The potential of midazolam for use as a sedative for blesbok (*Damaliscus pygargus phillipsi*)

by

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Thesis presented in fulfilment of the requirements for the degree of Master of Science in the Faculty of Agricultural Science at Stellenbosch University

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March 2018

Declaration

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Summary

Wildlife translocation results in stress in the animals, which impacts negatively on their welfare. Midazolam is used as a sedative in domestic species, with minimal cardiopulmonary side effects. Midazolam's effects in wildlife has not yet been determined. This study aimed to evaluate midazolam as a sedative in blesbok.

The first phase of the overall study entailed a pilot study using indigenous goats to determine the pharmacokinetic behavior of midazolam. Blood samples were collected at set time intervals following intramuscular (IM) midazolam administration in the goats. Resulting serum samples were analysed by means of gas chromatography-mass spectrophotometry, and a concentration-time profile of IM midazolam was compiled. Calculation of the pharmacokinetic parameters of midazolam indicated that it took approximately 36 min to reach a maximum serum concentration of 127.3 ng/L. Midazolam had a poor bioavailability and a relatively short elimination half-life.

In the second part of the study, the Equivital™ EQ02 biotelemetry system was validated for use in blesbok. On the first day of the validation study, two blesbok were immobilised, fitted with a biotelemetry belt, and translocated to a laboratory. The heart and respiration rate of each animal were individually recorded for 20 min using the Equivital™ system, a Cardell® monitor and a manual recording method. The accuracy of the Equivital™ system in detecting changes in heart and respiration rate caused by adrenaline and Dopram® administration respectively, was also assessed. After 20 min of recording, the animals were returned to the enclosure and the anaesthetic was reversed. The Equivital™ system remained on the animals for an additional 24 hrs to determine its accuracy in measuring physiological parameters and motion changes of conscious blesbok in captivity. After this 24 hr period, the experimental procedure was repeated. The agreement of the Equivital™ system with the Cardell® and the manual method for heart rate was moderate to excellent, while the agreement for respiration rate was poor to moderate. The Equivital™ system was accurate in measuring heart rate and detecting increases in heart rate resulting from adrenaline administration, but failed to accurately measure respiration rate and detect changes caused by Dopram®. The Equivital™ system successfully measured heart rate and motion changes of conscious blesbok in captivity.

In the third part of the study, the effect of three midazolam doses on behaviour, feed intake, heart rate, respiration rate, motion, and level of sedation in blesbok were studied. Four trials were conducted to establish the effect of four different dosages, i.e. a placebo, 0.6 mg midazolam/kg body weight (BWt), 0.4 mg midazolam/kg BWt or 0.2 mg midazolam/kg BWt. After immobilisation, the animals were fitted with the Equivital™ biotelemetry belts. After reversal of the anaesthetic, the specific dose was administered intramuscularly. Blesbok behaviour was recorded for 12 hrs using a CCTV system. The animals were stimulated and scored for sedation and response to stimulus for the first six hours after midazolam

administration. After an observation period of 24 hours, the animals were immobilised, the belts removed and the anaesthetic reversed. To determine the effects of midazolam on feed intake, the feed was weighed at the start of each trial and the end of each trial. Midazolam suppressed vigilance in blesbok. The lowest dose of midazolam decreased walking in blesbok, and increased standing and ruminating behaviour. Heart rate and respiration were decreased by the low dose when the animals were showing vigilance and trotting in alarm. The low dose did not affect heart rate and respiration when the animals were stimulated, but decreased both these parameters when the animals were not stimulated. The medium dose increased standing and ruminating behaviour, while it caused slower heart rate when the animals showed vigilance, trotting in alarm and avoidance. The high dose reduced grooming and agitation, increased walking and reduced standing and ruminating behaviour in blesbok. The high dose elevated the respiration rate of blesbok. Midazolam increased fast motion in stimulated blesbok. The low dose decreased motion in unstimulated blesbok. Midazolam treated via the IM route caused moderate sedation in blesbok. Midazolam decreased the response to stimulus of blesbok. The medium dose caused the least responsiveness to stimulation. Midazolam caused an increase in feed intake in blesbok. In conclusion, a dose of 0.2 mg midazolam/kg BWt was most effective in sedating blesbok without side effects and doses of 0.6 mg midazolam/kg BWt and higher should not be used on its own in blesbok to prevent the occurrence of extrapyramidal effects and severe ataxia. Higher doses of midazolam should rather be used in as adjuvants to anaesthetic immobilisation protocols in wild ungulates, but requires further research.

Opsomming

Die verskuiwing en aanhouding van wild belemmer dierewelsyn omdat dit stres veroorsaak. Midazolam is 'n doeltreffende kalmeermiddel vir plaasdiere, met minimale newe-effekte op die kardiopulmonêre stelsel, maar die effek daarvan in wild is nog nie bepaal nie. Hierdie studie het gepoog om midazolam te evalueer as 'n kalmeermiddel in blesbokke.

'n Loodsstudie is eerstens in inheemse bokke gedoen om midazolam se farmakokinetiese gedrag te bepaal. Bloedmonsters is per tydsinterval versamel na intramuskulêre behandeling van die bokke met midazolam en gesentrifugeer. Die serum vanaf die bloedmonsters is geanaliseer met gas kromatografie massa spektrometrie en 'n konsentrasie-tyd grafiek is getrek. Berekening van midazolam se farmakokinetiese parameters het getoon dat dit ongeveer 36 min geneem het om 'n maksimum serum konsentrasie van 127.3 ng/L te bereik. Midazolam se biobeskikbaarheid was laag en die eliminasie halfleeftyd was relatief kort.

In die tweede deel van die studie is die Equivital™ EQ02 biotelemetrie stelsel gevalideer vir gebruik in blesbokke. Op die eerste dag is twee blesbokke onder narkose geplaas, toegerus met 'n biotelemetrie belt en na 'n laboratorium gedra. Hartklop en asemhalings tempo van altwee diere is afsonderlik vir 20 min gemeet met die Equivital™ stelsel, 'n Cardell® monitor en per hand. Die akkuraatheid van die Equivital™ sisteem om veranderinge in hartklop en asemhaling tempo op te tel wat veroorsaak is deur adrenalien en Dopram® onderskeidelik, is ook bepaal. Na 20 min se data per dier versamel is, is hul terug geneem na die boma en die narkose is omgekeer. Die biotelemetrie belde is op die diere gelos vir nog 24 uur om die akkuraatheid daarvan in die meet van hart en asemhalings tempo veranderings, asook veranderinge in die beweging van die diere by hul volle bewussyn in aanhouding te bepaal. Na hierdie 24 uur is die eksperimentele prosedure herhaal. Die verwantskap van die Equivital™ sisteem met die Cardell® en die per hand metode was middelmatig tot uitstekend vir hart tempo, maar die verwantskap vir asemhalings tempo was swak tot middelmatig. Die Equivital™ stelsel was akkuraat in die meet van hartklop en hartklop stygings veroorsaak deur adrenalien behandeling, maar was onsuksesvol daarin om asemhalingstempo en veranderinge aangebring deur Dopram® akkuraat te meet. Die Equivital™ sisteem was suksesvol in die meet van hartklop en veranderinge in beweging van blesbokke by hul volle bewussyn in aanhouding.

In die derde deel van die studie is die effek van drie midazolam dosisse op die gedrag, voerinname, hartklop, asemhalings tempo en beweging, asook die vlak van verdowing in blesbokke bepaal. Vier proewe is gedoen om die effek van vier verskillende behandelings, naamlik 'n plasebo, 0.6 mg midazolam/kg liggaamsmassa, 0.4 mg midazolam/kg liggaamsmassa en 0.2 mg midazolam/kg liggaamsmassa, te bepaal. Nadat hulle onder narkose geplaas is, is elke dier toegerus met 'n Equivital™ biotelemetrie belt. Nadat die narkose omgekeer is, is die spesifieke behandeling binnespiers toegedien. Die gedrag van die

diere is opgeneem met CCTV vir 12 ure. Die diere is gestimuleer en tellings vir verdowingsvlak en reaksie tot stimulasie is toegeken vir die eerste ses ure. Die diere is 24 uur na behandeling weer onder narkose geplaas, die belde is verwyder en die narkose omgekeer. Om midazolam se effek op voerinname te bepaal is die voer aan die begin van elke proef en die voer aan die einde van elke proef geweeg. Midazolam het waaksaamheid in die blesbokke laat afneem. Die laagste dosis het veroorsaak dat die blesbokke minder rondloop en meer staan en herkou. Hartklop en asemhalings tempo is deur die lae dosis verlaag gedurende die toon van waaksaamheid en vlug gedrag in die belsbokke. Die lae dosis het geen effek gehad op die diere se hart en asemhalings tempo tydens stimulasies nie, maar het beide hierdie parameters verlaag toe die diere nie gestimuleer is nie. Die medium dosis het staan en herkou gedrag verhoog en hart tempo gedurende waaksaamheid, vlug gedrag en vermyding verlaag. Die hoë dosis het “grooming” en “agitation” verminder, loop gedrag verhoog en staan en herkou gedrag in blesbokke verminder. Die asemhalings tempo van blesbokke is deur die hoë dosis verhoog. Midazolam het vinnige beweging in blesbokke tydens stimulasies verhoog. Die lae dosis het beweging in blesbokke tydens tye van geen stimulasie verminder. ’n Midazolam dosis van 0.2 mg/kg liggaamsmassa was dus mees suksesvol daarin om blesbokke te verdoof sonder om ongewenste newe effekte te veroorsaak. Binnespierse midazolam het matige verdowing in blesbokke veroorsaak. Midazolam het die intensiteit van blesbokke se reaksie op stimulasie verminder. Die medium dosis het die reaksie tot stimulus die meeste verlaag. Midazolam het voerinname in blesbokke laat toeneem. Ten slotte, ’n dosis van 0.2 mg midazolam/kg liggaamsmassa was mees suksesvol daarin om blesbokke te verdoof sonder om ongewenste newe effekte te veroorsaak. Die gebruik van 0.6 mg midazolam/kg liggaamsmaasa dosisse en hoër word nie aanbeveel in blesbokke nie, om die voorkoms van ekstrapyramidale simptome en erge ataksie te verhoed. Hoë dosisse van midazolam moet eerder saam met narkose middels gebruik word as deel van immobiliserings protokolle in wilde boksoorte, maar dit verg verdere navorsing.

Acknowledgements

In memory of my late mother and uncle, Valerie du Plessis and Reynold du Plessis, who always believed in me, showed me what it means to love unconditionally and helped shape the person I am today.

Firstly, I want to give thanks and praise to Our Heavenly Father for guiding me to where I am today and giving me the strength and talents to be successful in anything I take on, including this thesis.

Then I am eternally grateful to my supervisor, Dr Helet Lambrechts, for encouraging me to take on this project, always taking the time to answer my questions and give advice, helping me to establish my scientific writing skills and ultimately helping me to become an independent researcher. Thank you for all your patience, time and kindness, I truly appreciate it.

I am also sincerely grateful to my co-supervisors, Prof Louw Hoffman and Dr Liesel Williams for making this project possible, encouraging me to be less timid, patiently answering my questions and developing my scientific thinking skills by giving me constructive criticism.

I would also like to thank Dr Silke Pfitzer for taking the time to help me with my project, teaching me about wildlife handling and encouraging me to speak up and be less timid.

Then I would like to thank Prof Martin Kidd for his assistance in the statistical analysis of my data. Thank you for your patience and taking the time to explain and answer all my questions.

I am very grateful to the employees of Wildlifevets.com who help me with my trials. I truly appreciate all your patience and help with the animals.

I also want to thank Wildlife Pharmaceuticals (Pty) Ltd, the South African Society for Animal Science and Stellenbosch University for their financial support. This research is also supported by the South African Research Chairs Initiative (SARChI) and funded by the South African Department of Science and Technology (UID: 84633), as administered by the National Research Foundation (NRF) of South Africa. The financial assistance of the NRF towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

To my friends in Animal Science and life, Chericke and Mari, I would like to thank you for all your support, advice, coffee, chats and encouragement. You always gave me the boost I needed to carry-on.

Thanks to my little brother and my father. *Dankie vir al jul liefde, ondersteuning en grappies wat my altyd laat lag. Ek is baie lief vir julle en sou nie dit sonder julle kon doen nie.*

Lastly, I would like to thank my wonderful fiancé, Lean. *Baie dankie vir jou oneindige geduld, aanmoediging en liefde. Jou geloof in my het my aangemoedig deur veral die moeilkste tye en is die rede vir my sukses. Ek is oneindig lief en dankbaar vir jou.*

Table of Contents

Alphabetical list of abbreviations

°C	Degrees Celsius
ACTH	Adrenocorticotropic hormone
AUC	Area under the curve
bpm	Beats per minute
breaths/min	Breaths per minute
BWt	Body weight
CCTV	Closed circuit television
CL	Clearance
CO_2	Carbon dioxide
COMT	catechol-O-methyltransferases
CRH	Corticotrophin releasing hormone
DOPA	Dihydroxyphenylalanine
e.g.	Exemplia gratia
ECG	Electrocardiogram
etc	Et cetera
F	Absolute bioavailability
GC-MS	Gas chromatography mass spectrometry
H_0	Null hypothesis
HR	Heart rate
HPA	Hypothalamic pituitary-adrenal axis
hr	hour
hrs	hours
HSD	Hydroxysteroid dehydrogenase
i.e	For example

ICC	Intraclass correlation coefficient
IM	Intramuscular
IV	Intravenous
k_e	Elimination rate constant
LSMean	Least square means
Ltd	Limited
mg/kg	Milligrams per kilogram
min	Minutes
MOA	Monamine oxidase
MRT	Mean residence time
NMDA	N-methyl-D-aspartate
PK	Pharmacokinetic
Pty	Proprietary company
PVC	Polyvinyl chloride
Ref nr	Reference number
REML	Restricted maximum likelihood estimation
rpm	Revolutions per minute
RR	Respiration rate
SABS	South African Bureau of Standards
sec	Second
secs	Seconds
SEM	Sensor electronic module / Standard error of the mean
SIM	Single ion monitoring
$t_{1/2}$	Terminal half-life
U.S.	United States

UK	United Kingdom
Vd	Volume of distribution

List of Figures

List of Tables

Chapter 1
General Introduction

South Africa's game farming industry has grown rapidly over the past 20 years, with the number of game farms estimated at 9000 in 2013, occupying ~20 million hectare of South Africa's total agriculture land (Mabunda, 2008; Boddington, 2010; Van Rooyen, 2013). Of these 9000 farms, ~5000 farms focus on game farming only, with the remainder (~4000 farms) accommodating a combination of game and livestock farming (Taylor *et al.*, 2016; WildlifeCampus, 2016b). According to Van Rooyen (2013), the wildlife industry is ranked as the fifth largest agricultural industry in South Africa, contributing approximately R10 billion annually to the country's general domestic product (GDP).

The four main economic pillars of the game industry include the breeding of game for live sale, ecotourism, production of game products, and hunting (Cloete *et al.*, 2007). Breeding, auctions, ecotourism and hunting have estimated total turnovers of R10 billion, R 2 billion, R 2 billion and R7.5 billion, respectively (Dry, 2012; Cloete, 2015; Janovsky, 2015). The increased scope of the South African wildlife industry, as evident in the income generated through ecotourism, hunting and associated products, has resulted in an increase in the breeding and trade, and thus translocation of wildlife. Translocation, which is defined as the process where wildlife species are transported from one location to be released in another, forms an important component of commercial wildlife production systems (Nielsen & Brown, 1988). Translocation is an important part of many wildlife management programs, such as the stocking of wildlife species, management of endangered or threatened species, the reintroduction of species eradicated from a certain area, and for the removal of animals that may be a nuisance in a certain area (Craven *et al.,* 1998). Transportation of wildlife contributes ~16% (R750 million - R 900 million) of the total turnover of the industry in South Africa, with ~300 000 animals estimated to be translocated per annum (National Agriculture Marketing Council, 2006).

The translocation process is a cause of concern for the welfare of the animals as it can be very stressful, resulting in reduced performance, injury and ultimately increase morbidity and mortality rate in the animals being transported (Knowles *et al.*, 1999; Knowles *et al.*, 1994). Transportation may also result in a decrease in meat quality due to bruising, and can potentially also contribute to the spread of diseases. Animals may be held in captivity following transportation. During this period in captivity, veterinary procedures are performed and when required, especially in cases of research, data is recorded. The captive environment itself represents a form of stress, and concerns regarding animal welfare during captivity are often raised. Various studies have found that wild animals in captivity tend to have a compromised reproduction potential, a shorter life span, and are more susceptible to diseases which result in higher mortalities, when compared to their free-ranging counterparts. Stress is hypothesized to be the major cause of the compromised

wellbeing and performance of animals in captivity (Munson, 1993; Munson *et al.*, 1999; Blay & Coˆte´, 2001; Terio *et al.,* 2004; Ellenberg *et al.*, 2006; Clauss *et al.*, 2007; Clubb *et al.*, 2008).

Regardless of the purpose for maintaining game species in captivity, it is essential that the captive environment accommodate the physiological and behavioural needs of the animal. The needs and thus welfare of animals in captivity can be met through the enrichment of enclosures, and maintaining an optimal standard of the most appropriate husbandry practices. The design of an enclosure will depend on the reason for maintaining the animals, the species, and the number of animals. As captivity is considered as a contributing factor to stress in wildlife, captive wildlife also require special handling and care. While in captivity, the animals should be provided with sufficient shelter, clean water and suitable feed. The diet of the animals should be established specifically for each species, with advice from a registered nutritionist with the experience in formulating wildlife diets (WildlifeCampus, 2016a). Furthermore, sufficient veterinary care should also be provided to the animals if and when needed whilst in captivity (South African Bureau of Standards, 2000). It is essential that the animals are adapted to the captive environment as soon as possible, as free-ranging animals often have reduced appetite in the first week following capture (Osofsky *et al.*, 1995). To aid in the adaptation process, the animals should be provided with natural feed or good quality hay for the first two to three weeks following capture, after which they should be accustomed to being fed artificially and a more balanced ration can be fed (Roosendaal, 1992).

Blesbok (*Damaliscus pygargus phillipsi*) is an antelope species that is abundant in South Africa (Lloyd & David, 2008). This antelope species is popular to farm with in South Africa as they are small to medium in size (55-80 kg), provide for easy handling and can be maintained in paddocks that is enclosed with normal livestock fencing (Frost, 2014; Wildlife South Africa, 2017). Blesbok is also a popular species to hunt for meat. Blesbok meat has a favourable fatty acid profile, low lipid and fat content, and higher amino acid values, when compared to that of duiker (*Sylvicapra grimmia*) and impala (*Aepyceros melampus*) meat (Hoffman *et al.*, 2008). Commercial production of blesbok meat has consequently increased, with various colour variants such as white-blesbok and yellow-blesbok being selectively bred by private game farmers (Taylor *et al.*, 2016).

The increased commercial production of blesbok has resulted in an increase in the transport of blesbok between locations. As previously stated, transportation can be very stressful for the animals, especially wildlife species. The primary causes of the stress during the translocation process is human contact, the noise and movement of the transport vehicle, withholding of food and water and in some cases, exposure to weather extremes (Broom, 2003). Stressed animals are difficult to handle, and tend to injure themselves, other animals or the handlers involved in the transport activities. Mortalities and reduction in meat quality of animals due to the stress associated with transport result in major financial losses (Knowles *et al.*, 1999; Fazio & Ferlazzo, 2003; Minka & Ayo, 2007). Transport stress thus needs to be managed to minimize the

possibility of injury and ensure animal welfare. A potential means to manage transport stress is the use of tranquilizers/neuroleptics and sedatives.

Tranquilizers and sedatives have similar pharmacological effects, and are used to calm animals. Tranquilizers administered at higher dosages than what is recommended by the manufacturer will not have an apparent increase in the degree of action, but will result in side effects. In contrast, sedatives can be administered at very high doses, which may result in what appears to be immobilisation (Gleed, 1987). Sedatives and tranquilizers are also commonly used as adjuvants to anaesthetics to improve immobilisation. Anaesthetics are pharmacological agents that affect inhibitory and excitatory transmission of synapses in the central nervous system and thereby cause animals to lose consciousness and not feel pain (Swan, 1993). Sedatives are used to alleviate undesirable symptoms of anaesthesia, with the latter that may include respiratory depression, hypotension and reduced cardiac output (Taylor, 1991). Some sedatives can however, sometimes cause adverse side effects. Xylazine, a sedative commonly used in both domestic and wildlife species, has been found to cause enhanced depression of the central nervous system, increased airway pressure, lung oedema and hypoxia at higher dosages (Prajapathi *et al.,* 1994).

Benzodiazepines such as midazolam and diazepam are preferred sedatives, for they can act as muscle relaxants, cause minimal effects on the cardiovascular system, and their action can be reversed by the administration of antagonists (West *et al.*, 2014). Benzodiazepines have therefore been proposed as alternative sedatives for animals. Midazolam is preferred to diazepam as a sedative, because it is short-acting, water-soluble at a pH below 4, and provides effective sedation (Nordt & Clarke, 1997; Stegmann, 1998). Midazolam has been successfully used in a number of wildlife species (Stegmann & Jago, 2006; King *et al.*, 2008; Mellish *et al.*, 2010; Olsson & Phalen, 2013; Wenger *et al.*, 2013; Fiorello *et al.*, 2014; Mortenson & Moriarty, 2015). In contrast to the neuroleptics commonly used to immobilise wildlife species, midazolam and other benzodiazepines are also known to stimulate appetite (Berridge & Peciña, 1995). This potentially makes midazolam an ideal sedative for use in captive wildlife species, as it may potentially stimulate appetite that may be reduced due to the stress of captivity.

Drugs can have various effects on the physiology and behaviour of an animal. It is thus essential that the effects of a drug in a specific animal species must be known prior to its use to ensure that it will not be harmful to the health and welfare of the animals. Before any pharmaceutical product can be approved for use in animals, specific pharmacodynamic and pharmacokinetic studies need to be carried out. Pharmacokinetics is defined as the study of a drug's fate from its administration to its elimination from the body (Benet & Zia-Amirhosseini, 1995). Pharmacodynamics is defined as the study of a drug's mechanism of action in the body (Lees *et al.*, 2004). Biotelemetry offers researchers the possibility to study factors that affect an animal's physiological and behavioural homeostasis (Cooke *et al.*, 2004). The Equivital™ EQ02 biotelemetry system, originally designed for use in humans, has been modified for use in blue wildebeest

(*Connochaetes taurinus*) (Laubscher *et al.*, 2015a). The system can measure heart rate, respiration rate and motion, as well as various other physiological parameters. The Equivital™ EQ02 system can therefore potentially be used for measuring these parameters of wildlife species, which could be useful in studying the effects of pharmacological products in ungulate species such as blesbok. The use of the Equivital™ system has however, not yet been validated for use in blesbok.

Even though the use of midazolam in several wildlife species has been documented (Stegmann & Jago, 2006; King *et al.*, 2008; Mellish *et al.*, 2010; Olsson & Phalen, 2013; Wenger *et al.*, 2013; Fiorello *et al.*, 2014; Mortenson & Moriarty, 2015), available information on the potential of midazolam to be used as a sedative/tranquilizer in wild ungulates during captivity and transport, is scarce.

The aim of this study is thus to evaluate the influence of midazolam on the normal physiological and behavioural parameters of blesbok during captivity, using the Equivital™ EQ02 biotelemetry system. To achieve this aim, a pilot study was first conducted using indigenous goats (*Capra hircus*) to determine the time-release profile of midazolam and the best dosages to use in blesbok. Secondly, a validation trial was carried out to determine the accuracy of the Equivital™ EQ02 biotelemetry system in measuring the physiological parameters of blesbok. Following success of the pilot and validation trials, the primary study was then conducted to determine the effects of different midazolam doses on the behaviour and physiology of blesbok by using the Equivital™ EQ02 system. The results from this study will contribute to the formulation of management protocols of blesbok in captivity to minimize stress, which will also assist in addressing animal welfare concerns in the wildlife industry.

Chapter 2
Literature Review

2.1 General overview of South Africa's game ranching industry

Wildlife has evolved from being considered an undesirable competitor for livestock farming in the 19th century to being seen to be a major contributor to South Africa's economy (National Agricultural Marketing Council, 2006; Carruthers, 2008; Dry, 2012). According to Dry (2012), South Africa's game ranching industry has expanded at a rate of 5% per year over the past ten years if measured in real terms. Regarding turnover, the industry has increased at an average of 20.3% per year (Dry, 2012). At present, the wildlife industry contributes ~R10 billion annually to South Africa's global domestic product (GDP). This makes the wildlife industry the agricultural industry that is fifth largest in rank in South Africa (Van Rooyen, 2013).

According to Boddington (2010) and Mabunda (2008), the number of game farms has increased considerably during the last two decades, and presently it is estimated that there are approximately 9000 game farms in South Africa. Of these 9000 farms, ~5 000 farms focus on game farming only, with the remainder (~4000 farms) accommodating a mixture of game and livestock farming (Taylor *et al.*, 2016; WildlifeCampus, 2016b). In South Africa, game farms currently occupy ~20 million ha of South Africa's total agricultural land, which is considerably more than the 14.7 million ha reported by Van Hoven in 2005 (Van der Merwe *et al.,* 2014). According to Van Rooyen (2013), the national wildlife population of South Africa comprises of approximately 21 million game animals. Of these 21 million animals, 16 million are owned privately and 5 million are detained in reserves and parks owned by the state.

Several factors contributed to an increase in game production activities in South Africa. One of the driving forces behind this shift in production can be ascribed to droughts and poor market prices, which resulted in livestock farmers investigating alternative approaches to ensure their commercial viability (Property24, 2015). Game farming was thus considered by an increasing number of farmers due to the lower input production costs and thus higher returns on invested capital, and the fact that most wildlife species are better adapted to arid conditions than livestock species. Game farming can utilize areas unsuitable for livestock farming. Stock theft is also experienced to a much lesser extent on game farms due to the danger associated with wildlife animals, as well as the remote location of most game farms. From these abovementioned factors, it is thus evident that game farming is considered to be more attractive than livestock farming (Otieno & Muchapondwa, 2016).

According to Dry (2016), the South African wildlife ranching industry provides 140 000 jobs, with remuneration being higher than that for jobs in livestock farming, due to the requirement for specialized skills in wildlife production activities. Dry (2016) stated that the local wildlife industry can be considered a major contributor to food security, with more than 150 000 tons of game meat produced per annum. According to Van der Merwe *et al.* (2014), game hunting and its related activities are responsible for 17 806 jobs in Limpopo, 4 558 jobs in the Free State and 9 072 jobs in the Northern Cape of South Africa. One drawback is that this study does not

provide information on the employment opportunities created in the other provinces of South Africa, which would have provided a more representative estimate of the impact of wildlife on job creation in South Africa.

The four main economic pillars of game ranching comprise of the breeding of game, ecotourism, value-adding of game products, and hunting. The breeding of game for auction purposes and related industries contributes more than R10 billion of the income generated by this industry and is also one of the major drivers for the increase in game ranching experienced and mentioned above (Cloete *et al.*, 2015). The local game hunting industry has a total turnover of ~R7.5 billion (Cloete, 2015; Janovsky, 2015), which is a substantial increase from the R6.3 billion reported in 2013 by Van der Merwe *et al.* (2014). From 2005 to 2014 the turnover of game animals sold at auctions have increased from R93 million to almost R 2 billion (Cloete, 2015). Ecotourism has a total turnover of R2 billion (Dry, 2012), with almost 50% of all tourists on holiday in South Africa including a wildlife experience as part of their visit (Janovsky, 2015). Saleable game products, i.e. hides, feathers, horns, eggs and the various manufactured products (e.g. handbags, curios, shoes, clothing, etc.) also contribute considerably to the overall income generated by game farming activities.

Game ranching is responsible for more than 20% of the red meat produced annually in South Africa. Of the >150 000 tons of game meat produced annually, only a small amount is being exported. The export of meat is a yet untapped market and with the country's wildlife numbers, South Africa should be able to establish itself in the international market. Other countries such as New Zealand, for example, exports R4 billion game meat to Europe annually (Dry, 2012). However, one of the major constraints to the export of game meat is the risks of diseases and the control thereof, with the Government being an important role player here through their oversight function. Unfortunately, the Government is not always able to fulfil their role as required by the importing countries. Game products have been calculated to contribute R1.2 billion towards South Africa's economy (Janovsky ErnstJanovsky, 2015). According to Janovsky (2015) South Africa's wildlife industry has a total turnover of R122.7 billion.

As a result of the increase in game farming and related activities in South Africa, the increase in the breeding and trade in wildlife is a logical consequence. An important component of the breeding and trade of wildlife is the translocation of wildlife species, and especially of wild ungulates. Translocation of wildlife species contributes majorly to the total turnover of the industry, with a contribution of ~16% (R750 million - R 900 million), and ~300 000 animals estimated to be translocated each year (National Agriculture Marketing Council, 2006).

Translocation, however, results in increased stress experienced by the animals due to amongst others, more than normal human contact, exposure to weather extremes during transport, the noise and movement of the transport vehicle, and withholding of food and water before and during transport (Broom, 2003). Animals maintained in captivity for the purpose of veterinary procedures, auctions or scientific research purposes also represents a source of stress for wildlife species. Studies by Munson (1993) and Munson *et al.* (1999) indicated a higher incidence of diseases in cheetahs in captivity, which resulted in morbidity and mortalities. Terio *et al.* (2004) found that cheetahs in captivity had higher faecal corticoid levels and lower testosterone levels than free-ranging cheetahs, which indicate a potential suppressive effect of stress on general health and

reproduction. Forest duikers in captivity were more prone to opportunistic infections and higher lamb mortalities occurred than in free-ranging contemporaries that were in good condition (Barnes *et al.*, 2002). Furthermore, giraffe (*Giraffa cameleopardalis*) and elephants (*Loxodonta africana* and *Elephas maximus*) also had shorter lifespans and poor reproduction in captivity versus their free-ranging counterparts (Ellenberg *et al.*, 2006; Clauss *et al.*, 2007; Clubb *et al.*, 2008).

Blesbok (*Damaliscus pygargus phillipsi*) is a common, endemic antelope species to South Africa that is popular to farm with due to the small size of the animal, the ease with which they can be handled and because they can be maintained using normal livestock fencing (Lloyd & David, 2008; Frost, 2014). Blesbok is a popular species to hunt for meat, with blesbok meat having a favourable fatty acid profile, low lipid and fat content, and higher amino acid values when compared to that of other wildlife species such as impala (Hoffman *et al.*, 2008). Commercial production of blesbok has consequently increased (Taylor *et al.*, 2016).

Blesbok are defined as a bastard hartebeest antelope species. These antelopes are commonly found in the plains or open veld of South Africa. Blesbok prefer an open grassland with water as their habitat (Frost, 2014). This species of antelope are grazers and require sufficient amounts of grasses, shade and water for their wellbeing to be maintained. The typical weight of blesbok ranges from 55 to 80 kilograms, and their shoulder height typically falls within the range of 85-100 cm. Adult male blesbok have an average weight of approximately 66 to 73 kg, whilst the females have an approximate average weight of 58 to 64 kg (Wildlife South Africa, 2017). Blesbok are gregarious and diurnal antelope (Wildlife South Africa, 2017). Male blesbok are territorial, but they do not maintain their territories when the concentration of animals formed is large, such as during winter and spring. Herds of blesbok usually consist of rams that are territorial, ewes that are nursing young, as well as herds of bachelors rams without territories (Frost, 2014). Blesbok are seasonal short-day breeders and mating of this species occurs in autumn, from March to May. Blesbok have a gestational period of 8 months and only one lamb is usually born per ewe. Most of the lambs are born between November and January, just after the first summer rains when nutrition is adequate and sufficiently available. Blesbok have an average lifespan of 11 years (Frost, 2014; Wildlife South Africa, 2017).

Wild ungulates, such as blesbok, are exceptionally prone to stress during transport, with transport stress that can manifest in a condition known as capture myopathy. Capture myopathy primarily results from damaged muscle due to overexertion and biochemical changes associated with stress during capture (Montané *et al.*, 2003; Kock & Meltzer, 2006) and has been linked to a high incidence of mortalities during transport of wildlife species (Ebedes *et al.*, 2006; Kleiman *et al.*, 2010). Injuries occurring during transport also result in a reduced meat quality due to bruising on carcasses, which in turn negatively affect the economic returns (Knowles *et al.*, 1999; Fazio & Ferlazzo, 2003; Minka & Ayo, 2007). To comply with animal welfare regulations and to ensure the commercial viability of game farming enterprises, it is imperative that approaches that can minimise the influence of transport stress, as well as stress experienced during maintenance in captivity, need to be developed.

Pharmaceutical intervention in the form of tranquilizers/neuroleptics and sedatives provides a potential approach to decrease the influence of stress on wildlife species in captivity or that are being transported. This

group of pharmaceuticals allows for the management of the stress response in animals by reducing the degree to which the animal responds to stressors. A long acting tranquilizer, zuclopenthixol acetate, has been successful in reducing the effects of stress and handling in elk (*Cervus canadensis*) (Read *et al.*, 2000; Woodbury *et al.*, 2001, 2002). Cattet *et al.* (2004) showed that the intranasal administration of xylazine also reduced stress in elk (*Cervus canadensis*) captured with the net gun method. Another long acting tranquilizer, perphenazine enanthate, has been found to rapidly reduce elevated cortisol levels and elevated heart rate in red deer (*Cervus elaphus*) after a stressor was experienced by the animals (Diverio *et al.*, 1996). Haloperidol has been found to be excellent in sedating fallow deer (*Dama dama*), blesbok (*Damaliscus pygargus phillipsi*), dik dik (*Madoqua kirki*), red hartebeest (*Alcelaphus buselaphus caama*), springbok (*Antidorcas marsupialis*), steenbok (*Raphicerus camperstris*) and duiker (*Sylvicapra grimmia*) (Hofmeyer, 1981). Xylazine has been used for mild to moderate sedation in white rhinoceros (*Ceratotherium simum*) (Raath, 1999). Azaperone tartrate has been found to be a safe tranquilizing agent for the African elephant (*Loxodonta africana*) (Silberman, 1977). The long acting neuroleptics, azaperone tartrate, zuclopenthixol and perhenazine enanthate, have been successfully used to reduce stress in captive rhinoceros (Portas, 2004). Long acting tranquilizers have also successfully reduced stress in captive blue wildebeest (*Connochaetes taurinus*) (Fick *et al.*, 2006; Laubscher, 2015).

The majority of the abovementioned studies, with the exception of that by Portas (2004), Fick *et al.* (2006) and Laubscher (2015), reported on the use of tranquilizers or sedatives in free-ranging animals in an effort to minimise the effect of transportation and handling on animal behaviour and stress. The available literature on the use of sedatives and/or tranquilizers in captive game species, specifically wild ungulates, is scarce. Seen against the background of the increase in commercial game farming activities, it is imperative to investigate the potential of such substances to reduce stress experienced by game species in captivity and especially during transport. It is therefore important to understand the stress response in animals and at which point pharmaceutical intervention can potentially minimise the effect of stress on animal welfare.

2.2 The stress response in mammals

Stress can be defined as the changes to an animal's behaviour and physiology that it uses to avoid or adapt to threats to its internal homeostasis (Wingfield & Kitaysky, 2002). According to Yousef (1988), the stress response is divided into three distinct stages, i.e. the alarm stage, during which the sympatho-adreno medullary axis (SAM) is immediately activated, the resistance stage that involves the activation of the hypothalamic pituitary-adrenal axis (HPA), and thirdly the exhaustion stage, during which the resulting and chronic high glucocorticoid levels will have negative effects, which may eventually lead to death. The way an animal responds to a stressor can be either acute or chronic. In response to a short-term stressor the acute stress response is activated, with the response having a definite start time and lasting for a couple of hours. The chronic stress response is activated when a frequent stressor or a multitude of stressors are perceived or if the exposure to a stressor continuous for a prolonged period of time (Cyr & Romero, 2009).

When a stressor is perceived, a signal of distress is sent by the amygdala to the hypothalamus. The amygdala is a part of the brain that plays a role in the processing of emotions. The hypothalamus communicates with the rest of the body via the autonomic nervous system. The autonomic nervous system controls bodily functions

that occur involuntarily, e.g. breathing, heart rate, dilation or constriction of blood vessels or bronchioles (Milosevic, 2015). The autonomic nervous system is comprised of the sympathetic and parasympathetic nervous systems. During periods of stress, the fight-or-flight response initiated by the sympathetic nervous system will provide more energy to the body, which is necessary for it to react to the perceived threat. The parasympathetic system has the opposite effect, as it triggers a "rest-and-digest" response once the threat has passed so that the body can return to a calm state (Jänig & McLachlan, 2013). Two groups of hormones, the catecholamines and glucocorticoids, have an important function in the ability of an animal to handle stress as well as the effects of stress. When the hypothalamus is triggered by the amygdala when a stressor is perceived, it sends signals to the adrenal glands via the autonomic nerves for the activation of the sympathetic nervous system. The adrenal glands respond to these signals through the release of catecholamines into the bloodstream (Dickens *et al.*, 2010).

Figure **2.1** provides a diagrammatic presentation of the stress response in mammals.

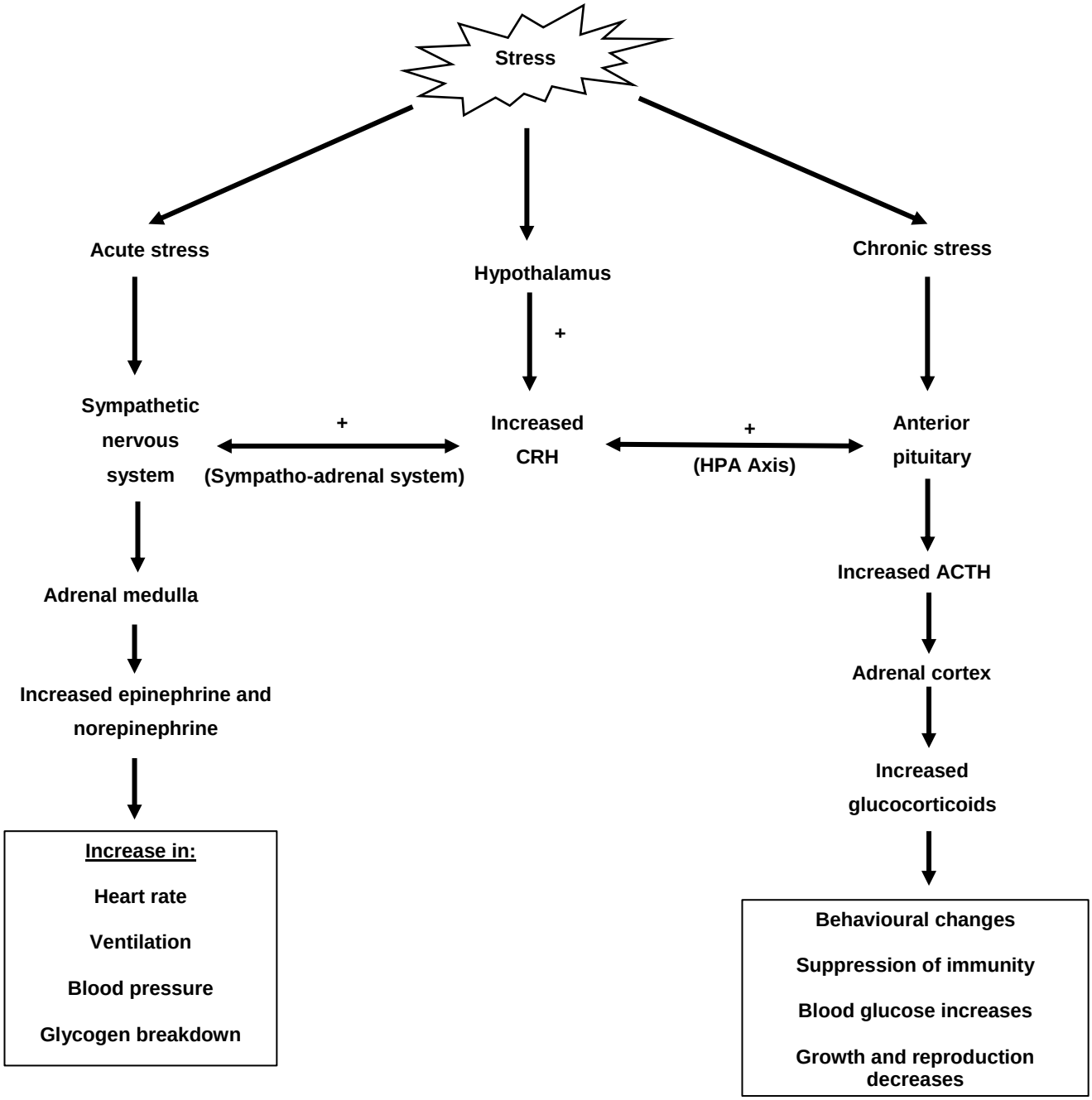

Figure 2.1 The stress response in mammals (adapted from Laubscher *et al.*, 2009).

The catecholamines dopamine, epinephrine and norepinephrine are synthesized from tyrosine by the chromaffin cells of the adrenal medulla as well as the postganglionic fibres of the sympathetic nervous system. Dopamine is the first catecholamine synthesized from the amino acid L-3,4-dihydroxyphenylalanine (DOPA). The further metabolic alteration of dopamine yields norepinephrine and epinephrine (Goldstein *et al.*, 2003). Norepinephrine and epinephrine, respectively also known as noradrenaline and adrenaline, are involved in the immediate fight-or-flight response when a stressor is perceived (Dickens *et al.*, 2010). As the sympathetic nervous system innervates various organs in the body, the catecholamines cause a multitude of physiological effects that include increased heart rate, increased ventilation, blood pressure changes and an increase in glycolysis (Reeder & Kramer, 2005). Catecholamines have a relatively short half-life after they are released, being degraded by catechol-O-methyltransferases (COMT) through a process of methylation or by monoamine oxidases (MAO) through a process of deamination. Monoamine oxidase inhibitors that can bind to MAO, may slow down the degradation of catecholamines (Goldstein *et al.*, 2003).

The fight-or-flight response caused by the catecholamines is followed by a slower hormonal response caused by the hormonal cascade along the hypothalamic pituitary-adrenal axis (HPA). The hypothalamus, pituitary gland and the adrenal glands collectively are referred to as the HPA axis. If a stressor is continuously perceived and the stress is thus chronic, corticotrophin releasing hormone (CRH) is released, which in turn stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). Adrenocorticotrophic hormone then stimulates the *zona fasciculata* cells of the adrenal cortex to increase the synthesis and release of glucocorticoids from the adrenal gland (Axelrod & Reisine, 1984). Glucocorticoids are steroid hormones that consist of a 4-ring carbon backbone, with variable carbon and hydroxyl side chains connected at various positions around these rings. Glucocorticoids promote the metabolism of protein and carbohydrates, exert significant effects on the deposition and metabolism of lipids, regulate the immune system and inflammatory response, increases blood pressure and are necessary for various processes associated with the protection of the body from stressors (Buckingham, 2006). The predominant glucocorticoid released in mammals is cortisol (Sapolsky *et al.* 2000; Dickens *et al.*, 2010). Cortisol is synthesized from cholesterol by the zona fasciculata cells of the adrenal cortex, and is metabolized by the 11-beta hydroxysteroid dehydrogenase (11-beta HSD) enzymes (Buckingham, 2006).

If glucocorticoids are elevated for long periods at a time, as is the case when an animal is under chronic stress, this results in initial hypertrophy followed by hyperplasia of the zona fasciculata cells, which leads to enlargement of the adrenal cortex (Axelrod & Reisine, 1984). Enlargement of the adrenal cortex can result in behavioural changes, increased blood glucose levels, suppression of the immune and reproduction systems, inhibited growth, elevated heart rate, and hypertension. These effects can eventually lead to loss of body weight and a shortening of the animal's lifespan (Munck *et al.*,1984; Sapolsky *et al.*, 2000; Romero, 2004). A study on adult male rats by Ulrich-Lai *et al.* (2006) indicated that chronic stress caused hyperplasia in the outer zona fasciculata cells and hypertrophy in the inner zona fasciculata cells and reduction of the sizes of glomerulosa cells. Adrenal cortical hyperplasia has been observed in captive cheetahs, but not in free-roaming cheetahs (Munson,1993; Munson *et al.*,1999). Thus, long-term stress can severely affect the size and function of the adrenal cortex, which will contribute to abnormalities in behaviour and physiology observed in stressed animals.

Translocation involves various processes causing stress in wildlife species, namely capture and handling, maintenance in captivity or long- term restraint, transportation and being released into a novel environment. Each of these processes elicits the prolonged stress necessary to cause a state of chronic stress in the animals (Dickens *et al.*, 2010). This can be detrimental to the health and thus welfare of the animals due to the abovementioned effects of chronic stress. As translocation is such an important tool in wildlife management, it is very important for those working with wildlife species to understand the stress response resulting from this process in order to prevent the adverse effects of chronic stress and hereby ensure the welfare of the animals when they are being translocated.

