Determining whether blood colour can be used to assess arterial blood oxygenation in immobilised impala (*Aepyceros melampus*).

by

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Table of contents

List of Tables

List of Figures

List of abbreviations

λ	-	Wavelength sign (lambda)
a*	-	Green to red colour spectrum
b*	-	Blue to yellow colour spectrum
CIE	-	International Commission on Illumination
cO_2Hb	-	Oxyhaemoglobin concentration
O_2Hb	-	Oxyhaemoglobin
cHHb	-	Deoxyhaemoglobin concentration
COHb	-	Carboxyhaemoglobin
ctHb	-	Total haemoglobin concentration measured by co-oximetry
$cSaO_2$	-	Functional arterial oxyhaemoglobin saturation calculated from the PaO_2 measured by the blood gas analyser
DOP	-	Delta-opioid receptor
FO_2Hb	-	Fractional oxyhaemoglobin saturation measured by co-oximetry
HHb	-	Deoxyhaemoglobin
KOP	-	Kappa-opioid receptor
L*	-	Luminosity or lightness of a colour
MetHb	-	Methaemoglobin
MOP	-	Mu-opioid receptor
ODC	-	Oxygen-haemoglobin dissociation curve
$PaCO_2$	-	Partial pressure of arterial carbon dioxide

PaO_2	-	Partial pressure of arterial oxygen
PO_2	-	Partial pressure of oxygen
SO_2	-	Functional oxyhaemoglobin saturation
SaO_2	-	Functional arterial oxyhaemoglobin saturation measured by co-oximetry
SD	-	Standard deviation
SHb	-	Sulfhaemoglobin
SpO_2	-	Functional peripheral oxyhaemoglobin saturation measured by pulse oximetry

Abstract

Hypoxaemia (oxyhaemoglobin saturation < 90%) often occurs during wildlife immobilisation and poses a risk of morbidity and mortality. Several methods have been used to assess blood oxygenation in immobilised impala (*Aepyceros melampus*). Pulse oximetry has been shown to be unreliable, co-oximetry and blood gas analysis are the gold standard but are limited by practicality and cost. With the advent of digital cameras and spectrocolourimeters the assessment of blood colour could be of value for determining blood oxygenation. This study set out to determine whether there is good association between arterial blood colour, as assessed by CIE L*a*b* (Commission on international illumination; L*: luminosity; a*: green to red; b*: blue to yellow) colour components, and blood oxygenation, as determined by functional oxyhaemoglobin saturation (SaO_2) and fractional oxyhaemoglobin saturation (FO_2Hb). To obtain arterial blood samples with different blood oxygen levels 11 impala were immobilised with either etorphine or thiafentanil. Arterial blood samples were collected from the auricular artery at five-minute intervals and immediately analysed by means of co-oximetry to measure blood oxygenation, and spectrocolourimetry to measure the CIE L*a*b* colour components. The colour components associated better with blood oxygenation (SaO_2 and FO_2Hb) using a quadratic rather than a linear model ($p < 0.001$). The association was strong for each of the colour components (CIE L*a*b*). Therefore both SaO_2 and FO_2Hb are reliable predictors of all three CIE L*a*b* components of arterial blood colour, and hence blood colour can be used to reliably estimate arterial blood oxygenation of impala. These findings could pave the way for developing colour charts and devices that can be used in the field to inexpensively

determine blood oxygenation, and detect hypoxaemia, in immobilised or anaesthetised animals.

Keywords: immobilisation, impala, blood colour, CIE L*a*b*, oxyhaemoglobin saturation.

Chapter 1

Introduction

Hypoxaemia is regularly encountered during chemical capture (immobilisation) of wildlife (van Zijll Langhout et al. 2016). Potent opioids such as etorphine and thiafentanil are the drugs of choice for the chemical immobilisation of impala (Zeiler & Meyer 2017b). Although they are very effective for this purpose they cause respiratory compromise which often results in severe hypoxaemia (Meyer et al. 2015; Zeiler & Meyer 2017a). Hypoxaemia results in tissue hypoxia and lactic acidosis and can lead to tissue damage, organ failure and finally in death (Read 2003). Therefore the detection of hypoxaemia is of utmost importance. Clinical signs of hypoxaemia are not always a reliable indicator of the degree of hypoxaemia in animals, nor are they specific as they include tachycardia or bradycardia, tachypnoea, dyspnoea and cyanosis.

The most regularly used methods for quantifying arterial blood oxygenation are blood gas analysis, co-oximetry and pulse oximetry. Blood gas analysis is the gold standard for determining partial pressure of oxygen (PO_2), whereas co-oximetry is the gold standard for determining oxyhaemoglobin saturation (SO_2 & FO_2Hb). However, co-oximetry and blood gas analysis are costly with regards to procuring the analysers and analysing individual samples. Furthermore, they are light and temperature-sensitive, respectively, and only give point estimates making them less attractive in a field immobilisation situation. Pulse oximetry, on the other hand, is a useful patient-side

modality for the continual monitoring of arterial blood oxygenation. However, pulse oximetry has been deemed mostly unreliable in impala (Mtetwa 2019).

Therefore, the challenge remains on how to accurately and effectively detect and monitor hypoxaemia during the chemical immobilisation of wild ungulates such as impala. Cyanosis (bluish tinged mucous membranes) is a specific clinical sign indicating possible underlying hypoxaemia. The bluish colour is the result of the increased deoxyhaemoglobin (HHb) fraction within the tissue's blood vessels. However, in some species, especially those with dark mucous membranes, it is often difficult to detect, and it is also a biomarker that only becomes apparent later as the degree of hypoxaemia progresses.

Even when using modalities such as pulse oximetry, veterinarians still utilise mucous membrane colour to check whether capillary blood oxygenation corresponds with the pulse oximetry values. Furthermore, the visual assessment of the colour of arterial blood, either drawn into a syringe or from an intra-arterial catheter, is frequently used by wildlife veterinarians as a rough indicator of the animal's oxygenation status. Therefore, the question of whether colour can be used to accurately determine the oxygenation of a patient, more specifically arterial blood oxygenation, still needs to be answered. Impala are prone to developing hypoxaemia during chemical immobilisation with potent opioids (Meyer et al. 2010, Buck et al. 2017) and therefore were chosen as the model species for this study.

The aim of this study is to determine whether there is an association between arterial blood colour and oxyhaemoglobin saturation and if so, to determine if arterial blood colour can be used to successfully predict arterial blood oxygenation to develop a colourimetric scale for use in the field. Thereby forming the platform for developing a new field-ready and inexpensive way of determining arterial blood oxygenation in wildlife, such as impala.

Chapter 2

Literature Review

There is a substantial expansion of the wildlife industry and thus an increased need for human intervention in medical, research and translocation procedures. Most of these procedures encompass chemical immobilisation of wild animals (Allen 2017; Zeiler & Meyer 2017b). Clinical monitoring is a vital component when animals are immobilised so that complications can be detected and treated early. Hyperthermia, acidosis and capture myopathy sometimes result from the chase that occurs in the capture process (Zeiler & Meyer 2017a). However, the primary complication that occurs during chemical immobilisation of wildlife is hypoxaemia (Read 2003; Portas 2004; Mich et al. 2008). Although often unavoidable, hypoxaemia can result in death, especially when encountered together with other complications like hyperthermia, hypercapnia, acidosis or capture myopathy (Read 2003; Mich et al. 2008). Therefore, there is a considerable need for an inexpensive, field-ready and accurate modality to determine arterial blood oxygenation in chemically immobilised wildlife.

2.1 Hypoxaemia

Hypoxaemia is defined as subnormal arterial blood oxygenation (Hall JE 2016). Oxygen in blood is transported in two states, 97% of the total oxygen is bound to haemoglobin (referred to as oxyhaemoglobin) and about 3% of the total oxygen is dissolved in the plasma (which is directly proportional to the partial pressure of oxygen) and cytoplasm of blood cells (Hall JE 2016). The relationship between haemoglobin bound and dissolved oxygen can be explained by the oxygen-

haemoglobin dissociation curve (ODC) and this curve differs among species (Clerbaux et al. 1993). The normal partial pressure of arterial oxygen (PaO_2) is around 100 mmHg when breathing room air (fractional inspiration of oxygen = 0.21) at sea level (Dugdale 2010). A range of threshold levels of PaO_2 have been stated to define hypoxaemia across a range of species; a PaO_2 of < 60mmHg (Koenig et al. 2003) in horses, PaO_2 < 70 mmHg in rhinoceros (van Zijll Langhout et al. 2016) and PaO_2 < 60 mmHg in impala (Buck et al. 2017) However, further research is required to properly qualify which values represent the threshold for clinically relevant hypoxaemia in different species. As PaO_2 decreases below 60 mmHg haemoglobin desaturation accelerates (Koenig et al. 2003).Hypoxaemia can also be defined by means of oxyhaemoglobin saturation and it might be more correct to do so as it represents the larger oxygen fraction (97%), provided the animal is not anaemic. The World Health Organisation currently defines hypoxaemia as a SaO_2 < 90% (Mwaniki et al. 2009). Furthermore, different species haemoglobin has different affinities for oxygen and hence different oxygen dissociation curves (Figure 2.1) and p50 values (partial pressure of oxygen where 50% oxyhaemoglobin saturation occurs). Haemoglobin of larger species seems to have a higher affinity for oxygen (i.e. lower p50) than that of smaller species (Figure 2.2, Clerbaux et al. 1993; Wenger et al. 2007). Furthermore, within an individual, a higher affinity for oxygen can also be brought about by changes in the structure of haemoglobin molecules that are exposed to hypoxic conditions for long periods, like those animals that live at high altitudes (Weber 2007). Therefore, to better account for differences in this affinity, other physiological shifts in the oxygen dissociation curve, and the intrinsic colour properties of haemoglobin, we believe that for this study it is better to define

hypoxaemia by means of oxyhaemoglobin saturation rather than partial pressure of oxygen, especially as a generalisation over a wide range of species.

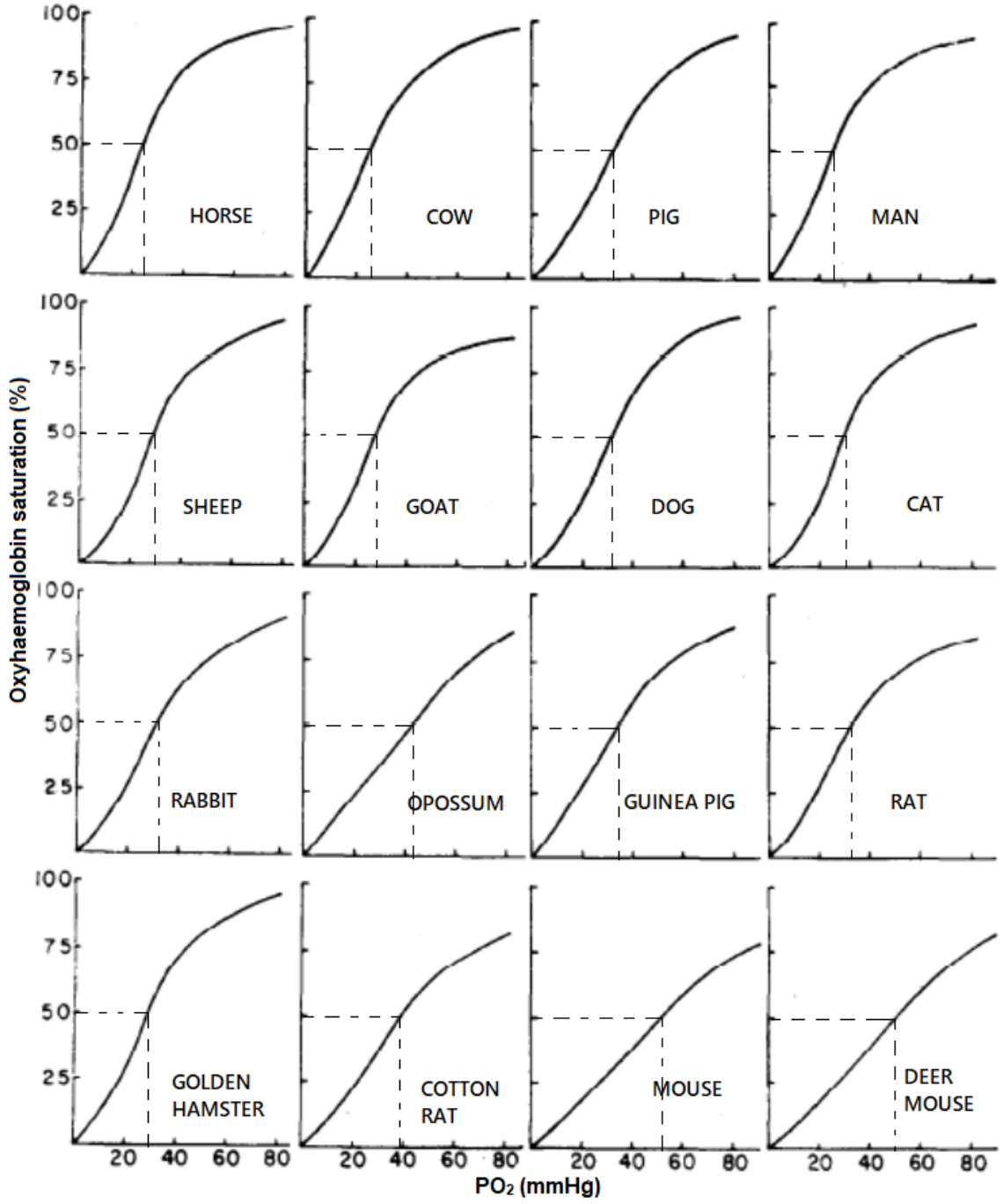


Figure 2.1 Oxyhaemoglobin saturation versus partial pressure of oxygen (PO_2) forming the oxygen-haemoglobin dissociation curve (ODC) of whole unbuffered blood from various mammals. Broken lines indicate the p50 - partial pressure of oxygen (PO_2) at 50% oxyhaemoglobin saturation (modified from Schmidt-Neilsen & Larimer 1958).

