs, 2012; Read, 2002).

The most commonly used LANs in the South African wildlife industry today are zuclopenthixol acetate (Clopixol Acuphase®) and perphenazine enanthate (Trilafon LA®), and both LANs are used successfully to minimise animal stress during capture and relocation in a variety of species (Fick *et al.*, 2006; McKenzie, 1993; Raath, personal communication, 17 April, 2013). Although there is no doubt about the contribution of LANs to the successful capture and relocation of free-ranging species, the effect of LANs on behavioural and physiological responses is poorly documented, with limited published research available. This is particularly true for Southern African wildlife species, and specifically for species where LANs are the most commonly used.

1.2 PROBLEM STATEMENT

The development of a new LAN for use in wildlife capture and relocation operations will greatly benefit the wildlife industry by providing an alternative to those drugs already commonly used. However, the development of such a drug requires its validation for use in wildlife species in order to show that it not only minimises animal stress but also does not cause any side effect or adverse reactions in a treated animal.

In order to evaluate the success of a LAN in minimising the stress associated with capture and translocation, it is necessary to evaluate the various components that form part of the stress response, i.e. behaviour, the autonomic nervous system and the neuroendocrine system, all of which are involved when an animal is confronted with a stressful situation (Moberg, 2001; Read *et al.*, 2000). These three components are involved in the stress response in differing ways and cause a cascade of variable changes in the body in response to stress. Each component contributes in a different way to an animal's ability to cope with stress and this makes the quantification of stress difficult since the components work together in a collaborative manner, and not each on their own.

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The aim of the study was therefore to determine the influence of a newly-developed LAN, Acunil® (Wildlife Pharmaceutical SA (Pty) Ltd., Rocky's Drift, Mpumalanga, South Africa) on the components of the neuroendocrine and autonomic nervous systems involved in stress, and the potential of Acunil® to minimize stress in blue wildebeest in captivity.

The action of Acunil®, a chemical specifically developed to allow for the more consistent slow release of the active drug ingredient, zuclopenthixol acetate, is based on the dissolution of the fatty acid ester in a 72 hour slow-release polymer, which is thought to produce a more constant and predictable release profile. The polymer has been used in other slow-release drug formulations, and was found to result in maximum serum concentrations of the active drug ingredient within 6 hours after administration. Additionally, serum concentrations of the active drug ingredient are maintained above therapeutic levels for up to 72 hours and peak drug effects are seen at 4 hours after treatment (Carbone, Lindstrom, Diep, & Carbone, 2012; Clark, Clark, & Hoyt, 2014; Foley, Liang, & Crichlow, 2011; Healy *et al.*, 2014; SR Veterinary Technologies, 2011, 2012). The aim of the study was not to investigate the exact release profile of Acunil®, but rather to determine if it provided adequate sedation in blue wildebeest. In order to show that Acunil® produces adequate long-term sedation, it was compared to with zuclopenthixol acetate (Clopixol Acuphase®) as well as a control (placebo) to establish whether the use of Acunil® or Clopixol Acuphase® will be effective as tranquilizers.

This project was executed in collaboration with Wildlife Pharmaceuticals SA (Pty) Ltd.[1] The bulk of the funding for equipment, labour, animals and research facilities was provided by Wildlife Pharmaceuticals SA, and the results from this study will be used for applications to the South African Medicine Control Council for registration of Acunil® for use in wildlife. The capture and relocation of animals used in the study were also executed by Wildlifevets.com, an affiliate company of Wildlife Pharmaceuticals SA. Further funding was received from the University of Stellenbosch, the University of the Witwatersrand, the South African Veterinary Foundation and the South African National Research Foundation (NRF).

[1] In accordance with scientific ethics, the results presented in this dissertation are reported as they were noted. As per the research agreement with Wildlife Pharmaceuticals SA (Pty) Ltd., the researcher maintains the right to publish the results as found.

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CHAPTER 2

Literature Review Part I

Quantification of the mammalian stress response

2.1 AN INTRODUCTION TO STRESS AND THE STRESS RESPONSE IN MAMMALS

The term ‘stress’ is often poorly defined in literature, with many authors struggling to make a distinction between ‘stress’ as a state of being and a ‘stressor’, which is the force that causes a disruption in homeostasis (Reeder & Kramer, 2005). Walter Cannon (Cannon, 1914) was the first to identify the body’s response to a stimulus in order to maintain what he refer to as ‘homeostasis’. Cannon called this response the ‘fight-or-flight’ response, which involved the activation of the sympatho-adrenal-medullary axis (SAM axis), after noting increases in adrenal medullary secretions in response to pain and major emotions that resulted in profound bodily changes (Cannon, 1914; Griffin & Thomson, 1998; Stott, 1981; Yousef, 1988). The concept ‘stress’ was thereafter introduced by Selye in the 1930s when he defined ‘stress’ as the non-specific response of the body to noxious environmental factors such as pathogens or a harsh physical environment (Dantzer & Mormède, 1983; Selye, 1936, 1978). He stated that the body responds in specific ways to ‘stressors’ in an attempt to return to ‘normalcy’,and named these responses the general adaptation syndrome (Dantzer & Mormède, 1983; Griffin & Thomson, 1998; Selye, 1978; Yousef, 1988). The general adaptation syndrome states that the response would be the same regardless of what the stressor is (Veissier & Boissy, 2007). For example, when the hypothalamic-pituitary-adrenocortical (HPA) axis is activated in response to any stressor that causes the body to want to return to its ‘normal’ state, regardless of what the stressor is (Selye, 1936, 1978).

Stress physiology can thus be viewed as the physiology of adaptation with physiological responses to stress, generally occurring when the homeostasis of the animal is threatened (Veissier & Boissy, 2007). Selye (1978) proposed the following stages of the stress response when an animal first encounters a stressor in the form of a novelty or

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threat (Ayala *et al.*, 2011; Griffin & Thomson, 1998; Reeder & Kramer, 2005; Yousef, 1988):

- the 'alarm reaction' characterised by an immediate activation of the sympatho-adreno medullary axis (SAM);
- a 'resistance phase' characterised by hypothalamic pituitary-adrenal axis (HPA) activation; and
- an 'exhaustion' phase during which elevated glucocorticoids begin to have deleterious effects, eventually resulting in death.

As the field of neuroendocrinology matured, the concept of a non-specific stress response was challenged, as findings suggested that the pattern of hormonal responses differed between types of stressors (Yousef, 1988). Selye's stress concept was thus followed by the idea that the greater the difference between the animal's actual environment and the ideal environment in which homeostasis is maintained, the greater the stress response that is triggered (Veissier & Boissy, 2007).

Following Selye's widely used stress concept, the concept of 'allostasis' was introduced by Sterling and Eyer (1988), referring to the modification of the functioning of an animal in response to a challenge so that the modification prepares the animal to cope better with that challenge (Veissier & Boissy, 2007). Furthermore, McEwen and Wingfield (2003) proposed two additional concepts, namely 'allostatic load' (the measure of how hard an individual must work to accomplish normal life-history tasks such as breeding) and 'allostatic overload' (the state in which energy requirements exceed the capacity of the animal to replace that energy from environmental resources) (Romero, 2004). McEwen and Wingfield (2003) also propose that the term 'stress' should only be used to refer to stimuli that require an emergency energetic response, although their new nomenclature remains controversial (McEwen & Wingfield, 2003; Romero, 2004).

According to Mills (2007), the term 'stress' essentially encompasses three related topics, namely the stimuli or changes in environment that disrupt homeostasis (referred to as the 'stressor'), the physiological and psychological responses to these stimuli (referred to as the 'stress response') and the diseases that result from an overstimulation of the physiological and psychological responses (referred to as 'chronic stress effects'). When assessing 'stress' in an individual, Johnstone, Reina, and Lill (2012) define 'stress' as the level of HPA axis activation and note that 'stress metrics' can be defined as quantifiable, physiological measurements used for estimating HPA axis activation, and that such metrics can encompass a variety of parameters related to this activation.

2.2 THE ENDOCRINE RESPONSE TO STRESS AND HOW IT IS MEASURED

The response to stress can be divided into an acute or chronic response, depending on the duration of the stressor (Dickens, Delehanty, & Romero, 2010). **Acute responses** are those that take place in response to short-term stressors or stressful events. Such responses have a definitive onset and last for only a few hours. **Chronic stress** on the other hand, is defined as either multiple, frequent and/or long-term constant exposure to stressors (Cyr & Romero, 2009). Accordingly, the hormonal response to a stressor involves the functioning of two sets of hormones that differ in terms of the time it takes for them to be released and take effect, namely the catecholamines and the glucocorticoids (Figure 2.1).

2.2.1 How does the endocrine system respond to stress

The catecholamines are responsible for the immediately initiated fight-or-flight response that is fast-acting and mediated by the sympathetic nervous system (SNS) (Dickens *et al.*, 2010). The most important of the catecholamines are epinephrine and norepinephrine, with epinephrine often reflecting psychological stress while norepinephrine is more closely correlated with the physical activity of the animal (Hattingh, 1988; Manteca, 1998; Mills, 2007; Möstl & Palme, 2002). Together, they produce the body's most rapid hormonal response to a stressor, with their release occurring within 1–2 seconds after the stimulus (Manteca, 1998). The catecholamines are produced beforehand and are stored in secretory vesicles so that they can be released rapidly by both the adrenal medulla (epinephrine) and peripheral nerve terminals (norepinephrine) of the SNS upon detection of a stressor (Mills, 2007; Reeder & Kramer, 2005). When a stressor is perceived, the paraventricular nucleus of the hypothalamus projects to the hindbrain and then to the spinal cord to activate the SNS, resulting in the secretion of the catecholamines (Reeder & Kramer, 2005). This is often referred to as the activation of the sympatho-adrenal-medullary axis (SAM axis) (Beerda, Schilder, Janssen, & Mol 1996). The SNS innervates multiple organs, including the adrenal gland, and the catecholamines generate a number of physiological effects such as increased visual acuity, increased blood pressure, increased gas exchange efficiency in the lungs, increased heart rate, and an increase in the breakdown of glycogen to release glucose stores (Dickens *et al.*, 2010; Mills, 2007; Reeder & Kramer, 2005). According to Reeder and Kramer (2005), each organ that is innervated by the SNS is dually innervated by the parasympathetic nervous system which serves to down-regulate

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the SNS (and vice versa) so that there is a dynamic balance between the two opposing and complementary systems.

The second set of hormones, the glucocorticoids (GCs), consists of the steroid hormones, cortisol (primarily relied upon by mammals and fish) and corticosterone (primarily relied upon by birds, amphibians and reptiles) (Dickens *et al.*, 2010). While GCs are typically considered as 'stress hormones', their primary role in the body is basic energy regulation (acquisition, deposition and mobilisation). It is only at high levels that they actually orchestrate the changes associated with stress (Busch & Hayward, 2009). Their release is preceded by a cascade of events starting with the detection of a stressor and the stimulation of the hypothalamus by neural signals sent from the different areas of the brain (e.g. the hippocampus and amygdala) (Manteca, 1998; Mills, 2007). At this time, the energy utilisation of the body shifts to focus energy on coping with a short-term threat to survival by curtailing long-term investments in functions such as courtship, territorial defence, reproduction, growth and/or immune defence (Busch & Hayward, 2009). This happens simultaneous to the activation of the SNS so that the paraventricular nucleus also projects to the anterior pituitary via the hypophyseal portal system (Reeder & Kramer, 2005; Sheriff, Dantzer, Delehanty, Palme, & Boonstra, 2011). The cells in the paraventricular nucleus of the hypothalamus release a number of hormones and neurotransmitters, including corticotrophin-releasing hormone (CRH) and vasopressin (VP) (Minton, 1994). Both these hormones travel to the anterior pituitary where they stimulate the synthesis and release of adrenocorticotropic hormone (ACTH) and form the first step in the activation of a list of events (Sheriff *et al.*, 2011; Von Borell, 1995). CRH also functions as a neurotransmitter in the brain so that it also activates the SAM axis when a stressor is perceived (Von Borell, 1995). In most animals, it is a combination of CRH and VP that regulates the release of ACTH although the ratio between the two hormones and their effect may vary between species (Hart, 2012; Minton, 1994). Adrenocorticotropic hormone is the principle regulator of glucocorticoid synthesis and secretion (McEwen *et al.*, 1997) and once it is released from the pituitary gland, it travels to the adrenal cortex where it stimulates the production and secretion of glucocorticoids into systemic circulation well above basal levels (Hart, 2012; Sheriff *et al.*, 2011). Because glucocorticoids (like all steroid hormones) are not stored, their production means an automatic increase in their release into the systematic and peripheral circulation (Mills, 2007). This whole cascade of events is widely known as the hypothalamic-pituitary-adrenocortical axis (HPA axis) (Griffin & Thomson, 1998; Johnstone *et al.*, 2012; Mills, 2007; Minton, 1994).

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Glucocorticoids are lipophilic, and therefore during a stress response, a large amount of GCs travel through the circulatory system attached to corticosteroid-binding globulins or CBGs (Romero, 2004; Sheriff *et al.*, 2011). In contrast to the catecholamines, the numerous steps of the HPA axis ensure that the release of GCs is much slower than catecholamines, and their effect can be seen as long as 20–30 minutes after the inception of a stressor (Mills, 2007). The physiological effects of GCs include notable changes in behaviour, increased blood glucose concentrations, inhibition of growth and reproduction, and eventually loss of body mass and a shortened lifespan (Chunwang, Zhigang, Songhua, & Yan, 2007; Mills, 2007). The most notable of these effects is the increased catabolism of protein via gluconeogenesis to produce glucose and increase blood glucose levels to provide an increased energy source for those parts of the body (e.g. muscles) that require increased energy in response to a stressor (Mills, 2007). Concurrently, GCs also cause a decrease in insulin sensitivity and an increase in fat catabolism (Reeder & Kramer, 2005). When the influence of the stressor decreases or is stopped, several negative feedback loops quickly suppress the release of GCs (Dickens *et al.*, 2010). These negative feedback loops ensure the maintenance of stable glucocorticoid levels while they also provide an emergency override in the brain in order to respond to perceived stressors. The central releasers and feedback loops of the HPA axis further interact with other hormonal control systems which may also eventually lead to some of the effects associate with chronic stress (Manteuffel, 2002).

Changes in the activity of the HPA axis are often the measurement of choice when investigating the animal stress response. However, caution must be taken when interpreting results as a number of authors have shown that the HPA axis exhibits changes caused by factors other than stressors (Ingram, Crockford, & Matthews, 1999). The HPA axis is not dormant, staying at a baseline level prior to being activated by a stressful event (Reeder & Kramer, 2005). On the contrary, it has been shown to secrete not only glucocorticoids in a pulsatile fashion with a periodicity of about 90 minutes but it also exhibits circadian (24-hour) and seasonal/circannual (yearly) rhythms of secretion (Hart, 2012; Ingram *et al.*, 1999). These rhythms are essential for the regulation of the body's energy balance, both in response to seasonal changes and in response to daily environmental changes (Reeder & Kramer, 2005; Von der Ohe & Servheen, 2002). Within a species, levels of GCs can also vary with sex, age, social status and breeding stage (Busch & Hayward, 2009).

The period during which the acute and chronic stress responses are activated, has been termed the "emergency life-history stage" (Wingfield *et al.,* 1998),and serves to suspend

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other normal day-to-day life-history functions so that the body can direct its behavioural and physiological focus to coping with the specific stressor (Bradshaw, 2007; Dickens *et al.*, 2010). This explains the suspension of the immune and reproductive function as these are deemed unnecessary for immediate survival. It is also because of this that prolonged exposure to stressors can have severe detrimental effects such as increased susceptibility to disease (Borysenko & Borysenko, 1982). In addition, the stress response itself can cause health risks such as cardiovascular problems because of the increased pressure on the cardiovascular system (Dickens *et al.*, 2010). Therefore, a consistent finding across various species has been that, whenever environmental stressors become too demanding and the individual cannot cope, its health is in danger (Busch & Hayward, 2009; Koolhaas *et al.*, 1999).

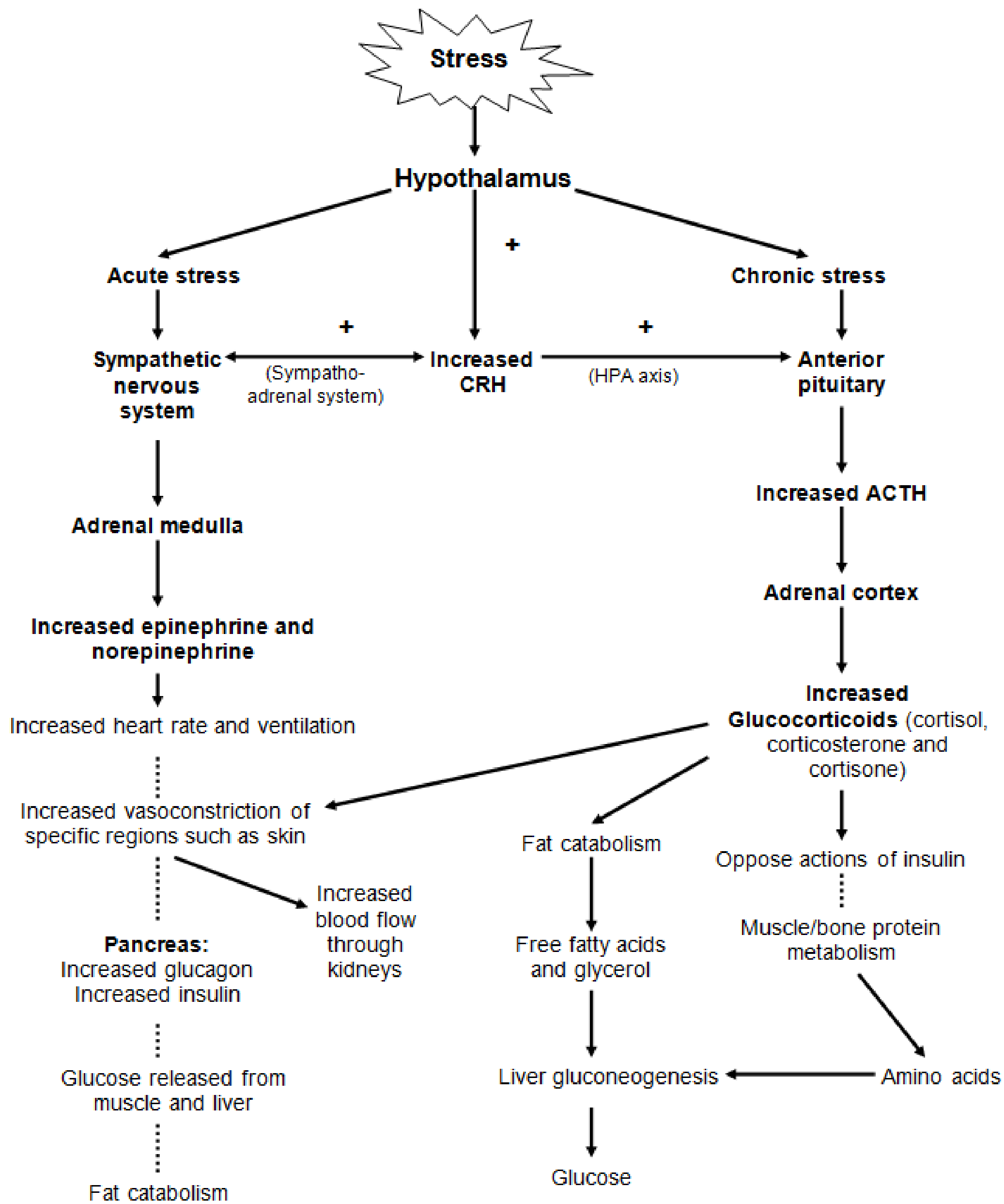

Figure 2.1 Diagrammatic representation of the stress response (Laubscher, 2009)

2.2.2 Measuring the endocrine response to stress in animals

Endocrine analyses are key to understanding the basic physiological functioning of animals and can be measured in several biological mediums including blood, urine, faeces, hair and saliva (Ganswindt *et al.*, 2012).

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2.2.2.1 Endocrinology

The discipline of field endocrinology aims to develop techniques that allow the collection of biological samples (e,g, blood, urine or faeces) from free-living animals for the analysis of various parameters such as hormones, receptors, and enzymes (Walker, Boersma, & Wingfield, 2005). The birth of this field can be traced back to as early as the 1800s although the major advancement of this field came in the 1970s when John C. Wingfield developed methods for measuring hormone concentrations in small blood samples from wild birds without killing the animals (Fusani, 2008).

According to Wingfield (1997), many factors can be classified as stressors that trigger a cascade of hormone secretions similar in all vertebrates. It is the measurement of these circulating hormones in free-living animals that will enable researchers to determine whether an individual is stressed. Both Wingfield (1997) and Walker *et al.* (2005) point out that the development of methods that quantify the physiological stress response without being debilitating to the animal itself, will not only give insight into the stress response of the animal but can also provide predictive information such as the animal's ability to tolerate or respond to a stressor.

Field endocrinology is presented with two major obstacles, namely which parameters to measure and the choice of sampling technique (Bradshaw, 2007). The former problem is complicated by the fact that, although each component of the stress response acts in concert with other components, the timescale for the actions of the various hormones is highly variable (Reeder & Kramer, 2005). Thus, concentrations of stress hormones, especially in the blood, can change rapidly in response to a stressor and thereafter, making it difficult to measure the concentrations of certain hormones accurately once samples have been taken (Johnstone *et al.*, 2012). This is especially true for hormones from the sympathetic nervous system (catecholamines), which respond almost instantly to a stressor, preparing the body for the fight-or-flight response (Mills, 2007). Concentration of these hormones increases rapidly and the hormones have a short half-life in the periphery, making sampling and measurement very difficult in wild, free-living animals (Reeder & Kramer, 2005). Hormones from the anterior pituitary on the other hand, respond to acute, long-term stressors so that they take a longer time to rise and will remain elevated for longer periods of time (Johnstone *et al.*, 2012; Reeder & Kramer, 2005; Sheriff *et al.*, 2011).

The choice of sampling technique is an important factor to consider since a number of factors will determine the suitability of a technique such as ease of sampling, implication

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to the animal, parameters that need to be measured and effect of the sampling technique on the parameters in question (Sheriff *et al.*, 2011).

2.2.2.2 Catecholamines

As previously discussed (see section 2.1.), the body's first hormonal response to stress is the activation of the SNS, which increases the secretion of the catecholamines, epinephrine and norepinephrine. Since the sampling procedure (blood sampling by venipuncture) itself is likely to elicit the secretion of these hormones, most authors advocate against the use of catecholamines as indicators of other stressors (Johnstone *et al.*, 2012). In addition, a prerequisite for the determination of the effect of a stressor on catecholamine concentrations is the determination of resting catecholamine concentrations which is problematic in animals since handling and blood sampling is likely to alter resting levels (Rulofson, Brown, & Bjur, 1988). According to Möstl and Palme (2002), data concerning catecholamine levels in animals are almost lacking with limited studies reporting on concentrations in blood samples from larger animals. However, a number of authors still use their measurement in combination with other endocrine parameters to provide a more comprehensive assessment of the stress response and the activity of the sympathetic nervous system (Althen, Ono, & Topel, 1977; Ganhao, Hattingh, Hurwitz, & Pitts, 1991; Linares, Bórnez, & Vergara, 2008; Nwe, Hori, Manda, & Watanabe, 1996).

Various analytical techniques can be used to determine catecholamine concentrations in blood samples, and include spectrophotometry, fluorometry, high-performance liquid chromatography (HPLC) and enzyme immunoassays (EIA kits) (Dehnhard, 2007; Immuno Biological Laboratories Inc., 2004; Nwe *et al.*, 1996; Rulofson *et al.*, 1988; Westermann, Hubl, Kaiser, & Salewski, 2002). Although many authors advise that blood samples should be frozen soon after sampling thus further complicating the difficulty of sampling under field conditions, both Goldstein, McCarty, Polinsk, and Kopin (1983) and Lay *et al.* (1992) report that catecholamines do not significantly degrade in plasma samples that are maintained at room temperature for a few hours. To a certain extent, the methods used for catecholamine determination are time-consuming and cumbersome and may actually be a more significant contributing factor to their limited use than the difficulty of sampling itself (Westermann *et al.*, 2002). In addition to blood sampling, catecholamine determination can also be done using urine samples if these samples can easily be collected (Beerda *et al.*, 1996; Dehnhard, 2007; Lowe, Devine, Wells, & Lynch, 2004).

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2.2.2.3 Glucocorticoids

Many authors make use of glucocorticoid (GC) concentrations as an indication of stress in animal studies since their levels remain elevated for longer periods of time (Franceschini, Rubenstein, Low, & Romero, 2008; Harper & Austad, 2001; Johnstone *et al.*, 2012; Merl, Scherzer, Palme, & Möstl, 2000; Millspaugh *et al.*, 2001; Mooring *et al.*, 2006). Indeed, Hart (2012) published a review on the advantages and disadvantages of using GCs as an objective assessment of stress in animals. The activation of the HPA axis results in the release of CRH, which stimulates the secretion of ACTH and, in turn, the secretion of glucocorticoids (cortisol in large mammals) in the blood with levels remaining elevated for up to several hours (Sheriff *et al.*, 2011). Glucocorticoids in response to stress can be measured directly from circulating peripheral blood, saliva, keratin, faeces or urine with blood and faecal samples being the most popular in animal studies (Johnstone *et al.*, 2012). Both have their advantages and disadvantages, and it is only recently that faecal glucocorticoids have become increasingly popular in animal field endocrinology studies (Tarlow & Blumstein, 2007).

Although measuring GCs holds numerous advantages over the use of catecholamines, a number of factors need to be kept in mind when interpreting their concentrations as an indication of the stress response. Firstly, GC secretion occurs in a pulsatile fashion that exhibits consistent ultradian rhythms (< 24-hour cyclicity) in almost all mammals as well as circadian rhythms (> 24-hour cyclicity) and seasonal rhythms in many species (Hart, 2012). Other factors that may influence GC levels include age, sex and reproductive status so it is important to ensure GC concentrations are measured over a series of similar individuals at the same time of day and within the same season (Tarlow & Blumstein, 2007).

Whenever discussing the use of GCs, it is important to distinguish between ‘free’ and ‘bound’ GCs and to report whether total, ‘free’ or ‘bound’ levels had been measured. In most species, a large proportion of the circulating GCs are bound to corticosteroid-binding globulin (CBG) so that it is thought that only ‘free’ unbound GCs are able to diffuse out of the capillaries and to reach their target tissues since CBG is too large to leave the capillaries under normal conditions (Johnstone *et al.*, 2012; Sheriff *et al.*, 2011). At high concentrations of GCs, such as during stress or ACTH stimulation, the amount of free GCs can increase to as much as 20–30% (Mormède *et al.*, 2007). According to Sheriff, Krebs, and Boonstra (2010), blood sampling not only provides total GC concentrations but also the amount that is free if the CBG levels are measured and the

binding coefficient is known. However, numerous authors have reported on the invasiveness of blood sampling to the extent that capture, handling and bleeding can result in rapid increases in circulating GCs within as little as three minutes (Hart, 2012; Sheriff *et al.*, 2010).

2.2.2.4 Invasive methods

Blood sampling is the most invasive method used to measure GCs in live animals although it is also the most popular (Førss & Munksgaard, 2008). As mentioned, the sampling procedure itself may elicit increases in plasma GCs although only a small amount of blood sample is required to make an accurate measurement (as little as 25–50 µl) (Tarlow & Blumstein, 2007; Wingfield, 1997). When blood samples are used, venous blood is usually taken although arterial blood has also been used (Sheriff *et al.*, 2011). The samples need to be cooled within 24 hours and cannot be stored at room temperature for prolonged periods of time (Mormède *et al.*, 2007). It is also important to note that samples that are defrosted long before being processed may have lower levels of GCs than at the time of collection (Mormède *et al.*, 2007). Both plasma and serum can be used to give the same results and GCs have been shown to be stable in both for very long periods of time if held at -20 °C (Sheriff *et al.*, 2011). Immunoassay is the most common method for measuring GC concentrations with the most frequently used being radio-immunoassays (RIA) and enzyme-immunoassays (EIA) (Ingram *et al.*, 1999; Nwe *et al.*, 1996; Yoshioka, Imaeda, Torimoto, Ohtani, & Hayashi, 2004). Sheriff *et al.* (2011) published a thorough review on the different techniques used to quantify GCs in wildlife as well as the particulars of each laboratory technique.

2.2.2.5 Non-invasive methods

During the past decade, determination of faecal GC metabolites (FGMs) has become an increasingly popular method amongst researchers investigating endocrine responses in animals. Schwarzenberger (2007) published an extensive review on the different uses of FGMs in different zoo and wildlife species, noting that the most important advantage of this technique is that it can be done non-invasively. In fact, numerous authors have investigated the use of FGMs in both domestic and wild species based purely on the non-invasive advantage FGMs holds (Franceschini *et al.*, 2008; Millspaugh *et al.*, 2001, 2002; Stubsjøen *et al.*, 2009; Von der Ohe & Servheen, 2002).

Glucocorticoids are metabolised chiefly in the liver although metabolism also occurs in the kidneys, adrenals, placenta, connective tissues, fibroblasts and muscles (Touma & Palme, 2005; Von der Ohe & Servheen, 2002). After extensive metabolism, a variety of GC metabolites are excreted in the faecal matter with little or no parent hormone remaining (Chinnadurai *et al.*, 2008). Because species-specific steroid metabolism and gut microflora can cause the assortment of FGMs to differ between species, it is important to validate the assays used to measure FGMs for a specific species (Chinnadurai *et al.*, 2008; Touma & Palme, 2005; Wasser *et al.*, 2000). This can be done through pharmacological administration of ACTH to stimulate adrenal hormone production or dexamethasone to supress adrenal function and then determining whether an assay is sensitive enough to detect FGM changes (Sheriff *et al.*, 2011; Touma & Palme, 2005; Wasser *et al.*, 2000). Alternatively, as may be the case with wildlife, biological validation can be accomplished by exposing animals to distinct stressful stimuli such as capture, and detecting changes in FGMs prior to capture and thereafter (Chinnadurai *et al.*, 2008). Touma and Palme (2005) published a comprehensive review on the importance of assay validation when measuring FGMs in mammals and birds, and compiled a list of the validation techniques used for various species (Touma & Palme, 2005).

It is assumed that only 'free' GCs are metabolised and thus the FGMs reflect the 'free' GC fraction of the total GCs (Sheriff *et al.*, 2011). As a result, faecal samples provide an integrated hormone profile over time with less interference from acute stressors (Sheriff *et al.*, 2010). In other words, FGMs reflect an average level of circulating GCs over a period of time rather than a point sample (as with a blood sample), and thus provide a more accurate assessment of long-term GC levels (Millspaugh & Washburn, 2004). A second advantage of FGMs is that they are an accurate reflection of an animal's physiological state and thus its ability to respond to a stressor (Sheriff *et al.*, 2010). Lastly, FGMs decrease the variability associated with blood samples from diurnal and pulsatile secretory patterns due to the pooling effect of the FGMs in a sample (Von der Ohe & Servheen, 2002). However, this technique is not without disadvantages and a number of factors can complicate results. Since GC secretion into urine is greater and more rapid than secretion into faeces, results may be confounded if faecal samples are contaminated by urine (Johnstone *et al.*, 2012). Other sampling issues and assay artefacts that may affect results include sample age, time of day the sample was obtained, size of the sample and storage techniques (Millspaugh & Washburn, 2004). Von der Ohe and Servheen (2002) reviewed a number of animal-specific factors that

may also influence results, and considered factors such as sex differences, reproductive status, diet and adaptation to repeated stressful events that may cause obscured results.

When faecal samples are taken, it is important to preserve them as soon as possible (usually by freezing) to prevent microbial or bacterial degradation of FGMs (Hunt & Wasser, 2003). Both wet and dry samples can be used but it is important to remove all undigested material to prevent large differences due to diet (Palme, Touma, Arias, Dominchin, & Lepschy, 2013). Complete homogenisation of the sample, rather than taking a sub-sample, will distribute FGMs more uniformly and ensure a more accurate estimation of FGM levels (Sheriff *et al.*, 2011). After methanol extraction, FGMs can be determined using either EIAs or RIAs (Mormède *et al.*, 2007). Sheriff *et al.* (2011) discuss the advantages of each method and note that both are equal in popularity in a variety of species.

2.2.2.6 ACTH

Numerous authors have used ACTH either on its own or in conjunction with GCs as an indication of the activation of the HPA axis in response to stressors (Andrés, Martí, & Armario 2007; Hattingh, Wright, De Vos, *et al.*, 1984; Knights & Smith, 2007; Mormède *et al.*, 2007; Van Reenen *et al.*, 2005; Von Borell, 2001). Mormède *et al.* (2007) suggested that the ACTH response may be more sensitive to the severity of a stressor than the GC response since dose–response studies have shown that the increase of plasma ACTH levels is much more graded than plasma GCs with stimulus intensity. Circulating levels of ACTH can be measured in plasma samples using commercially available RIA kits that need to be validated for use in the specific species, and this assay has been used successfully in various large animal species (Andrés *et al.*, 2007; Ayala *et al.*, 2011; Fazio, Medica, Cravana, Aveni, & Ferlazzo, 2013; Gupta, Earley, Ting, & Crowe, 2005; Knights & Smith, 2007). Adrenocorticotropic hormone is a relatively small protein hormone that is subject to rapid degradation so that samples should be taken in iced tubes or at least put on ice immediately after sampling until being centrifuged (Andrés *et al.*, 2007; Gupta *et al.*, 2005; Reeder & Kramer, 2005). Samples should be centrifuged and the plasma portion frozen as soon as possible after sampling if the assay is to be done later (Reeder & Kramer, 2005). Sampling tubes should contain ethylenediaminetetraacetic acid (EDTA) as an anticoagulant since heparin can interfere with the assay. It is furthermore important not to thaw and re-freeze samples repeatedly as this can lead to erroneous results (Mormède *et al.*, 2007; Reeder & Kramer, 2005).

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2.2.2.7 Blood composition changes in response to the endocrine stress response

As previously discussed, the activation of the SAM and HPA axis results in hormonal secretions that cause a cascade of events aimed at equipping the animal to cope (refer to Figure 2.1). Part of this cascade of events is a change in general substrate utilisation resulting in notable changes in blood composition (Hattingh & Petty, 1992). These changes include changes in total protein, glucose, lactate and total lipid levels in blood so that significant increases can be related back to the severity of the stress response (Hattingh, Wright, De Vos, Levine, *et al.*, 1984). Indeed, it is common amongst animal researchers to measure these variables in conjunction with hormonal responses to give a better indication of the stress response (Abeyesinghe, Goddard, & Cockram, 1997; Grigor, Goddard, Littlewood, & Macdonald, 1998; Knox & Hattingh, 1992; Säkkinen *et al.*, 2004; Stull & Rodiek, 2000).

Glucose and lactate are the most commonly used indicators of the activation of the HPA axis, as they are significantly affected due to increased breakdown of glycogen from the liver or the depletion of glycogen reserves from the skeletal muscle to provide a source of energy to the animal (Humann-Ziehank & Ganter, 2012; Minka & Ayo, 2009). For whole-blood glucose and lactate levels, it has been found that hand analysers used for human self-monitoring are suitable for detecting changes in animal blood glucose and lactate levels (Peng *et al.*, 2010; Rumsey, Kahl, & Elsasser, 1999; Zapata, Gimpel, Bonacic, & Bas, 2004). This is especially useful under field conditions since whole-blood glucose and lactate levels have been shown to decrease and increase, respectively, over time once the sample has been taken so that analyses need to be done as soon as possible (Peng *et al.*, 2010).

Blood protein consists of albumin and globulin, which are commonly measured together as total protein (Doumas, Bayse, Carter, Peters, & Schaffer, 1981; Hattingh *et al.*, 1983; Kingsley, 1939). A number of laboratory methods can be employed to measure total protein in serum with the spectrophotometric procedure based on the Biuret method, described by Kingsley (1939) and Gornall, Bardawill, and David (1949), being one of the most common (Doumas *et al.*, 1981; Laborde, Chapa, Burleigh, Salgado & Fernandez, 1995; Luca & Reis, 2004). Other spectrophotometric procedures include the Lowry and Coomassie Blue methods (Luca & Reis, 2004). Peters (1968) published an overview of the most popular methods used in clinical studies to measure total protein. Laborde *et al.* (1995) found that total protein levels in serum samples were affected by storage time

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when refrigerated and frozen so that samples should ideally not be stored for longer than 80 days before analysis.

2.3 THE IMMUNE RESPONSE TO STRESS AND THE WAY IT IS MEASURED

The relatively new interdisciplinary field of psychoneuroimmunology (PNI) was developed to investigate the interaction between the central nervous system (CNS), the endocrine system and the immune system, since the presence of hormones, neurotransmitters and receptors common to all three systems supports the view that communication exists between the three systems (Borysenko & Borysenko, 1982; Jordan, 2005; Padgett & Glaser, 2003; Vedhara, Fox, & Wang, 1999; Von Borell, 1995).

2.3.1 How does the immune system respond to stress?

Although there is overwhelming evidence linking stress and reduced immune function (Manteuffel, 2002), the modulation of the immune system by the CNS is not yet fully understood, as it is mediated by a complex network of signals that function in bi-directional communication between the nervous, endocrine and immune systems (Padgett & Glaser, 2003). Thus, not only does the CNS affect immune function but evidence reveals that the immune system influences brain functions as well, including structures involved in emotional processes (Boissy *et al.*, 2007).

The mechanisms by which the brain modulates the immune system involve both the HPA axis and the sympathetic nervous system (SAM) (McLaren *et al.*, 2003). To a large extent, modulation of the immune reaction in response to stress is controlled by the HPA axis (Manteuffel, 2002). In response to chronic stress, the hypothalamus secretes corticotrophin-releasing hormone, which subsequently stimulates the secretion of ACTH from the anterior pituitary. ACTH in turn induces the expression and secretion of glucocorticoids (GC) (cortisol in mammals), which function together with the nervous system to bring about an array of changes that assist the body in adequately responding to the stressor (Minton, 1994; Mormède *et al.*, 2007). Amongst other things, GCs also regulate a variety of immune-cell functions such as modulating cytokine expression, chemo-attractant expression, adhesion-molecule expression and immune-cell trafficking, proliferation, differentiation and effector function (Padgett & Glaser, 2003; Salak-Johnson & McGlone, 2007). The overall effect of increased circulating GCs is the suppression of immune function due to the anti-inflammatory activity of the GCs related to the down-regulation of interferon-γ (IFN-γ) (a cytokine critical for innate or adaptive

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immunity) and other pro-inflammatory cytokines (Amadori, Stefanon, Sgorlon, & Farinacci, 2007). This effect however, is not one-directional, and regulatory cytokines produced during infection, injury or inflammation can also stimulate the release of GCs from the pituitary–adrenal axis (Amadori *et al.*, 2007; Salak-Johnson & McGlone, 2007). In addition to GCs, ACTH appears to modulate the immune response via either direct or indirect effects at several levels (Manteuffel, 2002).

It is well known that activation of the HPA axis happens concurrently with the production of the catecholamines from the adrenal medulla in response to a stressor (Minton, 1994). In addition to the regulation of the immune function by GCs associated with stress, the catecholamines appear to modulate a range of immune functions. These include cell proliferation, cytokine and antibody production, cytolytic activity and cell trafficking so that – as part of the catecholamine's function of preparing to give the body a 'boost' in order to deal with a threat, they also up-regulate the functioning of the immune system to prepare the body for possible inflammation, infection or injury (Padgett & Glaser, 2003). However, after prolonged stress, evidence suggests that the immune-inhibiting effects of the GCs on leukocyte proliferation outweigh the promoting effect of catecholamines so that chronic stress has an inevitably negative effect on the immune response (Borysenko & Borysenko, 1982). Although the above review gives a simplified overview of the connection between the CNS and the immune system, this connection is in fact extremely intricate, and detailed reviews of the different links between stress and immune function have been published by a number of authors (Amadori *et al.*, 2007; Apanius, 1998; Kelley, 1980; Salak-Johnson & McGlone, 2007; Spencer, Kalman, & Dhabhar, 2001; Webster-Marketon & Glaser, 2008).

It is this link between stress and immune function that has led researchers to believe that measures of immune function can be used as a metric for stress (Johnstone *et al.*, 2012). Indeed, numerous studies have been performed in animals to investigate the effect of different stressors on the immune response of animals as well as the types of stressors that are particularly detrimental to immune function (Hulbert *et al.*, 2011; Minton & Blecha, 1990; Stanger *et al.*, 2005; Stull & Rodiek, 2000). Stressors that have been identified in domestic species include weaning, housing densities, handling, pen size and an array of other management practices (Carroll & Forsberg, 2007; Donovan, Jackson, Colahan, Norton, & Hurley, 2007; Fell, Colditz, Walker, & Watson, 1999; Geverink, Schouten, Gort, & Wiegant, 2002; Grasso *et al.*, 1999; Hanlon, Rhind, Reid, Burrells, & Lawrence, 1994; Kick, Tompkins, Flowers, Whisnant, & Almond, 2012). Kelley (1980) published a comprehensive review of stressors that have been shown to affect immune

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function in domestic animals. In addition, similar studies have been performed in wildlife species as well. As early as 1982, Rehbinder, Edqvist, and Lundström (1982) investigated the effect of different management stressors on a number of pathological, endocrinological and immunological parameters in reindeer (*Rangifer tarandus*). With regard to immune function, the authors report that capture and handling stress had a marked effect on the activation of the immune response and that prolonged stress had significant detrimental effects on the immune system. Other authors who have looked at the effect of capture and handling practices on immune responses in wildlife include Hanlon *et al.* (1994), Grasso *et al.* (1999), Marco and Lavín (1999), Montané *et al.* (2003) and Teixeira, Deazevedo, Mendl, Cipreste, and Young (2007). In South Africa, Kruger *et al.* (2010) developed a field assay for the evaluation of neutrophil function in white rhinocerosin response to capture and translocation. Kruger *et al.* (2010) intended to develop a field assay that could be used to evaluate the stress response of the rhinoceros to be able to predict adaptability problems such as decreased fertility and susceptibility to disease following translocation.

2.3.2 Measuring the immune response to stress in animals

The most numerous of the immune system cells are the various types of white blood cells, collectively known as leukocytes. Most mammals have five types of leukocytes, namely neutrophils, basophils, eosinophils, monocytes and lymphocytes, each with their own immunological function in immune defence (Davis, Maney, & Maerz, 2008; Windmaier, Raff, & Strang, 2004). As explained earlier, the stress response can alter the immune response and thus changes in the leukocyte profile of an animal may give a good indication of the effect of a stressor on such animal. Indeed, differential leukocyte cell counts from whole blood smears have been used in a variety of animal studies to evaluate the immune response to stress (Davis *et al.*, 2008; Gelling, McLaren, Mathews, Mian, & MacDonald, 2009; Hulbert *et al.*, 2011; Marco & Lavín, 1999; McLaren *et al.*, 2003; Mentaberre *et al.*, 2010; Montané, Marco, & Manteca, 2002; Zapata *et al.*, 2004).

According to Davis *et al.* (2008), neutrophils and lymphocytes make up the majority (up to 80%) of leukocytes in most animals,with neutrophils being the primary phagocytic leukocytes. Neutrophils proliferate in response to infection, inflammation and stress, while lymphocytes are involved in an array of other functions such as immunoglobulin production and modulation of the immune defence. According to Davis *et al.* (2008), leukocyte profiles can be extremely useful in the field of stress physiology as they are altered by stress and can be directly related to stress hormone levels. According to

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Borysenko and Borysenko (1982), lymphocytes have been demonstrated to have membrane surface receptors for a number of stress hormones that can either stimulate the proliferation and differentiation of immature cells or can have a distinct inhibitory effect on mature cells. Specifically, though, the changes brought about by stress result in an increase in the number of neutrophils (also known as neutrophilia) and a decrease in lymphocyte numbers (also known as lymphopenia) (Davis *et al.*, 2008). As a result, in mammals, the ratio of neutrophils to lymphocytes (N:L) is often used as an indication of circulating stress hormones and thus as an index of the stress response (Johnstone *et al.*, 2012; McLaren *et al.*, 2003). In a variety of species, treatment of animals with stress hormones has shown to induce significant alterations in leukocyte counts within 1–2 hours (Davis *et al.*, 2008). However, Johnstone *et al.* (2012) warn that in wild animals, the relationship between the N:L ratio and stress hormones is less clear and animals may respond differently to different environmental stressors so that the endocrine response should also be measured parallel to the N:L ratio. Davis *et al.* (2008) published a full review on the exact effects of stress hormones on the N:L ratio and on the various animal studies that have used this ratio to evaluate stress. Davis *et al.* explain that GCs cause a redistribution of lymphocytes from the blood to other bodily compartments while simultaneously causing an influx of circulating neutrophils from bone marrow. This is thought to ensure that the different immune cells are routed to where they are needed the most during the immune response (Dhabhar, Miller, McEwen, & Spencer, 1996).

Another relatively new technique for measuring neutrophil function is the use of a whole-blood chemi-luminescent (CL) assay. Although the use of this assay in humans has been around for a number of years (Thomas, Sanford, Driscoll, & Casto, 1988), its validity for use in animals has only recently received attention (Papp & Smits, 2007). The procedure takes advantage of the fact that an oxidative response, known as a respiratory or oxidative burst (McLaren *et al.*, 2003), occurs in phagocytic cells that are actively phagocytising particles (Thomas *et al.*, 1988). During a respiratory burst, oxygen uptake by leukocytes is accelerated in order to produce oxygen-free radicals (also known as reactive oxygen species or ROS) that destroy bacteria (McLaren *et al.*, 2003). Agonists that have been known to cause leukocytes to produce ROS include the activation of protein kinase-C with phorbol myristate acetate (PMA), bacterial peptides binding to receptors on their cell membranes as well as stress (Kruger *et al.*, 2010; McLaren *et al.*, 2008). In studies by Ellard, Castle, and Mian (2001), McLaren *et al.* (2003) and Gelling *et al.* (2009), the latter has been shown to have a marked effect on the respiratory burst activity of leukocytes. When leukocytes, particularly neutrophils in mammals, are

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stimulated, they produce ROS by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex and myeloperoxidase (Papp & Smits, 2007). It is believed that the stress-related release of these ROS may be a mechanism to prepare the body for injury and thus potential attack by bacteria (Gelling *et al.*, 2009). ROS are energetically costly to produce and thus, following a stressful event, there is usually a period when neutrophils reduce their usual respiratory burst response to a bacterial challenge with the result that animals are immuno-supressed (Gelling *et al.,* 2009). Thus, the ability of an animal's neutrophils to produce, *in vitro*, respiratory bursts in response to a bacterial challenge after a stressful event (defined as the 'leukocyte coping capacity' or LCC) has been used as measure of stress (Kruger *et al.*, 2010; McLaren *et al.*, 2003). The bacterial challenge can be stimulated chemically in whole-blood samples with the addition of PMA, which will induce respiratory bursts of ROS by neutrophils (Gelling *et al.*, 2009). Luminol (5-amino-2,3-dihydro-l,4-phthalazine-dione) is then added to the sample which amplifies the amount of light emitted by the respiratory burst and this light emission can then be measured with a luminometer (Kruger *et al.*, 2010; Papp & Smits, 2007). The LCC can be compared with values of non-stimulated blood, which are indicative of the individual's basal response level of the immune response (Gelling *et al.*, 2009). It has been concluded that animals with a high LCC will have a good capacity to produce a response against a bacterial challenge and thus an animal's LCC can give a good assessment of an animal's current physiological status and its ability to respond adequately to a stressor (Gelling *et al.*, 2009; McLaren *et al.*, 2003).

2.4 THE EFFECT OF STRESS ON PHYSICAL ANIMAL RESPONSES AND WAYS OF MEASURING THESE RESPONSES

According to Leiner and Fendt (2011), the animal stress response consists of patterns of behavioural and physical changes which can be species and individual specific. Physical changes that may occur in response to stress include changes in heart rate, respiration rate, and blood pressure as the body attempts to return to homeostasis (Stott, 1981).

2.4.1 The effect of stress on heart rate

The most notable of the physical responses is heart rate, which forms an invariable component of the stress response and has been shown in animals to be increased by a number of stressors, including noise, unpleasant experiences, unexpected stimulations

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and exposure to novel environments (Waynert, Stookey, Watts, & Waltz, 1999). Physiological responses associated with acute stress include the activation of the sympathetic nervous system so that catecholamines are secreted from the adrenal medulla, resulting in a noticeable increase in heart rate and respiration rate (Van Reenen *et al.*, 2005). In agreement with this, a number of authors have reported the normal heart rate of unstressed cattle to vary between 70 and 90 bpm (beats per minute) with an increase of between 30 and 40 bmp during stressful situations (Herskin, Munksgaard, & Kristensen, 2003; Lay *et al.*, 1992; Lefcourt, Kahl, & Akers, 1986; Waynert *et al.*, 1999). Jansen *et al.* (2009) found the heart rate of horses during the initial stage of their trial to be between 40 and 76 bpm when walking and to increase to between 83 and 122 bpm when trotting. Some authors have also found that an increase in heart rate due to a stressful stimulus correlates well with known behavioural responses, validating the use of heart rate as an indication of the stress response (Leiner & Fendt, 2011; Van Reenen *et al.*, 2005).

Generally, heart rate is believed to produce a good reflection of stress experienced as well as the severity of the stressful stimulation or situation (Schwartzkopf-Genswein, Booth-McLean, McAllister, & Mears, 2005). According to Visser *et al.* (2002), an increase in heart rate is just as likely to result from emotionality as from physical activity during the fight-or-flight response (Jansen *et al.*, 2009). Waas, Ingram, and Matthews (1999) found an expected increase in the heart rate of red deer during herding, which was explained as the result of exercise. However, Waas *et al.* also found secondary peaks in heart rate during physically un-taxing activities such as loading and offloading, and explained these peaks to be the result of the animals' emotional response to these aversive events.

2.4.2 The effect of stress on respiration rate

In response to acute stress, increased lung ventilation can be expected through the stimulation of the brain's breathing centres and the dilation of airways by epinephrine (Windmaier *et al.*, 2004). This results in an increased respiration rate or panting, a reaction that may be initiated in response to fear or the onset of a perceived threat (Gregory & Grandin, 1998). Additionally, depending on the stressor, the effect on the respiration rate may be determined by the animal's adaptation technique in an attempt to maintain homeostasis (Windmaier *et al.*, 2004). For example, high ambient temperatures may result in an increased respiration rate as part of the effort to dissipate body heat, while low environmental temperatures may have the opposite effect (Marai,

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El-Darawany, Fadiel, & Abdel-Hafez, 2007). According to Gregory and Grandin (1998), an extremely stressful situation, such as capture and constraint, may even cause some animals to go into an inactive catatonic state where the respiration rate is drastically subdued. Concurrent with the effect of stress on respiration, other aspects of the stress response may also be affected, such as heart rate, body temperature and blood variables (for example cortisol and catecholamines) (Barnett & Hemsworth, 1990; Stott, 1981). As a result, it may be just as relevant to measure respiration rate in animals in addition to more traditional parameters as a way of ascertaining the extent and type of stress response elicited.

According to Samson, Dumont, Specq, and Praud (2011), only limited research has been conducted on recorded respiration in non-sedated animals, mainly due to the limitation of available telemetric devices that are able to measure respiration rates in ambulatory animals. However, some authors have published literature on the respiration rate of certain domestic species, largely in conjunction with measurements of heart rate and/or other parameters such as behaviour. Hales (1969) investigated the effect of heat stress on the respiratory activity and body temperature of oxen and sheep and reported excessively high breaths per minute for heat-stressed animals as a result of panting. Andrade, Orihuela, Solano, and Galina (2001) used respiration rate, heart rate and blood cortisol levels to investigate the effect of handling procedures on the stress response of cattle and found that respiration rates decreased when animals were restrained after handling and that this decrease was greater if the animals were wearing masks than without masks.

2.4.3 The effect of stress on blood pressure

As previously described (see section 2.1.), the neural response to stress involves the release of catecholamines from the adrenal medulla through stimulation of the sympathetic nervous system (Mooring *et al.*, 2006). Concurrently, cortisol release from the adrenal cortex has permissive actions on the reactivity of muscle cells that surround blood vessels to epinephrine and norepinephrine (Windmaier *et al.*, 2004). As a result, the stress response results in increased vascular reactivity and an increase in blood pressure and heart rate in order to maximise the amount of circulating glucose in preparation for as well as the execution of the fight-or-flight response (Manteuffel, 2002). These changes in blood pressure in response to a stressor may thus be used as an indicator of the activation of the stress response and has been used in a number of studies (Peers, Mellor, Wintout, & Dodic, 2002; Gamallo, Alario, Gonzalez-Abad, &

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Villanua, 1992), alongside heart rate since an increase in heart rate is accompanied by an increase in blood pressure (Von Borell, 1995). In fact, it has been suggested that heart rate and blood pressure may give a more sensitive assessment of the immediate effect of a painful treatment on an animal than cortisol itself (Mellor & Stafford, 2004). In this regard, Peers *et al.* (2002) report significant increases in systolic, diastolic and mean arterial blood pressure in lambs in response to painful management procedures such as tail docking. Since blood pressure may also be affected by the administration of various pharmaceutical drugs used to minimise stress in animals, a number of authors also report on the effect of numerous tranquilisers and anaesthetics on the blood pressure of different species (Citino, Bush, Grobler, & Lance, 2001; Hattingh, Pitts, De Vos, Moyes, & Ganhao, 1991; Hattingh, Wright, De Vos, Levine *et al.*, 1984; Heard, Kollias, Buss, Caligiuri, & Coniglario, 1990; Howard, Kearns, Clippinger, Larsen, & Morris, 2004; Janssen *et al.*, 1993; Montané *et al.*, 2003; Pitts & Mitchell, 2002).

2.4.4 The effect of stress on body temperature

Stress-induced hyperthermia describes increases in body temperature in response to stressful stimuli and has been the focus of numerous animal studies because of the damaging effects of prolonged elevations in body temperature (Meyer *et al.*, 2008). Stress-induced hyperthermia seems to occur in preparation for the 'fight-or-flight' response, and Bouwknecht, Olivier, and Paylor (2007) published a full review on the phenomenon of stress-induced hyperthermia across a variety of species and the inducing stimuli. According to these authors, the hyperthermic response to a psychological stimulus is short-lasting although it may be accentuated by a number of factors such as the type and duration of a stimulus. In wildlife, some research has been conducted on minimising animal hyperthermia induced by capture stress since it has been proposed that capture-induced hyperthermia may play a role in capture myopathy and acute death during capture (Broekman, 2012; Mentaberre *et al.*, 2010). Although the mechanisms of capture-induced hyperthermia are not yet fully understood, there is a general assumption that capture-induced hyperthermia may be related to physical activity and high ambient temperatures (Cheney & Hattingh, 1988; Meyer, Fick, Mitchell, & Fuller, 2008). Meyer *et al.* (2008) showed that increases in body temperature occurred in impala (*Aepyceros melampus*) during capture irrespective of the level of activity and environmental temperature, and the authors concluded that the magnitude of the body temperature increase was predominantly influenced by the level of stress response during capture. Broekman (2012) argues that body temperature alongside other

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parameters can give a good estimation of the degree of stress in captured wild antelope. According to Mentaberre *et al.* (2010), stress-induced hyperthermia is an anticipatory response to stress and, although tranquilisers can modulate it, they cannot prevent it. The findings of Meyer *et al.* (2008) support this statement as the authors report that in their study, the magnitude of body temperature increase was similar for all drug combinations used to immobilise impala chemically although the duration of the hyperthermia was shorter for certain drug combinations. Future research into a complete understanding of this phenomenon may give more insight into the exact mechanisms that cause hypothermia and the treatments that may be employed to prevent it.