2.3 Qualification and quantification of stress and its influence on animal physiology and behaviour

When an animal perceives a stressor it can react by either adjusting its behaviour, physiology or both to alleviate the threat to its homeostasis. However, the set points for homeostasis that the stress response is aiming to maintain can shift with season, life history stage, or other internal (body condition, age, reproductive status) or external factors (weather, risk of predation). Stress is therefore multidimensional in nature, which makes it difficult to define (Dantzer *et al.*, 2014). Measuring stress in animals provides a further challenge. Various endocrine, behavioural, immunological and autonomic nervous system end points can be used to measure stress in animals, but none of these measures can be used as a definite measure of stress. One reason for the failure of these measures to give a clear measurement of stress is that the term stress can be applied to various situations that usually have nothing in common (Moberg, 2000). A single stress indicator may not necessarily be appropriate for all stressor types. The variability between animals in their response to stress proves a further complication in measuring stress.

In order to measure stress in animals, all the factors that contribute towards it should be considered and these may be based on both clinical and biochemical parameters. These parameters include activation of the HPA-axis, heart rate, respiration rate, changes in hormone levels and measures of behaviours. Stress can only be effectively analysed if information from many behavioural and physiological parameters are combined (National Research Council, 2008).

2.3.1 Animal behaviour as an indicator of stress

Exposure to prolonged stress can cause animals to exhibit abnormal behaviour (Capitanio, 1983; Dawkins, 1990) and increased self-harming behaviour (Reinhardt & Rossell, 2001; Bellanca & Crockett, 2002). If the normal behaviour of a specific animal species has been described, certain clinical signs in the animal or deviations from its usual behaviour can potentially be indicative of distress. Animals may show an increased frequency of abnormal motions e.g. head rubbing, or higher frequencies of specific behaviours e.g. scratching. Common behaviour used to study the effects of stress in animals are innate behaviour (e.g. motion, grooming and feeding behaviours), defensive and avoidance behaviour (Beck & Luine 2002; Laubscher, 2015). However, it is important that the context of the behaviour must also be taken into account as factors other than stress could be the cause.

Animal behaviour can differ within the species, gender, strain and physiological state of an animal. Female animals become less active on the first day post parturition for example and this is therefore an expected behaviour, not the result of stress (National Research Council, 2008). Another example of the complex nature of animal behaviour is tail biting in pigs. This behaviour can be caused by a number of factors such as dietary deficiencies, poor ventilation, overcrowding, lack of bedding, insufficient water, breed type, gene expression etc. (Sambraus, 1985; Smith & Penny, 1986; Beattie *et al.*, 2005; Breuer *et al.*, 2005). If these factors are taken into account, however, behaviour can be an accurate measurement of how an animal perceives changes in the environment and can be measured non-invasively.

2.3.1.1 Measurement of animal behaviour

The biological study of animal behaviour, ethology, is done through observing animal behaviour and movement (Ewer, 2013). Ethograms are commonly used to measure animal behaviour and consist of a complete list of behaviours or classes of functional behaviour. In ethograms, behaviour is classified as either an event or a state. An event or point is a short lasting behaviour that can be approximated as a point in time. A state is a longer lasting behaviour of which the initiation and end can be determined (Bowden *et al.*, 2008). Discretely categorized behaviours can thus be used to quantify behaviour by counting the number of times a specific behaviour occurs or the proportion of time the behaviour is exhibited (Ransom & Cade, 2009).

2.3.2 Physiological factors as an indicator of stress

The physiological effects of stress on an animal are mediated via the endocrine, immune and neural systems, and changes in the levels of stress hormones such as cortisol as well as changes in actions of the autonomic nervous system can be used to measure stress in animals (National Research Council, 2008). One of the most common ways to quantify physiological or long-term stress in animals that are not in captivity is by measuring the levels of glucocorticoids in the serum, faeces, urine, saliva, feathers or hair of the animals (Dantzer *et al.*, 2014).

2.3.2.1 Instantaneous measurements of stress

When experiencing chronic stress, individuals can have elevated baseline serum glucocorticoids. Baseline serum glucocorticoid level can be determined by collecting blood at ~3 minutes (min) (Romero & Reed, 2005) or the collection of saliva at 20 min (Kirschbaum & Hellhammer, 1989) following initial capture of the animals. However, a review on the effects of stress in animals by Dickens and Romero (2013) found that chronic stress can have ambiguous effects on baseline serum glucocorticoid levels. Colborn *et al.* (1991) for example found that similar amounts of cortisol were secreted by stallions whether the animals were restrained, exercised or allowed to mate with a mare. According to Dantzer *et al.* (2014) this ambiguity can be the result of the difficulty in obtaining blood samples from mammals within 3 min following capture or it may be due to the animals becoming habituated to the laboratory methodology used to measure long-term stress (Romero & Reed, 2005). The differences in the total (bound and unbound) glucocorticoid measurements versus the measurements of the free glucocorticoids (unbound) can further contribute to the ambiguity of results.

Exposure to chronic stress could for example increase the amount of free glucocorticoids, while causing no difference in total glucocorticoids (Boonstra *et al.*, 2001). This measurement of the severity of the stress

response is impractical in most wildlife species, because the live capture, restraint and anaesthesia of wild animals make measuring the plasma glucocorticoid levels, which must be acquired through the withdrawal of blood within 3 min of capture, difficult. This can result in further costs due to mortality associated with capture (Jacques *et al.*, 2009). The measurement of salivary glucocorticoids could prove to be a more reliable option for determining the magnitude of the stress response in wildlife species as they may still reflect the baseline glucocorticoid values without the need for blood to be drawn (Dantzer *et al.*, 2014).

2.3.2.2 Integrated measurements of stress

Glucocorticoids in faeces, urine, feathers or hair provide a reflection of the integrated average serum glucocorticoids that an animal has secreted, metabolized and excreted over a duration specific to a certain animal species (Sheriff *et al.*, 2011). Dickens and Romero (2013) found that the amounts of glucocorticoids in these integrated methods increased in almost all studied cases. The collection of faecal glucocorticoids is the least invasive method as they do not require capture for most species. Glucocorticoid levels in urine can be useful, but are very difficult to obtain from free-roaming animals. The collection of glucocorticoid levels in hair and feathers is still a relatively new method and there are various methodological problems associated with it. Hairs collected from different parts of the body may have different amounts of glucocorticoids and glucocorticoids in hair may not be representative of glucocorticoid levels circulating in plasma.

2.3.2.3 Downstream measurements of stress

Measures of glucose, fatty acids, immune responses, reproduction and body mass can also be used to determine the extent of the stress response in animals. Glucose levels have been found to increase (Boonstra *et al.*, 1998; Ruiz *et al.*, 2002; Clinchy *et al.*, 2004; Sheriff *et al.*, 2011), while fatty acids and haematocrit has been found to decrease in free-ranging animals experiencing chronic stress (Hellgren *et al.*, 1993; Boonstra *et al.*, 1998; Clinchy *et al.*, 2004; Sheriff *et al.*, 2011). Stress has been found to suppress the immune system (Kanitz *et al.*, 2004). Counts of immune cells, such as lymphocytes and leukocytes, have also been found to decline in free-ranging animals experiencing chronic stress (Baker *et al.*, 1998; Boonstra *et al.*, 1998; Hanssen *et al.*, 2003; Clinchy *et al.*, 2004; Davis, 2005; Lobato *et al.*, 2005; Travers *et al.*, 2010; Sheriff *et al.*, 2011). Stress has also been implicated in a reduction in reproductive performance (Tilbrook *et al.*, 2002; Moore & Jessop, 2003). Animals under conditions of chronic stress have been found to produce less reproductive hormones (Sapolsky, 1985; Boonstra *et al.*, 1998; Hackländer *et al.*, 2003; Delehanty *et al.*, 1997). A drawback of these downstream measurements for the determination of the stress response is that in most cases it requires blood samples to be collected, which involves the capture and restraint of the animals, a highly stressful event (Dantzer *et al.*, 2014).

2.3.2.4 Measuring stress via vital signs

The physiological responses of an animal to stress could also be determined by measuring an animal's vital signs, namely its heart rate and respiration rate following exposure to a stressor. Various studies have shown that the heart rate of animals increases when they experience stress (Stephens & Toner, 1975; Van Putten & Elshof, 1978; Syme & Elphic, 1982; Gomez *et al.,* 1989). The measurement of heart rate can be used to determine an animal's emotional response to an acute stressor, as long as metabolic and emotional effects are distinguished from each other and no disturbance to the animal is caused by the measuring method. Initial

studies of heart rate in free-roaming animals required the use of leads to connect the apparatus to the animal, which interfered with the reliability of the results as it hindered the animal. The use of telemetric systems is considered a much more suitable method for measuring heart rate in animals, as it causes minimal disturbance (Bohus, 1974; Adams *et al.*, 1988; Laubscher *et al.*, 2015a). Respiration rate also increases due to stress and can also be measured without disturbance to the animal via telemetry (Broom & Johnson, 1993).

2.4 The use of telemetry for measuring physiological parameters in animals

Telemetry can be defined as the remote measurement of a subject's physiology, behaviour or energetic status and is an especially useful tool for studying the factors that affect an animal's internal homeostasis, such as when new pharmaceuticals that have been developed to alleviate stress are evaluated (Cooke *et al.*, 2004). Of all the methods available for measuring an animal's physiological parameters, telemetry systems have generally been considered to be superior, as they measure the parameters of fully conscious animals and thus gives a better representation of animals in their normal state (Kramer *et al.*, 2001). Various authors consider telemetry to be the most accurate and reliable method for studying the effects of new pharmaceuticals in free-moving animals (Kramer *et al.*, 1993; Ando *et al.*, 2005; Hayashi *et al.*, 2005; Miyazaki *et al.*, 2005; Sasaki *et al.*, 2005; Laubscher *et al.*, 2015a).

Telemetry systems make use of a combination of small sensors and transmitters in order to detect and propagate biological signals from animals remotely. The analogue signal is then converted into a digital signal by the receiver so that it may be placed into a computerized data collection system, where the data can be manipulated and formatted according to the requirements of the user. Telemetry systems have the ability to measure blood pressure, heart rate, respiration rate, blood flow, pH, electrocardiogram indices and motion of animals (Kramer & Kinter, 2003). With telemetry, data can be continuously recorded for the duration of a stress response in an animal and can seamlessly be combined with the associated behavioural data of the animal (Sanford *et al.*, 2011).

Telemetry system can be invasive and non-invasive in nature. The non-invasive systems involve the use of externally placed surface electrodes for electrocardiogram (ECG) monitoring or cuffs placed on animal limbs (Kramer & Kinter, 2003). Non-invasive telemetry systems are easy to use and more cost-effective than invasive telemetry devices, but tend to cause a significant number of artefacts due to restraint stress. Such systems can also easily be damaged or move from their intended position on the body of the animal. Invasive telemetry systems refer to implantable sensors that can either be placed subcutaneously or within an animal's body cavities. Implantable telemetry systems have less chance of being removed during use and cause little discomfort to the animals (Kramer & Kinter, 2003; Cooke *et al.*, 2013). However, implantable telemetry devices are very expensive and may require surgery to be placed inside the animal. The choice of telemetry system will depend on the type of study and factors such as the amount of animals, study duration and the type of parameters to be measured.

According to Chui *et al.* (2009), conscious and unrestrained animals should be used when determining the safety of the use of new pharmaceutical products in animals. These authors also reported that the internal telemetry systems primarily used to determine the effects of a substance on the bodily functions of animals is

very resource intensive and require restraint of the animals, which has major disadvantages, such as handling stress and limited collection of data. Thus external systems are more suited for short-duration pharmacodynamic studies (Chui *et al.*, 2009; Braga & Burmeister, 2011). This argument was supported by Chiu *et al.* (2009) when these authors found that data collected by two external biotelemetry systems, the PhysioJacket™ and the Jet™, had sufficient sensitivity and comparable measurements for ECG values as an implantable telemetry system in beagle dogs following treatment with a pharmaceutical agent.

The Equivital™ EQ02 system is a biotelemetry system that consists of a belt that contains sensor pads that measure ECG values, and an elastic that measures respiration rate. This system can also measure various other physiological parameters, including motion and skin temperature. The Equivital™ system is not as expensive as implantable devices, easy to use, allows free movement of the animals during monitoring (less chance of causing experimental artefacts such as other non-invasive telemetry devices) and has replaceable batteries, which allow for prolonged use (Kramer & Kinter, 2003; Laubscher *et al.*, 2015a). The Equivital™ EQ02 biotelemetry system was intended for use in humans, but has been successfully modified for use in a wild antelope species, blue wildebeest (*Connochaetes taurinus*) by Laubscher *et al.* (2015a). This system has not yet been used in other wildlife, but holds great potential for the measurement of physiological parameters of especially wild antelope species, such as the blesbok (*Damaliscus pygargus phillipsi*).

2.5 Capture and relocation of wildlife

As previously mentioned, translocation of animals is an important part of the wildlife industry. Translocation of animals has various purposes, which include the introduction of species to previous or new habitats, the management of the environment and animal numbers, the monitoring of species and species health, habituation of injured or orphaned animals to captivity and also for research on wildlife species (Matson *et al.*, 2004; Parker, 2008; Lekolool, 2012; Seddon *et al.*, 2012). The first phase of the translocation process is the capture of the animals. Various capture methods for wildlife exist at present and have been modified according to the social hierarchy, habitat and behaviour of specific species.

Wildlife can be captured by either physical or chemical methods. Physical methods of capture have been found to have a more pronounced effect on the physiology of animals than chemical methods (Morton *et al.,* 1995). The capture method used will depend on the species, reason for capture, the amount of animals to be captured, availability of proper equipment, pharmaceuticals and personnel. In the past, wildlife was primarily captured through chasing them into snares or a noose (Carruthers, 2008; Lekolool, 2012). These methods often led to injuries and mortalities in the animals. Thus chemical immobilisation via the mixture of anaesthetics and tranquilizers is often used by researchers today to facilitate capture and handling of wildlife. Regardless of the method used, capture is a stressful event to wildlife and can often result in injury and mortalities. For ethical and animal welfare purposes it is essential that the method used to capture and handle wildlife minimizes stress, is safe, humane and ensures that the animals are released in the best condition possible (Schemnitz *et al.*, 2009).

2.5.1 Physical capture methods

Physical capture methods can include live traps (for example boxes, nets, snares, pitfalls), kill traps (such as rat traps and pitfalls) and traps that are designed for specific species (Schemnitz *et al.*, 2009; Sikes & Gannon, 2011). Traps and cages are generally used for the capture of hippos, birds of prey, crocodiles and predators (La Grange, 2006). Small ungulates can be herded along fences and into corrals or captured with nets. According to Broekman (2013), the use of drop-nets and net guns has been successful in the capture of small antelope such as nyala, blesbok, and springbok. The use of nets can be beneficial in areas where plastic bomas are difficult to camouflage due to lack of vegetation (Bothma, 2002; SANParks, 2013). The use of nets has however resulted in various injuries and mortalities due to suffocation. Capture via nets is very traumatic for the animals, resulting in higher levels of stress. Human handling is increased during net capture methods, which increase the levels of stress in the animals (SANParks, 2013).

Due to these reasons mass capture boma systems were developed (Dugmore, 2013). According to Dugmore (2013), mass capture systems involves driving species into a boma via helicopter. This has revolutionized game capture in South Africa and made the capture of a large number of animals at a time possible. Mass capture bomas are suitable for the capture of species that are very prone to stress (such as kudu) as they minimize trauma, require no handling and do not require immobilising drugs (SANParks, 2013).

Passive capture sites can be used for capturing animals as well. This method is similar to mass capture boma systems, but does not involve herding of the animals. The animals are lured via attractants, such as food or water, into plastic bomas or drop-nets that have been constructed at watering holes or feeding points. Once a sufficient number of animals has entered the boma, the wall of the boma is sealed or the drop-net is set free. The animals are then caught or herded into a vehicle for transport (Laubscher *et al.* 2015b). This approach is beneficial when there are constraints on time and causes less stress to the animals than methods requiring the animals to be chased. The only disadvantage of this method is that non-target species may also end up inside the boma or nets and will have to be removed (SANParks, 2013).

2.5.2 Capture via chemical immobilisation

Chemical immobilisation involves the use of immobilising drugs, usually in combination with tranquilizers and/or sedatives, via projectile darts shot from pneumatic dart guns or blow-pipes (Ebedes *et al.,* 2006; Kock & Meltzer, 2006). Darting of the animals can be done via helicopter, from a vehicle or on foot. Once the dart is injected, the induction phase follows until the animal enters recumbency. Once recumbent, the animal is held via its horns or head and blindfolded (Ebedes *et al.*, 2006). If the darted animal is a ruminant, its body is held in a sternal position to prevent regurgitation. Consistent monitoring of the animal's vital signs while it is chemically immobilised, is essential. Once the desired procedure(s) have been completed on the immobilised animal, the immobilisation drug's reversal agent is administered and once the animal has fully recovered, the blindfold is removed (Kock & Meltzer, 2006).

Chemical immobilisation requires a qualified veterinarian and adequate knowledge of proper dosage, reversal and the effect of the drugs used. The animal's location must be considered prior to administration of the drugs in light of the time till onset of sedation and recovery to prevent drowning or injury of the animal. If a drug and/or

drug combination takes long to take effect, the risk that an animal located near water will run into the water and drown is increased or the risk that an animal located in rocky terrain will run and break a leg is increased. It is essential that animals be carefully monitored during procedures and following release until their behaviour returns to normal. Animals should not be darted near water or areas where they are open to attack by predators. Care must also be taken to prevent accidental sedation of non-target animals or species (Ebedes *et al.,* 2006).

2.5.3 Transporting of animals following capture

The second phase of the translocation process is the transport of the animals, during which it is essential that animal welfare should be taken into consideration. Thus appropriate practices and equipment suiting the specific species being transported should be applied. It is essential that the guidelines provided by the South African Bureau of Standards (SABS) and the Animal Protection Act No. 71 of 1962 should be strictly followed when transporting wildlife, in order to prevent injury and potential losses (South African Bureau of Standards, 2007, 2008; Animal Protection Act, 1962). Guidelines for the design of pens for the short-lived detainment of animals and for the transit of specific wild herbivore species are given by the South African National Bureau of Standards (2000, 2007, 2008). Furthermore, it is also necessary for all transporters to be registered with the Wildlife Translocation Association. Members of the Wildlife Translocation Association capture and translocate ~130 000 wildlife animals annually (Wildlife Translocation Association, 2017).

When the animals are loaded onto the vehicle the process should not be rushed and if the animals appear exhausted, they should be allowed ample time to rest. Stressed or aggressive animals should preferably be tranquilized. To prevent fighting during transport, mature males should not be transported with younger males. To calm down animals that are stressed, motion and noise by staff should be kept to a minimum. Animals must be counted and grouped depending on the space available. Adequate ventilation and space must be available for the animals and protection of weather extremes must be provided. The design of cages or individual crates should be sufficiently strong, provide adequate ventilation and allow enough space for the specific animal species to move around easily while being transported. Materials used for bedding should mimic as closely as possible the natural environment of the specific captured animal species. In some cases, the animals may need to be treated for endo- and ectoparasites prior to transport. If the animals are not sufficiently calm, they must be offloaded and reloaded into the vehicle. The use of tranquilizers and sedatives to calm animals during transport play a cardinal role in preventing stress (Bothma, 2002; La Grange, 2006; Kock & Burroughs, 2012). The use of these drugs will be discussed in detail in 2.8.

2.6 Maintenance of wildlife species in captivity

Following transport, it is often necessary for the animals to be maintained in captivity for the conduction of veterinary procedures or the recording of data for scientific research, or until a suitable buyer is found. It is essential that the design of enclosures for captive animals should be practical while also taking animal welfare into consideration. Guidelines for the design of enclosures for wildlife species are given by the South African Bureau of Standards (2004).

2.6.1 General design of enclosures

Before a holding facility is constructed, the site of construction should be evaluated with regards to annual weather conditions, water availability and human disturbances (Kock & Burrows, 2012). An enclosure should meet the physiological, physical and behavioural requirements of all animals being housed by supplying sufficient space and adequate resources (National Research Council, 2011). These requirements include protection from rain, shade provision, adequate water troughs and feed containers that will not be a risk of injury for the animals, sufficient ventilation and an acceptable amount of space for animal movement. The facility should be built on a slope for adequate drainage of water and effluent. The design of a facility will also depend on the specific animal species being housed. An enclosure designed for giraffe will for example differ greatly from one designed for a rhino (Bothma, 2002).

2.7 The use of pharmaceutical products in the immobilisation and capture of game species

According to Fowler (2011), there are four basic principles restraint needs to adhere to. It must be safe for the handler, it must maximize the animal's safety, it must ensure that the required procedure can be done successfully, and it must be possible to constantly monitor and pay attention to the animal until it has fully recovered from the physical or chemical effects of the restraint. Fowler (2011) further states that it is essential to have an understanding of the behaviour, characteristics and psychological aspects of animals to ensure their welfare whilst working with them.

The use of chemical immobilisation allows for handling and capture of wildlife to be carried out safer and more cost effective, as compared to physical restraint methods. Prior to chemical immobilisation, it was very difficult to capture wildlife species, especially larger species such as elephant or rhino. Physical restraint of antelope was previously near impossible due to the mortality caused by the stress of the physical immobilisation (Swan, 1993), with capture myopathy contributing to mortalities experienced.

Techniques of sedation, tranquilization and anaesthesia have rapidly developed due to improvements in the performance of drugs and equipment under field conditions. The development of remote injection techniques, such as via blowpipes or pneumatic guns, has made it possible to reliably administer drugs via the intramuscular (IM) route. This development of improved methods for delivering drugs into animals coincides with the development of new drugs that have wider margins of safety and have less negative side effects. The availability of new drugs for immobilising animals has greatly reduced the cost of and improved the safety of handling, transport and adaptation following translocation in wild animals (Gales, 2009). Chemical immobilisation is often used in conjunction with physical methods of immobilisation, with the most common used drugs for the immobilisation and capture of wildlife species including anaesthetics, opioids, hypnotics, sedatives, tranquilizers and neuromuscular blocking agents (Swan, 1993).

The abovementioned pharmaceutical products all need to have certain characteristics to be considered for immobilisation and sedation purposes. According to Swan (1993), an ideal immobilisation drug has an effective dose that does not exceed the amount that can be contained in a dart of appropriate size, with a volume of preferably less than 3 mL. The drug must have a suitable stability, must be rapidly absorbed when administered

via the IM route, and should not cause any irritation or reaction at the site of administration. Drugs of this kind should have a fast onset of action to result in sufficient immobilisation, without having a supressing effect on the functioning of the respiratory and cardiovascular systems. Immobilising/sedating drugs also need to exert their effect for a sufficiently long enough period, and must have a wide safety margin. During cases when immobilising/sedating drugs are used, it is imperative that an antidote is available so that the effects of the drugs are reversed as quickly as possible to prevent any serious side effects. Pregnant animals should also not be negatively affected by the drug, with the drug not posing any danger to the mother and/or the foetus. There should be minimal risk towards personnel handling the drug and both the recovery and induction periods of the drug must be calm and rapid. An ideal immobilisation drug should reduce an animal's awareness of its surroundings, to ensure minimal fear, distress and pain in the animal (Swan, 1993; Walsh & Wilson, 2002).

2.8 Different categories of immobilising drugs and/or sedatives used in the immobilisation of wildlife species

2.8.1 Anaesthetics

Anaesthetics are primarily used to chemically restrain or immobilise animals. The main effects of anaesthetic agents in animals are that they cause recumbency, unawareness and the inability to feel pain. Thereby anaesthetics allow easy handling of the animals. Anaesthetics affect the peripheral and autonomic nervous systems simultaneously, and have both post- and pre-synaptic functions (Swan, 1993; Kock & Burrows, 2012). Anaesthetics can, however, cause complications that lead to difficulties during or after anaesthetic treatment. The main issue with anaesthetics is that they adversely affect the cardiovascular and respiratory systems. Due to these complications that can result from anaesthetics, they are usually used in combination with other drugs such as benzodiazepines and alpha-2-agonists that alleviate these effects. Opioids and cyclohexamines are the most commonly used anaesthetics in wildlife (Walsh & Wilson, 2002).

2.8.1.1 Opioids

Opioids are drugs that are chemically related to compounds that were derived from the seeds of a species of poppy*, Papaverum somniferum*. Opium contains about 20 pharmacologically active compounds, which includes morphine and codeine (Muir & Hubbell, 2014). Opioids interact with specific central and peripheral endorphin receptors that occur in the brain, the autonomic nervous system, the spinal cord, the gastro-intestinal tract, the heart and the kidneys. Opioids also cause species dependent sedation, immobilisation and/or side effects. Opioids may act as agonists or antagonists on the endorphin receptors, are potent analgesics and have various psychotropic effects in different animal species (Swan, 1993; Walsh & Wilson, 2002; Muir & Hubbell, 2014). As opioids have a high potency, they are administered in small volumes and have a rapid onset of action. The adverse side effects of opioids due to overdose include convulsions, catalepsy and muscle rigidity. Bradycardia, respiratory depression, hypertension, inhibition of gastric motility and the animal's thermoregulatory system, hackneyed gait and hyperactivity are other possible adverse side effects that can result from opioids. Opioids commonly used for immobilisation of wildlife species include thiafentanil, carfentanil and etorphine (Walsh & Wilson, 2002; Muir & Hubbell. 2014).

Etorphine is commonly used in ungulates, but is unsuitable for carnivores. Thiafentanil provides rapid knock down and sufficient anaesthesia, but has a very short duration and wide margins of safety. Carfentanil is a very potent opioid with a short phase of induction and may result in re-narcotisation due to its high potency. Carfentanil is not suitable for equid species. Common antagonists to opioids are naltrexone, nalmefene and diprenorphine (Swan, 1993; West *et al.*, 2014).

2.8.1.2 Cyclohexamines

Cyclohexamines cause catalepsy or dissociative anaesthesia by antagonizing the N-methyl-D-aspartate (NMDA) receptor in the central nervous system (Hirota & Lambert, 1996, Bryant, 2013). Drugs belonging to the cyclohexamine class include ketamine, phencyclidine and tiletamine.

Ketamine is lipid-soluble and has a rapid onset of action (Bryant, 2013). Along with being an anaesthetic, ketamine also inhibits pain by blocking impulses of pain conducted to the thalamic and cortical areas of the central nervous system (Sleigh *et al.*, 2014). Ketamine may cause adverse side effects that include depression of the respiratory system, hyperthermia, muscle tremors, cardiac arrest, high blood pressure and hyper salivation. Ketamine is often used in combination with etorphine and thiafentanil due to its adverse effects (West *et al.*, 2014). Phencyclidine has a slow induction period and causes slow recovery time. This drug can result in seizures and therefore phencyclidine is usually used in combination with muscle relaxants such as benzodiazepines. Tilatemine has effects similar to ketamine, but it causes good relaxation of the muscles and less apneustic breathing (Bryant, 2013). Adverse side effects of tilatemine include respiratory depression, tachycardia, hypersalivation, muscle twitches and lung oedema (Kock & Burrows, 2012).

Cyclohexamines typically do not result in the depression of the cardiovascular system, but can cause tachycardia and increases in blood pressure by indirectly stimulating the sympathetic nervous system (Bryant, 2013). Suleiman *et al.* (2012) found the heart rate and blood pressure of 52 patients were increased significantly above baseline values when ketamine was used to induce anaesthesia. In contrast to opioids, cyclohexamines have no antagonists and recovery from these drugs depends on the metabolism and elimination of the drug from the body. Due to their adverse effects when used alone, especially the muscle rigidity they cause, these drugs are often used in combination with benzodiazepines or alpha-2-agonists (West *et al.*, 2014).

2.8.2 Tranquilizers/ Neuroleptic agents

According to Walsh and Wilson (2002), neuroleptic agents are a group of drugs that cause therapeutic modification of behaviour. Neuroleptic agents generally cause a reduction in aggression and reaction without excessively depressing the central nervous system, while still maintaining reflexes and response to pain. Neuroleptics used commonly in wildlife are phenothiazines, butyrophenones and thioxanthine derivatives (Walsh & Wilson, 2002). These drugs cause sedation by blocking post-synaptic dopamine receptors, D2 dopaminergic receptors specifically, in the central nervous system. They thus inhibit or decrease dopamine neurotransmission in the forebrain (Seeman, 2002).

2.8.2.1 Butyrophenones, phenothiazine derivatives and thioxanthene derivatives

Butyrophenones, phenothiazine derivatives and thioxanthene derivatives cause sedation, reduction of anxiety and muscle relaxation, but generally do not inhibit pain. These drugs suppress movements but not spinal reflexes or nociceptive avoidance behaviours (Fish *et al.*, 2011).

Butyrophenones are neuroleptic drugs that cause their effect by blocking central dopaminergic receptors and peripheral adrenergic receptors (Swan, 1993). These drugs are very effective for treating vomiting or nausea, which usually results from the use of opioid analgesics (Clarke & Trim, 2013). Drugs belonging to the butyrophenones class include azaperone and haloperidol. Both azaperone and haloperidol can be administered via the IM or intravenous (IV) route (West *et al.*, 2014). Azaperone is a short acting neuroleptic that produces a dose dependent sedation and can potentiate anaesthesia and sedation resulting from other drugs. The effects of azaperone last three to six hours (hrs). Azaperone has minimal effects on the respiratory and cardiac systems and can alleviate respiratory depression (Clarke & Trim, 2013). Haloperidol is an intermediate butyrophenone as it effects last 12 to 18 hrs, and is very effective in tranquilizing antelope or gazelle species (Swan, 1993; West *et al.,* 2014). Adverse side effects resulting from the use of butyrophenones alone include hallucinations, mental agitation and aggression. Butyrophenones only have minor effects on the cardiovascular system, but hypotension may be caused by the blocking of α1-adrenergic receptors. If administered at high dosages, butyrophenones can sometimes result in the urge to consume abnormal foodstuffs such as dirt, ice, paint or clay (allotriaphagia). There are no reversal agents available for either azaperone or haloperidol (Swan, 1993).

2.8.2.2 Phenothiazine derivatives and thioxanthene derivatives

Phenothiazine derivatives effect either the central neurotransmission or neuronal terminal junctions in peripheral tissues. These drugs are antagonists of dopamine and their primary effects are therefore calming and mood alteration. Phenothiazine derivatives typically do not have pain inhibitory effects (Clarke & Trim, 2013). Adverse side effects of phenothiazines include hypotension, hypothermia and seizures (Muir & Hubbell, 2014). Phenothiazine derivatives are able to block α1-adrenoceptors and therefore they have an antiepinephrine effect, resulting in reduced blood pressure and associated side effects. Several phenothiazine derivatives can also cause prolapse of the penis (Clarke & Trim, 2013). There are no antidotes available for phenothiazines. Drugs belonging to the phenothiazine class include the short acting drugs acetylpromazine and propionyl promazine, and the long-acting drugs perphenazine enanate and pipotiazine palmitate (Swan, 1993; Walsh & Wilson, 2002).

Acetylpromazine is a competitive antagonist and it has receptors for both dopamine and acetylcholine. It has anti-histamine properties and can cause extrapyramidal effects at high dosages (Clarke & Trim, 2013). The sedation from acetylpromazine can last anything from 4 hrs to up to 24 hrs, depending on the dose. In horses it has been found that the sedation lasted for 12 hrs after an acetylpromazine dose of 0.1 mg/kg body weight (BWt) and lasted 16-24 hrs after an acetylpromazine dose of 0.15 mg/kg BWt (Parry *et al.*, 1982). Acetylpromazine can inhibit thermoregulation and cause hyperglaecemia. Propionyl promazine, perphenazine enanate and pipotiazine palmitate have very similar actions, sedation and effects as acetylpromazine (Swan, 1993; Clarke & Trim, 2013). Perphenazine enanthate has an onset of action of 12 to 16 hours and its effects

can last for 10 days. Pipotiazine palmitate has an onset of 72 hrs and its effects can last for up to 21 days (West *et al.*, 2014).

Thioxanthine derivatives are related to phenothiazines, and have long-lasting effects. Thioxanthine derivatives have anti-emetic, anti-histamine and anti-nausea properties. These drugs are also able to potentiate analgesics, sedatives as well as anaesthetic actions in general (Goodman, 1996). Post-synaptic dopamine receptors are blocked by thioxanthine derivatives in the central nervous system. Thioxanthene derivatives can block alpha-adrenergic receptors as well. Zuclopenthixol acetate (Acuphase) is a thioxanthine derivative that has a short onset of action and its effects can last for three to four days. Zuclopenthixol has very few side effects and a wide safety margin (Swan, 1993).

2.8.2.3 Long-acting neuroleptics

According to Ebedes (1993), long-acting neuroleptics such as haloperidol, perphenazine enanthate, pipothiazine palmitate and zuclopenthixol acetate are formulated by dissolving fatty acid esters of the basic tranquilizer in vegetable or medicinal oil. This causes a slow release of the drug that allows for the action of the drug to be longer in duration. For example, sedation from perphenazine lasts for 10 to 12 hrs and up to 72 hrs for pipothiazine palmitate when used in deer. Long-acting neuroleptics are used especially in wild animals such as deer to facilitate handling and reduce stress during capture as they reduce fear and anxiety for longer periods of time (Walsh & Wilson, 2002).

The use of long acting neuroleptics has raised concerns for animal welfare, as various studies have shown that certain long-acting neuroleptics tend to cause a reduction in feed intake in animals. For example, Fick *et al.* (2005) found that treatment with haloperidol, zuclopenthixol acetate and perphenanzine enanthate reduced feed intake in rats. According to West *et al.* (2014), the long-acting (oil based) formulation of haloperidol is not commonly used in gazelle and small antelope as it tends to lead to prolonged anorexia. According to Portas (2004), the use of perphenazine enanthate in white rhino (*Ceratotherium simum*) going into a boma should be avoided as this long-acting neuroleptic drug leads to reduced feed intake and thus anorexia in white rhinos. The use of long-acting neuroleptics has been implicated in anorexia in other wildlife species, such as giraffe (*Giraffa camelopardalis*) and cheetahs (*Acinonyx jubatus*) (Huber *et al.*, 2001; West *et al.*, 2014). It is thus clear that long-acting neuroleptics can cause anorexia in wildlife species and this can be especially detrimental for animals being kept in captivity for long periods of time, and that need to be sedated with these drugs.

2.8.3 Sedatives

Both tranquilizers and sedatives have the same effect, and are used to calm animals. Sedatives differ from tranquilizers in their effects at higher doses. When tranquilizers are administered at dosages higher than what is recommended by the manufacturer, they tend to cause side effects without an apparent increase in the degree of action. In contrast, sedatives can be administered at very high doses to the degree that they appear to cause immobilisation (Gleed, 1987). Alpha-2-agonists and benzodiazepines are the most common sedatives used for the immobilisation of animals.

2.8.3.1 Alpha-2 agonists

Alpha-2-agonists act on alpha-2-adrenoreceptors located in neuronal and non-neuronal tissues. Pre-synaptically, these receptors modulate neuron function, while post-synaptically they mediate vascular smooth muscle constriction in the periphery or on hormonal receptors outside of the central nervous system (Swan, 1993). Alpha-2-agonists stimulate presynaptic alpha-2-receptors, which results in neuron hyperpolarization and causes these receptors to exert negative feedback to inhibit the release of norepinephrine (Clarke & Trim, 2013). According to Walsh and Wilson (2002) alpha-2-agonists are potent agents that cause dose-dependent, predictable sedation and analgesia. Alpha-2-adrenoceptor agonists have been used in various wild herbivore and carnivore species as synergists in combination with opioids or cyclohexamines (West *et al.*, 2014).

As synergists, alpha-2-adrenoceptors reduce the required dosages of the drugs they are being used in conjunction with, they improve induction times and cause improved relaxation in the animals (Swan, 1993). According to Clarke and Trim (2013), common side effects resulting from alpha-2-agonists include modifications to the cardiovascular, pulmonary and gastrointestinal systems. Common alpha-2-adrenoceptor agonists include xylazine, detomidine and medetomidine. The antagonist drugs used for alpha-2-agonists are yohimbine and atipamizole (West *et al.*, 2014).
Xylazine causes sedation, analgesia and is a muscle relaxant (Muir & Hubbell, 2014). Xylazine is usually used in combination with opioids to enhance sedative and analgesic effects (West *et al.*, 2014). Its sedative effects tend to last up to two hours, but its analgesia effects only last 30 min. According to Clarke and Trim (2013), xylazine causes dose-dependent sedation in ruminants and the higher dosages usually used in these animals may result in recumbency and unconsciousness. Xylazine is considered as a safe sedative in various animal species, but severe reactions have still been observed, such as excessive excitement in horses and death in cattle due to hypoxaemia resulting from the drug. General adverse side effects of xylazine include hypotension, bradycardia and respiratory depression (Muir & Hubbell, 2014).

Both detomidine and medetomidine have the same effects as xylazine, but are higher in potency. Detomidine reduces electrical activity in the bovine uterus at lower dosages, whereas xylazine can cause contractions of the uterus. Furthermore, detomidine has the advantage over xylazine in that it reduces the occurrence of recumbency (Clarke & Trim, 2013). The effects of detomidine lasts much longer than that of xylazine and it is also often used in combination with opioids (Swan, 1993). Medetomidine is more selective than xylazine and thus it binds more strongly to adrenaline receptors (West *et al.*, 2014).

2.8.3.2 Benzodiazepines

Benzodiazepines are neuropsychoactive drugs whose chemical structure consists of a benzene ring fused with a diazepine ring. This group of drugs causes sedation by depressing the limbic system, and muscle relaxation by inhibiting internuncial neurons in the spinal cord (Clarke & Trim, 2013).

Neuropsychoactive drugs such as benzodiazepines act on Gamma-aminobutyric acid type A (GABAA) receptors in the central nervous system. Gamma-aminobutyric acid is an inhibitory neurotransmitter. Benzodiazepines do not directly activate the GABAA receptors, but they cause these receptors to become more responsive to GABA by increasing the frequency by which the chloride channels are opened, thereby

potentiating the inhibitory effect of GABA (Olkkola & Ahonen, 2008). Therefore, benzodiazepines have sedative, anxiolytic, anticonvulsant and muscle relaxant properties. Benzodiazepines have a favourable therapeutic index and a low toxicity. These drugs are used in veterinary medicine mainly as an adjunct to anaesthetics, in combination with drugs that cause muscle rigidity or to reduce doses of other anaesthetic drugs (Ferreri *et al.,* 2015).

The induction dose is reduced by benzodiazepines through various mechanisms, such as pharmacodynamic interactions with hypnotic drugs. Benzodiazepines can be administered orally, intranasally, rectally and by the IM route (if water soluble). An advantage of benzodiazepines is that they have been found to stimulate appetite (Van Miert *et al.*, 1989). Adverse side effects resulting from benzodiazepines include depression of the respiratory system, airway reflex impairment, depression of the cardiovascular system, consciousness impairment and coma (Heeremans & Absalom, 2010). Benzodiazepines commonly used as sedatives in animals are midazolam and diazepam. See Table 2.1 for the pharmacokinetic characteristics of commonly used benzodiazepines in humans (Heeremans & Absalom, 2010).

Table 2.1 The pharmacokinetic characteristics of commonly used benzodiazepines in humans (adapted from Heeremans & Absalom, 2010).

Drug	Bioavailability (Oral) %	Volume of distribution (liters/kg)	Distribution half-life ($t_{1/2\alpha}$) (min)	Elimination half-life ($t_{1/2\beta}$) (hrs)	Clearance (mL/min)	Active metabolites
Temazepam	80	1.3-1.5	48-162	5.1-15.3	60.9	No
Midazolam	48	0.8-1.14	Mar-38	2.1-2.4	202-324	No
Lorazepam	93	1.14-1.3	10-Mar	14.3-14.6	77	No
Diazepam	100	0.9-1	9-130	31.3-46.6	26-35	Yes

2.8.3.2.1 Diazepam

Diazepam is a benzodiazepine that is not water-soluble. It is anxiolytic, prevents convulsions, is a muscle relaxant and has a low toxicity (Clarke & Trim, 2013). This benzodiazepine has been found to have an elimination half-life of 3.2 hrs in dogs (Löscher & Frey, 1981) and in horses it has been found to have a half-life varying from between 2.5 and 21.6 hrs (Muir *et al.*, 1982). According to Klotz *et al.* (1976), diazepam has an elimination half-life of 32.9 hrs in humans and 7.6 hrs in dogs. According to more recent literature, diazepam in humans has an elimination half-life within the range of 31.3-46.6 hrs (Heeremans & Absalom, 2010). If administered by the IV route, diazepam can cause respiratory depression. If administered by the IM route, diazepam has variable pharmacokinetic effects (Swan, 1993). When used alone in healthy animals, diazepam's sedative effects seem to be minimal (Clarke & Trim, 2013). When used in ill animals, diazepam reduces the required anaesthetic dose and causes good stability of the cardiovascular system when combined with opioids for anaesthetic induction (Psatha *et al.*, 2011). Diazepam is especially beneficial when used before or in combination with ketamine, as it reduces the hallucinations that typically result from this anaesthetic and this combination of drugs has been used in various animal species (Clarke & Trim, 2013). According to Walsh and Wilson (2002), diazepam given at high doses orally made it possible to handle fallow deer. According to Muir and Hubbell (2014), diazepam causes minimal cardiovascular and respiratory suppression in comparison to other central nervous system depressants. Muir and Hubbell (2014) further stated that diazepam has minimal adverse side effects, but could lead to excitement in dogs and excessive suppression of the central nervous system if overdosed. Diazepam has been found to cause unpredictable tranquilization in red deer, resulting in aggression and abnormal behaviour (Walsh & Wilson, 2002). Walsh and Wilson (2002) argued that the reason for the unpredictable behaviour caused by diazepam in red deer could be due to the drug's anxiolytic effects, which removes inhibitions in the animals.

2.8.3.2.2 Midazolam

Midazolam is a benzodiazepine sedative that is four times more potent than diazepam. In contrast to diazepam, midazolam is water-soluble, short-acting, has a more constant absorption and a better bioavailability following IM administration (Reves *et al.,* 1985; Stegmann, 1998). The best benefit midazolam has over diazepam is its water-solubility and because of this no pain is also experienced during administration (Wright *et al.*,1993).

Midazolam has a pH of 3.5 and at pH levels above 4, midazolam becomes soluble in lipids (Clarke & Trim, 2013). In humans and dogs, midazolam has been found to have a shorter elimination half-life than diazepam. Elimination half-lives of midazolam administered via the IV route to humans have been reported to fall within the range of 1.3-3.4 hrs (Smith *et al.*, 1981; Puglisi *et al.*, 1978 Greenblatt *et al.*, 1981). According to recent literature, midazolam has an elimination half-life in humans of 2.1-2.4 hrs (Heeremans & Absalom, 2010). Midazolam had an elimination half-life of 77 to 98 min in dogs (Hall *et al.*, 1988; Court & Greenblatt, 1992).

When administered by non-IV routes, midazolam has a good bioavailability (Clarke & Trim, 2013). After IM administration midazolam is rapidly absorbed and rapidly penetrates the blood brain barrier. Midazolam is administered to facilitate restraint as well as to aid anaesthesia induction (e.g. isoflurane) in animals (Naritoku & Sinha, 2000). According to a study on goats by Dzikiti *et al.* (2011), midazolam resulted in a reduction in the minimum alveolar concentration of isoflurane and the quality of recovery from anaesthesia was good, thus proving that midazolam aids in anaesthesia induction and recovery. Midazolam is metabolized in the liver and the resulting metabolites are excreted via the kidneys. Elimination of midazolam occurs in a biphasic manner and consists of an initial rapid redistribution phase, followed by a slower terminal phase (Reves *et al.*, 1985). Infusing sedation in healthy animals with midazolam alone via the IV route is difficult and can result in abnormal excitement reactions in the animals (Covey-Crump & Murison, 2008). When used in ill animals or when combined with opioids or ketamine, midazolam provides good hypnosis.

The antagonist flumazenil is used to reverse midazolam (Clarke & Trim, 2013). In contrast to the long-acting neuroleptics commonly used in wildlife immobilisation, midazolam and other benzodiazepines are known to stimulate appetite in animals (Van Miert *et al.*, 1989; Berridge & Peciña, 1995). This makes midazolam a potentially ideal sedative for use in captive wildlife species. Due to sedation not always being guaranteed by using midazolam alone, there are a lack of studies in animals where midazolam is used as a sedative on its own. However, various studies have been done on the use of this benzodiazepine in combination with analgesic or anaesthetic drugs for sedation, as a premedication or for anaesthesia (Clarke & Trim, 2013).

Feed intake is essential for an animal's health and production, as all the nutrients (vitamins and minerals) necessary for the productivity of an animal are obtained from the feed it consumes. Productivity refers to growth, reproduction and the production of e.g. milk, draught strength, hair growth etc. of animals. Animals have a minimum protein and energy requirement for the maintenance of normal bodily functions. In ruminants, maximizing feed intake increases the provision of nutrients to rumen microbes, which in turn increases nutrients available to the animal for growth, reproduction, production and the maintenance of body condition (Naseri & Kabul-Afghanistan, 2005).