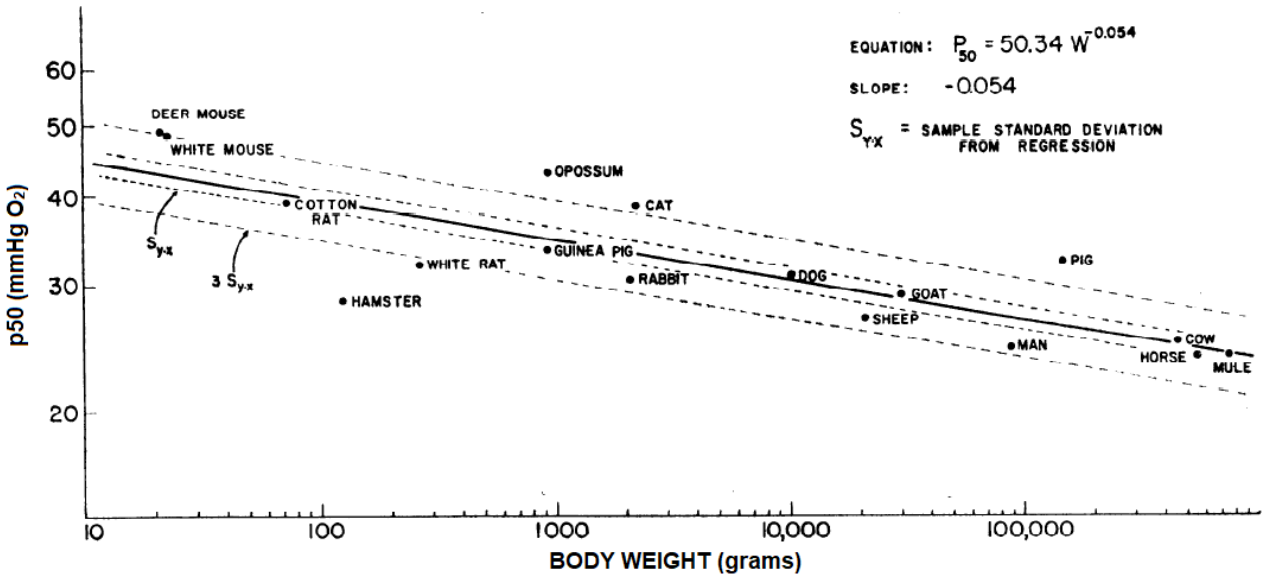


Figure 2.2 Partial pressure of oxygen at 50% oxyhaemoglobin saturation (p50) versus body weight from various mammals (modified from Schmidt-Neilsen & Larimer 1958)

2.2 Mechanisms of hypoxaemia

The mechanisms causing hypoxaemia include a low fraction of inspired oxygen, hypoventilation (respiratory muscle or central nervous system depression), ventilation to perfusion mismatch (positioning, shock etc.), shunts (right-to-left shunting), diffusion impairment (pulmonary oedema etc.), anaemia (Read 2003; Wenger et al. 2007). Most of these causes can be encountered when immobilising wildlife. The drugs used for chemical capture (immobilisation), especially the potent opioids, are the predominant cause of the resulting hypoxaemia in species such as rhinoceros (Fahlman et al. 2016; Buss et al. 2018), goat (Buss and Meltzer 2001; Meyer et al. 2015) and impala (Buck et al. 2017; Zeiler & Meyer 2017b).

During immobilisation with potent opioids, muscle tremors and rigidity is regularly observed in species such as rhinoceros (Buss et al. 2018) and impala (Buck et al. 2017). The muscle tremors increase in oxygen consumption (Buss et al. 2018) and hinder normal respiratory movements resulting in hypoventilation (van Zijll Langhout

et al. 2016). Like all other opioids, etorphine and thiafentanil depress respiratory rhythm at lower doses and tidal volume at higher doses (Pattinson 2008). Opioid receptors, MOP (mu - μ), KOP (kappa - κ) and DOP (delta - δ), are found in the respiratory centres within the brain and also in the central and peripheral chemoreceptors (Buss and Meltzer 2001; Pattinson 2008; Meyer et al. 2015). The main effect of potent opioids on the central respiratory centres is the suppression of neuronal firing in the pre-Bötzinger complex (Pattinson 2008) which is important for generating inspiratory signals. However, several studies (Zeiler et al. 2015; Buck et al. 2017) suggest that more mechanisms, other than just hypoventilation, contribute to the underlying hypoxaemia experienced during the immobilisation of impala. Buck et al. (2017) showed that during the anaesthetic maintenance of impala the mixed expired carbon dioxide tension remained constant whereas the arterial partial pressure of carbon dioxide increased throughout the anaesthetic period indicating either increased dead space ventilation or a right-to-left shunt occurred.

Etorphine has been shown to cause pulmonary hypertension in goats when injected intramuscularly at a dose of 0.1 mg kg^{-1} (Meyer et al. 2015). Meyer et al. (2015) speculated that the hypertension causes a hydrostatic fluid shift into the pulmonary interstitium resulting in a diffusion impairment thereby decreasing gas exchange in impala. Furthermore, the use of alpha-2 adrenergic agonists which are sometimes used in conjunction with the potent opioids are by themselves also known to precipitate alveolar and interstitial oedema in domestic and wild ruminants resulting in a diffusion impairment (Celly et al. 1999; Read 2003).

Incorrect positioning during recumbency also plays an important role in causing hypoxaemia by negatively influencing ventilation-perfusion ratios (Wenger et al. 2007; Caulkett et al. 1994). Immobilised animals should be corrected to sternal recumbency.

However, it is sometimes difficult to correctly position larger species, and thus ventilation perfusion mismatching may occur.

2.3 Treatment of hypoxaemia

The first line of treatment of hypoxaemia due to potent-opioid induced hypoventilation is increasing the fraction of inspired oxygen by means of supplemental oxygen. The most common supplemental method is intranasal oxygen insufflation from a flow-regulator fitted high-pressure oxygen cylinder (Fahlman 2014). Several studies suggest supplemental oxygen supply at a flow rate of 2 L minute^{-1} as a standard procedure during chemical immobilisation (Fahlman et al. 2014; Buck et al. 2017).

Furthermore, the administration of butorphanol partly antagonises etorphine and thiafentanil at the MOP, resulting in improved cardiopulmonary function (Buss et al. 2018). Butorphanol is partly a competitive MOP antagonist and KOP agonist (Wenger et al. 2007) The major effect of butorphanol in rhinoceros seems to be that it reduces metabolism and therefore oxygen consumption by reducing muscle tremors rather than improving ventilation (Buss et al. 2018). In impala immobilised with potent opioids, with apnoea, the administration of 1 mg of butorphanol to 1mg of potent opioid, given intravenously, results in an immediate increased respiratory rate and ventilation resulting in increased blood oxygen levels (Buck et al. 2017).

Respiratory stimulants such as doxapram, an analeptic, can also be used to increase minute ventilation. Doxapram improves minute ventilation within the first minute after administration, lowering the partial pressure of arterial carbon dioxide ($PaCO_2$), thereafter its effects decrease and disappear at about three minutes after administration (Meyer et al. 2010). The administration of doxapram intravenously at 1

mg kg^{-1} in impala only increased PaO_2 by 8-7 mmHg by three minutes after administration (Meyer et al. 2010). Serotonergic agonists have also been tested in immobilised animals, however, further research is required to determine their effectiveness in counteracting opioid-induced respiratory depression in wildlife (Meyer et al. 2010). Although there are a number of drug options that can be used to treat hypoxaemia their efficacy is not always certain and therefore it is not only important to detect hypoxaemia during immobilisation but also to monitor how it changes, especially when these treatments are given.

2.4 Detecting and quantifying hypoxaemia

Clinical signs of hypoxaemia include tachycardia or bradycardia, tachypnoea, dyspnoea and cyanosis (Fahlman 2014). Although these clinical signs are important indicators of a possible underlying hypoxaemia, they lack sensitivity. Fortunately, there are quantitative ways of determining arterial blood oxygenation. Modalities such as co-oximetry, pulse oximetry and arterial blood gas analysis have been the focus in detecting and quantifying hypoxaemia in both humans and animals (Table 2).

Table 2 Comparison of modalities commonly used in clinical practice to measure arterial blood oxygenation.

Device	Parameters	Advantages	Disadvantages
Blood gas analyser	PaO_2 – determined by electrical current changes measured by a Clark electrode. $cSaO_2$ - Calculated from standard ODC.	The accurate measure of PaO_2 Also measures other parameters such as haematocrit, pH, base excess, bicarbonate, PCO_2 and electrolytes. Oxygen content usually also calculated	Expensive, invasive and temperature-sensitive,
Co-oximeter	SaO_2, FO_2Hb – determined by the absorption of multiple wavelengths of light.	The accurate measure of SaO_2, especially during dyshaemoglobinaemia. Also reports HHb, COHb, MetHb, SHb and ctHb.	Expensive, invasive and light-sensitive.
Pulse oximeter	SpO_2 – determined by absorption at two wavelengths of light (660 nm and 940 nm).	Enables ontinuous patient-side monitoring. Non-invasive. Field ready.	Inaccurate at measuring oxyhaemoglobin saturation levels below 90% in impala and other wildlife species.

2.4.1 Blood gas analysis

As mentioned earlier, the oxygen in blood is either bound (98%) to haemoglobin or dissolved (2%) in the plasma. Blood gas analysis generally measures the smaller fraction that is dissolved within the plasma in either arterial or venous blood; referred to as partial pressure of oxygen (PO_2). Blood gas analysers mostly consist of electrodes that use changes in current at a Clark electrode to determine PO_2 and voltage changes at high-impedance electrodes to determine pH and partial pressure of carbon dioxide (PCO_2) (Haymond et al. 2005). Furthermore, some blood gas analysers also measure variables such as haematocrit, electrolytes (Na+, K+, Cl-) and metabolites (glucose, lactate, creatinine) and calculate variables such as haemoglobin concentration, bicarbonate, base excess and calculated functional arterial oxyhaemoglobin saturation ($cSaO_2$) (Gonzalez & Waddell 2016). Calculated functional arterial oxyhaemoglobin saturation ($cSaO_2$) is calculated from pH, PO_2, calculated haemoglobin and the standard ODC (Haymond et al. 2005; Gonzalez & Waddell 2016).

Two different types of analysers are available, benchtop and portable or point of care analysers. The most commonly used portable analysers are the EPOC (Heska/Cuattrro, Loveland, CO) and i-STAT (Abbott Laboratories, Abbott Park, IL). These analysers themselves are expensive and make use of a single-use self-calibrating cartridge to analyse each sample, making analysis expensive, especially if several samples require analysis throughout an immobilisation to monitor changes in arterial blood oxygenation (Gonzalez & Waddell 2016). Another downfall of these analysers is that they are temperature-sensitive, making them unreliable at times of extreme environmental temperatures. All of these attributes make the use of a blood gas analysis to monitor arterial blood oxygenation during routine chemical

immobilisation impractical. It is also important to keep in mind that different species have different oxy-haemoglobin dissociation curves (Clerbaux et al. 1993); therefore, quantifying hypoxaemia from calculated oxyhaemoglobin saturation, using a standard curve for many species, may be questionable without further research. Regardless, blood gas analysis remains the gold standard for determining partial pressure of oxygen (PO_2).

2.4.2 Co-oximetry

Co-oximetry is the gold standard for determining oxyhaemoglobin saturation which comprises the major fraction (98%) of arterial blood oxygen. Co-oximetry utilises spectrophotometry to measure different haemoglobin derivatives. Co-oximetry works on the principle of wavelength absorption. The first co-oximeters used the absorption of light at four different wavelengths and were further developed to use six different wavelengths to measure the different haemoglobin derivatives, namely: oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), carboxyhaemoglobin (COHb), methaemoglobin (MetHb) and sulfhaemoglobin (SHb) (Haymond et al. 2005). Modern co-oximeters are more accurate due to the fact that they measure absorbance at many different wavelengths (between 100-128 wavelengths) (Haymond et al. 2005). Absorbance is measured by transmitting a certain wavelength of light through the sample and measuring the amount of light on the other side (Haymond et al. 2005). Co-oximeters are able to measure two different oxyhaemoglobin entities used to define oxygenation, 1) functional saturation, and 2) fractional saturation. The functional saturation is the amount of oxygenated haemoglobin as compared to the total amount of haemoglobin that is able to transport oxygen (Haymond et al. 2005). Functional

saturation of arterial blood can be expressed by means of oxyhaemoglobin concentration (cO_2Hb) and deoxyhaemoglobin concentration (cHHb) as follows:

$$SaO_2 = \frac{cO2Hb}{cO2Hb+cHHb}$$

The second entity is referred to as fractional saturation (FO_2Hb) and can only be measured by co-oximetry. Fractional saturation is defined as the amount of oxyhaemoglobin as compared to the total haemoglobin (including the non-oxygen binding haemoglobin derivatives) present (Haymond et al. 2005) and can be expressed as follows:

$$FO_2Hb = \frac{cO2Hb}{ctHb}$$

Functional and fractional saturations are therefore two different measurements. In the case of dyshaemoglobinemias, such as methaemoglobinaemia or carboxyhaemoglobinaemia, fractional saturation will be much lower than functional saturation. Therefore, functional saturation should be interpreted with caution in instances of possible dyshaemoglobinaemia. Furthermore, we know that the absorption spectra of canine haemoglobin derivatives are similar to that of man (Zijlstra & Buursma 1987), however, some of the haemoglobin derivatives of bovine (Zijlstra & Buursma 1997) and rat (Zijlstra et al. 1994) seem to differ from human haemoglobin absorption spectra. Therefore, the absorption spectra of the different haemoglobin derivatives will have to be investigated in species of interest to validate the use of co-oximetry.

The fact that co-oximetry utilises light wavelengths to quantify haemoglobin derivatives, makes the analyser very sensitive to sunlight. Co-oximeters are also costly

and therefore it is not the most ideal field ready patient-side modality for the detection and monitoring of hypoxaemia.