2.4.5 Measuring physical changes in animals using biotelemetry

Biotelemetry originated from the word ‘telemetry’, which is derived from the Greek work *tele*, meaning ‘far’ and *metros*, meaning ‘measurement’ (Ropert-Coudert & Wilson, 2005). Thus, telemetry is the measurement of different variables that can be studied and transmitted over some distances with biotelemetry systems being able to measure a number of biological variables such as heart rate and respiration rate. In effect though, a biotelemetry system has no range limits as it makes use of both transmitting and recording devices with the device attached to the subject (in this case, the animal) itself (Ropert-Coudert & Wilson, 2005).

2.4.5.1 The history of biotelemetry

Originally, heart rate and respiration rate relating to behaviour were studied in humans with researchers gradually realising their importance in animal studies as well (Baldock, Sibly, & Penning, 1988). Since heart rate and respiration rate are influenced by both behaviour and the endocrine stress response, they quickly became reliable parameters for monitoring stress in a variety of species (Hooff, Van de Vries, & Mol, 1998; Nilsson *et al.*, 2006; Price & Sibly, 1993; Schmidt, Biau, Möstl, Becker-Birck & Morillon, 2010; Von Borell *et al.*, 2007). It has therefore become pertinent to be able to measure heart rate and respiration rate in free-ranging animals without hindering the animals’ movement or evoking a stress response in the process (Grenwis, 2010; Montané *et al.*, 2002; Signer *et al.*, 2010). It is generally accepted that physical measurements taken from conscious animals are superior to those taken when animals are immobilized since they are taken under conditions that best represent the normal state of the animal. Hence, the field of biotelemetry, in which telemetric systems are used to measure

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physical parameters in free-moving animals, has become an indispensable part of stress research (Kramer & Kinter, 2003).

According to Samson *et al.* (2011), telemetry systems used for recording cardiovascular variables such as heart rate and blood pressure, and obtaining an electrocardiogram (ECG) were initially developed for pharmaceutical companies who used them for drug research and development in response to requirements by the American Food and Drug Administration (FDA) to submit continuous measurements of vital functions with each new drug application. Thus, the term 'biotelemetry' was born, being defined as the remote measurement of behavioural, physiological and energetic data from live subjects (Cooke *et al.*, 2004; Ropert-Coudert & Wilson, 2005). A biotelemetry system combines miniature sensors and transmitters that broadcast biological signals from animals to a receiver, which in turn converts the analogue frequency signal into a digital signal, which can then be input into a computerised data acquisition system (Grenwis, 2010). Such acquisition systems can then format and manipulate the data to be output in different ways according to what the user requires (Kramer & Kinter, 2003). Although some researchers may refer to biotelemetry as 'physiological telemetry', this latter term limits its application to measuring physiological variables only. In fact, the true application of biotelemetry is much broader and includes any signal within an animal that requires amplification, such as heart rate, measurements of binary activities (such as wing beat), behavioural measurements (such as eating and vocalisation) and the measurement of environmental conditions (such as temperature), to name a few (Cooke *et al.*, 2004).

Biotelemetry in animal studies dates back over 50 years when Eliassen (1960) built and used a device that transmitted the heart rate and wing beat rate of ducks (Ropert-Coudert & Wilson, 2005). Although originally designed and used under laboratory conditions, by the 1970s, researchers were starting to investigate and discover its uses in free-living animals in their natural environments (Amlaner & McDonald, 1980). One of the first biotelemetry devices to be used on larger ambulatory animals, was designed and fitted by Johnson and Gessaman (1973) who developed an ECG telemetry device for large ungulates. However, the device had range limits, and similar devices were subsequently used by numerous other authors on captive animals (Syme & Elphick, 1982; Lyons & Price, 1987). A number of these studies (e.g. those by Davis, Von Recum, Smith, & Guynn, 1984 and Williams & Siniff, 1983) made use of surgically implanted devices, requiring the miniaturisation of the device which further hampered battery life and transmission range (Kiourti, 2010). To overcome the latter problem Cupal, Weeks, and Kaltenbach (1976) included an implantable transmitter to broadcast heart rate to a

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transmitter that was attached to a collar around the animal's neck (Johnston, MacArthur & Geist, 1980). In 1979, MacArthur, Johnston, and Geist designed a system that consisted of subcutaneous electrodes attached to an FM transmitter connected to a harness that was carried on the back of the animal. A remote receiver and tape recorder were also attached to the harness, which then detected and recorded the transmitted ECG signal.

Since, the 1980s, many advances have been made in the field of biotelemetry and by the 1990s, researchers were starting to investigate ways of measuring different physiological variables other than cardiac responses, such as respiration rate (Samson *et al.*, 2011). The International Society on Biotelemetry was founded as early as 1973 and holds symposia every two years, although presentations are focused predominantly on human biotelemetry systems (ISOP, 2014) In 2003, the first bio-logging symposium (focused on animal research) was held in Tokyo, although much of the research presented focused on the study of large marine mammals. However, by the second symposium in 2005, much more appreciation was being shown for the versatility and usefulness of the biotelemetry approach (ISOP, 2014; Ropert-Coudert & Wilson, 2005).

Although biotelemetry is becoming increasingly popular in animal research studies, it is still not as widely used in terrestrial species as was expected when these types of systems were first developed (Roupert-Coudert & Wilson, 2005). According to Cooke *et al.* (2004), the two most limiting factors to the use of biotelemetry is firstly the lack of commercial development for many applications, and secondly, the cost of such systems (Cooke *et al.*, 2004). Eloranta, Norberg, Nilsson, Pudas, and Säkkinen (2002) also state that the reliability of the few existing biotelemeters for free-ranging terrestrial animals has not been adequately established and they report and that literature regarding these systems is scarce.

2.4.5.2 Implantable versus external biotelemetry systems

Today, modern biotelemetry systems either consist of external devices fitted to the bodies of animals (Eloranta *et al.*, 2002) or implantable devices (Fick, Matthee, Mitchell, & Fuller, 2006). Both systems have their advantages and disadvantages, with external devices being a more cost-effective and a less risky alternative to implantable devices (Grenwis, 2010). Implantable devices act as sensors that detect signals within the body and then transmit these to an external device (Kiourti, 2010). The external device can consist either of a microcontroller, following the transponder principle, with a

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transmission range of about 50 cm or of an external repeater collar with a transmission range of around 20 m (Scheibe *et al.*, 1998). For example, Arnold *et al.* (2004) constructed a system that consisted of an implantable radio-transmitter with an external repeater unit located in a collar which measured heart rate and internal temperature in red deer.

Although external devices can be used without the risk of surgery, implantable devices can measure a larger number of variables more accurately than external devices and tend to cause little discomfort to the animals once implanted (Grenwis, 2010). In fact, advances in the development of microsurgical techniques and recovery procedures have greatly facilitated the use of implanted catheters, electrodes and other devices (Kramer & Kinter, 2003). Variables that can be measured with implanted systems include blood pressure and internal body temperature, parameters which cannot be measured with external devices (Kiourti, 2010). The smaller size of implantable telemetry systems, however, could also be a limiting factor since it may limit the energy source required to power it (Grenwis, 2010; Kiourti, 2010).

External biotelemetry devices are typically used for short-term studies with a high throughput and involving a large number of animals (Grenwis, 2010). As there are no surgical procedures involved, these devices provide a non-invasive alternative to implantable systems and can be used with single or group-housed animals (Eloranta *et al.*, 2002). However, as the device has to be fitted to the outside of the animals, animals often have to be trained to wear it in a jacketed or harness form before the study commences to prevent them from attempting to remove it or examining the devices on their counterparts (Grenwis, 2010). The use of external systems can also produce unnecessary artefacts since they require more technician interaction and may hinder animal movement if measures are put in place to ensure sensors do not move or get damaged (Kramer & Kinter, 2003). Implantable devices are mostly used with small, young animals and improvements in this field have made available smaller transmitters that are even less invasive (Fick *et al.*, 2006). However, these systems are often very expensive and more suitable for use under laboratory conditions (Grenwis, 2010; Kramer & Kinter, 2003). In the end, however, it is the objectives of the study and the study animals that will determine the system to be employed (Grenwis, 2010).

2.4.5.3 Polar biotelemetry monitors used in animal studies

Polar heart rate monitors have been popular choices for measuring heart rates in ambulatory animals over the last couple of decades (Stewart, Foster, & Waas, 2003). The first wireless wearable heart rate monitors were brought out by Polar in 1982 and, although they were originally developed for use in humans, they have become popular for use in animal research (Polar Electro, 2013). One of the first studies done with these heart monitors was by Lyons and Price (1987) who used the Sport Tester PE2000 from Polar® Electro in Finland. Lyons and Price (1987) investigated the relationship between heart rate and the behaviour of goats, and attached two mono-polar surface electrodes to the animal's body. The electrodes were then attached to a battery-pack transmitter and heart rate signals were transmitted to a receiver microcomputer which then stored the heart rate values at 30s intervals. The authors obtained good results with the system and reported mean heart rates for goats between 132 and 160 bpm. Thereafter, numerous studies were performed using different models of the Polar heart rate monitors. In 1994, Hopster and Blokhuis aimed to validate the use of the Polar® Sport Tester (PST) in cows using a girth belt that was manufactured by Polar®. Each PST consisted of the girth belt (supplied by the manufacturer for use in horses) to which a transmitter was connected as well as two circular, stainless steel plate electrodes covered by wash-leather pouches, which were in turn connected with the transmitter (Hopster & Blokhuis, 1994). One electrode was placed behind the scapula on the right withers and the other was situated on the left ventral abdomen. The wrist monitor, to which the heart rate signal was transmitted, was also attached to the girth belt as the transmitting range was only 1 m. Heart rates were stored at 5s intervals and the authors once again reported good results with mean heart rate values for the cows ranging from 65–92 bpm. Other studies have also reported good results with the PST system in a variety of species, including horses (Stewart *et al.*, 2003; Waran & Cuddeford, 1995), red deer (Abeyesinghe *et al.*, 1997; Carragher, Ingram, & Matthews, 1997), goats (Lyons & Price, 1987) and dairy cows (Waiblinger, Menke, Korff, & Bucher, 2004).

The Polar® Vantage NV™ HR monitor has also been used in a number of animal studies including species such as reindeer (Eloranta *et al.*, 2002; Nilsson *et al.*, 2006), horses (Visser *et al.*, 2002), southern chamois (*Rupicapra pyrenaica*) (Marco, Lavín, Mentaberre, Lopez-Olvera, & Casas-Díaz, 2010), and roe deer (Montané *et al.*, 2003; Montané *et al.*, 2002.). The system is similar to the PST, consisting of a girth belt with two sensors/electrodes attached to a transmitter and then a receiver in the form of a

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wristwatch, which most researchers then attach to the girth belt as the transmitting range is also only 1 m (Eloranta *et al.*, 2002). The advantage of the Polar® Vantage NV™ is that individual coding allows for simultaneous monitoring of animals in groups (Eloranta *et al.*, 2002).

Other Polar® models that have been used for animal research include the Polar S810i (Schmidt *et al.,* 2010), the Polar 800XS (Von Borstel, Pasing, & Gauly, 2011), and the Polar® RR Recorder (Rietmann *et al.*, 2004). More recently, Polar® also launched the Polar® Equine Heart Monitor, which comprises a girth belt specially designed for horses and a similar transmitter and receiver system as described previously (Jansen *et al.*, 2009; Leiner & Fendt, 2011; Von Borstel *et al.*, 2011).

2.4.5.4 Other commercially available systems that have been used in animal studies

One of the earliest studies with a commercial telemetric heart rate monitor was by Syme and Elphick (1982), who investigated the link between heart rate and behaviour in sheep. The authors measured heart rate using a commercial heart rate monitor from Exersentry Respironics Inc., Monroeville, USA. The monitor consisted of metal clip electrodes that attached to the animal’s skin at three different points (two at the thorax and one on the loose skin at the base of the tail), a monitor, a voltage control oscillator (VCO) and an FM transmitter. The monitor, VCO and transmitter were all attached to a harness on the animal’s back. An FM receiver was used to pick up the signal, and the heart rate was estimated in beats/minute by two observers using stop-watches until a stable level was reached. Using this method, the authors reported mean heart rates for sheep ranging between 92 and 154 bpm.

Tennessen, Price, and Berg (1984) who studied transport stress in bulls and steers, considered a number of different measurable responses, including respiration rate, rectal temperature, serum cortisol levels and heart rate. All of these variables were measured either before loading or after off-loading although heart rate was measured using a telemetric device during the transportation of the animals as well. The authors used a VHF radio telemetry system from Biotelemetry Systems Inc., New York, USA along with two self-adhesive Stress Test Electrodes from 3M Corporation™, Minneapolis, USA. The electrodes were fastened onto the skin – one under the harness between the scapula and the other 60 cm behind the harness – and this system allowed the researchers to monitor heart rate continuously during the tracking of the animals. They obtained reliable

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results with this system and found elevated heart rates during the loading, on and off, of the animals, which was expected (Tennessen *et al.*, 1984).

Pollard and Littlejohn (1995) used a heart rate monitor from Equine Electronics™, Kowloon, Hong Kong, to investigate the effects of social isolation and restraint on the heart rate and behaviour of alpacas. The system was also comprised of electrodes attached to a transmitter, which then transmitted the signal to a FM receiver. A videotape recorder was mounted beside the receiver and recorded the resulting beeps corresponding to the heart rate of the animal. The authors reported heart rates between 73 and 119 bpm.

Mosure, Meyer, Gudmundson, and Barth (1998) used the PARAGON™ 240 Cardiac monitoring system from Quinton Instruments, Seattle, USA with an additional radio transmitter also from Quinton Instruments on Hereford bulls. The researchers inserted two surgical staples under the skin of each animal, one on the upper right side of the animal at the caudal edge of the scapula and the other on the left side, 8 to 10 cm caudal to the olecranon, a week before fitting each bull with a heart monitor. The electrodes of the monitors were each attached to the staples using alligator clips (Mosure *et al.*, 1998). The researchers were investigating the effect of different electro-ejaculation treatments on the stress responses of the animals and measured baseline heart rates prior to each treatment, immediately after each treatment and at 2-minute intervals for 10 minutes after each treatment. The researchers obtained good responses in heart rate to treatments but concluded that some of the responses may have been due to muscle exertion and that heart rate may not necessarily give a good indication of pain experienced. Other authors that have used this system successfully in beef cattle include Mitchell *et al.* (2004), Mosure *et al.* (1998) and Waynert *et al.* (1999).

Hooff *et al.* (1998) used the Holter monitoring system from Biosensor, Maple Gove, USA, to measure heart rate in dogs. The system was attached to a jacket, which was fitted onto each dog prior to the trial to make the animals accustomed to wearing the apparatus. Inside the jackets, five electrodes were attached to the animals to obtain ECGs. The ECG data was then stored in a unit also mounted in the jacket and data was recorded continuously. Heart rate was calculated from the ECG data and the authors reported heart rates for dogs between 75 and 183 bpm.

De Jong *et al.* (2000) made use of a radio telemetry system from Data Science International (DataSci), USA, to measure heart rates in growing pigs. The system consisted of an implantable transmitter, implanted while animals were under

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anaesthesia, with electrode leads positioned caudal and cranial to the thorax. The receiver was connected to a multiplexer (also from DataSci) that was adapted for simultaneous data acquisition with LabPro analysis software and CARDIA software. The authors reported reliable results with this system with reported heart rates ranging between 118 and 176 bpm.

Schwartzkopf-Genswein *et al.* (2005) studied the physiological and behavioural changes of Holstein calves during certain management procedures, and used the heart rate monitoring system from Mini-mitter Inc. (also known as the Actical system from Camntech). This system consists of two external electrodes held in place by a girth belt, a transmitter and a data logger. The transmitter was attached to the girth belt while the data logger was placed in a padded case on top of the halter between the ears. The data logger collected heart rate data every minute and the authors reported heart rates between 92 and 105 bpm.

2.4.5.5 Custom-made systems that have been used in animal studies

Price and Sibly (1993) studied the effects of behaviour and handling on heart rate in farmed red deer by making use of a self-designed telemetry system consisting of a girth belt with two flat metal electrodes mounted on sponge blocks. The signals from the electrodes were taken through a pre-amplifier to a TT405 low-power transmitter from Wood and Douglas, Basingstoke, UK, and were recovered from a receiver up to 0.5 km away. Thereafter, the signal was processed to give a visual display of time between successive heartbeats. Poor skin contact resulted in 30% of data being lost but the authors still reported mean heart rates for the animals of between 56 and 118 bpm, depending on activity (Price & Sibly, 1993). Hargreaves and Hutson (1990) also fitted sheep with a self-constructed heart rate telemetry system consisting of a 300 MHz transmitter of limited range, which transmitted signals passed through a heartbeat amplifier and comparator. The transmitter unit was secured on the sheep's back by the two straps of a ram's raddle harness and two active lead disposable electrodes were attached to make skin contact. The receiver unit included an amplifier and ceramic transducer, which emitted heartbeats as audible clicks. The receiver was positioned next to a video camera, so that the sheep's heartbeats and treatment procedure were recorded simultaneously on videotape. Other authors that have made use of similar self-constructed external telemetry devices include Arnold *et al.* (2004) and Palestrini, Ferrante, Mattiello, Canali, and Carenzi (1998).

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Signer, Ruf, and Arnold (2011) studied hypometabolism and basking in Alpine ibex by fitting animals with a novel telemetry system that consists of a cylindrical ruminal unit and a collar unit with an expected lifespan of two years. Singer *et al.* (2010) had done a previous study to test the accuracy of the device. The ruminal unit was administered via the mouth after reversal of anaesthesia when the swallowing reflex was restored and the unit remained in the rumino-reticular tract so that it could be located in close proximity to the heart. The unit detected heartbeats with an integrated acceleration sensor that responded to the mechanical shock waves above a certain trigger level as well as ruminal temperature (Signer *et al.*, 2010). Twenty animals, ten males and ten females, were fitted with the devices for six months between June and November 2007. To maintain the battery life of the device, heart rate was only measured every 21 minutes for three minutes at a time while temperature was measured every three minutes. The authors reported good results using this system (Signer *et al.*, 2011).

2.4.5.6 Parameters other than heart rate measured with biotelemetry systems in animal studies

Respiration rate is frequently measured with telemetry systems in human patients (Mukai, Yonezawa, Ogawa, Maki, & Caldwell, 2009) although its use in animal studies is still limited (Kiourti, 2010). With many large animal studies, observations of flank movements are used to determine respiration rate in ambulatory animals (Hales, 1969). This is largely due to the lack of the availability of animal biotelemetry systems that are able to measure respiration rate, according to Samson *et al.* (2011), and these authors thus developed an implantable telemetry system which could measure respiratory rate amongst a number of other parameters for use in animal biomedical research. Reefmann, Bütikofer Kaszàs, Wechsler, and Gygax (2009) used an external system which consisted of an extendable belt (1132 Pneumotrace II, UFI, 280 mm × 25 mm × 3 mm) to measure respiration rate in sheep. The belt generated a continuous signal for its relative extension during inspiration and exhalation cycles.

Body temperature changes can also be measured with a variety of biotelemetry systems (Meyer *et al.*, 2008) and is often of interest in studies with large and wild animals because of the marked effect that factors such as drug administration, stress and environmental temperatures may have (Bouwknecht *et al.*, 2007; Fick *et al.*, 2006; Gill, 1993). According to Broekman (2012), the site at which body temperature changes are measured in response to different stimuli is influential on the results obtained since temperatures at different sites may be affected by different factors. Core body temperature is most

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notably affected by environmental factors such as stress, and thus researchers are generally concerned with an adequate representation of core body temperature changes (Fick *et al.*, 2006; Meyer, Fick, *et al.*, 2008; Montané *et al.*, 2002). Rectal temperature is commonly used in veterinary research as an indication of core temperature changes (Koga *et al.*, 2004) but may in fact reflect a temperature that is slightly lower than the actual core body temperature (Gill, 1993). Equally, skin temperature changes may not give a true reflection of core temperature changes in response to stressors since skin temperature changes are greatly affected by environmental temperatures, the wind chill factor and humidity (Broekman, 2012). As a result, researchers often make use of implantable biotelemetry systems to measure core body temperature changes with these systems usually consisting of implantable miniature temperature-sensitive data loggers (Meyer, Fick *et al.*, 2008). Fick *et al.* (2006), Hetem *et al.* (2009), Meyer, Hetem *et al.* (2008), Meyer, Fick *et al.* (2008), Signer *et al.* (2010) and Signer *et al.* (2011) all made use of such data loggers, implanted in the abdominal or ruminal cavity, to investigate core body temperature changes in free-ranging ruminants. Montané *et al.* (2002), Montané, Marco, Manteca *et al.* (2002) and Montané *et al.* (2003) on the other hand, made use of telemetric data logging temperature probes inserted into the rectum to measure body temperature changes in roe deer. Mitchell *et al.* (1997) made use of a similar probe but implanted it into the carotid artery and brain of free-ranging springbok using a guide tube to measure changes in blood and brain temperature. Arnold *et al.* (2004) used a self-constructed telemetry system with electrodes that were implanted subcutaneously to measure changes in subcutaneous skin temperature, and found small but significant changes in this temperature due to seasonal influences as well as correlations between heart rate and temperature changes.

The locomotor activity of free-ranging animals is also often measured, where activity can be defined as any condition different from physical rest (Scheibe *et al.*, 1998). Advances in biotelemetry have made it possible to record the activity patterns of various free-ranging species without doing direct observations of the animals (Fick *et al.*, 2006). This is particularly valuable in the long-term study of free-ranging animals where environmental and time constraints do not permit continuous observations. These biotelemetry systems can either make use of radio-tracking devices to determine spatial movements of animals or accelerometer devices that characterise the frequency of different activities such as feeding, movement and standing (Scheibe *et al.*, 1998; Winnie, Cross & Getz, 2008). Both Scheibe *et al.* (1998) and Pépin, Renaud, and Decuq (2006) made use of an external system consisting of a collar unit which recorded the

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frequency and intensity of activities such as grazing, ruminating, standing and total activity in free-ranging wild and domesticated species. Implantable activity sensors are also available, and Arnold *et al.* (2004), Fick *et al.* (2006), Signer *et al.* (2010) and Signer *et al.* (2011) all made use of such sensors to measure the activity patterns of various free-ranging species. However, it must be noted that these authors simultaneously used implantable biotelemetry devices that also recorded other variables such as heart rate and temperature so that surgical procedures were inevitable. Where surgery is not advisable, external activity sensors can be equally accurate (Scheibe *et al.*, 1998).

Lastly, although implantable devices are available to measure blood pressure in ambulatory animals (Stabenow, Schon, & Briissow, 1996), such devices are more appropriate for use under laboratory conditions and are not yet popular in studies with large, free-ranging animal species. Since changes is blood pressure are closely related to other parameters such as heart rate and endocrine responses as affected by different stimuli, it stands to reason that the development of a practical biotelemetry system for use in free-ranging species would be invaluable in animal research.

2.5 EFFECTS OF STRESS ON ANIMAL BEHAVIOUR AND MEASURING CHANGES IN BEHAVIOUR

By definition, the term ‘stress’ can be explained as the physiological and behavioural adjustments that an organism undergoes to adapt to or avoid any perceived threats that may challenge its internal homeostasis (Moberg, 2001; Swaisgood, 2007). According to Dantzer and Mormède (1983), hormonal and behavioural responses are closely related during stressful situations so that the perception of a stressor and the resulting behaviour of an individual are significant to the nature and intensity of the hormonal response (refer to Figure 2.2).

2.5.1 Changes in animal behaviour in response to stress

It has been proposed that the relationship between behavioural and physiological responses to stress may assume three different modes (Dantzer & Mormède, 1983; Leshner, 1978) namely:

- a given ‘brain state’ can trigger and coordinate certain endocrine and behavioural responses;
- specific hormones can affect behavioural attitudes; and

- the endocrine status of an individual can be modified if the individual engages in specific behaviours.

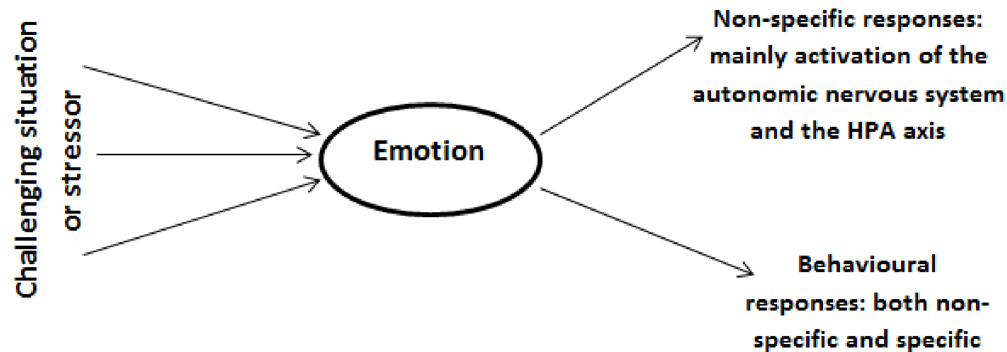

Figure 2.2 Stress model according to Dantzer and Mormède (1983) (adapted from Veissier and Boissy, 2007)

Dantzer and Mormède (1983) argue that the endocrine response to stress is non-specific while the behavioural response is specific and aimed at controlling the threatening stressor. For example, sheep and cattle may show evasive behaviour when confronted with a squeeze chute due to previous experiences that resulted in fearfulness of restraint (Grandin, 1997). Taking into consideration then that physiological and behavioural responses to stress are interrelated, the study of animal behaviour becomes as important as studying the underlying physiological changes. Therefore, an inclusive study of both animal behaviour and physiology provides a more comprehensive measurement of stress that avoids drawing ambiguous conclusions regarding the welfare of an animal by merely taking a single measurement (Young, Creighton, Smith, & Hosie 2012).

Animal behaviour can easily be measured non-invasively and can be a sensitive measure of an animal's perception of environmental changes so that any deviation from 'normal homeostatic behaviour' can indicate that the animal has perceived the possibility of a threat (Swaisgood, 2007). An animal's response to certain situations can be diverse and may be altered according to the stimuli received (Swaisgood, 2007). A typical initial response to an acute stressor may involve orientation towards the stimuli, suppression of normal activities and preparation for fight, flight or hiding (increased vigilance) (Hill & Broom, 2009; Morgan & Tromborg, 2007). According to Carlstead and Sliepherdson (1994), an animal may also have a 'conservation-withdrawal' response instead of a 'fight-

or-flight' response, which is characterised by a decrease in environmentally-directed activity (Carlstead & Sliepherdson, 1994). This latter response is behaviourally characterised by immobility and low levels of aggression as opposed to the 'fight-or-flight' response, which is behaviourally characterised by territorial control and aggression (Koolhaas *et al.*, 1999).

If the stressful situation persists and an intolerable threshold is reached, regulatory behaviour may occur (Broom, 1988b). For example, during extreme decreased environmental temperatures, animals may show prolonged huddling or shivering in an attempt to maintain body temperature (Broom, 1988a, 1988b). Short-term physiological changes may also be associated with these behavioural responses such as increased respiration and heart rate (Dantzer & Mormède, 1983). If the level of response is not successful in allowing adaptation to the stressful situation, the animal may evoke significant changes in the autonomic and neuroendocrine systems with concurrent behavioural changes (Barnett & Hemsworth, 1990). Animal behaviour typically indicative of chronic stress include increased aggression, changes in feeding or sexual behaviour, lack of responsiveness, reduced exploratory behaviour and increased behavioural inhibition, increased fearfulness and frequency of startle, increased abnormal behaviour and increased self-injurious behaviour (Carlstead & Sliepherdson, 1994; Morgan & Tromborg, 2007; Swaisgood, 2007). Stereotypical behaviour (also referred to as 'stereotypies') (Loeding, Thomas, Bernier, & Santymire, 2011) develops in animals in captivity and is a good indication that the animal has been experiencing chronic stress (Bashaw, Tarou, Maki, & Maple, 2001). Such behaviours are characterised by repeated sequences of movements without obvious function, and can differ between species (Broom, 2010). Although many 'normal behaviours' are performed in a stereotypical way (Mason & Rushen, 2005), the most striking feature of stereotypies is that there seems to be no functionality to them, implying a certain degree of abnormality with many authors suggesting that such behaviour assists the animal in coping with a chronically stressful situation (Mason & Rushen, 2005). For example, walruses (*Odobenus rosmarus*) in captivity stereotypically rub their tusks against concrete structures like pool edges, while captive giraffes (*Giraffa camelopardalis*) will show stereotypical oral behaviour such as wall-licking (Mason, 2010).

In the field of wildlife research, much emphasis is placed on the welfare of animals in captivity, with many researchers using animal behaviour studies to ascertain the welfare of the animals or the stress the animals endure (Clubb & Mason, 2003; Griffin & Thomson, 1998; Hanlon *et al.*, 1994; Loeding*et al.*, 2011; Mason, 2010; Mason &

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Veasey, 2010; Moll *et al.*, 2009; Morgan & Tromborg, 2007). According to Teixeira *et al.* (2007), various captive studies have shown that space reduction is one of the biggest stress-inducing factors, often resulting in increased aggression and disruption of normal social behaviour. All animals have 'behavioural needs' to engage in species-specific behaviours and these needs are often hampered by captivity and space restrictions (Reeder & Kramer, 2005). For example, housing a large number of animals together may lead to increased agonistic behaviour while small group sizes can lead to more normal, less adverse social interactions (Morgan & Tromborg, 2007). Under normal environmental conditions, many animals will spend a large part of their daily activity budget in search of food. This behaviour is hampered in captivity where food is generally provided (Herbers, 1981). Other factors that can place stress on animals in captivity include novel sounds, smells, lighting, mixing of social groups, food and human disturbances, all of which can result in changes in behaviour (Morgan & Tromborg, 2007). Thus, deviations in normal behaviour, displays of abnormal behaviour or signs of stereotypical behaviour can all be indicative of an animal's stress response and resultant underlying physiological changes.

2.5.2 Measuring behavioural changes in response to stress in animals

The study of animal behaviour is frequently used as a basis for assessing animal welfare (Broom, 1988b, 1991). Ethology is defined as the biological study of behaviour, and is characterised by an observable phenomenon (namely behaviour or movement) and by the type of approach or method of study (namely the biological method) (Tingergen, 1963). This field is concerned with the behaviour patterns of animals so that, together with anatomy and physiology, it gives a comprehensive and complete picture of the biology of the animal (Sambraus, 1998). Behavioural observations can be viewed as a type of 'assay' used to quantify animal biological responses and as such, methods of behavioural observations should be validated and selected based on the objectives of the particular study (Bowden, Karriker, Stalder, & Johnson, 2008). According to Martin and Bateson (2007), the following diagram is a representation of the steps involved in the studying of behaviour (Figure 2.3):

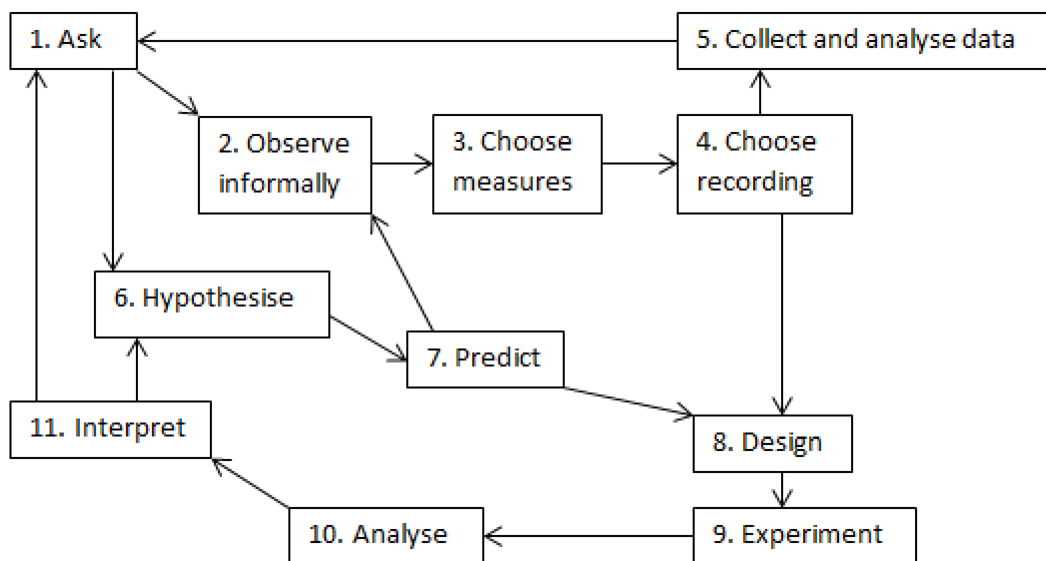

Figure 2.3 Steps involved in behaviour research. Some research will start at step 6 but would have to incorporate steps 2–4 before step 8. The progressive character of research may involve returning from step 11 to step 6 but in some cases may involve returning to step 1 (adapted from Martin & Bateson, 2007).

In animal studies, different ethological methods can be used to observe the behaviour of domestic, captive or wild animals and to assess the behavioural effects of changes in their environment such as diet, stress, climate, etc. (Goodwin, 1999). Ethograms are commonly used, consisting of a formal list of a species' behavioural repertoire or a large segment thereof (McDonnell & Haviland, 1995). This may be a complete list of behaviours or it may focus on functional classes of behaviours (McDonnell & Haviland, 1995). According to Sambraus (1998), when constructing an ethogram for a specific species, all functional circles should be included such as social behaviour, sexual behaviour and parent–offspring behaviour.. Within an ethogram, behaviours can be divided into two components, namely events or states (Noldus, Trienes, Jansen, & Jansen, 2000). **Events** are behaviours which are relatively short in duration and which can be approximated as points in time, while **states** are behaviours which are relatively long in duration and thus their onset and termination can be recorded (Bowden *et al.*, 2008; Martin & Bateson, 2007). This use of discretely defined categorical behaviours enables researchers to quantify behaviour in terms of counts of occurrences or proportions of time exhibited (Ransom & Cade, 2009). The types of behaviour patterns

to be investigated will also often determine the recording tool to be used (Bowden *et al.*, 2008).

Altmann (1974) presented a comprehensive and definitive review of the sampling methods that can be used in observational studies of behaviour. His review includes the advantages and disadvantages of both *ad libitum* sampling and instantaneous scan sampling where *ad libitum* sampling involves the recording of all behaviours that are visible and appear relevant (Banks, 1982). In other words, there are no systematic constraints and the observer merely notes down as much as he/she can (Altmann, 1974; Martin & Bateson, 2007). Scan sampling, on the other hand, involves the recording of the categorical behaviour of an animal at a precise point in time (for example at 1- or 5-minute intervals) (Altmann, 1974; Banks, 1982; Ransom & Cade, 2009). Scan sampling is popular in field studies, and a number of studies have found that where continuous observations of behaviour are often not possible due to time, budget or environmental constraints, scan sampling has proved equally effective, especially when animals are observed in their natural environments (Bowden *et al.*, 2008; Ransom & Cade, 2009; Winterbach & Bothma, 1998). Within scan sampling, one-zero sampling is often employed where a behaviour is scored a one or a zero depending on whether it occurs in the sample period (Banks, 1982). Thus behaviours are expressed as the proportion of all sample intervals during which the behaviour occurred (Altmann, 1974; Martin & Bateson, 2007). Focal sampling can also be used so that one individual is observed for a specified period and all instances of its behaviour are recorded (Altmann, 1974; Martin & Bateson, 2007). This method is commonly employed when studying groups of animals although it may be problematic in field studies where the individual may move out of sight or be obscured so that such interruptions are merely recorded at a 'time out' (Martin & Bateson, 2007; Noldus *et al.*, 2000). For studies of social behaviours, sequence sampling may be used in which the focus of an observation is an interaction sequence rather than an individual behaviour. The sample thus starts when a social interaction is initiated and ends when the sequence of behaviours within the interaction is terminated or interrupted (Altmann, 1974). If it is possible for the observer to perform continuous observations of an individual or a group of individuals, continuous sampling can be performed whereby the behaviour of each individual is recorded continuously, noting the point in time when a behaviour starts or stops (if it is an event behaviour) or occurs (if it is a state behaviour) (Ransom & Cade, 2009). This method enables the calculation of both frequencies and the duration of behaviours as well as the exact time at which the behaviours occurred (Martin & Bateson, 2007).

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The type of study will also determine the recording method to be used and a variety of recording medium options are currently available for field and laboratory studies. Video recordings are commonly used (where practical) in both field and laboratory studies with the added advantage that behaviours can later be slowed down for analysis (Ord, Martins, Thakur, Mane, & Börner, 2005). With both video recording and visual recording, event recorders, which make use of appropriate software, can be used. This will enable the observer to record behavioural observations directly onto a machine for analysis (Martin & Bateson, 2007; Noldus, 1991). Event recorders usually consist of portable or hand-held computer devices that allow the observer to record behaviours as key presses, making the observation of numerous behaviours or individuals less laborious and time-consuming (Noldus, 1991). Nowadays, many software packages are available with some software options even including the possibility of analysing and presenting behaviour data (Martin & Bateson, 2007). Noldus Information Technology (Noldus, Wageningen, The Netherlands) provides a number of popular software packages that have been used in a variety of animal behaviour studies (Abeyesinghe & Goddard, 1998; Abeyesinghe *et al.*, 1997; Grigor *et al.*, 1998; Hooff *et al.*, 1998; Waiblinger *et al.*, 2004).

2.6 THE NEGATIVE CONSEQUENCES OF STRESS IN WILDLIFE AND THE NECESSITY FOR RESEARCH INVOLVING THE REDUCTION OF STRESS

Translocation is an inevitable part of the wildlife industry, especially in South Africa where an estimated 300 000 head of wildlife is reported to be translocated annually although no official records are available so that the number may in fact be higher (Dugmore, 2013). According to Dickens *et al.* (2010), translocation of wild species may contain the following stressors:

- capture and handling;
- captivity or some form of prolonged restraint;
- transport; and
- release into an unfamiliar environment.

Any one of these stressors may elicit an acute stress response and may even result in a chronic state of stress (Dickens *et al.*, 2010). While short-term responses to stress may include increases in heart rate, respiratory rate and, to a lesser extent, an increase in body temperature, long-term effects may hold more deleterious consequences such as reduced reproductive rates, weight loss, impaired immune function and capture myopathy (Jordan, 2005). Dickens *et al.* (2010) published and in-depth review of the

effect of stress on the success of translocation of animals. The authors discuss the negative consequences of both acute and chronic stress on the success of translocation and note that chronic stress may have the largest influence on the failure rate of animal translocations (Figure 2.4). Teixeira *et al.* (2007) also reviewed the importance of considering stress during animal translocation programmes and note that subclinical symptoms of stress or mild stressors during translocations should not be underestimated as they may have a cumulative effect resulting in high mortality and morbidity. These authors also comment on very high mortality rates (as high as 85%) as reported for translocated deer species in the United States, concluding that the two main reasons were the presence of predators and stress. Monitoring of the stress response of wildlife during translocation as well as their acclimatisation thereafter is thus critical for assessing the true success of the translocation process and to minimise losses (Franceschini *et al.*, 2008).

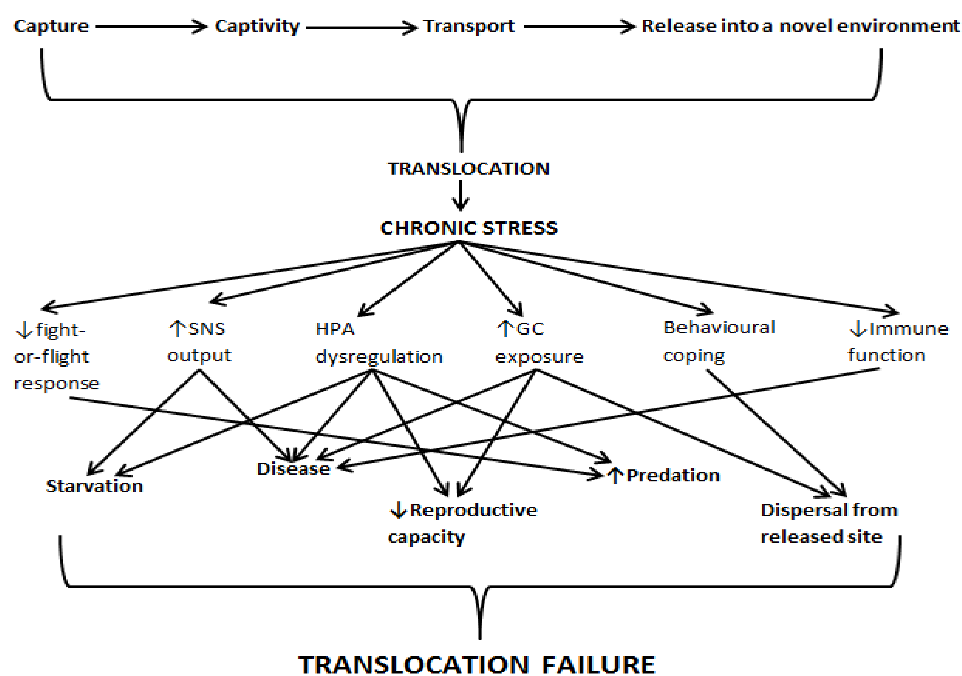

Figure 2.4 A schematic representation of the link between chronic stress and the failure of animal translocation (adapted from Dickens, Delehanty, & Romero, 2010)

In the South African wildlife industry, the most significant animal losses are due to capture myopathy (CM) (Broekman, 2012). According to Ebedes, Van Rooyen, and Du Toit (2006) more animals in Southern Africa died of CM in the last 30 years than from any

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other wildlife diseases (Broekman, 2012). Capture myopathy (CM) is known by an array of different names such as muscular dystrophy, white muscle disease, overstraining disease, transport myopathy and exertional rhabdomyolysis (West, Heard, & Caulkett, 2007). The condition has been described in a number of free-ranging and captive mammals, particularly in wild species that are commonly captured and relocated (Harthoorn & Van der Walt, 1974; Spraker, 1993). Spraker (1993) describes four primary syndromes associated with CM, namely

- acute death syndrome;
- ataxic myoglobinuric syndrome;
- ruptured muscle syndrome; and
- per-acute death syndrome.

According to Montané, Marco, Manteca, *et al.* (2002), the ataxic myoglobinuric syndrome probably occurs most commonly and can be seen several hours to several days after capture. Clinical symptoms may include mild to severe ataxia, torticollis and myoglobinuria with elevated serum enzymes and blood urea nitrogen levels. Animals with mild symptoms are likely to survive while moderate to severe symptoms may result in higher mortality (Montané, Marco, Manteca, *et al.*, 2002; West *et al.*, 2007). Wenker (1998) reviewed the syndrome with reference to the physiological mechanisms involved and concluded that it may be prevented by reducing capture stress, fear and exertion.

CM results in the dissolution of muscle fibres and affects both skeletal and cardiac muscles (Wenker, 1998). As a result, intracellular contents such as creatinine kinase and myoglobin are leaked into the blood resulting in fatal acid–base and electrolyte imbalances (Bonacic, Feber & Macdonald, 2006; West *et al.*, 2007). Predisposing factors that may contribute to the occurrence of CM include species, environment, other diseases and drugs. Some species are considered more susceptible to CM with highly susceptible African species including springbok, female impala, kudu (*Tragelaphus strepsiceros*) and nyala (*Tragelaphus angasii*) (Ebedes *et al.*, 2006; West *et al.*, 2007). Capture-related factors are likely to play the most significant role so that capture techniques that involve high chase speeds, prolonged exertion, excessive handling and restraint that promote struggling may predispose animals to CM. Any injuries resulting from capture techniques or induced by other animals can also increase the incidence of CM (West *et al.*, 2007).

Treatment is generally considered ineffective once CM is diagnosed and thus prevention of its onset should be the main objective when exposing wild animals to any causative

factors (Wenker, 1998). In addition to controlling environmental factors that may contribute to excessive stress and exertion, immobilisation drugs should also be tailored for rapid induction, rapid recovery, efficient delivery and physiological recovery (West *et al.*, 2007). Indeed, a number of researchers have investigated the use of several drug and drug combinations in an attempt to minimise the occurrence of CM (Bonacic & Macdonald, 2003; Bonacic *et al.*, 2006; Cheney & Hattingh, 1988; Citino, Bush, Grobler, & Lance, 2001, 2002; Diverio, Goddard, & Gordon, 1996; Marco *et al.*, 2010; Mentaberre *et al.*, 2010; Meyer, Fick *et al.*, 2008; Montané, Marco, Manteca, *et al.*, 2002, 2003), while others have investigated the effect of different capture methods (Bonacic *et al.*, 2006; Denicola & Swihart, 2012; Ganhao, Hattingh, & Pitts, 1988; Knox, Zeller, & Hattingh, 1992; Marco & Lavín, 1999). Since CM is however, still relatively poorly understood, there is a need for research involving wildlife and the stressors that may predispose animals to the onset of CM, as well as the formulation of preventative measures that may be taken to minimize the effects of CM.

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CHAPTER 3

Literature Review Part II

The wildlife industry and the translocation of wildlife in South Africa*

3.1 AN OVERVIEW OF THE WILDLIFE INDUSTRY IN SOUTH AFRICA

In literature and in practice, a variety of different terms are used in relation to wildlife so that some clarification is needed (Van Hoving, 2011). For this review, the term 'wildlife' will refer to all wild animals, including game (defined as wild animals that are hunted or subject to commercial use) and wild animals that are not hunted (Higginbottom & King, 2006; Van Hoving, 2011). The term 'wildlife industry' will refer to the industry as managed by both private wildlife producers and government conservation authorities while 'wildlife ranching' will refer to the management of the extensive production of free-living animals on large fenced or unfenced private or communal land, usually for the purposes of hunting, live sales, trophy hunting, wildlife meat or tourism (Bothma, 2002; Higginbottom & King, 2006).

3.1.1 A brief history

Toward the middle of the 19th century, wildlife had almost no economic value and was viewed as an undesirable competitor for limited livestock grazing land (Dry, 2012; NAMC, 2006). Wildlife was considered as belonging to no one (*res nullius*) and could as such be hunted and eliminated by anyone with an attitude towards wildlife as being one of utilisation for survival by meat consumption (Carruthers, 2008; Van Hoving, 2011). Since commercial farming with domesticated livestock was more profitable, the absence of wildlife was in fact viewed as an advantage when selling land (NAMC, 2006; Van Hoving, 2011). The national drive was aimed at putting land towards some kind of productive use

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* **A subsection of this review was published in:**
Laubscher, L.L., Hoffman, L.C., Pitts, N.I., & Raath, J.P. (2015). Non-chemical techniques used for the capture and relocation of wildlife in South Africa. *African Journal of Wildlife Research*, 45(2), *In Press.*

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with wildlife being viewed as an agricultural threat due to grazing competition and the spread of diseases to domestic livestock (Carruthers, 2008). Indeed, with commercial livestock farming and crop production being the major contributors to the country's gross domestic product (GDP), wildlife received no attention and was even eradicated by government in large numbers when viewed as threatening to livestock production systems (Carruthers, 2008; Van Hoving, 2011). However by 1894, drastically declining wildlife numbers and uncontrolled hunting began to receive government and public attention, and this resulted in the establishment of the first statutory game reserve by the South African government on marginal land not deemed suitable for agriculture or that was prone to tsetse fly and malaria (Dry, 2012; National Agricultural Marketing Council [NAMC], 2006; Van Hoving, 2011). By 1926, the first National Parks Act (No. 56 of ***1926***) was promulgated together with the establishment of the Kruger National Park and various other national parks (Dry, 2012; NAMC, 2006). Thereafter, considerably more attention was given to wildlife management practices with public perceptions slowly starting to change with more emphasis being placed on the conservation of wildlife populations (Dry, 2012; NAMC, 2006; Van Hoving, 2011). Carruthers (2008) compiled an in-depth review on the evolution of game ranching from the early 1900s and noted the trends toward adding value to wildlife.

3.1.2 Economics of the wildlife industry

Today, more than a century after the first game reserve[1] was established in South Africa, the wildlife industry has grown significantly and is still growing (Van der Merwe & Saayman, 2010). Over the past 40 years, the economic value of wildlife has gained momentum so that conservation efforts have resulted in there being more wildlife animals in South Africa now than there was 150 years ago (Dry, 2012; NAMC, 2006; Steyn, 2012). According to Cousins, Sadler, and Evans (2010), the decline in agricultural subsidies and the deregulation of the agricultural sectors as well as increased stock thefts and land claims also contributed to a movement towards wildlife production systems as an economic alternative to livestock production. Also, since only 17% of South Africa's agricultural land has a high production potential, wildlife ranching is seen as one of the best development options for marginal agricultural areas (Dry, 2012; NAMC, 2006).

[1] The Hluhluwe Umfolozi Game Reserve was proclaimed in 1895 (Brooks, 2000).

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Today, the wildlife ranching industry (consisting of both private and national parks) comprises more than 20% of South Africa's total land, utilising almost a third of the country's potential grazing land for wildlife and wildlife-related purposes (Cloete, Taljaard, & Grové, 2007). Over 28 million hectares of South Africa's land (22.9% of its total land) consists of either government-protected areas or commercial wildlife ranches, and 80% of South Africa's nature conservation takes place on privately owned land (Dry, 2010; Van der Merwe & Saayman, 2010).

In South Africa, private properties used for wildlife purposes are either classified as 'exempt farms', which are properties registered for wildlife ranching with the provincial authorities or 'mixed use farms', which are properties that are occupied by both domestic livestock and wildlife (Dry, 2010; Higginbottom & King, 2006). Exempt wildlife ranches are unique in the world since wildlife on these ranches belongs to the owner of the land so that loss of animals (due to escape, theft or death) results in financial loss to the owner (NAMC, 2006). Exemption entitles the owner to hunt, capture and sell specified and/or approved species all year round while non-exempted areas (areas without suitable fencing) are only allowed hunting mainly during the period March to September (hunting season) although each province has its own guidelines on when the hunting season is open and which species may be hunted (NAMC, 2006).

In 2000, there were 5 061 exempted wildlife ranches which represented a 47% increase in the area occupied by wildlife ranches since 1993 (Higginbottom & King, 2006). Today (2015), the total number of privately owned commercial wildlife ranches are estimated at more than 10 000 with over 6 000 of these being exempt ranches (Bothma, 2002; Dry, 2010, 2012; Muir-Leresche & Nelson, 2000). It is also estimated that more than 2.5 million head of wildlife are commercially owned, which is four times more than the wildlife in state-owned parks (Dry, 2010). Ellof (2002) estimated that by 2001–2002 the wildlife industry was growing at an annual rate of 5.6% in terms of area exempted for wildlife ranching although some slowing in growth was experienced due to the strengthening of the rand against the US dollar as well as drought (NAMC, 2006). However, by 2006 it was still recognised that wildlife ranching had been the fastest growing agricultural activity in South Africa in the previous three decades (1980–2010) (NAMC, 2006).

The economics of the wildlife industry rest on four pillars, namely hunting, live-game trade, processed game products and eco-tourism, with numerous sub-sectors within these sections (Cloete *et al.*, 2007; Van der Merwe & Saayman, 2010). In 2009, the total wildlife industry income in South Africa was estimated at R7.7 billion with the private

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wildlife ranches forming an important contributor to the South African economy with an income contribution of more than R4.7 billion per annum (Dry, 2010; NAMC, 2006; Van Hoving, 2011). It is estimated that wildlife ranches generate about R220 per hectare in economic output annually, as compared to an average of R80 per hectare for conventional livestock farming (Dry, 2010). When measured in terms of turnover, the wildlife industry has grown at an average of 20.3% per annum since 2000 (Dry, 2012). The major contributor to the total income of the wildlife industry is the biltong-hunting sub-sector, which is said to contribute about 66% of the income (Dry, 2010). The translocation of animals also generates a significant amount of money with a contribution of about 16% to the total turnover and it is estimated that capture and relocation operations generate an annual income of between R750 to R900 million (NAMC, 2006). In addition, the live sale of animals at auctions contribute around 2% of the total turnover with an estimated annual income of R94 million (Dry, 2010; NAMC, 2006; Saayman, Van der Merwe, & Rossouw, 2011; Steyn, 2012; Van Hoving, 2011).

3.1.3 Live game sales

Most wildlife ranches have conservation as one of their main objectives so that both restocking of animal populations as well as offtake (either in the form of hunting, capture for live sale or harvesting) to prevent overpopulation is necessary (Higginbottom & King, 2006; Muir-Leresche & Nelson, 2000). The trade of live animals has become a significant part of the wildlife industry and nowadays, both public and private wildlife ranching managers view the trade of live animals as meeting both the objectives of removing surplus animals and raising revenue (Higginbottom & King, 2006; Muir-Leresche & Nelson, 2000). Prior to 2006, it was estimated that around 70 000 animals were translocated annually (NAMC, 2006) but in 2010 alone, an estimated 167 400 head of wildlife were reported as having been translocated across the country (Dry, 2012). Currently, according to the Wildlife Translocation Association (WTA), there are between 80 and 100 wildlife capture operators in South Africa that move over 300 000 head of game per year (Dugmore, 2013). Both private owners and government conservation agencies buy and sell live animals, principally through auctions, transactions through agents or through private transactions directly from the seller (Higginbottom & King, 2006):

Wildlife auctions are a very popular way for government and private owners to generate income and can be done either through boma (enclosure) auctions, in which animals are captured and transported to a central point and kept in bomas for potential buyers to

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inspect or catalogue auctions where buyers can consider animals via brochures or electronic media (NAMC, 2006). Only about 10% of animals sold at auctions are sold through catalogue auctions with animal prices through catalogue auctions being about 12% lower than those at boma auctions (NAMC, 2006).

According to Van der Merwe and Saayman (2010), the analysis of game auction turnovers from 1991 to 2001 revealed an annual increase of 9% with the turnover per year growing from R9 million in 1991 to R105 million in 2002 (Van der Merwe & Saayman, 2010). According to Cloete (2011), about 15 000 animals were sold in 2010 at 56 wildlife auctions with a total turnover of R316 million that year. Disease-free buffalo and roan antelope prices alone increased by 72% and 178% respectively between the years of 1991 to 2001 (Weaver & Skyer, 2003). Bezuidenhout (2014) reports that during 2013, wildlife auctions reached a record high turnover of R1.029 billion with 23 963 animals being sold at 67 official South African wildlife auctions. Bezuidenhout further notes that this record turnover may have been the result of an increase in high quality animals, specifically rare species and colour variants that were being auctioned. In 2012, a record price of R26 million was paid for a young buffalo bull while a buffalo cow and her heifer calf sold for R20 million (Christie, 2012).

The live trade of wildlife in South Africa is limited principally to larger mammals that are considered valuable in terms of hunting, tourism and meat production (Higginbottom & King, 2006). These include common species such as impala, blesbok, springbok, blue wildebeest and eland as well as more rare species such as disease-free buffalo, sable antelope, white rhinoceros (*Ceratotherium simum*), Livingstone eland (*Tragelaphus oryx livingstonii*) and nyala (NAMC, 2006; Van Hoving, 2011). Steyn (2012) published an article on the profitability of the wildlife trade in South Africa and reasons why the sale of live animals has become increasingly popular.

3.1.4 Legislation regarding the South African wildlife industry

In South Africa, the legal framework regarding wildlife is set out at government level with a specific legal regime regarding wildlife being established at provincial level (Morgera, 2010). The Constitution of South Africa sets up three spheres of governance, namely national, provincial and local, with national parks being deemed national competencies while nature conservation and environmental management fall under both national and provincial competencies (NAMC, 2006). The Constitution sets up a system of cooperative governance between the provinces with the application of legislation

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differing between the provinces. As a result, this system has received some criticism owing to the lack of consistency between the provinces as a result of a lack of national standards and norms in both legislation and the implementation thereof (Cousins *et al.*, 2010; NAMC, 2006). Although the institutions responsible for wildlife are not specifically addressed in the legislation available, the principle ones involved are the Department of Environmental Affairs and Tourism (DEAT), South African National Parks (SANParks), provincial nature conservation authorities and departments of agriculture (Morgera, 2010; NAMC, 2006). The Centre for Human Rights (2006) published a compendium of South African legislation as pertaining to conservation in which the national legislation regarding animals and wildlife is also discussed.