According to Pulina *et al.* (2013), an animal's energy homeostasis is dependent on the integration of signals from the periphery. These signals are humoral, neurohumoral or neural (Seoane *et al.*, 1972). According to Bear *et al.* (2007), there exists both appetite stimulating neuropeptides and appetite suppressing neuropeptides. The secretion of leptin hormone from the animal's adipose tissue stimulates the secretion of these neuropeptides in accordance with the animal's energy status. If the animal's energy status is positive, leptin secretion will increase which will cause a certain group of neuropeptides to be secreted by the

hypothalamus, which in turn will increase the animal's metabolism by increasing the levels of thyroid stimulating hormone and adreno-corticotropic hormone secreted by the pituitary gland. This will decrease voluntary intake in the animal. In the case of a negative energy balance, leptin secretions will decrease, which will cause another set of neuropeptides to be secreted that inhibit thyroid stimulating hormone and adreno-corticotropic hormone secretion and thereby reducing metabolism. This then stimulates voluntary feed intake in the animal (Bear *et al.*,2007; Pulina *et al.*, 2013).

Midazolam and other pharmaceutical products can affect an animal's appetite and potentially reduce feed intake, which can thus be detrimental to an animal's productivity and health, especially if animals are kept in captivity or transported for long periods of time. Phenothiazine derivatives, which are commonly used for the immobilisation of wildlife species, have been found to cause anorexia (Knox *et al.*, 1990; Kock & Burroughs, 2012). Perphenazine is not recommended for use in white rhinoceros (*Ceratotherium simum*) as it causes anorexia in these animals (Portas, 2004; Kock & Burroughs, 2012; West *et al.*, 2014). The long acting formulation of haloperidol is not commonly used in gazelle due to the risk of causing anorexia (West *et al.*, 2014). Long acting neuroleptics have been found to cause anorexia in giraffe (*Giraffa camelopardalis*) (West *et al.*, 2014). Various studies have reported that benzodiazepines increase food intake in animals (e.g. Randall *et al.*, 1960; Bainbridge, 1968; Brown *et al.*, 1976; Fratta *et al.*, 1976; Baile & McLaughlin, 1979; Mereu *et al.*, 1979; Cooper,1980; Brown *et al.*, 1981; Berridge & Pacina, 1995; Ilkiw *et al.*, 1996). Ilkiw *et al.* (1996) found that midazolam specifically increased feed intake in cats in a dose dependent manner. A drug that stimulates appetite can thus be preferred in situations of captivity or transport. It is thus necessary for feed intake to also be measured when studying the effects of a drug on the physiology of an animal. According to Barboza *et al.* (2008), measurement techniques for feed intake can be either direct or indirect. Direct measurements include measuring the behaviour of the animal or measuring the mass difference between total feed given and feed left after the total feeding period. Digestible or indigestible markers incorporated into feed can be used to indirectly measure feed intake (Barboza *et al.* 2008).

2.9 Determining the pharmacokinetic values of pharmaceuticals

Pharmacokinetics refers to the complex set of events that occur following the administration of a drug into the body (Wanamaker & Massey, 2009). This refers to the drug's liberation from the administration form, absorption, bioavailability, distribution, metabolism and excretion. Pharmacokinetics makes it possible to quantify the absorption, distribution, metabolism and excretion of drugs (Nishant *et al.*, 2011). By quantifying these processes, it is possible to determine the concentration of a drug in the body. It is essential to have an understanding of these factors in order to design a pharmacokinetic study (Nishant *et al.*, 2011). Absorption indicates the movement of a drug from the area of administration to the blood. Distribution refers to the movement of drugs from the blood after absorption to tissues (Ciccone, 2015). Elimination is defined as the irreversible reduction in the concentration of a drug at the site of measurement (Tozer & Rowland, 2006). Elimination occurs via metabolism in the liver and excretion primarily via the kidneys (Tozer & Rowland, 2006). The absorption, distribution and elimination information of a drug can be determined by measuring the concentration of a drug in blood, urine or other fluids at different times following administration (Urso *et al.*, 2002). The point where the drug's absorption is equal to the drug's elimination is referred to as the steady state (Wanamaker & Massey, 2009).

2.10 Design of pharmacokinetic studies

The design of a pharmacokinetic study depends on what the researcher is investigating or assessing. An optimal experimental design for any pharmacokinetic study is one in which the variables chosen will maximize the information that can be obtained from an experiment (Aarons & Ogungbenro, 2010). According to Nishant *et al.* (2011) a pharmacokinetic study should be designed in a manner that a drug 's effects can be distinguished from effects resulting from other factors. Nishant *et al.* (2011) further states that for a study where two formulations are compared, a two period, two-sequence crossover design should be used, with an adequate washout period between the phases. For studies where only a single formulation is studied, a single centre, single dose, random crossover design should be used (Nishant *et al.,* 2011).

There are certain conditions that must be defined clearly before a pharmacokinetic experiment can be planned. These conditions include the administration route of the drug, the dosage, the tissues that will be sampled, time of sampling, analytic method and the species. This information and the purpose of the experiment should always be clearly given when designing a pharmacokinetic study (Urso *et al.*, 2002). There are various routes by which a drug can be administered. This includes IV, oral, IM and subcutaneous routes. Administration by the IV route allows for rapid onset of action, but causes a short duration of drug action. Administering a drug via the IM route results in a slower onset of action, but a much longer duration of action than the IV route (Wanamaker & Massey, 2009).

The number of subjects/animals used in a pharmacokinetic study should be statistically significant and allow for potential withdrawals or removals from the study (Nishant *et al.*, 2011). Aarons and Ogungbenro (2010) stated that optimal design will lead to lowered costs, as it may lead to a reduction in the number of animals/subjects required for the study. The number of animals used in the study should ensure results that are scientifically valid. There should also be justification for the gender composition of the animals in the study (U.S. Department of Health and Human Services, 2015).

It is also essential that variation be minimized. This can be achieved by ensuring that the subjects used in the study are acceptable and standardized (Nishant *et al.*, 2011). The subjects used in the study should be healthy and allow differences to be observed between drugs being studied (U.S. Department of Health and Human Services, 2015). Subjects can be either male or female, but the chosen gender should be consistent with safety and usage criteria. The study environment, the diet, water intake, posture post dosing, exercise and sampling schedules should all be standardized to minimize variation. In a single dose study, subjects should not receive food for at least 10 hrs prior to the study. The subjects may be allowed to consume water during the study (Nishant *et al.*, 2011).

With regards to sampling, at least three points of sampling should be present during absorption of the drug, three to four sampling points for the time to maximum concentration (T_{max}) and four sampling points during the elimination phase of the drug. Venous samples can be collected via an IV catheter or via direct venepuncture of an anticubital vein such as the jugular vein. The blood samples should be collected into tubes that contain heparin or other anticoagulants (Nishant *et al.,* 2011).

The bio-analytical method used to determine the drug or metabolites in the plasma, serum, blood or urine should be validated, standardized and well characterized to ensure that reliable results are obtained and can be sufficiently interpreted (U.S. Department of Health and Human Services, 2015). Mass spectrometry is generally used in pharmacokinetic studies as the matrix is complex in nature and a high sensitivity is required to determine concentrations following a low dose over a long time. Light chromatography mass spectrometry, gas chromatography mass spectrometry and high performance liquid chromatography are the most common bio-analytical methods used in pharmacokinetic studies (Nishant *et al.*, 2011). High performance liquid chromatography has been found to be more sensitive and accurate than a microbiological assay (El Badawy *et al.*, 2015).

The analysis of pharmacokinetic data is performed using compartmental or non-compartmental methods. Non-compartmental methods approximate the AUC of a concentration-time curve to determine the exposure to a drug. Compartmental methods use kinetic models to determine the concentration-time curve (Nishant *et al.,* 2011).

The size of a data set collected from a pharmacokinetic study is often very large. It is therefore beneficial to synthetize the data without losing information that is relevant. Thus the following few pharmacokinetic parameters can be defined: peak concentrations (C_{max}), T_{max}, terminal half-life (t ½) and the AUC. These four parameters can summarize a single kinetic profile. By having more than one profile (at least 8 parameters) the mean and standard deviation of these parameters can provide a summary of a drug's kinetics in the complete population (Urso *et al.*, 2002).

2.11 The basic pharmacokinetic parameters that are measured in a pharmacokinetic study

2.11.1 Time to peak concentration (T_{max}) and peak concentration (C_{max})

The T_{max} and the C_{max} can be determined directly from experimental observations of each subject (the concentration-time curve plotted from the data). Following IV administration of a drug, T_{max} and C_{max} closely depend on the protocol of the experiment, as the concentrations are continuously decreasing after the dose is administered. If the drug is infused intravenously at a constant rate, the peak concentration will correspond to the time of infusion. Following oral administration T_{max} and C_{max} depend on the absorption rate and extent, as well as on the drug's disposition profile (Urso *et al.*, 2002).

2.11.2 Area under the curve (AUC)

This parameter may be used as an index of the body's drug exposure or as an index of the drug exposure of specific tissues. Under general assumptions, the area under the plasma or blood concentrations is very dependent on the amount of the drug that enters systemic circulation and on the system's ability to eliminate the drug from the body. Thus AUC can be used to determine the amount of the drug that is absorbed or how efficiently it is eliminated. A sufficiently accurate estimation of the AUC can be achieved by using the trapezoidal rule. Following single drug administration, the AUC should be calculated from time 0 to infinity, while the AUC should be calculated within the dose interval following multiple drug administration (Urso *et al.*, 2002).

2.11.3 Volume of distribution and clearance

Even though the most commonly measured variables in pharmacokinetics is the concentration of the drug in the plasma or tissues, the researcher may also be interested in how much of the drug is present in the body or how much of the drug is eliminated from the body at a specific time after administration of a specific dose. Thus volume of distribution and clearance are measured (Urso *et al.*, 2002). The volume of distribution is the ratio of the amount of the administered drug to the plasma concentration of the drug. If the volume of distribution equates to the total plasma volume this will mean that the drug is poorly distributed in the tissues and occurs mainly in the plasma. If a drug were to be in equilibrium with all the body tissues, the apparent total volume of distribution would be defined as the volume of distribution at steady state (Vd_{ss}). Having knowledge of the apparent volume of distribution makes it possible to calculate initial dosages of a drug to administer and aids in achieving the desired concentration of the drug in the tissues and blood (Clarke & Trim, 2013). Ciccone (2015) defines clearance as the ability of all tissues or organs to eliminate a drug (systemic clearance), or the ability of one organ or tissue to eliminate a drug. Clearance depends on an organ or tissue's ability to remove the drug from the plasma and the organ's perfusion (Ciccone, 2015). Urso *et al.* (2002) stated that clearance is a measure of how efficiently a drug is eliminated from the body. The following equations are used to calculate the clearance (CL) and the volume of distribution (Vd) (Ciccone, 2015):

$$CL = Q \times \frac{(C_i - C_o)}{C_i}$$

Q – Blood flow to the original organ (determines the amount of the drug delivered to the organ for elimination)

C_i – Concentration of drug entering the organ

C_o – Concentration of drug exiting the organ

$$Vd = \frac{Amount\ of\ drug\ administered}{Plasma\ concentration\ of\ drug}$$

2.11.4 Biotransformation

Biotransformation refers to the chemical transformation of a drug from its form at administration to a form in which it can be eliminated from the body (Wanamaker & Massey, 2009). Biotransformation results from the oxidation, reduction, hydrolyses or conjugation of a drug. Biotransformation causes an altered version of a drug to be produced, which is called a metabolite (Ciccone, 2015). The majority of biotransformation occurs in the liver via cytochrome P450 enzymes (Wanamaker & Massey, 2009). These enzymes catalyze the changes in the structure of the drug and hereby reduces the drug's pharmacological effects. The pharmacological activity of a metabolite is greatly reduced from that of the original drug prior to biotransformation. The main function of biotransformation is termination of a drug (Ciccone, 2015).

2.11.5 Elimination

The main site of drug excretion is the kidneys. Once a drug has been metabolized, it enters the nephron, a kidney's functional unit, and undergoes filtration by the glomerulus. The filtered compound then crosses the proximal convoluted tubule, the loop of Henle, distal convoluted tubule and finally enters the collection ducts. From here it enters into the bladder and is ultimately excreted in the urine (Ciccone, 2015).

2.11.6 Elimination Half-life (t½)

The elimination half-life is a dependent variable defined as the time required to reduce a drug's plasma concentration to reach half of its original value (Clarke & Trim, 2013). Half-life is a function of the volume of distribution and clearance. The half-life can be estimated by visually inspecting the semi-log plot, using a ruler to trace the straight line which interpolate the data points on the plot (Urso *et al.*, 2002). Half-life is a useful parameter to calculate, as it can be used to determine for what length of time a drug should be stopped if a subject has toxic levels of the drug. The half-life can be calculated as follows (Nishant *et al.*, 2011):

$$t_{1/2} = \frac{0.693}{k}$$

k - Elimination rate constant

2.11.7 Bioavailability

Bioavailability is defined by Ciccone (2015) as the extent and rate of the absorption of a drug. Bioavailability is a parameter expressed as a percentage of the administered drug that enters systemic circulation and is dependent on the administration route and the ability of the drug to cross membrane barriers (Ciccone, 2015). A simple way to determine the bioavailability of a drug is to administer the drug by the IV route (to get the clearance) and via another route, e.g. orally or IM (to achieve the area under the curve). By giving the same dose in both routes of administration, the following equation can be used to determine bioavailability (Urso *et al.*, 2002):

$$F = \frac{CL\ x AUC_t}{CL\ x\ AUC_{iv}} = \frac{AUC_t}{AUC_{iv}}$$

F - Fraction of the dose which enters systemic circulation
AUC_t - Area under the curve estimated after the test administration (e.g. either orally or IM)
AUC_{iv} - Area under the curve estimated after IV administration

2.12 Aims of this study

The increase in the scope of the wildlife industry has led to an increased need for the translocation of wildlife. Concerns for animal welfare has increased due to the stress experienced in the animals during transportation and whilst in captivity. Various tranquilizers and sedatives are available for the management of stress in wildlife, but very little research on their solitary use in especially wild ungulate species is currently available. Each sedative and tranquilizer can have both desired and undesired effects when used in specific animal species. The effects of a drug in its target species must thus first be determined to ensure it is effective and safe when used in said species. Midazolam is a benzodiazepine sedative with benefits above other commonly used sedatives, e.g. alpha-2-agonists and other benzodiazepines. The blesbok is a commonly farmed wild ungulate species in South Africa that frequently undergoes translocation and the associated stress. Midazolam could prove to potentially be an ideal sedative for managing stress in blesbok, but its effects in this wild ungulate are not yet known.

A drug's pharmacokinetics must first be determined in order to explain its behaviour in the body, the reliability of different administration routes and the most suitable dosages to be administered. A pharmacokinetic study requires blood samples to be collected continuously at set time points following treatment, which is near

impossible and dangerous to do in wildlife without immobilisation. Immobilisation agents may also affect the results of the pharmacokinetic study. Due to these reasons, domestic species with a similar physiology to the target wildlife species are frequently used as a model for determining the pharmacokinetics of a drug. In this study the indigenous goat (*Capra hircus*) was thus used to determine the pharmacokinetics of midazolam to be used in blesbok. Further requirements for studying the effects of a drug is determining how it affects the behaviour, physiology and general welfare of a specific animal species. Studying the physiological parameters can be achieved via the use of various methods, but the Equivital™ EQ02 biotelemetry has been proven to be an effective tool for measuring these parameters in blue wildebeest and thus holds potential for use in blesbok. This biotelemetry system must, however, first be validated for use in blesbok before it can be used reliably.

The aim of this study is thus to determine the potential of midazolam as a sedative in captive blesbok. This will be achieved by first conducting a pilot pharmacokinetic study of midazolam using indigenous goats as a model. Furthermore, the accuracy of the Equivital™ EQ02 biotelemetry system in blesbok will be validated. Success of the pharmacokinetic study will make it possible to determine the best dosage of midazolam to be used in blesbok, while successful validation of the Equivital™ system will ensure a reliable and easy method for determining the effects of midazolam on the physiology of blesbok. Ultimately the effects of midazolam on the behaviour, heart rate, respiration rate and general welfare will be determined and provide an understanding of the safety and effectiveness of this drug for reducing the stress response in blesbok during translocation activities.

Chapter 3

The time-release profile of midazolam in indigenous goats (*Capra hircus*)

Abstract

Midazolam is a benzodiazepine sedative that has various benefits for use in wild antelope translocation activities. The time release-profile and therefore the pharmacokinetics of midazolam in wild antelope species are currently unknown. As it is difficult to draw blood from wildlife species, domestic animals with a similar physiology to wild antelope can be used as a model to determine the pharmacokinetics of a drug in wildlife. The purpose of this study was therefore to determine the time-release profile of midazolam in indigenous goats (*Capra hircus*), to serve as a pilot study for the use of midazolam in a wild ruminant species, blesbok (*Damaliscus pygargus phillipsi*). In this study midazolam was administered by the IM route to five female goats at a dose of 0.8 mg midazolam/kg body weight. Blood samples were collected from each of the goats immediately before administration, at 30 min, 1 hr, 2 hrs, 4 hrs, 6 hrs, 12 hrs and 24 hrs following midazolam administration. A concentration versus time profile was derived from the average plasma concentrations of each goat, which was used to calculate the maximum serum concentration, volume to bioavailability ratio, clearance to bioavailability ratio, elimination half-life and mean residence time. The absolute bioavailability was calculated from the area under the curve of the intravenously administered midazolam's serum concentrations. Midazolam administered by the IM route only caused sternal recumbency in one animal, was found to cause a maximum serum concentration of 127.3 ng/L after 36.2 min and had an elimination half-life of 46.8 min. Furthermore, midazolam was found to have a low bioavailability (58.1%), a low volume to bioavailability ratio (0.1 mg/(ng/L) and a low clearance to bioavailability ratio ((0.1 mg/(ng/L))/hr) in indigenous goats. Midazolam also appears to have stimulated appetite in the animals, although the increase in chewing activity observed may have been the result of extrapyramidal allotriophagia. Ultimately, midazolam administered via the IM route may be a viable option to sedate indigenous goats, but only if the bioavailability is improved. Further research on methods that can improve the bioavailability of midazolam administered IM, such as alternative drug formulation and study design, is required.

3.1 Introduction

Various animal management practices require the capture and restraint of animals, such as translocation, veterinary procedures or scientific research. These practices induce stress, which in turn often lead to injury and mortalities in the animals (Dickens *et al.*, 2010). This raises concerns regarding animal welfare and therefore methods of reducing the stress response in animals are continuously being developed and improved. Various pharmaceuticals are available for sedating or tranquilizing animals for the abovementioned practices, but each type of drug may have different side effects that need to be considered when it is to be used in a specific animal species. Tranquilizers may cause appetite suppression, hypotension, convulsions and inhibition of thermoregulation (Burroughs *et al.*, 2012b; Lamont & Grimm, 2014). Sedatives, e.g. benzodiazepines and α-2-adrenergic agonists, cause drowsiness and impair locomotor activity, but generally cause minimal side effects (Burroughs *et al.*, 2012b; Lamont & Grimm, 2014). It is essential that the time-release profile of a drug be known so that some basic pharmacokinetics of a drug, i.e. how the drug moves inside the body from its administration up to its elimination, can be determined before it can be safely used in a specific animal species. From drug's time-release profile it can be determined when a drug's onset will be, when it's maximum effects will be reached and how long its effects will last. This data can be used to determine the most effective dosages and route of administration to use for a drug.

Sedatives are commonly used to suppress an animal's stress response to ensure easy and safe handling, to increase the induction quality of anaesthesia and to reduce the adverse effects resulting from certain anaesthetics by reducing the amount of injected anaesthetic required to maintain anaesthesia (Kojima *et al.*, 2002). Alpha-2-adrenergic agonists, such as xylazine, have commonly been used as sedatives in sheep and goats. While increasing the dose of xylazine increases its depressant effects on the central nervous system, it has also been found to cause hypoxia, lung oedema and a decrease in cardiac output (Prajapathi *et al.,* 1994; Yamashita *et al.*, 2000). Benzodiazepines, such as midazolam and diazepam, have thus been used as alternative sedatives to xylazine in small ruminants since these drugs do not cause the abovementioned side effects when their dosages are increased. Benzodiazepines also have the added benefit of that they stimulate appetite in certain species (Van Miert *et al.*, 1989; Berridge & Peciña, 1995; Wanamaker & Massey, 2009).

Midazolam is a short-acting benzodiazepine that has a rapid onset after administration. Midazolam's salts are water-soluble and are stable in aqueous solutions (Heizmann *et al.*, 1983). Due to midazolam's water-solubility, appetite-stimulating effect and a short elimination half-life, it is an ideal sedative to administer via the IM route and it is thus preferred as a sedative to diazepam, which is lipid-soluble and not as potent (Swan, 1993). The pharmacokinetics of midazolam administered by the IM route have been described for humans (Hung *et al.*, 1996), dogs (Court & Greenblatt, 1992; Schwartz *et al.*, 2013), alpacas (Aarnes *et al.*, 2013), guinea pigs (Capacio *et al.*, 2004) and sheep (Simon *et al.*, 2017). Midazolam has also been studied in various wildlife species (Stegmann & Jago, 2006; King *et al.*, 2008; Mellish *et al.*, 2010; Olsson & Phalen, 2013; Wenger *et al.*, 2013; Fiorello *et al.*, 2014; Mortenson & Moriarty, 2015). Although the data has not been published, wildlife veterinarians in South Africa prefer to use midazolam doses of 0.1-0.2 mg/kg for the sedation of ungulate species and even lower dosages

when used as part of immobilisation protocols of these animals (Raath, personal communication, 3 November 2017). Published information on the use of this drug and specifically about its pharmacokinetic behaviour in wild ungulate species is, however, very limited.

To determine the pharmacokinetics of a drug, a time-release profile of the drug must be plotted. For this purpose, blood must be collected from the animal at set time points following administration of the drug to determine the serum concentrations of the drug at set time points after administration. However, it is very difficult to draw continuous blood samples from wild animals (Dantzer *et al.*, 2014). Domestic ruminants with a similar physiology to wild ruminants are frequently used as a model for determining the pharmacokinetic behaviour of a drug in wildlife. Thus the aim of this study was to determine the serum concentrations of midazolam to plot a preliminary time-release of the drug in indigenous goats in order to serve as a model for the potential use of this drug in similar sized wild ruminant species. The results from this study will also aid in refining studies on midazolam use in other animal species.

3.2 Materials and methods

Ethical approval for this study was obtained from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa. Protocol ethical approval number SU-ACUD16-00025.

3.2.1 Experimental location

This study was conducted on Ngongoni farm (25°31’25.2” S, 31°06’50.8” E), outside Nelspruit in Mpumalanga, South Africa. The region is characterized by an average temperature of 19.8 °C (minimum 14.6 °C and maximum 23.6 °C), and annual rainfall of 796 mm (summer rainfall region; range: 11 mm in June to 130 mm in January) (Climate: Nelspruit).

3.2.2 Experimental animals and husbandry

This study was carried out using six healthy female indigenous goats (*Capra hircus*), with an average weight of 20.8 ± 1.88 kg (range: 17.7 to 23 kg). Only six animals were used due to the high cost of the blood analysis. The number of animals were also based on that used in other pharmacokinetic studies, where six subjects were deemed sufficient to yield the desired pharmacokinetic results (Kreuder *et al*., 2012; Kuusela *et al*., 2000; Carroll *et al*., 2001; Cole *et al*., 2006; Shukla *et al*., 2007; Wasfi *et al*., 2012; Aarnes *et al*., 2013; Hubbell *et al*., 2013; Schwartz e*t al*., 2013). The animals were detained in a large pen surrounded by wire fencing (Figure 3.1A), which contained a small enclosure for the conduction of veterinary procedures (Figure 3.1B). The animals were provided with fresh water and lucerne hay *ad libitum* prior to the start of the trial.. Feed was withheld for 12 hrs prior to the start of the trial and during blood sampling to standardize conditions, while water was provided *ad libitum*. On the day of the trial, the goats were herded into the small enclosure within the pen. After blood sampling was completed, the animals received fresh lucerne hay *ad libitum*. The animals were checked for diseases before being brought onto the farm and were continuously monitored for their health via the veterinarian throughout their time in captivity.

Figure 3.1 (A) The pen used for housing indigenous goats used during the pharmacokinetic study of midazolam, and (B) the handling facility where veterinary procedures were performed.

3.2.3 Administration of pharmaceutical substances

Midazolam was administered by the IM route at a dose of 0.8 mg midazolam/kg body weight (BWt) and a concentration of 20 mg/mL (Wildlife Pharmaceuticals SA (Pty) Ltd, White River, South Africa) into the right quadriceps femoris muscle of five of the trial animals. The dosage was selected based on previous dosages of midazolam used in goats (Stegmann, 1998, 1999; Stegmann & Bester, 2001).

The remaining goat received the drug intravenously via the auricular vein in order to determine the absolute bioavailability. The volume (in mL) midazolam to be administered per goat was calculated based on their respective body weights as per guidelines of the manufacturer. All goats were weighed and ear-tagged prior to the study. Goats were selected at random and physically restrained for blood sampling.

3.2.4 Blood sampling and processing

Blood samples were collected from the jugular vein from each goat at time 0 (immediately prior to midazolam administration), 30 min, 1 hr, 2 hrs, 4 hrs, 6 hrs, 12 hrs and 24 hrs following midazolam administration. The blood samples were collected using an 18-gauge sterile needle and 20 mL syringe, with the blood being transferred to sterile 10 mL serum vacutainer tubes (BD Vacutainer® Plus plastic serum tubes, 16 mm x 100 mm, 10 mL, Clot Activator, Ref nr 367896). A total volume of 30 mL was collected from each goat per sampling session. Two extra 10 mL blood samples were collected per goat with sample 0 for calibration of the gas spectrometry machine at Labserve (Pty) Ltd (Nelspruit, Mpumalanga, South Africa). The time at which each sample was collected per goat was noted on a data sheet (*Appendix A*).

The blood samples were centrifuged (Heraeus Biofuge A 1217) at 5000 rpm for 10 min, as soon as possible after collection. The serum in each centrifuged blood sample was then drawn up using an 18-gauge sterile needle and a 3 mL syringe. The serum was then stored in a marked 5 mL cryotube at ~4ºC until collection. A minimum of 5 mL serum was collected per goat for each sampling interval. The serum samples were sent to Labserve (Pty) Ltd (Nelspruit, Mpumalanga, South Africa) for analyses with gas chromatography mass spectrometry (GC-MS).

3.2.5 Gas chromatography mass spectrometry assay

The goat serum samples were analysed via GC-MS by Labserve (Pty) Ltd. Due to the confidential nature of the protocol developed for the assay of midazolam, only a brief explanation is provided of the assay process. The analysis entailed the extraction of each serum sample using an organic solvent. Salt was used to remove moisture and proteins. Diazepam was used as an internal standard to compensate for any losses during extraction or on the instrument. GC-MS was performed with liquid injections. Each serum sample was extracted with acetonitrile in the presence of magnesium sulphate. An aliquot of the organic phase was analysed on the GC-MS in Single Ion Monitoring (SIM) mode. A split/splitless injector was used with helium serving as the mobile phase. Quantification was performed using the Internal Standard method (Immelman, personal communication, 4 August, 2016).

3.2.6 Statistical analyses

The serum concentrations of midazolam in the pharmacokinetic study were analysed with the PK solver add-on in Microsoft Excel (2016). A non-linear one-compartmental analysis was used to fit the mean serum time-concentration data to standard pharmacokinetic models and to generate pharmacokinetic data. Predicted versus observed concentrations as a function of time and estimates of pharmacokinetic parameters were generated from the analysis of the concentration-time data. The following pharmacokinetic parameters were generated: Elimination rate constant (k_e), ratio of clearance to bioavailability, the ratio of volume of distribution to bioavailability (CL/Vd), peak plasma concentration (C_{max}), time to peak plasma concentration (T_{max}), the area under the plasma concentration curve (AUCt), the area under the plasma concentration curve from zero to infinity ($AUC_{0-\infty}$), area under the first moment curve (AUMC) and the mean residence time (MRT).

The AUC following IV administration of midazolam to one of the goats was also obtained with the PK Solver add-on in Microsoft Excel (2016) via a one-compartmental analysis, for the purpose of calculating the absolute bioavailability of midazolam when administered by the IM route. The absolute bioavailability of the IM administered midazolam was calculated from the AUC ratio determined following IM and IV administration of midazolam using Equation 3.1:

Eq. 3.1:
$$F = \frac{AUC_{I.M.}}{AUC_{I.V.}}$$

The following pharmacokinetic parameters were calculated:

- Peak plasma concentration (C_{max}): The maximum concentration reached by a drug in the plasma of an animal following administration (Urso *et al.*, 2002).
- Time to peak plasma concentration (T_{max}): The amount of time it takes for the maximum plasma concertation of a drug to be reached in an animal (Urso *et al.*, 2002).
- Elimination rate constant (ke): The drug fraction that is eliminated per unit of time in an animal (Clarke & Trim, 2013).

- Elimination half-life ($t_{1/2\beta}$): The time necessary for a drug's plasma concentration to reach half its original value (Clarke & Trim, 2013).
- Ratio of volume of distribution to bioavailability (V/F): The apparent volume of distribution after non-IV administration of a drug. The apparent volume of distribution refers to the theoretical volume that would be required to contain the total amount of the drug at the same concentration observed in the plasma of an animal after administration (Gepts,1998). The volume of distribution and bioavailability (F) of drugs administered via non-IV routes cannot be determined on their own, only their ratio.
- Ratio of clearance to bioavailability (CL/F): The apparent total clearance of a drug from plasma after oral administration. The total clearance of a drug is the measurement of the volume of plasma from which a drug is completely eliminated per time unit. The total clearance and bioavailability (F) of drugs administered via non-IV routes cannot be determined on their own, only their ratio (Gepts, 1998)
- The area under the plasma concentration curve ($AUC_{0\text{-}t}$): The calculated area under the plasma drug-concentration profile of a drug, which is a reflection of the total drug exposure of an animal's body after administration (Urso *et al.*, 2002).
- The area under the plasma concentration curve from zero to infinity (AUC0-∞): The area under the plasma drug-concentration profile of a drug extrapolated to infinity (Urso *et al.*, 2002).
- The area under the first moment curve (AUMC): The area under the product of the concentration and time against the time curve (Gabrielsson & Weiner, 2012).
- The mean residence time (MRT): The mean amount of time a drug remains in an animal's body (Yamaoka *et al.*, 1978).
- Absolute bioavailability (F): A drug's bioavailability when administered via non-IV route in comparison with the bioavailability of the drug when administered via the IV route. Bioavailability refers to the fraction of the original drug that enters circulation after administration (Urso *et al.*, 2002).

The AUC following IV administration of midazolam was calculated using the PK Solver add-on in Microsoft Excel (2016) following a one-compartmental analysis approach for the purpose of calculating the absolute bioavailability of midazolam when administered by the IM route. The fit weight of the IV administered midazolam data was calculated from the observed plasma concentrations using the following equation:

$$W = \frac{1}{C_{Observed}}$$

The absolute bioavailability of the IM administered midazolam was calculated from the AUC ratio determined following IM and IV administration of midazolam using the following equation:

$$F = \frac{AUC_{I.M.}}{AUC_{I.V.}}$$

3.3 Results

Within 5 min of midazolam administration all of the goats appeared sedated, but only two became sternally recumbent. Goats 1, 2, 4 and 5 each stood with their heads and necks lowered, while Goat 3 and 6 became sternally recumbent within 5 min following the administration of midazolam.

Goat 6 was standing again 30 min after becoming recumbent, while Goat 3 was standing only at 50 min after becoming sternally recumbent. While Goats 3 and 6 were sternally recumbent, they were still conscious and able to stand when disturbed. No other side effects were observed following midazolam administration in any of the goats and there was no observed inflammation at the injection sites. The mean serum concentrations of midazolam administered IM (and thus its time-release profile) are depicted in Figure 3.2, while the pharmacokinetic parameters calculated for midazolam are presented in Table 3.1.

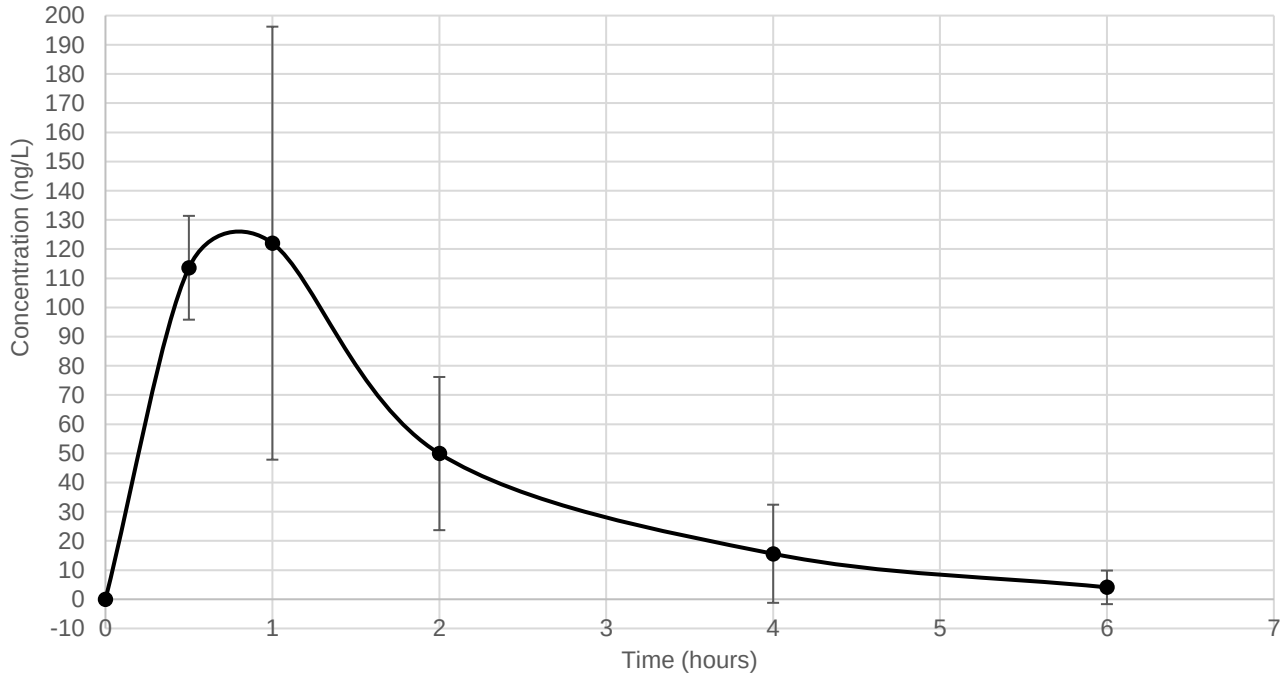

Figure 3.2 The change in the mean plasma concentration of midazolam administered intramuscularly to five healthy indigenous goats during a six-hour observation period.

Table 3.1 Pharmacokinetic parameters (mean ± SD) of midazolam administered intramuscularly at a dose of 0.8 mg midazolam/kg BWt to five indigenous goats.

Pharmacokinetic parameter of midazolam	Value of parameter in indigenous goats
T_{max}	0.6 ± 0.18 hrs
C_{max}	127.3 ± 42.23 ng/L
k_e	0.9 ± 0.20 1/hr
$t_{1/2\beta}$	0.8 ± 0.18 hr
AUC_{0-t}	251.8 ± 133.56 ng/L*hr
$AUC_{0-infinity}$	257.4 ± 133.26 ng/L*hr
AUMC	411.9 ± 281.82 ng/L*hr^2
MRT	1.5 ± 0.28 hrs
V/F	0.1 ± 0.04 ng/(ng/L)
CL/F	0.1 ± 0.03 (mg)/(ng/L)/hr
Absolute F	58.1%

3.4 Discussion

Midazolam administered IM to indigenous goats at a dose of 0.8 mg midazolam/kg BWt resulted in a maximum serum concentration of 127.5 ng/L after 0.6 hrs (~36 min). The mean t_{max} of midazolam administered by the IM route was longer than the mean t_{max} reported for humans (17.5 min) (Hung *et al.*, 1996), dogs (7.5 min and <15 min) (Court & Greenblatt, 1992; Schwartz *et al.*, 2013), guinea pigs (1.7 in animals not experiencing seizure and 2.9 min in animals experiencing seizure) (Capacio *et al.*, 2004) and sheep (28 min) (Simon *et al.,* 2017). The goats in this study appeared sedated within 5 min of IM midazolam administration, characterized by lowering of the head and neck. However, sedation scoring was not recorded in this study, and thus it is difficult to say when exactly the onset of sedation occurred. Midazolam has a rapid onset of action following IM administration (Niratoku & Sinha, 2000) due to its high lipophilicity at pH levels higher than 4 (Coleman & Temo, 1997). It would thus have been expected that a dose of 0.8 mg midazolam/kg BWt will result in rapid sedation of the indigenous goats. This may explain why the goats appeared sedated within 5 min of treatment. It is recommended that sedation scoring should be incorporated in future studies to accurately determine the onset of sedation of midazolam in indigenous goats.

The elimination half-life calculated for IM administered midazolam to goats in this study (0.8 hrs [~47 min]) was longer than the elimination half-life recorded in guinea pigs (19.5 min in animals not experiencing seizure and 21.9 min in animals experiencing seizure) (Capacio *et al.*, 2004), while it was much shorter than that observed in alpacas (98 min) (Aarnes *et al.*, 2013). The mean residence time for IM administered midazolam to goats in this study (1.5 hrs) was similar to that calculated for sheep (1.59 hrs) (Simon *et al.,* 2017).

The goat that received midazolam via the IV route (Goat 6) became sternally recumbent. After IV administration drugs reach circulation almost immediately, and therefore have an immediate and very potent effect when compared to IM administered drugs (Sakai, 2008). This explains why Goat 6 became sternally recumbent after treatment with midazolam. One of the five goats that received midazolam IM, Goat 3, became sternally recumbent within 5 min of midazolam administration for ~50 min. The animal was still conscious and able to stand after becoming sternally recumbent due to sedation. This is a common occurrence observed following treatment with sedatives, but not with tranquilizers. Higher doses of sedatives cause recumbency, but the animals are still able to stand when disturbed. At higher doses of tranquilizers, animals will be immobilised and unable to get up (Thurmon *et al.*, 1996; Hendrickson & Baird, 2013). It was thus expected that midazolam would result in sternal recumbency in all the goats since previous studies by Stegmann (1998) and Dzikiti *et al.* (2009) found lower doses of IM midazolam (0.4 mg midazolam/kg BWt and 0.3 mg midazolam/kg BWt, respectively) resulted in sternal recumbency in all goats.

Stegmann (2001) found that midazolam at a dose of 0.6 mg/kg BWt failed to cause sternal recumbency in goats, similar to the observations in the present study. Stegman (2001) hypothesized that this may be due the development of drug tolerance in the goats due to previous drug treatments. However, the goats in this study did not receive drug treatments prior to the start of the trial, and this could therefore not have been the cause for the failure of midazolam to induce recumbency in all the animals. Stegmann (2001) further argued that another reason for the failure of midazolam to cause recumbency in goats was that the separation of individual animals from the rest prior to drug administration results in anxiety, which may increase the dosage required to induce recumbency in the animals. This may have been the case in the present study, as individual goats were separated from the rest to be weighed before midazolam was administered. The goat that became recumbent after IM midazolam administration, Goat 3, was also the smallest of all the goats. Goat 3 only weighed 17.7 kg in contrast to the rest of the animals that all weighed more than 20 kg. According to Fowler & Cobas (2001) drugs are absorbed faster in smaller animals than larger animals due to a higher metabolic rate. Thus midazolam may have had a more immediate and potent effect in the smallest goat than the rest of the animals, which could offer another possible explanation as to why only one goat became sternally recumbent after IM treatment with midazolam.

Stegmann (1998) found that midazolam administered IM to goats with weights ranging between 18 and 26.6 kg at a dose of 0.4 mg/kg BWt resulted in reliable sedation after 5 min. This sedation was characterized by the lowering of the head and neck in the animals, and sternal recumbency occurred in all the animals within 16 min of administration. Similar to this study, the goats in Stegmann's (1998) study were still conscious while in sternal

recumbency and able to stand when disturbed. Stegmann (1998) further found that increasing the dose to 1 mg midazolam/kg BWt resulted in ataxia, lateral recumbency and loss of consciousness in the animals. A dose of 1 mg midazolam/kg BWt administered via the IM route is therefore only recommended if the purpose of the drug's administration is to immobilise and not sedate the animal. The study by Stegmann (1998) unfortunately only included two dosages, vastly different in concentration (0.4 mg midazolam/kg BWt and 1 mg midazolam/kg BWt) to what was investigated in the present study, and thus it is difficult to completely compare the results from this present study to those found by Stegmann (1998) or to determine the effect of dosage specifically.

Midazolam had an absolute bioavailability of 58.1% after IM administration to indigenous goats in this study, which is relatively low when compared to studies on dogs (>90%) (Court & Greenblatt, 1992), humans (106%) (Hung *et al.*, 1996), alpacas (92%) (Aarnes *et al.*, 2013) and sheep (352%) (Simon *et al.*, 2017). In a study by Schwartz *et al.* 2013), however, midazolam administered IM to dogs at a dose of 0.2 mg midazolam/kg BWt, was found to be incompletely absorbed resulting in a relatively low bioavailability (50%), which is comparable to that found in the goats in this study. Bioavailability is the portion of a drug dose that is absorbed at the administration site and enters circulation intact, and is thus available to cause a therapeutic effect (Urso *et al.*, 2002). The bioavailability of drugs administered via the IV route is usually 100%, which would be the ideal bioavailability to achieve maximum effect from the drug administered. If the bioavailability of a drug is very low, it means that very little of the drug is absorbed and available to cause an effect in the subject and thus the subject would be under-medicated. Low bioavailability thus results in the therapeutic failure of a drug (World Health Organization, 1974). The bioavailability of IM administered midazolam found in dogs, alpacas and humans were very close to the bioavailability of IV administration, which suggests that midazolam is almost completely available in the circulatory system after administration (i.e. 100%) via the IM route in various species. Thus midazolam administered by the IM route to goats in this study (58.1%) was only approximately half as effective as it would have been if administered via the IV route. This may also explain why the midazolam failed to cause sternal recumbency in almost all of the goats. The study by Schwartz *et al.* (2013) in dogs, which reported a similarly poor bioavailability to the present study, suggested that an increase in dose may potentially aid in increasing the bioavailability of IM midazolam.

According to Tuttle (1977), water-solubility, dissolution after injection and the blood flow at the injection site can influence the bioavailability of an IM administered drug. One method of improving bioavailability of a drug is the use of the drug in its salt form. Counter ions in the salt can improve the pH of the drug during dissolution in water, improving the solubility of the drug and thus the absorption of the drug into circulation (Kalepu & Nekkanti, 2015). The addition of co-solvents or surfactants to drug formulations increased the di-electric constant, which increase the solubilisation of drug molecules that are non-polar (Kwakami *et al.*, 2006). The use of polymeric micelles and cyclodextrins, which are aqueous vehicles, in drug formulation can improve a drug's water solubility and thus absorption. Nanonization, defined as the sub-micron reduction in the size of a drug's particles, can also be used to increase the cellular uptake of drugs and thus its absorption and bioavailability (Kraft *et al.*, 2004; Mouton *et al.*, 2006; Kalepu & Nekkanti, 2015). Solid lipid soluble nanoparticles are drug-vehicles that have been found to enhance the bioavailability of drugs by increasing the surface area of the particles and the rate of dissolution (Luo

et al., 2006; Chakraborty *et al.*, 2009). Other methods available for improving the solubility and thus bioavailability of drugs are lipids, micro-emulsions and self-emulsions (Müllertz *et al.*, 2010; Kalepu *et al.*, 2013; Kalepu & Nekkanti, 2015). Thus there are various options available for the improvement of the solubility and thus bioavailability of midazolam. Midazolam's formulation for use in goats may thus have to be reconsidered and further studies on different midazolam formulations in goats are required.

Another possible reason for the low bioavailability of midazolam reported in this study may be due to the study design. According to Chow and Liu (2008), bioavailability studies require 18-24 healthy subjects, which is substantially more than the six used in the present study. In this study, only one animal received the drug intravenously and thus the bioavailability of the IM administered midazolam was determined based on the area under the curve calculated from the midazolam serum concentration after IV administration to only a single animal. More animals would have made it possible to administer the midazolam by the IV route to more subjects, and thus improve accuracy of the calculation of the absolute bioavailability. Chow and Liu (2008) suggests that a crossover design should preferably be used in bioavailability studies as the parallel design, which was used in the present study, is not capable of removing variability within subjects. Furthermore, the crossover design is more favourable for bioavailability studies as it provides more unbiased results due to sufficient randomization.

Thus for a more accurate and possibly higher bioavailability determination of midazolam after IM administration to indigenous goats, the dosage, the drug formulation and the study design need to be reconsidered. However, this was only a pilot study for determining the time-release profile of midazolam in indigenous goats, and therefore the requirements for a proper bioavailability study were not within the scope of the present study. In order to do a proper pharmacokinetic study and to determine a drug's bioavailability, a much larger amount of animals is required and is therefore recommended in future studies to determine the plasma pharmacokinetics and bioavailability of midazolam in indigenous goats.