2.4.3 Pulse oximetry

Pulse oximeters are spectrophotometers that measure absorption at two light wavelengths (660 nm and 940 nm) through, or from, a well-vascularised area of the body, using a probe containing light-emitting diodes (Haymond et al. 2005; Chan et al. 2013). Haemoglobin that is oxygenated will allow most of the red light to pass through and absorb the infrared light (IR, 940 nm); whereas deoxygenated blood will absorb more red light (red, 660 nm). The photodetector on the probe of the pulse oximeter detects the unabsorbed light as either non-pulsatile direct current (DC) indicating venous and tissue origin or pulsatile alternating current (AC) indicating arterial origin (Chan et al. 2013). The absorbance ratio (R) is determined as follows

$$R = \frac{(Ared,AC/Ared,DC)}{(AIR,AC/AIR,DC)}$$

The pulse oximeter then uses a calibration curve to convert this ratio to peripheral functional saturation (SpO_2) (Chan et al. 2013). Pulse oximetry unfortunately also has limitations (Sinex 1999; Chan et al. 2013). Pulse oximetry has been shown to be inaccurate in immobilised wildlife especially at the lower (SaO_2 <90%, Mtetwa 2019) and higher (when supplemental oxygen was given) SaO_2 values where it over and underestimated SpO_2 values respectively (Fahlman et al. 2012).

In horses, there is a general trend of pulse oximeters to underestimate SpO_2 at values greater than 90% (Koenig et al. 2003). Furthermore, at SaO_2 < 90%, SpO_2 can either give an under- or overestimate of SaO_2. Similar results indicated that pulse oximetry

is inaccurate in detecting hypoxaemia in species like deer (Mich et al. 2008), dama gazelle (Schumacher et al. 1997), white rhinoceros (Haymerle et al. 2016) and brown bears (Fahlman et al. 2010). The reason for this unreliability is that the calibration ODC that is used in most pulse oximeters is derived from haemoglobin of healthy human individuals exposed to different PO_2 levels. Furthermore, human pulse oximeters are only calibrated to be accurate within oxygen saturation levels between 70% and 100% (Chan et al. 2013). Therefore, making measured SpO_2 mostly inaccurate in scenarios where severe hypoxaemia (SaO_2 < 70%) occurs. Furthermore, the use of drugs such as alpha-2 adrenergic agonists during chemical immobilisation makes reliable readings difficult to obtain with pulse oximetry because of drug-induced peripheral vasoconstriction and arrhythmias (Fahlman et al. 2012). For these reasons, pulse oximetry has been used with caution in wild ruminants. A recent study compared pulse oximeter readings at different attachment sites (tail, ear, rectum and cheek) and compared it to both PaO_2 and SaO_2. The purpose of the study was to validate the use of pulse oximetry in impala, and the findings deemed pulse oximetry to be mostly inaccurate (at SaO_2 < 90%, Mtetwa 2019).

2.5 Cyanosis and the colour of blood

The colour of arterial blood and mucous membranes is commonly used as a gross indicator of oxygenation status to detect hypoxaemia in patients (Mich et al. 2008; Allen 2017). However, the colour of blood seems to be more sensitive than that of mucous membranes in detecting hypoxaemia, as the colour is not hidden by the overlying tissue (Morgan-hughes & Bartlett, 1968). In immobilised deer, the use of arterial blood colour was successful as a crude indicator of oxygenation status (Mich et al. 2008). Morgan-hughes and Bartlett (1968) indicated that the visually observed colour of arterial blood in a syringe enabled the detection of hypoxaemia in 100% of patients with SaO_2 < 85% and in 75% of patients with SaO_2 of 85-94%.

Blood colour is very complex because of the large number of components influencing its scattering of light (Naumenko 1996). Thrombocytes and leukocytes only account for about 1% of the composition of blood and their influence on the absorption and scattering of light is negligible (Yim et al. 2012). Therefore, erythrocytes, being the dominant cell type, play a major role in the absorption spectra of blood (Naumenko 1996). Although the size, concentration and orientation of erythrocytes influence the reflection, absorption or refraction of light (Yim et al. 2012); the absorption or reflectance of light by erythrocytes can mostly be attributed to the various haemoglobin derivatives (Naumenko 1996).

Haematocrit also plays an important role in determining the hue saturation and brings about two phenomena, the Sieve and the Detour effects. The Sieve effect is the result of a very low haematocrit and results in decreased absorption of light by haemoglobin (Yim et al. 2012). The Detour effect is the result of a very high haematocrit and results

in more light being absorbed by intracellular haemoglobin (Yim et al. 2012). In human blood, investigators noted that while determining whether the colour of blood in a syringe can be used to detect hypoxaemia; a small number of anaemic samples, with functional oxyhaemoglobin saturations of more than 94%, were classified as desaturated (Morgan-hughes & Bartlett 1968). However, the effects of haemoglobin concentration and oxygen saturation on arterial blood colour in different wildlife species remains to be elucidated.

Immobilised free-living animals are prone to developing hypercapnia, acidosis and hyperthermia. Increases in $PaCO_2$, H^+ or body temperature results in increased oxygen offloading - a right shift in the ODC (Baumann et al. 1984; Collins 2015). Ruminants are also known to be chloride shifters, as their haemoglobin's affinity for oxygen decreases as Cl^- concentrations increase (Eike & Palmer 2004). In larger species, particularly rhinoceros, their p50 is lower (15 mmHg at pH 7.4) (Reiners et al. 2019) as compared to humans (26.3 mmHg at pH 7.4) (Kim et al. 1990). All these factors affect the configuration of the ODC and therefore the relationship between SaO_2 and PaO_2. Therefore, differences in SaO_2 within and between species at the same PaO_2 are possible. However, considering blood colour is primarily influenced by the amount of oxygen bound to haemoglobin, it is unlikely that these factors (i.e. $PaCO_2$, H^+, Cl^-, body temperature or p50) will change blood colour independently of their effects on oxygen haemoglobin binding. Therefore, these factors are unlikely confounding variables that need to be considered when assessing the relationship between colour and blood oxygenation.

2.6 Aspects of colour

Perceiving a colour is complicated and it depends on both the composition of the object, the light source and the observer (Melendez-Martınez et al. 2005). When looking at a blood sample the colour reported by an observer will be highly influenced by the observer's colour perception and how well the sample is illuminated. However, when a blood sample is compared to a colour chart, illumination and observer perception should be similar for both objects (Zuckerman et al. 1995; Lee et al. 2013; Park et al. 2015), thus possibly making the colour assessment of the blood sample more accurate. The colour that is perceived can be measured or described by three components or characteristics that contribute to the three-dimensionality of a colour (Melendez-Martınez et al. 2005). These components are hue, luminosity and saturation (colourfulness) (Melendez-Martınez et al. 2005). Hue is also referred to as the qualitative measure of chromaticity (quality of colour). Hue can be defined as the actual colour, for example red, yellow, blue or green and is determined by the light absorbed at a specific wavelength. Luminosity can be ascribed to the amount of black or white that is mixed into a colour or hue, for instance where the colour will be equivalent to a shade within the greyscale (0-100). Saturation (colourfulness) is referred to as the quantitative measure of chromaticity. Saturation (colourfulness) can be defined as the purity of a colour, the amount of light that is reflected at a specific wavelength; 100% saturation (colourfulness) indicates the purest form of a colour whereas 0% has a significant amount of grey in the colour (Melendez-Martınez et al. 2005).

2.6.1 Perceiving and measuring colour

Perceiving colour has intrigued scientists for years and as radiometry (the science of measuring wavelengths in the electromagnetic spectrum) developed it revealed that colours are produced by specific light wavelengths. However, scientists soon realised that colour is more complex than just a specified wavelength (Hasting & Rubin 2012). From there two colour theories developed, the trichromatic theory and the Herring's opponent pair's theory. The trichromatic theory is based on the mixing of colours to form a specific colour; whereas the Herring's opponent theory uses three functions namely luminosity (black to white) and two functions of chromaticity (red to green and yellow to blue) (Hasting & Rubin 2012). These theories influenced the development of ways to standardise, measure and describe colours, which overall are known as colour spaces. These colour spaces fall in one of two groups, namely; geometric spaces that are mathematically based or psychological spaces that are based on a collection of colours (Hasting & Rubin 2012). The focus of the current study was the mathematical measurement of colour and comparing that to oxygen-haemoglobin saturation values. Geometric colour spaces enable us to specify a colour by means of its co-ordinates and include the many colour spaces such as RGB (Red Green Blue), CMYK (Cyan Magenta Yellow and Black), HSL (Hue Saturation Lightness), YIQ (luminosity, orange – blue and purple - green), YUV (luminosity, blue and red), CIE Luv (luminosity, green – red, blue and purple) and CIE L*a*b* colour spaces. The most commonly used colour spaces are RGB, CMYK and CIE L*a*b*. RGB is an additive colour space and is based on the addition of red, green or blue to create the perceived colour for sources that emit light such as televisions (Hasting & Rubin 2012). CMYK is a subtractive colour space that was developed from RGB. The CMYK colour scale was created to produce a perceived colour form cyan, magenta, yellow and black on a reflective source such

as printed images (Hasting & Rubin 2012). The CIE L*a*b* colour space consists of a measure of luminosity and two measures of chromaticity and is believed to be one of the most accurate spaces in describing a colour (Hasting & Rubin 2012). Although CIE L*a*b* doesn't have a direct measure of saturation (colourfulness), a measure of saturation (colourfulness) seems to be included in the model (Schiller et al. 2018). Therefore it includes all three aspects of colour (hue, luminosity and saturation) in its three-dimensional co-ordinate system.

2.6.2 Colour measuring apparatus

Colour scales are probably the most practical way of determining the colour of an object, as they can be used to make a comparative visual assessment. Visual observation on its own is subjective and observer dependent. However, multiple investigators have shown that the incorporation of a colour scale increases observer objectivity (Zuckerman et al. 1995; Lee et al. 2013; Park et al. 2015). Psychological colour spaces, as mentioned earlier, consist of a collection of colours, and reference examples of these scales are the OCA-UCS system, the Farnsworth-Munsell system and the DIN system (Melendez-Martınez et al 2005). Over time, colour scales have proven to be effective analytical tools in several aspects of medical and veterinary sciences. Colour scales like the FAMACHA colour card has had a major impact on the small stock production industry. It gives farmers a practical, affordable and efficient means of assessing anaemia from mucous membrane colour in individual animals (Van Wyk & Bath 2002; Reynecke et al. 2011). Similarly, the World Health Organisation (WHO) developed a blood colour chart that aids as a patient-side test in assessing the degree of anaemia from blood in humans (Marn & Critchley 2016). A review of 14 studies using the WHO Haemoglobin colour scale revealed an overall

sensitivity and a specificity of 80% to diagnose anaemia by means of this scale (Marn & Critchley 2016).

There are several types of instruments that can also be used to measure colour and these devices can be divided into three groups, namely; spectroradiometers, colourimeters or spectrophotometers (Zwinkels 1996), of which spectrophotometers and colourimeters are the most widely used (Chu et al. 2010). Colourimeters directly measure tristimulus values (Red, Green, and Blue) to determine the colour, whereas spectrophotometers measure spectral reflectance (Zwinkels 1996; Chu et al. 2010). Spectrophotometers include instruments such as spectrocolourimeters and co-oximeters. Spectrocolourimeters are the most accurate instruments used to measure colour, as they do so by measuring within the visible light spectrum (400 nm to 700 nm) the amount of light reflected at regular wavelength intervals (1-25 nm) (Chu et al. 2010). With the use of spectrocolourimeters, it is important to specify the conditions under which the colour was observed (illuminant and observer view) (Zwinkels 1996). The most important condition is the illumination and is usually lit to a standardised level (called D65) which refers to the CIE standard daylight illuminant (Zwinkels 1996) which relates to a colour temperature of 6504 Kelvin. The observer field of view (angle of observation) should also be specified and can be classified as either 2^0 for small or 10^0 for large areas of colour.

Currently, there are several different models of these devices that are widely used, especially in the food industry. The LC100 (Lc100, Lovibond, Lasec, South Africa), is an example of a spectrocolourimeter that is portable, robust and designed for use on fluids, and therefore suited to quantify the colour of blood in the field. This handheld device also captures and stores images of samples for later assessment and was used in the current study.

Chapter 3

Materials and Methods

3.1 Animals and housing

Eleven healthy, adult female impala (*Aepyceros melampus*) were used in the study. The impala (34.1 ± 5.2 kg - mean ± SD body weight) were caught from the wild and transported to a purpose-built outdoor enclosure (boma complex) consisting of six pens (pen size: 6 x 8 meters) and allowed to acclimatise for eight weeks prior to the first data collection. The boma complex was situated on the Ngonigoni farm, Nelspruit, South Africa (25º31'25.2"S, 31º06'50.8"E) at an altitude of 812 m above sea level and an average barometric pressure of 699 mmHg. The impala were fed lucerne (*Medicago sativa*) and teff (*Eragrostis tef*) hay daily and received water *ad libitum* throughout the study.

3.2 Study design

Data was collected opportunistically during two other studies which aimed to investigate

- Whether a novel serotonergic drug could prevent potent opioid-induced respiratory compromise when combined with etorphine.
- How the immobilisation and physiological effects of etorphine and thiafentanil differ.

3.2.1 Sample size

Originally sixteen adult female impala (14 for data collection and 2 replacement animals) were used in the studies. However, due to difficulties with sample collection and equipment errors, we only included the 11 impala and trials with complete data sets. The studies which we opportunistically collected data from had sample sizes that were set for these studies. In order to make our study viable, we required as many samples over as wide a range of oxyhaemoglobin saturations as possible and applied modelling to account for repeated measures and pseudo-replication.

3.2.2 Randomisation and design

The original studies used prospective randomised crossover study designs. Each impala was immobilised on six occasions that were separated by a 14-day washout period. For this study, this design was not necessarily required as the samples were collected opportunistically.

3.3 Experimental procedures

During each trial, impala were immobilised, according to their previous weight, with 0.09 mg kg^{-1} etorphine HCl (Captivon 9.8 mg ml^{-1}; Wildlife Pharmaceuticals (Pty) Ltd, South Africa) or 0.09 mg kg^{-1} thiafentanil oxalate (Thianil 10 mg ml^{-1}; Wildlife Pharmaceuticals (Pty) Ltd, South Africa). Immobilisation drugs were administered via a 1.5 ml Pneudart type P dart with a 14 G ¾ inch barbed needle (Wildlife Pharmaceuticals (Pty) Ltd., South Africa) propelled by a gas-powered dart gun (X-Caliber, Wildlife Pharmaceuticals (Pty) Ltd., South Africa) from a distance that

ranged between 5 and 12 meters. All darts were placed in muscles of the pelvic girdle.