Legislation regulating the translocation of wild animals is covered by both nature conservation legislation at provincial level and animal welfare legislation at government level so that landowners and those involved in the translocation process are required to obtain the necessary permits and meet the criteria for certain permits (Higginbottom & King, 2006). However, administration within different provinces lacks consistency so that different permits are required within different provinces for the same species and activities (Van Hoving, 2011). Although there is currently (i.e. in 2015) no legislation governing the specific treatment of wild animals during translocation or the certification of animals to determine whether they are fit for travel, the South African Bureau of Standards (SABS) has published a code for the transportation of wild herbivores as well as for the care and use of animals for scientific purposes in which different capture methods are addressed (Higginbottom & King, 2006; South African Bureau of Standards, 2007, 2008; South African Veterinary Foundation, 2010). The Animal Protection Act, No. 71 of 1962 also provides a legislative basis for animal welfare standards for both domestic and wild species (Animal Protection Act, 1962). There is however, no government system in place for keeping national records of translocated animals (Higginbottom & King, 2006).

3.2 THE CAPTURE AND RELOCATION OF WILDLIFE IN SOUTH AFRICA

The translocation of wildlife is generally done for a number of reasons including the introduction of species into new or former habitats (Matson, Goldizen, & Jarman, 2004), the management of animal population numbers and the environment (Seddon, Strauss, & Innes, 2012), research and species monitoring activities (Parker, 2008), monitoring of population health (Bengis, 1996) or the habituation of orphaned or injured animals into

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captivity (International Union for Conservation of Nature [IUCN] Council, 1987; Lekolool, 2012). Initially, game capture methods included the use of dogs to ambush and drive frightened animals into pits or snares as well as chasing animals by vehicle and capturing them using a noose (referred to as the 'catch-and-rope' method) (JP Raath, personal communication, 8 August, 2013). These were methods which resulted in high mortalities and injuries (Carruthers, 2008; Lekolool, 2012). It was not until the work of Harthoorn (in Uganda) and Hofmeyr and Ebedes (in Namibia) that the use of tranquillising and immobilising drugs in the translocation of wildlife became an integral part of the wildlife industry (Carruthers, 2008). Historically, the capture of surplus animals in the Umfolozi, Hluhluwe and Mkuze reserves was the beginning of the development of various capture techniques (Oelofse, 2010). By 1968, different capture and relocation methods were being compared with each other although the safest and most reliable methods had not been determined yet (Carruthers, 2008; Visagie, 1968). In 1973, the milestone publication of the book *The capture and care of wild animals* by Dr Eddie Young[2] gave some of the first guidelines (based on comparative research and experience) on how to capture and relocate wild animals effectively (Young & Ebedes, 1973). Thereafter, numerous other books and publications have provided the basis for the effective capture and relocation of wildlife by minimising danger to both animals and personnel (Kock & Burroughs, 2012; Kreeger & Arnemo, 2012; La Grange, 2006; McKenzie, 1993). Techniques for the administration of tranquillising and immobilisation drugs as well as different combinations of drugs were also being investigated although this field was still relatively new in South Africa by the 1980s (Van Niekerk & Pienaar, 1962).

3.2.1 Guidelines and regulations

Translocation is defined as the physical movement of animals from one location and their free release into another, and involves the capture, transportation and release of wild animals (Craven, Barnes, & Kania, 1998; Dickens *et al.*, 2010; IUCN Council, 1987; Lekolool, 2012). Problems occurring during this process are almost always related to stress, often resulting in mortalities (Kock & Burroughs, 2012). Wildlife translocation in South Africa involves a number of role players, including private sector capture teams, SANParks, provincial capture teams, wildlife veterinarians as well as wildlife ranchers (NAMC, 2006). In the early 1990s, the Wildlife Translocation Association of South Africa (WTA) was established to bring together all these role players and raise the national

[2] Young, E., & Ebedes, H. (1973). *The capture and care of wild animals: The work of eighteen veterinary, medical and wildlife experts* (1st ed.). Cape Town: Human & Rousseau.

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standards of wildlife translocations (Higginbottom & King, 2006; NAMC, 2006). Membership is voluntary although anyone who wishes to tender for government or big business contracts must be a member and all members must adhere to the WTA's code of conduct with special reference to animal welfare standards (Higginbottom & King, 2006; NAMC, 2006).

3.2.2 Capture and handling methods not involving chemical immobilisation

Capture methods that are commonly used today are chemical immobilisation, traps and cages, drop-nets, net bomas, net guns, plastic bomas and passive capture sites (JP Raath, personal communication, August 8, 2013). The choice of a particular method will be dependent on a number of factors, such as species, reason for restraint, number of individuals to be captured, availability of appropriate drugs and equipment as well as personnel (Lekolool, 2012).

Traps and cages are specifically suited for the capture of hippo, predators, crocodiles and birds of prey, and a number of different traps can be employed, depending on the species (La Grange, 2006). With most traps, different attractants are used to lure the animals into the traps and these may either be olfactory, visual or audible in nature (De Wet, 1993). Kock and Burroughs (2012) reported that wild felids in North and South America as well as big African cats, such as leopard, cannot resist the smell of Calvin Klein Obsession for Men so that it has been used, rather successfully, to lure big cats into camera traps. Foot-hold traps can be used for predator species such as jackal and African wildcats (*Felis silvestris lybica*) and can be modified (padded) to make capture as humane as possible (Brand, 1993). Box traps are used for the capture of small mammals while cage traps are generally used for the capture of carnivore species, such as honey badgers (*Mellivora capensis*) and felids. Bait is attached to a trigger system which, when activated, causes a trapdoor or spring-loaded door to close, trapping the animal inside (Kock & Burroughs, 2012). The trap is usually designed to be twice the size of the animal with a framework that is covered with suitable wire mesh (Lekolool, 2012). For details on the construction and use of cage traps, refer to De Wet (1993) and La Grange (2006).

In South Africa, drop-nets can be used for small and medium-sized antelope species, warthog (*Phacochoerus africanus*) and wild dog (*Lycaon pictus*) while they are commonly used in Europe for various deer species (Denicola & Swihart, 2012). The system generally consists of a mesh nylon net erected in the field and camouflaged by

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both its colour and the natural vegetation. The nets are suspended by cables, metal poles (in grassland areas) or vegetation (in bushveld areas) so that when animals are herded (usually by vehicle, on horseback, on foot or by helicopter) into the nets, the attachments collapse and the nets fall down so that the animals become entangled in them (Bothma, 2002; Broekman, 2012; Lekolool, 2012). A cable that is released by an observer can also be used. In this case, the nets are dropped manually when animals enter the demarcated area (Kock & Burroughs, 2012). The height of the nets will be determined by the species but 2 m is usually sufficient for larger species (Bothma, 2002; La Grange, 2006). The bottom part of the net (usually about 1 m) lies flat on the ground so that animals cannot lift the nets with their horns and escape (Bothma, 2002). For illustrations of different drop-net systems used on African herbivore species, refer to Bothma (2002), Knox, Zeller, and Hattingh (1992), La Grange (2006) and Broekman (2012). The use of nets is advantageous in areas where there is not enough vegetation to camouflage plastic bomas, and the nets can be moved and set up easily between captures (Bothma, 2002; SANParks, 2013). However, it must be borne in mind that this system is a very rough and traumatic method of capture and numerous studies have found it to cause elevated stress response parameters (Broekman, 2012; Denicola & Swihart, 2012; Ganhao, Hattingh, & Pitts, 1988; Knox, Zeller, & Hattingh, 1992). The use of nets also involves increased handling of the animals, which may result in injuries or suffocation due to strangulation in the net and increasing the time taken to load animals (SANParks, 2013).

Net guns, used either from a vehicle or a helicopter, can be used for smaller plains game species that do not herd well in groups (Kock & Burroughs, 2012). A net gun is a hand-held physical restraint device that uses an explosive charge to project a net over an animal. The net gun was originally developed in New Zealand to capture deer in mountainous areas (Morkel & La Grange, 1993). Square or triangular nylon nets are loaded into a special canister which is then loaded into a 3- or 4-barrel firearm and fired with a blank cartridge (Bothma, 2002; La Grange, 2006). Although this system is a cheaper alternative to chemical immobilisation, the same risks, namely injury and an increased stress response, are associated with this system as with drop-nets, and the system requires a high degree of skill and coordination between the operator and the driver/pilot (Kock & Burroughs, 2012; Morkel & La Grange, 1993). For a full review on the use of net guns, see Bothma (2002) and Morkel and La Grange (1993).

Net or plastic bomas are generally used for the mass capture of species, and animals are driven into the bomas using a helicopter (Bothma, 2002). This is the most widely used method of mass capture in South Africa, and is suitable for use with most species,

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including impala, buffalo, giraffe and even wild dogs (*Lycaon pictus*) (JP Raath, personal communication, August 8, 2013). It has the advantage that highly stress-susceptible species, such as kudu, can be captured and transported successfully with minimal trauma and no handling and without the necessity of immobilising drugs (Kock & Burroughs, 2012; SANParks, 2013). Since the equipment required can be expensive and vehicles and containers need to be of a high quality, this system is only financially viable if large numbers of animals are to be captured (SANParks, 2013). Plastic sheeting or netting (in areas with limited natural camouflage) is used to construct a boma in the shape of a large funnel (Kock & Burroughs, 2012). The entrance should ideally be 100–120 m wide and well camouflaged to allow adequate space for the animals to enter (Openshaw, 1993a). The funnel consists of different segments separated by plastic curtains, which allow for the animals to be herded (since the animals view the sheeting as a solid wall) towards a ramp situated at the end of the funnel and leading into a transport truck (Figures 3.1 and 3.2) (Kock & Burroughs, 2012; La Grange, 2006). Once animals enter the boma, the curtains are systematically closed behind them by manual operators so that animals move forward into the boma (Bothma, 2002).

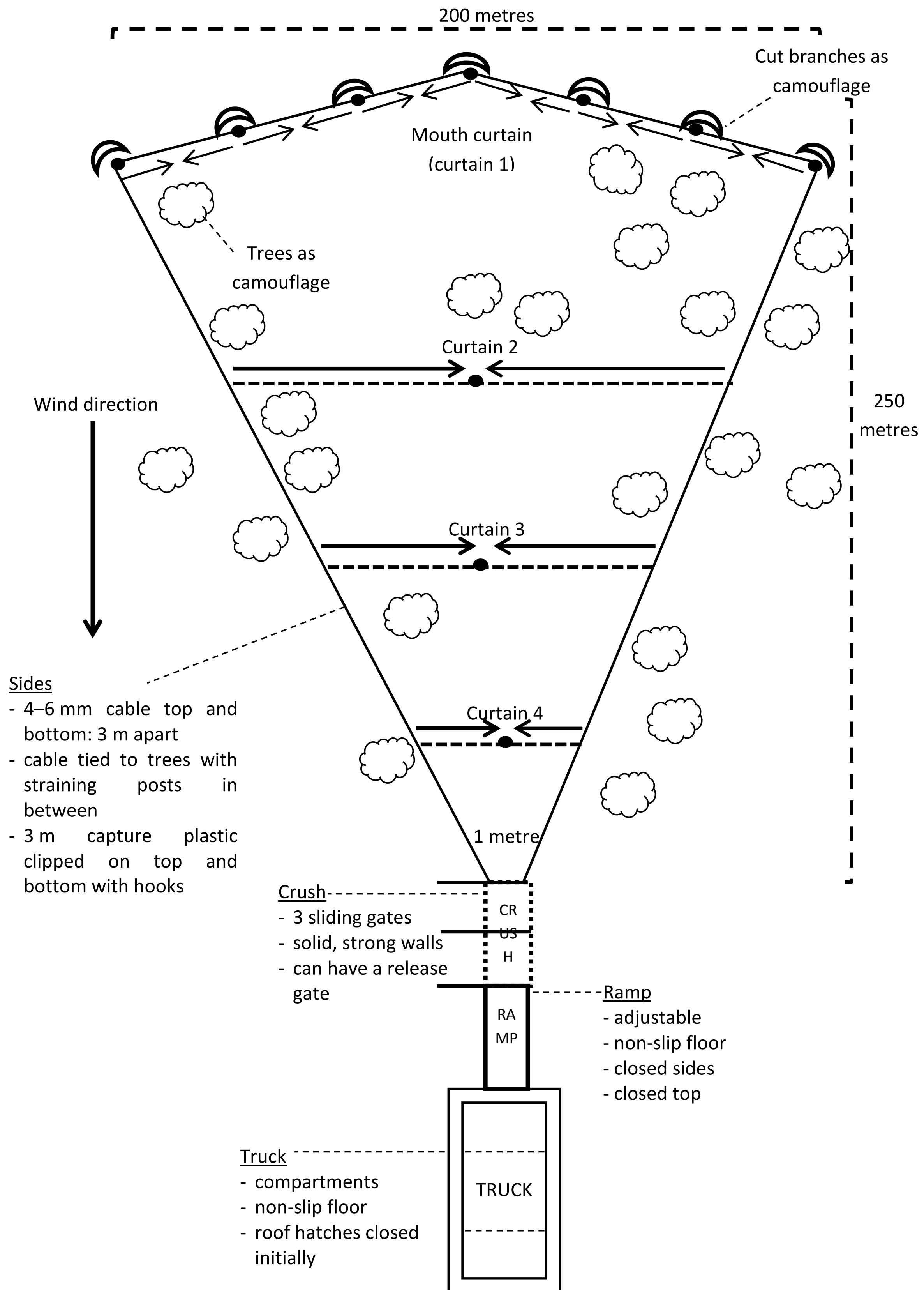

Figure 3.1 Diagrammatic representation of a mass capture boma (JP Raath, personal communication, 17 April, 2014)

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Figure 3.2 A plastic boma set up in the field for the mass capture of impala (courtesy: L Laubscher)

During the construction of the boma, the area in front of the entrance must be left undisturbed (free of human activity and vehicle movement) and the loading ramp and crush must be at ground level if possible, to prevent injury (Openshaw, 1993a). Other important factors that will affect the success of the system include the capability and experience of the helicopter pilot, the strength and direction of the wind, adequate camouflaging of the boma walls, the co-operation of the ground team, and communication between the pilot and the ground team (Bothma, 2002; La Grange, 2006).

Using similar techniques as described above, passive capture sites are also used to capture groups of animals. These may consist of drop nets, net or plastic bomas that are constructed at watering holes of feeding grounds and left there until enough animals have entered the passive capture sight (JP Raath, personal communication, 8 August, 2013). Once enough animals are inside the site, the boma walls are closed or the drop-nets released and the animals are then captured or chased into transport units. This method is suitable when there are no time constraints and is less stressful than other methods involving chasing the animals into a trap (SANParks, 2013).

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Although all the methods described above have been successfully used in game capture operations across South Africa, Kock and Burroughs (2012) note the following factors that are important to consider in order to guarantee the success of the capture operation:

1. **Condition of the animal**: poor body condition or pregnancy can increase the animal's stress susceptibility
2. **Age**: older or very young animals may be more stress-susceptible
3. **Ambient temperatures**: capture of wild animals should be carried out during winter or the cooler hours of the day to prevent the animals overheating
4. **Physical terrain**: animals may be reluctant to enter thick bush or cross a fence
5. **Boma site**: bomas should be located as close as possible to the animals being captured to prevent overexertion during the chase
6. **Time of capture**: this will be dependent on ambient temperatures
7. **Human contact**: increased human contact can lead to unnecessary stress on the animals
8. **Equipment**: equipment should be carefully maintained and well-designed and manufactured
9. **Disturbance**: minimal human disturbances prior to capture can help minimise the stress susceptibility of the animals
10. **Provision of food and water**: if the animals are to be transported over a long period of time, sufficient food and water should be supplied
11. **Mixing species**: species should never be mixed when transported
12. **Crowding**: overcrowding should be prevented to prevent injuries and stress to the animals
13. **Transport containers**: containers or crates should be well constructed and strong enough to prevent animals from breaking through them
14. **Off-loading**: planning and correct crate design can prevent any problems when off-loading animals
15. **Time of year**: the calving season of a species will determine when certain species can be captured since pregnant animals or very young animals are not suitable for translocation

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3.2.3 Transporting wildlife

During the transportation of wildlife, the most important aspect to take into consideration is the welfare of the animals and, as such, suitable equipment and practices should be employed as pertaining to the specific species being transported. Criteria that should be kept in mind when planning a transportation operation include the characteristics of the species (for example naturally aggressive species should be transported in individual crates and under tranquilisation), the life history and population dynamics of the species (for example some herbivore species require boma training before being transported), route (for example whether animals are being transported by road, air or sea) and costs (La Grange, 2006; Openshaw, 1993b). The SABS as well as the Animal Protection Act No. 71 of 1962 provides guidelines to capturers and owners of wildlife regarding equipment and appropriate procedures for the transportation of wildlife and it is in the interest of all parties concerned to follow these guidelines stringently to minimise potential losses (SABS, 2007, 2008; Animal Protection Act, 1962). Such losses may include injuries due to inadequate transport equipment being used or overloading of animals as well as potential death, resulting in substantial financial losses (JP Raath, personal communication, 8 August, 2013). Capture myopathy has been found to occur within hours and up to fourteen days after transportation with resulting mortalities up to four weeks after capture and transportation (Broekman, 2012; Harthoorn & Van der Walt, 1974).

The first aspect to take into considering is the loading of the animals and the following guidelines should be adhered to when loading animals to be transported (Kock & Burroughs, 2012; La Grange, 2006):

1. The operation should never be rushed.
2. Animals should be allowed to rest before loading when they appear exhausted and hot. It must be noted though that this may a problem in some species like zebra where loading animals as soon as they are caught is critical to avoid aggression between animals in the crush area.
3. Tranquilisation should be considered for apparently stressed or potentially aggressive animals.
4. Mature males should be removed prior to loading to avoid fighting during transportation.

5. Excessive movement and noise by staff and capturers should be avoided in order to calm stressed animals down.
6. Animals should be counted and loaded in groups according to space requirements.
7. When partitioning animals, appropriate animals should be put in each compartment and adequate space and ventilation should be provided.
8. If animals are not calmed and settled properly, they should be unloaded and reloaded again.

Crate design for the transportation of wildlife is of the utmost importance to prevent injury and unnecessary stress nimals can either be transported individually in single crates or in mass (in groups) or multiple crates (Bothma, 2002). The type of crate used will depend entirely on the species with naturally aggressive species that have the potential to injure their counterparts, having to be crated individually (JP Raath, personal communication, 8 August, 2013). Commonly, these species include gemsbok (*Oryx gazella*), roan antelope, sable antelope *(Hippotragus niger*), red hartebeest, eland and blue wildebeest, and Openshaw (1993b) and Bothma (2002) provide guidelines for the transportation of each specific species. Stringent guidelines should also be followed when considering crate designs so as to minimise animal stress and possible injury and to ensure proper ventilation during hot days and or heat retention during trips in cold conditions. General principles that apply to crate design include (Bothma, 2002; Kock & Burroughs, 2012; La Grange, 2006; Openshaw, 1993b):

1. The size and strength of the crate should be appropriate to accommodate and restrict the animal while still allowing the animal to stand or lie down as necessary
2. Ample ventilation is a strong pre-requisite but gaps at eye-level should be eliminated so that animals cannot sense movement outside
3. The floor should allow for drainage of urine and water whilst still providing a surface that is safe and secure for the animal to retain its footing
4. All doors, including partition doors between communal crates, should move freely and without hindrance and should be a minimum of 1 m wide to allow animals to pass through without feeling threatened
5. The tops of the crates should be accessible so that animals can be reached if necessary (for example for the administration of tranquilisers) without the animals being able to escape

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In addition, transport vehicles should adhere to specific designs and criteria and for both crates and vehicles, the WTA provides Certificates of Adequate Equipment to verify that their members' capture equipment meets certain standards (WTA, 2013). These permits are recognised by the South African government in relation to the issuing of transport permits (Higginbottom & King, 2006).

The use of both short- and long-term tranquilisers during the transportation of wildlife is common practice and these are used extensively on aggressive animals in mass crates and even in single crates (Openshaw, 1993b). The appropriate use of such tranquilisers will be discussed in detail later on in subsection 3.2.5.

3.2.4 Holding/boma facilities for wildlife

Extensive guidelines are available for the appropriate set-up of holding/boma facilities (La Grange, 2006). Prior to the construction of holding facilities, siting of the facility should be taken into account so that weather conditions throughout the year, the availability of water, and human disturbances (for example excessive traffic and noise that may stress animals) should be considered (Kock & Burroughs, 2012). When constructing such facilities, basic animal needs have to be addressed such as the adequate provision of shade and protection against rain, constructing the facilities on a slope to allow for drainage, sufficient ventilation, appropriate feed containers and water troughs that cannot be moved around by animals or cause injury (elevated hay racks will be needed for species like giraffe although most animals feed at or near ground level) and size that allows for enough movement (it is recommended that 1 m^2/50 kg body weight be provided) (Bothma, 2002; Ebedes, 1993). Depending on the species, other specifications will also have to be kept in mind so that, for example, the holding pens for giraffes will differ significantly from those for rhinos. The behaviour of a specific species will also determine whether groups can be kept together or whether dominant individuals must be kept separately. Such factors will therefore determine the size of the holding pen (Bothma, 2002; La Grange, 2006). Species such as kudu can jump over walls of up to 2,4 m and this should be taken into consideration (Kock & Burroughs, 2012). The holding pens and gates should also be designed to facilitate ease of movement and minimise stress of the animals, and no sharp or protruding structures should be found in or around the facilities as these may cause injury to the animals during movement (Kock & Burroughs, 2012). Holding pen walls are usually constructed from sturdy gum poles and depending on the construction methods, these walls can be lined internally or

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externally with sheeting so that newly introduced animals cannot see outside (Kock & Burroughs, 2012; La Grange, 2006).

3.2.5 The use of chemical restraint and tranquilisation in wildlife

The administration of chemical agents to wild animals for the purpose of capture is not a recent development in Africa, and was in fact first used by the Khoisan, who killed animals using poison arrows (Dewar *et al.*, 2006). The application of this principle ultimately led to the development of the dart, a device that injects the drug on impact (Kock & Burroughs, 2012). For the purpose of this review, drug delivery systems will not be discussed, and the review on chemical immobilisation will be limited only to the drugs used.

3.2.5.1 Basic nomenclature and classification of drugs used in wildlife translocations

Drugs used for the capture and translocation of wildlife can broadly be divided into those used for restraint, those used for the reduction of stress, and those used to reverse or reduce drug effects (Swan, 1993).

Drugs used predominantly for restraint are also known as immobilising agents where immobilisation refers to a state in which the animal is usually recumbent, anaesthetised and unaware of its surroundings, does not feel pain and can be handled easily (Knox, 1992). These drugs are used for the chemical restraint of animals and are classified as anaesthetics with various groups falling within this classification (see Table 3.1). Anaesthetics bring about their immobilising effect by interfering with neurotransmission and disrupting central nervous system function (Richards, 1983). Both the peripheral and autonomic nervous systems are usually affected at the same time (Kock & Burroughs, 2012). Anaesthetics have pre-synaptic and/or post-synaptic functions by either binding to synaptic receptors and modifying the response of the post-synaptic effector cells or by modifying the fate of the neurotransmitters within the synapse (as is the case with cyclohexylamines) (Kock & Burroughs, 2012; Swan, 1993). In wildlife, the most popular anaesthetics used are opioids and cyclohexylamines (JP Raath, personal communication, 3 July, 2012). Unlike opioids, cyclohexylamines have no antagonists to reverse their effects, and the recovery of the animal is linked to the metabolism and excretion of the drug by the body (Kock & Burroughs, 2012).

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When making use of chemical immobilisation in the capture of wild animals, a number of characteristics have been identified for the ideal drug (Knox, 1992; Kock & Burroughs, 2012; Kreeger & Armeno, 2012; Swan, 1993; Wenker, 1998). The ideal drug

1. should have a wide safety margin as doses are more often than not calculated on estimated body weight, which can be difficult to estimate accurately at a distance;
2. should be absorbed rapidly into the systemic circulation with a rapid onset of action (i.e. short induction time);
3. should have a high potency so that small volumes can be carried in an appropriately sized dart;
4. should have minimal side-effects;
5. should have a reliable antidote to reverse the drug if complications occur;
6. should have no negative effects on pregnant animals and cause no permanent damage to the animal;
7. should not cause tissue irritation when injected intramuscularly;
8. should have a minimal effect on cardiorespiratory function;
9. should be stable under a variety of conditions and be able to mix well with a variety of drugs; and
10. should keep animals calm during recovery and induction periods.

For the purpose of this review, details regarding the pharmacology of anaesthetics will be limited to the details given in Table 3.1.

Drugs used for the reduction of stress can be divided into tranquilisers/neuroleptics and sedatives (Swan, 1993). Neuroleptics or tranquilisers are psychotropic agents that result in the suppression of behavioural responses without affecting spinal and other reflexes (Swan, 1993). Although tranquilisers do not cause severe cortical depression (unconsciousness), they suppress spontaneous movements while sparing spinal reflexes and unconditioned pain reflexes (Read, 2002). Ideally, tranquilised animals will appear to lose their fear of humans and also their aggressiveness towards each other while still retaining the ability to react to noxious stimuli or noise (Knox, 1992). Tranquilisers can be categorised according to their potency (minor or major, based on the incidence of side-effects), structural similarities and duration of action (short-acting or long- acting) (Read, 2002). For details on these categories, see Table 3.1.

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Although sedatives and tranquilisers bring about similar effects, a distinction is made between sedatives and tranquilisers because of the different effects of the dosage of each. Tranquilisers, when administered at dosages higher than that recommended by the manufacturer, will have no apparent increase in the degree of action and will only induce side-effects (Swan, 1993). Sedatives, on the other hand, may be given in very high doses to the extent that they cause what appears to be immobilisation. However, if such animals are approached, they may shake off the effects of the drug and attempt to escape or attack (Kock & Burroughs, 2012). Sedatives and tranquilisers are often used as adjuvants to anaesthetics during the immobilisation of animals, to calm the animal further (Meyer *et al.*, 2008).

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Table 3.1 The classification of drugs used during the capture and transportation of wildlife

GROUP: Anaesthetics		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Class: *Opioids* – drugs derived from opium with opium- or morphine-like properties. Opioids work by binding to opioid receptors, which are found principally in the central and peripheral nervous system and the gastrointestinal tract. These receptors play an important role in the neuro-modulation of endocrine, cardiovascular, gastrointestinal and immune functions. Binding to opioid receptors inhibits the propagation of input through the higher levels of the pain system and therefore results in analgesia (the selective suppression of pain).		
Sub-class: *Opioid receptor agonist* – a substance that fully activates the neuronal receptor to which it attaches and causes a sedative effect		
Etorphine HCl (*morphine derivative*)	M99® (Novartis), Captivon® (Wildlife Pharmaceuticals)	Is used for most ungulate species. It is not suitable for carnivores and can be used alone or in combination with a tranquiliser or sedative, which works synergistically with etorphine HCl as well as reducing excitement and muscular hypertonicity caused by etorphine HCl. It causes respiratory depression and muscle tremors. It is commonly reversed by diprenorphine, naltrexone or naloxone
Thiafentanil oxalate	Thianil®	This is an opiate super drug that was only approved in South Africa in 2009. It induces quick knockdown and a good level of anaesthesia, but has a shorter duration with wide safety margins. There is less respiratory depression but the drug still causes muscle tremors. It is commonly reversed by naltrexone. Thiafentanil oxalate is not effective in zebras

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GROUP: Anaesthetics		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Carfentanil citrate (*opiate substitute*)	Wildnil®	This is a most potent opioid. It has a short induction phase (within 2–5 minutes), and may cause re-narcotisation due to its high potency and allotriophagia after the antidote has been administered. It is not recommended in equid species. Reversal with diprenorphine is not effective and it is thus commonly reversed by naltrexone.
Fentanyl citrate (*opiate substitute*)	Sublimaze®	Fentanyl citrate has the same side-effects as M99. It also has the same indications and precautions as M99 but it has a slower induction time. Fentanyl citrate is not effective in zebras. The drug is commonly reversed by naltrexone.
Sub-class: *Opioid receptor antagonists* – a substance that attaches to a receptor without activating it. Instead, it displaces an agonist at that receptor and so seemingly deactivates it, thereby reversing the effect of the agonist		
Complete antagonist		
Naloxone (*short-acting*)	Narcan®	This is a short-acting drug, and re-narcotisation can occur.
Nalmefene HCl (*intermediate acting*)	Revex®	This is a long-acting drug, but not as long-acting as naltrexone.
Naltrexone HCl (*long-acting*)	Trexonil®	The effects of the drug last for up to 24 hours. It is a highly specific opioid antagonist. It is the antidote of choice for carfentanil. Naltrexone HCl has non-opioid antagonistic effects against diazepinones and cyclohexamines. It is given to white rhino when they are released into the field. Naltrexone HCl is excellent when animals are to be released into a

GROUP: Anaesthetics		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
		high-predation area since reversal of opioid effect is almost immediate and long-lasting.
Agonistic antagonists/partial antagonists (*exhibits some properties of an agonist and some properties of an antagonist*)		
Diprenorphine	M5050, Activon®	Reversal of carfentanil may lead to re-narcotisation. Given as an intramuscular injection, it can take up to 20 minutes to take effect. The effects are not complete in bovids, rhino, giraffe and elephant. Diprenorphine is commonly used as an antidote for etorphine. It improves breathing within 20 seconds after administration.
Nalorphine	Nalline®	The drug causes partial reversal of opioid effects to improve respiration and cause partial arousal. It is an excellent mixed antagonist.
Nalbuphine	Nubain®	Nalbuphine plays a similar role as that of nalorphine, and is used in rhino to improve respiration and walk animals while they are sedated.

GROUP: Anaesthetics		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Mixed antagonist (*partial agonist and antagonist activity at opioid receptors*)		
Butorphanol tartrate	Turbogesic®	Butorphanol tartrate is used to reduce respiratory depression and causes partial arousal. It is commonly used in drug mixtures for the immobilisation of rhino, hippo, lion and wild dog, and is often used in boma and ground darting. Butorphanol tartrate is used for standing sedation in capture situations, and in large felids for reversible anaesthesia.
Class: *Cyclohexamines/dissociatives* – a group of drugs that produce a cataleptoid state of immobility referred to as 'dissociative anaesthesia', which is accompanied by somatic analgesia. There are no antagonists and recovery is dependent on metabolism and excretion of the drug.		
Ketamine	Ketaset®	Ketamine is often used for its synergistic effect with etorphine and thiafentanyl. It also inhibits pain receptors. The adverse effects of ketamine include respiratory depression, hyperthermia, seizures, muscle tremors, cardiac arrest, hypertension and hypersalivation. It may give rise to prolonged recovery.
Phencyclidine	Sernylan®	This drug shows very slow induction and very slow recovery with seizures. It is used with benzodiazepines.

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GROUP: Anaesthetics		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Tiletamine/zolazepam	Telazol®, Zoletil®	This drug results in a painful intramuscular injection. It may also cause hyperthermia. Its adverse effects include respiratory depression, tachycardia, excessive salivation, pulmonary oedema and muscle twitching. Its effects are non-reversible (except zolazepam). Tiletamine has a shorter half-life than zolazepam and causes difficult recoveries in some species.
Class: *Steroids* – the anaesthetic properties of steroids was first observed in 1941, and led to the development of steroidal anaesthetics devoid of hormonal activity. There are no antagonists.		
Alphaxalone/alphadalone	Saffan®	This drug has a wide margin of safety with almost no cardiovascular suppression. Its action is of short duration. It maintains respiration well, and the depth of anaesthesia is dose-dependent.
Class: *Barbiturates* – act as central nervous system depressants and can thus produce a wide spectrum of effects, from mild sedation to total anaesthesia		
Pentobarbitone	Sagatal®	Pentobarbitone can be used for euthanasia through respiratory arrest in both humans and animals, and is not commonly used.
Thiopentone	Intraval sodium®	This is seldom used in wildlife. The drug is used for euthanasia.
Class: *Neuromuscular blockers* – cause relaxation and paralysis of muscles by interfering with the ability of acetylcholine to activate nicotinic cholinergic receptors of the skeletal muscle cells. These drugs are still commonly used in reptiles but have been replaced by safer immobilising drugs in other species.		

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GROUP: Anaesthetics		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Sub-class: *Non-depolarising* – an antagonist that blocks neurotransmission at the neuromuscular junction, thereby causing muscle relaxation.		
Gallamine	Flaxedil®	Gallamine is the drug of choice in crocodiles. It takes effect within 20–30 minutes in crocodiles and lasts for up to 12 hours. It also blocks muscarinic receptors in the heart and so improves blood pressure and heart rate. Gallamine causes flaccid paralysis. The mouths remain open in crocodiles and salivation may occur. This can be reversed with neostigmine methylsulphate (Neostigmine®).
Sub-class: *Depolarising* – a substance that depolarises the muscle fibres by acting in the end-plate to cause neurotransmission, depolarisation of the fibres and then remain in place to prevent re-polarisation.		
Suxamethonium chloride (also known as succinylcholine chloride)	Suxamethonium-Fresenius® (*Old trade name: Scoline®*)	Suxamethonium Cl affects all skeletal muscles including respiration muscles. At high doses, prolonged paralysis, respiratory depression and apnoea may occur. No antidote is available, and there is a very small safety margin. It was routinely used in the culling operations of buffalo and elephant.

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GROUP: Tranquilisers (neuroleptics)		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Class: *Butyrophenones* – used to treat various psychiatric disorders in humans. They produce their effects through a central dopaminergic and a peripheral adrenergic blockade.		
Sub-class: Short-acting (*lasts 3–6 hours*)		
Azaperone	Stresnil®	Azaperone counteracts respiratory depression, but can cause allotriophagia at high doses. It decreases heart rate and causes peripheral vasodilation and hypotension. The drug potentiates anaesthesia and sedation, and has no known antidote.
Sub-class: Intermediate acting (*lasts 12–18 hours*)		
Haloperidol	Serenace®	Haloperidol is incompatible with etorphine and fentanyl. The onset takes 10 minutes plus. Extrapyramidal symptoms can occur with overdose such as allotriophagia, and there is no known antidote.
Class: *Phenothiazine derivatives* – the term 'phenothiazines' describes the largest of the five main classes of neuroleptic antipsychotic drugs. They are multi-potent receptor blockers that give rise to a large number of pharmacological responses. Responses are either due to their effects on central neurotransmission or their influences on neuronal terminal junctions in peripheral tissue. They have no known antagonists.		
Sub-class: Short-acting		
Acetyl promazine	ACP®, Neurotranq®	Acetyl promazine is a competitive antagonist with dopamine and acetylcholine receptors. It possesses anti-histaminic activity, and

GROUP: Tranquilisers (neuroleptics)		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
		extrapyramidal effects can occur in high doses. The drug causes inhibition of thermoregulation, and may cause hyperglycaemia.
Propionyl promazine	Combelen®	Propionyl promazine has the same effects and uses as ACP®, and may cause penile prolapse.
Sub-class: Long-acting (*long-acting tranquillisers consist of fatty acid esters of the basic tranquilliser compounds which by a process of esterification have been dissolved in vegetable or medicinal oils. The prolonged activity derives from the slow release of the esters as it diffuses from the solvent into the tissue fluid and is absorbed into the blood. During this process, there is a release of the active tranquilliser. The storage depot of the tranquilliser is at the injection site, which is usually intramuscular).*		
Pherphenazine enantate	Trilafon®	Same as ACP®
Pipothiazine palmitate	Piportal®	Same as ACP®
Class: *Thioxanthine derivatives* – also related to phenothiazine. Several of its derivatives are used as typical antipsychotics in the treatment of schizophrenia and other psychoses in humans. This drug has long-acting effects.		
Zuclopenthixol acetate	Acuphase®	Zuclopenthixol is similar to phenothiaxines. The onset occurs within one hour and can last up until 3–4 days. There are very few side-effects although extrapyramidal signs can sometimes be seen. The drug is very safe with a larger margin for error.
Class: *Serotonin antagonists* – inhibit the action of serotonin (5-HT) receptors, which are found in the central and peripheral nervous systems. These receptors modulate the release of many neurotransmitters and hormones and influence various biological and neurological functions.		

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GROUP: Tranquilisers (neuroleptics)		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Tameridone	R51163	Tameridone is still under development, and is known as the '“taming drug’. It blocks serotonin receptors and causes increased blood flow to muscles and sedation. It was originally developed for ‘Monday morning disease’ in horses (a low form of capture myopathy).

GROUP: Sedatives		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Class: *Alpha-2s.* They act on alpha-2-adrenoreceptors, which are found in both neuronal and non-neuronal tissues. These receptors act pre-synaptically by modulating the function of other neurons, by post-synaptic mediation of constriction of vascular smooth muscle in the periphery or on hormonal receptors situated outside the nervous system. They have varying effects on blood pressure as well as negative effects on respiration.		
Sub-class: Alpha-2 agonists		
Xylazine	Rompun®, Chanazine®	Xylazine is most commonly used with opiates or dissociates (e.g. ketamine). Its effects last 1–2 hours, but analgesia only lasts for 30 minutes. It is a strong potentiator of tranquilisers, opioids and dissociative agents. It depresses cardiovascular function and bradycardia, and causes regurgitation, especially in buffalo. The drug is ineffective in excited animals as adrenaline binds to the same receptors. Sudden movement can cause an adrenalin rush so that xylazine is displaced from the receptors and the animal gets up.

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GROUP: Tranquilisers (neuroleptics)		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Detomidine	Demosedan®	Duration is longer than with xylazine. The drug is used in combination with opioids.
Medetomidine	Domitor®	Medetomidine has 10 times the power of xylazine. It is more specific and therefore binds tighter to adrenalin receptors. The effects are reversible and the drug is thus very safe. It is frequently used in combination with ketamine.
Sub-class: Alpha-2 antagonists		
Atipamezole (*pure antagonist*)	Antisedan®	Atipamezole is alpha-2 receptor specific. It is rapidly absorbed after intramuscular injection and is specifically designed for use with medetomidine. The drug results in a smooth recovery, while overdosing will result in re-sedation. It is used with xylazine as well.
Yohimbine (*alpha2>alpha 1*)		Yohimbine has an antagonising effect on barbiturates, cyclohexylamines and diazepinones. The response times are short. Yohimbine enters the central nervous system and may cause increased heart rate and blood pressure. The required dosage is low, and must be given very slowly if given intravenously or it causes excitability.

Class: *Benzodiazepines* – their effect stems from their affinity for specific receptors in the brain and spinal cord so that it is to a large degree central in origin. They cause amnesia and their reversal has become possible with the introduction of flumazenil although the expense of these drugs and their antidote may reduce their use in wildlife.

Sub-class: Agonists

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GROUP: Tranquilisers (neuroleptics)		
DRUG	**COMMERCIAL NAME**	**EFFECTS AND USES**
Diazepam	Valium®	Diazepam takes effect within 1–2 minutes of intravenous injection and within 15–30 minutes if given intramuscularly. It should be used alone as it is incompatible with most aqueous solutions of other drugs. Its effects can be reversed with flumazenil. Diazepam is good muscle relaxant and anticonvulsant, and has an appetite-stimulating effect in some species. Diazepam causes excellent tranquilisation in ostriches.
Midazolam	Dormicum®	Midazolam is used for sedation and induction of anaesthesia. It is more potent than diazepam, and has a profound amnesic effect. Midazolam is not commonly used in wildlife immobilisation but is valuable when administered in meat used to bait large carnivores. The drug is useful as a short-acting sedative, particularly in rhino relocation.
Sub-class: Antagonist		
Flumazenil	Anexate®	Flumazenil binds competitively to benzodiazepine receptors to reverse the effect of benzodiazepines. Flumazenil has short-acting effect, but the sedative effects and respiratory effects may return.

Adapted from: (Bothma, 2002; Fahlman, 2008; Haigh, 1990; JP Raath, personal communication, 3 July, 2012; Kock & Burroughs, 2012; Kreeger & Arnemo, 2012; La Grange, 2006; Read, 2002; Reisine & Bell, 1993; Swan, 1993; Wenker, 1998; West *et al.*, 2007; Widmaier, Raff, & Strang, 2004)

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3.2.5.2 The use of tranquilisers/neuroleptics in wildlife translocations

Tranquilisation refers to a situation where an animal is in a state of calmness to the extent that it is aware of but indifferent to its surroundings (Knox, 1992). This state can be achieved through the antipsychotic action of dopamine antagonists that block dopamine receptors and increase the turn-over of dopamine in the limbic area of the central nervous system (Holz & Barnett, 1996). This produces a state of 'ataraxia' or behavioural quietening, which is characterised by decreased emotional reactivity and aggression and relative indifference to stressful stimuli (Read, 2002). These dopamine antagonists are known as tranquilisers or neuroleptics, and were first recognised in human medicine for their antipsychotic effects in the treatment of acute psychoses such as schizophrenia (Diverio, Goddard, & Gordon, 1996; Read, 2002). Neuroleptics produce a calming or tranquilising effect but do not cause immobilisation, and are primarily used as synergists with opioids or cyclohexylamines (Wenker, 1998). One characteristic of neuroleptics is that their effects are generally observed at low doses, while higher doses induce characteristic cataleptic immobility (Swan, 1993). However, even at high doses most neuroleptics do not induce coma and arousal from CNS depression is easily achieved (Swan, 1993). Care must be taken with species with darker pigmentation, such as sable antelope and waterbuck (*Kobus ellipsiprymnus*), as they are vulnerable to overheating and should thus not remain tranquilised for long periods of time (Bothma, 2002).

The synergistic effect of neuroleptics with anaesthetics has several advantages (Bothma, 2002; Knox, 1992; Wenker, 1998):

1. the combined effect of the drugs is greater than their individual effects;
2. neuroleptics can suppress some of the unwanted side-effects of anaesthetics;
3. required doses can be reduced so that safety margins are increased;
4. neuroleptics remain unaffected by the antidotes used to reverse anaesthetics so that a calming effect remains;
5. some species such as eland, kudu and gemsbok cannot be captured with anaesthetics alone, and it is thus essential to combine anaesthetics with neuroleptics; and
6. neuroleptics potentiate the effect of the anaesthetic agent and cause a smoother and speedier induction.

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As previously stated, neuroleptics can be classified according to the duration of their action (see Table 3.1.). Accordingly, most neuroleptics fall into either one of two categories, namely short-acting neuroleptics and long-acting neuroleptics (LANs) (JP Raath, personal communication, 3 July, 2012). The length of action of neuroleptics has been increased by the development of fatty acid ester formulations of the drug so that they are slowly released over time from the depot (injection) sites (Fick *et al.*, 2005). The use of these LANs has increased in popularity in the wildlife industry over the last decade (West *et al.*, 2007)

3.2.5.3 Short-acting neuroleptics

Short-acting neuroleptics have been used extensively in wildlife capture operations since the 1950s and are still commonly used today in a wide variety of animals for different purposes (JP Raath, personal communication, 3 July, 2012). These drugs have a relatively short-acting effect and are therefore of limited use in extended transportation procedures and acclimatisation programmes (Knox, 1992). Short-acting neuroleptics take effect within a couple of minutes, with effects lasting between 2 to 6 hours, depending on the drug. As per Table 3.1, these drugs are classified further according to the parent compound so that they fall either under phenothiazine derivatives or butyrophenone derivatives (Kock & Burroughs, 2012). Because short-acting neuroleptics take effect relatively quickly, they are useful in bridging the gap between anaesthetics and LANs under extended capture and handling procedures (Knox, 1992). Unlike LANs, which can only be injected intramuscularly (IM), short-acting neuroleptics can be administered effectively both IM and intravenously (IV) (West *et al.*, 2007).

Phenothiazine derivatives have no antidotes and they are multi-potent receptor blockers that give rise to a number of pharmacological effects (Swan, 1993). Although the exact mechanisms of the action of phenothiazine derivatives are not fully understood, phenothiazines block post-synaptic dopamine receptors in the central nervous system and may also inhibit the release of dopamine and increase its turnover rate. These drugs are thought to depress portions of the reticular activating system, which is important for the control of body temperature, basal metabolic rate, emesis, vasomotor tone, hormonal balance and alertness (Kock & Burrough, 2012; Swan, 1993). In addition to their dopamine-blocking actions, phenothiazines have varying degrees of anticholinergic, antihistaminic, antispasmodic and α-adrenergic-blocking effects (Montané *et al.*, 2003; Montané, Marco, & Manteca, 2002). They cause both central and peripheral effects (Swan, 1993) as summarised in Table 3.2.

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Table 3.2 A summary of the central and peripheral effects of phenothiazine derivatives (Swan, 1993)

Central effects	Peripheral effects
Sedation	Cardiovascular: negative inotropic effects, vasodilation, hypotension
Catalepsy and extrapyramidal effects at high dosages	Inhibition of ejaculation and prolapse of penis in equines
Potentiation of sedatives and anaesthetics	Mild anti-muscarinic effects, constipation, urinary retention
Anti-emetic	Mild hyperglycaemia
Thermoregulation inhibited	Residues in meat
Hormonal secretion inhibited	Potentiation of organophosphate toxicity
Prolactin release increased	Not Applicable (N/A)

The short-acting neuroleptics, acetyl promazine maleate (ACP®/Neurotranq®) and propionylpromazine (Combelen®) are the dominant drugs within this group used in wildlife (Swan, 1993). ACP® takes effect within 10–-20 minutes and lasts for up to 6–8 hours, depending on the species. It is used well in combination with other anaesthetic agents and was one of the first neuroleptics to be used in darting mixtures. It acts rapidly and effectively in small doses and can be used for most species (JP Raath, personal communication, 3 July, 2012). ACP® interferes with thermoregulation, however, so that care must be taken not to use it on hot days or with animals that have been chased for long distances as it may result in overheating (Bothma, 2002; Kock & Burroughs, 2012; Swan, 1993). For the same reason, caution should be taken when using ACP® in dark-pigmented species such as sable antelope and blue wildebeest and it is contra-indicated in waterbuck (Bothma, 2002). ACP® often causes extrapyramidal symptoms such as circling, chewing and convulsing as well as relaxation of the eyelids resulting in slight drooping of the upper eyelid (Kock & Burroughs, 2012; Swan, 1993). This drug is used prophylactically by some equine practitioners in horses prior to exercise to decrease the incidence of exertional rhabdomyolysis (Montané *et al.*, 2003).

Combelen® is widely used for various wild species and works well in combination with opioids in particular (although also in combination with cyclohexalymines). Its pharmacology is similar to that of ACP® and it is popular as a tranquiliser following physical capture and for the transportation of certain antelope species (Kock &

Burroughs, 2012; Swan, 1993). There is no specific antidote for Combelen®; however, doxapram (Dopram®) has been found to reverse the effects of phenothiazine derivatives quite effectively (Swan, 1993).

The butyrophenone derivatives are exclusively made up of short-acting neuroleptics, namely azaperone (Stresnil®) and haloperidol (Serenace®). The butyrophenones produce their effect through a central dopaminergic and peripheral adrenergic blockade and result in the central and peripheral effects summarised in Table 3.3. They may induce hypotension because of their alpha-adrenergic blockade action (Marco, Lavín, Mentaberre, López-Olvera, & Casas-Díaz, 2010; Swan, 1993).

Table 3.3 A summary of the central and peripheral effects of butyrophenones (Swan, 1993)

Central effects	Peripheral effects
Sedation	Vasodilation; hypotension
Catalepsy and extrapyramidal effects at high dosages	Decreased heart rate
Potentiation of sedatives and anaesthetics	Inhibition of ejaculation
Anti-emetic	N/A
Allotriophagia	N/A
Decreased aggression in swine but increased aggression in larger species	N/A
Reflex stimulation of respiration	N/A

The most common side-effects of butyrophenones are restlessness, hypertonia, catalepsy, stiffness, tremors, ataxia, allotriophagia and severe hypotension, normally associated with over-dosage (Marco *et al.*, 2010). Butyrophenones have minimal effects on respiration, and some studies have shown them to increase ventilation in pigs, horses and humans. Butyrophenones have also been proposed to inhibit some of the respiratory depressant actions of both opioids and other anaesthetics (Radcliffe, Ferrell, & Childs, 2000).

Azaperone is frequently used as an opioid synergist in many wildlife species or on its own as a short-acting neuroleptic in wild herbivores (Marco *et al.*, 2010; Portas, 2004). It is the neuroleptic of choice in many drug mixtures but has the disadvantage of not

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having an antagonist, particularly at high doses (Kock & Burroughs, 2012). It can be administered IM or IV and its initial effect can be seen within 15–20 minutes. Its effects can last for up to 4–6 hours (Kock & Burroughs, 2012; West *et al.*, 2007). It produces peripheral vasodilation, which affects thermoregulatory control and reduces the hypertensive effects of etorphine. Azaperone may even counteract the respiratory depression caused by fentanyl and etorphine (Bothma, 2002; Knox, 1992; Marco *et al.*, 2010; Meyer *et al.*, 2008). Extrapyramidal symptoms and allotriophagia as well as aggression in some species, such as gemsbok, are sometimes seen with the use of azaperone (Kock & Burroughs, 2012; Swan, 1993).

Haloperidol is a very effective neuroleptic used in numerous antelope and gazelle species. It has a relatively long half-life compared to azaperone, typically lasting up to 12 hours when given IV or IM although effects of up to 72 hours have been reported in some cases (Kock & Burroughs, 2012; West *et al.*, 2007). Its onset is within 10–15 minutes if given IM and < 5 minutes if given IV (Swan, 1993). Haloperidol appears to be more effective in small and medium-sized antelope species with variable results having been found in larger species such as gemsbok and sable antelope (Read, 2002). It has also bee successfully used during the transport of ostriches (Pfitzer & Lambrechts, 2001). Haloperidol does not mix well with opioids and is therefore not used in immobilising drug mixtures but rather on its own as a tranquiliser after capture for transportation and boma constraint (Kock & Burroughs, 2012). This drug is unique amongst neuroleptics in that it can be administered orally, which can be useful under a number of circumstances (Kock & Burroughs, 2012; Read, 2002). In the event of an overdose, extrapyramidal symptoms can be induced and these may be reflected as aggression in kudu, blesbok and red hartebeest (JP Raath, personal communication, 3 July, 2012). In such cases, biperiden (Akineton®) can be used as treatment as a single or in repeated doses (Kock & Burroughs, 2012).

3.2.5.4 Long-acting neuroleptics

The term 'long-acting neuroleptics' (LANs), is used to describe a member of a group of tranquilisers that have been used in wildlife over the past 20 years (Read, 2002). With the increased capture and relocation of wildlife species in southern Africa, it became apparent that the animals often struggled with long-distance transportation as well as to adapt to confinement which often resulted in exhaustion and a marked loss in condition due to refusal to eat and drink (Ebedes & Raath, 1999; Read, 2002). While shorter-acting tranquilisers were already being used and found to be useful in anxious and aggressive

animals, the desired effects only lasted for a couple of hours (Read, 2002). Thus, longer lasting tranquilisation was required to reduce anxiety during long-distance transportation by road or air, to assist in acclimatising animals recently captured and introduced into foreign situations, enclosures and new habitats, and to sedate animals sufficiently to enable them to withstand stressful activities during wildlife auctions (Read, 2002; West *et al.*, 2007). The term 'long-acting' has been used to describe any formulation that can be injected in such a manner that a single dose produces an effective tissue concentration for effects to be seen for up to 3 – 7 days. Irrespective of these definitions, a LAN can be seen as any tranquiliser that can produce an effect lasting from 3 to 30 days (Read, 2002; Swan, 1993). The following are some of the most notable effects seen in animals treated with LANs (Ebedes, 1993):

- Modification of the animal's disposition towards its immediate surroundings and also towards other captive animals
- The animals show an indifference to their captive surroundings and they start eating and drinking sooner than animals not tranquilised
- Animals that were apprehensive of humans lose their instinctive fear and allow humans to approach closely
- In losing their fear of humans, some individual animals may become reckless and attack a person who approaches too quickly

Ebedes (1993) also presented a comprehensive review of the use of LANs in various wildlife species as well as which situations and animals are best suited for its use. The pharmacology of all LANs is very similar to that of phenothiazine derivatives with the prolonged effect of LANs being achieved in any one of four ways (Ebedes, 1993; La Grange, 2006):

- by the slow release of the active ingredients from the injection site;
- by the slow, sustained absorption of the active ingredients or metabolites into the bloodstream;
- by the slow metabolism of the active drug in the tissue; and
- by the slow elimination of the drug and/or its metabolites from the tissues and the bloodstream.

The formulation of most LANs is similar in that they consist of a fatty acid ester of the active drug ingredient, which is then dissolved in vegetable or medicinal oil. Injected intramuscularly, this formulation forms a depot at the injection site (Fick *et al.*, 2005). With the slow breakdown of the oil solvent, the ester then diffuses out of the depot and

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into muscle and once absorbed into the blood, it is hydrolysed and the active ingredient is able to exert its effect (Read, 2002; Swan, 1993). This particular pharmacokinetic property accounts for the delayed onset of action of LANs. Because of different onsets and durations of action, a combination of tranquillisers is usually given simultaneously to an animal to achieve rapid, continual and long-lasting tranquilisation (Ebedes, 1993; Fick, Fuller, & Mitchell, 2005). All LANs are metabolised by the liver, and their metabolites are excreted in the faeces (Read, 2002; Swan, 1993).

LANs act primarily as antipsychotics by blocking dopamine receptors in the limbic system, and there are no specific pharmacological antidotes to LANs. In humans, their efficacy and success depend mainly on individual dosage after titration of the dose to the most effective dose regimen (Read, 2002). However, in wildlife this is rarely possible with the result that the potential for side-effects is higher in animals than in humans (JP Raath, personal communication, 3 July, 2012; Read, 2002). Well-documented side-effects include extrapyramidal symptoms such as allotriophagia (chewing), torticollis, tremors, shivering and catatonia (Kock & Burroughs, 2012). Treatment of these effects is generally symptomatic with sedatives such as xylazine, butorphanol and acepromazine having been used to limit the excitatory signs that may be seen (Ebedes, 1993). Anticonvulsants such as biperiden or diazepam may also be used with single or repeated doses of 10–20 mg (Kock & Burroughs, 2012; Read, 2002).

The most commonly used LANs in wildlife are zuclopenthixol acetate (Clopixol Acuphase®) and perphenazine enanthate (Trilafon LA®) (JP Raath, personal communication, 3 July, 2012). Other LANs include zuclopenthixol decanoate (Clopixol®) and pipothiazine palmitate (Piportil®) although these are often not recommended due to the excessively long duration of their effects and the practical complication that arises (refer to Table 3.4) (Swan, 1993).

Table 3.4 Characteristics of long-acting neuroleptics (Read, 2002)

Drug name	Trade name	Time to initial effect	Duration of action
Zuclopenthixol acetate	Clopixol Acuphase	1 hour	2–4 days
Zuclopenthixol decanoate	Clopixol	1 week	10–21 days
Perphenazine enanthate	Trilafon LA	12–16 hours	7–10 days
Pipothiazine palminate	Piportil	3 days	2–4 weeks

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Perphenazine enanthate is a phenothiazine derivative with a piperazine side chain. It is available in a 100 mg/ml injectable solution in the enanthate ester form, dissolved in sesame oil, which causes its slow absorption and long-acting effect (Ebedes, 1993; West *et al.*, 2007). As with all LANs, perphenazine enanthate must be given intramuscularly. However, its effects can only be seen after approximately 12–18 hours and it is thus often used in combination with other shorter-acting tranquilisers that take effect sooner. The effects of perphenazine enanthate peak at 36 hours and last for up to 7–10 days (Kock & Burroughs, 2012). It is recommended for the sedation and calming of a variety of herbivore species, including impala, deer species and equid species (Read, 2002).

Zuclopenthixol acetate is a thioxanthene similar to the phenothiazine derivatives. Its absorption and duration have been extended through esterification with the acetate and its dissolution in vegetable oil (West *et al.*, 2007). It is also available as a decanoate ester (Clopixol®), which has a longer effect of up to 21 days. With both forms of the drug, dissolution in oil delays the absorption of the drug from the injection site (Read, 2002). The effects of zuclopenthixol acetate can be seen within an hour after administration, peak at approximately 36 hours and last for up to 72 hours. It is often seen as an intermediate LAN, and is used either alone or to bridge the effects of shorter or longer-acting neuroleptic drugs when used in combination (Kock & Burroughs, 2012; Swan, 1993). Generally, very few side-effects are observed; however, as with all other LANs, extrapyramidal symptoms may occur (Ebedes, 1993). Zuclopenthixol acetate has been successfully used in a variety of species such as deer species, equid species, bison, wapiti, various antelope species and rhino (Kock & Burroughs, 2012; Read, 2002).