Following administration of midazolam, all the goats showed increased rumination and food seeking behaviour. Studies have reported that benzodiazepines increase feed intake by stimulating appetite in various mammalian species (Randall *et al.*, 1960; Brown *et al.*, 1976; Fratta *et al.*, 1976; Mereu *et al.*, 1976). Midazolam stimulated appetite in goats (Van Miert *et al.*, 1989), which may explain the increased chewing and ruminating behaviour in the indigenous goats in this study. It is also possible that the increase in chewing behaviour could have been the result of an extrapyramidal effect known as allotriophagia, which is the impulse of an animal to consume abnormal food items, e.g. clay, dirt, paint etc. and usually occurs when an animal experience a deficiency of a specific nutrient (Gordon *et al.*, 1954). This may have been the case in the present study as some of the goats were observed nibbling at the poles of the enclosure after treatment with midazolam.

3.5 Conclusion

Midazolam administered IM at a dose of 0.8 mg/kg BWt did not cause sternal recumbency in all the goats, which may be the result of anxiety caused by separation of the trial animals before midazolam was administered, and

poor bioavailability of the drug after administration. Midazolam also took a substantial amount of time to reach maximum serum concentrations compared to dogs, alpacas and guinea pigs. Although midazolam administered IM at a dose of 0.8 mg/kg may be a viable option for sedating the goats, an improvement in its bioavailability is required to ensure reliable sedation in this species. Possible methods of improvement may be an improved formulation of the drug for better solubility and absorption, and including more trial animals to be able to determine the bioavailability. Therefore, further studies on the use and effect of midazolam as a sedative in indigenous goats are recommended.

Chapter 4

Validating the use of the Equivital™ EQ02 biotelemetry system to measure physiological parameters of blesbok (*Damaliscus pygargus phillipsi*)

Abstract

Two blesbok (*Damaliscus pygargus phillipsi*) were fitted with the Equivital™ EQ02 wireless biotelemetry system to determine the potential of the system to accurately measure heart rate and respiration rate in the animals during periods of being immobilised, as well as while being fully awake in captivity. The animals were chemically immobilised and heart and respiration rate were monitored using the Equivital™ system, a Cardell® veterinary monitor and manual recordings, respectively. Doxapram hydrochloride (Dopram®) and adrenaline were administered intravenously at specific times to deliberately manipulate the respiration rate and heart rate of the animals, respectively. Immobilisation was reversed after 20 min, and the animals remained fitted with the Equivital™ belts for an additional 24hr. After this 24hr period, the experimental procedure was repeated. Intraclass correlation coefficients for absolute agreement were calculated for the three methods of monitoring. The Equivital™ system showed a moderate (ICC=0.50 - 0.75) to excellent (ICC>0.90) agreement for heart rate measurements recorded with the Cardell® monitor and the manual measurements. The Equivital™ system showed poor (ICC<0.50) to moderate agreement with the Cardell® monitor and manual measurements for respiration rate. The Equvital™ system could accurately detect changes in heart rate caused by adrenaline, but could not detect changes in respiration caused by Dopram®. The results showed that blesbok heart rate and motion can be quantified with good to excellent accuracy by the Equivital™ biotelemetry system but this system's accuracy in measuring respiration rate of sternally recumbent blesbok was poor. The poor respiration rate measurements could, however be attributed to pressure from the sternum of the animals on the respiration elastic of the biotelemetry system during immobilisation, preventing accurate measurement of respiration rate. Ultimately, the Equivital™ system can be used with reliability to measure physiological parameters of blesbok in captivity.

4.1 Introduction

South Africa's game farming industry has showed rapid growth over the past 20 years, with the industry contributing ~R10 billion to the South African economy (National Agricultural Marketing Council, 2006; Carruthers, 2008; Dry, 2012). Hunting, eco-tourism, breeding game animals for sale and game products form the four economic pillars of South Africa's game farming industry, and during recent years, wildlife breeding and trade activities have increased exponentially.

Translocation is an integral part of any wildlife breeding and trade operation, with translocation contributing an estimated 16% (R750 million - R 900 million) to the total turnover of the South African wildlife industry (National Agriculture Marketing Council, 2006). Translocation however, results in stress in the animals due to various environmental changes such as human contact, the noise and movement of the transport vehicle, withholding of food and water and in some cases, exposure to extreme weather conditions. Stressed animals are difficult to handle and tend to injure themselves or other animals. Mortalities can also occur due to transport-associated stress, which can cause major financial losses.

Stress during capture can cause a condition known as capture myopathy, which results in morbidity and potential mortality in both wild and domesticated animals. Wild ungulates are predisposed to this disease due to the stress caused by the processes of capture and restraint (Ebedes *et al.*, 2006). The characteristics of capture myopathy include muscle necrosis, metabolic acidosis and myoglobinuria (Paterson, 2007). Capture myopathy impacts negatively on the quality of meat produced from affected animals (Kleiman *et al.*, 2010). There is thus a need to qualify and quantify stress during the transport of game animals to ensure the animals' wellbeing during transport and to reduce potential mortalities. Methods thus need to be investigated that will allow for the monitoring of physiological parameters of game animals during capture and transport.

The most common methods for the measurement of physiological parameters in animals include manual measurements in trained or immobilised animals, the use of veterinary monitors which can be used in immobilised animals, and biotelemetry systems. Manual measurement is the least expensive method for measuring heart rate and respiration rate and does not require much experience, but this method has a high risk of inaccuracy due to human error, and can also be labour intensive. Accurate recording of respiration may also be difficult in animals with apnoea, shallow breathing or an apneustic breathing pattern. The Cardell® 9500 HD multi-parameter veterinary vital sign monitor (Kyron Laboratories (Pty) Ltd, Johannesburg, South Africa) measures the rate of respiration via a mainstream Capnostat® CO_2 probe attached to an endotracheal tube inserted into the trachea of the animal and measures heart rate by means of electrode clips attached to the animal (Laubscher *et al.*, 2015a). These electrode clips measure pulse oscillations and changes in the heart's electric conduction. Thereby the Cardell® is more sensitive to changes than can be manually detected and thus produces more accurate readings for immobilised animals. Veterinary monitors such as the Cardell® monitor are more time-efficient than for example, the Dinamap system. The Dinamap system is a non-invasive oscillometric blood pressure monitor that can also measure heart rate, but this monitor can only give readings every few minutes and not instantaneously

like the Cardell® monitor (Wadell, 2005). The Cardell® monitor is also much smaller in size than the Dinamap system and is therefore more portable. However, monitors such as the Cardell® monitor are usually expensive and require maintenance and often replacement of probes and electrodes which may be costly. A further disadvantage of the Cardell® monitor is that movements by the animal or significant arrhythmias disrupt monitoring by this system and makes measuring parameters difficult (Wadell, 2005, Laubscher *et al.* 2015a).

Biotelemetric systems that allow for the remote measurement of a subject's physiology, behaviour or energetic status, can potentially assist researchers in formulating the most appropriate protocols for the use of pharmaceuticals substances and their effect on wildlife (Cooke *et al.*, 2004). Biotelemetry systems can be external or internally positioned. External systems involve the placement of system components on the body surface of subjects, compared to internal or implantable systems where the components consist of electrodes that are placed inside the organ to be monitored (Bazaka & Jacob, 2012). Each system is characterized by its own advantages and disadvantages. Implantable telemetry systems can accurately measure a multitude of variables, and also cause very minor discomfort to the animals (Braga & Burmeister, 2011). However, implantable telemetry devices are expensive and usually require surgery for placement inside the animal. Furthermore, internal telemetry systems used primarily for determining drug pharmacodynamic effects are very resource intensive and require the animals to be restrained. This can cause issues such as handling stress and limited collection of data (Kramer & Kinter, 2003; Cooke *et al.*, 2013). The external telemetry systems overcome the abovementioned drawbacks of internal devices and have the extra benefit of being non-invasive (Chui *et al.*, 2009; Braga & Burmeister, 2011). The selection of a specific telemetry system will depend on the type of study and factors such as the amount of animals, duration of the study and the type of parameters to be measured. Both internal and external systems are commonly used, but external systems are more suited for short-duration pharmacodynamic studies (Chui *et al.*, 2009; Braga & Burmeister, 2011).

Biotelemetry systems are extremely valuable in that they allow for the quantification of the influence of factors in an animal's natural and immediate environment on internal homeostatic mechanisms (Cooke *et al.*, 2004). Thermometric telemetry has been used to understand endothermy in animals (Cooke *et al.*, 2004), and cardiovascular telemetry used in combination with thermometric loggers have been used to determine the mechanisms that large ungulates use to adapt to and survive under conditions such as cold and water and food shortages (Arnold *et al.*, 2004). In recent years, biotelemetry systems have also found application in animal pharmacodynamic studies where these systems provide data on the effect of pharmaceuticals substances on certain physiological and behavioural parameters in animals, once administered (Braga & Burmeister, 2011; Laubscher, 2015).

Laubscher *et al.* (2015a) found that a human biotelemetry system, the Equivital™ EQ02 biotelemetry belt (Hidalgo Limited, Unit F. Trinity Court, Buckingway Business Park, Cambridge, UK), could be modified and used to accurately measure the heart rate, respiration rate and motion of blue wildebeest (*Connochaetes taurinus*) in captivity. These results made it possible for Laubscher *et al.* (2015a) to use this biotelemetry system to study the

effects of the long acting neuroleptics Clopixol Acuphase® and Acunil® on the physiology and behaviour of blue wildebeest. An improved understanding of the behaviour and effect of pharmaceuticals substances can assist in the formulation of treatment protocols and implementation in animal production systems. Although the Equivital™ EQ02 biotelemetry system was successfully used in blue wildebeest, it may not necessarily work in other antelope species such as blesbok, and thus needs to first be validated in the specific species before it can be used.

The aim of this study was therefore to evaluate the potential of the Equivital™ EQ02 biotelemetry system to accurately measure heart rate and respiration rate, as well as changes in these vital signs caused by the administration of adrenaline and doxapram hydrochloride, respectively, in immobilised blesbok. Furthermore, the aim of this study was also to determine if the Equvital™ system could accurately measure changes in the vital signs of blesbok with changes in motion whilst the animals are fully conscious in captivity. Successful validation of the Equivital™ EQ02 biotelemetry system may provide an accurate, cost-effective biotelemetry system that is available for use in studying physiological parameters in immobilised and free-moving wild ungulate species.

4.2 Materials and methods

Ethical approval for this study was obtained from the Research Integrity and Ethics Committee: Animal Care and Use at Stellenbosch University, South Africa (Ethical Approval number: ACU-2017-0280-200). All pharmaceutical products used in this study was administered by a registered veterinarian.

4.2.1 Experimental location

This study was conducted on Ngongoni farm (25°31’25.2” S, 31°06’50.8” E), outside Nelspruit in Mpumalanga, South Africa. The region is characterized by an average temperature of 19.8 °C (minimum 14.6 °C and maximum 23.6 °C), and annual rainfall of 796 mm (summer rainfall region; range: 11 mm in June to 130 mm in January) (Climate: Nelspruit).

4.2.2 Experimental animals and husbandry

Two blesbok (*Damaliscus pygargus phillipsi*), one young ram (body weight = 43.1 kg) and a mature ewe (body weight = 51.7 kg), were used in this study. The animal numbers were based on those used in the similar study done in blue wildebeest by Laubscher *et al*. (2015a). The blesbok were detained in an enclosure of 6 x 8 m in size containing a group of 12 blesbok in total and referred to as the group enclosure (Figure 4.1). Water and lucerne hay were provided *ad libitum* to the animals in fixed cement water and feed troughs, respectively. A small door is located at the side of the enclosure from where old feed could be removed at the start of each day and fresh feed could be placed out for the animals. The water trough extends to the outside of the enclosure from where fresh water for the animals is added at the beginning of each new day.

All the animals used in this study were inspected for any diseases before arrival on the farm and were acclimatized to the enclosure for a period of at least two weeks prior to the start of each trial. To prevent animals from injuring

themselves or the handlers, PVC piping were placed on the horns of the animals. The animals were continuously monitored for health and wellbeing by the veterinarian throughout their time in captivity.

4.2.3 Experimental facilities

The blesbok were housed in an enclosure of 6 × 8 m, and the design of the enclosure prevented visual contact between animals in adjacent enclosures (Figure 4.1). Shade netting covered a third of each enclosure and the slight slope on which the enclosures were constructed ensured sufficient drainage. Every enclosure is connected to a passage way that has a width of 1.2 m, which ensured the animals could move with ease after being off-loaded from the transport vehicle. All the enclosures were equipped with concrete feed containers with smooth edges to prevent injury to animals during feeding. A completely equipped veterinary laboratory was available only 150 m away from the enclosure.

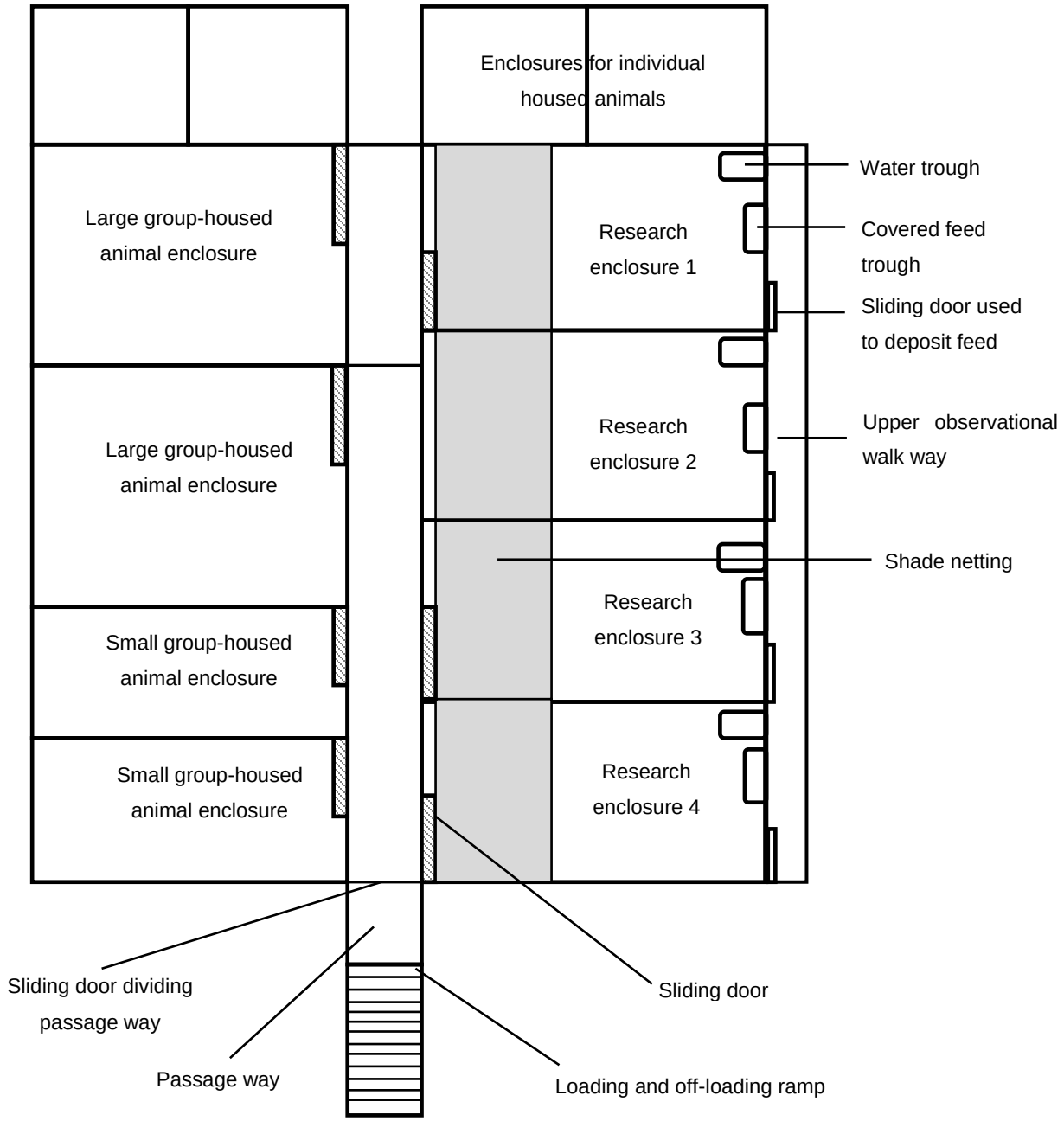

Figure 4.1 Diagram of the enclosure used to house blesbok during studies on the validation of the Equivital™ EQ02 biotelemetry system and the effects of midazolam in the animals (modified from Laubscher, 2015).

4.2.4 Design and fitment of the biotelemetry belts

This study made use of the Equivital™ EQ02 biotelemetry system (Hidalgo Limited, Unit F, Trinity Court, Buckingway Business Park, Cambridge, UK) (Figure 4.2) for the recording of heart rate, respiratory rate and movement data of the animals. The belts, originally designed to be used in humans, were modified for use in blue wildebeest (*Connochaetes taurinus*) (Laubscher *et al.*, 2015a).

The Equivitial™ EQ02 biotelemetry system consists of a belt with a chest band containing an elastic and two lead electrocardiogram (ECG) sensors that connect to a sensor electronic module (SEM) which logs the recorded data. The elastic measures respiration rate via the expansions it undergoes every time the animal breathes, and a stretch inhibitor on the belt prevents the elastic from overstretching. The two ECG sensors measure the number of heart beats if placed correctly over the heart of the animal. The recorded respiration rate and heart rate are logged every 15 secs by the SEM of this biotelemetry system and can be monitored by Bluetooth on a mobile device on which the corresponding application is installed. Additionally, the SEM also logs data that can be downloaded. The Equivital™ biotelemetry system also has the ability to detect and log an animal's motion according to three categories, namely stationary, moving slowly or moving fast via an accelerometer.

Modification to the belts included the extension of the chest bands with elastic material as well as replacement of the original stretch inhibitor with a sturdier stretch inhibitor that was placed inside the belt to reinforce the belt and prevent overstretching of the respiratory sensor. A SEM was secured to each belt with insulation tape, and double-sided tape was used to make sure that the belts were secured at the preferred position on the animal's body (Laubscher *et al.*, 2015a).

On the first day of the study (referred to as Day 1), both blesbok were individually immobilised with a reversible anaesthetic delivered by a projectile dart for the fitment of the biotelemetry belts. Each of the animals underwent the same experimental procedure in turn separate from each other. The male (Blesbok 1) was darted first with 3 mg thiafentanil oxalate (Thianil®, Wildlife Pharmaceuticals (Pty) Ltd). The female (Blesbok 2) was darted with 3.5 mg Thianil®. The drugs administered in this study are presented in **Table 4.1**. Once a respective animal was darted, it was carried via stretcher to the laboratory. All pharmaceutical products used in this study were administered by a registered veterinarian.

The blesbok were shaved around the chest before the belts were fitted. This was to ensure that the electrocardiogram (ECG) sensors made direct contact with clean skin. Whilst immobilised, each blesbok was fitted with an Equivital™ biotelemetry belt, and care was taken to position the SEM over the heart (Figure 4.3). Ultrasound transmission gel was applied to the ECG sensor pads on each belt. Locktite® Super Glue gel or Bostik® Super glue Blits Stick was applied to the double sided tape on the belts to further secure the belts to the

Drug	Dose	Route	Purpose	Frequency
Doxapram hydrochloride	2.5 mg	IV	Respiratory stimulation	Twice per animal within 24 hrs
Adrenaline	1 mL	IV	Cardiovascular stimulation	Twice per animal within 24 hrs
Thiafentanil oxalate	3-3.5 mg	IM	Immobilisation of animals	Twice per animal within 24 hrs
Diprenorphine hydrochloride	0.5 mL	IV	Reversal of immobilisation agent	Once per animal
Naltrexone hydrochloride	0.6 mL	IV	Reversal of immobilisation agent	Twice per animal within 24 hrs

skin of the animals. The ends of the belts were sutured together after the belts were placed round the chest of the animals to ensure that they would not fall off during the trial. The SEMs were connected to the belts and switched on immediately before the belts were fitted to the animals. The data loggers on each belt were covered with two layers of Opsite™ Flexigrip™ plastic film (Smith & Nephew (Pty) Ltd, Pinetown, South Africa) to provide protection from dirt and water (Figure 4.3). Following the fitment of the belts, the SEMs on each animal was checked with Bluetooth connection and EquiView Mobile software and compared to manual readings for their consistency in accurately measuring heart rate and respiration rate.

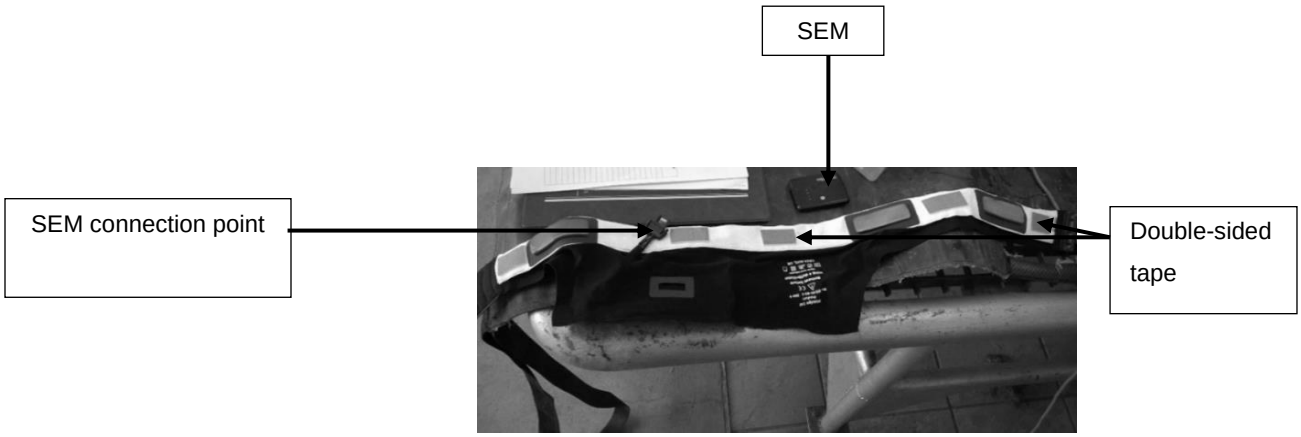

Figure 4.2 The Equivital™ EQ02 biotelemetry belt used to measure heart rate and respiration rate in blesbok.

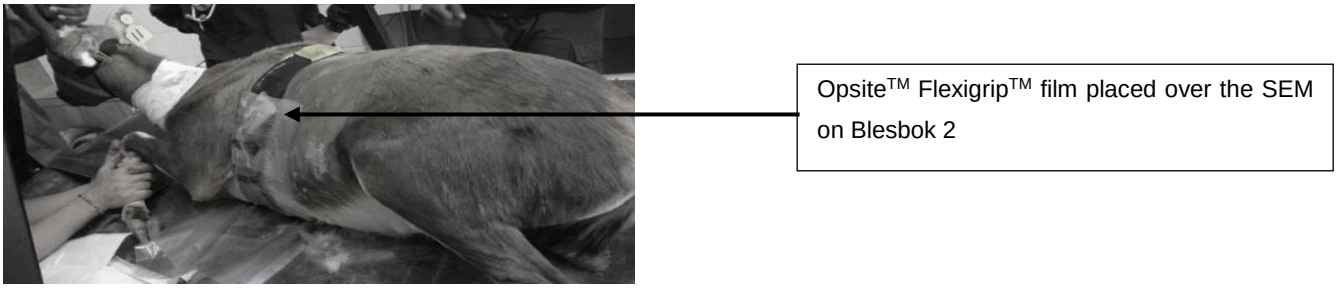

Figure 4.3 An Equivital™ biotelemetry belt fitted onto Blesbok 2 during the validation of the biotelemetry system.

Table 4.1 A summary of the pharmaceutical substances used in this study.

Drug	Dose	Route	Purpose	Frequency
Doxapram hydrochloride	2.5 mg	IV	Respiratory stimulation	Twice per animal within 24 hrs
Adrenaline	1 mL	IV	Cardiovascular stimulation	Twice per animal within 24 hrs
Thiafentanil oxalate	3-3.5 mg	IM	Immobilisation of animals	Twice per animal within 24 hrs
Diprenorphine hydrochloride	0.5 mL	IV	Reversal of immobilisation agent	Once per animal
Naltrexone hydrochloride	0.6 mL	IV	Reversal of immobilisation agent	Twice per animal within 24 hrs

4.2.5 Data recorded

After successful immobilisation of the blesbok on Day 1 one of this study, the animals were each fitted with an Equivital™ biotelemetry belt and in turn transported by stretcher to the laboratory. Measurements of the heart rate and respiration rate of the respective blesbok were compared between three different methods of measurement: manual measurements with a stethoscope for heart rate and the counting of the exhaled breaths of the animals, the Cardell® 9500 HD multi-parameter veterinary vital sign monitor (Kyron Laboratories (Pty) Ltd, Johannesburg, South Africa) and the Equivital™ EQ02 biotelemetry system.

Heart rate and respiration rate were recorded for a period of 5 min before the start of the trial to ensure that stable readings were obtained before the respective pharmaceutical substances were administered. All recordings were synchronized to ensure a comparison of the three methods.

Heart rate and respiration rate were recorded every 15 secs by the Equivital™ system and the Cardell® monitor. The Cardell® monitor has ECG sensors that attach to the animal with metal clips. A clip was attached to the skin underneath both the left front leg and left hind leg of the animal. Electrogel was applied to the skin before the clips were attached.

Manual measurements of heart rate (using a stethoscope) and respiration rate (using manual counts of exhaled breaths) were recorded, and a pulse oximeter (Nonin®, 2500A VET veterinary pulse oximeter, Kyron Laboratories (Pty) Ltd, Johannesburg, South Africa) was inserted into the rectum of the animal for additional monitoring. Respiration rate were counted manually every 15 secs and were multiplied by four to obtain the respiration rate per minute (breaths/min). Manual measurement of heart rate was performed every 30 secs and measurements were multiplied by two to obtain the heart beat per minute (bpm).

After 5 min of stable readings were recorded, the lead veterinarian administered 2.5 mg doxapram hydrochloride (Dopram®, Midlands Veterinary Wholesalers, South Africa) intravenously via the auricular vein. Doxapram hydrochloride (Dopram®) stimulates the respiratory system, and was thus used to elicit a respiratory response. Once the Dopram®'s effects had worn off after approximately 5-10 min, the lead veterinarian administered an additional 1 mL adrenaline intravenously via the auricular vein. Adrenaline was administered to stimulate the heart rate, and has a short half-life of approximately 1-2 min. Once the adrenalin's effect dissipated, recording of data continued for a total period of 20 min, with parameters noted every 15 secs.

The effects of thiafentanil oxalate (Thianil®, Wildlife pharmaceuticals (Pty) Ltd) begins to wear off after 30 min in the blesbok, resulting in the animal showing muscle tremors. This would complicate the accurate measurement of heart and respiration rate, and thus the observation period was shortened from 30 min to 20 min. The Cardell® monitor was disconnected by detaching the clips from the animal after the completion of the 20 min data recording period, and both of the blesbok were returned to the group enclosure with the rest of the blesbok they were initially housed in. The lead veterinarian reversed the anaesthetic by administering 0.5 mL diprenorphine (12 mg/mL

M5050®, Wildlife Pharmaceuticals (Pty) Ltd). Once the animals were fully awake, they were left together in the enclosure with the Equivital™ biotelemetry belt still attached and recording of the heart rate, respiration rate and movement (motion) of the animals were done for an additional 24 hr period whilst they were fully conscious in captivity. The Bluetooth functionality of the biotelemetry belt has a limited battery life, and thus the belt was fitted with an external battery pack to make monitoring for periods of longer than 24 hrs possible. The Bluetooth functionality was not used during this period even though it was on. Instead, data was logged and downloaded after the trial was completed. This whole process was repeated for Blesbok 2.

On the following day both blesbok were individually darted once more and the protocol of Day 1 was repeated. This was referred to as Day 2 of the study. The heart and respiration rate were recorded as previously explained. The anaesthetic was reversed by the administration of 0.6 mL naltrexone hydrochloride (50 mg/mL Trexonil®, Wildlife pharmaceuticals (Pty) Ltd) since no re-darting was required, and each Equivital™ biotelemetry belt was removed from the animals.

4.2.6 Statistical analysis

Statistica's Variance Estimation, Precision and Comparison module (Statistica version 13, Stat Soft, Inc., 2016) was used for statistical analysis. Data were analysed via a mixed model analysis of variance. The day, animal and method of measurement were included as fixed effects, whilst time was included as the random effect. Differences within the fixed effects were accepted as significant if the probability of rejecting the null hypothesis (H_0) was less than 5% ($P \leq 0.05$).

Two-way intraclass correlation coefficients for absolute agreement (ICCs) were calculated amongst the different methods of measurement on each day of the study and for each animal. The ICCs were used to determine the level of agreement between two different measurement methods. Coefficients were only considered to be significant at a confidence level of 5% ($P \leq 0.05$).

4.3 Results

4.3.1 Heart rate measurements

Whilst the blesbok were immobilised in the laboratory, the Equivital™ system measured mean heart rate (± SEM) of 99.4 ± 3.39 beats per minute (bpm), the Cardell® measured mean heart rate (± SEM) of 118.4 ± 2.56 bpm and the manual method measured mean heart rate (± SEM) of 75.8 ± 3.21 bpm. A distinct elevation in heart rate in response to adrenaline administration was observed with each of the three methods of measurement, indicating a good sensitivity to the drug. Following adrenaline administration, the Equivital™ system measured mean heart rate (± SEM) of 161.2 ± 13.03 bpm, the Cardell® measured mean heart rate (± SEM) of 179.2 ± 9.33 bpm and the manual method measured mean heart rate (± SEM) of 139.9 ± 17.54 bpm. During the period where the animals were fully conscious in the enclosure, the Equivital™ system recorded mean heart rate (mean ± SEM) of 93.2 ± 0.58 bpm for Blesbok 1 and 57.5 ± 0.24 bpm for Blesbok 2.

4.3.2 Respiration measurements

Whilst the blesbok were immobilised in the laboratory, the Equivital™ system measured mean respiration rate (± SEM) of 12.3 ± 0.26 breaths per min, the Cardell® measured mean respiration rate of 12.8 ± 0.30 breaths per min and the manual method measured mean respiration rate of 9.7 ± 0.28 breaths per min. A distinct elevation in respiration rate in response to Dopram® administration was not observed with any of the methods of measurement. Following Dopram® administration, the Equivital™ system measured mean respiration rate (± SEM) of 10.7 ± 0.52 breaths per min, the Cardell® measured respiration rate (mean ± SEM) of 11.7 ± 0.57 breaths per minute and the manual method measured respiration rate (mean ± SEM) of 9.3 ± 0.77 breaths per min. Whilst the animals were fully awake in the enclosure, the Equivital™ system measured respiration rate (mean ± SEM) of 21.1 ± 0.17 breaths/min for Blesbok 1 and 17.3 ± 0.12 breaths/min for Blesbok 2.

4.3.3 Measurements per animal

Each of the three recording methods measured lower mean heart rate on Day 1 of the study for Blesbok 1 than on Day 2. The same pattern was observed for Blesbok 2 (Table 4.2). It should be noted that the recordings of heart rate for Blesbok 1 on Day 1 (Figure 4.4) made via the Cardell® monitor were much higher than that of the other two methods. A possible reason for this could be that the Cardell® monitor double counted heart rate values. Each of the three methods of recording showed a distinct escalation in heart rate following adrenaline administration in both blesbok and on both study days. The increase in heart rate following adrenaline administration recorded by the Equivital™ system and the Cardell® monitor were both higher than those recorded manually for both blesbok on both days of the study (Figure 4.4 - 4.7).

The respiration measurements did not show as clear a pattern as the heart rate measurements for both blesbok and on both days of the study. The mean respiration rate measured via the Equivital™ system and the Cardell® monitor were slightly higher on Day 2 for both blesbok (Figures 4.6 & 4.7) when compared to that recorded on Day 1 (Figures 4.4 & 4.5). The manual recordings of respiration rate for Blesbok 1 were lower on Day 2 than Day 1 (Figure 4.4 & Figure 4.6), whilst the manual respiration readings recorded for Blesbok 2 where slightly higher on Day 2 than on Day 1 (Figure 4.5 & 4.7).

Table 4.2 Mean heart and respiration rate (LSMean ± standard error of the mean) measured via three different methods (Equivital™ EQ02 biotelemetry system, Cardell® monitor and manual measurement) for the two blesbok over a 20 min monitoring period on two different days.

	Day 1					
	Mean heart rate			Mean respiration rate		
	Equivital™	Cardell®	Manual	Equivital™	Cardell®	Manual
Blesbok 1	$58.4^{b} \pm 0.50$	$114.2^{a} \pm 1.11$	$59.7^{b} \pm 0.81$	$15.1^{b} \pm 0.50$	$16.7^{a} \pm 0.43$	$14.3^{b} \pm 0.57$
Blesbok 2	$80.8^{b} \pm 5.51$	$106.2^{a} \pm 5.69$	$77.9^{b} \pm 8.14$	$8.8^{a} \pm 0.29$	$8.5^{a} \pm 0.29$	$6.7^{b} \pm 0.21$
	Day 2					
	Mean heart rate			Mean respiration rate		
	Equivital™	Cardell®	Manual	Equivital™	Cardell®	Manual
Blesbok 1	$165.1^{a} \pm 6.60$	$132.6^{b} \pm 5.11$	$79.1^{c} \pm 6.67$	$15.3^{b} \pm 0.48$	$17.6^{a} \pm 0.42$	$11.6^{c} \pm 0.42$
Blesbok 2	$91.9^{b} \pm 5.45$	$121.2^{a} \pm 6.49$	$87.0^{b} \pm 6.78$	$9.8^{a} \pm 0.27$	$8.3^{b} \pm 0.31$	$7.4^{c} \pm 0.33$

[a,b,c] Column means with different superscripts indicate significant differences ($P \leq 0.05$)

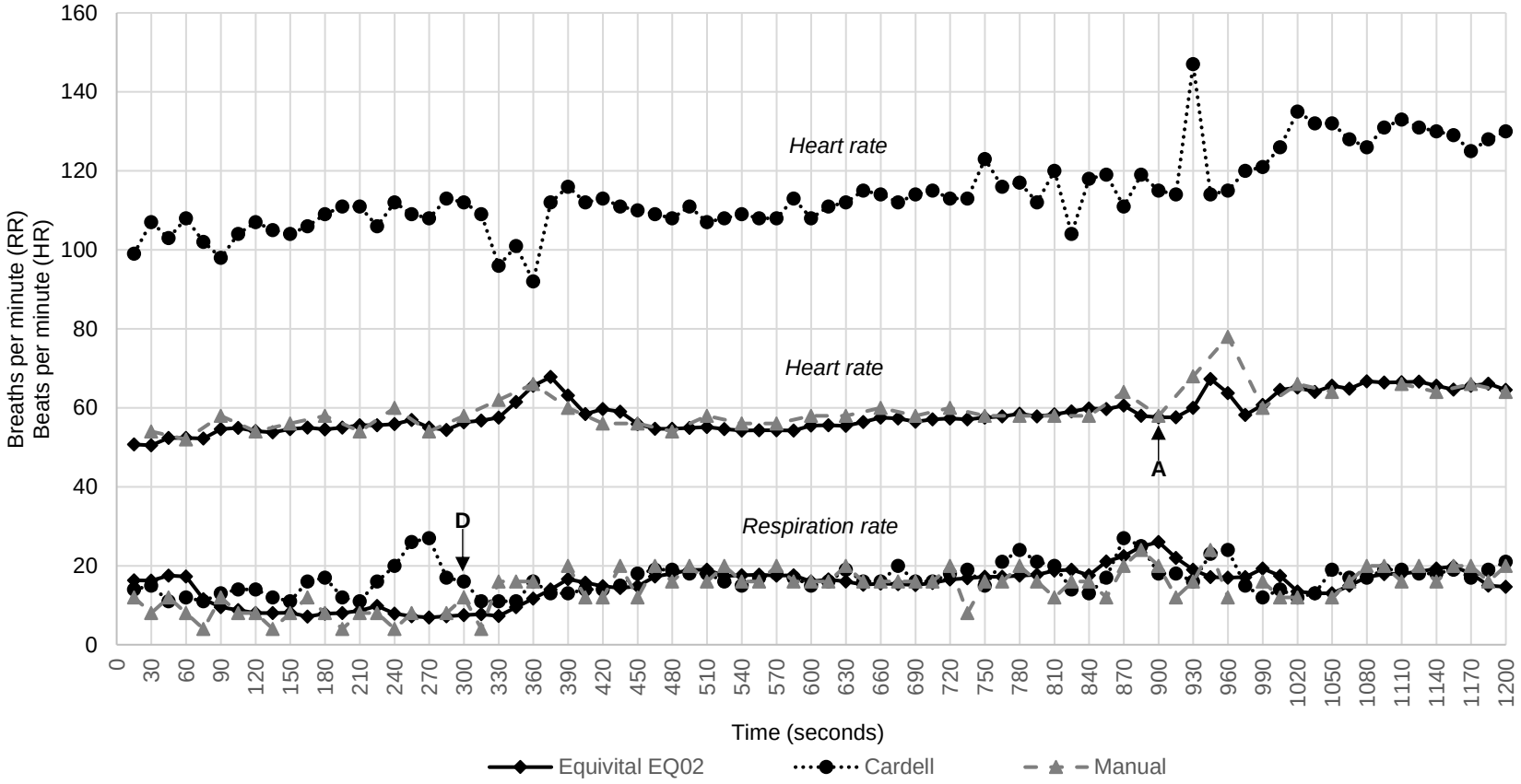

Figure 4.4 Heart rate and respiration rate recorded by means of the Equivital™ biotelemetry system, Cardell® monitor and manual measurement, respectively, for Blesbok 1 on Day 1 of the study. Dopram® (D) was administered after 300 secs and adrenaline (A) was administered after 900 secs.

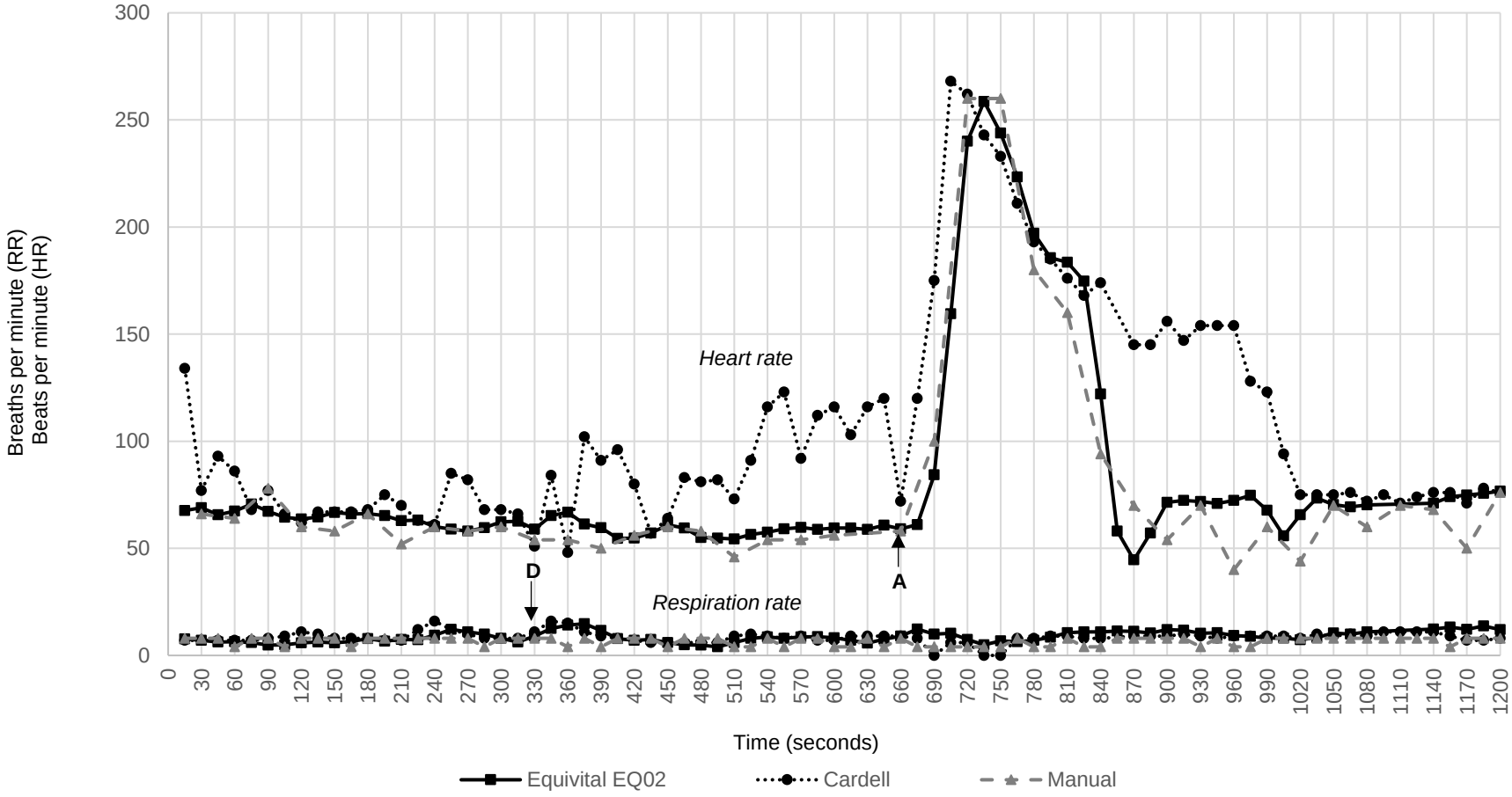

Figure 4.5 Heart rate and respiration rate recorded by means of the Equivital™ biotelemetry system, Cardell® monitor and manual measurements for Blesbok 2 on Day 1 of the study. Dopram® (D) was administered after 330 secs and adrenaline (A) was administered after 660 secs.

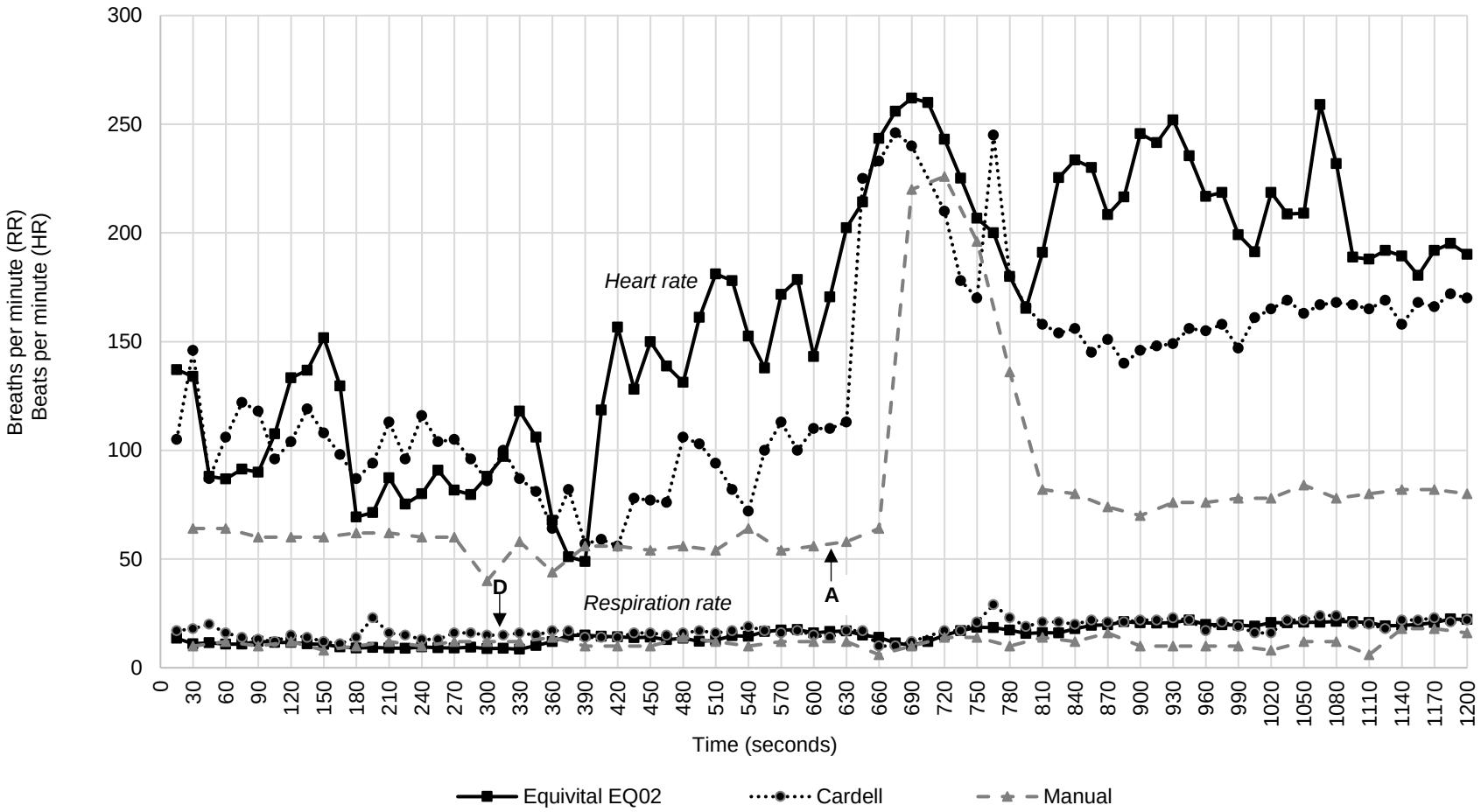

Figure 4.6 Measurements of both heart rate and respiration rate via three different measurement methods (Equivital™ biotelemetry system, Cardell® monitor and manual measurement) of Blesbok 1 on Day 2. Dopram® (D) was administered after 315 secs and adrenaline (A) was administered after 615 secs.