Once the impala became recumbent they were blind-folded and cotton wool was placed in the ear canals before they were moved to a close-by shaded data collection site where they were placed on a table in sternal recumbency. A catheter (Jelco 22 G; Smiths medical, Lancashire, United Kingdom) was placed aseptically and secured in the auricular artery. Clinical variables (heart rate, respiratory rate, temperature and peripheral oxyhaemoglobin saturation) were measured every 5 minutes of the immobilisation period.

3.3.1 Arterial blood analysis

Pre-heparinised 2ml blood gas syringes (BD A-Line™; Becton, Dickinson and Company) were used to collect arterial blood samples anaerobically at 5, 10, 15, 20 and 30 minutes after recumbency. The immobilising drugs caused varying degrees of hypoxaemia. Therefore, to ensure that some samples were taken during normoxaemic conditions some animals were insufflated with intranasal medical grade oxygen at 5 L min^{-1} from 40 minutes of immobilisation and additional samples were taken.

Each arterial blood sample was introduced immediately into the two separate machines, in quick succession (under 45 seconds), by different operators, in the following order after collection to measure the following variables:

- Spectrocolourimetry (Lc100, Lovibond, Lasec, South Africa) to determine CIE L*a*b* values at standard illumination (D65, 10°). CIE L*a*b* is a

colour space where the L* represents the luminosity or lightness of the colour and is ranged from 0 (black) – 100 (white). The a* describes the range from green to red and the b* describes the range from blue to yellow.

- Co-oximetry (Avoximeter 4000, Surgical Innovations (Pty) Ltd., Northriding, South Africa) to measure functional saturation (SaO_2), fractional saturation (FO_2Hb), HHb, COHb, MetHb, SHb and ctHb.

Blinding occurred as there were two different operators working independently on each machine. Randomisation between the machines may have been of value but samples were loaded in quick succession and therefore we don't believe that much difference would have occurred. These analyses were performed at ambient conditions below 25°C and under low light conditions (unmeasured but in a dimly illuminated room). The spectrocolourimeter light protective casing was used to block out ambient light during analysis. To prevent the influence of ambient air on the anaerobic blood sample, transparent adhesive tape (10mm) was used to seal the micro cuvettes (Cuvette Micro PS10X4X45mm 2ml, Lovibond, Lasec South Africa) which were filled with arterial blood and analysed immediately. The spectrocolourimeter was calibrated before the first sample and then at the 5 and 30-minute samples to ensure accuracy. The co-oximeter was calibrated daily, before the first data collection.

After the last sample, the arterial catheter and other monitoring devices were removed and the impala was moved back into the boma. Thereafter the cotton wool ear-plugs and blindfold were removed and the immobilising effects of the drugs were

antagonised with 20 mg or 10 mg of naltrexone intravenously (Trexonil 50 mg ml^{-1}; Wildlife Pharmaceuticals (Pty) Ltd) per mg etorphine or thiafentanil, respectively.

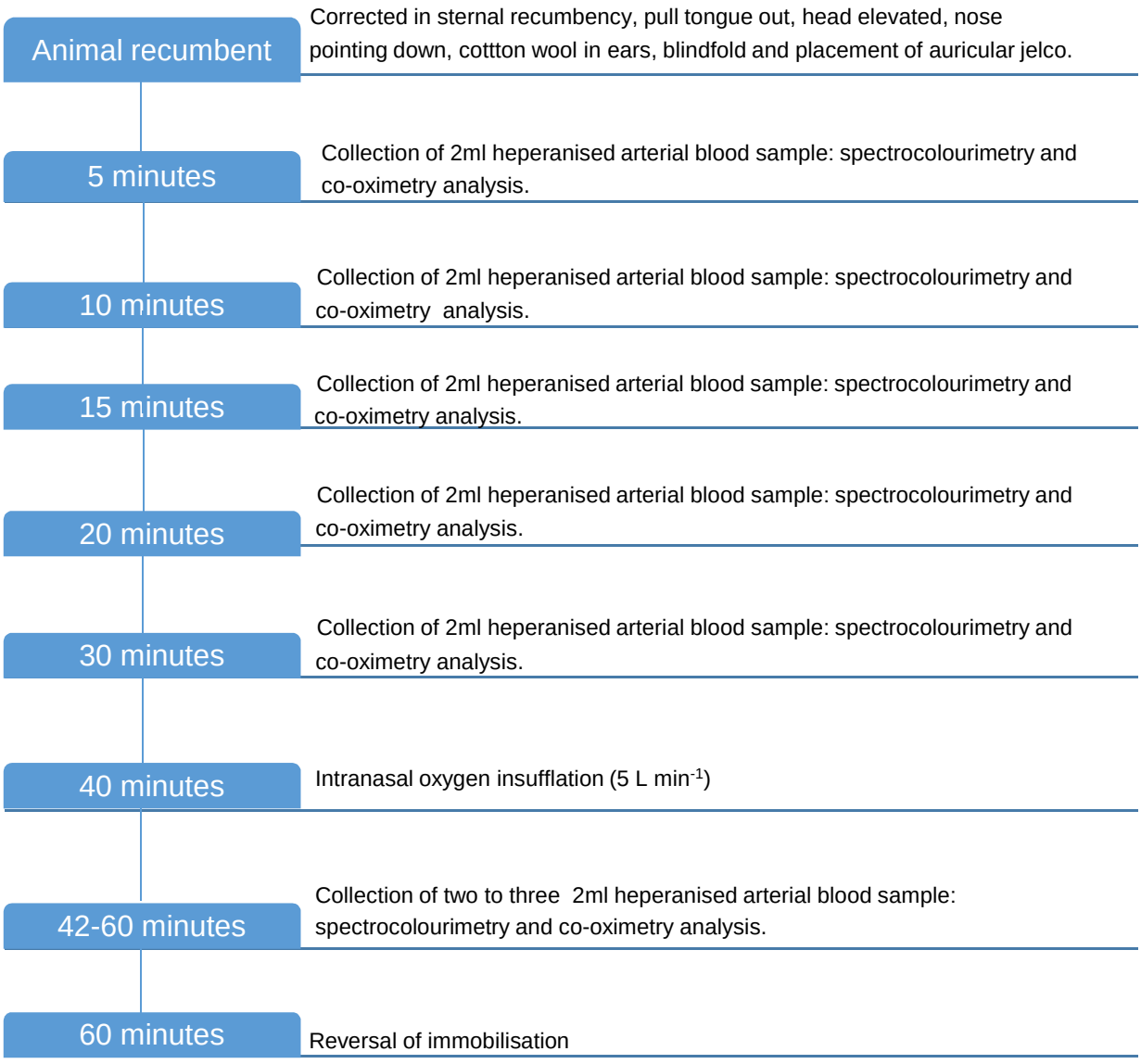


Figure 3 Time interval of experimental procedures indicating timing of arterial blood sample collection, analysis and oxygen supplementation.

3.4 Colour differentiation

This part of the study was a preliminary assessment to determine the value of developing a printed colour chart. We created 5 different arrangements of the colours representing different ranges of SaO_2. Twenty random untrained individuals were asked to arrange the colours for one of these arrangements from the darkest to the brightest on a tablet screen on 100% brightness at standard room lighting.

3.5 Ethical clearance

The study was approved by the Animal Ethics Committee of the University of Pretoria Approval number V035-17 (Addendum B).

3.6 Data analysis

The relationship between CIE L*a*b* values (L*, a* and b*) and functional saturation (SaO_2) and fractional saturation (FO_2Hb) were initially examined by means of exploratory plots, and then using linear modelling. In all cases, the individual CIE L*a*b* values were the dependent variables, and SaO_2 or FO_2Hb were the independent variables

Repeated samples were collected from individual animals and not all animals were used in every trial, therefore, a linear mixed model approach was used to account for these repeated measures, with animal ID used as the random effect, and SaO_2 or FO_2Hb as the fixed effect. Three regression models were created for each CIE L*a*b* component and SaO_2 or FO_2Hb. These models were a null model (intercept only), a first-order polynomial (a straight line), and a second-order polynomial (quadratic

curve). The goodness of fit of the first and second-order models were compared to the null model and to each other using likelihood ratio tests. The simplest model with the best fit was chosen for further analysis. Standard linear regression diagnostics were performed on this model to check for homoscedasticity and normality of fixed effect residuals, linearity of the relationship, and normality of the random effect intercept.

Initially, the confounding effects of haematocrit (Ht) and total haemoglobin concentration (ctHb) were included separately in these models, but had little influence, possibly because these values occurred in a narrow range, so these confounding variables were removed from the models.

The relationship between predicted CIE L*a*b* components and SaO_2 and FO_2Hb values were plotted (with 95% prediction intervals), and prediction values determined for the SaO_2 and FO_2Hb at the mid-point between each 10% range of these variables i.e. at the values 15, 25, 35, 45, 55, 65, 75, 85 and 95%. For the final part of the study, a modification of the Farnsworth-Munsell hue test was then analysed to validate if the colours at these mid-points were distinguishable enough from each other in order to predict the best associated oxyhaemoglobin saturation in each 10% range.

All data analyses were performed using R v3.5.2 (R Core Team, 2018) and $p < 0.05$ was determined significant.

Chapter 4

Results

4.1 Data

The quality and quantity of the data collected was sufficient for this study and no data was discarded unless there was no matching data. However more data in the 15 – 30% oxyhaemoglobin saturation range would have improved the results from the models. The final data set consisted of a total of 164 analysed samples from the 11 impala. Diagnostic plots show normal distribution of fixed effect residuals (addendum, Fig A), normality of random intercepts (addendum, Fig B) and homoscedasticity of fixed effect residuals (addendum, Fig C).

4.2 The relationship between CIE L*a*b* values and SaO_2 and FO_2Hb

Figure 4.1 and 4.2 show the linear and quadratic model relationships between fixed effects (SaO_2 and FO_2Hb) and the colour components (CIE L*a*b*). The chi-squared test showed that the linear (straight line) and quadratic (polynomial) models were a better fit than the null model and that the quadratic model was a better fit than the linear model for “luminosity” (L*, $p < 0.001$), “green to red” (a*; $p < 0.001$) and “blue to yellow” (b*; $p < 001$) values for both SaO_2 and FO_2Hb (Table 4.1).

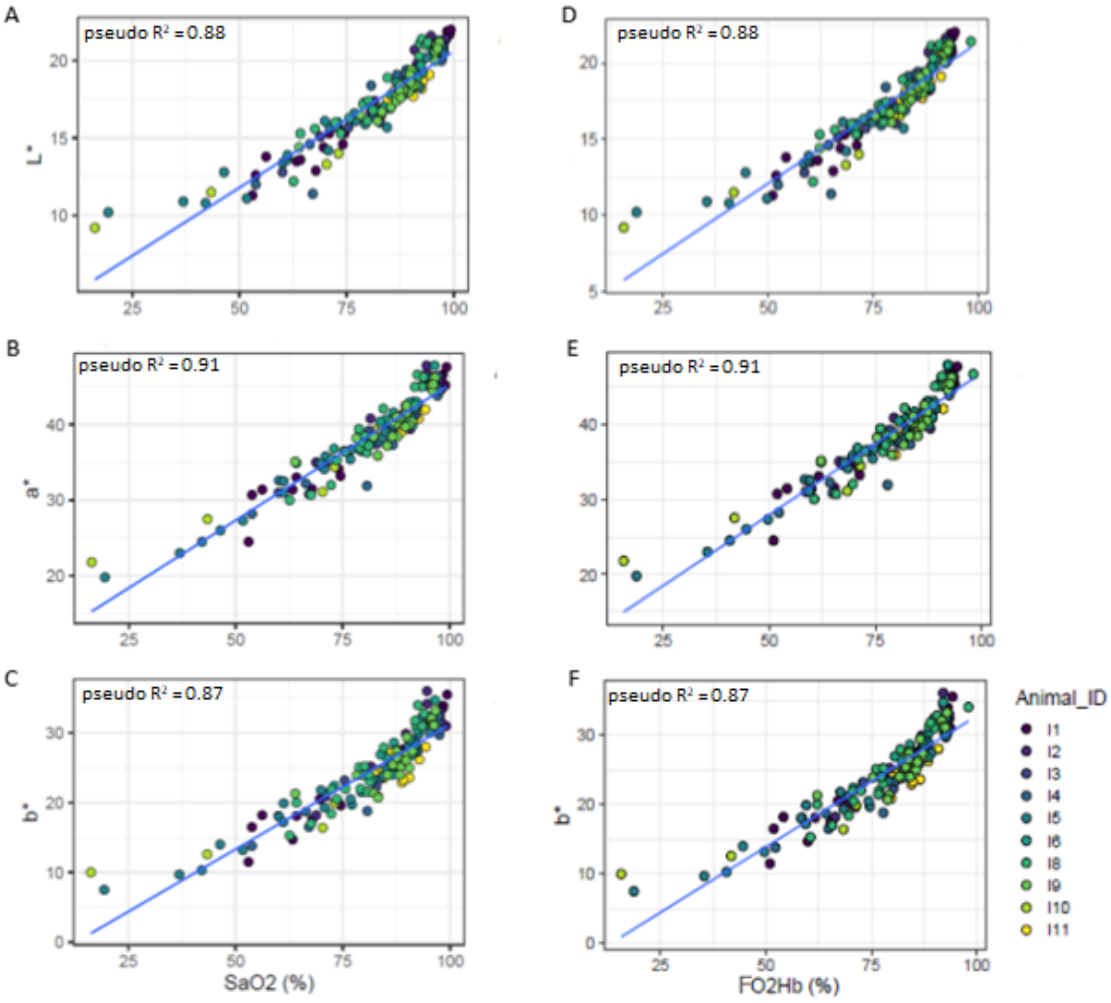


Figure 4.1 Functional saturation (SaO_2; A-C) and fractional saturation (FO_2Hb; D-F) versus the colour components luminosity (L*), green to red (a*) and blue to yellow (b*) as a simple linear model. Different colours represent data from the different animals (impala 1-11).