As previously mentioned (see section 2.5.1.), a distinction can be made between sedatives and tranquilisers. Unlike tranquilisers, the effect of sedatives is dose-dependent with only slight sedation at low doses (Walsh & Wilson, 2002). Increasing the dose will increase the sedation until an apparent state of immobilisation occurs. However, when such animals are stimulated, they can be aroused easily from this state of immobilisation and can quickly become respondent again (Kock & Burroughs, 2012; Swan, 1993). Sedatives are seldom used on their own, and are generally used in combination with immobilising drugs or immediately after restraint of an animal for their anticonvulsant, muscle relaxing and sedative qualities (Swan, 1993). Sedatives have minimal side-effects since they affect central nervous system function, the brain and the spinal cord and not the peripheral nervous system (Kock & Burroughs, 2012).

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There are two main groups of sedatives used in wildlife, namely the benzodiazepines and the α-2-adrenoreceptor agonists (La Grange, 2006). The benzodiazepines were originally used in human medicine for the treatment of anxiety but have become popular for animal use, as they produce a moderate sedation, cause profound muscle relaxation, have anticonvulsant activity with essentially no cardiopulmonary side-effects (Walsh & Wilson, 2002). The effects of benzodiazepines stem from their affinity for specific receptors in the brain and spinal cord, and responses after their administration are to a large degree central in origin (Swan, 1993). It is believed that there is some difference between the anxiolytic and the hypnotic-sedative effects of this class of drugs, as they produce variable degrees of sedation in most animal species (Walsh & Wilson, 2002). For example, it has been found in deer that the reduction of anxiety and the removal of inhibitions by administration of diazepam causes animals to become aggressive rather than quiet (Walsh & Wilson, 2002).

There are several benzodiazepines on the market for human use and for some time, diazepam was the only drug from this group used in wildlife (Kock & Burroughs, 2012). Diazepam (Valium®/Pax®) is a colourless, crystalline substance that is available in injectable solution in various formulations (Kock & Burroughs, 2012). If administered intravenously, the onset of effects occurs within 1–2 minutes and within 15–30 minutes if given intramuscularly (Swan, 1993). It is a good muscle relaxant and anticonvulsant and is often used to control extrapyramidal symptoms caused by other drugs, such as convulsions and muscular hypertonicity (Kock & Burroughs, 2012). Diazepam is well tolerated at high doses although respiratory depression may occur at very high doses (JP Raath, personal communication, 3 July, 2012). Reversal of the effects of diazepam can be achieved through the administration of flumazenil (Anexate®) (Swan, 1993).

Midazolam (Dormicum®) is another benzodiazepine that is used in wildlife. It is more potent than diazepam and has been used both for sedation and for induction of anaesthesia (Kock & Burroughs, 2012; Swan, 1993). It is highly lipophilic, resulting in rapid distribution into the central nervous system and adipose tissue (Malinovsky *et al.*, 1991). These properties allow the drug to reach the site of action rapidly and to take effect within 1–2 minutes (Gan, 2006). Although midazolam is not often used in wild animal immobilisations, it has a significant use as a short-acting sedative during translocations (JP Raath, personal communication, 3 July, 2012). The rapid recovery from sedation with midazolam results from the rapid redistribution of the drug from the brain to peripheral tissues (Gan, 2006; Kock & Burroughs, 2012). As with diazepam, the

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effects of midazolam can be reversed with flumazenil, which binds to the benzodiazepine receptors (Kock & Burroughs, 2012).

The second group of sedatives is the α-2-adrenoreceptor agonists. The α-2-adrenoreceptor is a distinct sub-class of α-adrenergic receptors, which are located in the central nervous system and virtually every peripheral tissue (Sinclair, 2003). The central α-2-adrenoreceptors in the locus coeruleus on the pons and lower brainstem are thought to be the mediators of the sedative effects of the α-2 agonists (Walsh & Wilson, 2002). The α-2-receptor subtypes in this region regulate the stages of awareness, arousal and vigilance in the brainstem as well as regulating peripheral vasoconstrictive effects (Sinclair, 2003). The α-2 agonists combine with these receptors at both the pre-synaptic and post-synaptic levels and have varying effects on blood pressure. As a result, an initial increase in blood pressure is seen due to vasoconstriction and an increase in peripheral resistance but thereafter, blood pressure decreases due to the baroreceptor reflex and the suppression of cardiac output (Bousquet, Feldman, & Schwartz, 1984). Notably, the α-2 agonists also have a significantly negative effect on respiration (Kock & Burroughs, 2012). Sinclair (2003) gives a complete and comprehensive review of the physiological effects of α-2 agonists.

Xylazine (Rompun 2%®, Chanazine 2%®, Xylavet 2%®) is an α-2 agonist that is commonly used, on its own or in combination with other drugs, during the immobilisation of wildlife (Wollen, 1978). As with most other sedatives, the effective dose varies markedly between species and individuals, with high doses being required to produce sedation in highly excited animals (Walsh & Wilson, 2002). It is available either in powder form or as injectable solution and effects can be seen within 3–5 minutes after intravenous administration or 10–15 minutes after intramuscular administration. Analgesia lasts for 15–30 minutes while sedation can last for up to 1–2 hours (Kock & Burroughs, 2012; Swan, 1993). Although it can be used on its own at high doses to induce immobilisation, it is more commonly used in combination with various anaesthetics and tranquilisers for its synergistic effect with these drugs, and it has been shown to be very effective in a number of species including rhino and lion (Fahlman, 2008; Swan, 1993). Its effects can be reversed with the administration of α-2 antagonists, yohimbine and atipamezole (Antisedan®).

Detomidine (Dormosedan®) and medetomidine (Domitor®) are α-2 agonists that display a greater affinity for all α2- adrenoreceptor subtypes than xylazine (Walsh & Wilson, 2002). Both these α-2 agonists are reported to be up to 10 times more potent than

xylazine (Kock & Burroughs, 2012). The chemical structure of medetomidine differs from that of detomidine by one methyl group in the CH_2-bridge.Although medetomidine's pharmacodynamic properties are similar to those of detomidine, medetomidine is more potent than detomidine and its mechanisms of action is more selective than those of detomidine (Swan, 1993). Both sedatives are available in aqueous injectable form and can be administered intramuscularly or through slow intravenous injection (Kock & Burroughs, 2012; Swan, 1993). Both detomidine and medetomidine are lipophilic and as a result are rapidly and completely absorbed after intramuscular injection (Sinclair, 2003). Effects can be anticipated within 1–5 minutes after administration, depending on the dose and can last for up to 6–8 hours at very high dose rates (Swan, 1993). As with xylazine, both medetomidine and detomidine can be safely reversed with the administration of the α-2 antagonists, yohimbine and atipamezole (Kock & Burroughs, 2012).

3.3 OVERVIEW OF EXISTING RESEARCH AND POTENTIAL RESEARCH OPPORTUNITIES ON THE EFFECT OF NEUROLEPTICS ON THE STRESS RESPONSE OF WILDLIFE

From the information presented in Table 3.1, it is evident that there are three classes of typical neuroleptics, all of which are currently used in wildlife management practices. The three classes are phenoxianines, thioxanthenes and butyrophenones, each with its own pharmacodynamics, and a particular drug is usually selected on the basis of the duration of tranquilisation required (Fick *et al.*, 2005). All of these neuroleptics have been used successfully worldwide to reduce the considerable losses associated with translocation stress in wildlife and considerable research has been done to establish their efficacy in various species (Diverio, Goddard, Gordon, & Elston, 1993).

The use of phenothiazine derivatives (both long-acting and short-acting) as tranquilisers has been investigated in a number of studies. Acetyl promazine is the most commonly used phenothiazine in veterinary medicine, and reports often show its use in combination with etorphine in the form of Immobilon® (Walsh & Wilson, 2002). Both Fong (1982) and Mckean, Stock and Magonigle (1978) report the suitability of its use in combination with etorphine in caribou and domestic goats respectively. According to Jones (1984), this combination is also suitable for use in sika (*Cervus nippon*) and red deer but is less suitable for other species of deer because the promazine derivative has been deemed ineffective in reducing the excitatory effect of etorphine (Walsh & Wilson, 2002).

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Montané *et al.* (2003) report on the suitability of acetyl promazine on its own during the capture of roe deer to reduce stress and prevent adverse effects. The authors found that heart rate stabilised sooner in treated animals than in untreated animals, and that blood parameters such as red blood cell count (RBC), lymphocyte count, haemoglobin concentration, packed cell volume (PCV), and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) were significantly lower in treated animals. Montané *et al.* (2002) investigated the suitability of acetyl promazine in reducing stress during the transport of roe deer and found similar decreases in blood parameters in treated animals.

Knox, Hattingh, and Raath (1990) report on the effects of the long-acting phenothiazine derivatives, perphenazine enanthate and pipothiazine palmitate, on the immediate responses of impala to capture and handling. The authors found no difference in the physiological responses of animals to capture and handling between treated and untreated groups. They did however, note that the tranquillisers produced sedation during the initial confinement and concluded that the effect of these tranquillisers may be to increase the threshold above which a stress response is elicited rather than to suppress the response itself. Diverio *et al.* (1996) report on the effects of perphenazine enanthate in red deer. The authors found that treated animals showed reduced behavioural changes due to stressors and that plasma cortisol levels returned to pre-treatment levels faster after the application of a stressor in treated animals than in untreated animals. In a similar study, Diverio *et al.* (1996) investigated the effect of the phenothiazine derivative, perphenazine enanthate and the thioxanthene derivative, zuclopenthixol acetate on stress in farmed red deer. The authors looked at behavioural responses to management practices and found that both LANs (perphenazine enanthate and zuclopenthixol acetate) promoted maintenance of a more normal activity pattern when the animals were disturbed.

Zuclopenthixol acetate is a thioxanthine derivative that shares general properties with the other phenothiazines (Swan, 1993). Zuclopenthixol acetate has been used highly successfully in a variety of species and is either used on its own or in combination with other tranquilisers such as azaperone (Read, 2002). In red deer, Read and McCorkell (2002) found that, used in combination, azaperone and zuclopenthixol acetate provided adequate relief from anxiety during translocation. The authors report that the tranquilisers allowed the animals to become gradually familiar with their new surroundings without excitement, injuries or mortalities. Read, Caulkett, and McCallister (2000) evaluated the effect of zuclopenthixol acetate on stress and activity in wild wapiti and found that treated

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animals had lower body temperatures, haemo-concentrations, serum cortisol and blood lactate and they were less metabolically acidotic. Read *et al.* (2002) conclude that the tranquiliser could successfully be used to reduce handling stress and activity in wapiti. Clippinger, Citino, and Wade (1998) found that Nile lechwe (*Kobus megaceros*) treated with zuclopenthixol acetate were easier to handle and manipulate, their flight distances were reduced, and they appeared subjectively less stressed during and after handling.

Butyrophenones, azaperone and haloperidol are commonly used for short-term tranquilisation of a number of wildlife species with haloperidol having the longest effect, lasting up to 12–18 hours (Swan, 1993). Mentaberre *et al.* (2010) investigated the effect of both tranquilisers on the stress response of drive-net captured roe deer. The authors found that the inter-individual variability of heart rate was lower in the treated animals, suggesting a palliative effect. They also found that erythrocyte and biochemical parameters indicated vasodilation, splenic sequestration, haemo-dilution, improvement of renal perfusion and a protective effect on muscle. They conclude that both tranquilisers could be used to reduce stress and prevent adverse effects in this species. Mentaberre *et al.* (2010) report similar results in Iberian ibexes (*Capra pyrenaica*) and conclude that both tranquilisers were suitable for reducing stress in this species. In southern chamois however, Marco *et al.* (2010) found that haloperidol and azaperone resulted in elevated heart rates, elevated body temperatures and elevated serum muscular enzymes, suggesting increased muscles stress, and the authors consequently do not recommend the use of either of these tranquilisers in this species.

The effect of neuroleptics on a number of physical responses in wildlife has also been investigated. Fick, Matthee, Mitchell, and Fuller (2006) investigated the effect of perphenazine enanthate and zuclopenthixol acetate on the body temperature, activity and food intake of blue wildebeest since neuroleptics have the potential to affect body temperature through several pathways. The authors conclude that neither LANs adversely affected any of these parameters. The same authors also investigated the effect of zuclopenthixol acetate and pherphenazine enanthate on the thermoregulation of goats. Again, they found that treatment with the LANs did not impair the activity or thermoregulation of the animals. Meyer *et al.* (2008) investigated the effect of chemical immobilisation using four different anaesthetic and sedative/tranquiliser combinations on thermal, cardiorespiratory and cortisol responses in impala. The authors found that changes in these parameters were largely due to the time it took for animals to reach recumbency (and thus the time exposed to the stress of capture) rather than being due to specific drug combinations and the effects of the drugs.

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3.4 CONCLUSIONS

Although the contribution of neuroleptics to the successful capture, relocation and confinement of wildlife is indisputable, the particular effects of these drugs on physiological and behavioural variables need further investigation (Fick, Mitchell, & Fuller, 2007; Swan, 1993). In the South African wildlife industry, these drugs are primarily used for the reduction of stress and as such, elicit an array of changes in the body. In an attempt to illustrate these potential changes, this review aimed to present a synopsis of the various facets that make up the mammalian stress response. In addition, the review has provided an examination of available literature in order to explain the different ways in which all the aspects of the stress response can be quantified in terrestrial species. It has also expanded on the wildlife industry in South Africa as well as the use of various capture and relocation techniques in order to emphasise the use of neuroleptics during these practices and the need for research into the exact effects that these drugs have on South African species. Currently, available literature on the use of neuroleptics in South African wildlife species is limited, with few studies focusing on more than one aspect of the stress response.

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CHAPTER 4

Materials and Methods

In order to avoid repetition between chapters in the dissertation, the materials and methods used in the study have been summarized in a single chapter (Chapter 4). References to this chapter are made in the chapters that follow and if any deviation occurred from the materials and methods described below, it is stated in the respective chapters. The research hypotheses are also stated within each chapter of the dissertation.

4.1 MATERIALS

Ethical approval for all aspects of the project was obtained from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa (Protocol Ethical Approval number SU-ACUM11-00005).

4.1.1 Animals

For this study, blue wildebeest (*Connochaetes taurinus*) were used. Preliminary trials on wildlife behaviour in captivity indicated that this species exhibited minimal behavioural problems when placed in groups in enclosures.

4.1.6.1 Species

Blue wildebeest are relatively abundant in the study area and are not as costly as some of the other similar-sized wildlife species. Blue wildebeest (*Connochaetes taurinus*) are among the most prominent grazers in the African savannah biome (Codron & Brink, 2006). Adult males weigh between 230 and 270 kg and have a shoulder height of 1.5 m, compared to females that generally weigh between 160 and 200 kg and have a shoulder height of 1.3 m (Wildlife South Africa, 2014).

Blue wildebeest are highly gregarious and territorial. They are usually found in herds of 20–30 females and young with an adult bull, or the latter is also found in bachelor herds. Sometimes, aggregations of thousands can occur (Apps, 1997; Wildlife South Africa, 2014). Reproduction of this species has been found to be timed with climate so that both

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rutting and calving occur when conditions are most favourable. Mating season occurs between March and June, once the summer rains have come to an end, and usually lasts for 3–4 weeks (Geraci, 2012). The gestation period is on average 8–8.5 months long, and calving occurs from mid-November to December (Estes, 1991; Skinner & Chimimba, 2005; Wildlife South Africa, 2014). The average lifespan of a wildebeest is estimated at 20 years (Estes, 1991; Geraci, 2012).

4.1.6.2 Origin of animals

Animals were acquired from the respective farms indicated in Table 4.1.

Table 4.1 The origin of the animals used for the experimental trials in this study

Trial	Tranquiliser administered	Number of animals	Genders	Source	Capture method
16–18 June 2012	Clopixol Acuphase®	6	6♀	Ngongoni Farm, Nelspruit	Darted from a helicopter
23–25 October 2012	Acunil®	6	3♀ : 3♂	Mataffin Eco-Estate, Nelspruit	Darted from a vehicle
24–26 April 2013	Acunil®	5	3♀ : 2♂	Shandon Eco-Estate, Nelspruit	Darted from a vehicle
2–4 May 2013	Acunil®	6	2♀ : 4♂	Ngongoni Farm, Nelspruit	Darted from a helicopter
1–3 August 2013	Placebo	6	3♀ : 3♂	Shandon Eco-Estate, Nelspruit	Darted from helicopter
4–6 August 2013	Clopixol Acuphase®	6	3♀ : 3♂	Shandon Eco-Estate, Nelspruit	Darted from helicopter
6–8 October 2014	Placebo	6	3♀ : 3♂	Ngongoni Farm, Nelspruit	Darted from a vehicle
9–11 October 2014	Clopixol Acuphase®	5	3♀ : 2♂	Mataffin Eco-Estate, Nelspruit	Darted from a vehicle

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During the April 2013 and October 2014 trials, one animal was removed from the boma (enclosure) within 24 hours after capture, as it was deemed a problem animal that could potentially injure itself or its contemporaries. These animals were highly volatile and consistently fought with other animals throughout the first 24 hours in captivity.

4.1.2 Transport and relocation

Animals were captured and transported by a professional game capture team from Wildlifevets.com. The animals were transported in mass crates as the loading and transportation process is much quicker and less labour-intensive compared to individual transportation. All recommended guidelines for transportation of wildlife were followed at all times and crates were also constructed according to these guidelines (McKenzie, 1993). A short-acting neuroleptic, azaperone tartrate (Wildlife Pharmaceuticals SA (Pty) Ltd., White River, South Africa) at a maximal dosage of 0.3 mg/kg to sedate the animals during the relocation. Azaperone is eliminated from the blood within 0.5 – 2.5 hours due to its rapid and extensive metabolism by the liver, and therefore no residual effect was expected after 24 hours (Food and Drug Administration of the United Nations, 1991). All drugs used were administered by a registered veterinarian.

4.1.3 Housing and management

Animals were housed in enclosures (also referred to as a boma) constructed from sturdy gumpoles, and were 6X8m in size (Figure 4.1). The gumpoles were positioned close to each other so that animals could not see into adjacent bomas. The bomas were located on Ngongoni Farm, outside Nelspruit (25°31’25.2”S, 31°06’50.8”E), on an open site that was easily accessible by game capture trucks, but far away enough from any commercial roads so that the animals would not be disturbed by the resulting noise. Only one boma was used at a time in each trial. A fully equipped veterinary laboratory was available within 150 m from the boma. One third of each boma was covered with shade netting and the bomas were built on a slight slope to ensure proper drainage. Each boma was connected to a 1.2 m wide passageway that allowed for easy movement of the animals when they were being off-loaded. At the end of the passage was an off-loading ramp which was 2.2 m wide so that it created a funnel for the animals when they were off-loaded. Solid feed containers on the ground were used, with smooth edges to prevent injury to the animals. Cement water troughs that could accommodate more than 50L of water, were sunk into the ground and extended to the outside of the bomas. Animals were fed lucerne hay *ad libitum*.

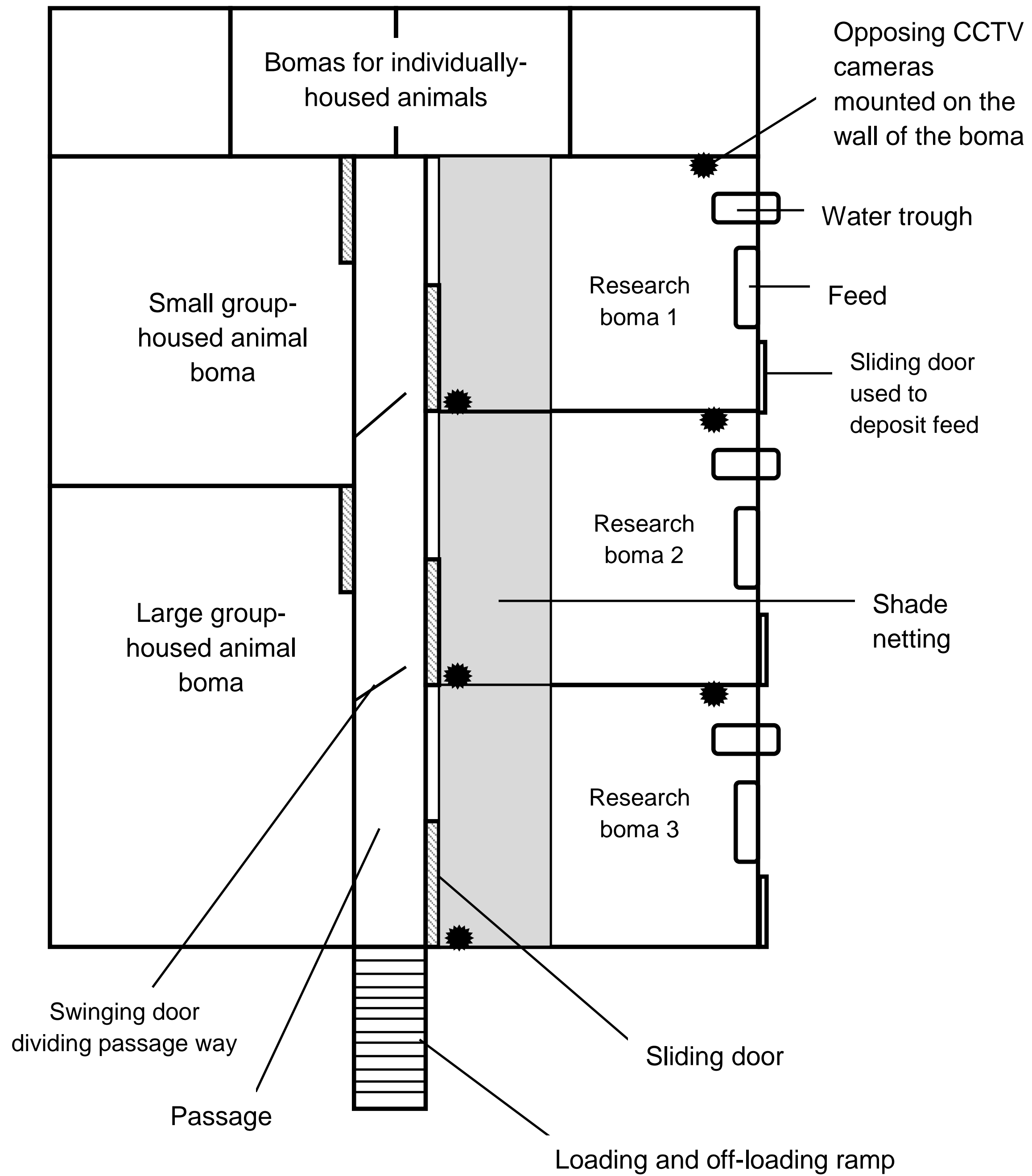

Figure 4.1 Diagrammatic illustration of the research enclosures used during the study

4.1.4 Drug administration

The pharmaceutical drugs summarised in Table 4.2 were administered during the capture, relocation and immobilisation of all animals. All pharmaceutical drugs were administered by registered veterinarians.

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Table 4.2 Details on the pharmaceutical drugs used during the study

Substance	Supplier	Route	Dosage/body mass	Frequency
Thiafentanil oxalate	Wildlife Pharmaceuticals SA (Pty) Ltd.	IM*	0.01–0.02 mg/kg	Four times in 7 days
Azaperone tartrate	Wildlife Pharmaceuticals SA (Pty) Ltd.	IM	Up to 0.3 mg/kg	Once in 72 hours
Naltrexone	Wildlife Pharmaceuticals SA (Pty) Ltd.	IM	10 times thiafentanil	Four times in 7 days

* Intramuscularly

A standard chemical capture datasheet (Appendix I) was used for the monitoring of all animals during capture and relocation as well as when animals were anaesthetised.

The effect of treatment with two long-acting neuroleptics on reducing stress in captive wildebeest was investigated (Table 4.3). One of these two neuroleptics was administered on the second day of each trial to all animals within that trial. In addition, animals were administered a placebo consisting of sterile water at the same volume as the neuroleptics in two additional and separate trials.

Table 4.3 Long-acting neuroleptics investigated and administered in the study

Substance	Source	Route	Dosage/body mass	Frequency
Acunil®	Wildlife Pharmaceuticals SA (Pty) Ltd.	IM	Up to 1.5 mg/kg	Once in 72 hours
Clopixol Acuphase®	Lundbeck, South Africa	IM	Up to 1.5 mg/kg	Once in 72 hours

The active drug ingredient in both Acunil® and Clopixol Acuphase® is zuclopenthixol acetate. However, Acunil® is zuclopenthixol acetate dissolved in a slow-release polymer while Clopixol Acuphase® is zuclopenthixol acetate dissolved in a vegetable oil. Both solvents allow for the slow release of the active drug component from the intramuscular site of injection, although the release profiles differ between the two formulations. Previous studies have shown that Clopixol Acuphase® produces a maximum serum

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concentration after 36 hours, and maximum sedation is achieved at 8 hours after administration (Aaes-Jørgensen, 1989; Amdisen *et al.*, 1987; Chakravarti, Muthu, Muthu, Naik, & Pinto, 1990; Lundbeck Canada Inc., 2011). Although no clinical research has been conducted on the use of Acunil®, studies performed with drug formulations in which the same slow-release polymer was used, indicated that maximum serum concentrations were achieved within 6 hours, and that serum concentrations were maintained above a therapeutic level for up to 72 hours. In addition, peak drug effects were observed at 4 hours after treatment (Carbone, Lindstrom, Diep, & Carbone, 2012; Clark, Clark, & Hoyt, 2014; Foley, Liang, & Crichlow, 2011; Healy *et al.*, 2014; SR Veterinary Technologies, 2011, 2012).

Both Clopixol Acuphase® and Acunil® are referred to as long-acting neuroleptics in the dissertation and in order to avoid confusion, the trade name will be used when referring to each drug.

4.1.5 Biotelemetry system

The Equivital™ EQ02 wireless biotelemetry system was used in the study. The fitting and modification of this system is described below. The accuracy of the belt was validated in the study of Laubscher *et al.* (2015).

4.1.5.1 Modification of the Equivital™EQ02 biotelemetry system

Six Equivital™ (Hidalgo Limited, Unit F. Trinity Court, Buckingway Business Park, Cambridge, UK) EQ02 sensor electronic modules (SEMs), weighing only 39 g each, were used in this study with the corresponding belts. The Equivital™ EQ02 wireless monitoring system was developed for use in humans and is capable of measuring an array of physiological parameters. For the purposes of this study, only heart rate, respiration rate, skin temperature and motion, were recorded. Although the Equivital™ system has the capacity to do live monitoring via Bluetooth technology, the data logging ability of the system was the preferred choice of recording data, since this resulted in a longer battery life.

Each belt consisted of a chest band with three sensors and an over-the-shoulder strap. The sensors act as a 2-lead electrocardiogram (ECG) so that heart rate is derived from a continuous ECG measurement. Each sensor is connected to an insulated wire that runs along the inside of the belt, and the wires from all three sensors lead to an attachment module on which the SEM is placed. On a human, the SEM would be located

directly above the heart. In animals, the design of the belt with the shoulder strap, however, does not allow this. Therefore, the shoulder straps on each of the belts were removed and the attachment module, to which the SEM is attached, was relocated from its original position and secured to run in line with the sensors. When the SEM was placed on the attachment module, it was secured to the belt using cable ties to ensure that it did not move or dislodge from its position (refer to Figures 4.2–4.3).

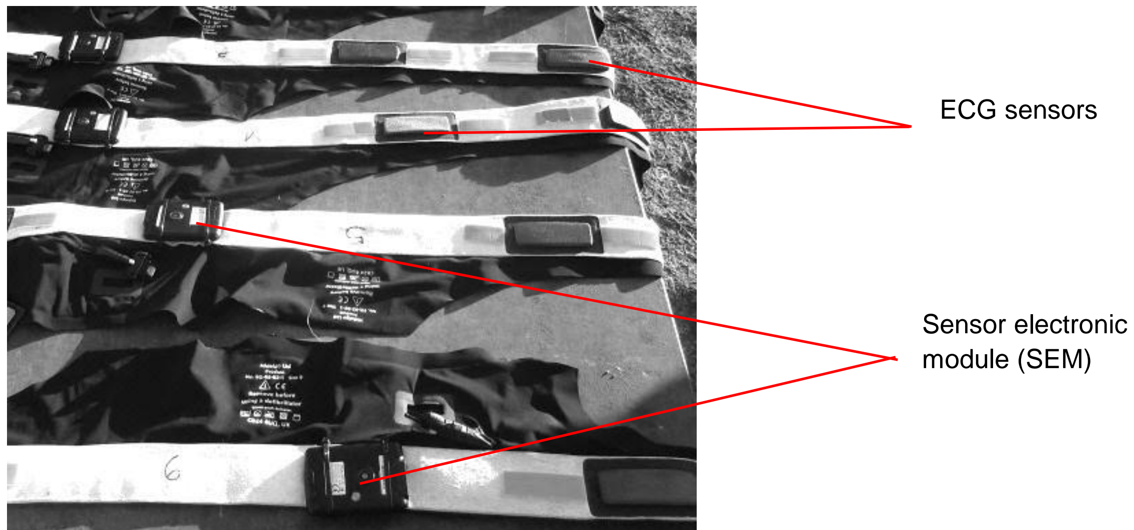

Figure 4.2 Modified Equivital™ EQ02 belts

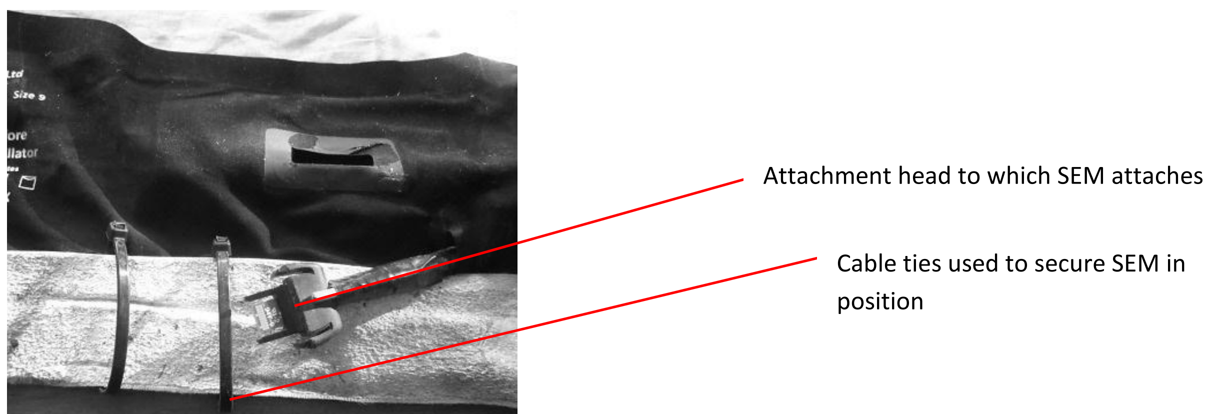

Figure 4.3 Modification of the SEM location on the Equivital™ EQ02 belts

Running along the sensor wires on the inside of the belt, is an elastic band with stretch sensors that record respiration rate through the counting of extensions. To prevent the elastic band from being over-extended, a stretch inhibitor runs alongside it consisting of a non-stretch fabric band that is secured to both ends of the belt (Refer to Figure 4.4). The stretch inhibitor proved to be too weak, and it was subsequently replaced with a

sturdier stretch inhibitor to prevent the belt from being over-extended, resulting in the wires and respiration elastic band breaking. We reinforced the attachment of the respiration elastic band to the belt with Dow Corning™ 1345, as we found this attachment to come undone quite easily when the belt is slightly over-extended.

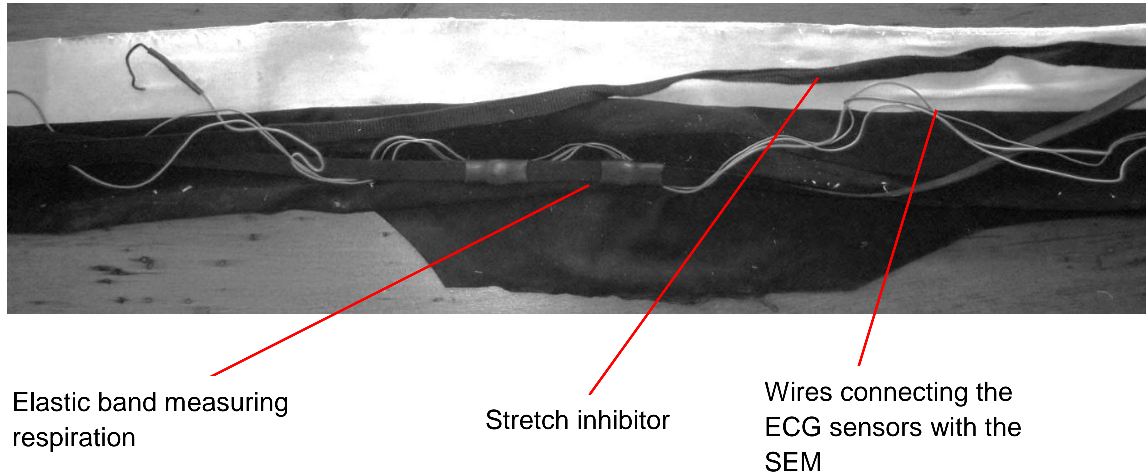

Figure 4.4 Sensors that run along the inside of the Equivital™ EQ02 belts

Lastly, the length of the belt was extended to fit around the chest circumference of a wildebeest.

4.1.5.2 Fitting the Equivital™ belts onto the animals

Whilst immobilised, each animal was kept in sternal recumbency with the head lifted to prevent regurgitation. The chest of each wildebeest was shaved and fitted with a modified Equivital™ belt so that the data-logger was positioned above the heart. Electro-gel was applied to each ECG sensor, and double-sided tape and Pattex Supergel® were used to secure the belt to the skin. Opsite™ Flexigrip™ transparent film dressings from Smith & Nephew (Smith & Nephew (Pty) Ltd., Pinetown, South Africa) were placed over the data-logger to protect it from dirt or water (Figure 4.5). Bluetooth technology and EquiView Mobile software (supplied by the manufacturer) were used to cross-check the readings of each of the belts with the readings of the pulse oximeter as well as manual heart rate and respiration rate readings.

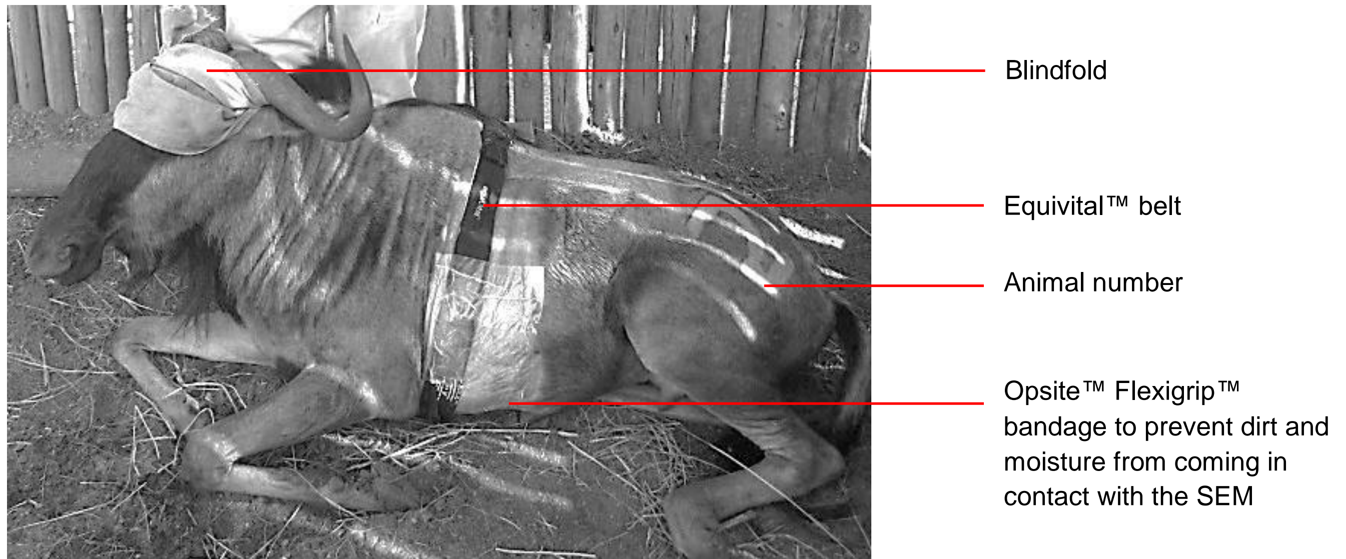

Figure 4.5 The fitted and secured Equivital™ EQ02 belt on a blue wildebeest

4.1.6 Behavioural monitoring

Continuous behavioural monitoring was done in order to quantify changes in animal behaviour.

4.1.6.1 CCTV cameras

The enclosure was fitted with a CCTV camera system (Nictec Radio Communications, Nelspruit, South Africa) consisting of two opposing cameras with infrared capabilities. The footage from these cameras was used for behavioural observations.

4.1.6.2 Noldus behavioural software

The Observer® XT11 behavioural software (Noldus Information Technology, Wageningen, the Netherlands) was used to perform behavioural analysis and to combine the biotelemetry data for each animal with its behavioural data.

4.2 EXPERIMENTAL DESIGN

Due to the high costs involved in the purchasing of the Equivital™ EQ02 belts, animal numbers were limited to only six animals per trial. As such, a repeated measures experimental design was used (Figure 4.6).

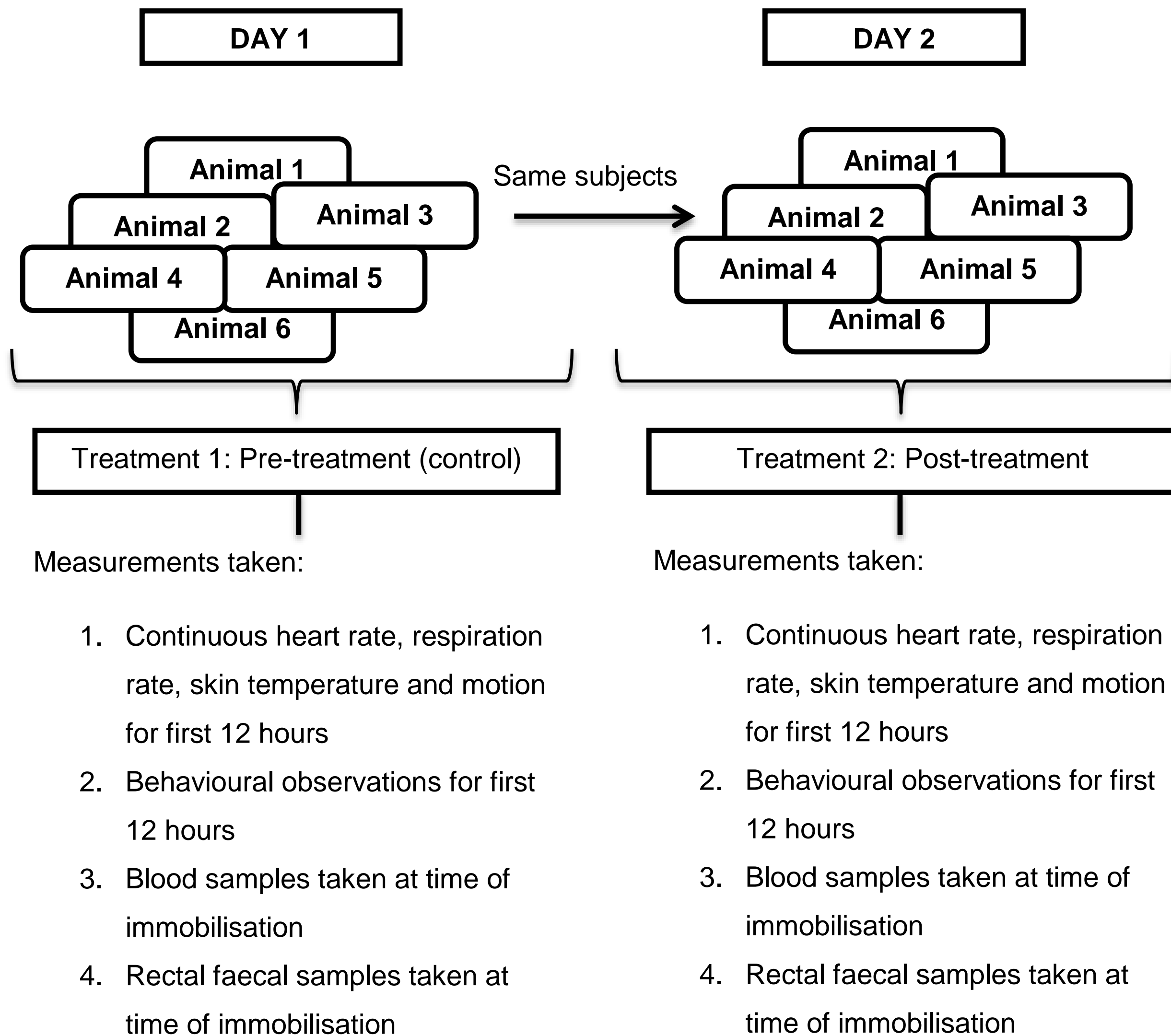

Figure 4.6 Schematic representation of experimental design

On the day of capture, the animals were darted from a vehicle or from a helicopter with 3.5–4.5 mg thiafentanil (Thianil®, Wildlife Pharmaceuticals SA (Pty) Ltd.) and transported to Ngongoni Farm. They were then left for up to 24-48 hours to ensure that they showed no behavioural problems such as fighting, and that all drugs used in the darting of the animals were completely eliminated from their systems. Figure 4.7 provides a schematic representation of the trial procedures.

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On day 1 (of a specific experiment), animals were anaesthetised early in the morning with thiafentanil (3.5–4.5 mg). Once animals were immobilised, they were blindfolded, the darts removed and dart wounds cleaned and treated. Each animal was weighed (using a hanging scale and stretcher) and consistently monitored with a pulse oximeter as well as manually measuring heart rate and respiration rate. Each animal was allocated a number corresponding to the number on the telemetry system and the number was spray-painted on both sides of the rear and sides of the animal for identification during video monitoring. A band of hair, approximately 20 cm in width, was shaved around the chest, all the way to the top of the spine. The cleanly shaven skin was also disinfected before fitting the biotelemetry belt.

After all the animals had been fitted with a biotelemetry belt, the CCTV cameras were switched on. The trial started once the reversal (35–45 mg naltrexone; Trexonil®, Wildlife Pharmaceuticals SA (Pty) Ltd.) was administered and all animals were standing, reactive and displaying normal behaviour. This was considered as Treatment 1, pre-tranquilisation. Previous studies have found that thiafentanil is rapidly and completely reversed by naltrexone and no residual effects or renarcotizations were observed in the current study (Arnemo, Kreeger, & Soveri, 2003; Kreeger, Cook, Piché, & Smith, 2001; Wolfe, Lance, & Miller, 2004).

On the following day, the process was repeated and belts were readjusted and fastened if they had loosened or moved. Before administering the reversal, either the tranquiliser being tested or a placebo was given intramuscularly to all the animals. This was considered as Treatment 2, post-tranquilisation. On day 3, the animals were darted one last time with thiafentanil in order for the belts to be taken off and the trial was completed. Throughout each treatment for the first 12 hours, animals were stimulated every two hours for a minute. This was achieved by a person entering the enclosure and moving around a radius of 1-2 meters from the enclosure door. A pipe was used to hit the enclosure wall in order to make a noise and frighten the animals. Since animals had no other human contact during the trials, it was thought that such stimulations would elicit a stress response and by doing so, minimize acclimatization to captivity. In addition, the elicitation of the stress response would enable the analysis of data within this stress response before and after administration of a neuroleptic. Each treatment and trial started at roughly the same time in the morning and stimulations within a trial occurred at roughly the same time each day.

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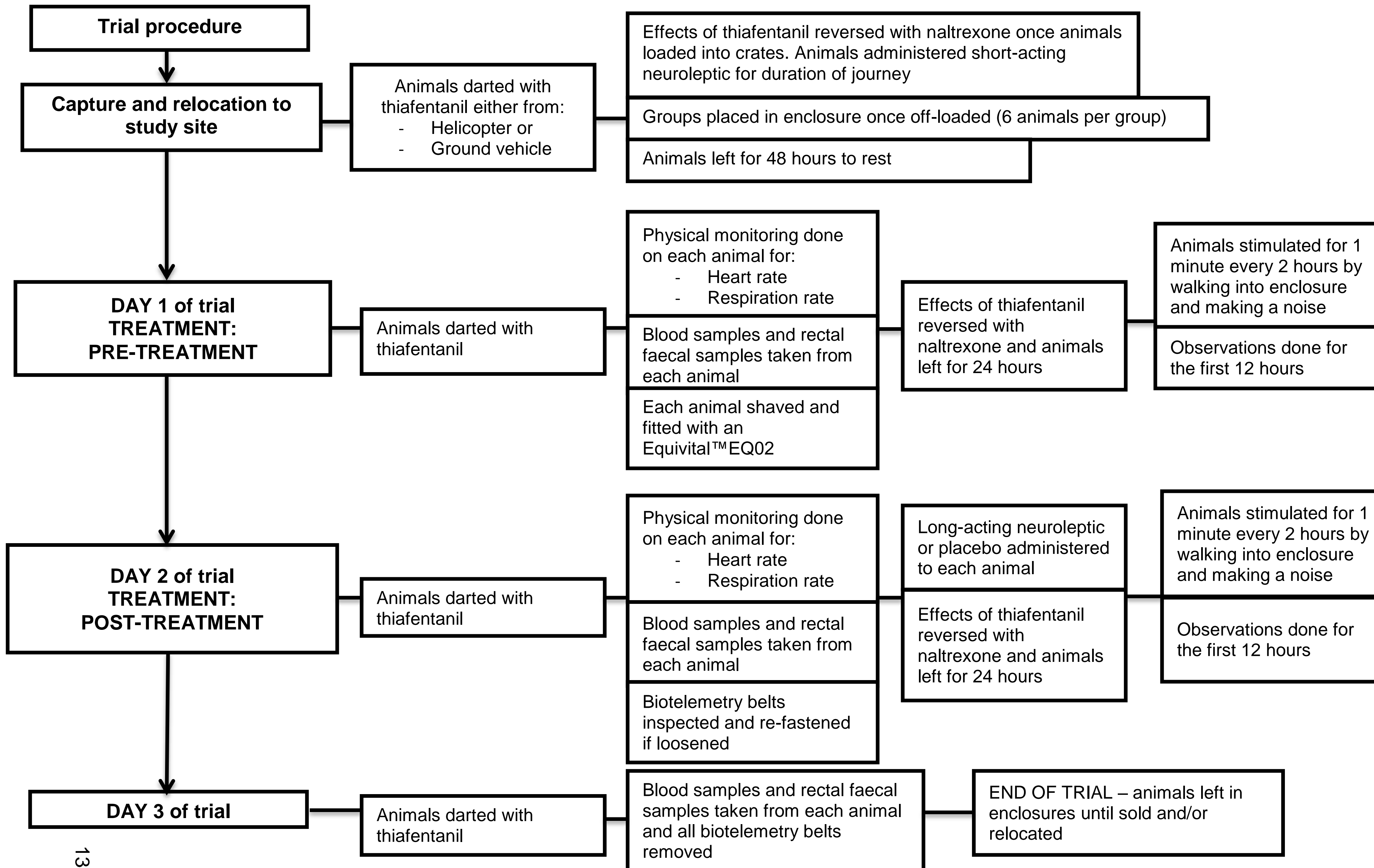
Each time the animals were anaesthetized, blood and rectal faecal samples were taken for analysis. A thermo-hygrometer data logger (Comet™, Comet Systems, Czech Republic) was placed outside the boma for the duration of each trial to measure environmental temperature. Only the first 12 hours of each trial day were used since the battery life of the Equivital™ system does not exceed 38 hours so that data logging stopped after the first 14 hours of the second trial day. The first 12 hours after treatment with both formulations of zuclopenthixol acetate, is also the period in which the drug takes full effect and causes the most notable changes in the animal. Although serum concentrations of Clopixol Acuphase® reach a maximum at 36 hours after its administration, sedation as a result of its administration reaches a maximum 8 hours after intramuscular injection (Amdisen *et al.*, 1987; Aaes-Jørgensen, 1989; Chakravarti *et al.*, 1990; Lundbeck Canada Inc., 2011). Therefore, no new changes can be expected to result from its administration after 12 hours post-treatment. Behavioural observations exceeding 12 hours also increased the risk of observational errors due to the difficulty in observing and identifying individual animals at night.

At the end of each trial, animals were kept in the boma for an additional 3-5 days in order to observe any possible adverse effects that may have resulted from the trials. No such effects were observed in any of the animals. Animals were thereafter either released onto Ngongoni farm or sold to game farms in the area, and relocated once sold.

Figure 4.7 Schematic representation of experimental trial design and procedures



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4.3 EXPERIMENTAL PROCEDURES

4.3.1 Sampling regimen

Both blood and faecal samples were collected from all animals during the study. The sample collection techniques are described below.

4.3.1.1 Faeces

Faecal samples were collected rectally from each individual animal every time the animals were anaesthetised, namely when the Equivital™ belt was fitted, prior to administration of the LAN, and on removal of the Equivital™ belt. To prevent contamination, faecal samples were collected with a new rectal examination glove for each animal. Samples were immediately placed in a collection bottle and frozen. The frozen samples were transported to the Department of Physiology at the University of the Witwatersrand for analysis.

4.3.1.2 Blood

Blood samples from the jugular vein were drawn into green-top lithium heparin tubes as well as red-top vacutainer tubes containing a clot activator (BD Vacutainer Systems, Plymouth, UK) on the same occasions that faecal samples were collected (Ganhao, Hattingh, & Pitts, 1988; Meyer *et al.*, 2008). Samples were taken in duplicate. Whole-blood samples were used for determination of blood glucose and lactate levels as well as neutrophil function. The remaining samples were centrifuged at 5000 rpm for 10 minutes and the serum transferred into cryotubes and frozen in liquid nitrogen. These samples were later transported to the Department of Physiology at the University of the Witwatersrand for endocrine analysis (as described below).

4.3.2 Blood analysis

A number of parameters were measured in the blood samples collected from each animal and the analysis techniques are described below.

4.3.2.1 Cortisol assay

The plasma was stored at -70 °C until thawed for cortisol concentrations to be measured by radio-immunoassay (Coat-a-Count®, Siemens Healthcare Diagnostics, Los Angeles,

Santa Ana, CA 90045, USA). The sera were incubated for 45 minutes at 37 °C with [^{125}I]-cortisol tracer in assay buffer in tubes coated with anti-cortisol antibody. After decantation, radioactivity in the tubes was measured. For each standard, the percentage bound was manually plotted against the cortisol concentration on logit-log graph paper provided with the kit and the unknown samples read from the curve (Meyer *et al.*, 2008; Wilke & Hirning, 1984).

4.3.2.2 Glucose

Whole-blood glucose was determined using the AccuTrend Plus® hand-held device from Roche. The device (115 × 62 × 18.5 mm) is a battery-driven (3 × 1.5 V batteries, type AAA) analyser that weighs approximately 100 g. Duplicate samples were tested for each animal. The device is a point-of-care device that returns whole-blood glucose levels instantly on instillation of a single drop of blood on a dedicated test strip. The AccuTrend Plus® device and test strips were calibrated every day. The AccuTrend Plus® reads the lot-specific characteristic of the test strips by means of a code strip. When a strip is inserted, the application area of the strip is illuminated by an LED (light-emitting diode) from below. Before sample application, the reflection behaviour of the test strip is determined by means of the light which is reflected from the application area (blank value). After the blood sample has been applied to the application area and the measurement chamber flap closed, the glucose undergoes an enzymatic reaction with the formation of dye. The amount of dye formed increases with the concentration of the glucose. After five seconds, the colour intensity is measured by illuminating the application area again from below using the LED. The intensity of the reflected light is measured with a detector (reflectance photometry). The measured value is determined from the signal strength of the reflected light, using the previously measured blank value and the lot-specific information from the code strip (Meynaar *et al.*, 2009; Roche Diagnostics, 2010).

4.3.4.1 Lactate

The AccuTrend Plus® hand analyser from Roche was used to measure whole-blood lactate. Duplicate samples were tested for each animal. The device measures whole-blood lactate values sampled from capillary blood, as suggested by the manufacturer, with the measuring range being 0.8–22 mmol/L. The sample is first applied to a coded yellow test strip with a reagent chemical substance. Then the strip is inserted into the analyser where the lactate concentration is determined by reflectance photometry via a

colorimetric lactate-oxidase mediator reaction. The results are displayed within about 60 seconds. When whole-blood is added to the strip, some penetrates the surface and thus only the plasma portion reaches chemicals that start the reactions processing the lactate. Built-in equations, according to the manufacturer, then calculate the concentration in whole-blood from the measured value in plasma. In this study, 25–50 µl of blood was used for each analysis, as recommended by the manufacturer. Whole-blood was added to the strip with the use of a sterilised pipette, making sure that the tip never touched the strip (Baldari *et al.*, 2009).

4.3.2.3 Neutrophil function

Neutrophil function was assayed according to the method developed McLaren *et al.* (2003) and adapted by Kruger *et al.* (2010), using a portable luminometer (3M Clean-Trace™, 3M, St. Paul, MN, USA). Blood samples were used to perform a whole-blood chemi-luminescent (CL) assay. The assay works on the principle that leukocytes – or more specifically neutrophils – react to a bacterial challenge (in the form of phorbol 12-myristate 13-acetate or PMA) and produce an oxidative response known as a respiratory burst (McLaren *et al.*, 2003). Luminol is added to the sample to amplify the amount of light emitted during the respiratory burst and this light emission is then measured in relative light units (RLUs) with a luminometer. The number of respiratory bursts (and thus neutrophil activity) can therefore be measured over time in order to calculate the extent to which the immune response is activated. Since stress has been shown to cause activation and eventually exhaustion of the immune response if the stressor persists (Johnstone, Reina, & Lill, 2012), changes in the immune response over time were investigated to determine whether tranquilisation had an effect.

For the reaction mixture, 500 µL of phosphate buffer solution (PBS) was incubated with 100 µL of fresh heparinised blood at 37.6 °C. After 5 minutes of incubation, 100 µL of luminol was added. The luminol (Sigma, A8511-5G) solution was prepared to have a concentration of 10^{-4} mol/L in PBS, and the solution (110 µL/aliquot) was then placed in labelled Eppendorf tubes and frozen to -20 °C. The phorbol 12-myristate 13-acetate (PMA, Sigma P8139) solution was initially diluted in dimethyl sulfoxide (DMSO; Sigma D 5879), and then to a concentration of 10^{-4} mol/L in PBS. The PMA solution was aliquoted, placed in labelled Eppendorf tubes and frozen to -20 °C. All luminol and PMA aliquots were kept frozen and protected from light.

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The first sample was read in the luminometer as a blank and then 100 µl of 12-O-tetradecanoylphorbol-13-acetate (TPA), also commonly known as phorbol 12-myristate 13-acetate (PMA), a diester of phorbol, was added to the sample. The luminescence readings or RLUs were read at 2.5 minutes and then 5 minutes after PMA addition. All samples were prepared in triplicate. The readings were then taken every 5 minutes for 75 minutes. Three samples of blood were collected and evaluated for each animal: the first whilst the Equivital™ belt was being fitted, the second on day 2 of the trial before administering the long-acting neuroleptic, and the third on day 3 of the trial, before removal of the Equivital™ belt. Three response curves were produced for each animal using the means of the triplicate readings. The curves were then transformed with a trend line, and the area under the curve (AUC) was calculated. The three AUCs for the animals were then compared statistically using Wilcoxon rank regression, with $P < 0.05$ considered as being statistically different (Kruger *et al.*, 2010).