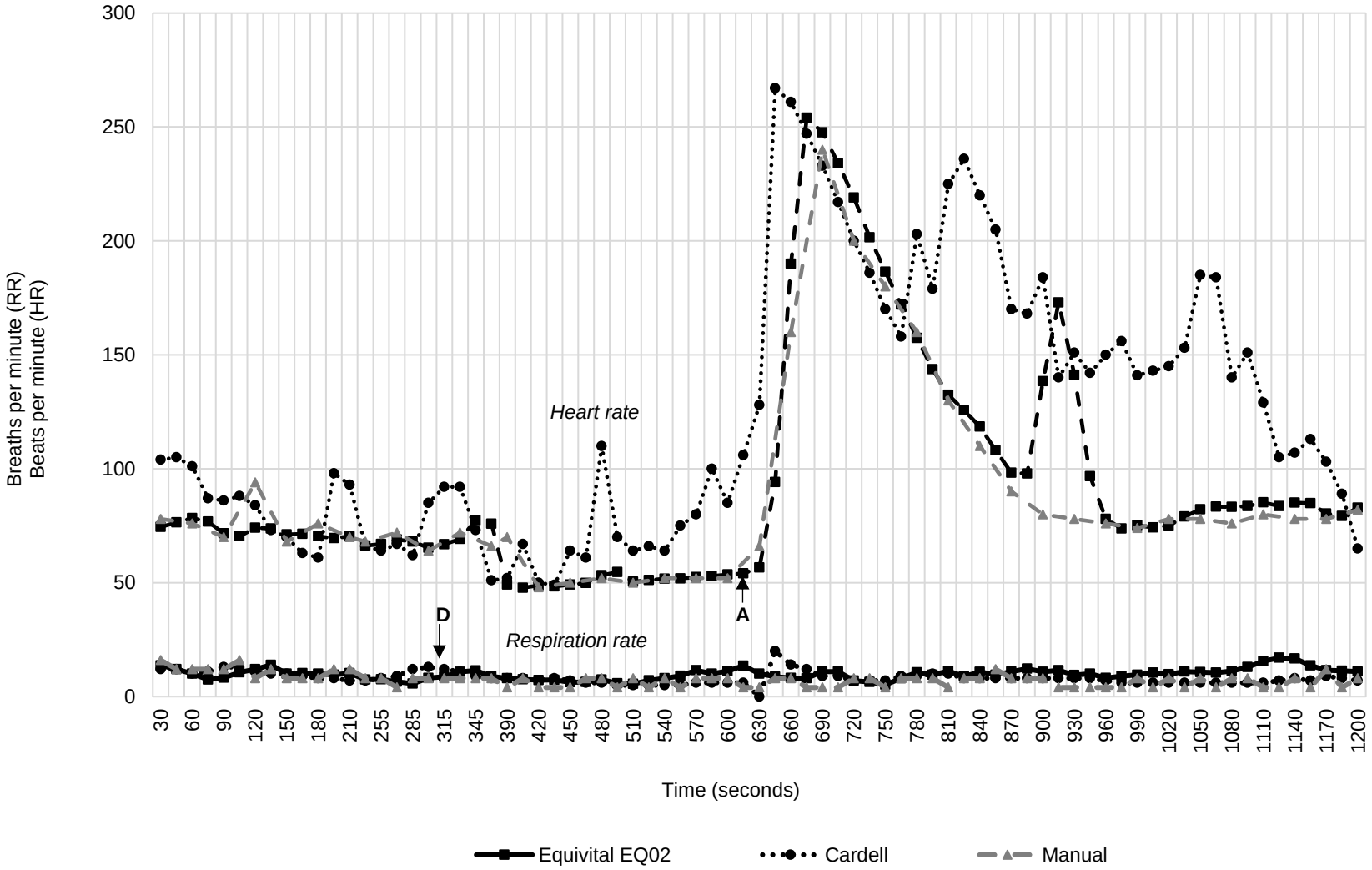

Figure 4.7 Measurements of both heart rate (H) and respiration rate (R) via three different measurement methods (Equivital™ biotelemetry system, Cardell® monitor and manual measurement) of Blesbok 2 on the second day of the study. Dopram® (D) was administered after 315 secs and adrenaline (A) was administered after 615 secs.

4.3.4 Agreements between the recording methods during the full 20 min of recording in the laboratory

According to the intraclass correlation coefficients (ICCs) for absolute agreement calculated between each of the methods of measurement (

Table 4.3), the Equivital™ system had a poor agreement (ICC<0.50) with the Cardell® monitor for the heart rate measurements of Blesbok 1 on Day 1 of the study, but on the second day of the study the agreement between these two recording methods was moderate (ICC = 0.50-0.75) for the heart rate of this animal. The ICCs calculated for the Equivital™ system and the manual method for the heart rate measurements showed a good agreement

	Day 1			
	Blesbok 1		Blesbok 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ EQ02 vs. Cardell®	0.018	0.284	0.707	0.391
Equivital™ EQ02 vs. Manual	0.751	0.646	0.966	0.003*
Cardell® vs. Manual	0.016	0.361	0.704	0.189
	Day 2			
	Blesbok 1		Blesbok 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ EQ02 vs. Cardell®	0.615	0.591	0.649	0.028*
Equivital™ EQ02 vs. Manual	0.198	0.110*	0.939	0.085*
Cardell® vs. Manual	0.384	0.163	0.589	0.414

(ICC = 0.75-0.90) on Day 1 for Blesbok 1, but the agreement between these two recording methods was poor on Day 2 for the heart rate of this animal. The agreement between the Cardell® monitor and the manual method for the heart rate recordings of Blesbok 1 was poor on both days of the study. The Equivital™ system had a moderate to excellent (ICC>0.90) agreement with the other two recording methods for the heart rate measurements of Blesbok 2 on both days of the study.

According to ICCs for absolute agreement calculated for the three methods of measurement (

	Day 1			
	Blesbok 1		Blesbok 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ EQ02 vs. Cardell®	0.018	0.284	0.707	0.391
Equivital™ EQ02 vs. Manual	0.751	0.646	0.966	0.003*
Cardell® vs. Manual	0.016	0.361	0.704	0.189
	Day 2			

	Blesbok 1		Blesbok 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ EQ02 vs. Cardell®	0.615	0.591	0.649	0.028*
Equivital™ EQ02 vs. Manual	0.198	0.110*	0.939	0.085*
Cardell® vs. Manual	0.384	0.163	0.589	0.414

Table 4.3), the Equivital™ system had a poor agreement with the Cardell® monitor for the respiration rate measurements of Blesbok 1 on Day 1 of the study, but there was a moderate agreement between these two methods on Day 2 for the respiration rate of this animal. The agreement between the Equivital™ system and the manual method for the respiration rate recordings of Blesbok 1 was moderate on Day 1, but the ICC calculated for these two recording methods for the respiration rate of this animal on Day 2 was not significant, indicating that the null hypothesis that ICC=0, is not rejected and thus no correlation exists. The agreement between the Cardell® monitor and the manual method for the respiration rate recordings of Blesbok 1 was poor on both days of the study. The agreement between the recording methods was either poor, or the ICC calculated was not significant (indicating that the null hypothesis that ICC=0, is not rejected and thus no correlation exists) for the respiration rate recordings of Blesbok 2 on both days of the study.

Table 4.3 Intraclass correlation coefficients for absolute agreement calculated to determine agreements between heart rate and respiration rate measurements via the three different measurement methods (Equivital™ EQ02 biotelemetry system, Cardell® monitor and manual measurements) for the two blesbok over a 20 min monitoring period on two different days.

	Day 1			
	Blesbok 1		Blesbok 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ EQ02 vs. Cardell®	0.018	0.284	0.707	0.391
Equivital™ EQ02 vs. Manual	0.751	0.646	0.966	0.003*
Cardell® vs. Manual	0.016	0.361	0.704	0.189
	Day 2			
	Blesbok 1		Blesbok 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ EQ02 vs. Cardell®	0.615	0.591	0.649	0.028*
Equivital™ EQ02 vs. Manual	0.198	0.110*	0.939	0.085*
Cardell® vs. Manual	0.384	0.163	0.589	0.414

*Intraclass correlation coefficient is not significant (P > 0.05), and thus no correlation exists

4.3.5 Agreements between the recording methods during the 2 min period following either adrenaline or Dopram® administration

The ICCs calculated for absolute agreement (Table 4.4) between the methods for the heart rate of Blesbok 1 following the administration of adrenaline were mostly not significant (P > 0.05) (indicating that the null hypothesis that ICC=0, is not rejected and thus no correlation exists), with the exception of the ICC calculated for the agreement between the Equivital™ system and the Cardell® monitor on Day 2. The ICC calculated for the Equivital™ system and the Cardell® monitor for Blesbok 1 on Day 2 indicated that there was a moderate agreement between these two methods for the heart rate measurements of this animal following adrenaline administration. The Equivital™ system had a good to excellent agreement with the other two methods of recording for the heart rate measurements of Blesbok 2 following adrenaline administration on Day 1 of the study. Only the ICC calculated for the agreement between the Equivital™ system and the manual recording method for the heart rate measurements of Blesbok 2 on Day 2 of the study were significant (P ≤ 0.05) and indicated an excellent agreement between these two recording methods for the heart rate measurements of this animal on the second day of the study.

The ICC were either not significant (indicating that the null hypothesis that ICC=0, is not rejected and thus no correlation exists) or indicated a very poor agreement between the methods of recording for the respiration rate measurements of both blesbok on both days of the study, with the exception of the Equivital™ and Cardell® monitor in Blesbok 2 on Day 1. The Equivital™ system and the Cardell® monitor had a good agreement with each other for the respiration rate measurements of Blesbok 2 on Day 1 of the study (Table 4.4).

Table 4.4 Intraclass correlation coefficients for absolute agreement calculated to determine agreements between heart rate and respiration rate measurements via three different measurement methods (Equivital™ EQ02 biotelemetry system, Cardell® monitor and manual measurements) for two blesbok for a two minute period following either Dopram® (influences respiration rate) or adrenaline administration (influences heart rate) on two different days.

	Day 1			
	Blesbok 1		Blesbok 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ EQ02 vs. Cardell®	0.002*	0.208*	0.763	0.814
Equivital™ EQ02 vs. Manual	0.355*	0.355*	0.985	-0.068*
Cardell® vs. Manual	0.004*	0.107*	0.902	-0.013*
	Day 2			
	Blesbok 1		Blesbok 2	
	Heart rate	Respiration rate	Heart rate	Respiration rate
Equivital™ EQ02 vs. Cardell®	0.601	-0.079*	0.533*	0.507*
Equivital™ EQ02 vs. Manual	0.191*	-0.762*	0.972	0.366*
Cardell® vs. Manual	0.418*	0.285	0.637*	0.284*

*Intraclass correlation coefficient is not significant (P > 0.05), and thus no correlation exists

4.3.6 Agreements between the recording methods over the whole study period without taking animal or day into account

The ICCs calculated for absolute agreement between the methods for the heart rate and respiration rate for the study as a whole, without taking day or animal into account (Table 4.5) were all significant (P ≤ 0.05). According to these ICCs, the agreement for both heart rate and respiration rate was highest between the Equivital™ system and the Cardell® monitor. The agreement for heart rate measurements was lowest between the Cardell® monitor and the manual recording method, whilst the lowest agreement for respiration rate measurements was between the Equivital™ system and the manual recording method. The agreement for heart rate measurements were moderate between the Equivital™ system and the other two methods, whilst the agreement between the Cardell® monitor and manual method for these recordings was poor. The agreement for respiration rate was moderate between the Equivital™ system and the other two methods, as well as between the Cardell® monitor and the manual recording method.

4.3.7 Motion measurements of the animals whilst in the enclosure

The mean heart rate and respiration rate of both blesbok measured by the Equivital™ system increased significantly when the animal moved slowly or fast in comparison to when the animals were not moving, as would be expected (

Table **4.6**). The mean heart rate of Blesbok 1 measured by the Equital™ system did not differ significantly between slow or fast movement of this animal although it did differ significantly when the animal was stationary compared to when it was moving. There was also no significant difference between the respiration rate recorded by the Equital™ system for Blesbok 1 during slow or fast motion of this animal although respiration was significantly lower when the animal was stationary. The mean heart rate and respiration rate of Blesbok 2 measured by the Equital™ system were significantly higher when this animal was moving fast compared to when the animal was moving slowly or the animal was stationary.

Blesbok 1 (the young ram) spent 67.5% of its time in the enclosure being motionless (stationary), 26.9% of the time moving slowly and 5.6% of the time moving fast. Blesbok 2, in comparison, spent 74.1% of its time in the enclosure being motionless, 20.9% of the time moving slowly and 5.0% of the time moving fast.

Table 4.5 Intraclass correlation coefficients for absolute agreement calculated to determine agreements between heart rate (bpm) and respiration rate (breaths/min) measurements via the three different measurement methods (Equital™ EQ02 biotelemetry system, Cardell® monitor and manual measurements) whilst in the laboratory, for the whole study period (without taking day or animal into account).

	Heart rate	**Respiration rate**
Equital™ EQ02 vs. Cardell®	0.601	0.685
Equital™ EQ02 vs. Manual	0.536	0.594
Cardell® vs. Manual	0.478	0.630

Table 4.6 The mean heart rate (bpm) and respiration rate (breaths/min) (mean ± standard error of the mean) of two blesbok per motion category (stationary, moving slowly or moving fast) whilst the animals were fully awake in captivity.

	Heart rate			**Respiration rate**		
	Stationary	Moving slowly	Moving fast	Stationary	Moving slowly	Moving fast
Blesbok 1	$85.6^{b} \pm 0.68$	$111.8^{a} \pm 1.19$	$111.4^{a} \pm 2.13$	$16.9^{b} \pm 0.15$	$30.1^{a} \pm 0.34$	$29.0^{a} \pm 0.83$
Blesbok 2	$53.1^{c} \pm 0.19$	$67.4^{b} \pm 0.60$	$89.4^{a} \pm 1.93$	$14.7^{c} \pm 0.11$	$24.0^{b} \pm 0.26$	$29.0^{a} \pm 0.41$

a,b,c Column means with different superscripts indicate significant differences between motion categories ($P \leq 0.05$)

4.4 Discussion

In this study the Equivital™ EQ02 biotelemetry system was capable of measuring heart rate with a reasonable amount of accuracy, both while the animals were under anaesthesia and whilst the animals were fully conscious in captivity. The Equivital™ EQ02 system measured similar mean heart rate to the manual method, whilst the Cardell® monitor measured higher mean heart rate values than both these methods. Laubscher *et al.* (2015a) also found that the heart rate measurements of blue wildebeest (*Connochaetes taurinus*) measured by the Equivital™ system agreed with the measurements of at least one of the other two methods used in this study, although this author did not find higher heart rate values measured via the Cardell® as was found in the present study. The intraclass correlation coefficients calculated per blesbok per day also showed that there was a higher agreement between the Equivital™ system and manual measurements for heart rate than between the Equivital™ system and the Cardell® monitor for these measurements. A possible reason for this is that heart rate values were recorded in duplicate by the Cardell® monitor. According to the American Heart Association (2006) an ECG monitor can mistake a tall T wave for an R wave, which results in the monitor displaying a value that is twice the intrinsic heart rate. Another possible reason for this could be due to the animal shivering and the resulting tremors interfering with the electrocardiogram measurements taken by the Cardell® monitor. According to Adam (2017), ECG monitors are unable to distinguish the muscle potentials (resulting from muscle contractions) from ventricular beats and therefore all large upward deflections are counted by these monitors which can therefore result in falsely elevated heart rate readings. Thus muscle tremors from the animal could have caused the heart rate readings measured by the Cardell® monitor to be higher than the other two recording methods.

Respiration rate did not show as similar a pattern in each of the three methods of measurements as was the case in the heart rate measurements. The Equivital™ EQ02 system measured similar mean respiration rate to the Cardell® monitor, whilst the manual readings of mean respiration rate were lower than both these two methods. Most of the intraclass correlation coefficients calculated per blesbok per day for agreement between the three recording methods for respiration rate were either low or indicated no correlation at all. Thus there was generally poor correlations between the three recording methods for respiration rate measurements. In a similar study, Laubscher *et al.* (2015a) also noted inconsistencies between the three methods of measurement for respiration rate in blue wildebeest (*Connochaetes taurinus*). Laubscher *et al.* (2015a) noted that the manual measurements are much less sensitive to subtle respiration changes as the number of breaths exhaled are counted to calculate the respiration rate and no fractional component is involved - as is the case in the calculation of the respiration rate by the Equivital™ system and the Cardell® monitor. This can potentially also account for the inconsistencies found between the respiration rate recorded manually and that recorded by the other two methods in this study. The Capnostat® probe of the Cardell® monitor's sensitivity to small changes in respiration could potentially have contributed to the inconsistencies between the respiration rate recorded by this method and that recorded by the other two methods in this study. Furthermore, the Equivital™ system measures respiration rate via a respiration elastic that measures chest movements when stretched during breathing. This function of the respiration elastic may be inhibited if an animal is lying sternally because of the pressure of the sternum and chest on the elastic -

this may have contributed to the poor agreement between this method and the other two methods for the respiration rate recorded for the animals in this study. Most of the manual measurements of both the respiration rate and heart rate of both animals were not comparable to that recorded by the Cardell® measurements - the only exception being the heart rate measurements for Blesbok 2. Laubscher *et al.* (2015a) also found weak agreements between manual measurements and Cardell® measurements in blue wildebeest. The author argued that this lack of agreement could be the result of erroneous measurements taken by one of these methods, which could also be the case in this study.

The mean respiration rate measured by the Equivital™ EQ02 system whilst the animals where being monitored in the laboratory (8.8–15.3 breaths/min) are similar to those found by Mortola and Lanthier (2005) for bovidae species. These authors found that ruminants with a similar weight class as the blesbok had a resting respiration rate ranging from 11 breaths/min in mule deer (*Odocoileus hemionus*) with an average weight of 72kg, and 15 breaths/min in alpaca (*Vicugna pacos*) with an average weight of 68kg.

All three methods of measurement showed a good sensitivity to adrenaline administration indicated by a significant increase in heart rate following adrenaline administration. The Equivital™ system measured similar mean heart rates to those recorded manually following adrenaline administration, whilst the Cardell® monitor measured higher mean heart rates than both these two methods following adrenaline administration. As previously stated, double counting of values could account for the higher heart rate recorded via the Cardell® monitor. A slight increase in heart rate can also be seen for both animals on both days following Dopram® administration. These results are similar to those of Lugliani *et al.* (1979), who reported a slight increase in heart rate in patients following doxapram hydrochloride infusion. Hsu *et al.* (1985) found that doxapram hydrochloride antagonized bradycardia (i.e. abnormally slow heart rate) caused by xylazine in dogs. Oikawa *et al.* (2014) indicated that doxapram hydrochloride resulted in tachycardia (i.e. an abnormal rapid heart rate) in rats.

In this study, all methods showed poor sensitivity to doxapram hydrochloride administration. A possible reason could be that blesbok as a species do not respond to Dopram®, and thus no increase in respiration rate was observed in these animals after treatment with this drug. There is no published data available on the use of Dopram® in blesbok and therefore it is unknown as to how this drug would affect this animal species. According to Golder *et al.* (2013) a patient may not respond to doxapram hydrochloride if the central nervous system is severely depressed. It is thus possible that the anaesthetic (thiafentanil oxalate) used to immobilise the blesbok in this study had suppressed the central nervous system of the animals to such an extent that Dopram® had no effect on the respiratory system. Further research on the effects of Dopram® in blesbok is required to completely understand its failure to cause a respiratory response in this study.

Despite these inconsistencies in the agreement between the three recording methods per blesbok per day, there exists a moderate agreement between the Equivital™ system and the other two recording methods for both heart

rate and respiration rate when the animal and day is not taken into account. This shows that the Equivital™ system can measure the heart rate and respiration rate with a reasonable amount of accuracy.

In order to further determine the accuracy of the Equivital™ system, the motion measured via the system whilst the animals were in the enclosure was compared to the heart rate and respiration rate of the animals during this time in captivity. An increase in motor activity results in an increase in heart rate and respiration rate (Price & Sibly, 1993), thus it is expected that the heart and respiration rate of the animals would increase if their motion increased. The Equivital™ system depicted elevations in heart and respiration rate when the animals showed an increase in motion, showing its reliability as a potential biotelemetry system for blesbok.

4.5 Conclusion

According to the results found in this study, the Equivital™ biotelemetry system can be successfully used to measure heart rate and motion in blesbok, but its respiration rate measurements in this species need to be improved should it be used in sternally recumbent animals. Overall the Equivital™ system shows great potential for the biotelemetric measurement of physiological parameters in wild antelope species both whilst immobilised and whilst fully conscious in captivity. The successful use of the Equivital™ biotelemetry system in wildlife can make it possible to study and understand factors that cause changes in the normal physiology of these animals without needing to constrain the animals. However, the Equivital™ system most likely does not have a high enough sensitivity to detect or measure minute respiration changes or changes caused by the administration of certain drugs such as Dopram®. The blesbok may not have responded to Dopram® and the functioning of its respiration elastic may have been hindered by the pressure of the sternum and chest of immobilised animals that are kept in sternal recumbency. It is therefore recommended that further investigation be conducted on how the Equivital™ biotelemetry system's sensitivity to respiratory changes could be increased and the design of its respiration elastic should be improved to ensure that the position of an animal do not influence its measurements of respiration rate.

Chapter 5

The influence of midazolam on the behaviour and wellbeing of blesbok (*Damaliscus pygargus phillipsi*) in captivity

Abstract

This study investigated the potential of midazolam to be used as a sedative in adult blesbok kept in captivity. Six adult female blesbok were each fitted with an Equivital™ EQ02 biotelemetry system that recorded respiration and heart rate during the treatment periods as well as motion. Midazolam was administered at a low (0.2 mg midazolam/kg BWt), medium (0.4 mg midazolam/kg BWt) and high dosage (0.6 mg midazolam/kg BWt) during three individual trials, whilst a placebo served as the control treatment. The behaviour of the animals during each trial was recorded for 12 hrs post-midazolam administration using CCTV cameras. Behavioural analysis included a comparison of the time spent on specific state and point behaviours, as well as on different motion categories by the animals during each trial. Feed consumption was measured to determine if midazolam influenced feed intake during the treatment intervals. Midazolam generally did not affect the behaviour of the blesbok, and any significant effects observed may have been the result of the paradoxical nature of benzodiazepines. The lowest midazolam dose was the most effective in reducing behaviours such as vigilance, walking and head swaying, which are commonly associated with stress. The medium dose of midazolam was only effective in increasing the "standing with head up ruminating" behaviour and decreasing vigilance in the blesbok. The high dose was only effective in reducing vigilance and agitation point behaviours, but caused a paradoxical increase in walking and a paradoxical reduction in "standing with head up ruminating" behaviour. The different doses of midazolam caused variable effects on the point behaviours of the blesbok, but the high dose had the most prominent effect on these behaviours as the counts for three out of the four point behaviours were lower compared to when animals were treated with a placebo. Midazolam caused a contradictory increase in fast motion during periods of stimulation. The high and medium doses of midazolam had no significant effect on motion during periods of no stimulation. The low dose of midazolam reduced the time spent on slow motion and resulted in the animals spending more time standing still during periods of stimulation when compared to the other treatments. Midazolam also appears to have increased feed intake per dose in blesbok. Ultimately, midazolam caused variable changes in behaviour of the blesbok. The low dose appears to have been most effective in calming the animals with minimal side effects, and is therefore recommended for use in blesbok that are exposed to minimal stress. Future research should focus on investigating the dosage of midazolam tested in different ungulate species.

5.1 Introduction

The blesbok is a common, endemic wild ungulate species in South Africa and is popular to farm with due to their small size, ease of handling and the capability to detain them within normal livestock fencing (Lloyd & David, 2008; Frost, 2014). Various wildlife farming and management practices such as capture, translocation, and performing veterinary or research procedures, require these animals to be handled. Handling induces stress and negatively affects the welfare of the animals (Dickens *et al.*, 2010). It is thus essential that the blesbok be sufficiently calm for the successful execution of these procedures and therefore stress in the animals must be managed. Sedatives and tranquilizers present a potential solution for the management of stress in blesbok.

Sedatives and tranquilizers are pharmaceuticals that suppress an animal's central nervous system, thereby having a calming effect and thus reducing stress. The development and consequent testing of new sedatives and tranquilizers under field conditions play an integral role in the design of drugs that can address specific issues related to the levels of stress in wildlife species. Tranquilizers and sedatives offer a solution for managing stress in wildlife, but their use can have both desired and undesired effects in the treated animals (Jones, 1972). Tranquilizers have been found to effectively reduce fear, anxiety and aggression in animals, but their use may also result in anorexia, hypotension, convulsions, and inhibition of thermoregulation (Burroughs *et al.*, 2012b; Lamont & Grimm, 2014). Sedatives, e.g. benzodiazepines and α-2-adrenergic agonists, result in drowsiness and reduced locomotor activity, with minimal side effects. Compared to tranquilizers, sedatives may result in sleep at high doses, are less selective and can be readily reversed (Burroughs *et al.*, 2012b; Lamont & Grimm, 2014; Wolfe, 2015).

Midazolam is a benzodiazepine sedative that is more potent, effective and has a more predictable absorption following IM administration than its counterpart, diazepam. Furthermore, midazolam is preferred to diazepam as it can be used as a top-up drug in wildlife, in bait or as a sedative that is short-acting for the relocation of certain wildlife species (e.g. rhinoceros) (Burroughs *et al.*, 2012b.; Miller & Buss, 2015). Midazolam has been successfully used for the sedation of various domestic species (Smith *et al.*,1991; Stegmann,1998; Stegmann, 1999; Stegmann, 2001; Upton *et al.*, 2009; Aarnes *et al.*, 2013; Schwartz *et al.*, 2013; Simon *et al.*, 2017). Midazolam is usually combined with other pharmaceuticals for the immobilisation of wildlife species (Laricchiuta *et al.*, 2012; Lapid & Shilo-Benjamini, 2015; Van Zijl *et al.*, 2016; Gerlach *et al.*, 2017). According to unpublished data, most wildlife veterinarians in South Africa use 0.1-0.2 mg midazolam/kg BWt for the sedation of antelope and even lower dosages if midazolam is used as part of immobilisation protocols for these animals (Raath, personal communication, 3 November 2017). No published literature is available on the use of midazolam on its own in wild ungulate species and specifically in blesbok, and its effect in this species is therefore unknown.

According to Dawkins (2004), animal behaviour and certain physiological parameters are considered valuable indicators of animal wellbeing. Therefore, it would be possible to determine the safety of a drug for use in a specific animal species by determining how it affects the normal behaviour of the animal species following treatment with the product. Behavioural indicators provide a potential means to assess the effects of stress associated with

capture, translocation, and handling. Stress results in changes in the normal behaviour of an animal, manifesting in abnormal behaviour that can be dangerous to the animal and its handlers. Common behaviour used to study animal welfare include innate behaviour (e.g. motion, grooming and feeding behaviour), defensive and avoidance behaviour (Beck & Luine 2002; Laubscher, 2015). Stress can result in an increase in the abnormal behaviour of an animal, including an increase in the frequency of abnormal motions e.g. increased head rubbing or scratching, all situations that impact negatively on the wellbeing of the animal. Animal behaviour can be quantified by visual observation following treatment with the test substance (Ransom & Cade, 2009).

Various behavioural studies reported on the influence of stress experienced during transport or maintenance in captivity on behaviour of animals. A study in beagle dogs showed for example that the animals spent more time being stationary and lying down during air transport, which indicated that the transport conditions were stressful to the animals (Bergeron *et al.*, 2002). Bergeron *et al.* (2002) showed that sedation of the dogs did not have any effect on the behaviour during the stress experienced by the animals while being transported. Bulitta (2012) found in swine that stress hormones and stress behaviours, namely rooting and vocalization, increased during transport. Various studies have shown that captivity causes animals to show abnormal (stereotypical) behaviours (Bergeron *et al.*, 2002; Clubb & Vickery, 2006; Würbel, 2006); Mason and Lathom (2004) found that more than 85 million laboratory, farm or zoo animals around the globe showed stereotypic behaviours and that this should be considered an indicator of poor animal welfare.

Telemetry is considered by various authors to be the most accurate and reliable method for studying the effects of new pharmaceuticals in free-moving animal, as they measure the parameters of fully conscious animals with minimal stress/interference, and thus give a better representation of animals in their normal state (Kramer *et al.*, 1993; Kramer *et al.*, 2001; Ando *et al.*, 2005; Hayashi *et al.*, 2005; Miyazaki *et al.*, 2005; Sasaki *et al.*, 2005; Laubscher, 2015). Both invasive and non-invasive telemetry systems can be used to study animal behaviour and physiology. Examples of non-invasive techniques include, among others, surface electrodes for electrocardiogram (ECG) monitoring or cuffs placed on animal limbs (Kramer & Kinter, 2003). Non-invasive telemetry systems are easy to use and more cost-effective than invasive telemetry devices, but tend to produce data artefacts due to restraint stress. Externally fitted devices or electrodes can also easily be damaged or move from their intended position on the body of the animal. Invasive telemetry systems refer to implantable sensors either placed subcutaneously or within an animal's body cavities. Implantable telemetry systems have a lower chance of being removed during their use, and cause only a minor level of discomfort to the animals. However, implantable telemetry devices are very expensive and may require surgery to be inserted into the animal. Furthermore, internal telemetry systems used to determine the effects of a substance on the bodily functions of animals are very resource intensive and require restraint of the animals, which can cause issues such as handling stress and limited collection of data (Kramer & Kinter, 2003; Cooke *et al.*, 2013).

The use of external telemetry systems has been recommended for use in animal studies as they overcome the abovementioned drawbacks of internal devices and have the extra benefit of being non-invasive (Chui *et al.*, 2009).

Laubscher (2015) reported that an external biotelemetry system, the Equivital™ EQ02 biotelemetry belt, successfully measured the effects of pharmaceuticals on the physiological parameters and behaviour of blue wildebeest (*Connochaetes taurinus*). Therefore, the Equivital™ system could also be used in determining how midazolam affects the behaviour, specifically motion, of blesbok.

The effects of certain drugs known to alter animal behaviour can be studied through observations of animal behaviour. The least invasive way of doing this is through the recording of animal behaviour via video recordings. Analysis of the recorded video footage then allows for the analysis of a drug's effect on animal well-being, with specific focus on behaviour known to occur as a result of stress. Analysis of the video footage can be used to determine the occurrence of different state and point behaviour of the animal after drug treatment, which can then be compared to the behaviour of the animal observed when treated with a control (Bowden *et al.*, 2008; Ransom & Cade, 2009; Laubscher, 2015). Point behaviour is short lasting behaviour that can be measured at a point in time. State behaviour is defined as longer lasting behaviour of which the start and end can be determined (Bowden *et al.,* 2008). Software, such as The Observer® XT (Noldus Information Technology, Wageningen, The Netherlands), is available for the analyses of video footage for behaviours via a pre-set coding scheme.

Feed intake is an important aspect of an animal's welfare, as the consumption of food ensures that essential nutrients are available to the animal for growth, reproduction and production (Naseri & Kabul-Afghanistan, 2005). Various tranquilizing agents have been found to reduce appetite in wildlife species, thereby reducing feed intake (Huber *et al.*, 2001; West *et al.*, 2014) which can be very concerning for animal welfare, especially for animals in captivity. Midazolam has, however, been found to stimulate appetite in animals (Van Miert *et al.*, 1989; Berridge & Peciña, 1995), but its specific effects on the appetite of blesbok has not yet been determined.

Midazolam's influence on the normal behaviour, motion and feed intake of blesbok has not yet been studied. Due to its previously stated attributes, midazolam holds potential as a sedative for use during the transport of blesbok or the maintenance of these animals in captivity. This study's aim was thus to evaluate the safety and effectiveness of midazolam in blesbok, by studying the effects of this sedative on the behaviour, motion and feed intake of this antelope species.

5.2 Materials and methods

Ethical approval for this study was obtained from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa. Protocol ethical approval number: ACU-2017-0279-422.

5.2.1 Experimental location

This study was conducted on Ngongoni farm (25°31'25.2" S, 31°06'50.8" E), outside Nelspruit in Mpumalanga, South Africa. The region is characterized by an average temperature of 19.8 °C (minimum 14.6 °C and maximum 23.6 °C), and annual rainfall of 796mm (summer rainfall region; range: 11mm in June to 130mm in January) (Climate: Nelspruit).

5.2.2 Experimental animals and husbandry

Six mature female blesbok, with an average weight of 58.5 ± 3.34 kg, were used in this study. The number of animals used in this study was determined by the number of available biotelemetry systems. Before the initiation of the study, the blesbok were detained in an enclosure of 6 x 8 m in size containing a group of 12 blesbok in total and referred to as the group enclosure. Clean, fresh water and lucerne hay were provided *ad libitum* to the animals. Fresh feed was provided to the blesbok at the beginning of each trial, while any remaining feed from the previous day was removed. The amount of feed weighed were noted on a datasheet (*Appendix B*).

The blesbok were housed in an experimental enclosure of 6 × 8 m, and the design of the enclosure prevented visual contact between animals in adjacent enclosures. Shade netting covered a third of each enclosure and sufficient drainage was ensured by the slope on which the enclosure was constructed. The enclosures were equipped with concrete feed containers with smooth rims to ensure that the animals would not be injured during feeding. The water containers were constructed from cement (capacity 50L), were sunk into the ground and also protruded on the outside of the enclosure to allow for easy cleaning. The enclosure was equipped with four CCTV video cameras (Nictec Radio Communications, Nelspruit, South Africa) to record the animals at each angle of the enclosure, in order to observe their behaviour throughout each trial. To ensure video footage recorded at night could be clearly observed, an infrared light was set up in the enclosure. A completely equipped veterinary laboratory was available only 150 m away from the enclosure. In an attempt to prevent the animals from becoming habituated, they were moved between enclosures and regrouped with other blesbok before and after each trial was conducted. Please refer to Chapter 4 for a detailed description of animal husbandry and the design of the enclosure used in this study.

5.2.3 The biotelemetry system and modifications

The Equivitial™ EQ02 biotelemetry system was used for measuring the vital signs and motion of the blesbok in this study. This biotelemetry system consists of a belt with a chest band containing a stretch inhibitor with an elastic which measures respiration, and two lead ECG sensors that measures heart rate. The Equivital™ EQ02 biotelemetry system also detects the position of an animal and records motion within three categories: stationary, slow motion and fast motion via an accelerometer. Each of the sensors on the belt connect to a sensor electronic module (SEM) which logs the recorded data.

The SEM was secured to each belt with insulation tape, and double-sided tape was used to ensure that the belts did not move from the preferred position on the animal's body. Please refer to Chapter 4 for a full description of the Equivital™ EQ02 biotelemetry system and its modification.

5.2.4 Experimental design

The experimental design to determine the effects of four different treatments on the behaviour, motion and feed intake of blesbok is presented in Figure 5.1.

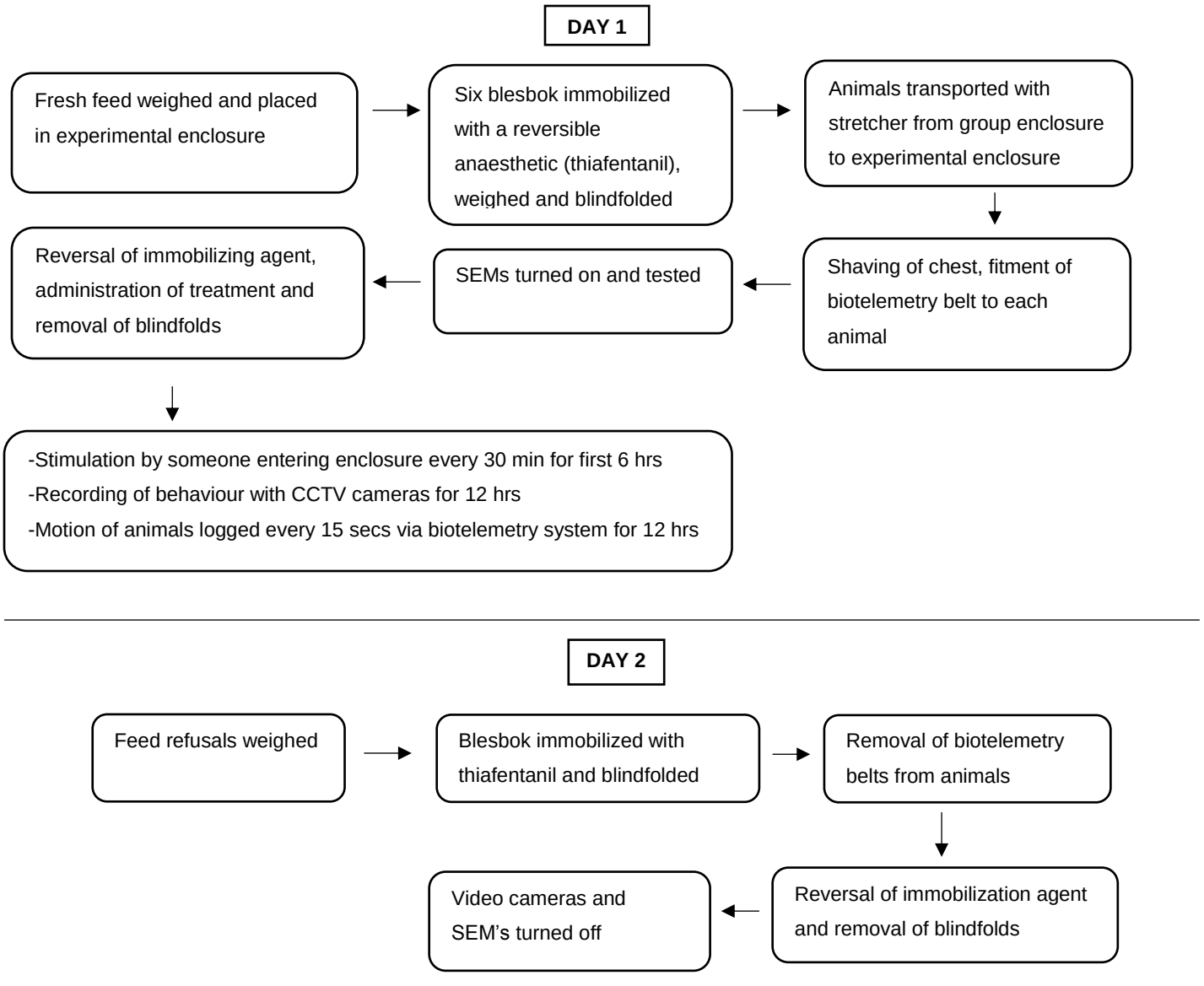

Figure 5.1 The experimental design for determining the effect of a placebo and three different midazolam treatments on the behaviour, motion and feed intake of blesbok.

5.2.5 Identification of the animals

An identification number was allocated to each blesbok, and spray-painted on both sides of each animal's rear and midsection to ensure easy identification. Piping made from PVC was also placed over the horns of each animal to prevent the animals from harming each other or risk harming the personnel handling them. In addition to the number spray-painted on the rear and midsection, rings of white duct tape, corresponding to the number of the

animal, were placed on the piping (Figure 5.2). The number allocated to each animal was noted on a datasheet (*Appendix B*).

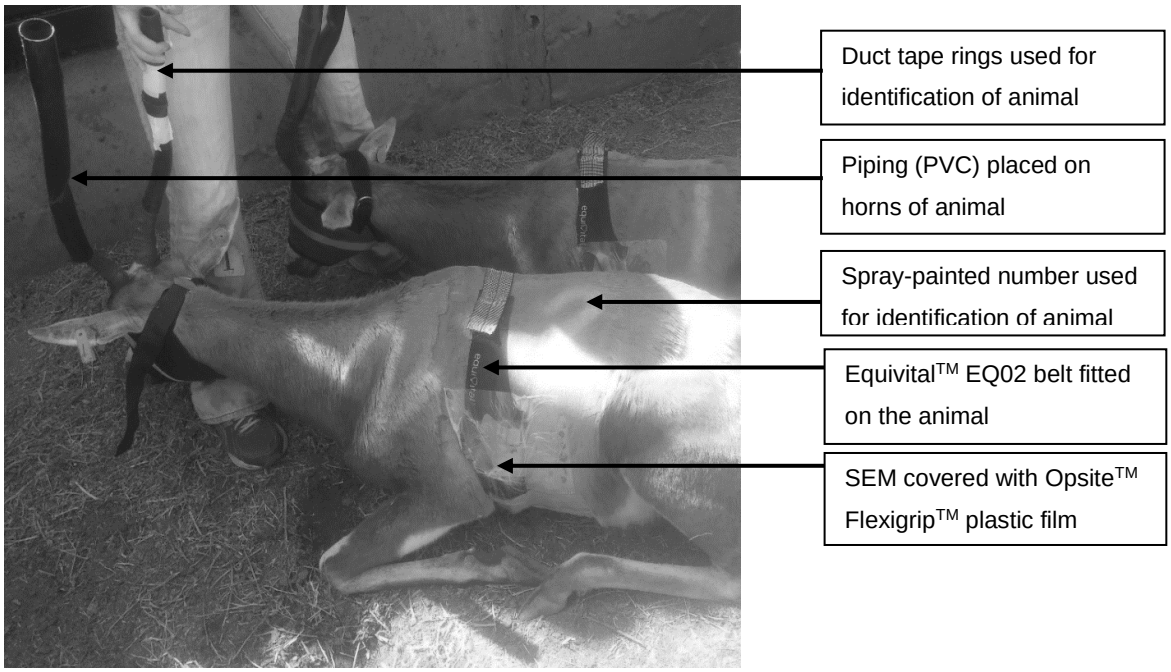

Figure 5.2 Two immobilised blesbok fitted with the Equivital™ biotelemetry belts.

5.2.6 Fitment of biotelemetry belts and the administration of pharmaceuticals

The animals were immobilised with 3 mg thiafentanil oxalate (a reversible anaesthetic) (Thianil® Wildlife pharmaceuticals (Pty) Ltd, White River, South Africa), placed on a stretcher and blindfolded. Once an animal was successfully immobilised, the darts were removed and the dart wounds were cleaned and disinfected. Each animal was then carefully transported from the group enclosure to the trial enclosure by trained personnel from Wildlifevets.com, where they were each weighed with a hanging scale in a stretcher (the weights were noted on the datasheet in *Appendix B*) and fitted with a biotelemetry belt. During their immobilisation, each blesbok was held by their horns and kept in a sternally recumbent position to prevent regurgitation (Figure 5.2).

The blesbok were shaved around the chest before the belts were fitted, to ensure that the ECG sensors made direct contact with clean skin. Each animal was then fitted with an Equivital™ EQ02 biotelemetry belt and care was taken to position the SEM over the heart. The belts were secured to the skin of the animals with double sided tape and superglue. To protect it from damage by dirt and water, the SEM on each belt was covered with Opsite™ Flexigrip™ plastic film. The ends of the belt were sutured together to hold it in place on the animal. After each animal was fitted with a biotelemetry belt, the accuracy of the sensor electronic module (SEM) of each belt was confirmed by comparing the measurements observed on a mobile device (connected to the SEM via Bluetooth technology) to manual measurements. Once each animal was fitted with a biotelemetry belt and the accuracy of each SEM confirmed, the anaesthetic was reversed with 0.6 mL naltrexone hydrochloride. The number of the SEM on the belt of each respective animal was noted on a datasheet (*Appendix B*). Please refer to Chapter 4 for a detailed description of the fitment of the biotelemetry belts to the blesbok.