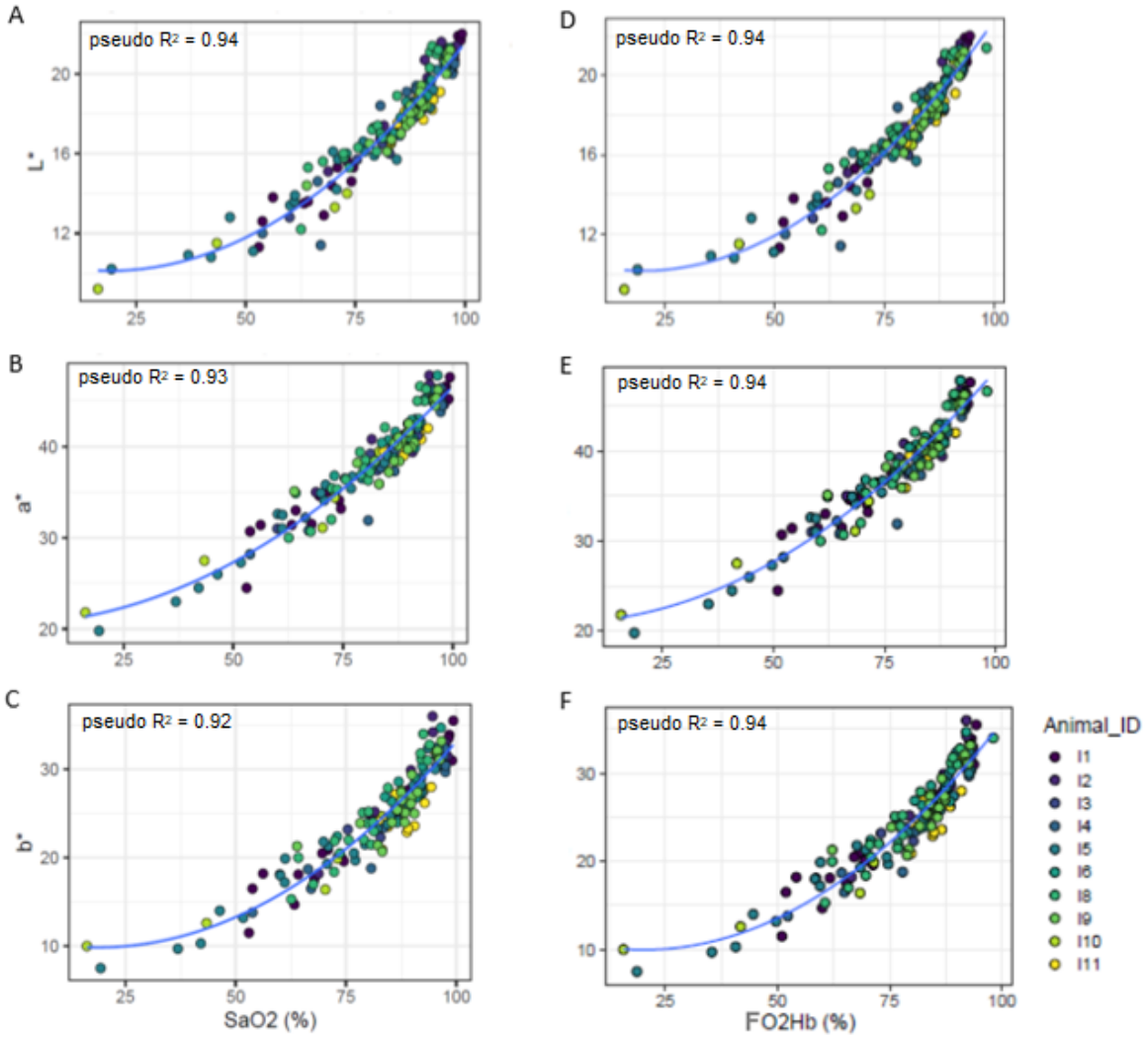


Figure 4.2 Functional saturation (SaO_2; A-C) and fractional saturation (FO_2Hb; D-F) versus the colour components luminosity (L*), green to red (a*) and blue to yellow (b*) as a quadratic model. Different colours represent data from the different animals (impala 1-11).

Table 4.1 Functional saturation (SaO_2) and fractional saturation (FO_2Hb) model comparisons. Null, linear (straight line) and quadratic curve models are compared to each other by means of likelihood ratio tests. Degrees of freedom (df), Akaike information criterion (AIC), Bayesian information criterion (BIC), log-likelihood ratio (logLik), Chi-square (Chisq), probability (Pr), significantly different (*)

SaO_2

Component	Model	df	AIC	BIC	logLik	deviance	Chisq	df	Pr(Chisq)	
L*	Null	3	798.66	807.94	-396.33	792.66				
	linear	4	463.12	475.50	-227.56	455.12	337.53	1	< 2.2e-16	*
L*	Null	3	798.66	807.94	-396.33	792.66				
	poly	5	361.75	377.22	-175.88	351.75	440.9	2	< 2.2e-16	*
L*	linear	4	463.12	475.50	-227.56	455.12				
	poly	5	361.75	377.22	-175.88	351.75	103.37	1	< 2.2e-16	*
a*	Null	3	1026.63	1035.91	-510.32	1020.63				
	linear	4	654.50	666.88	-323.25	646.50	374.13	1	< 2.2e-16	*
a*	Null	3	1026.63	1035.91	-510.32	1020.63				
	poly	5	604.82	620.29	-297.41	594.82	425.81	2	< 2.2e-16	*
a*	linear	4	654.50	666.88	-323.25	646.50				
	poly	5	604.82	620.29	-297.41	594.82	51.68	1	6.532e-13	*
b*	Null	3	1036.4	1045.7	-512.22	1030.44				
	linear	4	730.04	742.42	-361.02	722.04	308.4	1	< 2.2e-16	*
b*	Null	3	1036.4	1045.7	-512.22	1030.44				
	poly	5	659.79	675.26	-324.90	649.79	380.65	2	< 2.2e-16	*
b*	linear	4	730.04	742.42	-361.02	722.04				
	poly	5	659.79	675.26	-324.90	649.79	72.252	1	< 2.2e-16	*

(Table 4.1 continued)

FO_2Hb

Component	Model	df	AIC	BIC	logLik	deviance	Chisq	df	Pr(Chisq)	
L*	Null	3	798.66	807.94	-396.33	792.66				
	linear	4	465.72	478.09	-228.86	457.72	334.94	1	< 2.2e-16	*
L*	Null	3	798.66	807.94	-396.33	792.66				
	poly	5	362.57	378.04	-176.29	352.57	440.08	2	< 2.2e-16	*
L*	linear	4	465.72	478.09	-228.86	457.72				
	poly	5	362.57	378.04	-176.29	352.57	105.15	1	< 2.2e-16	*
a*	Null	3	1026.63	1035.91	-510.32	1020.63				
	linear	4	650.82	663.2	-321.41	642.82	377.81	1	< 2.2e-16	*
a*	Null	3	1026.63	1035.91	-510.32	1020.63				
	poly	5	592.68	608.15	-291.34	582.68	437.95	2	< 2.2e-16	*
a*	linear	4	650.82	663.2	-321.41	642.82				
	poly	5	592.68	608.15	-291.34	582.68	60.137	1	8.85e-15	*
b*	Null	3	1036.4	1045.7	-512.22	1030.44				
	linear	4	726.47	738.85	-359.24	718.47	311.97	1	< 2.2e-16	*
b*	Null	3	1036.4	1045.7	-512.22	1030.44				
	poly	5	646.58	662.05	-318.29	636.58	393.86	2	< 2.2e-16	*
b*	linear	4	726.47	738.85	-359.24	718.47				
	poly	5	646.58	662.05	-318.29	636.58	81.889	1	< 2.2e-16	*

The association between the blood colour components (CIE L*a*b*) and SaO_2 and FO_2Hb were best described by the quadratic model. There was a significant association between the blood colour components (CIE L*a*b*) and SaO_2 (Table 4.2) with pseudo R-squared (Nakagawa & Schielzeth 2013) values of 0.94 for L*, 0.93 for a* and 0.92 for b*. Similarly, for FO_2Hb there was also a significant association with the blood colour components (CIE L*a*b*, Table 4.2) and had similar pseudo R-squared values of 0.94 for L*, 0.94 for a* and 0.92 for b*.

Table 4.2 Contribution of each model component to the quadratic model for predicting L*(luminosity), a*(green to red) and b*(blue to yellow) for functional saturation (SaO_2) and fractional saturation (FO_2Hb). Showing estimate, standard error (Std. Error), degrees of freedom (df), t-value and the probability (Pr) for the Welch's t-test of each predictor and level of significance (*)

SaO_2

Predictor(s)	Estimate	Std. Error	df	t-value	Pr	
L*						
Intercept	17.5035	0.1266	9.2565	138.29	< 2.2e-16	*
linear component	33.3450	0.7193	160.2496	46.36	< 2.2e-16	*
quadratic component	8.5646	0.7048	159.1582	12.15	< 2.2e-16	*
a*						
Intercept	39.007	0.212	9.353	184.003	< 2.2e-16	*
linear component	68.374	1.524	162.491	44.856	< 2.2e-16	*
quadratic component	11.675	1.497	161.244	7.801	7.24e-13	*
b*						
Intercept	25.0024	0.2953	9.6667	84.681	3.33e-15	*
linear component	68.5032	1.7981	161.0661	38.097	< 2.2e-16	*
quadratic component	16.8702	1.7627	159.9209	9.571	< 2.2e-16	*

FO_2Hb

Predictor(s)	Estimate	Std. Error	df	t-value	Pr	
L*						
Intercept	17.5055	0.1052	8.4061	166.33	4.39e-16	*
linear component	33.2314	0.7236	161.9920	45.92	< 2.2e-16	*
quadratic component	8.7227	0.7095	160.3221	12.29	< 2.2e-16	*
a*						
Intercept	38.9938	0.1725	8.7863	226.036	< 2.2e-16	*
linear component	68.1708	1.4682	162.9597	46.432	< 2.2e-16	*
quadratic component	12.3180	1.4444	162.1422	8.528	9.95e-15	*
b*						
Intercept	24.9897	0.2537	9.5248	98.49	1.19e-15	*
linear component	68.4258	1.7290	162.0411	39.58	< 2.2e-16	*
quadratic component	17.5254	1.6950	160.5744	10.34	< 2.2e-16	*

The relatively narrow prediction intervals from the quadratic model (Fig 4.3) indicated that L* (luminosity), a* (green to red) and b* (blue to yellow) could be accurately predicted from both SaO_2 and FO_2Hb. The predicted L*, a* and b* components form the CIE L*a*b* co-ordinates for the SaO_2 or FO_2Hb value from which they were

predicted. Table 4.3 indicates the median predicted CIE L*a*b* co-ordinates that represent a 10% interval of SaO_2 and FO_2Hb respectively.

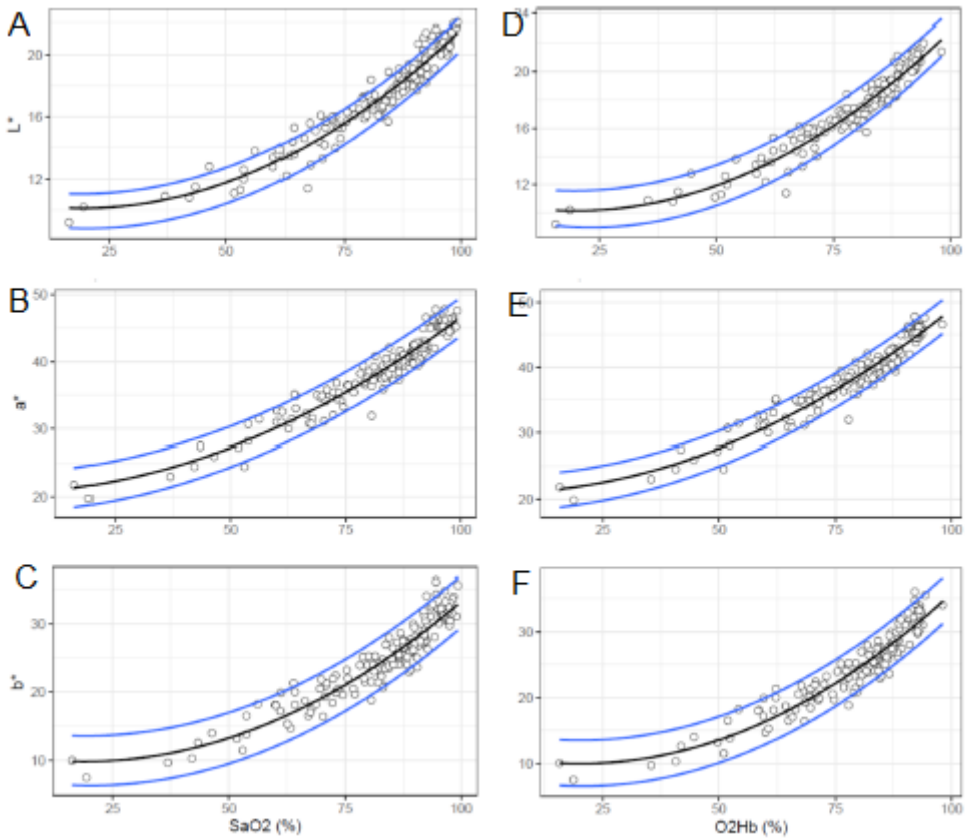


Figure 4.3 Prediction intervals (95% prediction interval indicated in blue) when functional saturation (SaO_2; A-C) and fractional saturation (FO_2Hb; D-F) are compared to the colour components luminosity (L*; A & D), green to red (a*; B & E) and blue to yellow (b*; C & F) in the quadratic model.