4.3.2.4 White blood cell counts

White blood cell (WBC) counts were performed by using whole-blood samples, and WBC were counted by using the haemocytometer technique described by Schalm, Jain, and Carrol (1975).

4.3.3 Faecal analysis

In preparation for the FGM extraction, each faecal sample was thawed at room temperature and mixed thoroughly. A random portion of 2.5 g (± 0.001g) was then placed in a conical tube with 10 mL 80% methanol (Palme, Touma, Arias, Dominchin, & Lepschy, 2013). Each sample was rotated at room temperature on a vertical plane for 16 hours and thereafter centrifuged at 1,000 x g for 10 minutes. The supernatant was then withdrawn and stored at -70°C. In order to determine the dry weight of each faecal sample, a further 2.5 g of the sample was placed in a foil cup and dried in an oven at 60˚C for 24 hours. The dry matter weight of the faeces was then recorded.

The measurement of FGM was performed with a 125I Corticosterone RIA kit (MP Biomedicals LLC, Santa Ana, CA 92707, USA) that was validated for use in blue wildebeest (Chapter 6). The methanol extracts were diluted 10:1 with the manufacturer's steroid diluent (Cat. No. 07-166196) and assayed as per instructions. Since the six corticosterone calibrators in the kit (0-1000 ng/ml) are pre-diluted to account for the 1:200 dilution prescribed for rat plasma corticosterone measurement, the effective calibration

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range extended from 0-5 ng/ml. The FGM concentrations thus determined were then corrected for dilution ratios and water content of the faeces and the results expressed as ng/g dry faeces. Serial dilutions of selected samples gave slopes of $R > 0.98$ which remained parallel to the standard curve.

4.3.4 Behavioural tests and observations

Animal behaviour was analysed using The Observer® XT11 behavioural software (Noldus Information Technology, Wageningen, the Netherlands). Animals were observed and observations of their behaviour entered in the form of codes that had previously been specified in the coding scheme created for this project. Once an observation had been completed, it was stored in a computer file. All physiological data collected with the Equivital™ system was then imported into The Observer® XT11. From that moment, the observational and physiological data, all or part of it, could be visualised and analysed and data could be exported into Excel for further analysis (Noldus, 2011).

Behaviours were classified as either state behaviours, which have a distinct onset and end time, or point behaviours, which are without measurable or relevant duration (Noldus, 2011). Modifying behaviours were used to record extra information about certain behaviours. Table 4.4 shows the behaviours that were identified and used in the construction of a coding scheme for this project.

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Table 4.4 Wildebeest behaviours used to create a coding scheme in the Observer®XT11 software

Behaviour	Modifying behaviour	Type of Event
Lying	Lying with head up	State event
	Lying with head folded back	State event
Walking		State event
Eating		State event
Drinking		State event
Grooming	Grooming while standing	Point event
	Grooming while lying down	Point event
Standing	Standing with head down	State event
	Standing with head up, ruminating	State event
Being vigilant		State event
Agitation	Head shaking	Point event
	Foot stomping	Point event
Interaction	Rubbing against other animal	State event
	Being rubbed by other animal	State event
	Fighting	State event
	Defending	State event
Trotting in alarm		State event
Exploring	Standing and sniffing surfaces	State event
	Walking and sniffing surfaces	State event
Other behaviour		State event

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Behaviours classified as “other”, were those behaviours observed only once or twice, or in only one group or individual, and did not occur frequently enough to warrant independent classification.

4.4 STATISTICAL ANALYSIS

Unless otherwise stated, all data was analysed using a restricted maximum likelihood estimation (REML), which included treatment, gender, animal weight and trial as fixed effects. For the analysis of vital sign data with behaviour data, behaviour and stimulation were included as fixed effects. Behaviour within periods of stimulation could not be analysed since not all behaviours occurred within a period of stimulation. However, changes in vital sign data were analysed within stimulations. All trials within a neuroleptic or placebo group started and ended at around the same time of day so that stimulations occurred around the same time each day. Data analysis was performed using the Variance Estimation and Precision module of Statistica (version 12) statistical software (StatSoft Inc., 2013). All results were considered significant if $P < 0.05$, and are expressed as least squared means (LSmeans) ± standard error of the mean (SEM). Pearson correlation coefficients were calculated where necessary and considered as significant if $P < 0.05$.

In order to minimise the effect of outliers in the data, the medians for each vital sign per animal were used for statistical analysis. Means for each group were then calculated from these medians.

Means between the three drug regimens (Acunil®, zuclopenthixol acetate or placebo) were not statistically analysed. The reason for this is that the aim of the study was to investigate whether a treatment resulted in any significant change not to which extent this change occurred.

4.5 RESEARCH LIMITATIONS

Animals, labour, equipment and drug costs as well as the high costs involved in the purchase, modification and validation of the Equivital™ biotelemetry system limited the number of animals that could be used per trial and the number of trials that could be conducted. Animal availability also limited the choice of genders per group so that some groups had unequal numbers of males and females. The inclusion of gender as a main effect in the statistical analysis of the data aimed to alleviate this bias.

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The Equivital™ EQ02 biotelemetry system was originally chosen because, at the onset of the study, Equivital™ had a distributor in Gauteng, South Africa so that the system could be purchased locally. In addition, the distributor allowed for the testing of the system prior to purchase. The average price of the system at first purchase was R2 500 per belt and R20 000 per data-logger. After the first year of study, the distributor stopped supplying Equivital™ products in South Africa and replacement belts had to be ordered from either the United Kingdom (Hidalgo™) or the United states (Philips Respironics). This not only substantially increased the cost of the belts but also the time taken until belts arrived due to shipping and customs clearing issues.

Another major limiting factor to the number of trials that could be conducted was that wildlife cannot be captured and transported in Mpumalanga during the summer months (beginning of November until end of February) and no translocation permits are issued during this time. This is due to excessively high environmental temperatures and humidity. As a result, trials were limited to mid-March until mid-October, giving some leeway for the organisation and issuing of permits.

Lastly, the battery life on the Equivital™ data-logger limited the length of each trial since data could only be collected for up to 38 hours. Although external battery packs are available, funding availability limited their use. However, it was decided that 12 hours per treatment was adequate since the most pertinent physiological and physical effects brought about by the administration of a LAN will occur within the first 12 hours after its administration (JP Raath, personal communication, 2 March, 2012).

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CHAPTER 5

Validating a human biotelemetry system for use in captive blue wildebeest *(Connochaetes taurinus)**

ABSTRACT

Two blue wildebeest (*Connochaetes taurinus*) were fitted with modified Equivital™ belts to evaluate the belt's accuracy in measuring heart and respiration rate in wildebeest whilst anaesthetized and fully conscious in captivity. During anaesthesia, each animal's heart and respiration rate was monitored using an Equivital™ belt, a Cardell® monitor and manual measurements. Doxapram hydrochloride (Dopram®) and adrenaline was administered intravenously to stimulate changes in respiration and heart rate, respectively. The anaesthetic was reversed after 30 minutes of monitoring and the animals left in captivity for 24 hours, wearing the Equivital™ belts. After 24 hours, the previous protocol was repeated. Intraclass correlation coefficients (ICC) calculated between all three monitoring methods showed moderate to excellent agreements for heart rate on both days (ICC: 0.73-0.98). ICCs calculated for respiration rate showed good to excellent agreement between the Equivital™ belt and the other two methods (0.82-0.92) with the exception when only poor to fair agreements were found between the Cardell® and manual measurements. Heart and respiration rates increased with motion while animals were in captivity. The results indicate that a modified version of the Equivital™ EQ02 system can be used as a potential biotelemetry device for measuring heart and respiration rate in captive blue wildebeest.

Keywords: biotelemetry, blue wildebeest, Equivital™, heart rate, respiration rate

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* **Published as:** Laubscher, L.L., Hoffman, L.C., Pitts, N.I., & Raath, J.P. (2015). Validating a Human Biotelemetry System for Use in Captive Blue Wildebeest (*Connochaetes taurinus*). *Zoo Biology*, 34, 321-327. doi: 10.1002/zoo.21222.

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5.1 INTRODUCTION

Telemetry systems used for recording cardiovascular variables such as heart rate, electrocardiogram (ECG) and blood pressure in animals were initially developed for pharmaceutical companies who used them for drug research and development (Samson *et al.*, 2011). In addition, numerous researchers and laboratories developed biotelemetry systems to measure physiological parameters in animals for other research purposes. As early as 1960, researchers built and used a device that transmitted the heart rate and wing beat rate of ducks (Eliassen, 1960; Ropert-Coudert and Wilson, 2005). Thus, the term biotelemetry was born, being defined as the remote measurement of behavioural, physiological and energetic data from live subjects (Cooke *et al.*, 2004; Ropert-Coudert & Wilson, 2005).

Today, modern biotelemetry systems either consist of external devices fitted to the bodies of animals or humans or implantable devices (Stabenow *et al.*, 1996). Both systems have advantages and disadvantages with external devices being more cost-effective and less invasive than implantable devices. External biotelemetry devices are typically used for short-term studies with a high throughput, involving a large number of animals. As there are no surgical procedures involved, they provide a non-invasive alternative to implantable devices and can be used with single or group-housed animals (Grenwis, 2010). Implantable devices however, are able to measure a larger number of variables more accurately, are less likely to be lost or removed and tend to cause little discomfort to animals once implanted (Grenwis, 2010; Wild *et al.*, 1995).

Although biotelemetry is becoming more popular in animal research studies, it is still not as widely used in large terrestrial mammals as was anticipated when these types of systems were first developed. According to Cooke *et al.* (2004), the two most limiting factors to its use in species other than bird and marine species are firstly, the lack of commercial development for many applications and secondly, the cost of such systems. Eloranta *et al.* (2002) also stated that the reliability of the few existing cardiac telemeters for free-ranging terrestrial animals has not been adequately established and reported. This may be because cardiac biotelemetry currently requires double measurement by a secondary device, typically one that is implanted, to validate externally worn telemeters.

The aim of this study was to modify a biotelemetry device originally developed for use in humans to be suitable for use in captive wildlife species during short-term studies. The

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biotelemetry device chosen provides a far less invasive method of measuring heart rate and respiration rate simultaneously without the need for surgically implanted electrodes. Our goal was to illustrate the accuracy of the system without the necessity of a secondary biotelemetry device by showing that the Equivital™ EQ02 belt could measure heat rate and respiration rate accurately during immobilization, both before and after the animal was left fully awake in captivity for 24 hours. This approach not only reduces costs but also minimizes the behavioural and physiological disturbances from the monitoring protocol itself. In addition, the study aimed to show that the Equivital™ belt was sensitive enough to detect changes in heart rate and respiration rate, induced by the administration of adrenaline and doxapram hydrochloride (Dopram®), respectively. Successful validation will enable researchers to perform more accurate, but less invasive, short-term continuous measurements of vital signs in wild mammals held in captivity for short periods of time.

5.2 METHODS

Ethical approval was received for all aspects of the trial from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa (Protocol Ethical Approval number SU-ACUM11-00005). Two qualified veterinarians handled and administered all the drugs used as well as consistently monitored the animals during anaesthesia.

Two male blue wildebeest (*Connochaetes taurinus*) were darted (3.5-4.5mg of Thiafentanil oxalate; Thianil®, Wildlife Pharmaceuticals, RSA) and transported to an enclosure (6 x 8 m in size) constructed of gum poles and equipped with two infrared surveillance cameras (Nictec Radio Communications, Nelspruit, South Africa) for monitoring. Food and water was provided *ad libitum*. The study occurred in the Lowveld area of Mpumalanga, South Africa which forms part of the species' native range.

After 2 days, each animal was darted separately, spray painted with a number, weighed and shaved around the chest to accommodate the electrocardiogram (ECG) sensors. Two Equivital™ EQ02 belts (Hidalgo Limited, Unit F. Trinity Court, Buckingway Business Park, Cambridge, UK) were used. For the purpose of this study, we were only interested in heart rate, respiration rate and motion although the system also has the capacity to measure other physiological parameters.

In animals, the design of the belt does not allow for the appropriate positioning of the data-logger and we therefore removed the shoulder straps on the Equivital™ belt, extended the chest bands with elastic material and reinforced the stretch inhibitor inside the belt to prevent over-stretching of the respiration sensor. Whilst immobilized, each wildebeest was fitted with a modified Equivital™ belt so that the data-logger was positioned above the heart. Electro-gel was applied to each ECG sensor and double-sided tape and Pattex Supergel® used to secure the belt to the skin. Opsite™ Flexigrip™ transparent film dressings from Smith & Nephew (Smith & Nephew (Pty) Ltd., Pinetown, South Africa) were placed over the data-logger to protect it from dirt or water (Figure 5.1).

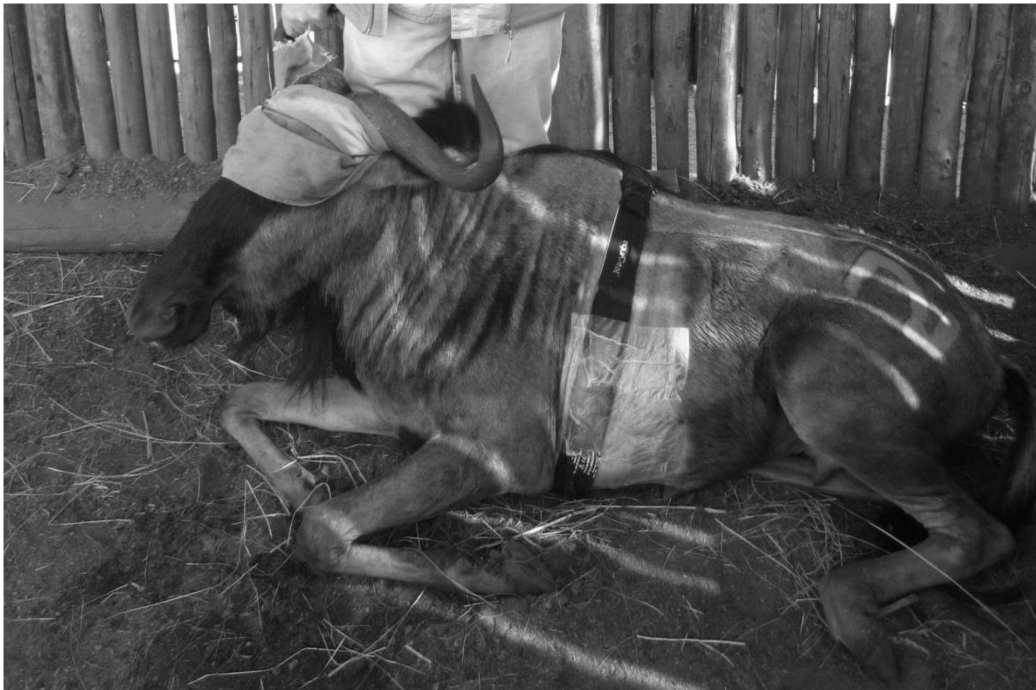

Figure 5.1 A wildebeest fitted with a modified Equivital™ belt

Once an animal was fitted with an Equivital™ belt, it was moved into a veterinary laboratory and the study began by recording heart rate and respiration rate every 15 seconds for 30 minutes using the following three methods:

1 Equivital™ monitoring via Bluetooth™

2 Cardell® 9500 HD multi-parameter veterinary vital sign monitor (Kyron Laboratories (Pty) Ltd., Johannesburg, South Africa).

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3 Manual monitoring of heart rate (via stethoscope) and respiration rate (by counting exhaled breaths)

This part of the study was referred to as Day 1 and all recordings were synchronized. For manual heart rate measurements, heart beats were counted as the number of heart beats/30 seconds since it was difficult to manually count consecutive heart beats in a smaller interval. These values were then multiplied by 2 to get an estimated beats/minute. For manual counts of respiration rate, breaths/15 second interval were counted and multiplied by 4 to get an estimated breaths/minute. The Equivital™ and Cardell® monitors reported estimated heart rates and respiration rates per minute every 15 seconds. The Cardell® monitor records heart rate with electrode clips attached to the animal that detect signals caused by changes of electrical conduction in the heart during the cardiac cycle. The system measures respiration rate directly through a Capnostat® mainstream CO2 probe.

Once enough stable recordings were made and the animal showed no negative reactions to the anaesthetic, the animal was injected intravenously (IV) via the ear vein with 100mg of doxapram hydrochloride (Dopram®). Doxapram hydrochloride is a respiratory stimulant that increases respiration and is often used in patients with respiratory depression or apnoea. The doxapram hydrochloride was administered in order to determine if all three methods of respiratory monitoring could detect possible changes due to its administration.

After the effects of the doxapram hydrochloride had completely worn off, the animal was injected with 2000 IU of adrenaline IV, again via the ear vein. Adrenaline is known to increase heart rate and has a short half-life so that its expected effect lasts for no longer than one minute. Again, this was done to determine if the three heart rate monitoring methods could accurately detect changes in heart rate due to the administration of the adrenaline.

Once heart rate stabilized, vital sign monitoring and recording continued until 30 minutes of data had been collected. It must be noted that during the monitoring of wildebeest 1, a problem occurred with the positioning of the Capnostat® probe that is used for the measurement of respiration rate with the Cardell® monitor. As a result, measurements were only taken with the Cardell® monitor for the first 7 minutes of the study period.

The Cardell® monitor was then disconnected, the animal moved back to the enclosure and the anaesthetic reversed (35-45mg naltrexone; Trexonil®, Wildlife Pharmaceuticals, RSA). Once awake, the animal was left in the enclosure for 24 hours. The process was

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repeated with the second wildebeest as well. It must be noted that battery life is a limiting factor when using the Bluetooth functionality of the system and thus it was decided to not exceed a 24 hour captivity period. External battery packs however, are available for the system and could be used should the captivity period exceed 24 hours.

The next day, the animals were darted individually again and the previous days' protocol was repeated. This part of the study was referred to as Day 2.

Heart rate and respiration rate was continuously logged by the Equivital™ device whilst the animals were fully awake and in captivity. This data was also used for analysis. In addition, the Equivital™ data-logger also detects the motion status of the animal via tri-axis accelerometry in terms of a.) Stationary; b.) Moving slowly; and c.) Moving fast. This motion status is logged every 15 seconds. Because the intent was to compare motion status with heart rate and respiration, the motion measured by the belt was not correlated to any continuous behavioural monitoring and wildebeest were observed only to note if any abnormal behaviours were present.

5.2.1 Statistical Analysis

The data was analysed using a restricted maximum likelihood estimation (REML) which included day and recording method as fixed effects. This was done using the Variance Estimation and Precision module of Statistica (version 12) statistical software (StatSoft Inc., 2013). Differences within the fixed effects were considered as being significant if the probability of rejection of H_o was less than 5% ($P < 0.05$). Two-way Intraclass Correlation Coefficients (ICC) for Absolute Agreement were calculated between the different methods of vital sign monitoring on both study days for each animal. This was done for the total 30 minute study period on each day as well as for the 2 minute period after the administration of adrenaline or doxapram hydrochloride. The Cardell® measurements for wildebeest 1 were included in the latter analysis as the doxapram hydrochloride was administered 4.5 minutes after the study period began and thus the Cardell® monitor was still measuring for the 2 minute interval after administration of the doxapram hydrochloride. ICCs are used for assessing agreement between two methods of measurement and we considered coefficients as significant at a confidence level of 5% ($P < 0.05$).

5.3 RESULTS

The results obtained in both animals, using all three monitoring methods on each of the two trial days are reported below.

5.3.1 Day 1

Figures 2 and 3 show the heart rate (beats/minute) and respiration rate (breaths/minute) of wildebeest 1 and wildebeest 2, respectively, as measured over the total 30 minute study period on Day 1. Wildebeest 1 showed a slight increase in heart rate followed by a noticeable decrease in response to the adrenaline given (Figure 5.2). The Cardell® measurements did not show as marked an increase as the other two methods. In comparison, wildebeest 2 showed a noticeable increase in heart rate directly following the administration of adrenaline and this is clearly illustrated by all three methods (Figure 5.3). None of the animals showed a marked increase in respiration rate in response to the doxapram hydrochloride, and all three monitoring methods showed varied respiration patterns.

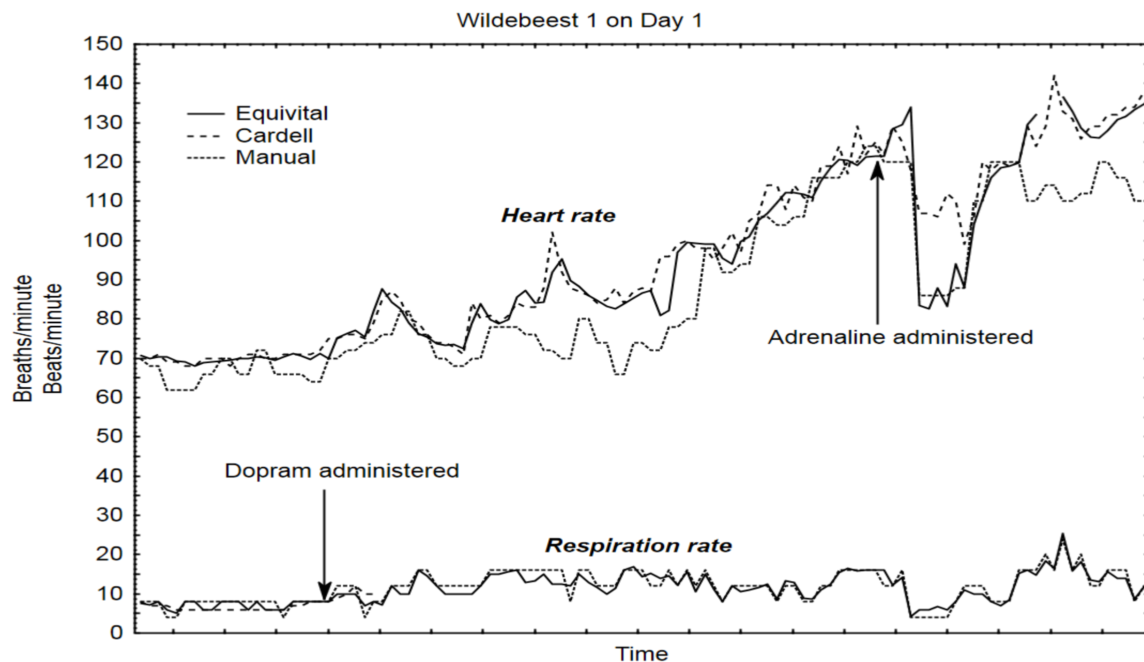

Figure 5.2 The heart rate (beats/minute) and respiration rate (breaths/minute) of wildebeest 1 on Day 1 over a 30 minute period as measured with the Equivital™ system, a Cardell® monitor and manually showing the response before and after the administration of adrenaline and Dopram®

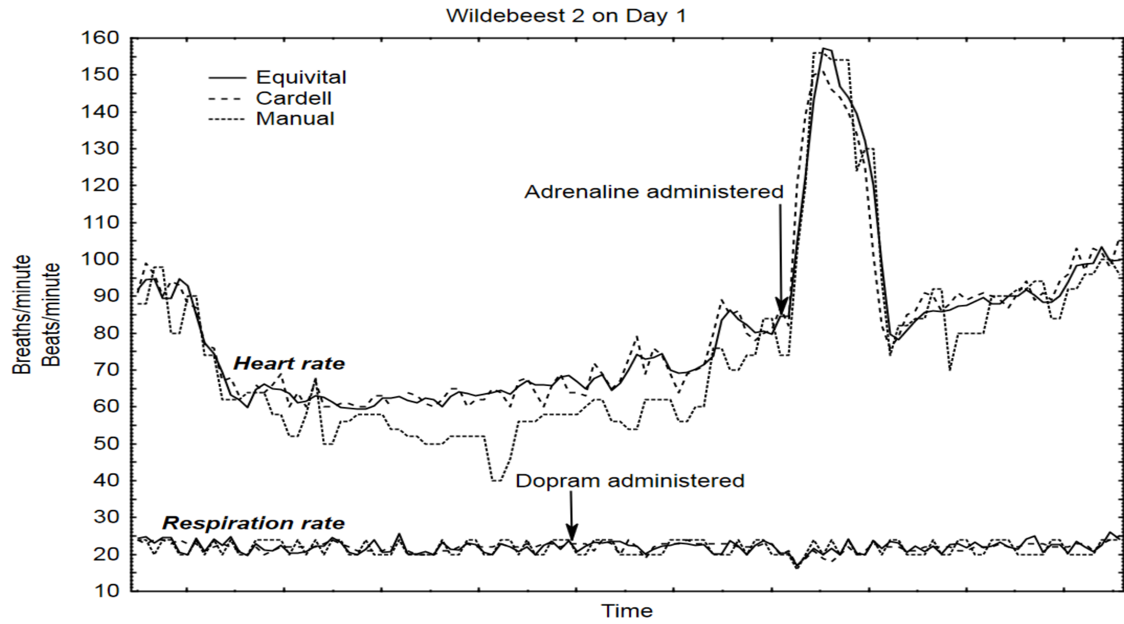

Figure 5.3 The heart rate (beats/minute) and respiration rate (breaths/minute) of wildebeest 2 on Day 1 over a 30 minute period as measured with the Equivital™ system, a Cardell® monitor and manually showing the response before and after the administration of adrenaline and Dopram®

ICCs for Absolute Agreement calculated for the total 30 minute study period for both animals on both days are given in Table 5.1. All ICCs were significant ($P < 0.05$).

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Table 5.1 ICCs for Absolute Agreement calculated between heart rates as well as respiration rates measured with the Equivital™ belt, a Cardell® monitor and manually for both wildebeest for the entire study period.

DAY 1				
	Wildebeest 1		Wildebeest 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ - Cardell®	0.96	*	0.98	0.58
Equivital™ - Manual	0.88	0.92	0.93	0.60
Cardell® - Manual	0.87	*	0.92	0.30
DAY 2				
Equivital™ - Cardell®	0.97	0.92	0.95	0.82
Equivital™ - Manual	0.76	0.89	0.78	0.55
Cardell® - Manual	0.73	0.82	0.73	0.53

* The ICC was not calculated since only 7 minutes of data was recorded with the Cardell® capnostat®

Mean heart rates and respiration rates calculated for both animals on both days are given in Table 5.2.

Table 5.2 Mean heart rates (beats/minute) and respiration rates (breaths/minute) ± standard error of the mean (S.E.M.) as measured by the three different methods on Day 1 and Day 2

DAY 1						
	Mean heart rate			Mean respiration rates		
	Equivital™	Cardell®	Manual	Equivital™	Cardell®	Manual
Wildebeest 1	91.2^{a} ± 1.61	92.3^{a} ± 1.61	84.6^{b} ± 1.61	10.9^{a} ± 0.33	*	11.2^{a} ± 0.33
Wildebeest 2	83.4^{a} ± 1.74	83.8^{a} ± 1.75	80.2^{a} ± 2.22	22.1^{a} ± 0.17	22.1^{a} ± 0.18	21.2^{a} ± 0.17
DAY 2						
Wildebeest 1	80.7^{a} ± 2.10	81.9^{a} ± 2.10	78.8^{a} ± 2.10	12.5^{a} ± 0.44	13.3^{a} ± 0.44	13.4^{a} ± 0.44
Wildebeest 2	69.8^{a} ± 1.65	70.8^{a} ± 1.65	62.2^{b} ± 1.65	23.8^{ab} ± 0.19	23.6^{b} ± 0.19	23.2^{bc} ± 0.19

^{ab} Means with the same letter between methods for a specific animal do not differ significantly (P > 0.05)

* The Cardell® measurements for respiration were excluded from this analysis as measurements were only taken for 7 minutes of the total study period

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ICCs for Absolute Agreement calculated for the 2 minute period after the administration of adrenaline or Dopram® for both animals on both days are given in table 3. All ICCs were significant (P < 0.05) except for those ICCs indicated in Table 5.3.

Table 5.3 ICCs for Absolute Agreement calculated between heart rates as well as respiration rates measured with the Equivital™ belt, a Cardell® monitor and manually for both wildebeest for the 2 minute period after the administration of adrenaline or Dopram®.

	Wildebeest 1		Wildebeest 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
DAY 1				
Equivital™ - Cardell®	0.42	0.45*	0.95	0.07*
Equivital™ - Manual	0.93	0.72	0.97	0.89
Cardell® - Manual	0.36	0.30*	0.92	0.04*
DAY 2				
Equivital™ - Cardell®	0.96	0.90	0.87	0.74
Equivital™ - Manual	0.69	0.71	0.71	0.39*
Cardell® - Manual	0.67	0.61	0.63	0.32*

* ICC is not significant (P > 0.05)

No significant differences (P > 0.05) were found between mean heart rates and respiration rates measured with each of the three methods during this period on either of the two study days. Thus, all three methods measured similar heart rates and respiration rates during this period but measurements did not follow the same pattern (Figures 5.2-5.5).

5.3.2 Day 2

Figures 5.4 and 5.5 show the heart rate (beats/minute) and respiration rate (breaths/minute) of wildebeest 1 and wildebeest 2, respectively, as measured over the 30 minute study period on Day 2. Again, Figure 5.4 shows that all three methods indicate a slight increase in wildebeest 1's heart rate followed by a noticeable decrease after the administration of adrenaline. Wildebeest 2, on the other hand showed a slight decrease

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in heart rate followed by a marked increase following the administration of adrenaline with all three methods indicating the same pattern. After the administration of doxapram hydrochloride, wildebeest 1 showed an increased respiration rate measured with all three methods although the Cardell® monitor measured the most marked increase. Wildebeest 2's respiration rate showed varied patterns between the three monitoring methods after the administration of doxapram hydrochloride with the Equivital™ and Cardell® measurements showing the most similar pattern while the manual measurements showed noticeable fluctuations.

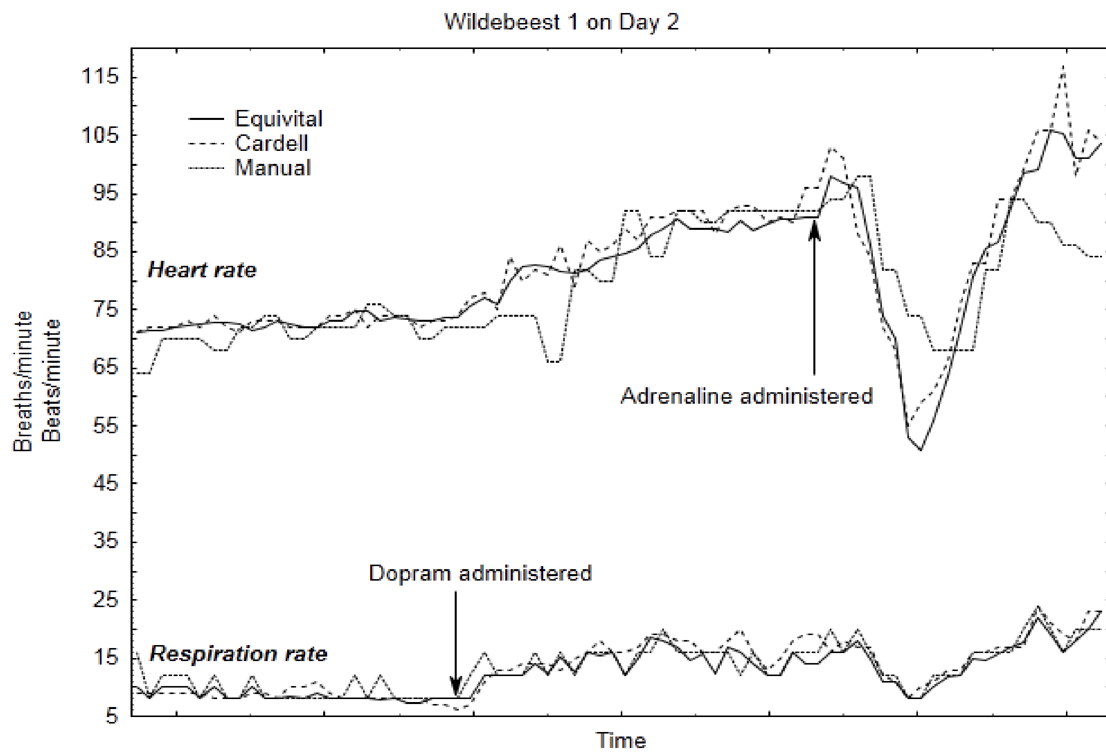

Figure 5.4 The heart rate (beats/minute) and respiration rate (breaths/minute) of wildebeest 1 on Day 2 over a 30 minute period as measured with the Equivital™ system, a Cardell® monitor and manually showing the response before and after the administration of adrenaline and Dopram®

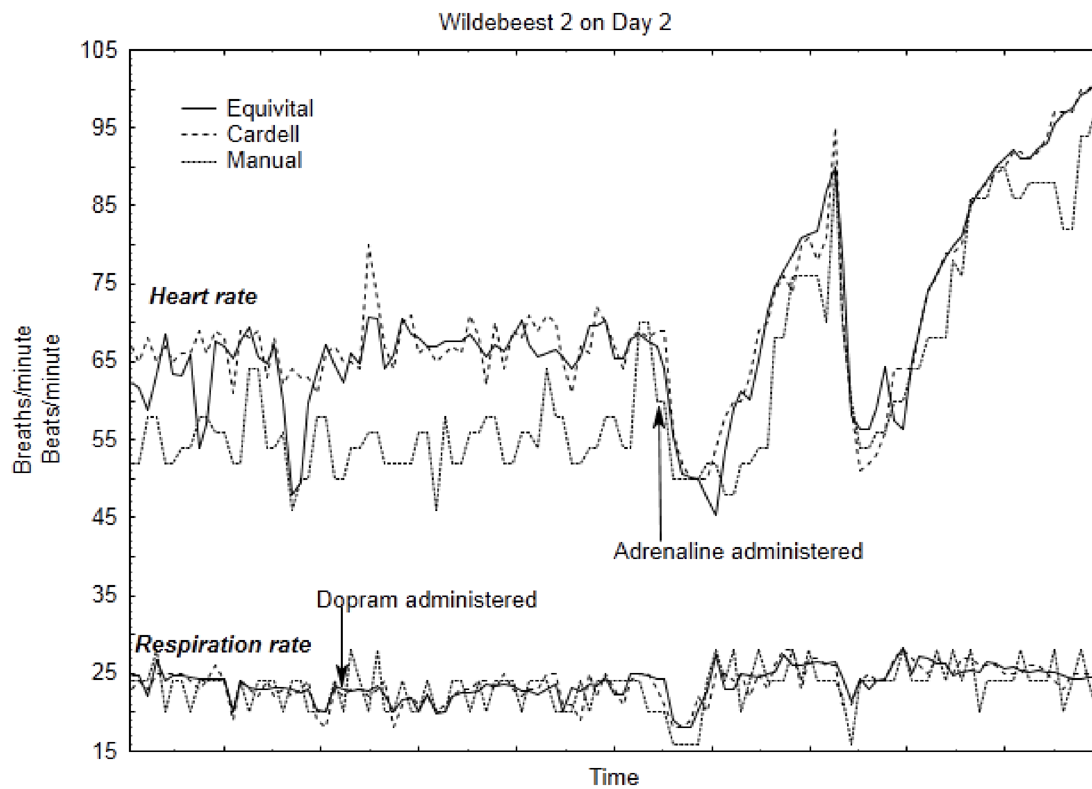

Figure 5.5 The heart rate (beats/minute) and respiration rate (breaths/minute) of wildebeest 2 on Day 2 over a 30 minute period as measured with the Equivital™ system, a Cardell® monitor and manually showing the response before and after the administration of adrenaline and Dopram®

ICCs for Absolute Agreement for Day 2 are given in Table 5.1. All ICCs were found to be significant ($P < 0.05$). Mean heart rates and respiration rates are given in Table 5.2. ICCs calculated for the 2 minute period after the administration of adrenaline or doxapram hydrochloride on Day 2 are given in Table 5.3.

5.3.3 Heart Rate and Respiration Rate while in the Enclosure

The mean heart rate and respiration rate of wildebeest 1 while in the enclosure was calculated as 65.39 ± 0.21 beats/min and 10.67 ± 0.12 breaths/min, respectively. The mean heart rate and respiration rate of wildebeest 2 while in the enclosure was calculated as 70.80 ± 0.36 beats/min and 13.30 ± 0.09 breaths/min, respectively.

We also calculated mean heart rates and respiration rates per animal for each motion category as measured by the Equivital™ belt. Both heart rate and respiration rate increased significantly ($P < 0.05$) with motion (Table 5.4).

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Table 5.4 Mean heart rates (beats/minute) and respiration rates (breaths/minute) ± standard error of the means (S.E.M.) as measured by the Equivital™ belt for both wildebeest per motion category

Motion	Wildebeest 1		Wildebeest 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Stationary	62.0 ± 0.24	7.9 ± 0.12	64.2 ± 0.36	12.0 ± 0.10
Moving slowly	71.7 ± 0.37	16.6 ± 0.19	89.7 ± 0.73	17.1 ± 0.20
Moving fast	82.5 ± 1.04	17.8 ± 0.53	103.7 ± 1.50	19.1 ± 0.41

The results showed that wildebeest 1 spent 68.79% of its time being stationary, 27.62% of its time moving slowly and 3.59% of its time moving fast. In comparison, wildebeest 2 spent 76.62% of its time being stationary, 18.86% of its time moving slowly and 4.52% of its time moving fast.

5.4 DISCUSSION

The results showed that the Equivital™ system could accurately measure heart rate, both before and after being worn by a fully conscious wildebeest in captivity for 24 hours. The Equivital™ belt also measured heart rates ranging from 69.8 – 91.2 beats/minute (bpm), which is similar to results reported by Dittberner (2011). This author reported mean heart rates ranging from 70.5 to 102.9 bpm for blue wildebeest immobilized with different variations of etorphine with hyaluronidase.

All three monitoring methods indicated that the heart rates of both animals responded differently to the administration of the adrenaline on both study days. These responses cannot be explained in this study but may be related to unknown underlying physiological mechanisms. Both the Cardell® monitor and those measurements taken manually showed a lack of sensitivity to heart rate changes in response to adrenaline on at least one of the study days, indicating that erroneous measurements may have been taken using these two methods. However, in both animals on both study days, the Equivital™ belt measurements had a good to excellent agreement with at least one of the other monitoring methods if not with both.

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Respiration rate results were more confounding with all three methods showing varied agreements with each other. In wildebeest 1 on both days it appeared that all three methods recorded accurate readings, showing excellent agreements with each other. However, in wildebeest 2 the three methods showed at best a moderate agreement with each other except for the Equivital™ belt measurements which were in good agreement with the Cardell® measurements on Day 2. This lack of consistency may be attributable to the sensitivity of the Cardell® capnostat® probe in picking up subtle changes in respiration such as an increase in shallow breaths induced by anaesthesia. These shallow breaths may not have been picked up by the Equivital™ respiration sensor or manual respiration counts. In addition, manual measurements of respiration rate were calculated from the counting of exhaled breaths with no fractional component (unlike the Equivital™ system) so that there was a lack of sensitivity in picking up changes in respiration.

This inconsistency between the three methods in measuring respiration was evident as well after the administration of doxapram hydrochloride. However, the Equivital™ belt showed at least a moderate to good agreement with one of the other two methods in each of the animals on both study days. Weaker agreements were found between the Cardell® and manual measurements during this time indicating again that erroneous readings were likely taken with one of these methods.

We found that mean respiration rates measured with the Equivital™ belt while the animals were in the enclosure ranged from 7.9 – 19.1 breaths/min. which is comparable to those reported by Mortola and Lanthier (2005) for blue wildebeest (17 breaths/min.). In agreement with our findings where the Equivital™ belt respiration rates for the anaesthetized animals ranged from 10.9 – 23.8 breaths/min., Dittberner (2011) reported respiration rates for immobilized blue wildebeest to range from 19-26 breaths/min.

The analysis of belt-measured motion served to further substantiate its measures of heart and respiration rates. We expected that moving fast would result in the highest mean heart rates and mean respiration rates since increased motor activity can result in increased respiratory and cardiovascular output (Price and Sibly, 1993).

Overall, the study found that the modified version of the Equivital™ belt can measure heart rate, respiration rate and motion in this species with a good degree of accuracy. The inconsistencies found in respiration rate using all three monitoring methods, requires further investigation in order to fully explain them. The successful use of such a

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biotelemetry system can greatly improve the understanding of factors that may cause changes in these parameters while animals are conscious and in captivity.

5.5 CONCLUSIONS

The results showed strong to near perfect agreements between heart rates measured with the Equivital™ system and those measured manually and with a Cardell® monitor, both before and after the belts have been worn for 24 hours. The results also showed strong to near perfect agreements between respiration rates measured with the Equivital™ system and those measured manually and with a Cardell® monitor, with the exception of instances where the latter two methods showed little to no agreement either. The study thus found that the biotelemetry belt still measures accurately after being worn by the animals for 24 hours in captivity. Supporting this evidence is the fact that the Equivital™ biotelemetry belts measured corresponding increases in heart and respiration rates with increased motion and all mean heart rates and mean respiration rates measured with the Equivital™ system fell within acceptable ranges as reported for this species. These results give good indication of the Equivital™ system's ability to measure heart rate and respiration rate accurately in conscious animals.

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CHAPTER 6

Validation of a faecal glucocorticoid assay for blue wildebeest *(Connochaetes taurinus)*

ABSTRACT

Blue wildebeest (*Connochaetes taurinus*) are frequently translocated, and the activities involved may cause a more pronounced increase in the stress response of the animals, with the latter that can be measured using faecal glucocorticoid metabolite (FGM) concentrations. The current study validated the use of a commercially available radio-immunoassay in detecting changes in FGMs after an ACTH challenge (1.0 IU/kg Synacthen®). Blood samples were collected every 10 minutes for 40 minutes and faecal samples were collected from the enclosure floor every 6 hours for 42 hours after the challenge. A blood and rectal faecal sample was also collected immediately prior to the challenge. Mean plasma cortisol significantly increased from 10.9 ± 1.44 nmol/l to 125.4 ± 11.25 nmol/l at 10 minutes and from 178.1 ± 19.6 nmol/l at 20 minutes. Mean FGM concentration showed a significant increase within the first 12 hours from 86.0 ± 5.33 ng/g to 477.9 ± 122.95 ng/g of dry faeces. An inert marker (20 g of glitter) was administered to each animal in order to estimate gut transit time. A peak in glitter in faeces was noted at about 12 hours after ACTH administration, corresponding to the peak in FGM concentration. The study adequately showed the precision and suitability of the assay in measuring FGM concentration in this species.

Keywords: assay validation, faecal glucocorticoid metabolites, radio-immunoassay, stress, blue wildebeest

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6.1 INTRODUCTION

The adrenal gland plays a pivotal role in the stress response (Maschke, Rupp, & Hecht, 2000) which involves both the hypothalamic–pituitary–adrenal (HPA) axis and the sympatho–adreno–medullary system (Dehnhard, Clauss, Lechner-Doll, Meyer, & Palme, 2001). The activation of the HPA axis results in the release of corticotrophin-releasing hormone, which stimulates the secretion of ACTH and in turn the secretion of glucocorticoids (cortisol in large mammals) in the blood. Glucocorticoid levels can remain elevated for up to several hours (Sheriff, Dantzer, Delehanty, Palme, & Boonstra, 2011). Previous studies investigated the use of glucocorticoid (GC) concentrations as an indication of adrenocortical activity, since GC levels remain elevated for longer periods of time compared to catecholamines (Franceschini, Rubenstein, Low, & Romero, 2008; Harper & Austad, 2001; Johnstone, Reina, & Lill, 2012; Merl, Scherzer, Palme, & Möstl, 2000; Millspaugh *et al.*, 2001; Mooring *et al.*, 2006). Glucocorticoids released in response to stress can be measured directly from circulating peripheral blood, saliva, faeces or urine, with blood and faecal samples being the most popular in animal studies (Johnstone *et al.*, 2012).

It is only recently that the measurement of faecal glucocorticoids has become increasingly prevalent in animal field endocrinology studies since blood sampling is a more invasive method (Tarlow & Blumstein, 2007). This is particularly true in free-ranging animal studies where capture, handling and blood collection is likely to induce a physiological stress response and complicate data interpretation (Chinnadurai *et al.*, 2008). In addition, faecal studies have the added benefit of showing cumulative effects of stressors because faecal samples contain metabolites of stress hormones produced over a period, whereas plasma samples only provide hormone levels at one point in time (Chinnadurai *et al.*, 2008; Millspaugh & Washburn, 2003; Wasser *et al.*, 2000).

GCs are metabolised chiefly in the liver although metabolism also occurs in the kidneys, adrenals, placenta, connective tissues, fibroblasts and muscles (Touma & Palme, 2005; Von der Ohe & Servheen, 2002). After extensive metabolism, a variety of faecal glucocorticoid metabolites (FGMs) are excreted in the faecal matter with little or no parent hormone remaining (Chinnadurai *et al.*, 2008). In animals that are confined, obtaining faecal samples can be relatively easy, so measurement of these FGMs has been used to evaluate veterinary procedures and anaesthetic handling, the effect of different types of facilities or environment enrichment practices, and even in assisted reproduction techniques (Brousset, Galindo, Valdez, Romano, & Schuneman, 2005).

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Because species-specific steroid metabolism and gut microflora can cause the assortment of these FGMs to differ between species, it is important to validate the assays used to measure FGMs for a specific species (Chinnadurai *et al.*, 2008; Touma & Palme, 2005; Wasser *et al.*, 2000). Validation can be done through pharmacological administration of ACTH to stimulate adrenal hormone production or dexamethasone to supress adrenal function, and then determining whether an assay is sensitive enough to detect FGM changes (Keay, Singh, Gaunt, & Kaur, 2006; Sheriff *et al.*, 2011; Touma & Palme, 2005; Wasser *et al.*, 2000). Alternatively, biological validation can be accomplished by exposing animals to distinct stressful stimuli such as capture, and detecting changes in FGMs prior to capture and thereafter (Chinnadurai *et al.*, 2008).

The aim of the current study was to validate the use of a commercially available radio-immunoassay (RIA) to detect changes in FGM in blue wildebeest (*Connochaetes taurinus*) faeces. This assay was biologically validated for use in this species by Chinnadurai *et al.* (2008), who investigated the sensitivity of the assay in detecting changes in FGM concentrations due to season. In the current investigation, an ACTH challenge was performed on two male and two female blue wildebeest in order to confirm that the assay was sensitive enough to detect changes in FGMs resulting from a physiologically relevant increase in blood cortisol. Since the measurement of FGMs provides a non-invasive method of investigating long-term stress in wildlife (Schwarzenberger, 2007), the validation of this assay provides a non-invasive method of evaluating the effects of long-acting neuroleptics (LANs) in minimising the stress of captivity and human disturbances in blue wildebeest. The results from studies performed using this assay, are discussed in Chapter 10 of this dissertation.

6.2 MATERIALS AND METHODS

Four blue wildebeest (*Connochaetes taurinus*) were used to investigate blood and FGM concentrations in response to an adrenocorticotropic hormone (ACTH) challenge (Table 6.1). Ethical approval for all aspects of the project was obtained from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa (Protocol Ethical Approval number SU-ACUM11-00005).

6.2.1 Animals

Animals were investigated on two separate occasions (Table 6.1) and were captured and relocated to the study site by a professional game capture team from

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Wildlifevets.com. The study site was located on Ngongoni Farm (25°31’25.2”S, 31°06’50.8”E), outside Nelspruit, Mpumalanga, South Africa, and forms part of this species’ natural range. Once at the study site, animals were off-loaded and kept in an enclosure, constructed as per industry guidelines (McKenzie, 1993). The animals formed part of a separate study and were held together in the enclosure for a minimum of 5 days prior to being part of the validation study. All animals received clean water and fresh lucerne hay *ad libitum*. Two qualified veterinarians handled and administered all the drugs used and also consistently monitored the animals during anaesthesia.

Table 6.1 The animal numbers, weights and genders for each trial performed

Animal number	Weight	Gender	Trial
1	240	Male	July 2014
2	180	Female	
3	90	Female	October 2014
4	81	Male	

For the current study, the animals were immobilised with a combination of 0.01–0.02 mg/kg thiafentanil oxalate (Thianil®, Wildlife Pharmaceuticals SA (Pty) Ltd., White River, South Africa) and up to 0.3 mg/kg azaperone tartrate (Wildlife Pharmaceuticals SA (Pty) Ltd., White River, South Africa) between 8:00 and 10:00 in the morning. Once immobilised, each animal was moved on a stretcher into a fully equipped veterinary laboratory adjacent to the enclosure and placed on a large animal surgery table. All animals were blindfolded and kept in sternal recumbency throughout the anaesthesia and were also consistently monitored throughout.

Animals remained immobilised for a maximum of 60 minutes. On completion, each animal was carried back into the enclosure and the anaesthetic was reversed with an intravenous injection of 35–45 mg naltrexone (Trexonil®, Wildlife Pharmaceuticals, SA (Pty) Ltd.).

6.2.2 Faecal sample identification and the determination of gut transit time

In order to determine at which time an induced increase in plasma cortisol concentration was reflected in the FGM levels in the faeces, the gut transit time was determined by

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administering an inert marker to each animal through a veterinary oesophageal tube (Palme, 2005). The inert marker was 20 ml non-toxic glitter suspended in 500 ml sterile water (Brown, Bellem, Fouraker, Wildt, & Roth, 2001; Fuller, Margulis, & Santymire, 2011), with a different colour given to each animal. The marker was administered just prior to the reversal of anaesthesia and also served to identify faecal samples collected from the enclosure floor where animals were housed together. The abundance of glitter in each sample was assessed when the samples were prepared for the extraction of the FGMs (see 6.2.5 below). A portion of the thoroughly mixed sample was examined under a dissecting microscope and the amount of glitter present was visually assessed and scored on a four-point linear scale. This information was used to establish at which time glitter concentration reached a peak in excretion and to estimate gut transit time.

6.2.3 Sample collection and ACTH challenge

Once the animal was recumbent and stable on the surgery table, the first blood sample was collected from the jugular vein and was labelled as time zero. To induce a physiologically relevant increase in plasma cortisol concentration, ACTH was administered to each immobilised animal after baseline blood samples had been drawn. The ACTH analogue Synacthen® (Novartis Pharma AG, CH-4002, Basel, Switzerland) was administered intra-muscularly at a dose of 1.0 IU/kg directly after the time zero samples had been taken. Thereafter, samples were collected from the jugular vein at ten or twenty minute intervals up to 40 minutes. Blood collection tubes were kept in ice water until sampling was completed. Plasma was harvested by centrifugation, and aliquots were stored at -20 °C until the assay was performed.

In order to confirm that an increase in the plasma cortisol concentration corresponded to a detectable change in the amount of FGMs, faecal samples were collected for analysis. The first fresh sample was collected rectally from each animal at the time of immobilisation (baseline). The samples thereafter (10–15 g) were collected from the enclosure floor and all enclosure floors were cleaned prior to placing the animals inside. This was done to ensure that any samples collected, originated from the animals after the administration of the Synacthen®. Two wildebeest were kept in the same enclosure and an adjacent enclosure was kept empty. Faecal samples were collected from the enclosure floor by moving animals into the empty, clean adjacent enclosure. The enclosure floor was cleaned after sample collection in order to move the same animals back during the next sample collection. The first faecal sample obtained from the enclosure was collected 12 hours after the administration of the Synacthen®. Thereafter,

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samples were collected every 6 hours with the exception of instances when a sample was collected in the evening and again the next morning (no samples collected at midnight). Sample collection ended when glitter was no longer visible in any of the samples.

In addition to faecal samples collected following the ACTH challenge, fresh faecal samples from two different free-ranging animals were collected from a feeding ground on the same property one year after the ACTH challenge had been performed. This was done to compare FGM levels of wild animals to those of boma-confined animals. One sample originated from an animal used in the ACTH challenge (Wildebeest 4) and which had been released after completion of the initial experiment. All faecal samples were frozen at -20 °C until FGM extractions were performed.

6.2.4 Plasma cortisol assay

Plasma cortisol was measured using a commercially available ^{125}I RIA kit (Coat-a-Count®, Siemens Healthcare Diagnostics, Los Angeles, Santa Ana, CA 90045, USA) designed for humans for the quantitative measurement of cortisol in serum, urine or heparinised plasma, but has wide species applicability.

6.2.5 FGM estimation and assay validation

In preparation for the FGM extraction, the entire faecal sample was thawed at room temperature and mixed thoroughly. A random portion of 2.5 g (± 0.001 g) was then placed in a conical tube with 10 ml 80% methanol (Palme, Touma, Arias, Dominchin, & Lepschy, 2013). The sample was rotated at room temperature on a vertical plane for 16 hours and thereafter centrifuged at 1 000 x g for 10 minutes. The supernatant was then removed, and stored at -70 °C. The sample weight used in this study differed from previous reports (Touma & Palme, 2005; Wasser *et al.*, 2000). Using a larger sample mass and volume of methanol ensures that a more accurate representation of the FGM is measured (Millspaugh & Washburn, 2003). In order to determine the dry weight of each faecal sample, a further 2.5 g of the sample was placed in a foil cup and dried in an oven at 60 °C for 24 hours. The dry matter weight of the faeces was then recorded.

The measurement of FGM was performed with a ^{125}I Corticosterone RIA kit (MP Biomedicals LLC, Santa Ana, CA 92707, USA) that has been validated for a number of South African herbivore species (Chinnadurai *et al.*, 2008). The methanol extracts were diluted 10:1 with the manufacturer's steroid diluent (Cat. No. 07-166196) and assayed

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as per the instructions. Since the six corticosterone calibrators in the kit (0–1000 ng/ml) are pre-diluted to account for the 1:200 dilution prescribed for rat plasma corticosterone measurement, the effective calibration range extended from 0–5 ng/ml. The FGM concentrations thus determined were then corrected for dilution ratios and water content of the faeces and the results expressed as ng/g dry faeces. Serial dilutions of selected samples gave slopes of $R > 0.98$, which remained parallel to the standard curve.

Possible sources of variation in this methodology include the within-assay variation in the FGM concentration (ng/ml) measured in the supernatants, and the repeatability of the extraction of the FGM from the faecal samples (ng/g dry faeces). To investigate the within-assay variation, the FGM concentration in a methanolic extract from a single faecal sample was measured eight times in the same assay. To investigate the repeatability of the extraction method, the FGM concentration (ng/g dry faeces) was determined from eight aliquots of the same faecal sample in the same assay. To investigate the efficiency of the extraction method, aliquots of the same faecal sample were spiked with a known quantity of corticosterone (Sigma cat. C2505; USA) before the addition of the methanol. Percent recovery was calculated from the observed and expected FGM concentrations.

6.2.6 Data analysis

All data is reported as least square mean (LSMean) ± standard error of the mean (SEM), where applicable. For the analysis of FGM data, the average FGM concentration for a specific animal was calculated within each time interval. This was done since more than one faecal sample was often collected for each animal during each period of faecal collections. Samples differed in freshness, and since the exact time of excretion was unknown, the average FGM concentration was therefore used. Responses over time intervals were analysed using a restricted maximum likelihood estimation (REML) model. Data analysis was performed using the Variance Estimation and Precision module of Statistica (version 12) software program (StatSoft Inc., 2013).

6.3 RESULTS

The repeatability of FGM extraction from faecal samples and the within-assay variation are presented in Table 6.1, and the efficiency of FGM extraction from faecal samples is presented in Table 6.2.

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Table 6.2 The within-assay variation and repeatability of faecal extraction for analysis of the FGM concentration (ng/ml) in repeated measurements of a single methanol supernatant.

	Within-assay variation	**Repeatability of faecal extraction**
Number of assessments	8	8
Mean FGM ± SEM (ng/g dry faeces)	303.3 ± 12.73	82.4 ± 2.29
% Coefficient of Variation (CV)	11.9	7.8

Table 6.3 The efficiency of the extraction of the FGM from the faecal samples (ng/g dry faeces). A single faecal sample was extracted seven times and five of the aliquots were spiked with different amounts of corticosterone before being extracted.