The treatment, which was either a placebo, 0.6 mg midazolam/kg BWt, 0.4 mg midazolam/kg BWt or 0.2 mg midazolam/kg BWt (Wildlife Pharmaceuticals (Pty) Ltd), was then administered via the IM route to each animal. The dosage and time of administration per animal was noted on a datasheet (*Appendix B*). The treatment dosages were selected based on published literature on the use of midazolam in similar sized domestic species or goats (Stegmann, 1998,199; Stegmann & Bester, 2001), results from the pharmacokinetic study of midazolam in indigenous goats (Chapter 4) and unpublished data of midazolam use in antelope species by wildlife veterinarians. In Chapter 4 it was found that a midazolam dose of 0.8 mg/kg BWt administered IM to indigenous goats had a poor bioavailability, but a short elimination rate. According to unpublished data, South African wildlife veterinarians in practice commonly use midazolam doses of 0.1-0.2 mg/kg BWt to sedate antelope via the IM or IV route, and they use even lower doses when this drug is used as part of immobilisation protocols for antelope (Raath, personal communication, 3 November 2017). Based off this information, it was decided that doses between those used in practice by veterinarians and those used in goats should be used for the sedation of the blesbok in this study. The thiafentanil oxalate was reversed prior to treatment of the animals with the specific treatment of the trial with 0.6 mL naltrexone hydrochloride (Trexonil®, Wildlife Pharmaceuticals (Pty) Ltd) intravenously via the auricular vein to reverse the effects of thiafentanil oxalate (Thianil®, Wildlife Pharmaceuticals (Pty) Ltd). According to Grimm *et al.* (2015), naltrexone rapidly and completely reverses thiafentanil oxalate. Once all of the animals were awake and showing no abnormal behaviour, the trial was initiated. The pharmaceuticals administered in this study are presented in Table 5.1 and the allocations of the treatments per trial are presented in Table 5.2. To prevent a carry-over effect, a minimum wahout period of 1 week was allowed between trials. All pharmaceuticals were administered by a registered veterinarian.

On Day 2 of each respective trial, the animals were once again immobilised with 3 mg thiafentanil so that the biotelemetry belts could be removed. The animals were blindfolded again and held in a sternally recumbent position to prevent regurgitation. Once all the belts were removed from animals, 0.6 mL naltrexone was administered to reverse the effects of the thiafentanil oxalate. Once each trial was completed, the animals were moved back in to the large group enclosure to prevent habituation to the facility and presence of humans.

Table 5.1 Drugs administered in this study to blesbok.

Drug	Dose	Route	Purpose	Frequency
Midazolam	0.2-0.8 mg/kg	IM	Experimental treatment	Once to all the animals in the associated trial
Thiafentanil oxalate	3-3.5 mg	IM	Immobilisation of animals	Twice per animal within 24 hrs
Naltrexone hydrochloride	0.6 mL	IV	Reversal of immobilisation agent	Twice per animal within 24 hrs

Table 5.2 The allocation of the different treatments over the 4 trials to determine the effects of midazolam in blesbok.

Trial	Treatment
1	Placebo administered to all 6 animals
2	0.6 mg midazolam/kg BWt administered to all 6 animals
3	0.4 mg midazolam/kg BWt administered to all 6 animals
4	0.2 mg midazolam/kg BWt administered to all 6 animals

5.2.7 Data recorded

5.2.7.1 Measurement of motion

The Equivital™ system measures motion via an accelerometer according to three different categories, namely “stationary”, “moving slowly” and “moving fast”. This data of the blesbok was logged by the SEM on the biotelemetry belt of each respective animal every 15 secs from the start of the trial for 12 hrs following treatment with the test substance. A person entered the enclosure every 30 min for the first 6 hrs following administration of midazolam in order to elicit a stress response in the animals. The change in the motion of the animals during these periods of stimulation was also recorded to determine the effect of each treatment during periods where the animals were experiencing a stressor.

5.2.7.2 Behavioural monitoring and analysis

Following the administration of the treatment during a specific trial (placebo, high dose, medium or low dose of midazolam), the animals were continuously monitored with the CCTV cameras inside the enclosure to quantify changes in their behaviour. The footage captured during each trial was analysed to determine the influence of midazolam administration on the behaviour of the animals.

The behaviour recorded for the animals were defined as being either state or point behaviour (

Table **5.3**). State behaviour is defined as behaviour with a clear start and end time and was measured as a percentage of the total measurement period of 12 hrs (Bowden *et al.*, 2008). Point behaviour is behaviour of which the duration is so short that it cannot be measured according to a start and end time (Bowden *et al.*, 2008), and were measured via the number of counts of their occurrence.

The Observer® XT 11 behavioural Software (Noldus Information Technology, Wageningen, The Netherlands, 2011) was used for the analyses of the behaviour of each animal from the CCTV video footage collected for each respective trial. The specific state and point behaviour of the animals (

Table **5.3**) were observed and each animal's behaviour was entered into the software with a coding scheme set up specifically for this study. The data from the Equivital™ biotelemetry systems was imported into the software and combined with the observed behavioural data.

It should be noted that behaviour classified as "other abnormal behaviour" refers to behaviour only observed a few times and/or only in a specific animal. These behaviour descriptions include skin trembling or stumbling/falling and were not observed enough times to be classified on their own.

Table 5.3 The behaviours of blesbok analysed via the Observer XT software.

Behaviour	Behaviour type
Standing with head up ruminating	State
Walking	State
Vigilance	State
Lying with head up	State
Eating	State
Lying with head down	State
Exploring (sniffing while standing)	State
Standing with head down	State
Trotting in alarm	State
Exploring (sniffing while walking around)	State
Fighting	State
Avoidance	State
Drinking	State
Other abnormal behaviour	State
Grooming - Nibbling skin	Point
Grooming - while lying down	Point
Grooming- scratching with hind leg	Point
Agitation - head swaying	Point
Agitation - feet stomping	Point

5.2.8 Measuring of feed intake

At the start of each trial, the animals were supplied with the same lucerne hay that they had been fed before the start of the experiment. The amount of feed supplied at the beginning of each trial and the amount of feed remaining at the end of each trial was weighed (Figures 5.1 & 5.3) so that the percentage of feed consumed by the animals per trial could be calculated. These percentages were used to determine whether midazolam increased or decreased appetite and thus feed consumption by blesbok.

Figure 5.3 A hanging scale and bag used to weigh the amount of feed given at the beginning and remaining at the end of each trial.

5.2.9 Statistical analysis

The data from this study was analysed using a restricted maximum likelihood estimation (REML) in Statistica's Variance Estimation, Precision and Comparison module (Statistica version 13, Stat Soft, Inc., 2016). Dose and behaviour were the fixed effects for behaviour data. Dose, motion and stimulation were the fixed effects used during the analyses of motion per period of stimulation data. Results were expressed as mean ± standard error of the mean (SEM) and were considered significant at $P \leq 0.05$.

5.3 Results

5.3.1 Localized reaction at administration sites

The injection sites were examined for swelling to ensure no adverse reactions occurred, as IM administration has been found to cause local irritation and inflammation if not performed correctly (Mark *et al.*, 1999). No adverse reaction was found at the injection sites during any of the trials.

5.3.2 Influence of midazolam on state behaviour

The influence of midazolam on the state behaviour of the blesbok treated with a placebo, low, high or medium dose of midazolam is presented in

Behaviour	Type	Placebo	High Dose (0.6 mg/kg BWt)	Medium Dose (0.4 mg/kg BWt)	Low Dose (0.2 mg/kg BWt)
Standing with head up ruminating (%)	State	32.4^{c} ± 4.33	24.5^{d} ± 5.67	40.0^{b} ± 5.60	56.6^{a} ± 2.59
Walking (%)	State	23.6^{b} ± 1.70	34.9^{a} ± 3.37	25.5^{b} ± 2.39	14.3^{c} ± 1.40
Vigilance (%)	State	22.5^{a} ± 2.12	11.5^{b} ± 1.44	10.0^{b} ± 1.95	11.6^{b} ± 2.08
Lying with head up (%)	State	6.6^{b} ± 3.39	12.7^{a} ± 2.01	7.9^{b} ±2.94	3.8^{b} ± 1.65

Eating (%)	State	6.6[a] ± 0.63	6.6[a] ± 1.03	5.8[a] ± 0.71	8.6[a] ± 0.77
Lying with head down (%)	State	3.9[a] ± 0.54	4.3[a] ± 1.11	5.5[a] ±1.82	3.7[a] ±0.38
Exploring (sniffing while standing) (%)	State	2.1[a] ± 0.83	1.3[a] ± 0.44	3.0[a] ± 1.12	1.5[a] ± 0.42
Standing with head down (%)	State	0.6[a] ± 0.31	0.3[a] ± 0.10	1.3[a] ± 0.39	1.6[a] ± 0.40
Trotting in alarm (%)	State	1.4[a] ± 0.44	1.5[a] ± 0.19	0.9[a] ± 0.09	0.5[a] ± 0.07
Exploring (sniffing while walking around) (%)	State	1.3[a] ± 0.62	1.6[a] ± 0.97	0.7[a] ± 0.27	0.2[a] ± 0.08
Fighting (%)	State	0.9[a] ± 0.28	0.5[a] ± 0.14	0.2[a] ± 0.08	0.0[a] ± 0.02
Avoidance (%)	State	0.5[a] ± 0.10	0.2[a] ± 0.06	0.1[a] ± 0.03	0.1[a] ± 0.005
Drinking (%)	State	0.1[a] ± 0.01	0.2[a] ± 0.03	0.1[a] ± 0.01	0.1[a] ± 0.01
Other abnormal behaviour (%)	State	0.1[a] ± 0.09	0.0[a] ± 0.02	0.0[a] ± 0.01	0.0[a] ± 0.02
Grooming - Nibbling skin (# of counts)	Point	6.1[a] ± 0.23	5.1[b] ± 0.26	6.3[a] ± 0.14	5.1[b] ± 0.29
Grooming - while lying down (# of counts)	Point	1.6[b] ± 0.63	3.1[a] ± 0.78	3.2[a] ± 0.59	1.7[b] ± 0.65
Grooming- scratching with hind leg (# of counts)	Point	1.7[a] ± 0.29	0.7[b] ± 0.35	2.4[a] ± 0.40	2.0[a] ± 0.38
Agitation - head swaying (# of counts)	Point	7.6[a] ± 0.19	6.7[b] ± 0.36	7.8[a] ± 0.39	6.8[b] ± 0.33
Agitation - feet stomping (# of counts)	Point	3.6[a] ± 0.65	2.2[b] ± 0.76	3.7[a] ± 0.60	3.2[a] ± 0.33

. Two exploratory behaviour patterns were observed in this study and categorized as either “sniffing while standing” or “sniffing while walking around”. The low and medium doses of midazolam did not suppress rumination in the blesbok, when compared to the placebo (

Behaviour	**Type**	**Placebo**	**High Dose (0.6 mg/kg BWt)**	**Medium Dose (0.4 mg/kg BWt)**	**Low Dose (0.2 mg/kg BWt)**
Standing with head up ruminating (%)	State	32.4[c] ± 4.33	24.5[d] ± 5.67	40.0[b] ± 5.60	56.6[a] ± 2.59
Walking (%)	State	23.6[b] ± 1.70	34.9[a] ± 3.37	25.5[b] ± 2.39	14.3[c] ± 1.40
Vigilance (%)	State	22.5[a] ± 2.12	11.5[b] ± 1.44	10.0[b] ± 1.95	11.6[b] ± 2.08
Lying with head up (%)	State	6.6[b] ± 3.39	12.7[a] ± 2.01	7.9[b] ±2.94	3.8[b] ± 1.65
Eating (%)	State	6.6[a] ± 0.63	6.6[a] ± 1.03	5.8[a] ± 0.71	8.6[a] ± 0.77
Lying with head down (%)	State	3.9[a] ± 0.54	4.3[a] ± 1.11	5.5[a] ±1.82	3.7[a] ±0.38
Exploring (sniffing while standing) (%)	State	2.1[a] ± 0.83	1.3[a] ± 0.44	3.0[a] ± 1.12	1.5[a] ± 0.42
Standing with head down (%)	State	0.6[a] ± 0.31	0.3[a] ± 0.10	1.3[a] ± 0.39	1.6[a] ± 0.40
Trotting in alarm (%)	State	1.4[a] ± 0.44	1.5[a] ± 0.19	0.9[a] ± 0.09	0.5[a] ± 0.07
Exploring (sniffing while walking around) (%)	State	1.3[a] ± 0.62	1.6[a] ± 0.97	0.7[a] ± 0.27	0.2[a] ± 0.08
Fighting (%)	State	0.9[a] ± 0.28	0.5[a] ± 0.14	0.2[a] ± 0.08	0.0[a] ± 0.02
Avoidance (%)	State	0.5[a] ± 0.10	0.2[a] ± 0.06	0.1[a] ± 0.03	0.1[a] ± 0.005
Drinking (%)	State	0.1[a] ± 0.01	0.2[a] ± 0.03	0.1[a] ± 0.01	0.1[a] ± 0.01
Other abnormal behaviour (%)	State	0.1[a] ± 0.09	0.0[a] ± 0.02	0.0[a] ± 0.01	0.0[a] ± 0.02
Grooming - Nibbling skin (# of counts)	Point	6.1[a] ± 0.23	5.1[b] ± 0.26	6.3[a] ± 0.14	5.1[b] ± 0.29
Grooming - while lying down (# of counts)	Point	1.6[b] ± 0.63	3.1[a] ± 0.78	3.2[a] ± 0.59	1.7[b] ± 0.65
Grooming- scratching with hind leg (# of counts)	Point	1.7[a] ± 0.29	0.7[b] ± 0.35	2.4[a] ± 0.40	2.0[a] ± 0.38
Agitation - head swaying (# of counts)	Point	7.6[a] ± 0.19	6.7[b] ± 0.36	7.8[a] ± 0.39	6.8[b] ± 0.33
Agitation - feet stomping (# of counts)	Point	3.6[a] ± 0.65	2.2[b] ± 0.76	3.7[a] ± 0.60	3.2[a] ± 0.33

). The animals spent a higher percentage of their time standing with their heads up ruminating following treatment with the low (56.6% ± 2.6, P ≤ 0.05) and medium (40.0% ± 5.6, P ≤ 0.05) doses of midazolam than when they received the placebo (32.4% ± 4.3). The animals spent a lower percentage of their time standing with their heads up ruminating following treatment with the high dose of midazolam than following the treatment with the placebo (24.5% ± 5.7 vs. 32.4% ± 4.3, P ≤ 0.05). Also, the animals spent a higher percentage of time walking after being treated with the high dose of midazolam (34.9% ± 3.4) than when they were treated with either the placebo (23.6% ± 1.7, P ≤ 0.05), the low dose (14.3% ± 1.4, P ≤ 0.05) or the medium doses of midazolam (25.5% ± 2.4, P ≤ 0.05), whilst the animals spent less time walking when treated with the low dose of midazolam (14.3% ± 1.4) than after treatment with either the placebo (23.6% ± 1.7, P ≤ 0.05), the medium dose (25.5% ± 2.4, P ≤ 0.05) or the high dose (34.9% ± 3.4, P ≤ 0.05) of midazolam. All three dosages resulted in the blesbok being less vigilant, when compared to the placebo (22.5% ± 2.1) (low dose: 11.6% ± 2.1; medium dose: 10.0% ± 1.9; high dose: 11.5% ± 1.4, P ≤ 0.05). The animals spent more time lying with their heads up following the treatment with the high dose of midazolam (12.7% ± 2.0) compared to the placebo (6.6% ± 3.4, P ≤ 0.05), low dose (3.8% ± 1.7, P ≤ 0.05) and medium dose (7.9% ± 2.9, P ≤ 0.05) of midazolam (

Behaviour	**Type**	**Placebo**	**High Dose (0.6 mg/kg BWt)**	**Medium Dose (0.4 mg/kg BWt)**	**Low Dose (0.2 mg/kg BWt)**
Standing with head up ruminating (%)	State	32.4^{c} ± 4.33	24.5^{d} ± 5.67	40.0^{b} ± 5.60	56.6^{a} ± 2.59
Walking (%)	State	23.6^{b} ± 1.70	34.9^{a} ± 3.37	25.5^{b} ± 2.39	14.3^{c} ± 1.40
Vigilance (%)	State	22.5^{a} ± 2.12	11.5^{b} ± 1.44	10.0^{b} ± 1.95	11.6^{b} ± 2.08
Lying with head up (%)	State	6.6^{b} ± 3.39	12.7^{a} ± 2.01	7.9^{b} ±2.94	3.8^{b} ± 1.65
Eating (%)	State	6.6^{a} ± 0.63	6.6^{a} ± 1.03	5.8^{a} ± 0.71	8.6^{a} ± 0.77
Lying with head down (%)	State	3.9^{a} ± 0.54	4.3^{a} ± 1.11	5.5^{a} ±1.82	3.7^{a} ±0.38
Exploring (sniffing while standing) (%)	State	2.1^{a} ± 0.83	1.3^{a} ± 0.44	3.0^{a} ± 1.12	1.5^{a} ± 0.42
Standing with head down (%)	State	0.6^{a} ± 0.31	0.3^{a} ± 0.10	1.3^{a} ± 0.39	1.6^{a} ± 0.40
Trotting in alarm (%)	State	1.4^{a} ± 0.44	1.5^{a} ± 0.19	0.9^{a} ± 0.09	0.5^{a} ± 0.07
Exploring (sniffing while walking around) (%)	State	1.3^{a} ± 0.62	1.6^{a} ± 0.97	0.7^{a} ± 0.27	0.2^{a} ± 0.08
Fighting (%)	State	0.9^{a} ± 0.28	0.5^{a} ± 0.14	0.2^{a} ± 0.08	0.0^{a} ± 0.02
Avoidance (%)	State	0.5^{a} ± 0.10	0.2^{a} ± 0.06	0.1^{a} ± 0.03	0.1^{a} ± 0.005
Drinking (%)	State	0.1^{a} ± 0.01	0.2^{a} ± 0.03	0.1^{a} ± 0.01	0.1^{a} ± 0.01
Other abnormal behaviour (%)	State	0.1^{a} ± 0.09	0.0^{a} ± 0.02	0.0^{a} ± 0.01	0.0^{a} ± 0.02
Grooming - Nibbling skin (# of counts)	Point	6.1^{a} ± 0.23	5.1^{b} ± 0.26	6.3^{a} ± 0.14	5.1^{b} ± 0.29
Grooming - while lying down (# of counts)	Point	1.6^{b} ± 0.63	3.1^{a} ± 0.78	3.2^{a} ± 0.59	1.7^{b} ± 0.65
Grooming- scratching with hind leg (# of counts)	Point	1.7^{a} ± 0.29	0.7^{b} ± 0.35	2.4^{a} ± 0.40	2.0^{a} ± 0.38
Agitation - head swaying (# of counts)	Point	7.6^{a} ± 0.19	6.7^{b} ± 0.36	7.8^{a} ± 0.39	6.8^{b} ± 0.33
Agitation - feet stomping (# of counts)	Point	3.6^{a} ± 0.65	2.2^{b} ± 0.76	3.7^{a} ± 0.60	3.2^{a} ± 0.33

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5.3.3 Point behaviour

There were two point behaviour activities that were measured in this study, namely grooming (categorized as either skin nibbling, grooming while laying down or scratching with hind leg) and agitation (divided into head swaying and foot stomping). A boxcox transformation was applied to the data of the point behaviour activities to obtain normality, as the original data were characterized by a large degree of variation. The changes in point behaviour activities of the animals during each of the respective treatments are presented in

Behaviour	Type	Placebo	High Dose (0.6 mg/kg BWt)	Medium Dose (0.4 mg/kg BWt)	Low Dose (0.2 mg/kg BWt)
Standing with head up ruminating (%)	State	32.4^{c} ± 4.33	24.5^{d} ± 5.67	40.0^{b} ± 5.60	56.6^{a} ± 2.59
Walking (%)	State	23.6^{b} ± 1.70	34.9^{a} ± 3.37	25.5^{b} ± 2.39	14.3^{c} ± 1.40
Vigilance (%)	State	22.5^{a} ± 2.12	11.5^{b} ± 1.44	10.0^{b} ± 1.95	11.6^{b} ± 2.08
Lying with head up (%)	State	6.6^{b} ± 3.39	12.7^{a} ± 2.01	7.9^{b} ±2.94	3.8^{b} ± 1.65
Eating (%)	State	6.6^{a} ± 0.63	6.6^{a} ± 1.03	5.8^{a} ± 0.71	8.6^{a} ± 0.77
Lying with head down (%)	State	3.9^{a} ± 0.54	4.3^{a} ± 1.11	5.5^{a} ±1.82	3.7^{a} ±0.38
Exploring (sniffing while standing) (%)	State	2.1^{a} ± 0.83	1.3^{a} ± 0.44	3.0^{a} ± 1.12	1.5^{a} ± 0.42
Standing with head down (%)	State	0.6^{a} ± 0.31	0.3^{a} ± 0.10	1.3^{a} ± 0.39	1.6^{a} ± 0.40
Trotting in alarm (%)	State	1.4^{a} ± 0.44	1.5^{a} ± 0.19	0.9^{a} ± 0.09	0.5^{a} ± 0.07
Exploring (sniffing while walking around) (%)	State	1.3^{a} ± 0.62	1.6^{a} ± 0.97	0.7^{a} ± 0.27	0.2^{a} ± 0.08
Fighting (%)	State	0.9^{a} ± 0.28	0.5^{a} ± 0.14	0.2^{a} ± 0.08	0.0^{a} ± 0.02
Avoidance (%)	State	0.5^{a} ± 0.10	0.2^{a} ± 0.06	0.1^{a} ± 0.03	0.1^{a} ± 0.005
Drinking (%)	State	0.1^{a} ± 0.01	0.2^{a} ± 0.03	0.1^{a} ± 0.01	0.1^{a} ± 0.01
Other abnormal behaviour (%)	State	0.1^{a} ± 0.09	0.0^{a} ± 0.02	0.0^{a} ± 0.01	0.0^{a} ± 0.02
Grooming - Nibbling skin (# of counts)	Point	6.1^{a} ± 0.23	5.1^{b} ± 0.26	6.3^{a} ± 0.14	5.1^{b} ± 0.29
Grooming - while lying down (# of counts)	Point	1.6^{b} ± 0.63	3.1^{a} ± 0.78	3.2^{a} ± 0.59	1.7^{b} ± 0.65
Grooming- scratching with hind leg (# of counts)	Point	1.7^{a} ± 0.29	0.7^{b} ± 0.35	2.4^{a} ± 0.40	2.0^{a} ± 0.38
Agitation - head swaying (# of counts)	Point	7.6^{a} ± 0.19	6.7^{b} ± 0.36	7.8^{a} ± 0.39	6.8^{b} ± 0.33
Agitation - feet stomping (# of counts)	Point	3.6^{a} ± 0.65	2.2^{b} ± 0.76	3.7^{a} ± 0.60	3.2^{a} ± 0.33

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The animals had lower counts of the “skin nibbling” behaviour following treatment with the low dose of midazolam (5.1 ± 0.29) than following treatment with either the placebo (6.1 ± 0.23, P = 0.017) or the medium dose (6.3 ± 0.14, P = 0.009) of midazolam. Counts of the “skin nibbling” behaviour in the animals were higher (P = 0.009) after treatment with the medium dose of midazolam than after treatment with the high dose of midazolam. The animals had lower counts (P = 0.017) of the “skin nibbling” behaviour when they were treated with the high dose of midazolam than when they were treated with the placebo.

Counts of the “grooming while lying down” behaviour when the animals were treated with the low dose of midazolam (1.7 ± 0.65) were lower than when they were treated with either the medium dose (3.2 ± 0.59, P =

0.001) or the high dose (3.1 ± 0.78, P = 0.005) of midazolam. Also, the animals had higher (P < 0.001) counts of the "grooming while lying down" behaviour after treatment with the medium dose of midazolam than after treatment with the placebo. Counts of the "grooming while lying down" behaviour when the animals were treated with the high dose of midazolam were also higher (P = 0.003) than when the they were treated with the placebo (

Behaviour	Type	Placebo	High Dose (0.6 mg/kg BWt)	Medium Dose (0.4 mg/kg BWt)	Low Dose (0.2 mg/kg BWt)
Standing with head up ruminating (%)	State	32.4^{c} ± 4.33	24.5^{d} ± 5.67	40.0^{b} ± 5.60	56.6^{a} ± 2.59
Walking (%)	State	23.6^{b} ± 1.70	34.9^{a} ± 3.37	25.5^{b} ± 2.39	14.3^{c} ± 1.40
Vigilance (%)	State	22.5^{a} ± 2.12	11.5^{b} ± 1.44	10.0^{b} ± 1.95	11.6^{b} ± 2.08
Lying with head up (%)	State	6.6^{b} ± 3.39	12.7^{a} ± 2.01	7.9^{b} ±2.94	3.8^{b} ± 1.65
Eating (%)	State	6.6^{a} ± 0.63	6.6^{a} ± 1.03	5.8^{a} ± 0.71	8.6^{a} ± 0.77
Lying with head down (%)	State	3.9^{a} ± 0.54	4.3^{a} ± 1.11	5.5^{a} ±1.82	3.7^{a} ±0.38
Exploring (sniffing while standing) (%)	State	2.1^{a} ± 0.83	1.3^{a} ± 0.44	3.0^{a} ± 1.12	1.5^{a} ± 0.42
Standing with head down (%)	State	0.6^{a} ± 0.31	0.3^{a} ± 0.10	1.3^{a} ± 0.39	1.6^{a} ± 0.40
Trotting in alarm (%)	State	1.4^{a} ± 0.44	1.5^{a} ± 0.19	0.9^{a} ± 0.09	0.5^{a} ± 0.07
Exploring (sniffing while walking around) (%)	State	1.3^{a} ± 0.62	1.6^{a} ± 0.97	0.7^{a} ± 0.27	0.2^{a} ± 0.08
Fighting (%)	State	0.9^{a} ± 0.28	0.5^{a} ± 0.14	0.2^{a} ± 0.08	0.0^{a} ± 0.02
Avoidance (%)	State	0.5^{a} ± 0.10	0.2^{a} ± 0.06	0.1^{a} ± 0.03	0.1^{a} ± 0.005
Drinking (%)	State	0.1^{a} ± 0.01	0.2^{a} ± 0.03	0.1^{a} ± 0.01	0.1^{a} ± 0.01
Other abnormal behaviour (%)	State	0.1^{a} ± 0.09	0.0^{a} ± 0.02	0.0^{a} ± 0.01	0.0^{a} ± 0.02
Grooming - Nibbling skin (# of counts)	Point	6.1^{a} ± 0.23	5.1^{b} ± 0.26	6.3^{a} ± 0.14	5.1^{b} ± 0.29
Grooming - while lying down (# of counts)	Point	1.6^{b} ± 0.63	3.1^{a} ± 0.78	3.2^{a} ± 0.59	1.7^{b} ± 0.65
Grooming- scratching with hind leg (# of counts)	Point	1.7^{a} ± 0.29	0.7^{b} ± 0.35	2.4^{a} ± 0.40	2.0^{a} ± 0.38
Agitation - head swaying (# of counts)	Point	7.6^{a} ± 0.19	6.7^{b} ± 0.36	7.8^{a} ± 0.39	6.8^{b} ± 0.33
Agitation - feet stomping (# of counts)	Point	3.6^{a} ± 0.65	2.2^{b} ± 0.76	3.7^{a} ± 0.60	3.2^{a} ± 0.33

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The animals had higher counts (P = 0.005) of the "scratching with hind leg" behaviour after treatment with the low dose of midazolam than after they were treated with the high dose of midazolam. Counts of the "scratching with hind leg" behaviour in the animals following treatment with the medium dose of midazolam was also higher (P <0.001) than following treatment with the high dose of midazolam. The animals had lower counts (P = 0.016) of the "scratching with hind leg" behaviour following treatment with the high dose midazolam treatment than following treatment of the animals with the placebo.

The counts for the "head swaying" behaviour after treatment of the animals with the low dose of midazolam (6.8 ± 0.33) was lower than following treatment of the animals with the placebo (7.6 ± 0.19, P = 0.045) and medium dose (7.8 ± 0.39, P = 0.015) of midazolam. The animals had higher counts (P = 0.011) of the "head swaying" behaviour after treatment with the medium dose of midazolam than after treatment with the high dose of midazolam. The

counts for the “head swaying” behaviour of the animals after treatment of the animals with the high dose of midazolam were lower (P = 0.033) than following treatment of the animals with the placebo (

Behaviour	Type	Placebo	High Dose (0.6 mg/kg BWt)	Medium Dose (0.4 mg/kg BWt)	Low Dose (0.2 mg/kg BWt)
Standing with head up ruminating (%)	State	32.4^{c} ± 4.33	24.5^{d} ± 5.67	40.0^{b} ± 5.60	56.6^{a} ± 2.59
Walking (%)	State	23.6^{b} ± 1.70	34.9^{a} ± 3.37	25.5^{b} ± 2.39	14.3^{c} ± 1.40
Vigilance (%)	State	22.5^{a} ± 2.12	11.5^{b} ± 1.44	10.0^{b} ± 1.95	11.6^{b} ± 2.08
Lying with head up (%)	State	6.6^{b} ± 3.39	12.7^{a} ± 2.01	7.9^{b} ±2.94	3.8^{b} ± 1.65
Eating (%)	State	6.6^{a} ± 0.63	6.6^{a} ± 1.03	5.8^{a} ± 0.71	8.6^{a} ± 0.77
Lying with head down (%)	State	3.9^{a} ± 0.54	4.3^{a} ± 1.11	5.5^{a} ±1.82	3.7^{a} ±0.38
Exploring (sniffing while standing) (%)	State	2.1^{a} ± 0.83	1.3^{a} ± 0.44	3.0^{a} ± 1.12	1.5^{a} ± 0.42
Standing with head down (%)	State	0.6^{a} ± 0.31	0.3^{a} ± 0.10	1.3^{a} ± 0.39	1.6^{a} ± 0.40
Trotting in alarm (%)	State	1.4^{a} ± 0.44	1.5^{a} ± 0.19	0.9^{a} ± 0.09	0.5^{a} ± 0.07
Exploring (sniffing while walking around) (%)	State	1.3^{a} ± 0.62	1.6^{a} ± 0.97	0.7^{a} ± 0.27	0.2^{a} ± 0.08
Fighting (%)	State	0.9^{a} ± 0.28	0.5^{a} ± 0.14	0.2^{a} ± 0.08	0.0^{a} ± 0.02
Avoidance (%)	State	0.5^{a} ± 0.10	0.2^{a} ± 0.06	0.1^{a} ± 0.03	0.1^{a} ± 0.005
Drinking (%)	State	0.1^{a} ± 0.01	0.2^{a} ± 0.03	0.1^{a} ± 0.01	0.1^{a} ± 0.01
Other abnormal behaviour (%)	State	0.1^{a} ± 0.09	0.0^{a} ± 0.02	0.0^{a} ± 0.01	0.0^{a} ± 0.02
Grooming - Nibbling skin (# of counts)	Point	6.1^{a} ± 0.23	5.1^{b} ± 0.26	6.3^{a} ± 0.14	5.1^{b} ± 0.29
Grooming - while lying down (# of counts)	Point	1.6^{b} ± 0.63	3.1^{a} ± 0.78	3.2^{a} ± 0.59	1.7^{b} ± 0.65
Grooming- scratching with hind leg (# of counts)	Point	1.7^{a} ± 0.29	0.7^{b} ± 0.35	2.4^{a} ± 0.40	2.0^{a} ± 0.38
Agitation - head swaying (# of counts)	Point	7.6^{a} ± 0.19	6.7^{b} ± 0.36	7.8^{a} ± 0.39	6.8^{b} ± 0.33
Agitation - feet stomping (# of counts)	Point	3.6^{a} ± 0.65	2.2^{b} ± 0.76	3.7^{a} ± 0.60	3.2^{a} ± 0.33

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The animals had higher counts (P < 0.001) of the “feet stomping” behaviour following treatment with the low dose midazolam treatment than following treatment with the high dose of midazolam. Counts of the “feet stomping” behaviour in the animals following treatment with the medium dose midazolam was higher (P < 0.001) than following treatment of the animals with the high dose of midazolam. The animals had lower counts (P < 0.001). of the “feet stomping” behaviour after treatment with the high dose of midazolam than after treatment with the placebo.

Table 5.4 The percentage time spent per state behaviour (mean ± SEM) and the number of counts per point behaviour observed in blesbok treated with either a placebo, high dose, medium dose or low dose of midazolam.

Behaviour	Type	Placebo	High Dose (0.6 mg/kg BWt)	Medium Dose (0.4 mg/kg BWt)	Low Dose (0.2 mg/kg BWt)
Standing with head up ruminating (%)	State	32.4^{c} ± 4.33	24.5^{d} ± 5.67	40.0^{b} ± 5.60	56.6^{a} ± 2.59

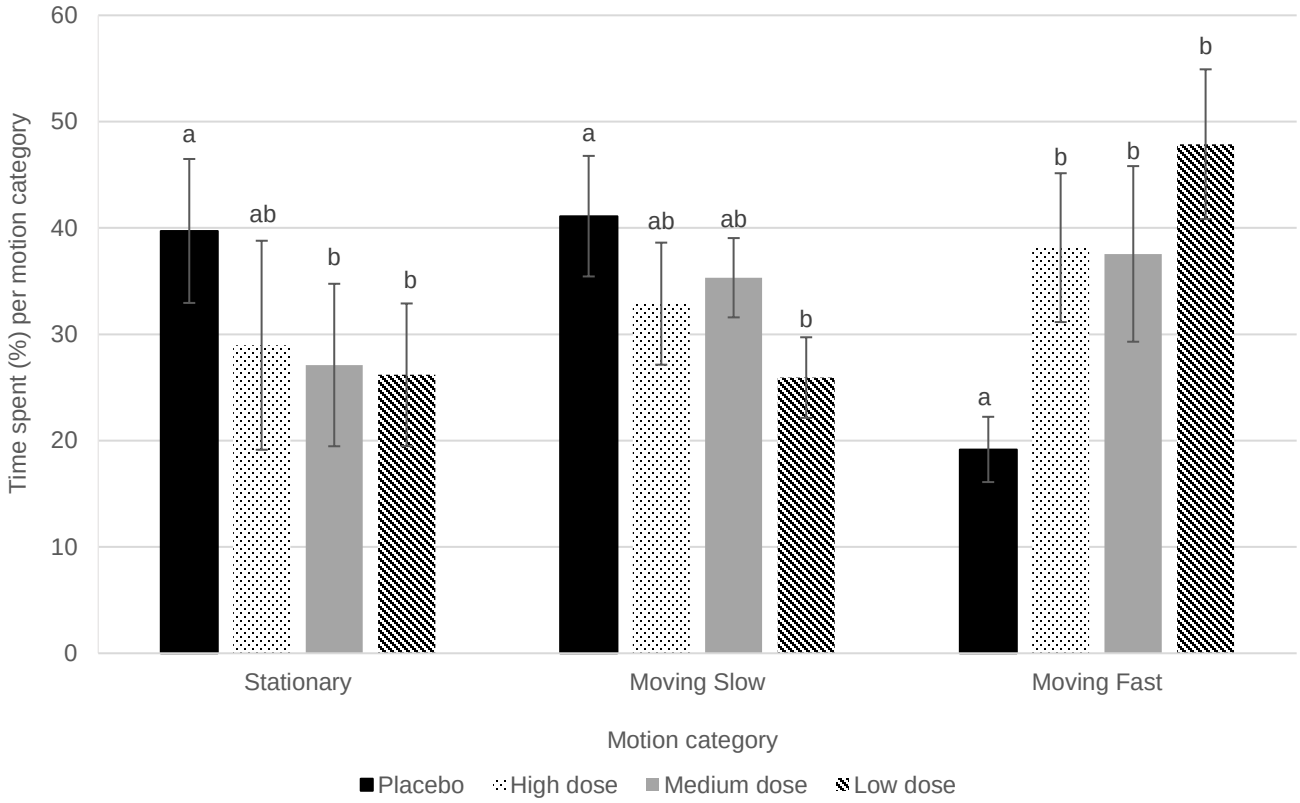
Walking (%)	State	$23.6^{b} \pm 1.70$	$34.9^{a} \pm 3.37$	$25.5^{b} \pm 2.39$	$14.3^{c} \pm 1.40$
Vigilance (%)	State	$22.5^{a} \pm 2.12$	$11.5^{b} \pm 1.44$	$10.0^{b} \pm 1.95$	$11.6^{b} \pm 2.08$
Lying with head up (%)	State	$6.6^{b} \pm 3.39$	$12.7^{a} \pm 2.01$	$7.9^{b} \pm 2.94$	$3.8^{b} \pm 1.65$
Eating (%)	State	$6.6^{a} \pm 0.63$	$6.6^{a} \pm 1.03$	$5.8^{a} \pm 0.71$	$8.6^{a} \pm 0.77$
Lying with head down (%)	State	$3.9^{a} \pm 0.54$	$4.3^{a} \pm 1.11$	$5.5^{a} \pm 1.82$	$3.7^{a} \pm 0.38$
Exploring (sniffing while standing) (%)	State	$2.1^{a} \pm 0.83$	$1.3^{a} \pm 0.44$	$3.0^{a} \pm 1.12$	$1.5^{a} \pm 0.42$
Standing with head down (%)	State	$0.6^{a} \pm 0.31$	$0.3^{a} \pm 0.10$	$1.3^{a} \pm 0.39$	$1.6^{a} \pm 0.40$
Trotting in alarm (%)	State	$1.4^{a} \pm 0.44$	$1.5^{a} \pm 0.19$	$0.9^{a} \pm 0.09$	$0.5^{a} \pm 0.07$
Exploring (sniffing while walking around) (%)	State	$1.3^{a} \pm 0.62$	$1.6^{a} \pm 0.97$	$0.7^{a} \pm 0.27$	$0.2^{a} \pm 0.08$
Fighting (%)	State	$0.9^{a} \pm 0.28$	$0.5^{a} \pm 0.14$	$0.2^{a} \pm 0.08$	$0.0^{a} \pm 0.02$
Avoidance (%)	State	$0.5^{a} \pm 0.10$	$0.2^{a} \pm 0.06$	$0.1^{a} \pm 0.03$	$0.1^{a} \pm 0.005$
Drinking (%)	State	$0.1^{a} \pm 0.01$	$0.2^{a} \pm 0.03$	$0.1^{a} \pm 0.01$	$0.1^{a} \pm 0.01$
Other abnormal behaviour (%)	State	$0.1^{a} \pm 0.09$	$0.0^{a} \pm 0.02$	$0.0^{a} \pm 0.01$	$0.0^{a} \pm 0.02$
Grooming - Nibbling skin (# of counts)	Point	$6.1^{a} \pm 0.23$	$5.1^{b} \pm 0.26$	$6.3^{a} \pm 0.14$	$5.1^{b} \pm 0.29$
Grooming - while lying down (# of counts)	Point	$1.6^{b} \pm 0.63$	$3.1^{a} \pm 0.78$	$3.2^{a} \pm 0.59$	$1.7^{b} \pm 0.65$
Grooming- scratching with hind leg (# of counts)	Point	$1.7^{a} \pm 0.29$	$0.7^{b} \pm 0.35$	$2.4^{a} \pm 0.40$	$2.0^{a} \pm 0.38$
Agitation - head swaying (# of counts)	Point	$7.6^{a} \pm 0.19$	$6.7^{b} \pm 0.36$	$7.8^{a} \pm 0.39$	$6.8^{b} \pm 0.33$
Agitation - feet stomping (# of counts)	Point	$3.6^{a} \pm 0.65$	$2.2^{b} \pm 0.76$	$3.7^{a} \pm 0.60$	$3.2^{a} \pm 0.33$

^{a,b,c,d} Column means with different superscripts indicate significant differences ($P \leq 0.05$)

5.3.4 Motion

The Equivital™ EQ02 biotelemetry system measured the motion of the blesbok every 15 secs during each trial and in three different categories, namely “stationary”, “moving slowly” and “moving fast”. The comparisons of the time spent on each motion category by the blesbok within periods of either stimulation or no stimulation are presented in Figures 5.4 and 5.5, respectively.

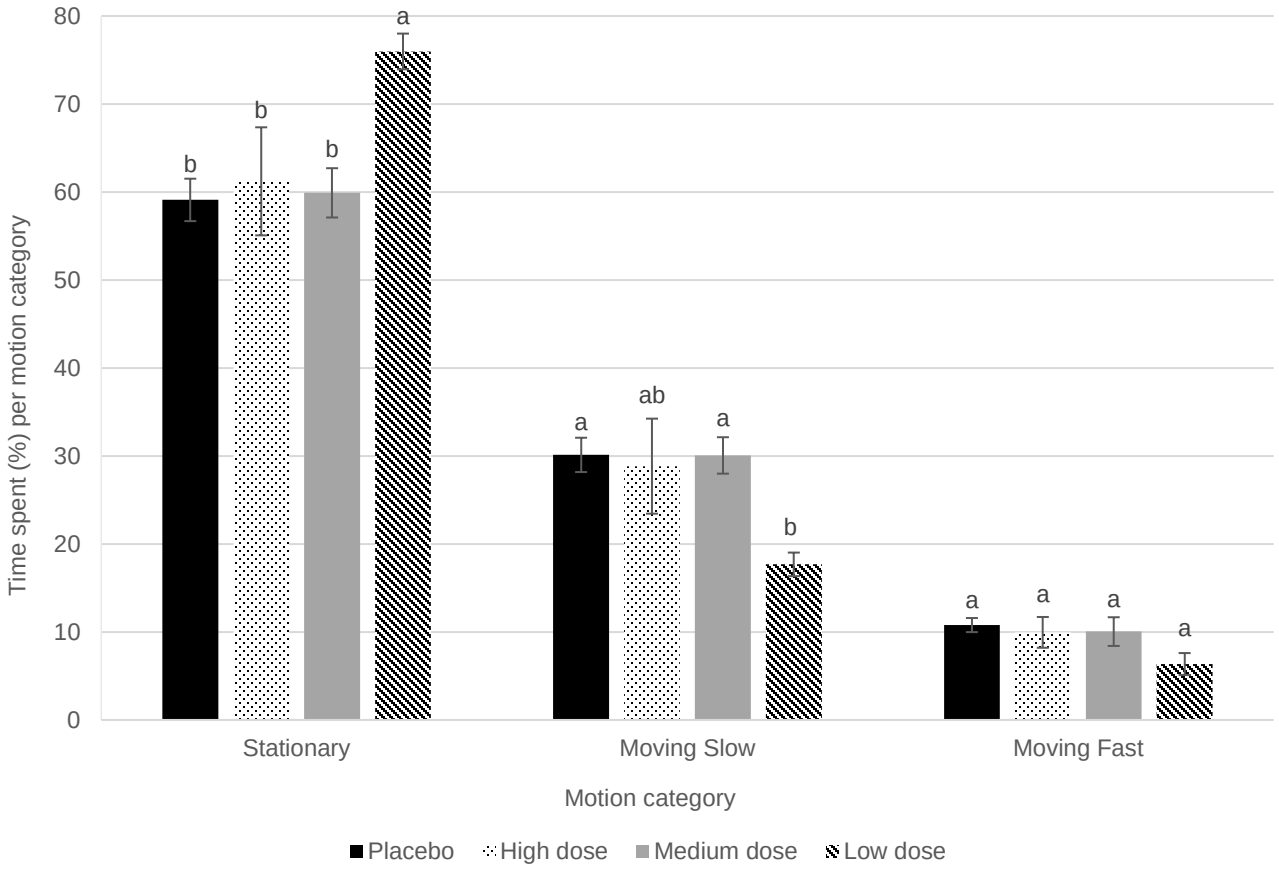
The animals spent less time being stationary when treated with the low (26.2% ± 6.7, P = 0.026) and medium (27.1% ± 7.6, P = 0.037) doses of midazolam than when they were treated with the placebo (39.7% ± 6.8) during periods of stimulation. The dose of midazolam did not affect the time spent by the animals on fast motion during stimulation, but the animals did spend more time moving fast when treated with each midazolam dose than when treated with the placebo (19.2% ± 3.1) during periods of stimulation (low dose: 47.9% ± 7.0, P < 0.001; medium dose: 37.6% ± 8.3, P = 0.003; high dose: 38.2% ± 7.0, P = 0.003). The animals spent less time (P = 0.014) on slow motion during periods of stimulation following treatment with the low dose of midazolam than when treated with the placebo (Figure 5.4).



[a,b] Means with different superscripts within a motion category differ significantly (P ≤ 0.05)

Figure 5.4 The time spent (mean ± SEM) on the three different motion categories (stationary, moving fast and moving slowly) by blesbok (expressed as percentage of the total motion measured within a period) during periods of stimulation following treatment with either a placebo or three different doses of midazolam (high, medium or low).

During periods of no stimulation the animals spent most of their time being stationary and the least amount of their time moving fast, regardless of treatment including the placebo (Figure 5.5). Only the low dose of midazolam (76% ± 2.0) affected stationary motion in blesbok during periods of no stimulation by increasing the time spent in this motion category when compared to the placebo (59.1% ± 2.4, P = 0.007), the medium dose (59.9% ± 2.8, P = 0.009) and the high dose (61.2% ± 6.1, P = 0.016) of midazolam. The animals spent less time (P = 0.040) on slow motion during periods when they were not being stimulated following treatment with the low dose of midazolam when compared to when they were treated with the placebo (Figure 5.5).



[a,b] Means with different superscripts within a motion category differ significantly (P ≤ 0.05)

Figure 5.5 The time spent (LSMeans ± SEM) on the three different motion categories (stationary, moving fast and moving slowly) by blesbok (expressed as percentage of the total motion measured within a period) during periods of no stimulation following treatment with either a placebo or three different doses of midazolam (high, medium or low).

5.3.5 Feed intake per treatment

The amount of feed consumed by the blesbok per specific treatment are presented in Table 5.5.