Table 4.3 Predicted L* (luminosity), a* (green to red) and b* (blue to yellow) values at the mid-point of each 10% range for functional saturation (SaO_2) and fractional saturation (FO_2Hb)

SaO_2

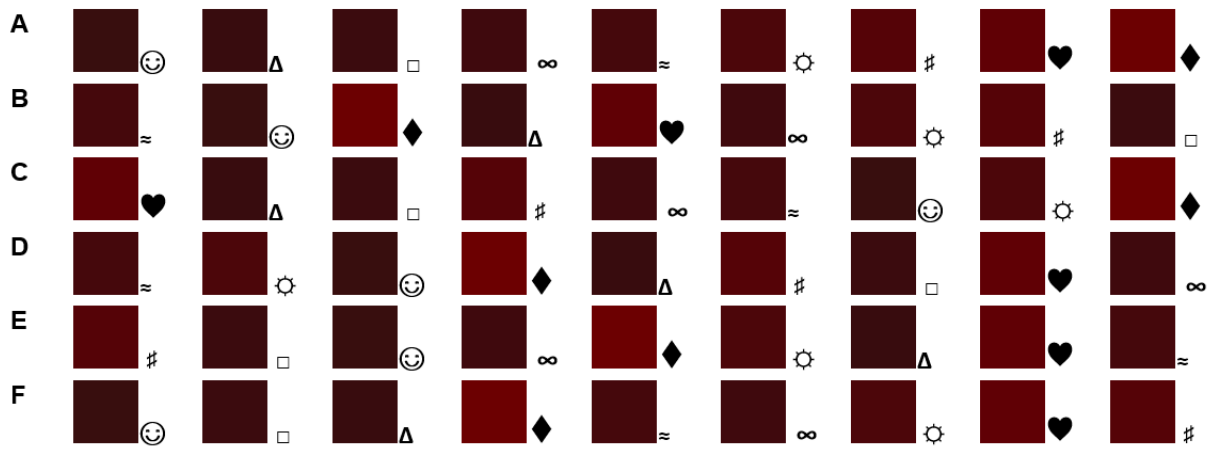
SaO_2 (%)	L*	a*	b*
15	10.3	21.0	9.8
25	10.2	22.1	9.8
35	10.6	23.8	10.6
45	11.2	25.9	12.1
55	12.3	28.6	14.3
65	13.7	31.7	17.3
75	15.5	35.4	21.0
85	17.7	39.6	25.4
95	20.2	44.2	30.5

FO_2Hb

FO_2Hb (%)	L*	a*	b*
15	10.3	21.2	9.9
25	10.2	22.3	9.9
35	10.6	24	10.7
45	11.4	26.2	12.3
55	12.5	29.1	14.8
65	14.1	32.5	18.0
75	16.1	36.5	22.1
85	18.5	41.1	27.0
95	21.3	46.2	32.8

4.3 Farnsworth-Munsell like hue tests

The results from the Farnsworth-Munsell like hue tests (Fig 4.4 & 4.5) indicated that the three darkest colours (predicted from 15, 25 and 35% functional oxyhaemoglobin saturation, respectively) could not be easily distinguished and were mixed up in the first three colour positions. This finding resulted in these combined colours (in the first three colour positions) representing a mean oxyhaemoglobin saturation of 22, 21 and 32% SaO_2, respectively (Fig 4.5). All twenty individuals successfully arranged the other six colours (predicted from 45, 55, 65, 75, 85 and 95% SaO_2) in their correct positions.



Colours will appear different on different screens. Therefore, this chart may not seem an accurate representation of the actual colours unless the screen colour is adjusted.

Figure 4.4 Functional saturation (SaO2) Farnsworth-Munsell like hue tests. The top row represents the correct order (A) and the following rows represent the different arrangements (B-F) given as tests.

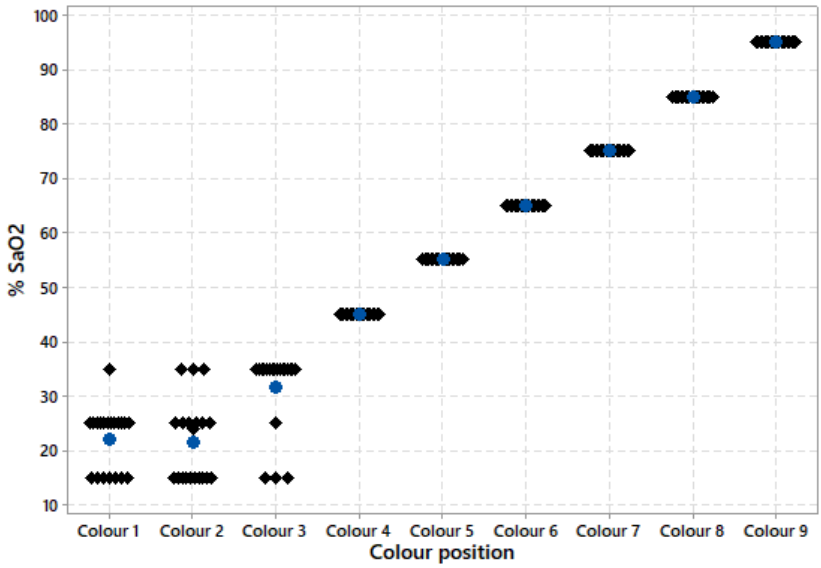


Figure 4.5 Individual interval plot of the Farnsworth-Munsell hue like test by 20 individuals. The plot indicates the specific functional saturation (SaO_2) of the colours in relevant nine colour positions. The mean SaO_2 of each group is indicated by a blue dot.

4.4 Haemoglobin variations

Carboxyhaemoglobin and methaemoglobin formed a small portion of the total haemoglobin measured by the co-oximeter from all the samples (COHb = 1.79 ± 1.15% and MetHb = 1.98 ± 0.68%; mean ± SD). The average total haemoglobin determined by co-oximeter was 10.3 g dL^{-1} at 5 minutes, 9.2 g dl^{-1} at 10 minutes, 8.6 g dl^{-1} at 15 minutes, 8.2 g dl^{-1} at 20 minutes, 7.7 g dl^{-1} at 30 minutes and 9.0 g dl^{-1} for the samples after 35 minutes.

Chapter 5

Discussion and Conclusion

5.1 Discussion

There was a good association between arterial blood colour and oxyhaemoglobin saturation. Furthermore, arterial blood colour could be used to successfully predict arterial blood oxygenation. These results allowed for the development of basic colourimetric scale. Initially, in the field, clinical visual assessment of the arterial blood colour indicated that there was a possible association between the colour of blood and arterial blood oxygenation. Initial cursory field assessment of the results also suggested that it would be relatively easy to predict whether arterial oxygenation was higher or lower as compared to other samples by means of either visual colour assessment or measures from the spectrocolourimeter (i.e. CIE L*a*b* components); as the CIE L*a*b* values increased or decreased for a subsequent sample so did the arterial blood oxygenation (SaO_2). These cursory clinical observations were confirmed by our models and the strong association between CIE L*a*b* components and the variables used to measure blood oxygenation i.e. SaO_2 and FO_2Hb. Although these associations could be determined by a linear model, a quadratic model gave a better fit for each colour component.

Both SaO_2 (functional saturation) and FO_2Hb (fractional saturation) can be used as a reliable predictor of all three CIE L*a*b* components of arterial blood colour. Therefore, because of these good predictions and strong associations, we believe that

it is appropriate to use measured colour components (i.e. CIE L*a*b*) from arterial blood to reliably indicate the level of arterial blood oxygenation in impala.

Predicted L* (luminosity), a* (green to red) and b* (blue to yellow) values can be used to form the colour that predicts specific SaO_2 and FO_2Hb values. However, when doing visual assessments it would not be practical to create a colour chart which is a continuum of these predictors. Therefore, we defined oxyhaemoglobin saturation ranges from our data and used the smallest oxyhaemoglobin saturation intervals with the least overlap, being 10% ranges of this saturation. Therefore, median SaO_2 (functional saturation) and FO_2Hb (fractional saturation) values representing 10% saturation intervals (15, 25, 35, 45, 55, 65, 75, 85 and 95% saturation) were used to predict the CIE L*a*b* values to represent their respective 10% saturation interval.

The Farnsworth-Munsell like hue test helped to identify up to what point the human eye will be able to distinguish these colours for the more regularly used functional saturation. The original Farnsworth-Munsell 100 hue test is a well-known test that examines an individual's ability to discriminate between different hues (Hardy et al. 1992). In the present study we produced five different arrangements of the nine functional saturation range colours, displayed on a tablet screen on 100% brightness and asked 20 random individuals to arrange one of the five arrangement groups from the darkest to the brightest colour. We asked our participants if they were colour blind or not and at this stage we only had participants that weren't colour blind. This part of the study was a preliminary assessment to determine the value of possibly developing a printed colour chart so using participants that weren't colour blind was ideal. However, in future studies we will assess the usefulness of this colour chart used by people in the field and this assessment will require using a more heterogeneous population of people which will include those people with colour blindness and other

visual disorders. The results showed that most individuals can easily differentiate each of the six brightest functional saturation colour ranges (SaO_2 of 40 to 100%). However, most individuals misarranged the three darker functional saturation colour ranges (SaO_2 of 10 to 40%). There was more confusion between the two darkest colours representing 15% and 25% SaO_2 as the first two colour positions each had a mean SaO_2 of 22% and 21.5% respectively. Whereas the third colour position had a mean of 32% SaO_2; therefore, standing slightly out from the other two colours. While doing the test most individuals commented that they were unsure whether they arranged this third colour correctly. Therefore, in order to finalise a colour chart for practical use in the field we decided to reduce our colour chart to seven colours by using the 35% colour (SaO_2 of 30 to 40%) to represent SaO_2 values below 40%, which means that any darker colour than the 35% colour would also represent SaO_2 values below 40%.

Although the study was focussed on the relationship between arterial blood colour and oxyhaemoglobin saturation, arterial blood colour may not only be influenced by oxygen levels. Haemoglobin concentration does have an effect on the colour of blood and haemoglobin colour charts have been utilised before in several different species as patient-side tests to determine the degree of anaemia from blood (Marn & Critchley 2016) or mucous membrane colour (Grace et al. 2007; Storey et al. 2017). The total haemoglobin concentration in the immobilised impala changed throughout the immobilisation period. Although including total haemoglobin as a co-variate in the quadratic model slightly improved the model, the complexity it added in predicting the CIE L*a*b* components outweighed the small degree of improvement that it added to the predictive model. The primary purpose of this study was to determine if there is a clinically relevant relationship between blood colour and oxyhaemoglobin saturation (SaO_2 & FO_2Hb). Therefore, the effects of confounding variables, like haemoglobin

concentration, did not fall into the scope of this initial study. It was shown that when using blood colour to assess arterial blood oxygenation, the change in colour was more marked with a lower haemoglobin concentration (Morgan-hughes & Bartlett 1968). In cases of severe anaemia, less oxygen will reach tissues even with good SaO_2 and PaO_2 values. Therefore, arterial blood colour could be a better measure of the true arterial oxygenation status as it might be possible to use colour to measure both the capacity (haemoglobin concentration) and the state (oxygen saturation) of oxygenation. However, the effect of large differences in haemoglobin concentration or haematocrit on arterial blood colour should be determined in the future and may need to be co-measured and factored into future models or devices that use colour as a determinant of blood oxygenation.

This study focused on assessing oxyhaemoglobin saturation only. Therefore, the residual haemoglobin fraction was assumed to be mostly deoxyhaemoglobin. In the field, in healthy animals, the proportion of other haemoglobin derivatives are assumed to be small, and this was confirmed in our study by the co-oximeter. Although moderate amounts of carboxyhaemoglobin and methaemoglobin are normally rare anomalies in animals in the field they cannot be completely ignored. Therefore, it might be advisable to further investigate the addition of some colour tinges (brown for MetHb and cherry red for COHb) to blood colour charts and models in future studies.

We believe that the ODC and the spectrophotometric characteristics of impala haemoglobin are similar to those of humans based on the similarity of measured SaO_2 in impala, determined by co-oximetry, and the calculated SaO_2 as determined by a human calibrated blood gas analyser (Mtetwa 2019). Therefore, the use of the co-oximeter (Avoximeter 4000, Surgical Innovations (Pty) Ltd., Northriding, South Africa), which is designed for human use, was likely adequate to properly asses blood

oxygenation in the impala. These similarities can be supported by another study that found differences in spectral properties between human and bovine oxyhaemoglobin, deoxyhaemoglobin and carboxyhaemoglobin to be minute, however, only that of methaemoglobin was found to have different spectral properties. (Zijlstra & Buursma 1997). However, this might not be the case for all species as the light absorption spectrums of the different haemoglobin derivatives for different species is yet to be determined. Furthermore, as mentioned previously, oxygen haemoglobin binding affinity differ among species (Baumann et al. 1984) as different species have different ODCs (Clerbaux et al. 1993). Therefore, the relationship between blood colour and oxygenation may differ between species, but this needs to be properly determined.

A number of methods have been used clinically to assess blood oxygenation in immobilised wildlife, but the efficacy of these methods have not been established in most wildlife species. Co-oximetry, which uses the absorption of different wavelengths of light to determine the fractions of the different oxygen-haemoglobin derivatives (Haymond et al. 2005) may be a promising alternative to blood gas analysis. During the present study, we discovered how sensitive co-oximeters are to light, samples had to be analysed in a room with low ambient light. Furthermore, other than practicality, co-oximeters may also have cost and species-specific limitations. It is important to note that both spectrocolourimeters and co-oximeters are spectrophotometers. Spectrocolourimeters, being more widely used for industrial purposes are more readily available and inexpensive compared to co-oximeters. Therefore, there is great value in further investigating the visual spectrum properties of haemoglobin; the most important transporter of oxygen.

Haemoglobin (Marn & Critchley, 2016), more specifically the haemoglobin derivatives (Park et al. 2015) are the major contributors to the colour of blood. Bovine haemoglobin

shows minimal light absorption in the range of λ = 640-800 nm (Zijlstra & Buursma 1997). Therefore, all light in this wavelength range (640-800 nm) is reflected. The wavelength interval of 640-700 nm is the visible light spectrum of red thus giving blood a red appearance. However, during data collection, several different shades of red were observed. The different shades are a result of the reflection of different wavelengths of light by each of the derivatives. Changes in either the iron state itself or different bindings to the iron results in the formation of the common derivatives of haemoglobin (Zijlstra & Buursma 1997). This difference in shades among the derivatives is the principal reason why spectrophotometers can be used for both co-oximetry (Haymond et al. 2005) and colourimetry (Zwinkels 1996).