Number of samples	**Corticosterone added to faeces (ng)**	**Observed FGM (ng/g dry faeces)**	**% CV**	**Expected FGM (ng/g dry faeces)**	**% recovery**
2	0	1.1 ± 0.02	2.3	-	-
5	200	3.1 ± 0.10	7.3	3.1	97.9

6.3.1 Plasma cortisol

Figure 6.1 shows the plasma cortisol concentrations over time for the four blue wildebeest (1–4) in response to Synacthen®. The administration of Synacthen® induced a significant increase ($P < 0.05$) in plasma cortisol. The initial increase occurred within 10 minutes after the administration of the Synacthen® in all four of the animals ($P < 0.01$; 10.9 ± 1.44 nmol/l at Time 0 to 125.4 ± 11.25 nmol/l at 10 minutes). Plasma cortisol further increased ($P = 0.01$) between 10 minutes and 20 minutes (178.1 ± 19.6 nmol/l). Thereafter, no significant increase ($P > 0.05$) was noted between 20 minutes and 30 minutes or between 20 minutes and 40 minutes after administration.

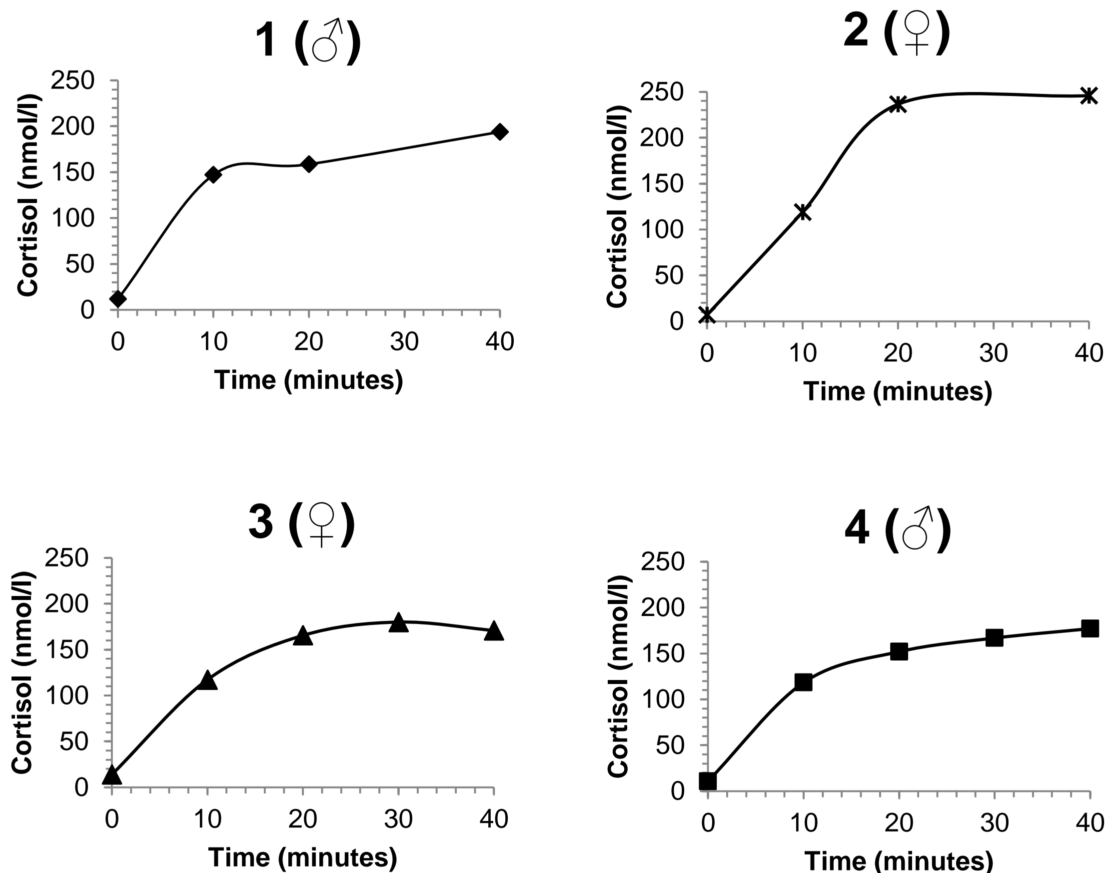

Figure 6.1 Plasma cortisol concentrations (nmol/ml) for four blue wildebeest (1, 2, 3 and 4) following an ACTH challenge. Blood samples were obtained from all the animals at time zero after recumbency (immobilising drugs 0.01–0.02 mg/kg thiafentanil oxalate plus 0.3 mg/kg azaperone tartrate) and at 10 or 20 –minute intervals thereafter. The animals received 1.0 IU/kg Synacthen® IM immediately after the first blood sample had been obtained.

6.3.2 Faecal glucocorticoid metabolites

Figure 6.2 presents the FGM concentration over time in the four blue wildebeest (1, 2, 3 and 4) in response to the administration of Synacthen®. There was a significant increase ($P < 0.05$) in FGM concentrations from Time 0 (rectal sample prior to Synacthen® administration) to Time 0–12 (first sample collected after Synacthen® administration) in all four animals so that FGM reached a peak at this latter time. Mean FGM concentration increased from 86.0 ± 5.33 ng/g of dry faeces at Time 0 to 477.9 ± 122.95 ng/g of dry

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faeces at Time 0–12. FGM concentration decreased back down to baseline level at Time 36–42 with no significant difference ($P = 0.83$) being observed between mean FGM concentration at this time (92.9 ± 23.56 ng/g of dry faeces) and at Time 0. Thus, pharmacologically elevating the plasma cortisol concentration resulted in at least a five-fold increase in FGM within the first 12 hours, which was detectable with the ^{125}I Corticosterone RIA kit.

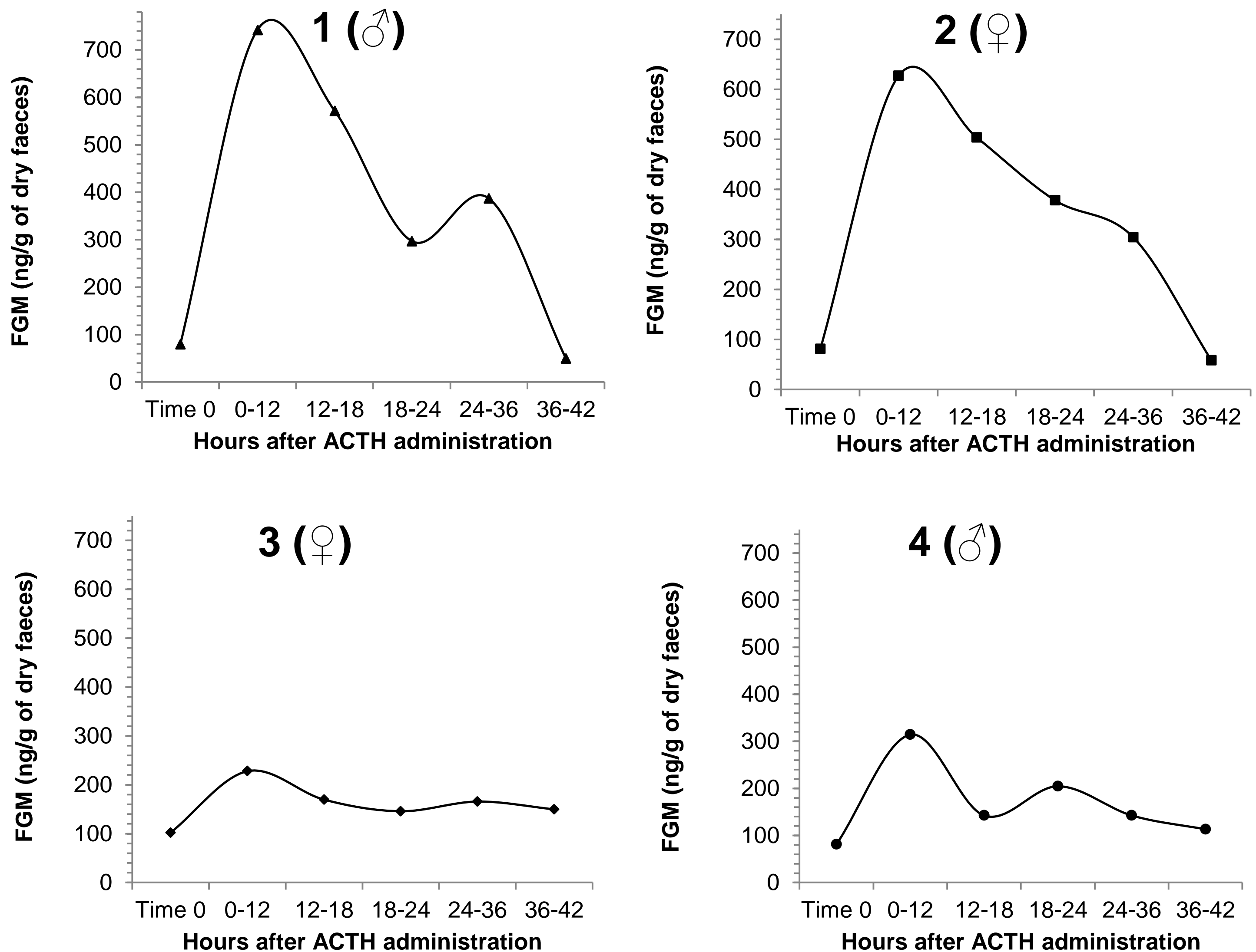

Figure 6.2 Faecal glucocorticoid metabolite concentrations (FGM ng/g dry faeces) for four blue wildebeest (1, 2, 3 and 4) following an ACTH challenge. An initial rectal faecal sample was collected from each individual animal at the time of the Synacthen® administration (Time 0) and thereafter from the boma floor every 6 to 12 hours until no glitter was visible in the faeces.

After the administration of Synacthen®, glitter was present in the first samples collected in the enclosure, with the peak passage of the inert marker occurring in the freshest samples obtained at the first collection (i.e. closest to 12 hours).

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Fresh faecal samples collected from two wildebeest in the wild had FGM concentrations of 31.0 ng/g and 26.1 ng/g of dry faeces. The latter sample was obtained from Wildebeest 4, one year after the ACTH challenge.

6.4 DISCUSSION

The results of the study show that a single intramuscular (IM) injection of a synthetic ACTH analogue (Synacthen®) produced a biologically significant increase in plasma cortisol within the first 10 minutes after its administration. This increase continued until 20 minutes after its administration, after which time, plasma cortisol started to plateau. These plasma cortisol response curves were greater than reported for this species by Schiewe, Bush, De Vos, Brown, and Wildt (1991). These authors reported a maximum plasma cortisol response of 110 nmol/l after ACTH administration. Schiewe *et al.* (1991) also reported a five-fold increase in plasma cortisol within 30 minutes after an ACTH challenge. However, Schiewe *et al.* (1991) only administered 50.0 IU of ACTH to each animal in comparison to the 1.0 IU/kg administered in the current study. In addition, these authors used an alternative ACTH derivative (Cortrosyn®, Organon, New Jersey, USA), which may account for the differences in results obtained. The maximum plasma cortisol response in the current study was also greater than was reported by Morton, Anderson, Foggin, Kock, and Tiran (1995) (24.6–87.5 nmol/l) for physically restrained wildebeest. It can be concluded that the plasma cortisol concentration that was achieved with this dose of Synacthen® is similar if not greater than the response of this species to acute stress. Due to the small sample size in the study, no comparison could be drawn between animals of different ages or genders or between samples obtained in different seasons.

Faecal glucocorticoid metabolite concentrations in all four animals showed a maximum peak within 12 hours after the administration of Synacthen®, indicating that acute elevation of circulating cortisol can be detected within 12–24 hours using the ^{125}I Corticosterone RIA kit. This peak was at least five-fold greater than the initial FGM concentration from the faecal sample collected from each animal rectally. It must be noted that the residual peak seen in some of the animals between Time 18–24 and Time 24–36 may be the result of artefact since Synacthen® is only supplied in a depot preparation, thus prolonging the return to baseline. A return to baseline concentrations of FGM was noted within 42 hours after the administration of Synacthen®.

The gut transit time was estimated to coincide with the peak passage of the marker, which occurred close to the 12-hour mark. However, caution must be taken when these

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results are interpreted since gut transit time is likely affected by diet, e.g. a diet high in fibre may result in an increase in gut transit time (Keay *et al.*, 2006).

Chinnadurai *et al.* (2008) noted FGM concentrations of 22–30 ng/g of dry faeces from free-ranging wildebeest, whilst Stabach, Boone, Worden, and Florant (2015) reported values ranging from 19.3–23.4 ng/g of dry faeces. These results are much lower than the baseline concentrations found in the current study (see Figure 6.2). This is an indication that baseline values were also higher in the current study than has been reported for this species in the wild. This is substantiated by the fact that FGM concentrations in faecal samples from two wild wildebeest in the current study were found to be almost three times lower than the baseline values observed before the administration of Synacthen®. This increase in FGMs in samples obtained from the captive wildebeest can be explained by the activation of the chronic stress response to the novelty of captivity as well as the change in diet while animals were in captivity prior to the start of the trials. Animals were fed high-quality lucerne hay whilst in the enclosures so that less feed was required to provide the same amount of nutrients as natural grazing in the wild. As a result of the decrease in the amount of feed ingested, it could be expected that the volume of faeces excreted would decrease as well. Assuming a constant output of excreted hormones, a lower concentration of hormone metabolites could be expected in the wild when animals are bulk-feeding. Such results have been reported by Goymann (2005) in avian species with the author concluding that similar results are likely to be found in mammal species as well.

The assay kit produced an acceptable coefficient of variation for a biological assay (11.9%). The repeatability of faecal extraction was also found to be acceptable with a coefficient of variation of 7.8%, although future studies may improve this coefficient of variation if the faecal sample size is increased to more than 2.5 g of faeces.

6.5 CONCLUSIONS

Blue wildebeest are frequently captured and relocated, or held in captivity at game auctions in South Africa because of their value for breeding of colour variants, live hunting and meat production (National Agricultural Marketing Council [NAMC], 2006; Van Hoving, 2011). These practices are highly stressful for any wild species with a number of negative consequences if stress is not minimised where possible (Dickens, Delehanty, & Romero, 2010). The measurement of FGM concentrations in faeces collected in the wild or from enclosure floors provides a non-invasive method of

quantifying long-term stress in captive wildlife species. It is believed that the current study has adequately validated the performance of the ^{125}I Corticosterone radio-immunoassay in terms of its suitability, consistency and precision, which has previously not been done using an ACTH challenge in blue wildebeest. The study did however note that results should be interpreted with caution since the baseline FGM concentrations of captive wildebeest appear to be much higher than was found for animals in the wild. This is probably due to the stress of captivity as well as a change in diet compared to animals in the wild.

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CHAPTER 7

Changes in behavioural and physiological responses in blue wildebeest *(Connochaetes taurinus)* due to tranquilisation with Clopixol Acuphase®*

ABSTRACT

The effect of zuclopenthixol acetate (Clopixol Acuphase®) on blue wildebeest (*Connochaetes taurinus*) in captivity was investigated. Animals (n = 17) were observed for 12 hours prior to being treated (pre-treatment) and for 12 hours after treatment (post-treatment) with Acuphase® (1.5 mg/kg body weight). During both treatments, animals were stimulated every two hours by a person entering the enclosure (referred to as 'periods of stimulation'). Behaviour, continuous heart rate, respiration rate and motion were measured throughout. Treated animals spent more time ($P < 0.05$) lying with their heads up and less time ($P < 0.01$) being vigilant and standing with their heads up, ruminating than untreated animals. Acuphase® lowered mean respiration rate ($P < 0.01$; pre-treatment: 17.79 ± 0.96 breaths/minute vs post-treatment: 14.64 ± 0.99 breaths/minute) and during periods of stimulation and no stimulation. The respiration rate of treated animals did not increase ($P > 0.05$) when animals were stimulated. Acuphase® also lowered respiration rate ($P < 0.05$) during certain active behaviours. Acuphase® resulted in animals spending less time moving fast when they were stimulated compared to when animals were untreated. Overall, Acuphase® caused a desirable palliative effect pertaining to wildlife management, with no observable adverse reactions.

Keywords: blue wildebeest, Clopixol Acuphase®, behaviour, heart rate, respiration rate, skin temperature, motion

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[*] **Results from this chapter in conjuction with results from Chapter 9 were submitted to the *African Journal of Wildlife Research* as:**
Laubscher, L.L., Hoffman, L.C., Pitts, N.I. & Raath, J.P. Behavioural and physiological responses to zuclopenthixol acetate in blue wildebeest (*Connochaetes taurinus*) (under review).

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7.1 INTRODUCTION

In South Africa, it is estimated that more than 300 000 head of game are translocated each year (Dugmore, 2013). A large number of these animals are kept in enclosures prior to being transported to their destination where they are subjected to stressful conditions that frequently end up in mortalities. The benefit of the long-lasting effects of long-acting neuroleptics (LANs) after a single administration has greatly improved the outcome of wildlife translocations by decreasing stress-related mortality (Ebedes, 1993; Fick, Mitchell, & Fuller, 2007). Although there is no double about the contribution of LANs to the successful capture, relocation and confinement of wildlife, the possible effects of LANs on other physiological and behavioural variables need further investigation (Fick *et al.*, 2007; Swan, 1993). This is particularly true for Southern African wildlife species, for which research regarding the use of LANs is limited.

Neuroleptic drugs suppress behavioural responses without affecting spinal and other reflexes (Read, 2002). In humans, LANs are prescribed for their anti-psychotic effects and for the suppression of alarm reactions, anxiety and psychomotor agitation (Huber, Walzer, & Slotta-Bachmayr, 2001). LANs can be administered in such a form and manner that a single dose gives a therapeutically effective tissue concentration for up to seven days (Huber *et al.*, 2001; Read, 2002). This is achieved by the binding of the esterificated tranquiliser to vegetable oils so that, injected intramuscularly, the formulation forms a depot at the injection site. With the slow breakdown of the oil solvent, the ester diffuses out of the depot and once absorbed into the blood, it is hydrolysed and the active ingredient is able to exert its effect (Huber *et al.*, 2001; Read, 2002; Swan, 1993). Zuclopenthixol acetate is a thioxanthene similar to the phenothiazine derivatives. In Clopixol Acuphase®, its absorption and duration has been extended through esterification with the acetate and its dissolution in vegetable oil (West, Heard, & Caulkett, 2007). It is also available as a decanoate ester (Clopixol®) which has a longer effect of up to 21 days. The effects of Clopixol Acuphase® can be seen within an hour after administration and last for up to 72 hours (Kock & Burroughs, 2012). Although serum concentrations of zuclopenthixol acetate are expected to reach a maximum at 36 hours after treatment with Clopixol Acuphase®, maximum sedation is achieved around 8 hours after its administration (Aaes-Jørgensen, 1989; Amdisen *et al.*, 1987; Chakravarti, Muthu, Muthu, Naik, & Pinto, 1990; Lundbeck Canada Inc., 2011). Clopixol Acuphase® is often seen as an intermediate LAN, and is used either alone or to bridge the effects of shorter or longer acting neuroleptic drugs when used in combination (Kock

& Burroughs, 2012; Swan, 1993). Generally, very few side-effects are observed; however as with all other LANs, extrapyramidal symptoms may occur (Kock & Burroughs, 2012). It has been successfully used in a variety of species such as deer, equid, bison, elk, various African antelope and rhino (Kock & Burroughs, 2012; Read, 2002).

The aim of the current study was to evaluate the use of Clopixol Acuphase®[1] (Lundbeck, Randburg, South Africa) in reducing both the behavioural and physical stress responses of blue wildebeest (*Connochaetes taurinus*) to captivity and human interferences.

7.2 MATERIALS AND METHODS

For the current study, the materials and methods as outlined in the materials and methods section of the dissertation were used.

A control study was also performed in which animals were administered 1.0 – 3.0 ml of sterile water (placebo). The purpose of the control study was to determine if changes in animal behavioural and physiological responses occurred in the absence of tranquilisation. In order to avoid repetition between chapters, the results from the control study, the results from the results from this study (Chapter 7) and Chapter 8, are compared in Chapter 9 of the dissertation. As such, the results obtained before and after treatment with Acuphase® will be reported and discussed in this chapter as they were observed without a comparison being drawn.

Ethical approval for all aspects of the trial was received from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa (Protocol Ethical Approval number SU-ACUM11-00005). Two qualified veterinarians handled and administered all the drugs used and also consistently monitored the animals during anaesthesia.

7.2.1 Animals

Three trials were conducted with the animal numbers, genders and weights indicated in Table 7.1. Prior to the start of each trial, genders were determined and each animal was weighed. Each trial consisted of a new group of animals, captured at a different location.

[1] The particular formulation of zuclopenthixol acetate, namely Clopixol Acuphase®, used in the study, is dissolved in vegetable oil in order to allow for delayed absorption (Lundbeck Canada Inc., 2011). To prevent any confusion between Chapters 7 and 8 of the dissertation, the trade name, Acuphase®, will be used when discussing results.

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All capture locations were within a one-hour drive from the study area (refer to Table 4.1).

Table 7.1 The animal numbers, genders and weights within each trial conducted to investigate the effect of Acuphase® on blue wildebeest behaviour and physiological responses

Trial	Month	Animal number	Gender	Weight (kg)
Trial 1	June	1	Female	170
		2	Female	146
		3	Female	155
		4	Female	164
		5	Female	176
		6	Female	123
Trial 2	August	1	Male	166
		2	Female	166
		3	Male	124
		4	Female	158
		5	Female	158
		6	Male	126
Trial 3	October	1	Male	170
		2	Female	165
		3	Female	120
		4	Female	150
		5	Male	101
TOTAL		17 Wildebeest	12 Females 5 Males	

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7.2.2 Statistical analysis

The data was analysed using a restricted maximum likelihood estimation (REML), which included treatment, gender, animal weight and trial (October, April and May trials) as fixed effects. For the analysis of vital sign data with behaviour data, behaviour and stimulation were included as fixed effects. Behaviour within periods of stimulation could not be analysed since not all behaviours occurred within a period of stimulation. However, changes in biotelemetry data were analysed within stimulations. Data analysis was performed using the Variance Estimation and Precision module of Statistica (version 12) statistical software (StatSoft Inc., 2013). All results were considered significant if $P < 0.05$ and are expressed as least squared means (LSMeans) ± standard error of the mean (SEM).

In order to minimise the effect of outliers in the data, the medians for each vital sign per animal were used for statistical analysis. Means for each group were calculated from these medians.

7.3 RESULTS

No extrapyramidal symptoms or side-effects were observed in any of the animals during any of the trials. There were also no observable adverse reactions at injection sites.

7.3.1 Behaviour

The results of the behaviours and the mean time spent on each of these behaviours (expressed as a percentage) prior to administration of Acuphase® (pre-treatment) and after the administration of Acuphase® (post-treatment) are indicated in Table 7.2.

Table 7.2 Time spent (LSMean ± SEM) per behaviour by blue wildebeest within a treatment (pre-treatment [PreT] and post-treatment [PostT]) in descending order, expressed as a percentage

Behaviour	PreT	PostT
Lying with head up	24.0[a] ± 4.43	34.1[b] ± 3.31
Being vigilant	26.2[a] ± 2.77	17.2[b] ± 1.69
Standing with head up, ruminating	21.3[a] ± 3.00	18.5[b] ± 2.53
Eating	19.6[a] ± 1.96	20.2[a] ± 1.92
Walking	3.8[a] ± 1.12	3.5[a] ± 0.68
Lying with head folded back	1.3[a] ± 0.35	2.5[a] ± 0.69
Standing with head down	1.2[a] ± 0.44	2.5[a] ± 0.34
Drinking	0.7[a] ± 0.25	0.5[a] ± 0.09
Exploring (standing and sniffing surfaces)	0.5[a] ± 0.13	0.3[a] ± 0.06
Exploring (walking and sniffing surface)	0.4[a] ± 0.25	0.2[a] ± 0.15
Fighting	0.3[a] ± 0.08	0.4[a] ± 0.06
Defending	0.3[a] ± 0.07	0.3[a] ± 0.06
Being rubbed by another animal	0.3[a] ± 0.10	0.2[a] ± 0.08
Rubbing against another animal	0.2[a] ± 0.05	0.2[a] ± 0.06
Trotting	0.2[a] ± 0.05	0.2[a] ± 0.04
Other	0.2[a] ± 0.10	0.2[a] ± 0.06

[ab] Means within a behaviour and between treatments with the same letter do not differ significantly ($P > 0.05$)

It must be noted that 0.2% of the time was spent on behaviours classified as “other”. “Other” behaviours were those behaviours observed only once or twice, or only in one group or individual. Examples of such behaviours are climbing into the feed trough, mounting or being mounted, bucking or kneeling (usually in response to aggression or sexual harassment) (Estes, 1991). In addition, behaviours that could not be recognised or appropriately described (such as an unfamiliar gait), were also grouped under “other”. These “other” behaviours were considered as abnormal as they were almost always brought on in response to some form of stressor, and were not observed often enough to be classified independently.

Two point behaviours were measured, namely grooming (sub-categorised into grooming while lying down and grooming while standing up) and agitation (sub-categorised into head shaking and feet stomping). The administration of Acuphase® had no effect (P > 0.05) on the mean number of occurrences of these behaviours.

7.3.2 Heart rate and respiration rate per behaviour

Acuphase® had no effect (P > 0.05) on heart rate during any of the behaviours (Table 7.3).

Table 7.3 The heart rates (bpm) (LSMean ± SEM) of blue wildebeest per behaviour between treatments (PreT and PostT) in descending order

Behaviour	PreT	PostT
Other	98.6[a] ± 5.08	95.7[a] ± 4.31
Fighting	91.5[a] ± 4.67	81.5[a] ± 4.38
Being vigilant	75.7[a] ± 3.72	75.4[a] ± 3.78
Defending	73.9[a] ± 4.72	81.0[a] ± 4.23
Trotting	74.7[a] ± 4.17	76.2[a] ± 3.97
Walking	73.3[a] ± 3.78	75.1[a] ± 3.81
Exploring while standing	63.5[a] ± 4.46	72.7[a] ± 4.46
Exploring while walking	62.5[a] ± 4.48	70.1[a] ± 4.40
Rubbing against other animal	62.7[a] ± 4.85	66.1[a] ± 4.59
Eating	64.2[a] ± 4.51	67.7[a] ± 4.56
Drinking	64.4[a]± 4.32	62.7[a] ± 4.69
Being rubbed by other animal	62.5[a] ± 4.52	63.6[a] ± 4.88
Standing with head up ruminating	60.9[a] ± 4.14	65.5[a] ± 4.18
Standing with head down	64.7[a] ± 5.39	58.4[a] ± 4.49
Lying with head up	57.0[a] ± 4.46	58.5[a] ± 4.13
Lying head folded back	52.6[a] ± 4.60	61.1[a] ± 4.54

[ab] Means within a behaviour and between treatments with the same letter do not differ significantly (P > 0.05)

The administration of Acuphase® was found to decrease (P < 0.05) respiration rate when animals were performing a number of behaviours (Table 7.4).

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Table 7.4 The respiration rates (breaths/minute) (LSMean ± SEM) of blue wildebeest per behaviour between treatments (PreT and PostT) in descending order

Behaviour	PreT	PostT
Fighting	27.7[a] ± 1.44	18.1[b] ± 1.29
Other	26.5[a] ± 1.68	19.3[b] ± 1.45
Defending	23.2[a] ± 1.47	19.0[b] ± 1.34
Trotting	18.1[a] ± 1.29	16.2[a] ± 1.24
Walking	20.0[a] ± 1.16	16.1[b] ± 1.18
Drinking	19.0[a] ± 1.36	19.3[a] ± 1.50
Being vigilant	19.0[a] ± 1.14	14.2[b] ± 1.17
Exploring while walking	18.3[a] ± 1.41	16.5[a] ± 1.41
Exploring while standing	17.2[a] ± 1.40	17.3[a] ± 1.43
Being rubbed by other animal	17.3[a] ± 1.42	14.9[a] ± 1.57
Standing head down	17.9[a] ± 1.74	12.3[b] ± 1.43
Rubbing against other animal	16.4[a] ± 1.52	16.1[a] ± 1.46
Eating	16.2[a] ± 1.41	15.9[a] ± 1.44
Standing head up ruminating	13.9[a] ± 1.29	12.3[a] ± 1.32
Lying head up	11.1[a] ± 1.40	10.5[a] ± 1.30
Lying head folded back	10.6[a] ± 1.45	9.4[a] ± 1.44

[ab] Means within a behaviour and between treatments with the same letter do not differ significantly ($P > 0.05$)

7.3.3 Motion

The motion of each animal was measured every 15 seconds by the Equivital™ belt in terms of the categories ‘stationary’, ‘moving slowly’ and ‘moving fast’. Treatment with Acuphase® had no effect ($P > 0.05$) on the frequency at which each motion category was measured in the animals. However, differences ($P < 0.05$) were found when data was analysed between periods of stimulation and periods of no stimulation (Table 7.5).

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Table 7.5 The frequency at which each motion was measured (LSMean ± SEM) in blue wildebeest within periods of stimulation (S) and periods of no stimulation (NS) between PreT and PostT, expressed as a percentage of total motion measured within a period

Treatment	Period	Stationary	Moving slowly	Moving fast
PreT	NS	$91.4^{a} \pm 0.98$	$7.9^{a} \pm 0.89$	$0.7^{b} \pm 0.15$
	S	$76.0^{b} \pm 2.67$	$18.9^{b} \pm 2.24$	$5.1^{a} \pm 1.14$
PostT	NS	$93.6^{a} \pm 0.74$	$6.0^{b} \pm 0.63$	$0.4^{b} \pm 0.14$
	S	$80.4^{b} \pm 3.32$	$17.1^{a} \pm 2.83$	$2.6^{b} \pm 0.91$

[abcd] Means within a motion category with the same letter do not differ significantly ($P > 0.05$)

As expected, more time ($P < 0.05$) was spent being stationary and less time ($P < 0.05$) was spent moving slowly during periods of no stimulation than during periods of stimulation in both treatments. No differences ($P > 0.05$) were found within a stimulation period between treatments for the motion categories ‘stationary’ and ‘moving slowly’. However, animals spent nearly double the time ($P = 0.04$) moving fast during periods of stimulation prior to the administration of Acuphase® compared to after the administration. In addition, Acuphase®-treated animals showed a tendency ($P = 0.08$) to spend more time moving fast during periods of stimulation compared to during periods of no stimulation.

When analysed within a motion category, mean heart rate did not differ ($P > 0.05$) between treatments for any of the motion categories. However, mean respiration rate was lower after treatment when animals were stationary ($P = 0.03$; PreT = 13.8 ± 1.06 breaths/minute; PostT = 10.7 ± 1.08 breaths/minute) and moving fast ($P < 0.01$; PreT = 20.9 ± 1.07 breaths/minute; PostT = 17.3 ± 1.19 breaths/minute).

7.3.4 Heart rate and respiration rate between periods of stimulation and periods of no stimulation

Mean heart rates (bpm) and respiration rates (breaths/minute) between treatments, for periods of stimulation and periods of no stimulation, are indicated in Table 7.6.

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Table 7.6 The heart rate (bpm) and respiration rate (breaths/minute) (LSMean ± SEM) of blue wildebeest between treatments (PreT and PostT) and between periods where animals were stimulated (S) and not stimulated (NS)

Treatment	Period	Heart rate	Respiration rate
PreT	NS	$67.8^{b} \pm 0.96$	$18.5^{b} \pm 0.43$
	S	$80.7^{a} \pm 2.99$	$20.7^{a} \pm 1.02$
PostT	NS	$70.8^{b} \pm 1.34$	$15.7^{c} \pm 0.40$
	S	$78.9^{a} \pm 3.23$	$16.3^{bc} \pm 0.84$

^{abc} Heart rate or respiration rate means between periods and treatments with the same letter do not differ significantly ($P > 0.05$)

As expected, heart rate was higher ($P < 0.05$) during periods of stimulation than during periods of no stimulation, both before and after treatment, although there was no difference ($P > 0.05$) between treatments for a specific period. Respiration rate was also higher ($P = 0.002$) during periods of stimulation than during periods of no stimulation but only before Acuphase® was given. After administering Acuphase®, stimulation had no effect ($P > 0.05$) on respiration rate. Unlike heart rate, respiration rate during both periods was higher ($P < 0.05$) prior to the administration of Acuphase® compared to after.

Treatment had no effect ($P = 0.43$) on overall mean heart rate (PreT: 70.3 ± 3.05 bpm; PostT: 73.4 ± 3.16 bpm). However, overall mean respiration rate was higher ($P = 0.03$) before treatment (17.8 ± 0.96 breaths/minute) than after treatment (14.6 ± 0.99 breaths/minute).

A significant but very weak correlation ($R^2 = 0.40$; $P < 0.05$) was found between mean heart rate and mean respiration rate so that mean respiration rate increased as mean heart rate increased.

7.3.5 Skin temperature

Skin temperature, overall or during a specific behaviour, was unaffected by treatment, stimulation or motion. It did, however show a positive correlation ($R^2 = 0.53$; $P < 0.05$) with environmental temperature. No such correlation was found between mean heart rate or respiration rate and environmental temperature.

Mean skin temperature also differed ($P < 0.05$) between trials (Figure 7.1) with mean skin temperature being lowest during Trial 1.

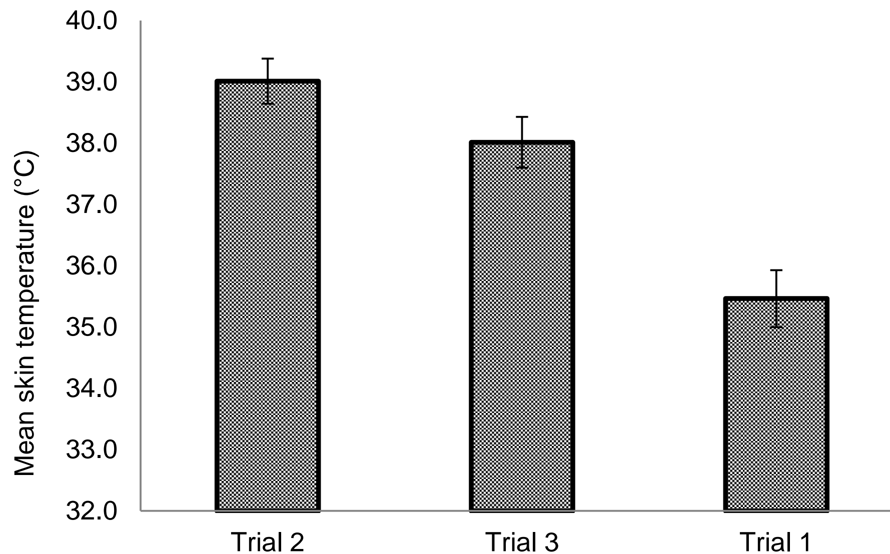

Figure 7.1 Skin temperature (°C) (LSMean ± SEM) between trials

7.4 DISCUSSION

Treatment with Acuphase® caused beneficial behavioural changes and decreased the respiration rate of the animals. In addition, Acuphase® caused a decrease in respiration rate for certain behaviours. Treated animals were also less active during periods of stimulation, and respiration rate was lower after treatment with Acuphase® when the animals were stationary and when they were moving fast. Skin temperature was unaffected by treatment with Acuphase®, behaviour or motion but was significantly correlated with environmental temperature. The latter suggests that skin temperature is not a good indicator of an animal's response to stress, as skin temperature is likely to be affected by environmental temperature.

Acuphase® caused an increase ($P < 0.01$) in the amount of time the animals spent resting by lying with their heads up as well as a decrease in the amount of time spent being vigilant. In wildebeest, the study observed that vigilant behaviour is characterized by an alert, upright posture with the head lifted and the ears moving. The findings are in agreement with Read, Caulkett, and McCallister (2000), who reported that wapiti (*Cervus canadensis*) treated with Clopixol Acuphase® were less active than untreated animals. Fick *et al.* (2007) and Fick, Matthee, Mitchell, and Fuller (2006) also reported a significant decrease in activity due to the administration of Acuphase® in goats and blue wildebeest, respectively. After the administration of Acuphase®, animals spent similar amounts of time lying down (34.1% lying with heads up and 2.5% lying with heads folded back) as

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reported by Berry, Siegfried, and Crowe (1982) for wildebeest in the wild. These authors reported that wildebeest spent 32% of their time lying down while Ben-Shahar and Fairall (1987) reported a much higher value of 44% for wild wildebeest. Captivity and stress are likely to result in abnormal behaviour patterns but the results indicate that Acuphase®-treated animals were more relaxed and spent similar amounts of time lying down than they would under natural conditions.

Treatment with Acuphase® had no effect ($P = 0.43$) on overall heart rate. Read *et al.* (2000) reported similar findings and found no significant difference in the heart rates of wapiti (*Cervus canadensis*) treated with Clopixol Acuphase® and untreated wapiti. However, these authors also reported no significant difference in mean respiration rates between treated and untreated animals whereas the current study found that animals had a lower ($P = 0.03$) overall respiration rate after the administration of Acuphase®. This may be attributable to the palliative effect of Acuphase® and is reflected in the decrease in respiration rate found for certain behaviours.

Diverio, Goddard, and Gordon (1996) reported that red deer treated with Acuphase® showed a greater increase in heart rate when a stressor was applied than untreated animals. They attributed this to reflex tachycardia, which is commonly seen after administration of a LAN because of a lowered peripheral resistance and resulting hypotension. This hypotension is thought to result from a peripheral α-adrenergic blockade since both phenothiazine and thioxanthine derivatives have high affinities for α1-adenoceptors (Walsh & Wilson, 2002). This may explain the lack of significant difference ($P > 0.05$) in mean heart rates, both overall (during periods of stimulation and periods of no stimulation) between tranquilised and non-tranquilised animals in the current study. Other authors have reported similar findings with the use of a variety of tranquilisers in wildlife (Diverio, Goddard, Gordon, & Elston, 1993; Marco, Lavín, Mentaberre, López-Olvera, & Casas-Díaz, 2010; Mentaberre, López-Olvera, Casas-Díaz, Bach-Raich *et al.*, 2010; Mentaberre, López-Olvera, Casas-Díaz, Fernández-Sirera *et al.*, 2010; Montané, Marco, & Manteca, 2002).

During both treatments, heart rate (bpm) was higher ($P < 0.05$) during periods of stimulation compared to periods of no stimulation, which is to be expected since increased motor activity and the stress evoked during these periods can be expected to elevate heart rate (Bonacic, Feber, & Macdonald, 2006; Price & Sibly, 1993; Waran & Cuddeford, 1995). The increase in respiration rate during periods of stimulation is in agreement with the findings of Reefmann, Bütikofer Kaszàs, Wechsler, and Gygax

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(2009) who reported that situations evoking negative emotional responses caused elevations in respiration rate in sheep. Likewise, Boiten, Frijda, and Wientjes (1994) reviewed the link between emotional stressors and respiration rate, and concluded that a negative emotional response resulted in a corresponding increase in respiration rate. These increases in both heart rate and respiration rate may partly explain the correlation (albeit a weak correlation) between these two parameters.

Dittberner (2011) reported mean heart rates ranging from 70.5 to 102.9 bpm for blue wildebeest immobilised with different variations of etorphine with hyaluronidase. These findings are similar to the ranges reported in the current study. To our knowledge, there is presently no published data available for the heart rates of free-ranging, untreated blue wildebeest.

In the current study, respiration rates for treated animals were lower than rates previously reported by Mortola and Lanthier (2005) for blue wildebeest in the wild. These authors measured respiration rate in a single standing, untreated male by counting chest movements over a 30-minute period, and reported an average of 17 breaths/minute. Dittberner (2011) reported respiration rates for blue wildebeest immobilised with etorphine and hyaluronidase to range from 19–26 breaths/minute, which is similar to untreated animals in the current study.

In terms of motion, it could be expected that moving fast would result in the highest heart and respiration rates since increased motor activity can result in increased respiratory and cardiovascular output (Price & Sibly, 1993). Although heart rate was unaffected, tranquilisation with Acuphase® resulted in a decrease in respiration rate during periods when animals were stationary ($P = 0.03$) and moving fast ($P < 0.01$). The former may be related to a lowered respiration rate of the animals while they were being vigilant. Both results suggest a calming effect resulting from the administration of Acuphase®.

It is also not surprising that animals moved most during periods of stimulation as compared to periods of no stimulation as stimulation would initiate the ‘fight-or-flight’ response of the animals (Uetake, Morita, Hoshiba, & Tanaka, 2002). Treatment with Acuphase® resulted in animals moving fast less often ($P = 0.04$) during periods of stimulation, indicating a reduction in the flight-and-activity response of the animals. Substantiating this is the fact that there was no increase ($P = 0.08$) in the percentage of time spent moving fast during periods of stimulation compared to periods of no stimulation in treated animals.

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All the behaviours that showed the highest mean heart rates and respiration rates are behaviours that occur in response to a stressor or which can induce stress, so it is expected that these would coincide with elevations in these parameters (Barnett & Hemsworth, 1990). These findings are consistent with the fact that the four behaviours with the lowest mean heart and respiration rates are all non-alert, resting behaviours. Price and Sibly (1993) reported similar results in farmed red deer where the mean heart rates for the behaviours trotting and walking were 120 bpm and 108 bpm, respectively. These authors found that the behaviours lying and lying ruminating had the lowest mean heart rates at 56 bpm and 58 bpm, respectively, and noted that this is due to the increased energy needs during locomotor activities (Price & Sibly, 1993). Other authors have reported similar results for a number of behaviours in various domestic species (Baldock, Sibly, & Penning, 1988; Jansen *et al.*, 2009; Macarthur, Johnston, & Geist, 1979). Administration of Acuphase® caused a lower ($P < 0.05$) respiration rate when animals were fighting, defending themselves, walking and being vigilant as well as performing "other" behaviours. Increased energy requirements during stressful or active activities can result in increased respiratory and cardiovascular output (Barnett & Hemsworth, 1990; Boiten *et al.*, 1994). All these behaviours are active behaviours so that a decrease in respiration can be an indication of lowered energy expenditure during these behaviours caused by the administration of Acuphase® (Green, 2011).

7.5 CONCLUSIONS

The measurement of a heart rate and respiration rate in addition to and combined with behavioural responses provided insight into the response of blue wildebeest to treatment with Acuphase® whilst in captivity. The use of Acuphase® was found to be effective in minimising the amount of time animals spent being vigilant and increasing time spent resting. No adverse effects due to the administration of Acuphase® were observed in any of the animals and all heart and respiration rate measurements fell within acceptable ranges. Although administration of Acuphase® had no effect ($P = 0.43$) on overall heart rate, it caused a lower ($P = 0.03$) overall respiration rate in the animals. The lack of effect on heart rate may be related to reflex tachycardia in response to hypotension induced by the administration of Acuphase® as previously mentioned (see section 7.4). Treatment also caused a lowered ($P < 0.05$) respiration rate during periods of stimulation and periods of no stimulation compared to when animals were untreated. In addition, treatment with Acuphase® resulted in a lower ($P < 0.05$) respiration rate for certain behaviours, particularly active behaviours. Treated animals also showed a reduction in

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the time spent moving fast during periods of stimulation, indicating a reduction in activity in response to a stressor. Overall, Acuphase® produced a suitable palliative effect in the animals with no observable side-effects.

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CHAPTER 8

Changes in behavioural and physiological parameters in blue wildebeest *(Connochaetes taurinus)* due to tranquilisation with Acunil®*

ABSTRACT

The study investigated the potential of a new long-acting neuroleptic (LAN), Acunil®, to be used as a tranquilizer in blue wildebeest in captivity. Animals (n = 17) were observed for 12 hours prior to treatment with Acunil® (pre-treatment). Twenty four hours after the onset of the trial, Acunil® was administered (1.5 mg/kg body weight) and animals were observed for another 12 hours (post-treatment). Animals were stimulated every two hours by a person entering the enclosure (referred to as ‘periods of stimulation’). Behaviour, continuous heart rate, respiration rate and motion were measured throughout. Time spent lying with heads folded back, eating and standing with heads down increased and time spent being vigilant and exploring while walking decreased after treatment. Animals groomed less while standing and performed less head shaking after treatment. Acunil® had no effect (P = 0.72) on mean heart rate (pre-treatment: 69.71 ± 4.06 beats/minute (bpm); post-treatment: 68.46 ± 4.05 bpm); however, mean respiration rate was lowered (P = 0.02) (pre-treatment: 14.51 ± 0.82 breaths/minute; post-treatment: 12.47 ± 0.83 breaths/minute). Acunil® also lowered (P < 0.05) respiration rate during periods of stimulation (pre-treatment: 16.17 ± 0.87 breaths/minute; post-treatment: 13.67 ± 0.87 breaths/minute) and when animals were trotting and being vigilant. Motion was affected by treatment with Acunil®. Overall, treatment with Acunil® caused a desirable palliative effect.

Keywords: blue wildebeest, Acunil®, behaviour, heart rate, respiration rate, skin temperature, motion

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[*] **Results from this chapter in conjuction with results from Chapter 9 were submitted to the *Journal of Zoo and Wildlife Medicine* as:**
Laubscher, L.L., Hoffman, L.C., Pitts, N.I. & Raath, J.P. The effect of a slow-release formulation of zuclopenthixol acetate (Acunil®) on captive blue wildebeest (*Connochaetes taurinus*) behavior and physiological responses (under review).

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8.1 INTRODUCTION

South Africa has a rapidly growing wildlife industry, with a resulting increased demand for wildlife capture and relocation across the country (Dry, 2010; National Agricultural Marketing Council [NAMC], 2006; Saayman, Van der Merwe, & Rossouw, 2011; Steyn, 2012; Van Hoving, 2011). In capture and relocation activities, animals are often kept in captivity for a number of related reasons, such as the holding of animals at game auctions, the collecting and keeping of animals prior to translocation, the adaptation of animals prior to release or the quarantining of animals (Bothma, 2002). The use of long-acting neuroleptics (LANs) has become increasingly popular since they can induce long-lasting sedation that can reduce animal anxiety during captivity, especially when animals are to be kept for periods exceeding 24 hours.

Long-acting neuroleptics were initially developed for the treatment of psychoses in non-compliant psychiatric patients (Fick, Mitchell, & Fuller, 2007). The formulation of most LANs is similar, with the drugs that they consist of a fatty acid ester of the active drug ingredient that is dissolved in vegetable or medicinal oil. Intramuscular administration of such LANs result in the formation of a depot at the injection site (Read, 2002). With the slow breakdown of the oil solvent, the ester diffuses out of the depot and once absorbed into the blood, it is hydrolysed and the active ingredient is able to exert its effect (Fick, Matthee, Mitchell, & Fuller, 2006; Read, 2002; Swan, 1993). Long-acting neuroleptics act primarily as antipsychotics by blocking dopamine receptors in the limbic system, and there are no specific pharmacological antidotes to LANs.

The benefit of the long-lasting effects of LANs after a single administration has greatly improved the outcome of wildlife capture and relocations, specifically by decreasing stress-related mortalities associated with capture, transport and captivity (Ebedes, 1993; Fick *et al.*, 2007). Although the contribution of LANs to the successful capture, relocation and confinement of wildlife is indisputable, their specific effects on physiological and behavioural variables require further investigation to ensure for the sae and responsible use of the drugs (Fick *et al.*, 2007; Swan, 1993). In Southern Africa, there is limited research on the use of LANs in wildlife species, and the practical application thereof.

In South Africa, one of the most commonly used LANs in wildlife is Clopixol Acuphase® (zuclopenthixol acetate). In Clopixol Acuphase®, zuclopenthixol acetate is modified so that its absorption and duration have been extended through esterification with acetate,

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and its dissolution in vegetable oil (West, Heard, & Caulkett, 2007). A new LAN, Acunil®,[1] is currently being developed with the aim of producing a more consistent and predictable slow release.[2] The premises that the new drug is based on is the exclusion of the traditional vegetable oil vehicle, and alternatively the dissolution of a fatty acid ester of zuclopenthixol acetate to yield a 72-hour slow-release polymer. Although this polymer is thought to produce a more consistent release profile, the effect and duration of action of Acunil® is expected to be the same as Clopixol Acuphase®. Previous studies, in which the same polymer was used in other drug formulations, showed that maximum serum concentrations of the active drug ingredient are reached within 6 hours, and that serum concentrations are maintained above a therapeutic level for up to 72 hours after administration. In addition, peak drug effects were observed at 4 hours after treatment (Carbone, Lindstrom, Diep, & Carbone, 2012; Clark, Clark, & Hoyt, 2014; Foley, Liang, & Crichlow, 2011; Healy *et al.*, 2014; SR Veterinary Technologies, 2011, 2012).

The aim of this study was to investigate the use of Acunil® (Wildlife Pharmaceutical SA (Pty) Ltd., Rocky's Drift, Mpumalanga, South Africa) in reducing both the behavioural and physical stress responses induced in blue wildebeest (*Connochaetes taurinus*) in reaction to being in captivity, and associated human interference.

8.2 MATERIALS AND METHODS

Refer to Chapter 4 for a detailed explanation of the materials and methods used in this study.

A control study was also performed in which animals were administered 1.0 – 3.0 ml of sterile water (placebo). The purpose of the control study was to determine if changes in animal behavioural and physiological responses occurred in the absence of tranquilisation. In order to avoid repetition between chapters, the results from the control study, the results from Chapter 7 and the results from this study (Chapter 8), are compared in Chapter 9 of the dissertation. As such, the results obtained before and after treatment with Acunil® will be reported and discussed in this chapter as they were observed without a comparison being drawn.

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[1] The particular formulation of zuclopenthixol acetate, namely Acunil®, used in the study is dissolved in a slow-release polymer in order to allow for delayed absorption. To prevent any confusion between Chapters 7 and 8 of the dissertation, the trade name Acunil® will be used when discussing results.

[2] Acunil® is under development by Wildlife Pharmaceuticals South Africa Pty (Ltd.), Rocky Drift, Mpumalanga, South Africa.

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Ethical approval for all aspects of the project was obtained from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa (Protocol Ethical Approval number SU-ACUM11-00005). Two qualified veterinarians handled and administered all the drugs used as well as consistently monitored the animals during anaesthesia.

8.2.1 Animals

Three trials were conducted with the animal numbers, genders and live weights given in Table 8.1. Prior to the start of each trial, genders were determined and each animal was weighed. Each trial consisted of a new group of animals, captured at a different location because of limitations in animal availability. All animals were wild and had no previous experience of captivity and human interaction. All capture locations were within a one-hour drive from the study area (refer to Table 4.1).

Table 8.1 The animal numbers, genders and weights within each trial conducted to investigate the effect of Acunil® on blue wildebeest behaviour and physiological responses

Trial	Month	Animal number	Gender	Weight (kg)
Trial 1	October	1	Male	142
		2	Male	204
		3	Male	186
		4	Female	176
		5	Female	156
		6	Female	136
Trial 2	April	1	Male	211
		2	Female	147
		3	Female	162
		4	Female	167
		5	Male	163
Trial 3	May	1	Female	86
		2	Male	78
		3	Male	80
		4	Female	138
		5	Male	96
		6	Male	190
TOTAL		17 Wildebeest	8 Females 9 Males	

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8.2.2 Statistical analysis

The data was analysed using a restricted maximum likelihood estimation (REML), which included treatment, gender, animal weight and trial (October, April and May trials) as fixed effects. For the analysis of vital sign data with behaviour data, behaviour and stimulation were included as fixed effects. Behaviour within periods of stimulation could not be analysed since not all behaviours occurred within a period of stimulation. However, changes in biotelemetry data were analysed within stimulations. Data analysis was performed using the Variance Estimation and Precision module of Statistica (version 12) statistical software (StatSoft Inc., 2013). All results were considered significant if $P < 0.05$, and are expressed as least squared means (LSMeans) ± standard error of the mean (SEM).

In order to minimise the effect of outliers in the data, the medians for each vital sign per animal were used for statistical analysis. Means for each group were calculated from these medians. Results are reported as LSMean ± SEM.

8.3 RESULTS

No extrapyramidal symptoms or side-effects were observed in any of the animals during any of the trials. There were also no observable adverse reactions at injection sites.

8.3.1 Behaviour

The percentage of time spent on each behaviour before and after treatment with Acunil® is indicated in Table 8.2. It must be noted that 1.7% of the time was spent on behaviours classified as ‘other’.

Two point behaviours (without a measurable duration) were observed, namely grooming (sub-categorised into grooming while lying down and grooming while standing up) and agitation (sub-categorised into head shaking and feet stomping). It was found that animals groomed less ($P = 0.03$) while standing after the administration of Acunil® (19.2 ± 4.25%) than before (27.1 ± 4.14%). In addition, animals also shook their heads less often ($P = 0.001$) after the administration of Acunil® (27.7 ± 3.30%) than before (46.4 ± 4.23%).

Table 8.2 The time spent (LSMean ± SEM) per behaviour by blue wildebeest within a treatment (pre-tranquilisation [PreT] and post-tranquilisation [PostT]) in descending order, expressed as a percentage

Behaviour	PreT	PostT
Lying with head up	$33.4^{a} \pm 2.60$	$33.8^{a} \pm 2.74$
Being vigilant	$25.3^{a} \pm 2.23$	$13.9^{b} \pm 1.55$
Eating	$12.1^{a} \pm 1.57$	$15.9^{b} \pm 1.40$
Standing with head up, ruminating	$10.4^{a} \pm 1.17$	$9.8^{a} \pm 1.04$
Walking	$6.5^{a} \pm 0.67$	$8.9^{a} \pm 1.50$
Standing with head down	$2.5^{a} \pm 0.95$	$5.6^{b} \pm 1.54$
Lying with head folded back	$2.7^{a} \pm 0.45$	$5.6^{b} \pm 1.03$
Other	$1.7^{a} \pm 0.19$	$1.7^{a} \pm 0.21$
Exploring (walking and sniffing surfaces)	$1.2^{a} \pm 0.32$	$0.6^{b} \pm 0.14$
Fighting	$1.1^{a} \pm 0.21$	$1.0^{a} \pm 0.16$
Exploring (standing and sniffing surfaces)	$0.8^{a} \pm 0.10$	$0.7^{a} \pm 0.08$
Defending	$0.7^{a} \pm 0.15$	$0.7^{a} \pm 0.13$
Rubbing against another animal	$0.4^{a} \pm 0.15$	$0.6^{a} \pm 0.16$
Drinking	$0.5^{a} \pm 0.05$	$0.7^{a} \pm 0.10$
Being rubbed by another animal	$0.3^{a} \pm 0.09$	$0.6^{a} \pm 0.13$
Trotting	$0.3^{a} \pm 0.07$	$0.2^{a} \pm 0.04$

[ab] Means within a behaviour and between treatments with the same letter do not differ significantly ($P > 0.05$)

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8.3.2 Heart rate and respiration rate per behaviour

The administration of Acunil® was found to have no effect (P > 0.05) on mean heart rate per behaviour (Table 8.3).

Table 8.3 The heart rates (bpm) (LSMean ± SEM) of blue wildebeest per behaviour between treatments (PreT and PostT) in descending order

Behaviour	PreT	PostT
Trotting	92.7 ± 6.16	85.9 ± 6.27
Fighting	79.9 ± 6.44	89.0 ± 6.48
Other	80.7 ± 6.64	86.9 ± 6.53
Defending	79.5 ± 6.44	85.1 ± 6.38
Drinking	74.9 ± 6.57	78.4 ± 6.57
Walking	73.7 ± 6.10	78.7 ± 6.04
Rubbing against another animal	73.0 ± 6.65	73.1 ± 6.75
Exploring (walking and sniffing surfaces)	77.4 ± 6.49	68.2 ± 6.59
Eating	72.8 ± 6.57	70.8 ± 6.49
Being rubbed by another animal	69.8 ± 6.57	71.6 ± 6.65
Exploring (standing and sniffing surfaces)	72.1 ± 6.49	69.9 ± 6.57
Being vigilant	70.6 ± 6.02	69.2 ± 6.00
Standing with head down	62.9 ± 6.61	66.1 ± 6.62
Standing with head up, ruminating	65.2 ± 6.57	63.4 ± 6.22
Lying head folded back	61.7 ± 6.65	60.4 ± 6.44
Lying with head up	61.2 ± 6.51	59.5 ± 6.28

However, respiration rate was lower (P < 0.05) in treated animals for certain behaviours than in untreated animals (Table 8.4).