Table 5.5 Comparison of the amount of feed consumed by blesbok when treated with either a placebo, 0.2 mg midazolam/kg BWt or 0.6 mg midazolam/kg BWt.

	Placebo	**Low dose (0.2 mg/kg BWt)**	**High dose (0.6 mg/kg BWt)**
Amount of feed fed at start of trial (kg)	19.3	13.3	14.4
Amount of feed remaining after 24 hr (kg)	13.1	7.3	6.8
Difference (kg)	6.2	6.0	7.6
Feed consumed (%)	32.1	45.1	52.8

Feed intake was higher when the animals were treated with midazolam compared to the placebo. It also appears that feed intake increased with midazolam dose. However, it needs to be noted that an unknown amount of feed was mistakenly added to the original feed without permission during the medium dose (0.4 mg midazolam/kg BWt) trial. The influence of the medium dose midazolam on feed intake could thus not be accurately determined. However, the feed intake was higher when the animals received midazolam, even without data from the medium midazolam dose trial, and therefore midazolam did stimulate appetite in blesbok.

5.4 Discussion

No adverse side effects were observed in any of the animals during any of the trials in this study. Extrapyramidal symptoms, in the form of hyperactivity and disorientation, were observed in some animals during the high dose midazolam trial. It should also be noted that the animals tended to fall or stumble quite easily, and were thus very ataxic, when startled following treatment with the high dose of midazolam. This occurred to a much lesser extent or not at all during the medium or low dose midazolam trials. According to Smith and Wesson (2012), benzodiazepines at high doses impair motor coordination, resulting in ataxia. A dose of 0.6 mg midazolam/kg BWt is high enough to cause ataxia in blesbok, while ataxia does not appear to occur in this antelope species at doses smaller or equal to 0.4 mg midazolam/kg BWt.

When treated with the placebo, the blesbok spent the majority of their time standing with their heads up ruminating (32.4%), followed by walking (23.6%) and vigilance (22.5%). According to Estes (1999), free roaming female Blesbok spend approximately 67% of their day eating and approximately 33% of their day ruminating and resting, while they spend a very small percentage of time on other activities. Thus the results of this study show a similar amount of time spent ruminating (32.4%) by the blesbok following treatment with the placebo, but higher levels of walking and vigilance than those reported for free-roaming blesbok. The higher level of walking and vigilant behaviour observed in this study may be due to the animals being held in captivity surrounded by human activity, which are not experienced by free-roaming blesbok and may have resulted in the blesbok in this study being more restless and wary of their surroundings than their free-roaming counterparts.

Regarding motion, the blesbok mostly moved slowly or were stationary when experiencing stimulation following treatment with the placebo. When free-ranging blesbok perceive a threat they tend to initially mill around, and will then run away from the threat for a safe distance then stop and mill around once more (Frost, 2014). In this study, the blesbok did show the milling around behaviour when stimulated, followed by them trying to move away from the cause of the stimulus and then milling around again. Due to the constraints of the enclosure size, however, the blesbok could only move short distances between milling activities. When the animals were not being stimulated, they spent the majority of their time being stationary following treatment with the placebo. These results agree with that reported for free-roaming blesbok in that these animals spent most of their time being stationary, either by eating, ruminating or resting when not experiencing a stressor (Estes, 1999).

When treated with the high dose (0.6 mg midazolam/kg BWt), the blesbok spent the majority of their time walking (34.9%), followed by standing and ruminating (24.5%) and lying with their head up (12.7%). The animals thus spent more time walking (34.9% vs. 23.6%), less time standing with their heads up ruminating (24.5% vs. 32.4%) and less time being vigilant (11.5% vs. 22.5%) when treated with the high dose than when treated with the placebo. Thus it appears as though the animals moved around more, but were less vigilant when treated with the high dose of midazolam than when treated with the placebo. The increase in walking behaviour observed may have been due to the animals being more ataxic and thus struggling to stand still after treatment with the high dose than when they were treated with the placebo. The increase in activity during the high dose trial may also be the result of extrapyramidal symptoms, such as hyperactivity and disorientation that occurred in some animals after treatment with the high midazolam dose.

When treated with the medium dose (0.4 mg midazolam/kg BWt) the blesbok spent the majority of their time standing with their heads up ruminating (40%), followed by walking (25.5%) and showing vigilance (10%). This pattern echoes that of when the animals were treated with the placebo, but the animals spent a significantly larger fraction of their time standing with their heads up ruminating when treated with the medium dose compared to when they were treated with the placebo (40% vs. 32.4%) and the high dose (40% vs. 24.5%) of midazolam. The medium dose did not significantly affect the time spent by the animals on walking behaviour compared to when they were treated with the placebo (25.5% vs. 23.6%) and also thus did not result in hyperactivity observed when the animals were treated with the high dose of midazolam. Thus the medium dose did have a calming effect on the animals, but mostly resulted in the animals showing similar behaviour to when they were treated with the placebo.

When treated with the low dose (0.2 mg midazolam/kg BWt), the blesbok spent most of their time standing and ruminating (56.6%), followed by walking (14.3%) and being vigilant (11.6%). The proportion of time spent standing and ruminating following treatment with the low dose of midazolam, was however significantly higher than when the animals were treated with either the placebo (56.6% vs. 32.4%), the medium dose of midazolam (56.6% vs. 40%) or the high dose of midazolam (56.6% vs. 24.5%) or for free-roaming female blesbok (56.6% vs. 33%) reported by Estes (1999). Furthermore, the animals spent the least amount of time on walking when treated with the low dose of midazolam compared to when they were treated with any of the other treatments, indicating that the low dose may have had the most calming effect on the animals of all the treatments. Thus

the low dose is effective enough to calm the animals without causing hyperactivity. However, the low dose trial was the last trial to be done and therefore it is possible that the animals may have become habituated to the trial process at that stage and therefore no longer felt the same amount of anxiety observed during the placebo trials, which was the very first of the trials to be conducted; this aspect warrants further research. It was attempted to minimise the habituation by moving the animals to other enclosures and regrouping with other blesbok between trials.

Various studies have reported paradoxical reactions following treatment with benzodiazepines (Hall & Zisook, 1981; File and Wilks, 1986; Honan, 1994; Ilkiw *et al.*, 1996, Ben-Porath & Taylor, 2002; Smith & Wesson, 2012). A study on deer showed that a high dose of diazepam administered orally made handling of fallow deer possible (Done *et al.,* 1975), while a study by Wilson (unpublished, In Walsh & Wilson, 2002) found that diazepam resulted in unpredictable behaviour in red deer (*Cervus elaphus*), such as aggression and a disregard of fences. According to Cabrera *et al.* (2010), midazolam can result in paradoxical excitement that includes side effects such as restlessness, agitation, uncontrollable shaking and aggression. The occurrence of contradictory excitement due to midazolam is not common however, and Cabrera *et al.* (2010) found that such symptoms occur in less than 1 % of human patients.

This type of excitement also occurs when benzodiazepines are used in animals and are thought to be associated with the anxiolytic effects of these drugs (Welsh, 2013). According to Welsh (2013), the combination of benzodiazepines with opioids can aid in reducing the risk of excitement occurring, but mild levels of excitement may still occur in healthy animals after the drugs have been administered. Various theories of the cause of contradictory reactions to drugs exist, but the precise mechanisms behind these reactions are not yet known. Benzodiazepines increase acetylcholine concentrations in the central nervous system, reduce the uptake of catecholamines and decrease serotonin re-uptake in the cortex (Ladinsky *et al.*, 1973; Taylor & Laverty, 1973; Lidbrink *et al.*, 1973). As all these substances are neurotransmitters, any alteration in their levels in the brain can potentially result in the undesired or contradictory effects witnessed when benzodiazepines are administered (Hall & Zisook, 1981).

According to Shin *et al.* (2013), the risk of paradoxical effects from midazolam is increased with an increased dose of the benzodiazepine. The results in this study appear to confirm this, as paradoxical hyperactivity was only observed in the blesbok when they were treated with a dose of 0.6 mg midazolam/kg BWt, while doses of 0.4 mg midazolam/kg BWt and 0.2 mg midazolam/kg BWt appears to have a more prominent calming effect in blesbok. It is thus possible that doses higher than 0.4 mg midazolam/kg BWt will result in contradictory effects in blesbok, while doses up to 0.4 mg midazolam/kg BWt may have a calming effect on the animals. However, it should be noted that the study by Shin *et al.* (2013) was done in humans and there are limited studies available on the effects of benzodiazepine dose, or specifically midazolam dose, on the occurrence of hyperactivity in animals and specifically in wild antelope species.

Furthermore, benzodiazepines remove or decrease the inhibitory effect of fear or frustration on the behaviour of an animal and thus causes animals to show atypical behaviours such as verbal or motor behaviour that are

spontaneously produced, fixation memory loss and aggression (Haefely, 1982; Senninger & Laxenaire, 1995; Bond, 1998; Smith & Wesson, 2012). For example, Walsh and Wilson (2002) argued that the reason for the abnormal behaviour caused in red deer, e.g. aggression and a disregard of fences when treated with diazepam, was due to the anxiolytic properties of this drug which had resulted in behavioural disinhibition in the animals. Behavioural disinhibition is thus a manifestation of impulse driven behaviours due to the attenuation of fear and anxiety caused by benzodiazepines. Behavioural disinhibition in animals following treatment with benzodiazepines has been reported in various studies (Fava & Borofsky, 1991; Reiter & Kutcher, 1991; Fiset *et al.*, 1992). Thus it is possible that the high dose of midazolam may have resulted in behavioural disinhibition, which in turn led to paradoxical hyperactivity in the blesbok following treatment. According to Bond (1998), the risk of behavioural disinhibition occurring increases with an increase in benzodiazepine dosage, which may explain why the animals appear to have shown less signs of behavioural disinhibition when treated with the medium and low doses of midazolam than when treated with the high midazolam dose.

Regarding point behaviour activities, the blesbok showed less grooming (with the exception of grooming while lying down) and agitation behaviour when treated with the high dose of midazolam than when they were treated with the placebo. According to Frost (2014), head shaking is a common behaviour in free-roaming blesbok that may indicate nervousness or joy. Blesbok are also known to contain nasal parasites (Horak *et al.*, 1982) and head shaking behaviour may thus also be due to irritancy caused by these parasites. According to Hart and Hart (1988) most antelope species spend a significant time of their day grooming, with females performing 600-1000 grooms to their body in a period of 12 hrs per day. Thus grooming and head swaying is a frequent and normal behaviour in free-ranging antelope that are not experiencing stress. According to Hart *et al.* (1992), grooming by antelope can make the animals less vigilant and thus more susceptible to predation. The opposite may therefore also be true - an increase in vigilance or anxiety would thus reduce grooming behaviour. Although the vigilance of the blesbok was reduced following treatment with the high dose of midazolam compared to when the animals were treated with the placebo in the present study, behavioural disinhibition may have been caused by this dosage and thus resulted in hyperactivity and higher levels of anxiety in the blesbok. This in turn may thus have reduced grooming and head swaying behaviour in the blesbok following treatment with the high dose of midazolam. Furthermore, another possible explanation of the decreased grooming behaviour may be that the high dose of midazolam affected specific neuropeptides in the brain, which are responsible for causing animals to groom themselves. ACTH and beta-endorphin are neuropeptides that have been found to increase grooming behaviour in rats (Gispen *et al.*, 1975; Gispen & Isaacson, 1981). James *et al.* (1979) suggest that benzodiazepines antagonize the function of ACTH. Dunn *et al.* (1981) showed that benzodiazepines decreased ACTH and β-endorphin concentrations, thereby decreasing the excessive grooming behaviour in rats when introduced to a novel environment. Thus it is possible that the high dose of midazolam had perhaps inhibited or reduced the concentrations of these neuropeptides in the central nervous system and thereby decreased grooming behaviour in the blesbok. However, no previous studies could be sourced that have evaluated the effects of benzodiazepines on neuropeptides that modulate grooming behaviour in animal species outside of rodents. Thus more research on this topic is necessary to have a clear understanding as to why grooming behaviour was reduced by the high dose of midazolam in this study.

The medium dose of midazolam generally did not affect the grooming (with the exception of grooming while lying down) and agitation behaviour activities of the blesbok compared to that of when the animals were treated with the placebo. The low dose of midazolam reduced skin nibbling, but did not affect the other two grooming behaviour activities. Thus the medium and low doses of midazolam generally did not decrease grooming behaviour in the blesbok. Both these doses also caused the animals to show less vigilant behaviour when compared to the placebo. This agrees with the findings by Hart *et al.* (1992); that increased grooming in antelope will decrease vigilance. This therefore indicates that the animals were much less wary of threats and much less anxious following treatment with the medium and low doses of midazolam than when treated with the high dose of midazolam. Furthermore, the low dose reduced the head swaying behaviour, but did not affect the foot stomping behaviour when compared to the placebo. The high dose of midazolam was thus most successful in reducing point behaviour activities, but this may only have been due to the paradoxical hyperactivity or behavioural disinhibition resulting from this dose which prevented the animals from being in the state of calm necessary to show normal grooming or head swaying behaviour.

When being stimulated, the animals spent most of their time moving fast following treatment with the high dose of midazolam. However, the high dose of midazolam caused no significant difference in the motion of the blesbok compared to the placebo during periods of no stimulation. The increase in fast motion during periods of stimulation observed when the animals were treated with the high dose of midazolam was also observed in the blesbok when they were treated with the medium and low doses of this benzodiazepine. Thus it appears as though midazolam generally tends to increase motion during periods when stress is experienced. According to Smith and Wesson (2012), the effects of benzodiazepines on the autonomic nervous system are absent or miniscule in stress-free situations, while both the endocrine and autonomic responses to stress are increased by benzodiazepines during conditions where stress is experienced. This may explain the increase in motion observed in the blesbok during periods of stimulation following midazolam treatment. Furthermore, a study by Munerato *et al.* (2010) in brown brocket deer (*Mazama gouazoubira*) found that there were no statistically significant differences between the protocols and times of collection of cortisol concentrations in the animals following treatment with midazolam. Munerato *et al.* (2010) argued that the reason for the lack of significant differences could potentially have been due to the small sample size and the large variation in the stress response in individual animals and not due to the failure of midazolam to reduce the concentrations of cortisol. Thus in the present study the failure of statistically significant differences observed between the midazolam doses for motion during periods of stimulation may also have been due to the limited number of animals and the variation in hormonal stress response in individual animals in this study and not due to the difference in midazolam dosage itself. Unfortunately, due to financial constraints only six blesbok could be obtained for this study. In future studies it is thus suggested that midazolam's effects should be studied (and quantified by measuring for e.g. cortisol) in a larger population of blesbok or antelope species.

As seen following treatment of the animals with the high dose of midazolam, the medium dose of midazolam also did not significantly affect the motion of the blesbok during periods of no stimulation when compared to the placebo. This agrees with the argument by Smith and Wesson (2012) that benzodiazepines primarily have no effect on the endocrine and autonomic nervous systems when stress is not experienced. The low dose,

however, increased the time spent being stationary by the animals, and decreased the time spent by the animals moving slowly when the animals were not being stimulated. When treated with the low dose of midazolam, the animals spent the least amount of their time moving fast, in a similar manner to the other treatments. Thus the low dose of midazolam generally decreased motion in the animals when they were not being stimulated. The low dose appears to have had the most profound calming effect on the animals and reduced activity in the animals more than any of the other treatments, without causing paradoxical reactions or behavioural disinhibition. As previously stated, wildlife veterinarians prefer to use dosages of 0.1-0.2 mg midazolam/kg BWt to sedate antelope species (Raath, personal communication, 3 November 2017). The current industry protocol for the use of midazolam provides further support that a dose of 0.2 mg midazolam/kg BWt may be best suited use in for blesbok in captivity.

Various studies have reported that benzodiazepines increase food intake in animals (e.g. Randall *et al.*, 1960; Bainbridge, 1968; Brown *et al.*, 1976; Fratta *et al.*, 1976; Baile & McLaughlin, 1979; Mereu *et al.*, 1979; Cooper,1980; Brown *et al.*, 1981; Berridge & Pacina, 1995; Ilkiw *et al.*, 1996). According to Welsh (2013), small doses of midazolam administered by the IV route have been used to manage anorexia in cats. Benzodiazepines have also been found to be more beneficial when used in the immobilisation of wild ruminants than phenothiazine derivatives which, although commonly used for the immobilisation of wild antelope species, have been found to cause anorexia (Knox *et al.*, 1990; Kock & Burroughs, 2012). According to Cooper (1980, 1989), the feed intake resulting from benzodiazepines is due to these drugs enhancing the hedonic quality of taste stimuli above sedative anxiolytic effects and thus increasing an animal's desire to consume feed. Similar to the results found in this study, Foltin *et al.* (1989) found benzodiazepines caused a dose dependent increase in feed consumption in baboons. Furthermore, Ilkiw *et al.* (1996) also found that midazolam specifically increased feed intake in cats in a dose dependent manner. The feed intake could not be measured with accuracy in this study due to the addition of an unknown amount of feed after the medium dose trial had already started as well as a lack of infrastructure/equipment to monitor individual feed intake. It did however, appear as if feed intake increased per midazolam dose when only comparing results from the high dose and low dose midazolam trials to the placebo dose trial, as the intake was highest during the high dose trial followed by the low dose trial and lastly the placebo trial. In future studies, the effects of feed intake after treatment with a dose of 0.4 mg midazolam/kg BWt should be included and treatments must be randomised in order to confirm that the increase in feed intake in blesbokis a direct effect of an increase in the dose of midazolam administered.

Should high doses of midazolam (0.6 mg midazolam/kg BWt and higher) be used, it is recommended that they be used as part of an immobilisation protocol where it can be combined with anaesthetics which will mask the undesirable effects of midazolam at high doses. In combination with an anaesthetic, the sedative properties of midazolam may improve the quality of immobilisation. Benzodiazepines are often used in combination with anaesthetics in animals to overcome adverse side effects from both types of drug in the animals (Schroeder & Smith, 2011). For example, a combination of 0.31 mg midazolam/kg BWt, 0.20 mg butorphanol/kg BWt and 0.20 mg detomidine/kg BWt was found to be effective in immobilising Nile lechwe (*Kobus magaceros*) with minimum effects on the cardiovascular and respiratory systems of the animals (Laricchiuta *et al.*, 2012). A combination of 0.13 mg butorphanol/kg BWt, 0.13 mg midazolam/kg BWt and 0.13 mg medetomidine/kg BWt

has been found to be superior to a combination of 0.11 mg butorphanol/kg BWt, 0.11 mg medetomidine/kg BWt and 0.22 mg azaperone/kg BWt for the short term immobilisation of captive Nubian Ibex (*Capra nubiana*) (Lapid & Shilo-Benjamini, 2015). To aid in reducing the risk of paradoxical excitement, higher doses of midazolam should be considered in immobilising mixtures where the initial side effects of midazolam may be minimized by the time the anaesthesia is reversed.

The combination of 4 mg etorphine and 35-40 mg midazolam has been found to effectively and safely immobilise white rhinoceros (*Ceratotherium simum*) (Van Zijl *et al.*, 2016). A combination of 0.38 mg etorphine/kg BWt, 0.20 mg medetomidine/kg BWt and 0.20 mg midazolam/kg BWt resulted in faster induction and recovery of impala (*Aepyceros melampus*) from anaesthesia than a combination of 4 mg ketamine/kg BWt, 0.15 mg butorphanol/kg BWt and 0.20 mg medetomidine/kg BWt, but caused cardiopulmonary side effects (Gerlach *et al.*, 2017). These drug combinations were however, primarily used for immobilising animals, and not only sedating them. They do however show that these combinations are effective in specific wild antelope species. The advantage of using midazolam might therefore lie in using it as an adjunct to immobilising mixtures to improve immobilisation instead of as a sedative on its own

The effects of the abovementioned drug combinations are not yet known in blesbok, and thus more research on their use and the use of other drug combinations in this antelope species is warranted. Furthermore, no published studies are available on the effect of midazolam on animal behaviour and physiological responses in antelope when used on its own. Therefore, the potential of midazolam as a sedative in other antelope species needs to be investigated.

5.5 Conclusion

Midazolam generally influenced ruminating, walking and vigilant behaviour in blesbok. Midazolam appears to influence the motion of the animals during periods of no stimulation, thus when stress was not experienced. Vigilance in blesbok was decreased by midazolam regardless of dose, while it appeared that feed intake increased in a dose-dependent manner. The dosage of midazolam appears to determine the sedative potential of the drug in the blesbok, with the dose of 0.6 mg midazolam/kg BWt resulting in paradoxical hyperactivity, while the dose of 0.2 mg midazolam/kg BWt was most effective in calming and reducing activity of the blesbok. The dose of 0.2 mg midazolam/kg BWt used in this study is also in agreement with unpublished data of midazolam doses commonly use in practice to sedate ungulates. Further research on different dosages of midazolam and combinations of midazolam with other pharmaceuticals, as well as midazolam's effects on feed intake in wild antelope species is necessary to improve its potential for use as a sedative in these animals and to reduce the occurrence of paradoxical effects.

Chapter 6

The influence of midazolam on physiological and behavioural parameters of blesbok (*Damaliscus pygargus phillipsi*) in captivity

Abstract

In this study six female blesbok were fitted with the Equivital™ EQ02 biotelemetry system and treated with a placebo and three different doses (0.2 mg midazolam/kg BWt, 0.4 mg midazolam/kg BWt and 0.6 mg midazolam/kg BWt) of midazolam to investigate its effect on heart rate and respiration rate of blesbok in captivity. The heart rate and respiration rate of each animal were recorded every 15 secs for a period of 12 hrs following treatment with midazolam. The animals were scored according to a predetermined system for level of sedation and response to stimulus at set time points after midazolam administration. In order to elicit a stress response in the animals, a person entered the enclosure and deliberately made a noise for 1 min, every half-hour for the first 6 hrs following administration of a respective treatment. The low dose of midazolam (0.2 mg/kg BWt) lowered the heart rate and did not affect the respiration rate for most of the behaviours of the blesbok, while neither physiological parameter was affected by the low midazolam dose when the animals were being stimulated. When the animals were not being stimulated, the low dose of midazolam lowered both the heart and respiration rate of the blesbok. The medium dose of midazolam lowered the heart rate during most of the behaviour and caused an overall increase in the respiration rate of the blesbok throughout the trial. The high dose of midazolam (0.6 mg/kg BWt) resulted in a faster heart rate in the blesbok during periods when the animals exhibited avoidance behaviour, but did not affect heart rate during any of the other observed behaviour of the blesbok or throughout the trial overall. The high dose of midazolam increased the respiration rate during most behaviour of the animals, and during both periods of stimulation and no stimulation. Mean sedation scores increased with an increase in the midazolam dosage and a moderate level of sedation (sedation score of 2-3) was achieved. Response to stimulus decreased over time regardless of treatment. The lowest dosage of midazolam (0.2 mg midazolam/kg BWt) is recommended for sedating blesbok in captivity when they are experiencing minimal stress. In future studies, faecal glucocorticoids should be measured in addition to the other parameters in order to also assess the effects of midazolam on the endocrine system and not just on the cardiopulmonary system of blesbok during stress.

6.1 Introduction

Stress resulting from transport and maintenance of wildlife in captivity can have severe consequences for the welfare of the animals. Diseases, opportunistic infections, reduced reproductive performance, mortality and morbidity have been found to be more prominent in captive wildlife species than their free-roaming counterparts (Munson,1993; Munson *et al.*, 1999; Blay & Coˆte´, 2001; Barnes *et al.*, 2002; Terio *et al.*, 2004; Ellenberg *et al.*, 2006; Clauss *et al.*, 2007; Clubb *et al.*, 2008). Stress during transportation can result in capture myopathy (Montané *et al.*, 2003; Kock & Meltzer, 2006), which contributes to mortalities in wildlife species (Ebedes *et al.*, 2006; Kleiman *et al.*, 2010). Methods for managing stress of the animals during transport or whilst in captivity are required to ensure the welfare of the animals.

Sedatives and tranquilizers are commonly used to reduce stress in wildlife species. Despite being known to reduce stress, sedatives and tranquilizers may have various side effects in a specific species. Some of these side effects may be adverse and thus negatively affect the welfare of an animal. Even though tranquilizers reduce fear, anxiety and aggression for example, they have also been found to result in anorexia, convulsions, inhibition of thermoregulation and hypotension (Burroughs *et al.*, 2012a; Lamont & Grimm, 2014). Sedatives, e.g. benzodiazepines and α-2-adrenergic agonists, cause drowsiness and impair locomotor activity, but generally cause minimal side effects (Burroughs *et al.*, 2012a; Lamont & Grimm, 2014). Pharmaceutical products such as sedatives are constantly being improved in order to increase their desired effects and to reduce their adverse side effects. Prior to the use of new pharmaceuticals in a specific animal species, their effects on the welfare of the target species must be studied. Stress can impair normal physiological processes of an animal, thus disturbing homeostasis.

The cardiovascular and respiratory systems are important for the regulation of homeostasis, and normal functioning and regulation may be influenced by pharmaceutical products (Stephens & Toner,1975; Van Putten & Elshof, 1978; Syme & Elphic, 1982; Gomez *et al.*, 1989; Broom & Johnson, 1993). According to the National Research Council (2008), the physiology of an animal can be considered as a primary indicator of its welfare. Assessment of the influence of a pharmaceutical product on, amongst others, the heart rate and respiration rate of an animal can potentially indicate whether such a product is safe for use in the particular species. Telemetry provides a potential means to measure heart rate and respiration rate in animals remotely after the administration of a pharmaceutical product, and thus a way to establish the influence of the drug on the animal's physiology and welfare (Cooke *et al.*, 2004).

Telemetry is the preferred method for measuring the effects of pharmaceuticals in animals, as it measures parameters in fully conscious, free roaming animals. Such measurements more closely resemble that of animals in their natural state (Kramer *et al.*, 1993; Kramer *et al.*, 2001; Ando *et al.*, 2005; Hayashi *et al.*, 2005; Miyazaki *et al.*, 2005; Sasaki *et al.*, 2005; Laubscher, 2015). Although both internal and external telemetry systems are available, each has benefits and drawbacks to consider if they are to be used. The choice of system will be dependent of study type, number of animals, study duration and specific parameters to be measured. External systems have been found to be better suited for use in short lasting pharmacodynamic studies in animals (Chui *et al.*, 2009; Brag & Burmeister, 2011). An external biotelemetry system, the Equivital™

EQ02 biotelemetry belt, has been successfully used to study the effects of pharmaceuticals on the physiology and behaviour of blue wildebeest (*Connochaetes taurinus*) by Laubscher (2015). Therefore, the Equivital™ system could potentially be used in determining how pharmaceuticals affect the physiology of other wild antelope species.

Midazolam is a benzodiazepine sedative that is more potent than diazepam and has less risk of causing a painful injection due to its water solubility (Reves *et al.*, 1985; Wright *et al.*, 1993; Stegmann, 1998). Although midazolam has been successfully used as a sedative on its own in various domestic species (Smith *et al.*,1991; Stegmann,1998; Stegmann, 1999; Stegmann & Bester, 2001; Upton *et al.*, 2009; Aarnes *et al.*, 2013; Schwartz *et al.*, 2013; Simon *et al.*, 2017), it is usually combined with other pharmaceuticals when used in wildlife for the purpose of immobilisation (Laricchiuta *et al.*, 2012; Lapid & Shilo-Benjamini, 2015; Van Zijl *et al.*, 2016; Gerlach *et al.*, 2017). No studies on the use of midazolam as a sedative on its own in wild antelope species has been reported as of yet.

The blesbok is a common and endemic wild antelope species in South Africa. Due to its small size, easy handling and the fact that they can be maintained within normal livestock fencing, it is a popular species to farm with (Lloyd & David, 2008; Frost, 2014). Due to their popularity in South Africa and their importance to the game farming industry, the breeding and trade of blesbok has increased and thus also the translocation activities associated with this species (Taylor *et al.*, 2016). The stress of the translocation processes holds concern for blesbok welfare and needs to be managed. Midazolam holds potential as a sedative for managing the stress of transport and captivity in blesbok due to its abovementioned benefits, however it has not yet been previously used on its own in this antelope species and its effects on especially the physiology of these animals are thus unknown. The aim of this study is therefore to investigate the effect of midazolam on the physiology of blesbok, specifically the heart rate and respiration rate, with the use of the Equivital™ EQ02 biotelemetry system. Furthermore, the degree of sedation and response to stimulus of the animals after treatment will also be measured. The results from this study will aid in determining the level of safety and effectiveness of midazolam use as a sedative in blesbok.

6.2 Materials and methods

Ethical approval for this study was obtained from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa. Protocol ethical approval number: ACU-2017-0279-422.

As this forms part of the same study, the experimental location, animals, husbandry, facilities, the biotelemetry system, its fitment and modification, and the administered pharmaceuticals of this study are described in Chapter 5 of this dissertation. The experimental design of this study is presented in **Error! Reference source not found.**.

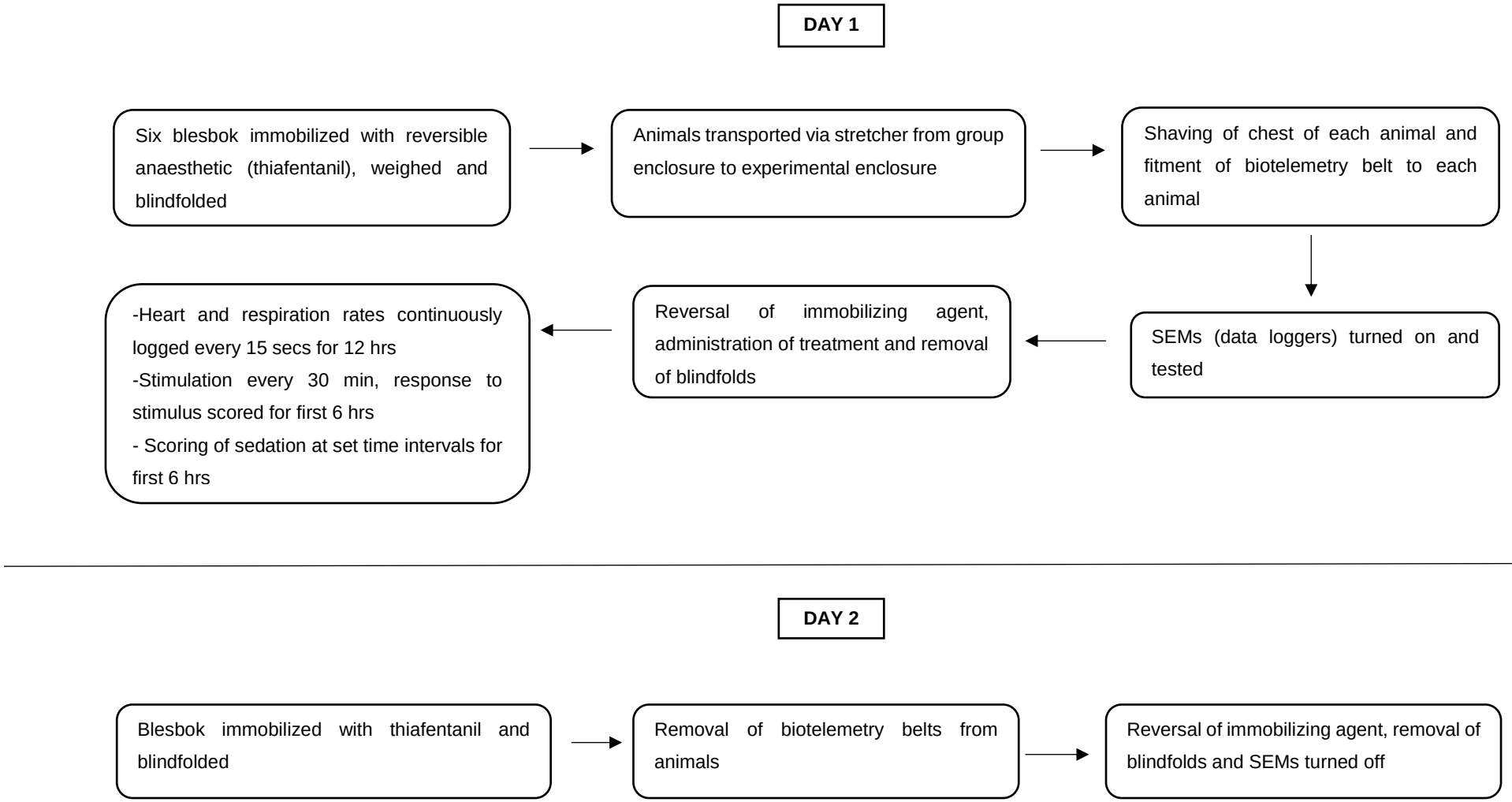


Figure 6.1 The experimental design for determining the effect of a placebo and three different midazolam treatments on the physiology of blesbok.

6.2.1 Data recorded

6.2.1.1 Heart rate

Heart rate is defined as the number of times an animal's heart beats per minute. From the start of each trial, the heart rate of each respective blesbok was measured every 15 secs with the ECG sensors of the biotelemetry system and logged by the SEM on each belt for 12 hrs following treatment.

6.2.1.2 Respiration rate

Respiration rate is defined as the number of breaths exhaled by the animal per minute. From the start of each trial, the respiration rate of each respective blesbok was measured every 15 secs via the expansion of the elastic of the biotelemetry system. This data is then logged by the SEM on each belt for 12 hrs following treatment.

6.2.1.3 Sedation scores

A scoring system was used to subjectively score the degree of sedation. Sedation scores ranging from 0 to 6 were awarded to each animal at set time intervals following administration of the treatment (Table 6.1). Degree of sedation refers to how severely the treatment suppressed an animal's central nervous system. A score of 0 was awarded if the animal was fully awake and showing normal behaviour and thus its central nervous system was not suppressed, while a score of 6 would be awarded to an animal if it was in lateral recumbency and completely unresponsive due to severe suppression of its central nervous system.

Table 6.1 Explanations of sedation scores awarded.

	Score	Explanations
Sedation score	0	No sign of sedation, a normal alert attitude
	1	Initial sign of sedation; depressed attitude, slight stumble when walking
	2	Mild to moderate sedation with depressed attitude, may stumble when walking or running, movements slower, braced stance, or lowered head
	3	Moderate to good sedation with depressed attitude; shows periods of sternal recumbency and periods where animal is standing, severe ataxia and instability
	4	Good to complete sedation with depressed attitude and complete sternal recumbency; still able to hold head up and eyes are open
	5	Complete sedation; unable to stand; recumbency varies between sternal and lateral but able to hold head up; no purposeful movements; eyes occasionally close
	6	Profound sedation; unable to hold head up; lateral recumbency, eyes closed

6.2.1.4 Response to stimulus scores

A person entered the enclosure every 30 min and stimulated the animals by banging a plastic clipboard against the enclosure wall to deliberately produce noise. This caused a stress response in the animals, as no other human contact was experienced by the animals during each trial. Each animal's response to this stimulus was also scored within a range of 0 to 4 (Table 6.2). Response to stimulus refers to the level of reactivity an animal

shows when startled by the stimulus. A score of 0 was awarded if the animal reacted immediately by moving rapidly away from the source of the stimulus. A score of 4 would be awarded if an animal showed very little or no response to the stimulus.

The sedation and response to stimulus of each animal was scored for the first 6 hours following placebo or midazolam administration, as midazolam has been found to have an elimination half-life of 2-6 hours (Naritoku & Sinha, 2000). The heart rate, respiration rate, skin temperature and motion of the animals were continuously logged by the SEM of the biotelemetry system every 15 secs for the first 12 hrs of a respective trial following administration of the treatment. The time intervals of sedation and reaction to stimulus scoring, as well as the duration of measurements were derived from the results of the pharmacokinetic study of midazolam conducted in the indigenous goats (Chapter 3). Each trial commenced at approximately the same time of the morning, and the stimulations occurred at approximately the same time of the day in each trial.

Table 6.2 Explanations for response to stimulus scores awarded to blesbok following treatment with either a high, medium or low dose of midazolam.

	Score	Explanation
Response to stimulation score	0	Full response. Immediately trots in alarm or walks away quickly
	1	Slightly delayed response, fast trotting in alarm or walks away quickly, mild ataxia when running, slightly unstable
	2	Delayed response, slow trotting, severe ataxia when running, very unstable
	3	Very delayed response, walks or just stands still
	4	No response. Recumbent or standing with head down

6.2.2 Statistical analysis

The data from this study was analysed using a restricted maximum likelihood estimation (REML) in Statistica's Variance Estimation, Precision and Comparison module (Statistica version 13, Stat Soft, Inc., 2016). Dose and behaviour were the fixed effects for the analyses of vital sign data per behaviour, while dose and stimulation were the fixed effects for the analyses of vital sign data per stimulation/no stimulation. Results for vital sign data per behavioural data were expressed as least square means (LSMeans ± SEM). The results of scoring data are expressed as means ± standard error of the mean (SEM). Results were considered significant at $P \leq 0.05$.

6.3 Results

No adverse side effects were witnessed in any of the animals during any of the trials in this study. Extrapyramidal symptoms, in the form of hyperactivity and disorientation, were witnessed in some animals during the high dose midazolam trial. Furthermore, no adverse reactions were observed at the injection sites.

6.3.1 Heart rate per behaviour

The results of the mean heart rate per behaviour are presented in Table 6.3.

Table 6.3 The mean (LSMean ± SEM) heart rate (bpm) per behaviour of blesbok when treated with either a placebo or three different doses of midazolam (high, medium or low).

Behaviour	Placebo	High Dose (0.6 mg/kg BWt)	Medium Dose (0.4 mg/kg BWt)	Low Dose (0.2 mg/kg BWt)
Standing with head up ruminating	122.2^{a}±0.64	119.8^{a}±0.67	83.8^{b}±0.44	78.9^{b}±0.31
Walking	128.1^{a}±0.82	128.0^{a}±0.61	92.8^{b}±0.52	86.1^{b}±0.66
Vigilance	126.8^{a}±0.87	122.8^{ab}±0.90	100.1^{bc}±0.95	91.4^{c}±0.84
Lying with head up	103.8^{a}±1.42	83.2^{ab}±0.82	63.4^{b}±0.79	65.0^{b}±0.54
Eating	129.8^{a}±1.53	132.8^{a}±1.83	84.3^{b}±1.10	80.4^{b}±0.63
Lying with head down	97.2^{a}±1.93	75.0^{ab}±1.08	60.4^{b}±0.92	65.2^{b}±0.61
Sniffing while standing	123.7^{a}±3.43	124.9^{a}±2.65	90.2^{b}±1.50	82.1^{b}±1.27
Standing with head down	114.1^{ab}±3.19	132.2^{a}±6.03	104.0^{bc}±2.61	85.5^{c}±1.74
Trotting in alarm	125.9^{ab}±3.02	142.5^{a}±2.67	117.7^{b}±3.00	109.1^{b}±4.32
Sniffing while walking around	117.4^{a}±3.90	116.9^{a}±1.40	90.5^{b}±2.99	88.7^{b}±3.32
Fighting	132.7^{a}±4.65	148.0^{a}±6.18	107.7^{ab}±8.68	98.8^{b}±15.00
Avoidance	119.8^{b}±6.26	156.8^{a}±6.49	120.2^{b}±12.49	103.3^{b}±5.97
Drinking	153.4^{a}±14.83	130.0^{a}±8.49	97.2^{b}±10.89	77.3^{b}±7.61
Other abnormal behaviour	106.9^{ab}±29.08	139.0^{a}±11.95	121.2^{a}±9.94	70.3^{b}±14.36

^{a,b,c} Column means with different superscripts indicate significant differences ($P \leq 0.05$)

The mean heart rate of the blesbok that received the low dose (0.2 mg midazolam/kg BWt) was lower than that following treatment of the animals with the placebo during the behaviours “standing with head up ruminating” ($P < 0.001$), “walking” ($P < 0.001$), “vigilance” ($P = 0.003$), “lying with head up” ($P = 0.001$), “eating” ($P < 0.001$), “lying with head down” ($P = 0.009$), “sniffing while standing” ($P = 0.001$), “standing with head down” ($P = 0.022$), “sniffing while walking around” ($P = 0.034$), “fighting” ($P = 0.027$) and “drinking” ($P < 0.001$). Heart rate during all unmentioned behaviours did not differ ($P > 0.05$) between the low dose treatment and the placebo (Table 6.3).

The mean heart rate of the blesbok resulting from treatment with the medium dose (0.4 mg midazolam/kg BWt) was lower than that following treatment of the animals with the placebo during the behaviours “standing with head up ruminating” ($P = 0.001$), “walking” ($P = 0.003$), “vigilance” ($P = 0.027$), “lying with head up” ($P = 0.001$), “eating” ($P < 0.001$), “lying with head down” ($P = 0.003$), “sniffing while standing” ($P = 0.006$), “sniffing while

walking around" (P = 0.034) and "drinking" (P = 0.001). Heart rate during all unmentioned behaviours did not differ (P > 0.05) between the medium dose treatment and the placebo (Table 6.3).

The mean heart rate following treatment with the high dose (0.6 mg midazolam/kg BWt) was higher than the heart rate following treatment with the placebo during "avoidance" behaviour (P = 0.006). Heart rate during all unmentioned behaviours did not differ (P > 0.05) between the high dose treatment and the placebo.

The mean heart rate per behaviour resulting from the low dose of midazolam was lower than those resulting from the medium dose of midazolam during "other abnormal behaviour" (P = 0.033). Heart rate during all unmentioned behaviours did not differ (P > 0.05) between the low dose treatment and the medium dose treatment.

The mean heart rate per behaviour resulting from the low dose of midazolam was lower than those resulting from the high dose of midazolam during the behaviours "standing with head up ruminating" (P = 0.001), "walking" (P = 0.001), "vigilance" (P = 0.009), "eating" (P < 0.001), "sniffing while standing" (P = 0.001), "standing with head down" (P < 0.001), "trotting in alarm" (P = 0.008), "sniffing while walking around" (P = 0.035), "fighting" (P = 0.002), "avoidance" (P = 0.001), "drinking" (P = 0.002) and "other abnormal behaviour" (P = 0.003). Heart rate during all unmentioned behaviours did not differ (P > 0.05) between the low dose treatment and the high dose treatment (Table 6.3).

The mean heart rate per behaviour resulting from the medium midazolam dose was lower than that following treatment of the animals with the high dose of midazolam during the behaviours "standing with head up ruminating" (P = 0.003), "walking" (P = 0.004), "eating" (P < 0.001), "sniffing while standing" (P = 0.005), "standing with head down" (P < 0.001), "trotting in alarm" (P = 0.046), "sniffing while walking around" (P = 0.035), "avoidance" (P = 0.013) and "drinking" (P = 0.034). Heart rate during all unmentioned behaviours did not differ (P > 0.05) between the medium dose treatment and the high dose treatment.

6.3.2 Respiration measurements per behaviour

The results of the mean respiration rate per behaviour are presented in Table 6.4.

The mean respiration rate when treated with the low dose of midazolam was lower than that resulting from treatment with the placebo during the behaviours "standing with head up ruminating" (P = 0.022), "walking" (P = 0.002), "vigilance" (P < 0.001) "standing with head down" (P < 0.001), "trotting in alarm" (P = 0.004) and "fighting" (P = 0.001). Respiration rate during all unmentioned behaviours did not differ (P > 0.05) between the low dose treatment and the placebo (Table 6.4).

The mean respiration rate when treated with the medium dose of midazolam was higher than that resulting from treatment of the animals with the placebo during the behaviours "standing with head up ruminating" (P < 0.001), "walking" (P < 0.001), "vigilance" (P < 0.001), "lying with head up" (P = 0.024), "eating" (P < 0.001), "sniffing while standing" (P < 0.001), "trotting in alarm" (P < 0.001), "sniffing while walking around" (P < 0.001), "fighting" (P = 0.014), "avoidance" (P = 0.004) and "other abnormal behaviours" (P = 0.006). Respiration rate

during all unmentioned behaviours did not differ ($P > 0.05$) between the medium dose treatment and the placebo.

The mean respiration rate of the blesbok when treated with the high dose of midazolam was higher compared to that when they were treated with the placebo for the behaviours “standing with head up ruminating” ($P < 0.001$), “walking” ($P < 0.001$), “vigilance” ($P < 0.001$), “eating” ($P< 0.001$), “sniffing while standing” ($P < 0.001$), “trotting in alarm” ($P < 0.001$), “sniffing while walking around” ($P < 0.001$), “fighting” ($P = 0.001$), “avoidance” ($P < 0.001$) and “other abnormal behaviours” ($P < 0.001$). Respiration rate during all unmentioned behaviours did not differ ($P > 0.05$) between the two specific doses being compared (Table 6.4).

The mean respiration rate of the blesbok when treated with the low dose of midazolam was lower than that following treatment of the animals with the medium dose for the behaviours “standing with head up ruminating” ($P < 0.001$), “walking” ($P < 0.001$), “vigilance” ($P < 0.001$), “lying with head up” ($P < 0.001$), “eating” ($P = 0.001$) “sniffing while standing” ($P < 0.001$), “standing with head down” ($P < 0.001$), “trotting in alarm” ($P < 0.001$), “sniffing while walking around” ($P = 0.005$), “fighting” ($P < 0.001$) and “avoidance” ($P = 0.011$). Respiration rate during all unmentioned behaviours did not differ ($P > 0.05$) between the low dose treatment and the medium dose treatment.