Since the 1900's scientist like Theodor Tallqvist described the possible application of colour scales to determine the state of haemoglobin (Lewis 2002). This general understanding that blood scales with blood oxygenation has been used clinically for years by veterinarians, especially wildlife veterinarians who often use free-flowing blood from a catheter to get a sense of changing blood oxygenation during immobilisation. Park et al. (2015) investigated the possibility of using blood colour brightness, seen by observers, to directly differentiate arterial (oxygenated) from venous (deoxygenated) blood in humans. These researchers also quantified blood colour by cross-matching blood in a gas syringe with the best-suited colour using the RGB blood brightness scale. The observers were able to accurately differentiate arterial from venous blood with a specificity of 84.9% and a sensitivity of 97.0%. These researchers also quantified blood colour by cross-matching blood in a gas syringe with the best-suited colour using the RGB blood brightness (Park et al. 2015).

The foremost challenge in this study was determining how to accurately measure the colour of blood. As discussed, there are many different colour grading systems which

are termed colour spaces. RGB or CMYK are colour spaces that are commonly used in light-emitting (television) and printing systems, respectively, and are good for use for a wide range of general colours. However, their specificity and accuracy in narrow colour ranges are limited. Therefore, we realised that comparing colour bands within the narrow visible red hue range (640-700 nm) that a more specific and accurate colour space, like CIE L*a*b*, was required (Ford & Roberts 1998). CIE L*a*b* works on the basis of a “three-dimensional co-ordinate” system. It places a colour, using three points namely L* (luminosity), a* (green to red) and b* (blue to yellow), in a three-dimensional scale (Hasting & Rubin 2012), thereby factoring in all of the colour characteristics (hue, luminosity and saturation), rather than just indicating the amount of each colour. As stated by others (Hasting & Rubin 2012) the downside of the CIE L*a*b* colour space is that it is difficult to precisely reproduce colours in printed media.

Although blood colour charts may still be of value, they may have limitations based on observer error and the ability of observers to distinguish similar shades of colour, thus limiting the accuracy and specific blood oxygen levels across a larger range. However, with the advent of smartphone digital cameras, and inexpensive spectrocolourimeters, the assessment of blood colour could be of more value than originally anticipated; because these modalities, combined with modelling of large data sets could provide a cost-effective, continuous, accurate and precise method of determining blood oxygenation. Although not specifically related to oxyhaemoglobin saturation there are several smartphone-based examples of applications (Apps) to measure blood haemoglobin concentrations. Some examples include a non-invasive fingertip smartphone-based system which determines haemoglobin levels by means of colour in humans (Hasan et al. 2018) and a smartphone based biosensor that enables the quantification of haemoglobin from a drop of blood in dogs (Farrell et al. 2017).

During chemical immobilisation, it is relatively easy to find an artery to obtain arterial blood samples in antelope and other large mammals. Drawing one sample at a time for colour and blood oxygen determination may prove tedious and will not provide continuous readings. To overcome these limitations an arterial-venous shunt incorporating a colour detector port could be placed for continuous measures, or a suction and injection device with a detector could be developed to help with making fairly regular but non-continuous measures.

5.2 Conclusion

We have shown that oxyhaemoglobin saturation (both SaO_2 and FO_2Hb) can reliably predict arterial blood colour. Therefore, when assessed by a specifically developed colour chart or spectrocolourimeter, blood colour can be used to reliably estimate arterial blood oxygenation of impala. This study highlights that there is value in pursuing further studies on the use of colour to determine arterial blood oxygenation, especially in other species. With the advent of modalities that can accurately measure colour cost-effectively, these findings could pave the way for developing an affordable, accurate and practical field method of assessing arterial blood oxygenation and detecting hypoxaemia in immobilised or anaesthetised wildlife.

References

(Veterinary Anaesthesia and Analgesia formatting)

Allen JL (2017) Immobilization of hartmann's mountain zebras (*Equus zebra hartmannae*) with carfentanil and antagonism with naltrexone or nalmefene. J Zoo Wildl Med 25, 205–208.

Baumann R, Mazur G, Braunitzer G (1984) Oxygen binding properties of hemoglobin from the white rhinoceros (β2-glu) and the tapir. Respir Physiol 56, 1–9.

Buck RK, Meyer LRC, Stegmann GF et al. (2017) Propofol–medetomidine–ketamine total intravenous anaesthesia in thiafentanil–medetomidine-immobilized impala (*Aepyceros melampus*). Vet Anaesth Analg 44, 138–143.

Buss P, Miller M, Fuller A et al. (2018) Postinduction butorphanol administration alters oxygen consumption to improve blood gases in etorphine-immobilized white rhinoceros. Vet Anaesth Analg 45, 57–67.

Buss PE, Meltzer DG (2001) Changes in respiratory function following the intramuscular administration of etorphine to boer goats (*Capra hircus*). J S Afr Vet Assoc 72, 137–42.

Caulkett NA, Cribb PH, Duke T (1994) Cardiopulmonary effects of medetomidine-ketamine immobilization with atipamezole reversal and carfentanil-xylazine immobilization with naltrexone reversal: a comparative study in domestic sheep (*Ovis ovis*). J Zoo Wildl Med 25, 376–389.

Celly CS, Atwal OS, McDonell WN et al. (1999) Histopathologic alterations induced in the lungs of sheep by use of alpha2-adrenergic receptor agonists. Am J Vet Res 60, 154-61.

Chan ED, Chan MM, Chan MM (2013) Pulse oximetry: understanding its basic principles facilitates appreciation of its limitations. Respir Med 107, 789–799.

Chu SJ, Trushkowsky RD, Paravina RD (2010) Dental color matching instruments and systems. Review of clinical and research aspects. J Dent 38, e2-16.

Clerbaux T, Gustin P, Detry B et al. (1993) Comparative study of the oxyhaemoglobin dissociation curve of four mammals: man, dog, horse and cattle. Comp Biochem Physiol 106a, 687–694.

Collins J, Rudenski A, Gibson J et al. (2015) Relating oxygen partial pressure, saturation and content: the haemoglobin–oxygen dissociation curve. Breathe 11, 194–201.

Dugdale A (2010) Veterinary anaesthesia principles to practice. (1st edn). Blackwell publishing, Oxford, United Kingdom. pp. 64-65.

Eike JH, Palmer AF (2004) Effect of Cl^- and H^+ on the oxygen binding properties of glutaraldehyde-polymerized bovine hemoglobin-based blood substitutes. Biotechnol Prog 20, 1543-1549.

Fahlman Å, Pringle J, Arnemo JM et al. (2010) Treatment of hypoxemia during anesthesia of brown bears (*Ursus arctos*). J Zoo Wildl Med 41, 161–164.

Fahlman Å, Caulkett N, Arnemo JM et al. (2012) Efficacy of a portable oxygen concentrator with pulsed delivery for treatment of hypoxemia during anesthesia of wildlife. J Zoo Wildl Med 43, 67–76.

Fahlman Å, Caulkett N, Woodbury M et al. (2014) Low flow oxygen therapy from a portable oxygen concentrator or an oxygen cylinder effectively treats hypoxemia in anesthetized white-tailed deer (*Odocoileus Virginianus*). J Zoo Wildl Med 45, 272–

277.

Fahlman Å (2014) Oxygen Therapy. In Zoo Animal and Wildlife Immobilization and Anesthesia (2nd edn) West G, Heard D, Caulkett N (eds). John Wiley & Sons Inc, USA. pp. 69–81.

Fahlman Å, Edner A, Wenger S et al. (2016) Pulmonary gas exchange and acid–base status during immobilisation of black rhinoceroses (*Diceros bicornis*) in Zimbabwe. J S Afr Vet Assoc 87, 1–9.

Farrell KS, Chapman PL, Sullivan LA (2017) Validation of a smartphone-based point-of-care hemoglobin assay for use in dogs. J Vet Emerg Crit Care 27, 206–211.

Gonzalez AL, Waddell LS (2016) Blood gas analyzers. Top Companion Anim Med 31, 27–34.

Grace D, Himstedt H, Sidibe I et al. (2007) Comparing FAMACHA© eye color chart and hemoglobin color Scale tests for detecting anemia and improving treatment of bovine trypanosomosis in West Africa. Vet Parasitol 147, 26–39.

Hall JE (2016) Transport of oxygen and carbon dioxide in blood and tissue fluids. In: Guyton and Hall Textbook of Medical Physiology (13th edn). Elsevier, Philadelphia. pp 527-537.

Hardy KJ, Lipton J, Scase MO et al. (1992) Detection of colour vision abnormalities in uncomplicated type 1 diabetic patients with angiographically normal retinas. Br J Ophthalmol 76, 461–464.

Hasan MK, Haque M, Sakib N et al. (2018) Smartphone-based human hemoglobin level measurement analyzing pixel intensity of a fingertip video on different color spaces. Smart Heal 5, 26–39.

Hasting GD, Rubin A (2012) Colour spaces - a review of historic and modern colour models. African Vis Eye Heal 71, 133–143.

Haymerle A, Knauer F, Walzer C (2016) Two methods to adapt the human haemoglobin-oxygen dissociation algorithm to the blood of white rhinoceros (*Ceratotherium simum*) and to determine the accuracy of pulse oximetry. Vet Anaesth Analg 43, 566–570.

Haymond S, Cariappa R, Eby CS et al. (2005) Laboratory assessment of oxygenation in methemoglobinemia. Clin Chem 51, 434–444.

Kim SJ, Salem MR, Joseph NJ et al. (1990) Contrast media adversely affect oxyhemoglobin dissociation. Anesth Analg 71, 73-76.

Koenig J, McDonell W, Valverde A (2003) Accuracy of pulse oximetry and capnography in healthy and compromised horses during spontaneous and controlled ventilation. Can J Vet Res 67, 169–174.

Lee JY, Chang JS, Koo KC et al. (2013) Hematuria grading scale: a new tool for gross hematuria. Urology 82, 284–289.

Lewis SM (2002) Looking backwards to the future: The WHO haemoglobin colour scale. Turkish J Haematol 19, 185–189.

Marn H, Critchley JA (2016) Accuracy of the WHO haemoglobin colour scale for the diagnosis of anaemia in primary health care settings in low-income countries: a systematic review and meta-analysis. Lancet Glob Heal 4, e251–e265.

Melendez-Martınez AJ, Vicario IM, Heredia FJ (2005) Instrumental measurement of orange juice colour: a review. J Sci Food Agric 85, 894–901.

Meyer LCR, Hetem RS, Fick LG et al. (2010) Effects of serotonin agonists and

doxapram on respiratory depression and hypoxemia in etorphine-immobilized impala (*Aepyceros melampus*). J Wildl Dis 46, 514–524.

Meyer LCR, Hetem RS, Mitchell D et al. (2015) Hypoxia following etorphine administration in goats (*Capra hircus*) results more from pulmonary hypertension than from hypoventilation. BMC Vet Res 11, 1–9.

Mich PM, Wolfe LL, Sirochman TM et al. (2008) Evaluation of intramuscular butorphanol, azaperone, and medetomidine and nasal oxygen insufflation for the chemical immobilization of white-tailed deer, *Odocoileus virginianus*. J Zoo Wildl Med 39, 480–487.

Morgan-hughes JO, Bartlett MC (1968) The colour of blood in syringes as a guide to hypoxaemia. Br J Anaesth 40, 310–314.

Mtetwa TK (2019) Effectiveness of pulse oximetry to detect hypoxaemia in immobilized impala (*Aepyceros melampus*). MSc thesis, University of Pretoria. Department of Paraclinical Sciences.

Mwaniki MK, Nokes DJ, Ignas J et al. (2009) Emergency triage assessment for hypoxaemia in neonates and young children in a Kenyan hospital: An observational study. Bull World Health Organ 87, 263–270.

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining r2 from generalized linear mixed-effects models. Methods Ecol Evol 4, 133–142.

Naumenko EK (1996) Choice of wavelengths for stable determination of concentrations of hemoglobin derivative from absorption spectra of erythrocytes. J Appl Spectrosc 63, 43–48.

Park JS, Lee BK, Jeung KW et al. (2015) Reliability of blood color and blood gases in discriminating arterial from venous puncture during cardiopulmonary resuscitation. Am J Emerg Med 33, 553–558.

Pattinson KTS (2008) Opioids and the control of respiration. Br J Anaesth 100, 747–758.

Portas TJ (2004) A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species. Aust Vet J 82, 542–549.

Read MR (2003) A review of alpha 2 adrenoreceptor agonists and the development of hypoxemia in domestic and wild ruminants. J Zoo Wildl Med 34, 134–138.

Reiners JK, Hellmann N, Schmidt J et al. (2019) Odd haemoglobins in odd-toed ungulates: Impact of selected haemoglobin characteristics of the white rhinoceros

(*Ceratotherium simum*) on the monitoring of the arterial oxygen saturation of haemoglobin. PLOS ONE 14, e0226851.

Reynecke DP, Van Wyk JA, Gummow B et al. (2011) Validation of the FAMACHA© eye colour chart using sensitivity/specificity analysis on two South African sheep farms. Vet Parasitol 177, 203–211.

Schiller F, Valsecchi M, Gegenfurtner KR (2018) An evaluation of different measures of color saturation. Vis Res 151, 117–134.

Schmidt-Neilsen K, Larimer JL (1958) Oxygen dissociation curves of mammalian blood in relation to body size. Am J Physiol 195, 424-428.

Schumacher J, Heard DJ, Young L et al. (1997) Cardiopulmonary Effects of Carfentanil in Dama Gazelles (*Gazella dama*). J Zoo Wildl Med 28, 166–170.

Sinex JE (1999) Pulse oximetry: principles and limitations. Am J Emerg Med 17, 59–66.

Storey BE, Williamson LH, Howell SB et al. (2017) Validation of the FAMACHA© system in South American camelids. Vet Parasitol 243, 85–91.

Van Wyk JA, Bath GF (2002) The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet Res 33, 509-529.

van Zijll Langhout M, Caraguel CGB, Raath JP et al. (2016) Evaluation of etorphine and midazolam anesthesia, and the effect of intravenous butorphanol on cardiopulmonary parameters in game-ranched white rhinoceroses (*Ceratotherium simum*). J Zoo Wildl Med 47, 827–833.

Weber RE (2007) High-altitude adaptations in vertebrate hemoglobins. Respir Physiol Neurobiol 158, 132-142.

Wenger S, Boardman W, Buss P et al. (2007) The cardiopulmonary effects of etorphine, azaperone, detomidine, and butorphanol in field-anesthetized white rhinoceroses (*Ceratotherium simum*). J Zoo Wildl Med 38, 380–387.