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Table 8.4 The respiration rates (breaths/minute) (LSMean ± SEM) of blue wildebeest per behaviour between treatments (PreT and PostT) in descending order

Behaviour	PreT	PostT
Other	$20.4^{a} \pm 1.26$	$20.2^{a} \pm 1.24$
Trotting	$19.1^{a} \pm 1.04$	$16.5^{b} \pm 1.12$
Defending from other animal	$17.1^{a} \pm 1.17$	$18.7^{a} \pm 1.17$
Fighting with other animal	$17.0^{a} \pm 1.17$	$17.6^{a} \pm 1.24$
Walking	$17.0^{a} \pm 1.02$	$17.1^{a} \pm 1.02$
Drinking	$17.1^{a} \pm 1.23$	$16.6^{a} \pm 1.26$
Rubbing against other animal	$16.1^{a} \pm 1.26$	$16.5^{a} \pm 1.30$
Exploring (standing and sniffing surfaces)	$16.5^{a} \pm 1.20$	$14.5^{a} \pm 1.27$
Exploring (walking and sniffing surfaces)	$17.0^{a} \pm 1.20$	$15.6^{a} \pm 1.26$
Eating	$16.1^{a} \pm 1.23$	$14.3^{a} \pm 1.23$
Being rubbed by other animal	$12.7^{a} \pm 1.23$	$15.6^{a} \pm 1.30$
Being vigilant	$15.1^{a} \pm 0.98$	$12.8^{b} \pm 1.00$
Standing head down	$13.9^{a} \pm 1.24$	$10.7^{b} \pm 1.27$
Standing head up ruminating	$13.7^{a} \pm 1.23$	$10.8^{b} \pm 1.11$
Lying head up	$9.9^{a} \pm 1.20$	$9.2^{a} \pm 1.14$
Lying head folded back	$7.9^{a} \pm 1.26$	$7.5^{a} \pm 1.20$

^{ab} Means within a behaviour and between treatments with the same letter do not differ significantly ($P > 0.05$)

8.3.3 Motion

The motion of each animal was measured every 15 seconds by the Equivital™ belt in terms of the categories ‘stationary’, ‘moving slowly’ and ‘moving fast’. After the administration of Acunil®, animals spent more time ($P < 0.05$) being stationary (82.8 ± 1.70%) and less time moving fast (4.8 ± 1.80%) than before (stationary: 75.1 ± 1.70%; moving fast: 10.2 ± 1.72%).

Differences ($P < 0.05$) were also found when motion data was analysed between periods of stimulation and periods of no stimulation (Table 8.5).

Table 8.5 The frequency at which each motion was measured (LSMean ± SEM) in blue wildebeest within periods of stimulation (S) and periods of no stimulation (NS) between PreT and PostT, expressed as a percentage of total motion measured within a period

Treatment	Period	Stationary	Moving slowly	Moving fast
PreT	NS	87.3[a] ± 0.96	11.3[a] ± 0.79	1.4[a] ± 0.24
	S	63.0[b] ± 2.43	18.9[b] ± 2.94	19.2[b] ± 2.94
PostT	NS	86.8[a] ± 1.83	11.9[ab] ± 1.62	1.3[a] ± 1.33
	S	78.8[a] ± 2.70	15.0[ab] ± 1.24	8.4[c] ± 1.74

[abc] Means within a motion category and between treatments and stimulation periods with the same letter do not differ significantly ($P > 0.05$)

Mean heart rates per motion category were unaffected ($P > 0.05$) by treatment with Acunil®. However, mean respiration rate was lower ($P = 0.04$) in treated animals when they were stationary (10.3 ± 1.31 breaths/minute) compared to when they were untreated (12.3 ± 1.29 breaths/minute).

8.3.4 Heart rate and respiration rate between periods of stimulation and periods of no stimulation

Mean heart rate (bpm) and respiration rate (breaths/minute) for periods of stimulation and periods of no stimulation are reported in Table 8.6.

Table 8.6 Heart rate (bpm) and respiration rate (breaths/minute) (LSMean ± SEM) of blue wildebeest between treatments (PreT and PostT), and between periods where animals were stimulated (S) and not stimulated (NS)

Treatment	Period	Heart rate	Respiration rate
PreT	NS	60.1[a] ± 4.44	12.9[a] ± 0.80
	S	79.4[b] ± 3.24	16.2[b] ± 0.94
PostT	NS	64.4[a] ± 3.25	11.3[a] ± 0.73
	S	72.5[b] ± 4.60	13.7[c] ± 0.98

[abc] Heart rate or respiration rate means between periods and treatments with the same letter do not differ significantly ($P > 0.05$)

Treatment with Acunil® had no effect (P = 0.72) on the overall mean heart rate (PreT: 69.7 ± 4.06 bpm; PostT: 68.5 ± 4.05 bpm). However, overall mean respiration rate was significantly higher (P = 0.02) prior to treatment (14.5 ± 0.82 breaths/minute) compared to after treatment (12.5 ± 0.83 breaths/minute).

A significant correlation (R^2 = 0.70; P < 0.05) was found between mean heart rate and mean respiration rate in that mean respiration rate increased as mean heart rate increased.

8.3.5 Skin temperature

Skin temperature, overall or during a specific behaviour, was unaffected by treatment, stimulation or motion. It did, however, show a positive correlation (R^2 = 0.59; P < 0.05) with environmental temperature. No such correlations were found between environmental temperature and mean heart rate or respiration rate.

Mean skin temperature also differed (P < 0.05) between trials (Figure 8.1) with mean skin temperature being lowest during Trial 1.

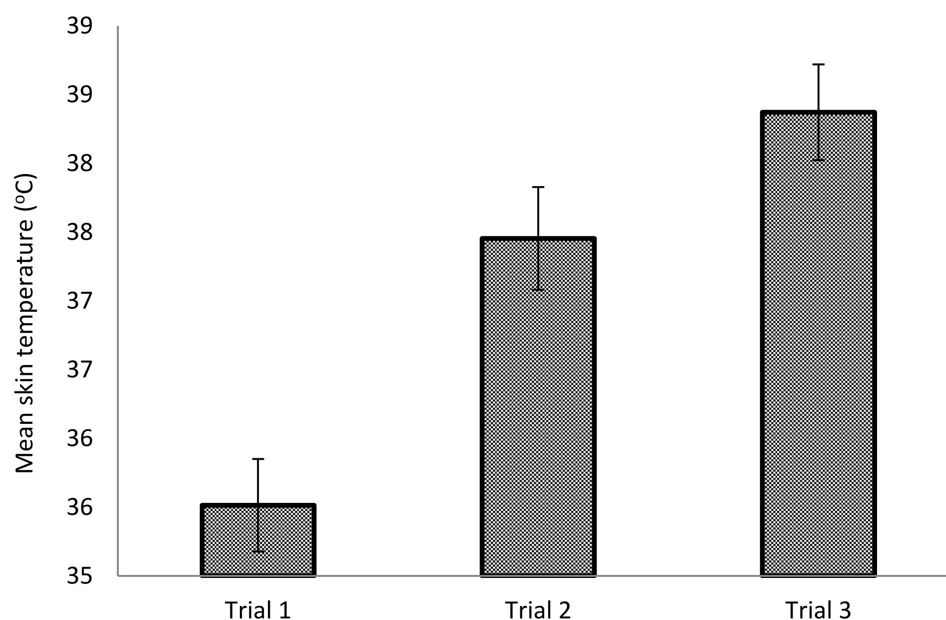

Figure 8.1 The skin temperature (°C) (LSMean ± SEM) of animals within each trial

As could be expected, mean skin temperature also differed (P < 0.001) between day and night with mean skin temperature being lower at night (36.36 ± 0.35 °C) than during the day (37.29 ± 0.34 °C).

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8.4 DISCUSSION

The results show that treatment with Acunil® resulted in behavioural changes and a slower respiration rate of the animals, both overall as well as whilst exhibiting certain behaviours. These changes are reflected in the analysis of motion, where treated animals spent more time being stationary, specifically during periods of stimulation when a person entered the enclosure. Acunil®-treated animals also showed decreased respiration rates whilst being stationary. Skin temperature was unaffected by treatment with Acunil®, behaviour or motion but was significantly correlated to environmental temperature. The latter suggests that skin temperature is not a good indicator of an animal's response to stress, as it is more likely to be affected by environmental temperature.

When animals were tranquilised, they ate significantly more ($P < 0.001$). It may be that since the animals spent less time performing behaviours such as exploring and being vigilant, they could allocate more time to eating during this period. Blue wildebeest in the wild have previously been reported to spend between 25 and 42% of their time grazing, which is much higher than the animals in this study (Ben-Shahar & Fairall, 1987; Berry, Siegfried, & Crowe, 1982; Knight, 1991). The constant availability of high quality feed in captivity likely resulted in the wildebeest (which are bulk feeders), requiring less time to fill their digestive tracts (Ben-Shahar & Fairall, 1987; Estes, 1991). In addition, their energy requirements may have been more effectively met with the high quality feed being constantly available.

It was observed that tranquilised wildebeest spent more time lying with their heads folded back as well as standing with their heads down, indicating that these animals spent more time resting. Read, Caulkett, and McCallister (2000) reported that wapiti (*Cervus canadensis*) treated with zuclopenthixol acetate (Clopixol-Acuphase®) were also less active than untreated animals (Read *et al.*, 2000). Fick *et al.* (2007) and Fick *et al.* (2006) also reported a significant decrease in activity due to the administration of zuclopenthixol acetate (Clopixol-Acuphase®) in goats and blue wildebeest, respectively (Fick *et al.*, 2006, 2007). This difference between the treatments may also partly be related to the increased time spent eating after treatment since other authors have noted that increased grazing time in wildebeest may correspond to increased resting time, giving the animals adequate time to digest (Ben-Shahar & Fairall, 1987).

In addition to the increase in the time spent on resting behaviours, treatment with Acunil® also resulted in animals spending almost 50% less time being vigilant as well as 50%

less time exploring while walking around the enclosure. In wildebeest, the study observed that vigilant behaviour is characterized by an alert, upright posture with the head lifted and the ears moving. These decreases in alert and explorative behaviour may indicate an increased disinterest in their surroundings.

Lastly, animals groomed less while standing after the administration of Acunil®. According to Estes (1991), this behaviour forms part of displacement activities, which are maintenance activities performed under stressful situations. In addition, there was a significant decrease in head shaking in treated animals. Head shaking was observed in this study to occur in response to stressful stimuli such as fighting, aggravation by another animal or the presence of a person in the enclosure during stimulations.

Heart rate was unaffected by the administration of Acunil®. Read *et al.* (2000) also reported no significant changes in heart rates between wapiti treated with zuclopenthixol acetate (Clopixol Acuphase®) and untreated wapiti. However, unlike the current investigation, these authors also reported no significant change in mean respiration rates due to tranquilisation (Read *et al.*, 2000).

In contrast to the current investigation, Diverio, Goddard, and Gordon (1996) reported that red deer (*Cervus elaphus*) treated with zuclopenthixol acetate (Clopixol Acuphase®) showed a greater increase in heart rate when a stressor was applied than untreated animals. They attributed this to reflex tachycardia, which has been noted in other studies after treatment with a LAN (Diverio, Goddard, Gordon, & Elston, 1993; Marco, Lavín, Mentaberre, López-Olvera, & Casas-Díaz, 2010; Mentaberre, López-Olvera, Casas-Díaz, Bach-Raich *et al.*, 2010; Mentaberre, López-Olvera, Casas-Díaz, Fernández-Sirera *et al.*, 2010; Montané, Marco, & Manteca, 2002). Reflex tachycardia is due to a lowered peripheral resistance resulting in hypotension as a result of peripheral α-adrenergic blockade (Diverio *et al.*, 1996; Walsh & Wilson, 2002). Thisreflex tachycardia may explain the lack of significant differences ($P > 0.05$) in mean heart rates, both during periods of stimulation and no stimulation, as well as overall in the current study. Other authors have reported similar findings with the use of a variety of tranquilisers in wildlife (Diverio, Goddard, Gordon, & Elston, 1993; Marco, Lavín, Mentaberre, López-Olvera, & Casas-Díaz, 2010; Mentaberre, López-Olvera, Casas-Díaz, Bach-Raich *et al.*, 2010; Mentaberre, López-Olvera, Casas-Díaz, Fernández-Sirera *et al.*, 2010; Montané, Marco, & Manteca, 2002).

The increase in heart rate during periods of stimulation was expected to occur when an increase in motor activity and the stress evoked during such periods, will result inan

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increase in heart rate (Bonacic, Feber, & Macdonald, 2006; Price & Sibly, 1993; Waran & Cuddeford, 1995). The increase in mean respiration rate during periods of stimulation, when animals were most stressed, is also expected to occur. Reefmann, Bütikofer Kaszàs, Wechsler, and Gygax (2009) reported that situations that evoke negative emotional responses resulted in an elevation of respiration rate in sheep.

Although treatment with Acunil® had no effect on heart rate, it did result in a lowered (P = 0.02) overall mean respiration rate, which may be attributable to the sedative effect of Acunil®. Mean heart and respiration rates were similar to those reported for blue wildebeest and other African ungulate species, although no published data is available for the heart rate of untreated animals (Ancrenaz, Ostrowski, Anagariyah, & Delhomme, 1996; Chittick, Horne, Wolfe, Sladky, & Loomis, 2001; Citino, Bush, Grobler, & Lance, 2002; Dittberner, 2011; Howard, Kearns, Clippinger, Larsen, & Morris, 2004; Meyer, Hetem, Fick, Matthee, Mitchell, & Fuller, 2008; Mortola & Lanthier, 2005).

The behaviours that were associated with the highest mean heart and respiration rates are behaviour that occured in response to a stressor or were behaviours which could induce stress. It was therefore expected that these behaviours coincide with elevations in heart rate and respiration rate (Barnett & Hemsworth, 1990). The findings are also consistent with the fact that the four behaviours with the lowest mean heart and respiration rates were all non-alert, resting behaviours. Price and Sibly (1993) reported similar results in farmed red deer where the mean heart rates for the behaviours trotting and walking were 120 bpm and 108 bpm, respectively. These authors found that the behaviours lying as well as lying and ruminating, had the lowest mean heart rates at 56 bpm and 58 bpm, respectively and noted that this is due to the increased energy needs during locomotor activities (Price & Sibly, 1993). Other authors reported similar results for a number of behaviours in various domestic species (Baldock, Sibly, & Penning, 1988; Jansen *et al.*, 2009; Macarthur, Johnston, & Geist, 1979).

Treated animals had lower respiration rates when they were trotting, being vigilant, standing with their heads down and standing with their heads up, ruminating. While the latter two behaviours are non-alert behaviours, trotting and being vigilant are considered alarm behaviours; thus, indicating the subdued state of treated animals.

In terms of motion, it was expected that moving fast would result in the highest mean heart and respiration rates since increased motor activity can result in increased respiratory and cardiovascular output (Price & Sibly, 1993). It was also not surprising that animals spent more time moving fast during periods of stimulation as compared to

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periods of no stimulation since stimulations would initiate the 'fight-or-flight' response of the animals (Uetake, Morita, Hoshiba, & Tanaka, 2002). It is interesting to note that tranquilisation caused a decrease in the time spent moving fast, and an increase in the time spent being stationary during periods of stimulation. This, together with the fact that tranquilised animals spent the same amount of time being stationary and moving slowly regardless of whether they were being stimulated, indicates that the tranquiliser was affecting the animals' flight response to stimulation so that tranquilised animals moved less when stimulated.

Treated animals had lower respiration rates at times when they were stationary compared to when they were not tranquilised. Similar results were reflected in the analysis of respiration rate per behaviour with tranquilisation causing a lowered respiration rate for certain stationary behaviours. This is likely the result of the palliative effect of tranquilisation, specifically when animals were least active.

8.5 CONCLUSIONS

The use of Acunil® was found to be effective in minimising stressor-induced behaviour as well as increasing resting behaviours. In addition, treated animals spent more time eating than when they were untreated. No deleterious effects due to administration of Acunil® were observed, and all heart and respiration rates measured fell within acceptable ranges. Although treatment with Acunil® had no significant effect on overall mean heart rate, it caused a significantly lower overall mean respiration rate in the animals. The lack of effect on heart rate may be related to reflex tachycardia in response to hypotension induced by the administration of the tranquiliser as previously mentioned (see Discussion). Treatment also caused a lower mean respiration rate during periods of stimulation compared to when animals were untreated. In addition, treatment with Acunil® resulted in a lower respiration rate for the stress-induced behaviours (being vigilant and trotting) as well as the stationary behaviours, standing with head down and standing with head up, ruminating. As indicated by the motion results measured with the biotelemetry system, the flight response of the animals was also reduced after the administration of Acunil®. Overall, Acunil® produced a suitable palliative effect in the animals with no observable side-effects. The drug shows potential for use in this species, and future investigations should focus on its use in other species and species in non-captive environments.

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CHAPTER 9

Comparing the effect of treatment with Acunil® or Clopixol Acuphase® with that of a placebo on blue wildebeest *(Connochaetes taurinus)* behaviour and physiology

ABSTRACT

Treatment with a placebo, Acunil® or Acuphase® on blue wildebeest in captivity was compared. Animals (n = 12) were observed for 12 hours prior to treatment with a placebo (pre-treatment) as well as for 12 hours after treatment (post-treatment). Animals were stimulated every two hours by a person entering the enclosure (referred to as 'periods of stimulation'). Behaviour, continuous heart rate, respiration rate and motion were recorded throughout. Placebo-treated animals spent more time ($P < 0.001$) lying with their heads up and less time being vigilant ($P < 0.001$) than untreated animals. Acuphase® caused similar results, with Acunil®-treated animals spending more time ($P < 0.05$) lying with their heads folded back and eating than untreated animals. Heart rate was unaffected by all three treatments. Respiration rate was unaffected by treatment with the placebo, with the exception of a decrease ($P = 0.003$) when animals were walking and an increase ($P = 0.001$) when animals were lying with their heads up. Acuphase® and Acunil® lowered respiration rate during a number of different behaviours ($P < 0.05$). Motion was affected in different ways by all three treatments but indicated that Acuphase® and Acunil® caused animals to spend less time moving fast during periods of stimulation.

Keywords: blue wildebeest, captivity, behaviour, heart rate, respiration rate, motion, placebo

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9.1 INTRODUCTION

Fear is an emotion consisting of a species-specific pattern of physiological and behavioural changes (Leiner & Fendt, 2011). Certain species are more susceptible than others to sustained environmental stress, which result in more intense behavioural and physiological changes and a decreased threshold for inducing such changes (Leiner & Fendt, 2011). Repeated stressors of low intensity, such as novel housing environments, have been found to lead to a decreased stress response intensity eventually. Therefore, animals might adapt to long-acting or repeated stressors that are presumed to be stressful, and may in fact not respond at all with alterations in the various intrinsic stress response systems (Schrader & Ladewig, 1999; Von Borell, 2001). According to Knight and Cole (1991), there are three learned responses that wildlife may have to human disturbances, namely avoidance, attraction and habituation. Habituation is defined as the weakening of a response to a repeated stimulus that is not associated with either a positive or negative reward. Although there is abundant anecdotal evidence that animals do habituate to humans, little experimental data on wildlife populations exist (Knight & Gutzwiller, 1995). Espmark and Langvatn (1985) measured cardiac responses in red deer calves and observed that animals became habituated to repeated stimulations so that there was a weakening of the bradycardia response, and a decrease in the frequency of startle responses. However, no other published studies could be found that elaborate on this and what the extensive effects of habituation are on the stress response of wildlife in captivity.

Blue wildebeest (*Connochaetes taurinus*) is a game species, that due to its value in hunting, tourism and meat production activities, is commonly traded and translocated across South Africa (Higginbottom & King, 2006). Blue wildebeest therefore are more often held in captivity for reasons such as game auctions, disease testing and treatment than other game species (Dittberner, 2011). It is common for such animals to be treated with long-acting neuroleptics (LANs) in order to minimise stress during captivity and prevent injury as well as losses due to stress-related mortality (Fick, Matthee, Mitchell, & Fuller, 2006). Since there is little information available regarding the use of LANs in this species, blue wildebeest are an ideal candidate species for the testing of LANs with potential results providing valuable information regarding the management and care of this species.

The aim of the current study was to establish what the baseline stress responses are for blue wildebeest in captivity by measuring a variety of parameters, namely behavioural

and physiological responses before and after treatment with a placebo. In doing so, it could be ascertained whether significant changes in these parameters could be brought about by the administration of an LAN, i.e. either Acunil® or Clopixol Acuphase®.[1] Treatment with a placebo would allow verification of whether changes that occurred after the administration of a neuroleptic were due to the sedative effects of the neuroleptic or whether they were merely due to the habituation of these animals. To our knowledge, no such placebo study has been performed previously.

9.2 MATERIALS AND METHODS

Ethical approval for all aspects of the trial was received from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa (Protocol Ethical Approval number SU-ACUM11-00005). Two qualified veterinarians handled and administered all the drugs used and also consistently monitored the animals during anaesthesia.

9.2.1 Animals

For this study, the same materials and methods were used as outlined in the materials and methods section of the dissertation (see Chapter 4). Two trials were conducted using a placebo. The weights and genders of the animals used in this investigation are presented in Table 9.1.

[1] Acunil® is zuclopenthixol acetate dissolved in a slow-release polymer while Clopixol Acuphase® is zuclopenthixol acetate dissolved in vegetable oil. Both solvents allow for the slow release of the active drug component from the intramuscular site of administration but produce different release curves. In order to avoid confusion, only the trade names will be used when referring to these drugs.

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Table 9.1 The animal numbers, genders and weights within each trial conducted using a placebo

Trial		Animal	Gender	Weight (kg)
Trial 1	August	1	Female	164
		2	Male	144
		3	Female	158
		4	Female	192
		5	Male	142
		6	Male	112
Trial 2	October	1	Male	227
		2	Female	130
		3	Male	123
		4	Female	167
		5	Female	90
		6	Male	81
TOTAL		12	6 Females 6 Males	

9.2.2 Study design

Two identical trials were conducted as described in Chapters 7 and 8 of this dissertation. This allowed for a comparison of the results with those from the previous trials. A placebo was administered on Day 2 of each trial instead of an LAN. As before, Day 1 of the trial was referred to as pre-treatment (PreT) and consisted of a 24-hour period in which animals received no treatment. Only the first 12-hour period of this period was used for analysis of behaviour and biotelemetry data. Post-treatment (PostT) occurred immediately after this 24-hour period. During this time, animals were administered a placebo intramuscularly consisting of sterile water, at a dose of 1.0–3.0 ml. Again, this period lasted for 24 hours, of which only the first 12 hours were used for analysis of behaviour and biotelemetry data. During each treatment, animals were stimulated for one minute every 2 hours by a person walking into the enclosure and making a noise.

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The purpose of these trials was to establish the validity of any behavioural and/or physiological changes that may have resulted from the administration of Acunil® or Acuphase® in previous trials. As such, results are discussed in the context of drawing a comparison with previous results, which are repeated where appropriate.

9.2.3 Statistical analysis

The data was analysed using a restricted maximum likelihood estimation (REML), which included treatment, gender, animal weight and trial (1 and 2) as fixed effects. For the analysis of vital sign data with behaviour data, behaviour and stimulation were included as fixed effects. Behaviour within periods of stimulation could not be analysed since not all behaviours occurred within a period of stimulation. However, changes in biotelemetry data were analysed within stimulations. Data analysis was performed using the Variance Estimation and Precision module of Statistica (version 12) statistical software (StatSoft Inc., 2013). All results were considered significant if $P < 0.05$, and are expressed as least squared means (LSMeans) ± standard error of the mean (SEM). In order to minimise the effect of outliers in the data, the medians for each vital sign per animal were used for statistical analysis. Means for each group were calculated from these medians.

Means between trials performed with Acunil®, Acuphase® and the placebo were not compared statistically. The purpose of the current study was to establish whether statistically significant changes occurred in the absence of a tranquiliser and not to which extent these changes occurred, since each group of animals was expected to react differently to captivity and to human disturbances (as had been noted in Chapters 7 and 8). The administration of the placebo served to validate whether changes observed after the administration of Acunil® or Acuphase® were due exclusively to tranquilisation and not partly due to habituation of the animals to captivity and human disturbances.

9.3 RESULTS

The results obtained after treatment with the placebo are reported as part of a comparison with those reported in Chapters 7 and 8 of this dissertation. As a result, there is some repetition between this chapter and previous chapters as some results from previous chapters are reported again where applicable.

9.3.1 Behaviour

Changes in behaviour after the administration of the placebo are indicated in Table 9.2. From the results in Chapters 7 and 8, it is evident that animals treated with Acuphase® and Acunil® also spent less time being vigilant (Acuphase®: PreT = 26.2 ± 2.77% vs. PostT = 17.2 ± 1.69%; Acunil®: PreT = 25.3 ± 2.23% vs. PostT = 13.9 ± 1.55%) as was observed when animals were treated with a placebo. In addition, as with placebo-treated animals, Acuphase®-treated animals spent more time ($P < 0.001$) lying with their heads up (PreT = 24.0 ± 4.43% vs. PostT = 34.1 ± 3.31%). However, Acunil®-treated animals spent more time lying with their heads folded back ($P = 0.003$; PreT = 2.7 ± 0.45% vs. PostT = 5.6 ± 1.03%) and eating ($P < 0.001$: PreT = 12.1 ± 1.57% vs. PostT = 15.9 ± 1.40%) while also spending less time exploring while walking around ($P = 0.005$; PreT = 1.2 ± 0.32% vs. PostT = 0.6 ± 0.14%). In addition, Acuphase®-treated animals spent less time standing with their heads up, ruminating ($P < 0.001$; PreT = 21.3 ± 3.00% vs. PostT = 18.5 ± 2.53%). These changes in behaviour were not observed when animals were treated with the placebo (Table 9.2).

Table 9.2 The time spent (LSMean ± SEM) per behaviour by blue wildebeest within a treatment (pre-treatment [PreT] and post-treatment [PostT]) in descending order, expressed as a percentage

Main behaviour	PreT	PostT
Being vigilant	$42.7^{a} \pm 2.62$	$29.0^{b} \pm 3.36$
Lying with head up	$14.0^{a} \pm 2.46$	$24.5^{b} \pm 2.20$
Standing with head up, ruminating	$16.4^{a} \pm 2.55$	$19.1^{a} \pm 3.88$
Eating	$14.4^{a} \pm 2.21$	$16.4^{a} \pm 2.53$
Walking	$7.4^{a} \pm 0.78$	$4.9^{a} \pm 0.67$
Lying with head folded back	$1.0^{a} \pm 0.24$	$2.5^{a} \pm 0.77$
Standing with head down	$1.3^{a} \pm 0.76$	$0.5^{a} \pm 0.44$
Fighting	$0.9^{a} \pm 0.25$	$0.8^{a} \pm 0.24$
Exploring (walking and sniffing surface)	$0.9^{a} \pm 0.14$	$0.6^{a} \pm 0.16$
Defending	$0.7^{a} \pm 0.20$	$0.7^{a} \pm 0.17$

Main behaviour	PreT	PostT
Exploring (standing and sniffing surface)	0.5[a] ± 0.11	0.5[a] ± 0.11
Being rubbed by another animal	0.2[a] ± 0.08	0.3[a] ± 0.07
Rubbing against another animal	0.2[a] ± 0.05	0.3[a] ± 0.11
Drinking	0.2[a] ± 0.03	0.2[a] ± 0.03
Other	0.2[a] ± 0.04	0.2[a] ± 0.09
Trotting	0.2[a] ± 0.01	0.1[a] ± 0.01

[ab] Means within a behaviour and between treatments with the same letter do not differ significantly (P > 0.05)

Two point behaviours (see Chapter 4) were measured, namely grooming (sub-categorised into grooming while lying down and grooming while standing up) and agitation (sub-categorised into head shaking and feet stomping). The administration of the placebo had no effect (P > 0.05) on the frequency at which these behaviours occurred (Table 9.3). The same results were obtained after the administration of Acuphase® with no significant effects observed after tranquilisation. Acunil®-treated animals however, groomed more while standing than untreated animals (P = 0.003; PreT = 27.1 ± 4.25 vs PostT = 19.2 ± 4.14) and shook their heads less often (P = 0.001; PreT = 46.4 ± 3.30 vs. PostT = 27.7 ± 4.23).

Table 9.3 The frequency (LSMean ± SEM) at which each point behaviour occurred within a treatment (PreT and PostT)

Behaviour	Modifying behaviour	PreT	PostT
Grooming	Grooming while lying down	3.1[a] ± 1.13	5.5[a] ± 2.28
	Grooming while standing	28.7[a] ± 8.37	27.6[a] ± 7.44
Agitation	Head swaying or shaking	81.6[a] ± 12.31	65.9[a] ± 7.95
	Stomping feet	21.6[a] ± 6.08	20.3[a] ± 6.63

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9.3.2 Heart rate and respiration rate per behaviour

Mean heart rate per behaviour was unaffected by the administration of the placebo (Table 9.4). The same results were obtained after the administration of Acunil® and Acuphase®.

Table 9.4 The heart rates (bpm) (LSMean ± SEM) of blue wildebeest per behaviour between treatments (PreT and PostT) in descending order

Behaviour	PreT	PostT
Trotting	93.1 ± 6.56	84.3 ± 7.50
Walking	84.9 ± 6.10	77.5 ± 6.33
Fighting	82.4 ± 7.10	75.8 ± 6.87
Other	81.8 ± 7.96	79.1 ± 1.97
Being rubbed by another animal	79.6 ± 7.46	72.7 ± 7.41
Being vigilant	78.2 ± 6.06	75.8 ± 6.21
Defending	78.0 ± 6.76	73.8 ± 7.29
Drinking	77.0 ± 7.20	80.6 ± 7.41
Eating	76.9 ± 7.00	73.0 ± 7.22
Exploring (sniffing while standing)	73.3 ± 7.20	74.9 ± 7.22
Exploring (sniffing while walking)	72.5 ± 7.20	70.2 ± 7.12
Lying head folded back	71.9 ± 7.90	69.1 ± 8.33
Lying with head up	71.7 ± 7.00	67.4 ± 7.22
Standing with head down	71.1 ± 7.90	72.5 ± 8.88
Standing with head up, ruminating	65.5 ± 7.00	69.7 ± 6.93
Rubbing against another animal	65.2 ± 7.20	75.2 ± 7.50

Respiration rate was lowered ($P = 0.003$) after administration of the placebo while animals were walking. However, respiration rate was also higher ($P = 0.01$) while animals were lying down, with their heads up (Table 9.5). This is in contrast to the results obtained for animals treated with Acunil® and Acuphase®. In the Acunil® trials, respiration rate decreased after treatment ($P < 0.05$) when animals were trotting (PreT = 19.1 ± 1.04 breaths/minute vs. PostT = 16.5 ± 1.12 breaths/minute), being vigilant (PreT = 15.1 ±

0.98 breaths/minute vs. PostT = 12.8 ± 1.00 breaths/minute), standing with their heads down (PreT = 13.9 ± 1.24 breaths/minute vs. PostT = 10.7 ± 1.27 breaths/minute) and standing with their heads up, ruminating (PreT = 13.7 ± 1.23 breaths/minute vs. PostT = 10.8 ± 1.11 breaths/minute). Acuphase®-treated animals also showed lower ($P < 0.05$) respiration rates when animals were fighting (PreT = 27.7 ± 1.44 breaths/minute vs. PostT = 18.1 ± 1.29 breaths/minute), defending (PreT = 23.2 ± 1.47 breaths/minute vs. PostT = 19.0 ± 1.34 breaths/minute), being vigilant (PreT = 19.0 ± 1.14 breaths/minute vs. PostT = 14.2 ± 1.17 breaths/minute), standing with their heads down (PreT = 17.9 ± 1.74 breaths/minute vs. PostT = 12.3 ± 1.43 breaths/minute) or performing “other” behaviours (PreT = 26.5 ± 1.68 breaths/minute vs. PostT = 19.3 ± 1.45 breaths/minute).

Table 9.5 The respiration rates (breaths/minute) (LSMean ± SEM) of blue wildebeest per behaviour between treatments (PreT and PostT) in descending order

Behaviour	PreT	PostT
Trotting	$17.8^{a} \pm 1.21$	$16.3^{a} \pm 1.39$
Walking	$17.8^{a} \pm 1.13$	$14.4^{b} \pm 1.17$
Eating	$16.9^{a} \pm 1.29$	$14.5^{a} \pm 1.34$
Fighting with other animal	$16.6^{a} \pm 1.32$	$14.6^{a} \pm 1.28$
Defending from other animal	$16.5^{a} \pm 1.25$	$13.9^{a} \pm 1.35$
Other	$16.4^{a} \pm 1.05$	$15.2^{a} \pm 1.05$
Being vigilant	$16.3^{a} \pm 1.12$	$14.7^{a} \pm 1.15$
Exploring (sniffing while walking)	$15.9^{a} \pm 1.33$	$13.7^{a} \pm 1.32$
Drinking	$14.9^{a} \pm 1.33$	$13.7^{a} \pm 1.37$
Rubbing against other animal	$14.9^{a} \pm 1.33$	$14.3^{a} \pm 1.39$
Exploring (sniffing while standing)	$14.3^{a} \pm 1.33$	$15.9^{a} \pm 1.34$
Standing head down	$13.7^{a} \pm 1.47$	$12.3^{a} \pm 1.66$
Being rubbed by other animal	$13.1^{a} \pm 1.38$	$12.1^{a} \pm 1.37$
Standing head up ruminating	$13.1^{a} \pm 1.29$	$12.3^{a} \pm 1.28$
Lying head up	$9.9^{a} \pm 1.29$	$13.5^{b} \pm 1.34$
Lying head down	$9.6^{a} \pm 1.55$	$11.4^{a} \pm 1.55$

[ab] Means within a behaviour and between treatments with the same letter do not differ significantly (P > 0.05)

9.3.3 Motion

The motion of each animal was measured every 15 seconds by the Equivital™ belt in terms of the categories 'stationary', 'moving slowly' and 'moving fast'. The frequency at which each motion was measured changed (P < 0.05) after the placebo was administered. Animals spent less time moving slowly (P = 0.005) and more time (P = 0.002) being stationary (Table 9.6). Contrasting results were found when Acuphase® was administered and treated animals spent the same amount of time being stationary (PreT = 83.7 ± 1.74% vs. PostT = 87.0 ± 1.89%), moving slowly (PreT = 13.4 ± 1.44% vs. PostT = 11.5 ± 1.56%) and moving fast (PreT = 2.9 ± 0.62% vs. PostT = 1.5 ± 0.67%) as untreated animals. Treatment with Acunil®, on the other hand, resulted in animals spending more time being stationary (P = 0.02; PreT = 75.1 ± 1.70% vs. PostT = 82.8 ± 1.70%) and less time moving fast (P = 0.001; PreT = 10.2 ± 1.72% vs. PostT = 4.8 ± 1.80%).

Table 9.6 The frequency at which each motion was measured (LSMean ± SEM) between PreT and PostT, expressed as a percentage of total motion measured within a treatment

Motion	PreT	PostT
Stationary	75.8[a] ± 1.98	86.8[b] ± 2.08
Moving slowly	15.7[a] ± 1.69	10.7[b] ± 1.78
Moving fast	3.5[a] ± 0.58	2.5[a] ± 0.61

[ab] Means within a motion category and between treatments with the same letter do no differ significantly (P > 0.05)

Within a stimulation period, motion also differed (P < 0.05) between treatments (Table 9.7). Both placebo-treated and Acunil®-treated animals spent more time being stationary during periods when they were stimulated compared to when they were untreated. Untreated animals in the Acunil® trials spent 63.0 ± 2.43% of their time being stationary when they were stimulated compared to 78.8 ± 2.70% after they had been treated with Acunil®. However, animals in the Acunil® trials spent the same amount of time moving slowly (P = 0.28) regardless of whether they had been stimulated (15.0 ± 1.24%) or not

(11.9 ± 1.62%) after being tranquilised. In addition, Acunil®-treated animals spent less time moving fast when they were stimulated (P = 0.002; 8.4 ± 1.74%) compared to when they were untreated (19.2 ± 2.94%). When animals were treated with Acuphase®, they also spent less time moving fast during periods when they had been stimulated (P = 0.004; PreT = 5.1 ± 1.14% vs. PostT = 2.6 ± 0.91%) but there were no differences (P > 0.05) in the amount of time they spent stationary or moving slowly during periods of stimulation compared to untreated animals.

Table 9.7 The frequency at which each motion was measured (LSMean ± SEM) in blue wildebeest within periods of stimulation (S) and periods of no stimulation (NS) between PreT and PostT, expressed as a percentage of total motion measured within a period

Treatment	Period	Stationary	Moving slowly	Moving fast
PreT	NS	86.8^{a} ± 0.73	12.2^{a} ± 0.69	1.0^{a} ± 0.10
	S	74.8^{b} ± 4.59	19.2^{b} ± 3.42	6.0^{b} ± 1.61
PostT	NS	90.9^{a} ± 1.53	8.4^{a} ± 1.35	0.6^{a} ± 0.21
	S	82.7^{a} ± 3.98	13.0^{b} ± 3.74	4.4^{b} ± 0.43

[ab] Means within a motion category and between treatments and stimulation periods with the same letter do not differ significantly (P > 0.05)

As with Acunil® and Acuphase®, heart rate within a motion category did not change (P > 0.05) after placebo treatment. However, there were also no changes (P > 0.05) in respiration rate. In contrast, the administration of Acunil® caused respiration rate to be lower while animals were being stationary (P = 0.04; PreT = 12.3 ± 1.29 breaths/minute vs. PostT = 10.3 ± 1.31 breaths/minute). After the administration of Acuphase®, respiration rate was also lower while animals were being stationary (P = 0.03; PreT = 13.8 ± 1.06 breaths/minute vs. PostT = 10.7 ± 1.08 breaths/minute) as well as while they were moving fast (P = 0.02; PreT = 20.9 ± 1.07 breaths/minute vs. PostT = 17.3 ± 1.19 breaths/minute).

9.3.4 Heart rate and respiration rate between periods of stimulation and periods of no stimulation

The administration of the placebo had no effect (P > 0.05) on overall heart rate (PreT: 84.48 ± 6.28 bpm; PostT: 86.57 ± 6.45 bpm) or respiration rate (PreT: 16.42 ± 1.04

breaths/minute; PostT: 16.75 ± 1.08 breaths/minute). Treatment with Acunil® and Acuphase® also had no effect ($P > 0.05$) on overall heart rate but did, however, result in a lowered ($P < 0.05$) overall respiration rate. Prior to tranquilisation, animals in the Acunil® trials showed a mean respiration rate of 14.5 ± 0.82 breaths/minute compared to when they were tranquilised (12.5 ± 0.83 breaths/minute). Animals in the Acuphase® trials showed mean respiration rates of 17.8 ± 0.96 breaths/minute and 14.6 ± 0.99 breaths/minute before and after being administered Acuphase®, respectively.

In the control trials, heart and respiration rates increased ($P < 0.05$) when animals were stimulated but did not differ ($P > 0.05$) within stimulation periods between treatments (Table 9.8). Similar results were reported for heart rate after the administration of Acunil® and Acuphase®. However, animals treated with both Acunil® and Acuphase® showed lower ($P < 0.05$) respiration rates during periods of stimulation (Acunil® = 13.7 ± 0.87 breaths/minute; Acuphase® = 16.3 ± 1.10 breaths/minute) compared to when they were not treated (Acunil® = 16.2 ± 0.87 breaths/minute; Acuphase® = 20.7 ± 1.15 breaths/minute).

Table 9.8 The heart rate (bpm) and respiration rate (breaths/minute) (LSMean ±SEM) of blue wildebeest between treatments (PreT and PostT) and between periods where animals were stimulated (S) and not stimulated (NS)

Treatment	Period	Heart rate	Respiration rate
PreT	NS	73.2^a ± 1.76	14.7^a ± 0.39
	S	96.6^b ± 6.27	19.1^b ± 0.87
PostT	NS	72.6^a ± 1.84	13.4^a ± 0.41
	S	81.4^b ± 4.06	17.3^b ± 0.95

[ab] Heart rate or respiration rate means between periods and treatments with the same letter do not differ significantly ($P > 0.05$)

As with the Acunil® and Acuphase® trials, there was a weak but significant correlation between heart rate and respiration rate ($R^2 = 0.53$; $P < 0.05$) observed with the placebo treatment.

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9.3.5 Skin temperature

As with the Acuphase® and Acunil® trials, skin temperature was unaffected by behaviour, treatment or stimulation. Again, skin temperature was found to be weakly correlated with environmental temperature ($R^2 = 0.44$; $P < 0.05$) although no difference ($P > 0.05$) was found between trials as with previous results.

9.4 DISCUSSION

The results indicated that even though animals were treated with a placebo, there were still significant changes in behaviour and biotelemetry measurements. The most notable are the changes in behaviour since these changes partly echo the results found with Acunil® and Acuphase®. The changes are likely related to acclimatisation of animals to captivity and to interactions with humans so that this, to some extent, masks the actual effect of tranquilisation.

After all three treatments, animals spent less time being vigilant, which indicates that the decrease in vigilance is more likely due to habituation than to the administration of the tranquiliser. In wildebeest, observation of this type of behaviour was associated with an alert, upright posture with the head lifted and the ears moving. According to Lind (2010), vigilant behaviour is related to the gathering of information about an animal's environment, and the animal's response in increasing or decreasing being vigilant. It is likely that after the first day of the trials, animals became accustomed to their surroundings and to the human stimulations so that on the second day, they were less reactive.

As with Acuphase®-treated animals, placebo-treated animals spent more time lying with their heads up compared to before being treated. However when animals were treated with Acunil®, they spent more time lying with their head folded back compared to before being treated. It was observed that while animals were lying with their heads up, they could still ruminate and groom so that even though they were resting, they were still awake and responsive. This was not true when animals folded their heads back and closed their eyes. Treatment with Acunil® therefore resulted in animals spending more time asleep and unresponsive to stimuli compared to before being treated. Treatment with Acunil® also resulted in animals eating more and spending more time with their heads folded back than prior to treatment. Both these behaviours are non-alert behaviours and were unaffected when animals were administered the placebo.

Treatment with Acuphase® on the other hand, resulted in animals spending less time standing with their heads up and ruminating, than before the treatment was administered.

None of the treatments resulted in any changes in heart rate per behaviour. Respiration rate was lower ($P = 0.003$) when animals were walking after they had been given the placebo compared to before. However, respiration was also higher when they were lying down with their heads up ($P = 0.001$) after treatment compared to before. This result is interesting since animals in the placebo group spent more time lying with their heads up after receiving the placebo. Therefore, although the amount of time spent on this behaviour may have increased, respiration rates indicate that animals were still reactive during this behaviour. In contrast, animals treated with Acunil® and Acuphase® showed lowered respiration rates for a number of different behaviours compared to before receiving treatment and neither treatment resulted in increases in respiration. Brosh (2007) reviewed the link between oxygen consumption, heart rate and energy expenditure, and noted that energy expenditure is strongly correlated with both heart rate and oxygen consumption. The results observed in the current research when animals were treated with Acunil® and with Acuphase®, indicated that tranquilised animals may have used less energy whilst performing certain behaviour than they were treated with the placebo.

Heart rate was unaffected by the administration of the placebo. The same results were obtained when animals were treated with either Acunil® or Acuphase®. However, a number of authors have reported that treatment with an LAN may result in reflex tachycardia in response to hypotension so that a treated animal's heart rate may remain unchanged or even become elevated compared to when it was untreated (Diverio, Goddard, & Gordon, 1996; Marco, Lavín, Mentaberre, Lopez-Olvera, & Casas-Díaz, 2010). As a result, the lack of effect on heart rate by both neuroleptics may not be a good representation of Acuphase® or Acunil®'s sedative effect on the animals.

Respiration rate also remained unchanged after the animals had received the placebo. Acunil® and Acuphase®, on the other hand, had a pronounced effect on respiration. Both neuroleptics resulted in a lowered ($P < 0.05$) overall respiration rates and a lowered ($P < 0.05$) respiration rate during periods when animals were stimulated. These results suggest that the effect on respiration was the result of the administration of the drugs and not due to the habituation of the animals.

Motion measured in the animals was affected ($P < 0.05$) after the animals had received the placebo. Animals spent more time ($P = 0.002$) being stationary and less time moving

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slowly (P = 0.005) following administration of the placebo. In addition, placebo-treated animals spent more time being stationary (P = 0.001) when they were stimulated compared to before administration of the placebo. As with the behavioural results, these changes may in part be due to the habituation of the animals to their captive environment. In addition, on the second day of captivity and after 12 hours of stimulation by human interference, animals may have become exhausted so that they spent more time performing less active, less energy-consuming activities than on the first day. When animals were treated with Acuphase®, motion remained unaffected, and when animals were treated with Acunil®, they spent less time moving fast (P = 0.02) and more time being stationary (P = 0.001) than before treatment. However, both Acunil® and Acuphase®-treated animals showed a reduction in the percentage time they spent moving slowly or moving fast during periods when they were stimulated, suggesting a reduction in flight response. Overall, the results suggest that motion on its own is not a ideal indicator of the sedative effect of a tranquiliser, and that assessment of the sedation effect requires evaluation in combination with other parameters such as behaviour, heart rate and respiration rate. This is further substantiated by the fact that the placebo produced no effect (P > 0.05) on respiration rate per motion category but that a decrease was seen when animals were treated with both Acunil® and Acuphase®.

In a study performed in horses, Leiner and Fendt (2011) noted two important results. The authors reported that behavioural and physiological responses (respiration and heart rate) correlated well during fearful behavioural responses to novel objects. In addition, the authors reported that these measurable parameters were diminished when animals became habituated to the novel objects that initially had elicited a fear response. The correlations noted between behavioural and physiological responses by Leiner and Fendt (2011) were corroborated in the current study, where increases in heart rate and respiration rate correlated well with increases in fearful behaviours and stimuli. The findings regarding habituation by Leiner and Fendt (2011) may explain why certain results obtained in the current study after treatment with the placebo were similar to those obtained with both tranquilisers.

Similar findings were reported by Waynert, Stookey, Watts, and Waltz (1999), where the authors found that habituation in cattle to their treatment protocol resulted in a gradual decrease in fearful responses over time. Schrader and Ladewig (1999) reviewed the stress response of pigs to repeated exposure to stressors. These authors interestingly noted that repetitive exposure to a stressor resulted in a continuous decline in hormonal responses, namely cortisol and ACTH, whilst heart rate, adrenaline and behavioural

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responses remained constant. These results are in contrast to the current study where changes in behaviour were observed even in the absence of a tranquiliser, suggesting a diminished stress response to repeated exposure to a stressor. However, as Schrader and Ladewig (1999) also concluded, the results of the current study show that different stress response systems can differ in their temporal patterns of response towards a repeated stressor. This is evidenced by the fact that respiration rate responses remained constant when a placebo was given. The results again indicate that it is important that the assessment of stress be based on a wide range of variables describing the entire process (Jensen *et al.*, 1996).

9.5 CONCLUSIONS

The results obtained after the administration of a placebo indicate that some of the changes in behaviour observed after the administration of both Acuphase® and Acunil® are likely partially due to the habituation and desensitisation of the animals to captivity and human disturbances. However, biotelemetry results suggest that changes in respiration rate and motion, specifically during periods when animals were stimulated, were exclusively due to the administration of the tranquilisers. Overall, the results indicate that, although certain stress assessment parameters are indeed affected by habituation of the animals, others are not, so that the assessment of more than one response is required in order to draw adequate conclusions regarding the effect of tranquilisation on the stress response of animals.

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CHAPTER 10

The effects of treatment with Acunil®, Clopixol Acuphase® or a placebo on blue wildebeest *(Connochaetes taurinus)* blood and faecal parameters

ABSTRACT

The effect of treatment with Acuphase®, Acunil® or a placebo on blue wildebeest blood and faecal parameters was investigated. Animals were anaesthetised on three consecutive days and blood and faecal samples were collected during each anaesthetization. Animals were treated with Acuphase®, Acunil® or a placebo on Day 2. Plasma cortisol remained unchanged over the three days in the Acuphase® group but decreased on Days 2 and 3 in the Acunil® and control groups. Faecal glucocorticoid metabolites remained unchanged over the three days in the Acuphase® and Acunil® groups and differed between trials within the control group. Blood lactate and glucose decreased from Day 1 to Day 3 in the Acuphase® and Acunil® groups. Blood glucose levels remained stable, whereas lactate levels increased from Day 1 to Day 3 in the control group. Neutrophil function was measured over time, and the area under the curve (AUC) calculated. The mean AUC remained unchanged in both the Acuphase® and control groups, and decreased from Day 1 to Days 2 and 3 in the Acunil® group. White blood cell count (WBC) did not change within any of the treatments. Overall, Acunil® and Acuphase® had little effect on the endocrine and immune responses within the first 24 hours after their administration.

Keywords: long-acting neuroleptic, endocrine, glucose, lactate, immune function, ungulates, wildlife

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10.1 INTRODUCTION

Long-acting neuroleptics (LANs) have been used for quite some time in South Africa to assist in the capture and relocation of wildlife (Fick, Matthee, Mitchell, & Fuller, 2006). Such neuroleptics provide numerous benefits related to the reduction of stress in animals during these processes, stemming from the long-term sedation they provide (Fick, Mitchell, & Fuller, 2007; Read, 2002). The effect of the drug is extended through its esterification and suspension in a vegetable oil so that, when it is injected intramuscularly, the rate at which it takes effect, is dependent on the absorption rate of the oil from the muscle tissue into the blood (Huber, Walzer, & Slotta-Bachmayr, 2001).

Stress is a generalised term used to describe environmental factors that solicit adaptation mechanisms in response to factors (Mormède *et al.*, 2007). Vertebrates cope with stressors (i.e. unpredictable and noxious stimuli) by initiating a stress response, which involves an evolved suite of physiological, hormonal and behavioural responses (Romero, 2004). Although usually most practical, it is difficult to assess animal stress from a purely behavioural point of view. This is because, although displacement and other activities may be present, animals may react in unpredictable manners depending on factors such as life history stages and previous experiences (Hattingh, 1988).

The hormonal response to stress is made possible by two types of hormones, namely glucocorticoids and the catecholamines (Mormède *et al.*, 2007). Both types of hormones, depending on the duration of the stressor, elicit physiological effects that result in a cascade of changes in the body (Mills, 2007). Measurement of the levels of these hormones has been widely used in animal studies to evaluate the extent of the effect of a stressor (Minton, 1994). However, challenges in interpretation can occur as hormone levels may change rapidly in response to a stressor, and it is therefore often advised to qualify and quantify the resulting changes that occur in response to these hormones in order to establish the effect of the stressors (Johnstone, Reina, & Lill, 2012).

When an animal is confronted with a stressful stimulus, the sympathetic part of the autonomic nervous system is stimulated to secrete catecholamines from the adrenal medulla (Mormède *et al.*, 2007). If exposure to the stressor continues, the catecholamines facilitate the secretion of corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus, which in turn stimulates the secretion of adrenocorticotropin hormone (ACTH). Adrenocorticotropin hormone in turn induces the secretion of glucocorticoids from the adrenal cortex (Minton, 1994). While the catecholamines are short-acting and result in changes such as increased

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gluconeogenesis (whereby liver glycogen is converted into glucose), the action of the glucocorticoids (GCs) are longer in duration, which can result in immunosuppression and the suppression of less vital physiological processes such as reproduction (Johnstone *et al.*, 2012). As a result these changes, blood glucose and lactate levels may deviate from baseline values in response to stress, providing a quantitative assessment of stress in conjunction with the measurement of stress hormones (Hattingh, 1988). Measurements of immune function may also provide additional information about the long-term effect of stressors on an animal (Apanius, 1998).

The aim of this study was to evaluate and compare the effects of two LANs, Acunil® and Clopixol Acuphase®, in reducing animal stress. This was achieved through the measurement of a number of blood and faecal stress indices. The active drug ingredient in both Acunil® and Clopixol Acuphase® is zuclopenthixol acetate. However, Acunil® is zuclopenthixol acetate dissolved in a slow-release polymer while Clopixol Acuphase® is zuclopenthixol acetate dissolved in vegetable oil (Read, Caulkett, & McCallister, 2000; SR Veterinary Technologies, 2001). Both solvents allow for the slow release of the active drug component from the intramuscular site of injection but produce different release profiles (Aaes-Jørgensen, 1989; Clark, Clark, & Hoyt, 2014). Both drugs are classified as long-term neuroleptics and in order to avoid confusion, the trade name will be used when referring to each drug. A control study was also performed in which a group of animals were administered a placebo (control) during an identical protocol. The results from the control study are also compared with those obtained when Acunil® or Acuphase® was administered.

10.2 MATERIALS AND METHODS

The same materials and methods as outlined in the materials and methods section of the dissertation were used (Chapter 4). Three different treatments were investigated, namely treatment with Acuphase®, treatment with Acunil® and treatment with a placebo (control). Three trials were performed using Acuphase®, and three trials were performed using Acunil® (Table 10.1). Two further trials were performed in which animals were administered a placebo in the form of an intramuscular injection of sterile water (1.0–3.0 ml). Each trial within a treatment occurred over three consecutive days. Prior to the start of a trial, all animals were anaesthetised with thiafentanil oxalate (Thianil®, Wildlife Pharmaceutical SA (Pty) Ltd., Rocky's Drift, Mpumalanga, South Africa) at a dose of 3.5–4.5 mg in order to collect blood and rectal faecal samples and fit each animal with a

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biotelemetry belt (see Chapter 4). Anaesthesia was reversed with 35–45 mg naltrexone (Trexonil®, Wildlife Pharmaceutical SA (Pty) Ltd., Rocky's Drift, Mpumalanga, South Africa). After 24 hours, animals were again anaesthetised with thiafentanil and treated with Acuphase®, Acunil® or a placebo, depending on the treatment group, before the anaesthesia was reversed. The next day, animals were anaesthetised one last time before the trial was completed. Blood and rectal faecal samples were collected at each immobilisation for the determination of plasma cortisol, blood glucose and lactate, neutrophil function and faecal glucocorticoid metabolites.

Table 10.1 Blue wildebeest numbers and genders for each trial within a treatment

Trial	Tranquiliser administered	Number of animals	Genders
Trial 1	Clopixol Acuphase®	6	6♀
Trial 2		6	3♀ : 3♂
Trial 3		5	3♀ : 2♂
Trial 1	Acunil®	6	3♀ : 3♂
Trial 2		5	3♀ : 2♂
Trial 3		6	2♀ : 4♂
Trial 1	Placebo (control)	6	3♀ : 3♂
Trial 2		6	3♀ : 3♂

Plasma cortisol was measured using a commercially available ^{125}I RIA kit (Coat-a-Count®, Siemens Healthcare Diagnostics, Los Angeles, Santa Ana, CA 90045, USA) designed for humans for the quantitative measurement of cortisol in serum, urine or heparinised plasma, although it has wide species applicability (Brown, Bellem, Fouraker, Wildt, & Roth, 2001; Meyer *et al.*, 2008; Nogueira & Silva, 1997). Plasma glucose and lactate levels (mmol/L) were measured using the AccuTrend Plus® hand analyser from Roche, South Africa. Neutrophil function was measured as per the method described by Kruger *et al.* (2010). The whole-blood chemi-luminescent (CL) assay has been shown to be a reliable and rapid method of assessing the production of reactive oxygen species (ROS) by circulating phagocytes, or more specifically neutrophils and thus gives a good indication of the extent of the immune response (Papp & Smits, 2007). Faecal glucocorticoid metabolites (FGMs) were assayed using a double-antibody ^{125}I-

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corticosterone radioimmunoassay kit (MP Biomedicals LLC, Santa Ana, CA 92707, USA). The assay was validated for use in blue wildebeest and the results are reported in Chapter 6 of the dissertation.

10.2.1 Study design

As with Chapter 9 of the dissertation, the purpose of the trials conducted using a placebo (control) was to establish the validity of any changes that may have resulted from the administration of Acunil® or Acuphase® in previous trials. As such, results will be discussed with the purpose of drawing a comparison between the results obtained within each of the treatments (Acuphase®, Acunil® or control).