The mean respiration rate of the blesbok when treated with the low dose of midazolam was lower than that following treatment of the animals with the high dose of midazolam for the behaviours “standing with head up ruminating” ($P < 0.001$), “walking” ($P < 0.001$), “vigilance” ($P < 0.001$), “lying with head up” ($P = 0.036$), “eating” ($P < 0.001$), “sniffing while standing” ($P < 0.001$), “standing with head down” ($P < 0.001$), “trotting in alarm” ($P < 0.001$), “fighting” ($P < 0.001$) and “avoidance” ($P = 0.005$). Respiration rate during all unmentioned behaviours did not differ ($P > 0.05$) between the low dose treatment and the high dose treatment.

The mean respiration rate of the blesbok when treated with the medium dose of midazolam was lower than that following treatment with the high dose for the behaviours “standing with head up ruminating” ($P = 0.041$) and “vigilance” ($P = 0.004$). Respiration rate during all unmentioned behaviours did not differ ($P > 0.05$) between the medium dose treatment and the high dose treatment (Table 6.4).

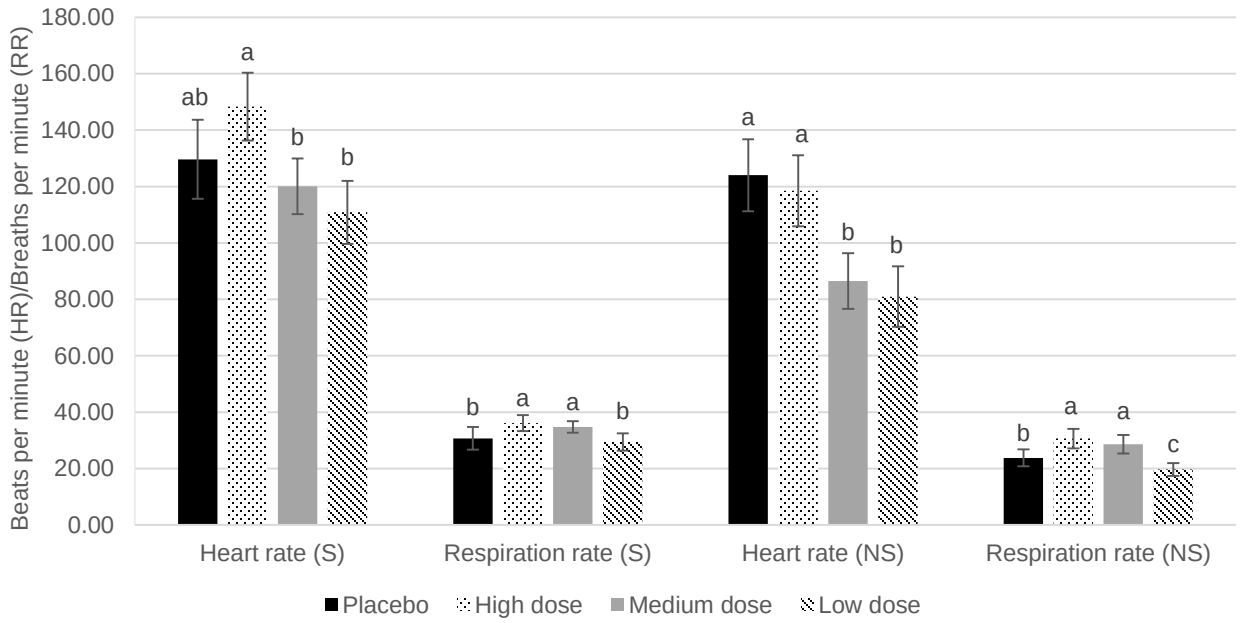
Table 6.4 The mean (LSMean ± SEM) respiration rate (breaths/min) per behaviour of blesbok when treated with either a placebo or three different doses of midazolam (high, medium or low).

Behaviour	Placebo	High Dose (0.6 mg/kg BWt)	Medium Dose (0.4 mg/kg BWt)	Low Dose (0.2 mg/kg BWt)
Standing with head up ruminating	21.4^{c}±0.13	29.8^{a}±0.18	27.2^{b}±0.14	18.5^{d}±0.06
Walking	27.7^{b}±0.15	36.4^{a}±0.14	34.2^{a}±0.13	23.7^{c}±0.15
Vigilance	28.2^{c}±0.15	36.4^{a}±0.21	32.7^{b}±0.21	21.7^{d}±0.16
Lying with head up	12.7^{ac}±0.22	13.6^{ab}±0.09	15.7^{b}±0.23	10.8^{c}±0.23
Eating	20.7^{b}±0.23	27.4^{a}±0.32	26.9^{a}±0.27	22.7^{b}±0.15
Lying with head down	12.6^{a}±0.28	13.6^{a}±0.25	12.6^{a}±0.20	11.2^{a}±0.28
Sniffing while standing	23.9^{b}±0.51	32.0^{a}±0.70	31.7^{a}±0.46	23.9^{b}±0.43
Standing with head down	32.7^{a}±0.68	35.0^{a}±1.51	33.3^{a}±0.78	20.5^{b}±0.38
Trotting in alarm	29.8^{b}±0.63	36.3^{a}±0.48	35.9^{a}±0.64	25.1^{c}±0.72
Sniffing while walking around	22.1^{c}±0.50	28.2^{ab}±0.53	31.4^{a}±0.75	25.4^{bc}±1.52
Fighting	29.2^{b}±0.84	35.3^{a}±1.13	34.8^{a}±1.55	19.5^{c}±1.53
Avoidance	29.9^{b}±1.00	37.2^{a}±0.68	37.5^{a}±2.17	28.9^{b}±2.21
Drinking	22.0^{a}±1.19	24.6^{a}±1.84	26.7^{a}±1.97	24.4^{a}±2.24
Other abnormal behaviour	15.3^{b}±1.51	37.6^{a}±2.26	30.1^{a}±4.05	20.7^{ab}±1.83

^{a,b,c,d} Column means with different superscripts indicate significant differences ($P \leq 0.05$)

6.3.3 Heart rate per period of stimulation/no stimulation

The heart rate of the blesbok when being either stimulated (S) or not stimulated (NS) are shown in Figure 6.2.



[a,b,c] Means for a specific parameter with different superscripts indicate significant differences (P ≤ 0.05)

Figure 6.2 The mean (LSMean ± SEM) heart rate (beats per min) and respiration rate (breaths/min) of blesbok within periods of either stimulation (S) or no stimulation (NS) when treated with either a placebo or three different doses of midazolam (high 0.6 mg midazolam/kg BWt, medium 0.4 mg midazolam/kg BWt or low 0.2 mg midazolam/kg BWt).

The mean heart rate of the blesbok treated with midazolam, regardless of dose, did not differ significantly from the heart rate recorded in animals receiving the placebo during periods of stimulation. However, when comparing midazolam doses, the animals had a lower mean heart rate during periods of stimulation when they were treated with the low midazolam dose than when they were treated with the high midazolam dose (110.8 ± 11.18 bpm vs. 148.3 ± 12.07 bpm, P = 0.009). The mean heart rate of the blesbok when they were treated with the low midazolam dose did not differ (P > 0.05) from that when they were treated with the medium midazolam dose during periods of stimulation. The mean heart rate of the blesbok when they were treated with the medium dose of midazolam was lower than that when they were treated with the high dose of midazolam during periods of stimulation (120.1 ± 9.91 bpm vs. 148.3 ± 12.07 bpm, P = 0.040) (Figure 6.2).

The mean heart rate of the blesbok during periods of no stimulation when treated with the low dose of midazolam (81.0 ± 10.76 bpm) was lower than that when they were treated with the placebo (124.0 ± 12.8

bpm, P = 0.004) and the high dose of midazolam (118.5 ± 12.60 bpm, P = 0.009). However, the mean heart rate of the blesbok during periods of no stimulation when treated with the low dose of midazolam was not different (P > 0.05) from that when they were treated with the medium dose. The mean heart rate of the blesbok during periods of no stimulation when treated with the medium dose (86.5 ± 9.84 bpm) was lower than that when they were treated with the placebo (124.0 ± 12.77 bpm, P = 0.009) and the high dose of midazolam (118.5 ± 12.60 bpm, P = 0.022). The mean heart rate of the blesbok during periods of no stimulation when treated with the high dose of midazolam did not differ (P > 0.05) from that when they were treated with the placebo (Figure 6.2).

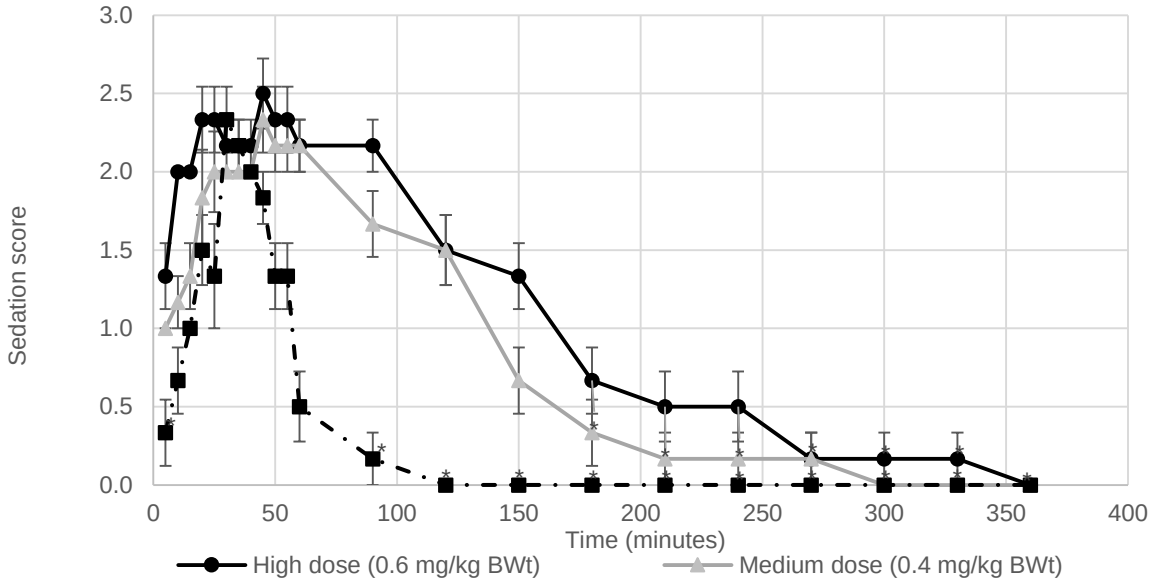
6.3.4 Respiration rate per period of stimulation/no stimulation

Respiration rate of the blesbok during periods of stimulation and no stimulation are shown in Figure 6.2. The mean respiration rate of the blesbok during periods of stimulation when treated with the low dose of midazolam (29.4 ± 3.05 breaths/min) was not different (P > 0.05) than that when they were treated with the placebo, but was lower than that when they were treated with either the medium dose (34.8 ± 2.03 breaths/min, P = 0.001) or the high dose of midazolam (36.1 ± 2.86 breaths/min, P < 0.001). The mean respiration rate of the blesbok during periods of stimulation when treated with the medium dose of midazolam was higher than that when they were treated with the placebo (34.8 ± 2.03 breaths/min vs. 30.7 ± 4.04 breaths/min, P = 0.008), but did not differ (P > 0.05) from that when they were treated with the high dose of midazolam. The mean respiration rate of the blesbok during periods of stimulation when treated with the high dose of midazolam was higher than that when they were treated with the placebo (36.1 ± 2.86 breaths/min vs. 30.7 ± 4.04 breaths/min, P = 0.009).

The mean respiration rate of the blesbok during periods of no stimulation when treated with the low dose of midazolam (19.7 ± 2.22 breaths/min) was lower than that when they were treated with the placebo (23.8 ± 3.02 breaths/min, P = 0.007), the medium dose (28.6 ± 3.27 breaths/min, P < 0.001) and the high dose (30.6 ± 3.43 breaths/min, P < 0.001) of midazolam. The mean respiration rate of the blesbok during periods of no stimulation when treated with the medium dose of midazolam was higher than that when they were treated with the placebo (28.6 ± 3.27 breaths/min vs. 23.8 ± 3.02 breaths/min, P = 0.002), but did not differ (P > 0.05) from that when they were treated with the high dose of midazolam. The mean respiration rate of the blesbok during periods of no stimulation when treated with the high dose of midazolam was higher than that when they were treated with the placebo (30.6 ± 3.43 breaths/min vs. 23.8 ± 3.02 breaths/min, P < 0.001) (Figure 6.2).

6.3.5 Sedation scores per treatment

The mean values of the sedation scores scored for the blesbok at set time intervals following administration of each respective treatment (placebo, high midazolam dose, medium midazolam dose or low midazolam dose) are given in Figure 6.3.



***Not significantly different from zero (P > 0.05)**

Figure 6.3 Comparison of mean sedation score (± SEM) values of blesbok following treatment with three different doses of midazolam.

During the placebo trial, all the blesbok had sedation scores of 0 for the full duration of the trial and this was considered the baseline value. Peak sedation was achieved after 30 min for the low dose, and after 45 min for the medium dose and high dose midazolam dose trials, respectively. The maximum sedation score scored after midazolam administration ranged from 2 to 3 (Table 6.1), with the highest mean sedation score (2.5) being awarded during the high midazolam dose trial (Figure 6.3).

The mean sedation score of the low dose of midazolam differed ($P \leq 0.05$) from 0 (placebo) at 10 min-60 min following treatment. The mean sedation scores of the medium dose midazolam trial differed ($P \leq 0.05$) from 0 at 5-150 min after treatment. The mean sedation scores of the high dose trial differed significantly from 0 at 5-240 min after treatment. The mean sedation scores and period of sedation of the blesbok increased with an increase in midazolam dosage. The medium dose of midazolam resulted in a sedation pattern more similar to the high midazolam dose than the low midazolam dose (Figure 6.3).

6.3.6 Response to stimulus scores per treatment

The mean values of the response to stimulus scores scored for the blesbok at set time intervals following administration of each respective treatment can be observed in Figure 6.4.

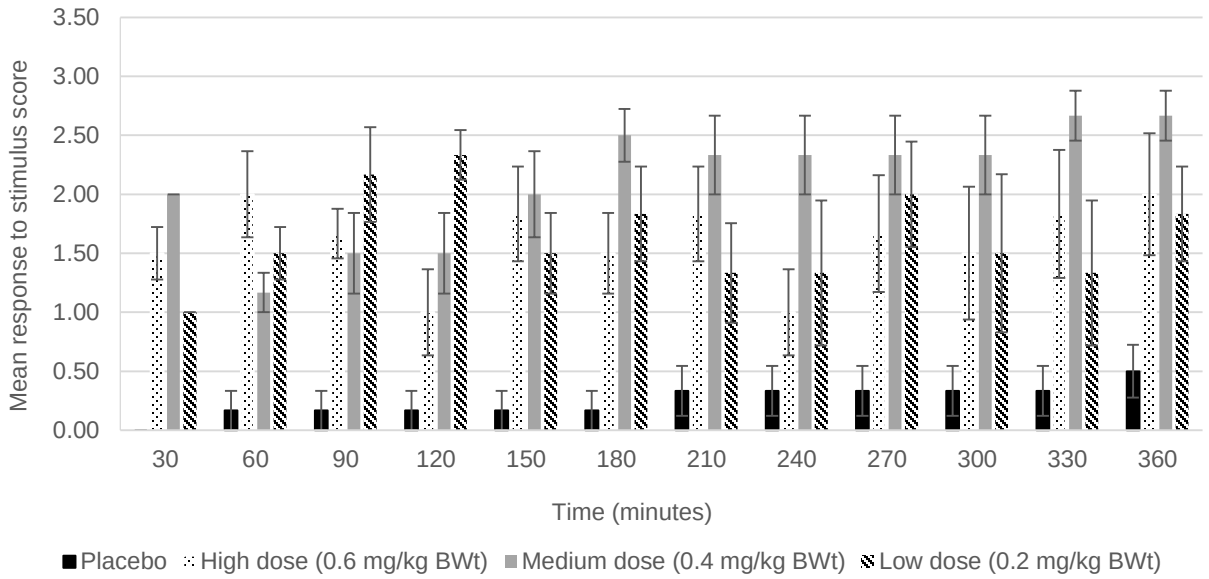


Figure 6.4 Comparison of mean response to stimulus score values of blesbok following treatment with three different doses of midazolam (a higher value equates less responsiveness).

It must be noted that an increase in response score indicates reduced responsiveness. The maximum response to stimulus score achieved in the blesbok treated with midazolam was 3 (Table 6.2), and the highest mean response to stimulus score (2.7) was awarded during the medium midazolam dose trial (Figure 6.4). The response to stimulus scores following treatment with each dose of midazolam differed ($P \leq 0.05$) from the placebo at all time points following treatment.

The animals were the most responsive after treatment with the placebo and least responsive (highest response to stimulus scores) after treatment with the medium (0.4 mg midazolam/kg BWt) dose. It also appears that the animals had become less responsive to the stimulus as time passed, which were specifically observed after treatment of the animals with the placebo and the medium midazolam dose (Figure 6.4).

6.4 Discussion

In the present study a low dose (0.2 mg midazolam/kg BWt) of midazolam administered IM resulted in a slower heart rate of the blesbok during most of the behaviours. When compared to the placebo, the heart rate of the animals was not affected after treatment with the low dose of midazolam during behaviour commonly associated with stress, namely trotting in alarm, avoidance and other abnormal behaviour (Oken *et al.*, 2006).The respiration rate of the blesbok was unaffected by the low dose of midazolam during most of the behaviours, but was decreased by this dose when the animals were standing with their heads up ruminating, walking, being vigilant, standing with their heads down, trotting in alarm and fighting compared to the placebo treatment.

The medium dose (0.4 mg midazolam/kg BWt) did not affect the heart rate of the blesbok when the animals were standing with their heads down, fighting, showing avoidance and showing other abnormal behaviour when compared to the placebo treatment. The heart rate of the blesbok was slower for all other behaviour following treatment with the medium dose of midazolam in a similar manner to the low dose, including the vigilance and trotting in alarm behaviour associated with stress and anxiety, when compared to the placebo treatment. The respiration rate of the animals was not affected ($P > 0.05$) by the medium dose of midazolam when the animals were lying down, standing with their head down and drinking, but was increased during the rest of the behaviours when compared to the placebo treatment.

The high dose (0.6 mg midazolam/kg BWt) administered by the IM route resulted in a faster heart rate in the blesbok during avoidance behaviour, but the heart rate of the blesbok during any of the other behaviour was unaffected by this dose when compared to the placebo treatment. Similar to when the animals were treated with the medium dose of midazolam, the respiration rate of the animals was unaffected by the high dose of midazolam when the animals were lying with their heads up or down, standing with their heads down and drinking compared to when they were treated with the placebo. The respiration rate of the blesbok was increased during all of the remaining behaviours following treatment with the high dose of midazolam when compared to the placebo treatment. Thus the high dose did not affect the heart rate of the blesbok per behaviour overall, including during behaviour associated with stress or anxiety: vigilance and trotting in alarm. The high dose increased the respiration rate during most of the behaviours of the animals, including the abovementioned behaviour commonly associated with stress or anxiety. Both the medium and high doses of midazolam appeared to have had a respiratory stimulatory effect in the blesbok.

Regarding the physiological parameters of the blesbok per period of stimulation, the low dose of midazolam did not affect either the heart rate or the respiration rate of the blesbok. The medium and high doses of midazolam did not affect the heart rate of the blesbok, but both these doses increased the respiration rate of the animals while they were being stimulated compared to the placebo treatment. Thus only the respiration rate of the blesbok was affected by midazolam, specifically by the high and medium doses, during periods of stimulation and the effects were paradoxical.

Various studies have found that benzodiazepines cause minor depression of the cardiac and respiratory systems (Jones *et al.*, 1979; Reves *et al.*, 1985; Wright *et al.*, 1990; Bailey *et al.*, 1990; Yaster *et al.*,1990; Heniff *et al.*, 1997; Paddleford, 1999; Lukasik & Gillies, 2003; Becker, 2012). In the present study the medium and high doses of midazolam had no effect on the heart rate and caused an increase in respiration rate of blesbok, which is thus contradictory to that found in previous studies. Stegmann (1998), however, found that the heart rate of immobilised goats under laboratory conditions did increase after treatment with midazolam at a dose of either 0.4 mg/kg BWt or 1 mg/kg BWt by the IM route, and the 0.4 mg/kg BWt dose also increased the respiration rate of the animals. The increase in heart rate found in the study by Stegmann (1998) was argued to be the result of the preservation of the baroreflex by midazolam, which caused a

decline in blood pressure and thus a reflexive increase in heart rate. Stegmann (1998) did not, however, offer an explanation for the elevation in respiratory rate in their study.

According to Simon *et al.* (2017), midazolam administered via the IV route resulted in a higher mean heart rate and respiratory rate in sheep than midazolam administered via the IM route. Simon *et al.* (2017) argued that the elevation in values may be due to the stress from environmental conditions and restraint due to a lack of randomization in the order of which the animals received the treatment. In their study, the animals were first exposed to the experimental conditions during the trial where midazolam was administered intravenously, and thus they were more stressed or excited than during the subsequent trial where midazolam was administered by the IM route. Simon *et al.* (2017) physically restrained the animals in their study to administer the midazolam however, which is much more stressful to the animals than the chemical immobilisation used in the present study. As a result, the order in which the animals received the treatments would not have affected the stress experienced by the animals during the present study. In the present study the animals were first exposed to the experimental conditions during the placebo trial, followed by the high dose trial, then the medium dose trial and lastly the low dose trial. The first introduction of the animals to midazolam was thus with the highest dose. The effects of such a high midazolam dose experienced for the first time may therefore have caused the animals to be stressed and excited. During the high dose midazolam trial, some of the animals appeared very unstable on their feet due to severe ataxia and these animals would fall over or run into the enclosure wall when running away – this behaviour was not observed in the medium and low dose midazolam trials. Falling over or running into walls would have caused stress to both the animals falling and the other animals observing them. This could therefore explain why the heart rate of the blesbok was faster after treatment with the high dose of midazolam compared to when they were treated with both the other two doses of midazolam. Stress due to some animals running into the walls of the enclosure or falling due to severe ataxia may also explain the observed elevation of respiratory rate in the animals following treatment with the high dose of midazolam. Support for the argument of stress or excitement experienced by the animals during the high dose trial is further reinforced by the results in Chapter 5, where it was found that the walking behaviour of the blesbok increased and the animals showed more fast motion per period of stimulation after treatment with the high dose of midazolam.

The animals may potentially have adapted to the experimental conditions by the time the low dose trial was conducted, even though an attempt to prevent adaptation was made by moving the animals between different enclosures and regrouping with other blesbok in between trials. Thus it is possible that adaptation had caused the respiration rate of the animals to be less elevated after treatment with the low dose of midazolam, when compared to the other two midazolam treatments. The stress from the stimulation process on the animals may also have overcome the depressant effect midazolam potentially could have had on the cardiopulmonary system of blesbok and thus none of the doses were able to have the previously reported depressant effect of benzodiazepines on the heart rate and respiratory rate of the animals during periods of stimulation. According to Pierce and Cheney (2013), if an environmental aspect is consistently

present following administration of a drug, e.g. a person stimulating the animals, it will become a stimulus that is conditioned. This conditioned stimulus has the ability to cause a conditioned response that may be compensatory or preparatory, and this learned response will then be used in order to counteract the unlearned effects of the drug in order to maintain homeostasis. This homeostatic counteraction can however be of such a magnitude that the effects of a drug are cancelled out. This process is called tolerance (Pierce & Cheney, 2013). The constant stimulus experienced by the animals for the first 6 hrs of this study may thus have become a conditioned stimulus and have resulted in a homeostatic counteraction that was of such an extent that the drug had no effect on the physiological parameters of the animals when they were being stimulated.

The low and medium doses of midazolam both caused a slower heart rate in the animals during periods of no stimulation when compared to the placebo treatment. The low dose of midazolam slowed down the respiration rate, while the medium dose increased the respiration rate of the animals when compared to the placebo treatment during periods of no stimulation. The increase in respiration resulting from the medium dose of midazolam may be due to the stress experienced in the medium dose trial similar to that previously discussed for the high dose trial. The results of the low midazolam dose trial are in accordance with that previously found for benzodiazepines, namely that they can result in a mild depression of the cardiopulmonary system (Jones *et al.*, 1979; Reves *et al.*, 1985; Wright *et al.*, 1990; Bailey *et al.*, 1990; Yaster *et al.*, 1990; Heniff *et al.*, 1997; Paddleford, 1999; Lukasik & Gillies., 2003; Becker, 2012). A study on swine by Smith *et al.* (1991) found that 0.1 mg/kg BWt midazolam administered via the IM route decreased the heart and respiration rate of pigs, similarly to the 0.2 mg midazolam/kg BWt (low dose) used in the current study.

The high dose of midazolam did not affect the heart rate of the blesbok, but increased respiration rate when compared to the placebo treatment during periods of no stimulation. Thus the high dose did not affect the heart rate of the blesbok throughout the trial, but did elevate the respiration rate of the animals. As previously explained, this increased rate of ventilation observed in the animals following treatment with the high dose may be due to the stress experienced as a result of severe ataxia in some animals. Studies by Simon *et al.* (2017), Stegmann (1999) and Stegmann and Bester (2001) found that midazolam doses (0.5 mg/kg BWt and 0.6 mg/kg BWt) similar to the high dose (0.6 mg midazolam/kg BWt) used in the present study, had no effect on either the heart rate or respiration rate of the animals. Furthermore, a study of midazolam in horses (Hubbell *et al.*, 2013) also found that midazolam did not affect the heart rate of the animals, similar to the high dose in this study. Thus it is not uncommon for midazolam doses of 0.5-0.6 mg/kg BWt to have no effect on the cardiopulmonary system. According to Stegmann (1998), the haemodynamic effects of midazolam may be dose-dependent, but a ceiling effect is reached above which an increased dosage will not increase the side effects of a drug on the cardiovascular system. It is thus possible that midazolam also reached a ceiling effect in the blesbok and thus the high dose did not affect the heart rate of the animals during periods of no stimulation. Sunzel *et al.* (1988) found that no correlation

existed between the dose of midazolam and the drug's respiratory effect, while Becker (2012) states that respiratory depression due to benzodiazepines is dose dependent. The results found in the present study agree more with Sunzel *et al.* (1988) than with Becker (2012), as the lowest of the three doses caused a slight decrease in respiration rate and an increase in dose resulted in increased respiration.

Undisturbed blesbok in captivity with a similar weight to that in the current study have been found to have a mean heart rate of 57.50 ± 0.24 bpm and a mean respiration rate of 17.3 ± 0.12 breaths/min (refer to Chapter 4, 4.3.1 and 4.3.2). The mean heart rate and respiration rate of the blesbok in this study were most similar to the values for unstimulated blesbok in captivity following treatment of the animals with the low dose of midazolam (80.98 ± 10.76 bpm and 19.66 ± 2.22 breaths/min respectively). The mean heart rate and respiration rate during periods of no stimulation resulting from treatment with the placebo (124.04 ± 12.77 bpm and 23.79 ± 3.02 breaths/min respectively), medium dose (86.49 ± 9.84 bpm and 28.64 ± 3.27 breaths/min respectively) and high dose (118.49 ± 12.60 bpm and 30.64 ± 3.43 breaths/min respectively) of midazolam were much higher than those reported for undisturbed blesbok in captivity (Chapter 4, 4.3.1 and 4.3.2). Thus despite the variation in heart and respiration rate in this study, the heart and specifically the respiration rate observed after the animals were treated with the low dose of midazolam during periods of no stimulation agreed most with the reference values reported for healthy blesbok in captivity (Chapter 4).

All the animals in the abovementioned studies to which the results of the present study were compared to, with the exception of that of Stegmann (2001), were done on immobilised animals and not in fully awake animals as in the present study. This makes it difficult to reliably compare previous results with those found in the present study. Thus, there is a clear lack of studies of pharmaceuticals such as midazolam in fully conscious wild antelope species in captivity. In order to sufficiently compare the results from this study, more studies on the use of especially sedative agents in free-roaming wild antelope species needs to be conducted to ensure a better representation of results from animals in their natural state. However, from these results combined with that found in the present study, it generally appears that doses of up to 0.2 mg midazolam/kg BWt tends to depress the cardiovascular and respiratory systems of blesbok that are not experiencing stimulation, while doses higher than 0.2 mg midazolam/kg BWt tends to have a minimal effect or no effect on the heart rate of blesbok, but tend to increase respiratory rate of the animals. The latter effect may be related to adverse effects at these dosages such as increased excitatory behaviour. Thus the effects of midazolam on the cardiovascular system are specifically related to dose, but appear to reach a limit above which an increase in dose does not increase the side-effects of the drug on the cardiovascular system. This argument is supported in a previous study by Hall *et al.* (1987) on the use midazolam in dogs, where it was found that even though midazolam's sedative effects were dose-dependent, a ceiling was reached beyond which increasing the dose did not have an increased effect on the sedative properties of this drug. The changes in the cardiopulmonary system following the treatments in this study would perhaps have been better explained if blood and faecal samples could be collected such as blood glucose, plasma

cortisol, neutrophil function and metabolites of faecal glucocorticoids. Blood samples are difficult to collect from animals that are not immobilised, but faecal samples can be collected for both animals that are immobilised and those that are not. The equipment and validation necessary for the collection of these samples were however not within the scope or budget of this study.

Peak sedation was reached at 30 min after treatment with the low midazolam dose, while it took 45 min to be reached after treatment with the medium and high doses of midazolam. Thus time to maximum sedation increased with dose in the blesbok. Significant signs of sedation were present in the animals for up to 1 hr following treatment with the low dose of midazolam, for up to 2.5 hrs following administration of the medium dose of midazolam and up to 4 hrs following administration of the high dose of midazolam. Thus sedation lasted longer with an increase in midazolam dose, as would be expected. According to Stegmann & Bester (2001), midazolam at a dose of 0.6 mg/kg BWt caused maximum sedation at 20 min after IM administration in goats, which is 25 minutes faster than that found when the same dose of 0.6 mg midazolam/kg BWt was administered to blesbok in the present study. Furthermore, Stegmann (1998) found that IM midazolam at a dose of 0.4 mg/kg BWt caused reliable sedation within 5 min. Reliable signs of sedation (mean sedation score of > 1.5) in the blesbok after treatment with the same dose as Stegmann (1998) (0.4 mg midazolam/kg BWt) was only observed after 15 min in the present study. According to Simon *et al.* (2017) the sedative effects of a dose of 0.5 mg midazolam/kg BWt are most prominent at 30 min following treatment in sheep, which is 15 min faster than that found in blesbok treated with similar doses in the present study. Thus midazolam appears to take longer to cause reliable sedation and to reach maximum sedation in blesbok than in domestic species of similar physiology.

None of the midazolam doses resulted in mean sedation scores above 3. Even though an ideal sedation score after midazolam treatment would have been between 3 and 4, the scores found in the present study are still very acceptable. Sedation scores below 2 would have meant the animals were under-sedated and would thus still be prone to stress and levels above 4 would have meant the animals would be immobilised rather than sedated. The primary purpose for the use of midazolam on its own is to calm the animals and not necessarily to fully immobilise. In the present study midazolam decreased vigilant behaviour in the blesbok (Chapter 5) and the low and medium doses caused a decrease in the heart rate of blesbok, which shows that midazolam did have a calming effect in the animals. The moderate level of sedation achieved by midazolam in the present study was thus acceptable.

The medium and high doses of midazolam resulted in similar mean sedation score values, and these sedation values were moderate to good (2-3). Simon *et al.* (2017) found that sedation was mild to moderate when midazolam was administered intramuscularly at a dose of 0.5 mg midazolam/kg BWt to sheep, in agreement with the results of the medium and high dose trials of the present study. The low dose of midazolam had the lowest sedation scores, with only mild sedation being observed at best after treatment with this dose. Thus it appears that in order for sufficient sedation to be achieved in blesbok, a midazolam

dose of higher than 0.2 mg/kg BWt would need to be administered. However, based on the results discussed in Chapter 5, the 0.6 mg/kg BWt dosage of midazolam appears to cause paradoxical effects such as excitement. The high dose of midazolam was also found to cause severe ataxia, which is both a source of stress and risk for injury to the animals. Thus increasing the midazolam dose to 0.6 mg/kg BWt or above would not be recommended in blesbok.

When compared to the placebo trial the animals were much less responsive to the stimulus during the midazolam trials. This further supports the argument that midazolam resulted in acceptable sedation in the blesbok. The animals became less responsive to stimulation as time passed, especially during the placebo, medium midazolam dose and low midazolam dose trials. It is possible that the animals had become habituated to the stimulation process, therefore the animals responded with less intensity as time went by. The animals were least responsive to the stimulus during the medium dose trial, while there was no difference in the response to stimulus scores of the low and high dose midazolam trials. As discussed previously, the blesbok may have been stressed during the high dose trial due to severe ataxia experienced by some animals and thus the high dose did not have the expected effect of causing less responsiveness during stimulation than the two lower midazolam doses.

6.5 Conclusion

Midazolam administered IM to blesbok at a low and medium dose (0.2 mg midazolam/kg BWt and 0.4 mg midazolam/kg BWt, respectively) decreased heart rate of blesbok overall, regardless of the stress level the animal experienced. The low midazolam dose also reduced respiration rate of blesbok for behaviours commonly associated with stress. The high (0.6 mg midazolam/kg BWt) and medium doses of midazolam both caused unexpected respiratory stimulation in the blesbok, which may be the result of stress due to the animals being treated with these two higher midazolam doses first, instead of with the low dose, and due to severe ataxia experienced by some animals during the high dose trials specifically. The high dose of midazolam did not affect the heart rate of the animals throughout this study. Midazolam did not affect the heart rate of blesbok during periods of stimulation, regardless of dose. The low and medium doses of midazolam doses both decreased the heart rate of blesbok when no stimulation was experienced. Ultimately, a dose of 0.2 mg midazolam/kg BWt caused results that agreed most with previous studies, in that it decreased both physiological parameters during periods of no stimulation.

Midazolam treatment resulted in moderate sedation in blesbok, but doses of above 0.4 mg midazolam/kg BWt is not recommended in blesbok. Furthermore, the measurement of faecal glucocorticoids should also be considered in future studies of midazolam in wild ungulate species to achieve a better understanding of the effect of this benzodiazepine on the endocrine system of these animals.

Chapter 7

General conclusions and recommendations

Game farming has experienced rapid growth during recent years, and is considered as an integral contributor to South Africa's economy. The increase in game farming activities consequently resulted in an increase in the trade and translocation of wildlife species, especially that of wild ungulates. The unfamiliar environment, noises and human contact associated with translocation represent stressors, with the resulting stress negatively impacting the welfare of translocated animals. Wild ungulates are especially prone to stress during translocation. Unfortunately, diseases, injuries, morbidity and mortality are all associated with increased stress in animals during translocation. Incorrect translocation methods and failure to adhere to proper management principles negatively impact on the welfare of the animals, which in turn can also result in financial losses. There is thus a need for the development of methods or protocols that can reduce and/or prevent the stress caused by translocation.

A large variety of pharmaceutical products is currently available for managing stress in wildlife, specifically tranquilizers and sedatives, although these are not always ideal due to side effects that may occur. There is a need for new drugs to be developed that will have the desired effect, and that will reduce unwanted side effects. The effects of many new drugs are unfortunately not yet known in various wildlife species. Midazolam is a benzodiazepine sedative that has several benefits when compared to other commonly used sedatives such as xylazine and diazepam. Midazolam has proven to have minimal effects on the cardiopulmonary system. Midazolam is often used, in combination with anaesthetics such as opioids, for the immobilisation of wildlife species. There are currently no reports on the use of midazolam administered intramuscularly on its own in wild ungulate species.

The blesbok is a wild ungulate species commonly farmed with in South Africa and which thus frequently undergoes translocation. Midazolam appears to have the potential to be used as a sedative for stress management in blesbok during translocation, but there is a lack of data available on its use in this species. The aim of this study was therefore to determine the efficacy and safety of midazolam as a sedative in blesbok by studying the time-release profile of this drug, its effects on the behaviour and on the physiology of the animals via the use of biotelemetry.

This first part of this study involved the determination of the time-release profile and some basic plasma pharmacokinetics of midazolam in indigenous goats (*Capra hircus*) in order to serve as a model for understanding the absorption, distribution, metabolism and elimination of this drug. Sternal recumbency was not observed in all but one animal after IM treatment with midazolam, which was in contrast to previous findings of midazolam use in goats and may have been the result of separation anxiety. Midazolam administered IM at a dose of 0.8 mg/kg BWt took 36 min to reach a peak serum concentration compared to that found in various other animal species. The elimination half-life of 47 min for IM administered midazolam in goats indicates a fast elimination rate of the drug from the body. The apparent volume of distribution and apparent total clearance

of IM midazolam were found to be low in goats, indicating poor distribution to tissues and poor clearance from plasma respectively, of the drug. Intramuscularly administered midazolam had a relatively low absolute bioavailability of 58.1%, when compared to that found in other animal species. The poor bioavailability indicates a relatively small fraction of the originally administered midazolam reached circulation to have a therapeutic effect in the goats. Midazolam appears to have stimulated the appetite in indigenous goats, observed as increased ruminating and chewing behaviour in the animals following treatment, but this may also be due to a pyramidal side effect known as allotriophagia. However, no feed intake was measured in this trial and therefore it could not be confirmed that midazolam had increased feed intake in indigenous goats. An appetite stimulation effect is typical of benzodiazepines such as midazolam and has been observed in other animals.

The second part of this study investigated the accuracy of a biotelemetry system initially intended for use in humans, the Equivital™ EQ02 biotelemetry belt, for the measurement of the heart rate, respiration rate and motion of blesbok. Heart rate and respiration rate measurements obtained by the Equivital™ system were compared to those simultaneously obtained via a Cardell® 9500 HD multi-parameter veterinary vital sign monitor (Kyron Laboratories (Pty) Ltd., Johannesburg, South Africa) and those manually measured while the blesbok were immobilised in a laboratory. The Equivital™ system's ability to measure changes in vital signs with changes in motion of fully conscious blesbok in captivity was also determined. The results confirmed that the Equivital™ EQ02 system could measure the heart rate and motion of blesbok with accuracy both while the animals were immobilised and fully conscious in captivity. However, the Equivital™ system's measurement of respiration rate in immobilised blesbok was poor. The failure of the Equivital™ system to measure respiration rate with accuracy may have been due to the pressure of the animal's sternum while it was in a sternallly recumbent position on the elastic of the belt preventing the elastic of the biotelemetry system from expanding properly as the animals exhaled, and thus recording inaccurate respiration rate measurements. Further investigation on improving the Equivital™ system's sensitivity to minute respiration changes and the design of its elastic to prevent the position of an immobilised animal from influencing its ability to measure respiration with accuracy is required.

In the third part of the study, the effects of midazolam on the behaviour, motion and feed intake of blesbok was investigated. Three different doses of midazolam, based on previous doses of midazolam used in similar domestic species and unpublished data of midazolam use in ungulate species by South African veterinarians in practice, were administered to blesbok via the IM route to determine the effects of this sedative on the abovementioned parameters of the animals. Results on the analyses of state behaviour showed that midazolam successfully reduced the time spent on "vigilance" behaviour by the blesbok regardless of dose. A midazolam dose of 0.2 mg/kg BWt reduced the time spent by the blesbok on the "walking" behaviour, a dose of 0.4 mg midazolam/kg BWt reduced the time spent by the animals on "standing with head up ruminating" behaviour and a dose of 0.6 mg midazolam/kg BWt caused a paradoxical increase in the time spent on "walking" behaviour. This contradictory effect of the 0.6 mg midazolam/kg BWt dose may be the result of ataxia preventing the animals from standing still after treatment or because of the extrapyramidal symptoms, namely hyperactivity and disorientation that occurred in the animals after treatment with the high dose of midazolam. Results of the analyses of point behaviour was variable amongst doses, but a dose of 0.6 mg midazolam/kg BWt was most successful in reducing the occurrence of both grooming and agitation behaviour. The reduction

in grooming behaviour may be due to a decrease in neuropeptides involved in the modulation of grooming behaviour by a midazolam dose of 0.6 mg/kg BWt. The midazolam doses of 0.2 mg/kg BWt and 0.4 mg/kg BWt did not decrease grooming behaviour overall, which could be attributed to the antagonistic relationship between vigilant behaviour and grooming behaviour typical to antelope species. The blesbok were stimulated at set time points throughout each trial, for the determination of changes in motion underwent by the animals when experiencing a stressor after treatment with a specific dose. Results indicated that midazolam increases fast motion in blesbok regardless of dose during periods of stimulation, and that motion during periods of no stimulation is only affected by a midazolam dose of 0.2 mg/kg BWt, observed as a reduction in motion after treatment with this dose. The contradictory increase in fast motion during stimulation could be attributed to an increase in the endocrine and autonomic responses benzodiazepines have been known to cause when stress is experienced. Measurements of feed intake indicated that midazolam appeared to increase feed intake in blesbok and in a dose dependent manner. Appetite stimulation is a known effect of midazolam and has been observed in animals previously. Ultimately, the low dose of midazolam was most effective in calming the animals, without causing side effects. This is in agreement with doses currently used in practice by wildlife veterinarians in South Africa, although no published data is available.

The second part of the third part of the overall study investigated the influence of three different midazolam dosages on the heart rate and respiration rate of blesbok, as measured using the Equivital™ EQ02 biotelemetry system, as well as its efficacy in achieving sedation in these animals**.** Results showed that even though a midazolam dose of 0.2 mg/kg BWt had no observable effect overall on the heart rate and respiration rate per behaviour of the animals, it was successful in decreasing both these parameters during “vigilant” behaviour. This dose also decreased the respiration rats during “trotting in alarm” behaviour. Thus a midazolam dose of 0.2 mg/kg BWt was successful overall in reducing the physiological parameters in the animals during the typical fight and flight response of blesbok. A midazolam dose of 0.4 mg/kg BWt caused a decrease in the heart rate of the animals per behaviour overall, including during behaviour commonly associated with stress, i.e. vigilance, avoidance and trotting in alarm. This dose generally caused an elevation in respiration rate per behaviour of the animals. A midazolam dose of 0.6 mg/kg BWt increased heart rate of the animals only when showing avoidance behaviour and caused an overall increase in respiration rate per behaviour. The increase in respiration rate observed in blesbok following treatment of the animals with both the 0.4 mg midazolam/kg BWt and 0.6 mg midazolam/kg BWt doses was contradictory to that previously reported for benzodiazepines, and may be the result of stress caused by some animals falling due to severe ataxia after treatment with the 0.6 mg/kg midazolam specifically. Paradoxical effects have been found previously after the use of benzodiazepines, but is not common. The animals were also stimulated at set time points after treatment and their cardiopulmonary responses during stimulations measured. Results showed that midazolam had no effect on heart rate, regardless of dose. The 0.2 mg midazolam/kg BWt dose had no effect on respiration rate, but both the 0.4 mg midazolam/kg BWt and 0.6 mg midazolam/kg BWt doses caused an increase in respiration rate during stimulations. This increase in respiration rate may be the result stress due to the animals not having been sufficiently adapted to the experimental conditions and midazolam treatment, because they received the highest midazolam dosage first after the placebo trial and because of the severe ataxia experienced by some animals, specifically when they received the high dose. Midazolam caused a moderate level of sedation in

blesbok, which is acceptable as it calmed the animals sufficiently to reduce vigilant behaviour and heart rate after treatment with doses of 0.2 mg midazolam/kg BWt and 0.4 mg midazolam/kg BWt.

In conclusion, a midazolam dosage of 0.6 mg/kg and higher is not recommended as a sedative in blesbok to prevent the occurrence of side effects such as extrapyramidal symptoms and severe ataxia. When a more pronounced effect of midazolam is required, it can be considered to use the midazolam in an adjuvant capacity in immobilisation protocols. Future studies should thus investigate the potential of midazolam to be used as adjuvant as part of immobilisation protocols, in combination with anaesthetics. The potential of midazolam to be used as sedative in other wildlife species also warrants further investigation.

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Appendix A

Date of weighing				
Date of midazolam administration				
Animal number	**Weight(kg)**	**Description**	**Midazolam dose in mg**	**Midazolam dose in mL**
1				
2				
3				
4				
5				
6				

Time	**Goat # 1**	**Goat # 2**	**Goat # 3**	**Goat # 4**	**Goat # 5**	**Goat # 6**
Sample 0						
Time midazolam given						
Sample 30						
Sample 60 (1 hr)						
Sample 120 (2 hrs)						
Sample 240 (4 hrs)						
Sample 360 (6 hrs)						
Sample 720 (12 hrs)						
Sample 1440 (24 hrs)						

Appendix B

Date				**Amount of feed fed initially (kg)**			
Test substance				**Amount of feed remaining after 24 hr (kg)**			
Drug concentration (mg/mL)				**Difference (kg)**			
Animal #	**Description**	**Weight (kg)**	**SEM #**	**Time of drug administration**	**Dose (mg/kg)**	**Dose in mg**	**Dose in mL**
1							
2							
3							
4							
5							
6							