Yim D, Baranoski GVG, Kimmel BW et al. (2012) A cell-based light interaction model for human blood. Comput Graph Forum 31, 845–854.

Zeiler GE, Stegman GF, Fosgate G (2015) Etorphine–ketamine–medetomidine total intravenous anesthesia in wild impala (*Aepyceros melampus*) of 120-minute duration. J Zoo Wild Med 46, 755-766.

Zeiler GE, Meyer LCR (2017a) Chemical capture of impala (*Aepyceros melampus*): a review of factors contributing to morbidity and mortality. Vet Anaesth Analg 44, 991–1006.

Zeiler GE, Meyer LCR (2017b) Comparison of thiafentanil-medetomidine to etorphine-medetomidine immobilisation of impalas (*Aepyceros melampus*). J S Afr Vet Assoc 88, a1520.

Zijlstra WG, Buursma A (1987) Spectrophotometry of hemoglobin: a comparison of dog and man. Comp Biochem Physiol 88, 251–255.

Zijlstra WG, Buursma A, Falke HE (1994) Spectrophotometry of hemoglobin: absorption spectra of rat oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and methemoglobin. Comp Biochem Physiol 107, 161–166.

Zijlstra WG, Buursma A (1997) Spectrophotometry of hemoglobin: absorption spectra of bovine oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and methemoglobin. Comp Biochem Physiol 118, 743–749.

Zuckerman GR, Trellis DR, Sherman TM et al. (1995) An objective measure of stool color for differentiating upper from lower gastrointestinal bleeding. Dig Dis Sci 40, 1614–1621.

Zwinkels JC (1996) Colour-measuring instruments and their calibration. Displays 16,163–171.

Addendum A

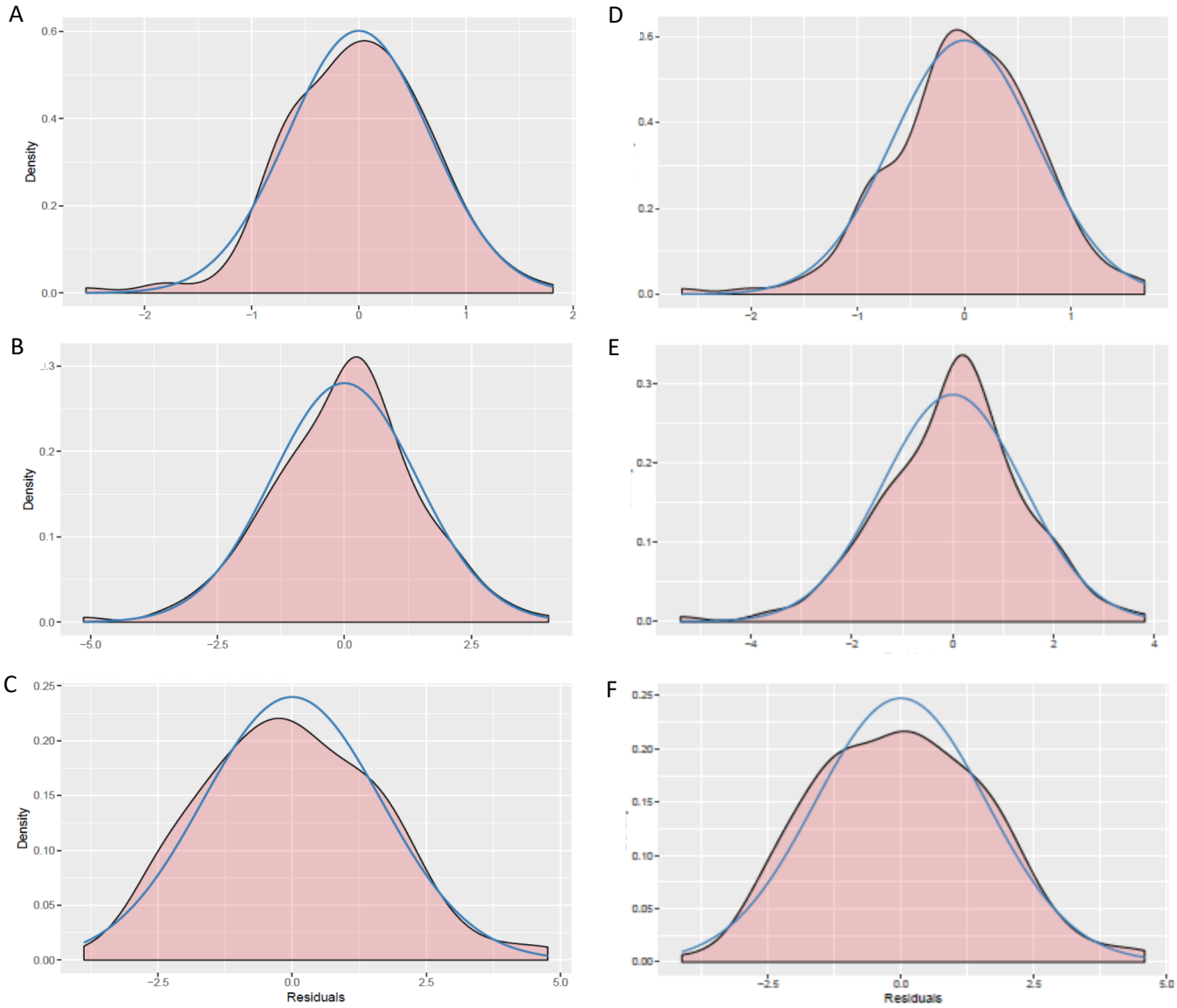


Figure A Residuals distribution of luminosity (L*; A & D), green to red (a*; B & E) and blue to yellow (b*; C & F) for both functional saturation (SaO_2; A-C) and fractional saturation (FO_2Hb; D-F).

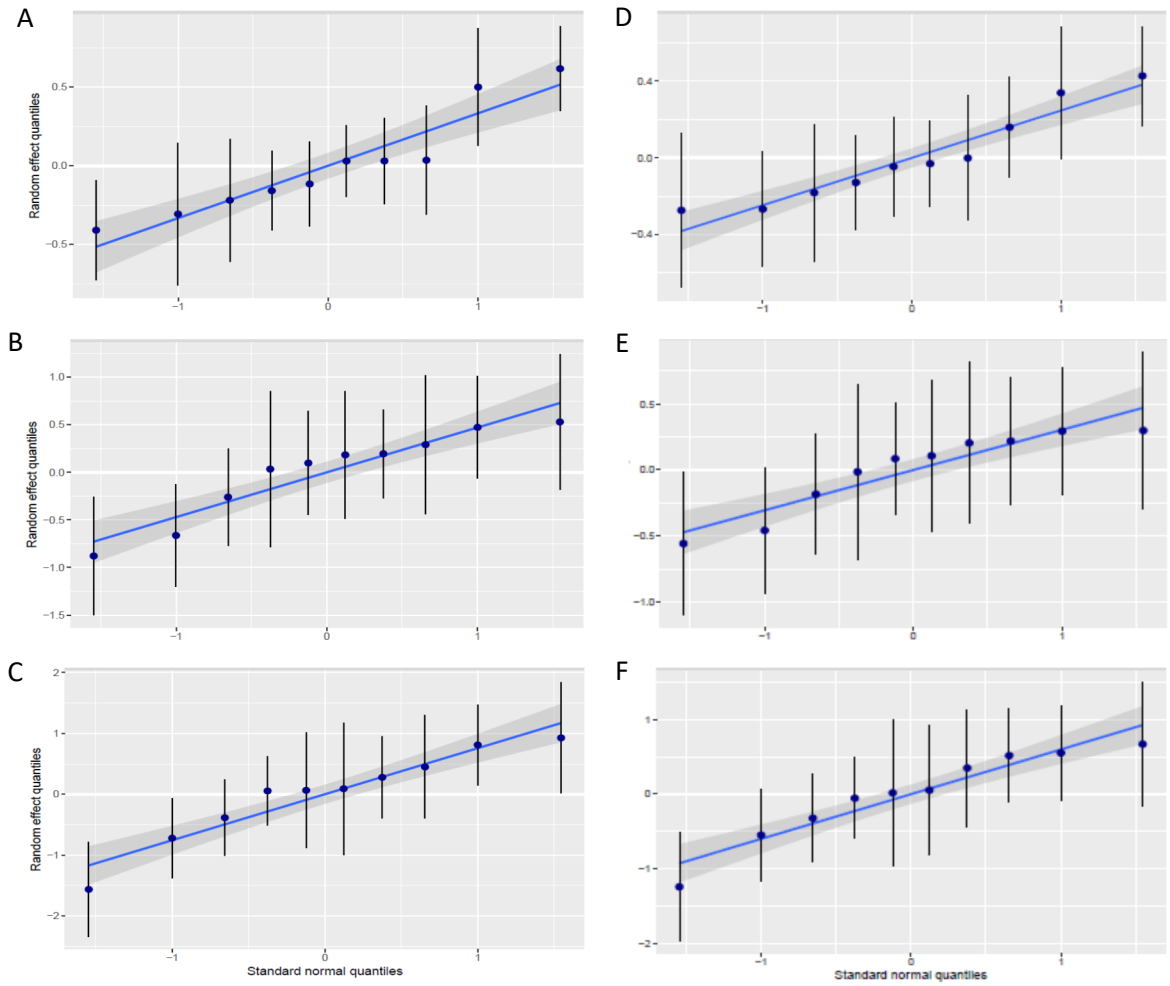


Figure B Normality of the random intercepts (Animal ID) for luminosity (L*; A & D), green to red (a*; B & E) and blue to yellow (b*; C & F) for both functional saturation (SaO2; A-C) and fractional saturation (FO2Hb; D-F).

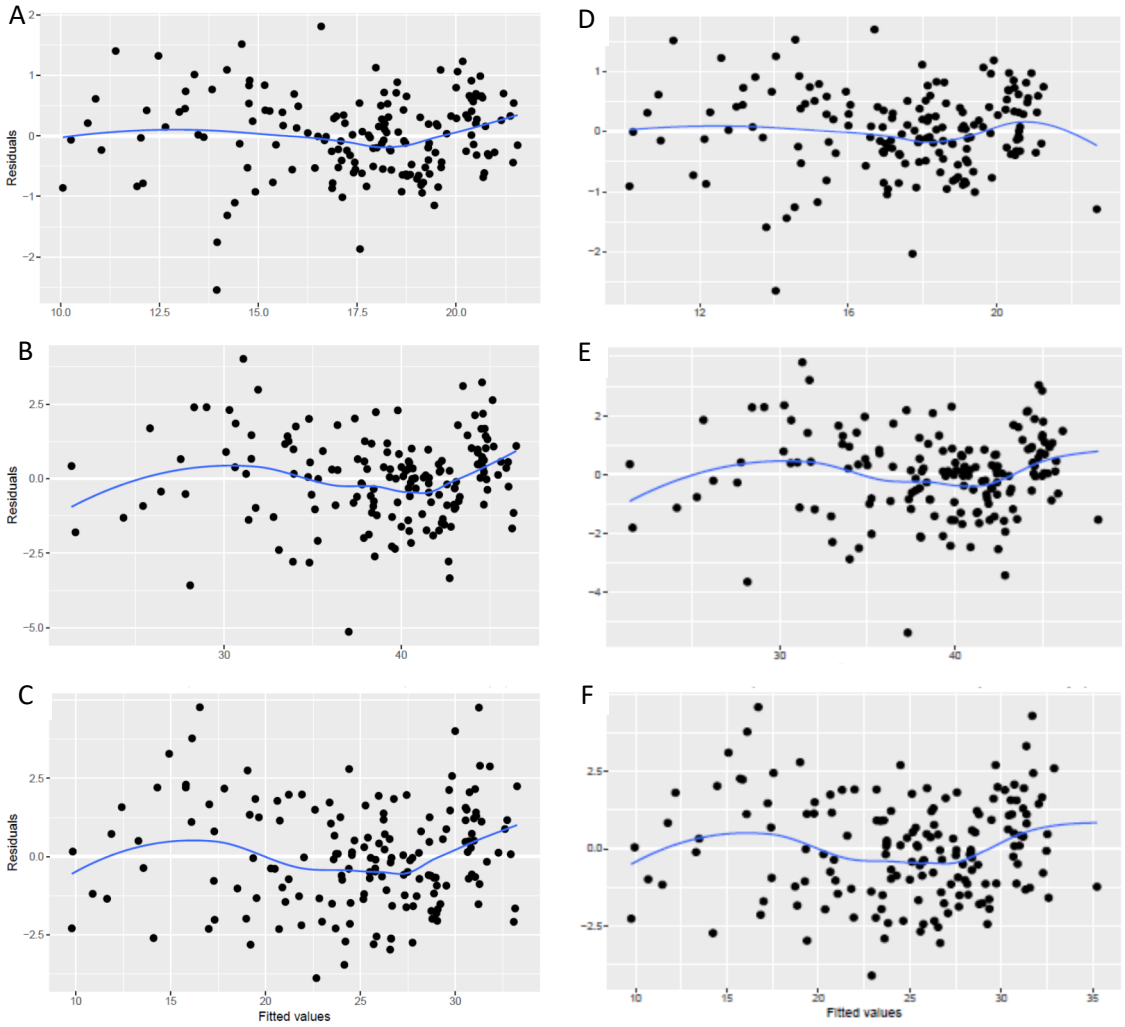


Figure C Homoscedasticity (constant variance of residuals) for luminosity (L*; A & D), green to red (a*; B & E) and blue to yellow (b*; C & F) for both functional saturation (SaO2; A-C) and fractional saturation (FO2Hb; D-F). The qual and random spread of residuals across the range of fitted values indicates homoscedasticity.

Addendum B

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Assessment of hypoxaemia during chemical immobilization of wildlife**
PROJECT NUMBER	**V035-17 (Amendment 1)**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Prof. L Meyer**

STUDENT NUMBER (where applicable)	----------
DISSERTATION/THESIS SUBMITTED FOR	**Academic**

ANIMAL SPECIES	**Blesbok**	**Impala**
NUMBER OF SAMPLES	**12**	**12**
Approval period to use animals for research/testing purposes		**July 2017 - July2018**
SUPERVISOR	**Prof. L Meyer**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 31 July 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15