10.2.2 Statistical analysis

Data was pooled for each treatment group (Acunil®, Acuphase® or control). The data was analysed using a restricted maximum likelihood estimation (REML), which included gender, animal weight, sampling day and trial as fixed effects. Data analysis was performed using the Variance Estimation and Precision module of Statistica (version 12) statistical software (StatSoft Inc., 2013). All results were considered significant if $P < 0.05$, and are expressed as least squared means (LSMeans) ± standard error of the mean (SEM).

Three response curves (Day 1 vs. Day 2 vs. Day 3) were produced for neutrophil function for each animal using the means of the triplicate readings from the chemi-luminescent (CL) assay as described by Kruger *et al.* (2010). On Day 1 and Day 2, animals received no treatment while on Day 3, all animals were administered either Acunil®, Acuphase® or a placebo, as described in Chapter 4 of the dissertation. The curves were transformed with a trend line, and the area under the curve (AUC) was calculated. The 3 AUCs for the animals were then compared statistically using Wilcoxon rank regression, with $P < 0.05$ considered statistically different (Kruger *et al.*, 2010).

Due to the limitation of animal and space availability as well as the amount of biotelemetry belts available, two to three trials were performed per treatment group (Table 10.1). As a result, the term ‘trial’ refers to the different trials within each treatment group while the term ‘treatment’ refers to the treatment administered to the animals (namely Acuphase®, Acunil® or a placebo).

Means between treatment groups (Acunil®, Acuphase® and the placebo) were not compared statistically. The purpose of the control study was to establish whether

statistically significant changes occurred in the absence of a tranquiliser and not to which extent these changes occurred. Each group of animals was expected to react differently to captivity and to human disturbances (especially on the first day of each trial), and therefore comparing means between treatments would not provide valuable information. The administration of the placebo served to validate whether changes observed after the administration of Acunil® or Acuphase® were due exclusively to tranquilisation and not in part due to habituation of the animals to captivity and human disturbances.

10.3 RESULTS

The results are reported as they were observed within each specific treatment group.

10.3.1 Plasma cortisol

The plasma cortisol results for each of the treatment groups are given in Table 10.2. Plasma cortisol did not change ($P > 0.05$) over the three sampling days when animals were treated with Acuphase®. However, differences were observed ($P < 0.05$) in groups treated with both Acunil® and a placebo so that plasma cortisol decreased on Day 2 and Day 3 compared to Day 1.

Table 10.2 Plasma cortisol (nmol/l) (LSMean ± SEM) in blood taken on three consecutive sampling days, within each treatment group

Day	Treatment		
	Acuphase®	**Acunil®**	**Control**
Day 1	$67.8^{a} \pm 7.15$	$61.1^{a} \pm 7.18$	$93.1^{a} \pm 8.38$
Day 2	$58.9^{a} \pm 10.46$	$44.3^{b} \pm 7.56$	$51.9^{b} \pm 9.16$
Day 3	$53.0^{a} \pm 8.89$	$46.7^{ab} \pm 5.44$	$57.5^{b} \pm 5.79$

[ab] Means between days within a treatment with the same letter are not significantly different ($P > 0.05$)

10.3.2 Faecal glucocorticoid metabolites (FGMs)

FGMs did not change ($P > 0.05$) over the sampling days within the Acuphase® and Acunil® treatment groups. However, FGMs increased ($P < 0.05$) on Day 2 and Day 3 in the control group (Table 10.3).

Table 10.3 Faecal glucocorticoid (FGM) levels (ng/g of dried faeces) (LSMean ± SEM) over the three consecutive sampling days for each treatment group

Day	Treatment		
	Acuphase®	**Acunil®**	**Control**
Day 1	87.8[a] ± 12.24	95.7[a] ± 13.38	160.7[a] ± 21.54
Day 2	104.0[a] ± 11.34	79.1[a] ± 13.47	265.7[b] ± 53.10
Day 3	88.0[a] ± 18.30	81.1[a] ± 12.74	269.3[b] ± 37.01

[ab] Means between days within a treatment with the same letter are not significantly different ($P > 0.05$)

Within the control group (placebo treatment group), there was an interaction between trial and sampling day ($P = 0.03$) so that FGM levels were higher in Trial 1 on Day 3 than in Trial 2 on the same day (Figure 10.1).

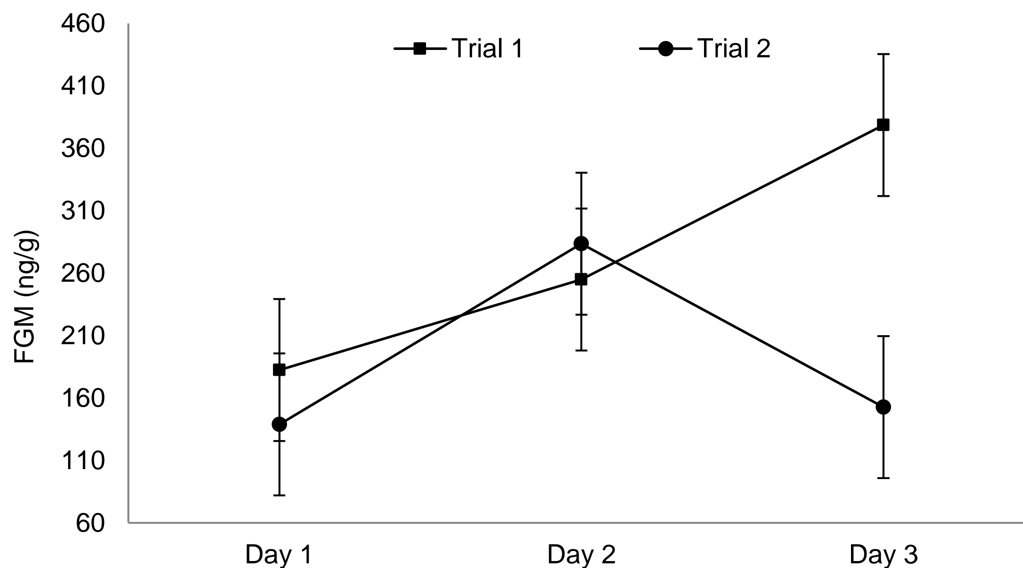

Figure 10.1 The interaction between trial and sampling day for FGMs within the control group

10.3.3 Plasma lactate and glucose

The mean glucose and lactate results for each of the three treatment groups are given in Table 10.4. It must be noted that, due to technical difficulties with equipment, blood

glucose and lactate levels were not measured in Trial 2 of the Acuphase® treatment group and Trial 1 of the control group.

Table 10.4 Plasma glucose and lactate (mmol/l) (LSMean ± SEM) in blood taken on three consecutive sampling days, within each treatment group

	Treatment					
	Acuphase®		Acunil®		Control	
Day	**Glucose**	**Lactate**	**Glucose**	**Lactate**	**Glucose**	**Lactate**
Day 1	6.0[a] ± 0.28	3.8[a] ± 0.30	6.7[a] ± 0.51	5.0[a] ± 0.40	3.5[a] ± 0.47	3.9[a] ± 0.16
Day 2	4.7[b] ± 0.84	3.6[a] ± 0.46	5.6[b] ± 0.43	3.8[b] ± 0.29	3.4[a] ± 0.66	3.0[ab] ± 0.46
Day 3	4.7[b] ± 0.75	3.0[b] ± 0.54	5.3[b] ± 0.30	3.3[b] ± 0.18	3.9[a] ± 0.65	4.6[ac] ± 0.18

[abc] Means between days and within a treatment and parameter with the same letter are not significantly different ($P > 0.05$)

In the control group, there was no decrease in blood glucose over the three consecutive days as was seen in the Acuphase® and Acunil® treatment groups. In addition, there was also an increase in blood lactate on Day 3, which was not seen in the Acuphase® and Acunil® treatment groups.

Within the Acuphase® treatment group, there was an interaction between trial and sampling day ($P < 0.05$) so that lactate and glucose levels showed differing changes over the three sampling days between the two Acuphase® trials (Figure 10.2).

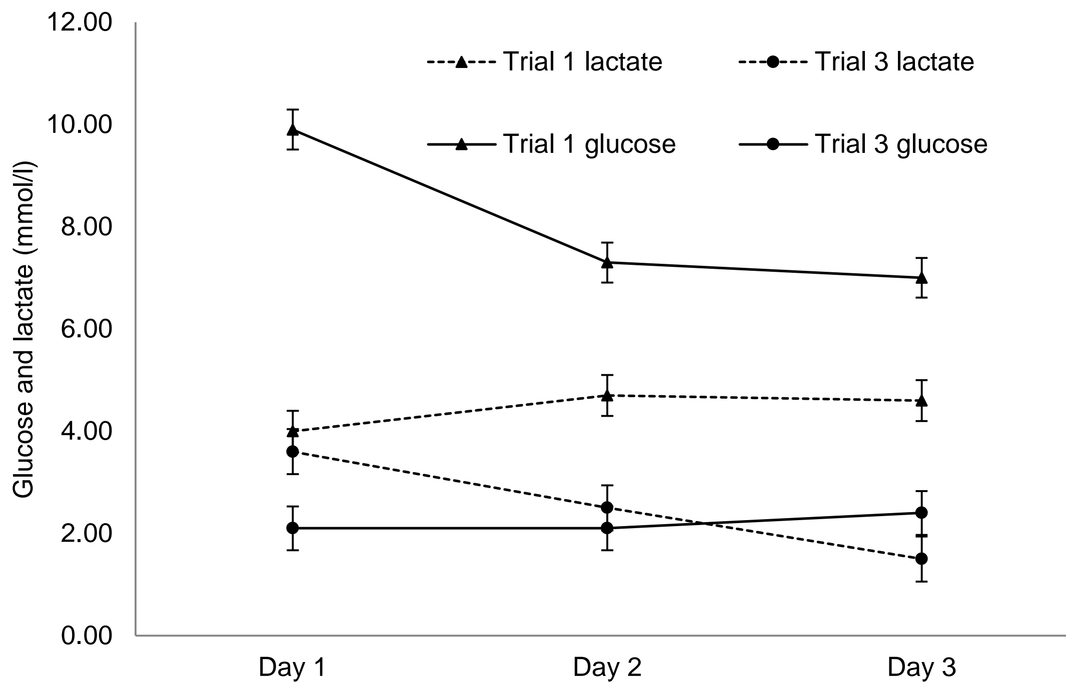

Figure 10.2 The interaction between trial and sampling day for glucose and lactate within the Acuphase® treatment group

10.3.4 Neutrophil function

Neutrophil response curves were plotted for all three sampling days within all three treatment groups.

10.3.4.1 Acuphase®

Figure 10.3 reflects the mean neutrophil response curves of the animals on the three sampling days within the Acuphase® treatment group. It must be noted that, due to equipment failures, the neutrophil function assay was not performed on blood samples from animals in Trial 2 within this treatment group.

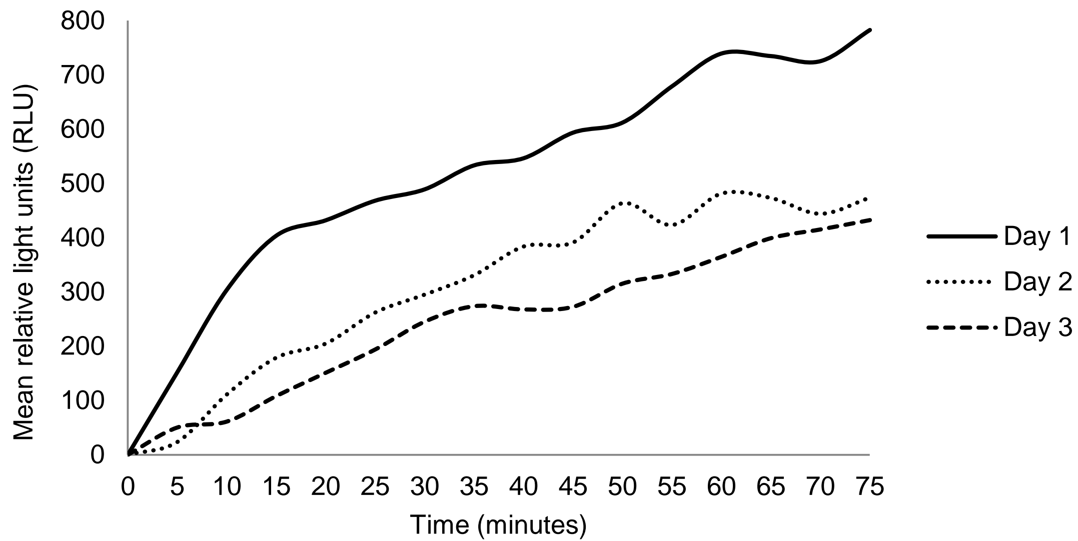

Figure 10.3 Mean neutrophil response curves over time for each of the three sampling days from trials conducted with Acuphase® on blue wildebeest

The mean area under the curve (AUC) did not differ ($P > 0.05$) between the three sampling days and are reflected in Figure 10.4.

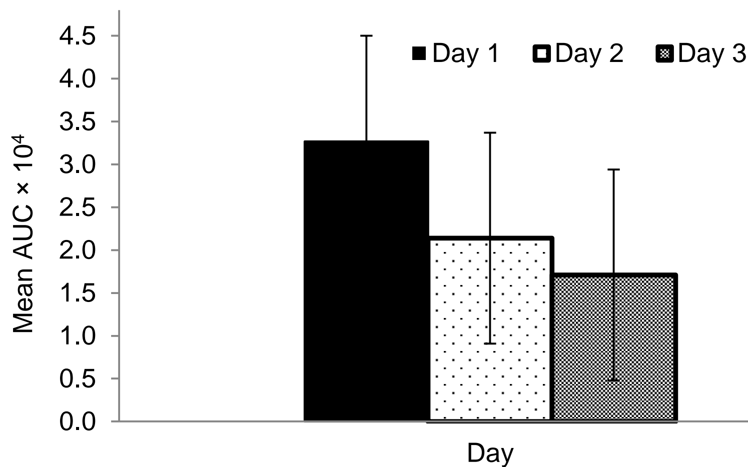

Figure 10.4 AUC (LSMean ± SEM) on the three consecutive sampling days from trials conducted with Acuphase® on blue wildebeest

10.3.4.2 Acunil®

Figure 10.5 reflects the mean neutrophil response curves for the animals on each of the three sampling days within the Acunil® treatment group.

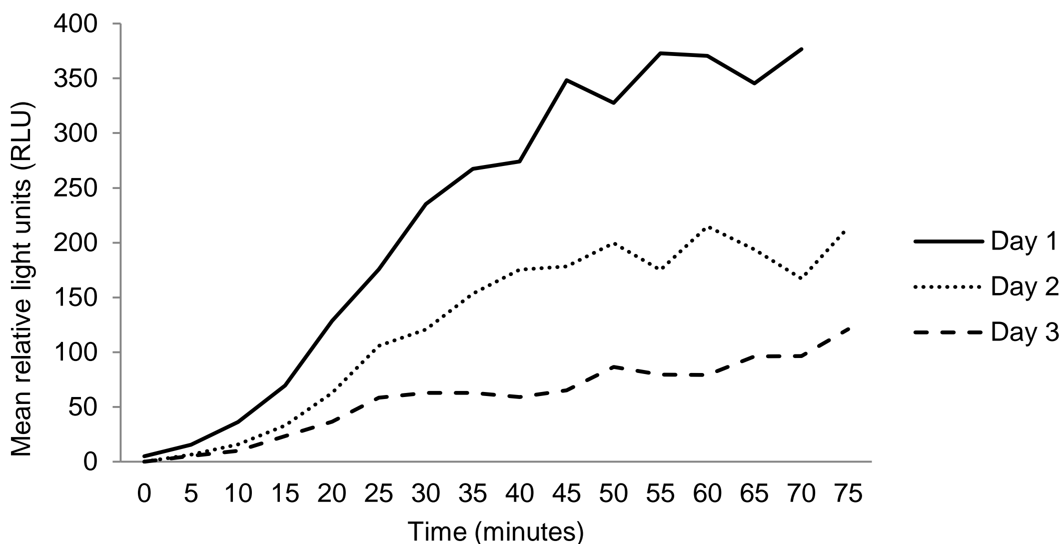

Figure 10.5 Mean neutrophil response curves over time for each of the three sampling days from trials conducted with Acunil® on blue wildebeest

The mean AUC for Day 1 was higher (P = 0.009) than that for Day 3 (Figure 10.6) although no differences in AUC were found between Day 1 and Day 2, or Day 2 and Day 3.

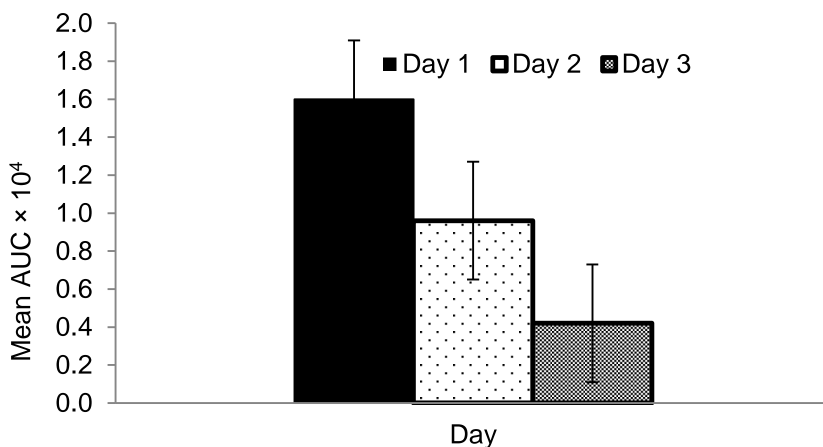

Figure 10.6 AUC (LSMean ± SEM) on the three consecutive sampling days from trials conducted with Acunil® on blue wildebeest

10.3.4.3 Placebo

Figure 10.7 presents the mean neutrophil response curves of the animals over the three sampling days in the control group. It must be noted that, due to equipment failure,

neutrophil function was not measured in the blood samples from animals in Trial 1 within this treatment group.

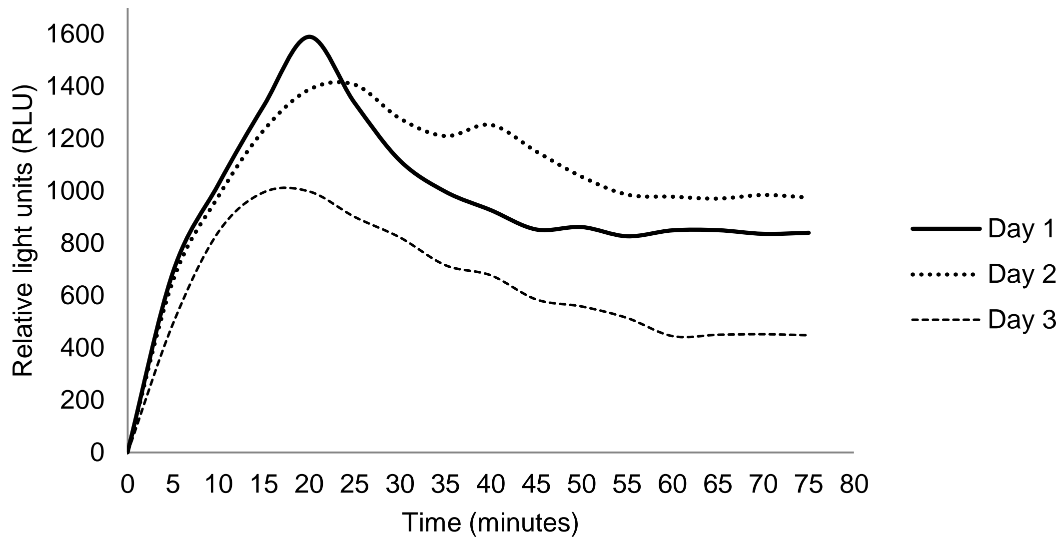

Figure 10.7 Mean neutrophil response curves over time for each of the three sampling days from trials conducted with a placebo in blue wildebeest

As when animals were treated with Acuphase®, the mean AUC did not differ ($P > 0.05$) between the three sampling days (Figure 10.8).

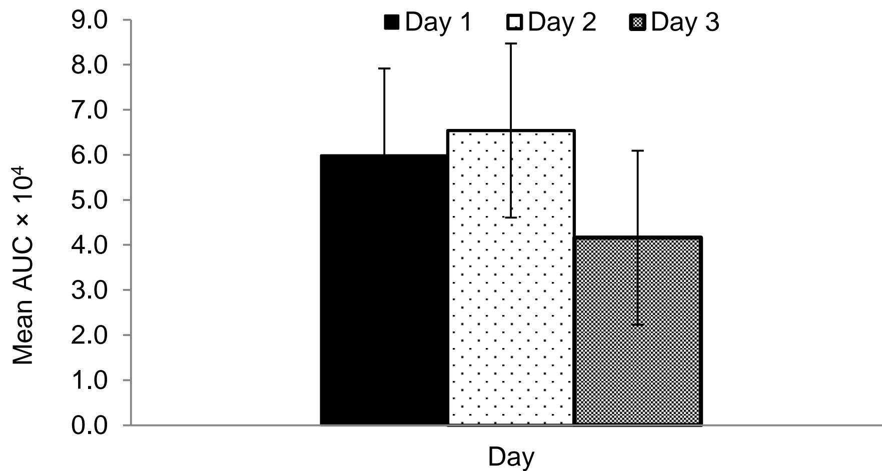

Figure 10.8 AUC (LSMean ± SEM) on the three consecutive sampling days from trials conducted with a placebo on blue wildebeest

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10.3.5 White blood cell counts (WBCs)

The WBCs from each of the three sampling days and within each treatment group are given in Table 10.5. No difference was observed ($P > 0.05$) between any of the sampling days within any of the treatment groups.

Table 10.5 Blue wildebeest white blood cell counts (WBCs × 10^6/ml) (LSMean ± SEM) from blood taken on three consecutive sampling days, within each treatment group

Day	Treatment		
	Acuphase®	**Acunil®**	**Placebo**
Day 1	5.6 ± 0.35	9.0 ± 0.95	5.8 ± 0.79
Day 2	6.5 ± 0.56	7.9 ± 0.71	6.5 ± 1.02
Day 3	5.7 ± 0.56	7.7 ± 0.51	6.3 ± 0.66

No correlations were found between WBC and the mean AUC for neutrophil response curves in any of the treatment groups.

10.4 DISCUSSION

The results in this study indicated both similar as well as dissimilar results obtained within the three treatment groups. Both measures of endocrine response as well as immune function indicated that treatment with Acunil®, Acuphase® or a placebo resulted in varying changes over the three sampling days.

Chemical restraint has been noted to result in an increase in plasma cortisol in both ungulate and felid species (Meyer *et al.*, 2008; Nogueira & Silva, 1997; Wesson, Scanlon, Kirkpatrick, Mosby, & Butche, 1979) while tranquilisation has been found to decrease plasma cortisol levels in response to capture in ungulate species (Mentaberre *et al.*, 2010; Montané *et al.*, 2003). It therefore stands to reason, that LAN-treated animals may show a lowered cortisol response to chemical immobilisation. However, plasma cortisol levels remained unaffected ($P > 0.05$) over the three sampling days in the Acuphase® treatment group, while animals in the Acunil® treatment group showed a decrease ($P < 0.05$) from Day 1 to Day 2 and then again an increase on Day 3. Similar results were observed in blood samples collected from animals in the control group where the most significant decrease in cortisol occurred on Day 2 of sampling. These

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results indicate that animal habituation to being immobilised most likely occurred and affected the changes in blood cortisol more than treatment with the LAN itself. Read, Caulkett, and McCallister (2000) reported conflicting results in wapiti (*Cervus elaphus*) treated with Clopixol Acuphase®. These authors found that treated animals showed a decrease in serum cortisol from Day 1 to Day 2 and Day 4 of handling. In comparison, animals that received a saline injection (control) showed no such decrease.

A lowered cortisol response to repeated handling has been reported in livestock studies, with authors attributing this to habituation (Andrade, Orihuela, Solano, & Galina, 2001). Results reported by Knox and Hattingh (1992), indicated that impala exposed to repeated capture also showed a high initial increase in blood cortisol (Capture 1 = 102 nmol/l) followed by a decrease in cortisol response at subsequent captures (Capture 2 = 91 nmol/l; Capture 3 = 74 nmol/l; Capture 4 = 75 nmol/l; Capture 5 = 70 nmol/l). Although these authors did not comment on the cause of the decrease, it could be speculated that the animals also became habituated to repeated capture in a similar way that livestock becomes habituated to handling. Meyer *et al.* (2008) also noted a correlation between time until recumbency and plasma cortisol, and it may be that an individual's response to the immobilisation drug (i.e. the time each individual took to respond to the drug), affected the plasma cortisol levels more than tranquilisation itself. It is worth noting that cortisol levels in all three treatment groups fell within ranges reported for physically restrained wild blue wildebeest by Morton, Anderson, Foggin, Kock, & Tiran (1995) (24.6–87.5 nmol/l), indicating that the animals' mean cortisol responses fell within ranges that could be considered as stressful during all three immobilisations, regardless of treatment.

Faecal glucocorticoid metabolite (FGM) analysis provides a non-invasive method for studying the physiological response of wildlife to a variety of long-term stressors such as relocation and acclimatisation to new environments (Millspaugh *et al.*, 2002; Romero, Meister, Cyr, Kenagy, & Wingfield, 2008). Unlike plasma cortisol, FGM levels are most affected by long-term chronic stressors so that, in the current study, they are more likely affected by the chronic stress of captivity as well as by stressful events (such as immobilisation) that occurred up to 24 hours prior to sampling (Dickens, Delehanty, & Romero, 2010). Interestingly, Franceschini, Rubenstein, Low, and Romero (2008) found that in zebra (*Equues grevyi*), captivity caused a greater increase in FGM than the capture process itself did. In the current study, Day 1 FGM concentrations provided information on the level of stress experienced by the animals prior to the start of the trials and as such, a comparison between treatments provides inconsequential information.

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However, the changes in FGM levels within a treatment and between sampling days provide significant information regarding the effect of treatment on chronic stress. When FGMs were measured in rectal faecal samples at each of the three immobilisations, no differences ($P < 0.05$) were noted between the three sampling days within the Acuphase® and Acunil® treatment groups. Clauss, Lechner-Doll, Palme, Rohleder and Dehnhard (2000) reported contrasting results, and found that treatment with a LAN (perphenazine enanthate) resulted in a significant decrease in FGM concentrations in roe deer (*Capreolus capreolus*) within 10–24 hours after its administration. However, it must be borne in mind that animals in the current study were constantly stimulated for the first 12 hours of each treatment and this may have contributed to why no decrease in FGM was observed. In the control group, mean FGM levels on Day 2 and Day 3 of immobilisation were more than one and a half times higher ($P < 0.05$) than on Day 1 (Table 10.3). However, in the current research, an interaction was also noted in this treatment group between trial and sampling day (Figure 10.1). In Trial 1, mean FGM was higher ($P = 0.01$) on Day 3 than on Day 1 but did not differ between Day 3 and Day 2, indicating that animals experienced a greater degree of stress during the trial than before the start of the trial. In Trial 2, mean FGM was higher ($P = 0.04$) on Day 2 than on Day 1 but not differ ($P = 0.06$) between Day 1 and Day 3. It is possible that the animals experienced a greater degree of stress during the first part of the trial than after the placebo had been administered; however, the reason for this is unknown. The results from all three treatments were much higher than have previously been reported for free-roaming wild wildebeest by Stabach, Boone, Worden, and Florant (2015) (19.3–23.4 ng/g of dry faeces) and Chinnadurai *et al.* (2008) (21–30 ng/g of dry faeces). Similarly high FGM levels were noted in Chapter 6 of the dissertation. These high levels are most likely related to the stress of captivity and human interference, as well as the effect of diet on the concentration of hormones excreted. This is because animals were provided with high quality lucerne hay *ad libitum* which may have resulted in a decrease in feed ingested compared to animals in the wild and a resultant lowered faecal output (see Chapter 6) (Goymann, 2005).

Both blood glucose and lactate levels have been found to increase with the activation of the sympathetic nervous system and the HPA axis in response to stress. This is due to an increase in gluconeogenesis, aimed at providing the body with additional energy (Barnett & Hemsworth, 1990; Dantzer & Mormède, 1983; Mormède *et al.*, 2007). As a result, the measurement of blood glucose and lactate levels has been used by various authors in animal stress response studies (Ganhao, Hattingh, & Pitts, 1988; Mitchell,

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Hattingh, & Ganhao, 1988; Montané *et al.*, 2003; Montané, Marco, & Manteca, 2002; Petty, Hattingh, Ganhao, & Bezuidenhout, 1994; Read *et al.*, 2000; Zapata, Gimpel, Bonacic, & Bas, 2004). In the Acuphase® treatment group, the results showed that blood glucose and lactate levels were highest on the first day of immobilisation ($P < 0.05$), and thereafter, levels decreased with each immobilisation. However, an interaction was also noted between trial and sampling day within this treatment group for both glucose and lactate so that differences ($P > 0.05$) between trials affected the results between sampling days (refer to Figure 10.2). This is likely explained by the body condition of the animals in each trial, since animals in Trial 1 had consistently higher glucose and lactate levels than those in Trial 3. Caldeira, Belo, Santos, Vazques, and Portugal (2007) reviewed the effect of body condition in ewes on various blood metabolites, and found that lower body condition scores and undernutrition correlated with lowered blood glucose levels. In the current study, it is possible that a particular group of animals had a poorer body condition, relating to the quality of feed on the property from which they were sourced prior to the trials.

In the Acunil® treatment group, both blood glucose and lactate levels differed between Day 1 and Day 2 of sampling. However, no such difference was found between Day 3 (after treatment with Acunil®) and Day 2 of sampling. On both these days, blood glucose and lactate levels appeared to have returned to normal so that treatment with Acunil® had a minimal effect. In contrast, animal blood glucose levels in the control group did not decrease on Day 2 and Day 3 of sampling. In addition, blood lactate showed a significant elevation in the control group on Day 3, possibly indicating an increased stress response to immobilisation on this day. Fourie and Van Ouwerkerk (1984) reported glucose levels for culled, wild wildebeest ranging from 3.8–4.9 mmol/l, which is similar to the range reported for the control group over all three sampling days. In comparison, glucose levels were much higher on Day 1 of sampling in both the Acuphase® and Acunil® treatment groups compared to in the control group. These results suggest that animals in the control group showed no elevated lactate and glucose response (i.e. levels were at baseline) to the first immobilisation, which may have affected their response to immobilisations thereafter.

The measurement of neutrophil function provides a quantitative measurement of the extent to which the immune response is activated (Van Eeden, Klut, Walker, & Hogg, 1999). Short-term, acute stressors have been shown to result in significant increases in neutrophil activation (Ellard, Castle, & Mian, 2001). Repeated or long-term stressors on the other hand (such as darting and blood sampling as well as captivity), have been

shown to cause immuno-suppression and thus a concurrent decrease in neutrophil function (Davis, Maney, & Maerz, 2008; Johnstone *et al.*, 2012; Stanger *et al.*, 2005). Both Gelling, Mclaren, Mathews, Mian, and Macdonald (2009) and Moorhouse, Gelling, McLaren, Mian, and Macdonald (2007) reported that handling and captivity resulted in a decrease in neutrophil response curves in bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*). It is thus not surprising that the neutrophil response curves during all three treatments in the current research showed a decreasing trend over the three sampling days. However, the results indicate that only animals that were treated with Acunil® showed a significant difference in the extent to which the response curves (mean AUC) changed between Day 1 and Day 3. This treatment group also showed the largest decrease in FGMs over the three sampling days and since glucocorticoids are known to activate neutrophils, these results are not surprising (Amadori, Stefanon, Sgorlon, & Farinacci, 2007). No such differences were observed when animals were treated with either Acuphase® or a placebo. In fact, when animals were treated with a placebo, the mean AUC showed a different pattern (increasing) on Day 2 of sampling. As previously stated, the neutrophil response pattern observed in the control group is only from animals in Trial 2. During this trial, FGMs were highest on Day 2 of sampling which would account for the elevation in neutrophil response on this day. It is also worth noting though that the extent of the neutrophil response was greater in the control group than in the other two treatment groups. This is likely related to the higher FGM response observed in this group which was not seen in either the Acuphase® or Acunil® groups.

White blood cells (WBC) can respond rapidly to a variety of stimuli, such as stress and disease (Davis *et al.*, 2008). Therefore, a WBC count provides an easy, quick and inexpensive method for assessing the health of an animal clinically (Bubenik & Brownlee, 1987). In the current study, total WBC counts were higher than reported by Sleeman and Widdowson (1993). Sleeman and Widdoson (1993) reported mean WBC for culled, wild blue wildebeest of 2.36×10^6/ml. However, Pospisil, Kase, Vahala and Mouchova (1984a) reported mean WBC for immobilised, wild wildebeest ranging from 3.2 to 6.2×10^6/ml, while Gamble, Anderson and Foggin (1994) reported the mean WBC of physically restrained blue wildebeest to be 7.2×10^6/ml. Drevemo, Grootenhuis and Karstad (1974) and Fourie and Van Ouwerkerk (1984) reported mean WBCs of 4.61×10^6/ml (immobilised, wild wildebeest) and 5.2×10^6/ml (culled, wild wildebeest), respectively for this species. Fourie and Van Ouwerkerk (1984) noted seasonal differences in WBC, which may account for the effect of trial on WBCs in the Acuphase® treatment group. It

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was also noted by Pospisil *et al.* (1984b) that WBCs are extremely variable in each individual and are influenced by many physical and perhaps psychological factors. This may partly explain the lack of difference (P > 0.05) between the WBC on each of the three sampling days and why none of the treatments were observed to have any significant effect on WBC. Interestingly, the WBC in the current study had no effect on neutrophil responses over time in any of the treatment groups.

10.5 CONCLUSION

The study aimed to compare blood and faecal results obtained before and after animals had been treated with either Acuphase®, Acunil® or a placebo. Although the results provided extensive information about the animals' responses to the stress of captivity and chemical immobilisation, they showed that the administration of Acuphase® and Acunil® had very little effect on blood and faecal parameters in this species within the first 24 hours after administration. When groups of animals were treated with Acuphase® on the second day of the trials, blood and faecal parameters showed no change over the three sampling days. Only blood glucose showed a decrease on Day 2 of sampling although an interaction between treatment and trial within the Acuphase® group revealed that the higher blood glucose levels of animals in Trial 1 affected this significant decrease on Day 2. In the Acunil® treatment group, plasma cortisol, blood lactate and glucose decreased on Day 2 of sampling. Since Day 2 blood samples were taken prior to treatment with Acunil®, these results indicate that differences were not attributable to treatment with Acunil® but rather to other factors, such as habituation to repeated immobilization. This is particularly true for plasma cortisol results since similar differences were observed in the control group on Day 2 of sampling. FGM concentration provides a good indication of the level of chronic stress experienced by the animals. FGM levels differed only between the three sampling days within the control group; however, an interaction between trial and sampling day revealed this to be related to differences between the trials. These results reveal that neither treatment with Acunil® nor treatment with Acuphase® resulted in any substantial effect on chronic stress although animals in the Acuphase® and Acunil® groups had a suppressed HPA response overall compared to the control group. In terms of immune function, WBC counts indicated that treatment with an LAN had no significant immuno-supressing effects on the animals in captivity. These results were echoed in the analysis of neutrophil function, where neutrophil response curves revealed a steady decrease over all three sampling days with no influence of any of the three treatments. Overall, future studies should focus on the

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effects of these two LANs on blood and faecal parameters over a longer period and within a single, larger group of animals (in order to minimise the effect of different trials and groups). Since both LANs have a palliative effect for up to three days, it could be postulated that their effect on these parameters only becomes apparent when measured in periods exceeding 24 hours after administration. This is particularly true for measures of chronic stress, such as immune function and faecal glucocorticoids.

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CHAPTER 11

General discussion and conclusions

11.1 DISCUSSION AND CONCLUSIONS

The South African game ranching industry is a fast-expanding industry, relying heavily on the capture and relocation of game animals across the country. This is evidenced by the fact that a recorded 300 000 head of game are translocated across South Africa annually, although the exact number is probably much higher (Dugmore, 2013). In addition, there has been a simultaneous increase in concern for the welfare of translocated animals as well as the minimisation of animal and financial losses due to stress-related mortality (Teixeira, Deazevedo, Mendl, Cipreste, & Young, 2007; Van Niekerk & Pienaar, 1962). Long-acting neuroleptics (LANs) have become increasingly popular and have been used effectively to control psychomotor excitement during the translocation of wildlife, as well as to reduce animal stress during translocation procedures (Huber, Walzer, & Slotta-Bachmayr, 2001). Long-acting neuroleptics produce a long-term effect, which is specifically useful during long-distance transportation and when animals are to be kept in captivity for extended periods of time (Diverio, Goddard, & Gordon, 1996; Gandini, Ebedes, & Burroughs, 1989; Knox, 1992). It has been observed that treatment with a LAN results in a modification of mood or attitude, an indifference to an animal's surroundings and a loss of fear for humans (Diverio *et al.*, 1996). LANs are formulated by combining the active drug with a long-chain fatty acid (Huber *et al.,* 2001). As a result, the combination is slowly hydrolysed in the body tissues, releasing the neuroleptic drug into the vascular system over a prolonged period of time (Huber *et al.*, 2001). These drugs have been used successfully to reduce the considerable losses associated with stress-related mortality during the translocation of wild ungulates (Raath, personal communication, 17 April, 2013), although very little research is available on their exact effect on the animal stress response (Diverio, Goddard, Gordon, & Elston, 1993). In addition, very few of these LANs are developed and manufactured locally so that any research regarding their use is primarily focused on wildlife species other than those found in South Africa.

In order to investigate factors that may minimise animal stress during capture and relocation procedures in depth, it is important that the animals' varying responses to

stress be fully understood. It has long been assumed that measuring stress in animals would provide an accurate indication of well-being (Von Borell, 2001). However, difficulties arise when attempts are made to quantify stress since there is no accepted definitive definition of stress and even more poorly defined parameters to measure it (Read, Caulkett, & McCallister, 2000; Reeder & Kramer, 2005). Traditionally, increased plasma levels of corticosteroids have been used as indication of stress although the adrenal cortical response does not always occur, therefore suggesting an over-reliance on this parameter as a sole measure of stress (Morton, Anderson, Foggin, Kock, & Tiran, 1995; Nwe, Hori, Manda, & Watanabe, 1996; Read *et al.*, 2000). As a result, animal stress is best assessed by examination of more than one of the systems used to cope with homeostatic disruption (Huber *et al.*, 2001). These systems are the autonomic nervous system, the neuroendocrine system as well as the animal's behaviour (Huber *et al.*, 2001).

The purpose for the current study was to investigate the effect of LANs on a wide array of animal responses since no such all-encompassing investigation has yet been performed on any Southern African wild ungulate species. The study was carried out in conjunction with Wildlife Pharmaceuticals SA (Pty) Ltd. in order not only to evaluate the behavioural and physiological effects of a well-known LAN, Clopixol Acuphase®[1] in blue wildebeest (*Connochaetes taurinus*), but also to evaluate the effects of a newly developed LAN, Acunil®[2], in this species. This specific species was chosen because it is native to the study area, relatively abundant, and is commonly translocated across South Africa because of its value in hunting and game meat production (Hoffman & Wiklund, 2006). The aim was to determine which behavioural and physiological effects are produced after administration of Acuphase® in blue wildebeest and whether similar effects could be achieved when animals were treated with Acunil®. In addition, two groups of animals were treated with a placebo (control treatment group) in order to determine whether any behavioural or physiological changes occurred in the absence of any neuroleptic.

The first undertaking of this study was to investigate the use of a human biotelemetry system in blue wildebeest as a means of measuring continuous heart rate, respiration rate, skin temperature and motion in this species (Chapter 5). This was done since all

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[1] Clopixol Acuphase® is zuclopenthixol acetate dissolved in vegetable oil which allows for the slow-release of zuclopenthixol acetate from the intramuscular site of injection.
[2] Acunil® is zuclopenthixol acetate dissolved in a slow-release polymer with similar expected effects as Clopixol Acuphase®

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these parameters are known to be affected by the stress response. Specifically, heart rate and respiration rate are most affected during the initial fight-or-flight response so it was of value to the study to be able to measure these parameters in ambulatory animals (Reefmann, Bütikofer Kaszàs, Wechsler, & Gygax, 2009; Von Borell, 2001). To our knowledge, no large animal biotelemetry system has previously been investigated in blue wildebeest, and this study thus provided some of the first data on what the heart rate and respiration rate of this species are when fully conscious. At the onset of the first trials, numerous human and animals biotelemetry systems were investigated and it was decided that the Equivital™ EQ02 wireless biotelemetry belt (Hidalgo Limited, Unit F. Trinity Court, Buckingway Business Park, Cambridge, UK) would provide the most effective option. This was decided for a number of different reasons including cost, the use of Bluetooth technology to validate the accuracy of the belts as well as the need for minimal modification. A trial was conducted on two different blue wildebeest to validate the accuracy of the belts. Measurements of heart rate and respiration rate taken with the Equivital™ belt were compared to those taken with a Cardell® 9500 HD multi-parameter veterinary vital sign monitor (Kyron Laboratories (Pty) Ltd., Johannesburg, South Africa) as well as those taken manually while animals were immobilised. Whilst immobilised, animals were also treated with doxapram hydrochloride (Dopram®) and adrenaline to induce and measure changes in respiration and heart rate, respectively. Results between the three monitoring methods were compared before and after the belts had been worn by the animals in captivity for 24 hours. Results obtained with the belts while being worn for 24 hours, were also analysed. The results showed that the Equivital™ EQ02 belt could accurately measure heart rate and respiration rate in blue wildebeest, both before and after being worn in captivity for 24 hours. In addition, heart rate and respiration rate increased concurrently with motion in the animals, indicating that motion (as measured with a tri-accelerometer) was also fairly accurate. Skin temperature was not validated as no external temperature logger was available to do so. In addition, preliminary studies showed that skin temperature was least affected by the stress response of the animals and instead rather correlated with environmental temperatures.

The second undertaking of the study was to validate the use of a commercially available faecal glucocorticoid metabolite assay (^{125}I Corticosterone RIA kit; MP Biomedicals LLC, Santa Ana, CA 92707, USA) for use in blue wildebeest (Chapter 6). A standard ACTH challenge was performed on four blue wildebeest. Blood samples were collected immediately prior to and every 10 minutes after administration of the ACTH analogue (1.0 IU/kg Synacthen®) for a total of 40 minutes. Faecal samples were also collected

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rectally prior to the administration of Synacthen® and every 6 hours thereafter over a 42-hour period once the animals had been taken back to enclosures (Nakagawa, Mostl, & Waas, 2003). This was done to validate whether the assay was sensitive enough to detect elevations in faecal glucocorticoid metabolites (FGMs) due to the administration of Synacthen® (Chinnadurai *et al.*, 2008). The results of the validation study found that mean plasma cortisol levels in the four animals, in response to the administration of Synacthen, increased from 10.9 ± 11.37 nmol/l to 125.4 ± 11.37 nmol/l at 10 minutes and continued to increase to 178.1 ± 11.37 nmol/l at 20 minutes, after which time no significant increase was noted. Mean FGM concentration also showed a significant increase within the first 12 hours after Synacthen® administration, from 86.0 ± 21.99 ng/g (in the rectal/baseline sample) to 477.9 ± 21.99 ng/g of dry faeces. An inert marker (20 g of glitter) was administered to each animal in order to determine gut transit time. A peak in gut transit was noted at around 12 hours, corresponding to the peak in FGM concentration at this time. The study adequately showed the precision and suitability of the assay in detecting changes in FGM concentration in this species.

This first trials conducted to address the objective of the study investigated the effect of zuclopenthixol acetate (Clopixol Acuphase®) on the behavioural and physiological responses of blue wildebeest (Chapter 7). The results showed that Acuphase® was effective in minimising the amount of time animals spent being vigilant as well as increased the time they spent resting (lying with their heads up and standing with their heads up, ruminating). Although it had no observable effect on overall heart rate, the administration of Acuphase® caused a lower overall respiration rate in the animals compared to prior to treatment. The lack of effect on heart rate may be related to reflex tachycardia in response to hypotension with a number of other authors having reported similar results with the use of LANs (Marco, Lavín, Mentaberre, Lopez-Olvera, & Casas-Díaz, 2010). This hypotension is thought to result from peripheral α-adrenergic blockade since both phenothiazine and thioxanthine derivatives have high affinities for α1-adenoceptors (Walsh & Wilson, 2002). Treatment with Acuphase® also resulted in lowered respiration rates during periods when animals were stimulated as well as periods when they were not, compared to when animals were untreated. The respiration rate of treated animals was also lowered during certain behaviours, particularly active behaviours such as walking, fighting and defending. Lastly, treated animals showed a reduction in the time they spent moving fast during periods when they were stimulated, indicating a reduction in activity in response to a stressor.

The second set of trials repeated the procedures of the first with the exclusion of Acuphase®, and instead involving treatment with a newly developed LAN from Wildlife Pharmaceuticals SA (Pty) Ltd., Acunil® (Chapter 8). This LAN replaced the traditional vegetable oil vehicle in which zuclopenthixol acetate is dissolved with a 72-hour slow-release polymer. Although the onset and duration of effect were expected to be the same as for Clopixol Acuphase®, this formulation was anticipated to produce a more consistent release profile. The aim of these trials was to ascertain whether Acunil® could elicit at least a similar reduction in the stress response as observed when animals were treated with Acuphase®. The results showed that treatment with Acunil® resulted in a reduction in time spent on certain behaviours, including being vigilant, as well as an increase in the amount of time spent on resting behaviours, particularly lying with their heads folded back. In addition, treated animals spent more time eating than when they were untreated. Although treatment with Acunil® had no significant effect on overall heart rate (as with Acuphase®), it caused a lowered overall respiration rate in the animals. Again, the lack of effect on heart rate may have been related to reflex tachycardia in response to hypotension induced by the administration of the tranquiliser. Treatment also caused a decrease in respiration rate during periods when animals were stimulated compared to when animals were untreated. In addition, treatment with Acunil® resulted in lower respiration rates for the stress-induced behaviours, such as being vigilant and trotting as well as the stationary behaviours, such as standing with head down and standing with head up, ruminating. As with Acuphase®, the measurement of motion indicated that the flight response of the animals was also reduced when they were treated with Acunil®.

The study also aimed to establish whether changes observed in behaviour, heart rate, respiration rate, skin temperature and motion in the previous trials were a direct result of the administration of the LANs or whether, to some extent, changes occurred due to the habituation of the animals to captivity and human interference. To this end, duplicate trials were performed with novel groups of animals and, instead of receiving any tranquilisation, animals were injected with sterile water on Day 2 of each trial. The results from these trials indicated that even though animals were treated with a placebo, there were still significant changes in behaviour and biotelemetry measurements. The most notable were the changes observed in behaviour since these changes echo the results found with both Acunil® and Acuphase®. Animals treated with the placebo also showed a reduction in the time they spent being vigilant as well as an increase in the time they spent lying down with their heads up. The measurement of motion indicated that even though animals only received a placebo, they were less active on Day 2 of the trials. In

addition, animals spent more time being stationary during periods when they were stimulated after they had received the placebo compared to before. Similar results were observed when animals were treated with Acunil®. These changes are probably related to acclimatisation of animals to captivity and to interactions with humans so that this, to some extent, may mask the actual effect of tranquilisation. However, unlike animals that had been treated with the placebo, animals treated with both Acuphase® and Acunil®, spent less time moving fast during periods when they were stimulated. These results indicate a reduction in the flight reaction of the animals, which was not observed in the control group. Furthermore, treatment with the placebo resulted in less consistent changes in respiration compared to when animals were treated with either Acuphase® or Acunil®, as well as no changes in heart rate. Although respiration rate decreased in placebo-treated animals when they were walking, respiration rate increased in treated animals while they were lying down with their heads up. These results indicate that changes in respiration after the administration of both Acunil® and Acuphase® are directly related to the sedative effect of these LANs and not as a result of the habituation of the animals.

Lastly, traditional blood and faecal endocrine responses were also measured in all the animals in all three treatment groups (Acuphase®, Acunil® and the placebo). Blood and rectal faecal samples were collected on three consecutive days (during each immobilisation) and analysed for plasma cortisol, whole-blood glucose and lactate, white blood cell counts (WBC), whole-blood neutrophil function and faecal glucocorticoid (FGM) concentrations. The results showed that plasma cortisol did not change over the three sampling days in the Acuphase® treatment group, while cortisol decreased on Days 2 and 3 in the Acunil® and control treatment groups. Consequently, changes in plasma cortisol were most likely attributable to the adaptation of the animals to captivity and repeated immobilisation and not to treatment with Acunil®. FGM concentrations increased in the control group on Day 2 and 3 of sampling while no such change was observed in the Acuphase® and Acunil® treatment groups. However, an interaction was found between trial and treatment in the control group, indicating that this change in the control group was possibly related to season or pre-trial stressors experienced by a particular group of animals rather than as a result of treatment. Lactate and glucose levels decreased from Day 1 to Day 3 in both the Acuphase® and Acunil® groups.

However, an interaction between trial and treatment within the Acuphase® group revealed that these changes in the Acuphase® group were not solely due to treatment with Acuphase®. In the control group, glucose showed no change over the three

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sampling days while lactate increased from Day 1 to Day 3. Neutrophil function was measured over time using a chemi-luminescent assay and the area under the curve (AUC) calculated. The mean AUC showed no change between the sampling days in both the Acuphase® and control treatment groups but did decrease from Day 1 to Days 2 and 3 in the Acunil® treatment groups. However, WBC did not differ between any of the sampling days within any of the three treatments, thereby indicating a minimal effect on immune function by all three treatments. Overall, the results revealed that Acunil® and Acuphase® had very little effect on the endocrine and immune responses of blue wildebeest within the first 24 hours after their administration although animals in these treatment groups showed an overall suppressed hypo-thalamic-adrenal (HPA) response compared to animals in the control group. Future studies could possibly focus on measuring these parameters over a longer period of time since their effects may only be noticeable after periods exceeding 24 hours after their administration.

11.2 STUDY LIMITATIONS AND FUTURE RECOMMENDATIONS

Although the study measured a wide variety of behavioural and physiological responses in blue wildebeest in order to evaluate the effect of two LANs, there are indeed limitations that need to be considered in the design of future studies.

Firstly, the use of a novel biotelemetry system in this species provided essential baseline data regarding the heart and respiration rates of free-moving wildebeest. In addition, the combination of this data with behavioural data further strengthened the knowledge on what these parameters are for particular behaviours in this species – something that had previously not been established. However, the use of an external battery pack to extend the battery life of the biotelemetry system would provide more valuable data in terms of the long-term effects of LANs in this species. In addition, the results indicated that the effects of the LANs investigated on chronic endocrine and immune responses may also be more apparent if measured over periods exceeding 24 hours after treatment. Therefore, future studies focusing on the long-term effects of Acuphase® and Acunil® will greatly strengthen the knowledge gained during the current investigation.

The measurement of endocrine and immune responses over three consecutive days provided valuable data on the response of this species to captivity, human disturbances and repeated immobilisation. Again though, the effects of both Acuphase® and Acunil® over this period appeared to be minimal. However, this protocol could provide essential information on the effect of shorter-duration neuroleptics in wildebeest and other wildlife

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species. This is particularly true for the use of neutrophil function, which has previously not been measured in wildebeest or any other African ungulate species. Neutrophil function showed an apparent decrease over the three sampling days within all three treatment groups and therefore may provide useful information regarding extrinsic and intrinsic factors that affect the immune response of ungulate species in captivity.

Lastly, the use of larger groups of animals would eliminate the need for repeated trials within a treatment. The current study was restricted by animal availability and costs, and therefore trials within a particular treatment occurred at different times of the year. The effect of season and intrinsic factors within a particular group of animals was apparent in measures of endocrine responses and could therefore be eliminated should a single, larger group of animals be studied.

This study in its entirety provides essential baseline data regarding the behavioural and physiological responses of blue wildebeest to captivity, human disturbance, repeated immobilisation and treatment with two LANs. Ultimately, this research provides a practical template for the investigation of factors that can reduce the stress response of wildebeest and other wildlife species in captivity.

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APPENDIX I

Chemical immobilisation sheet

<table>
<tr><td colspan="8">DATE:</td></tr>
<tr><td rowspan="11">ANIMAL INFORMATION</td><td>Species</td><td colspan="6"></td></tr>
<tr><td>Animal number/ID</td><td colspan="6"></td></tr>
<tr><td>Sex (M / F)</td><td colspan="6"></td></tr>
<tr><td>Age</td><td colspan="6"></td></tr>
<tr><td>Location</td><td colspan="6"></td></tr>
<tr><td>Capture methods (Boma / Truck / Chopper)</td><td colspan="6"></td></tr>
<tr><td>Estimated body weight (kg)</td><td colspan="6"></td></tr>
<tr><td>Actual body weight (kg)</td><td colspan="6"></td></tr>
<tr><td>Girth (cm)</td><td colspan="6"></td></tr>
<tr><td>Activity level (Calm / Active / Excited)</td><td colspan="6"></td></tr>
<tr><td>Health (Normal / Abnormal)</td><td colspan="6"></td></tr>
<tr><td rowspan="11">DRUG DATA</td><td>Immobilising drugs</td><td>mg/ml</td><td>Total mg</td><td>Dose in ml</td><td>Estimated dosage mg/kg</td><td>Actual dosage (mg/ml)</td><td>Time given</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Dart type</td><td colspan="6"></td></tr>
<tr><td>Injection site</td><td colspan="6"></td></tr>
<tr><td>Delivery method</td><td colspan="6"></td></tr>
<tr><td colspan="7">Comments:</td></tr>
<tr><td>Reversal</td><td>Volume</td><td>Dosage mg/kg</td><td>Total mg</td><td>Time</td><td>Route</td><td>Comments</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td rowspan="2"></td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

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<table>
<tr><td colspan="8">DATE:</td></tr>
<tr><td rowspan="4">TIMES</td><td></td><td>First effect</td><td>First down</td><td>Immobile</td><td>Antagonist injection</td><td>Standing</td><td>Comments</td></tr>
<tr><td>Time</td><td></td><td></td><td></td><td></td><td></td><td rowspan="2"></td></tr>
<tr><td>Stage</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="7">Stages of immobilization:
1 – Normal; 2 – Slightly ataxic; 3 – Very ataxic but standing; 4 - Falls down but can rise; 5 – Struggles to rise but cannot; 6 – Down, moves with stimulation; 7 – Down, slight movement with strong stimulation; 8 – No movement with strong stimulation; RR > 4/min.; 9 – No movement; RR < 4/min.; 10 – Respiratory collapse; RR = 0/min.; 11 - Death</td></tr>
<tr><td rowspan="11">MEASUREMENTS</td><td>Time from down</td><td>5 mins</td><td>10mins</td><td>15 mins</td><td>20 mins</td><td>25 mins</td><td>30 mins</td></tr>
<tr><td>Temp (°C)</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Pulse (bpm)</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Respiration (breaths/min)</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Systolic B.P.</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Diastolic B.P.</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Mean A.P.</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Oximetry SpO_2</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Venous sample</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>EKG</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="7"></td></tr>
<tr><td rowspan="3">REACTIVITY</td><td>Response to noise</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="7">Comments:</td></tr>
<tr><td colspan="7">Response to stimulation
1 – No response; 2 – Slight muscular tensing; 3 – Head movement; 4 – Increasing general body movement; 5 – Struggling to rise; 6 - Standing</td></tr>
<tr><td rowspan="4">OVERALL</td><td>Overall (Good / Acceptable / Fair / Poor)</td><td colspan="6"></td></tr>
<tr><td>Induction (Uneventful / Eventful - explain)</td><td colspan="6"></td></tr>
<tr><td>Immobilization (Uneventful / Eventful - explain)</td><td colspan="6"></td></tr>
<tr><td>Recovery (Uneventful / Eventful - explain)</td><td colspan="6"></td></tr>
</table>