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Reliability of calculated arterial oxygen-haemoglobin saturation in determining blood oxygenation of immobilised white rhinoceros (*Ceratotherium simum*)

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Abstract

Background Arterial oxygen-haemoglobin saturation (SaO_2) is an important biomarker of hypoxaemia, but is difficult to measure accurately in rhinoceros, especially during severe hypoxaemia. Pulse oximetry can reliably estimate SaO_2 in rhinoceros when attached to the third eyelid but only at SaO_2 ≥70%. The need for alternative methods led us to assess the reliability of the following approaches in calculating SaO_2 ($cSaO_2$) from conventional blood gas analysis: (i) Hill equation when the body mass-estimated P_{50} (PO_2 when haemoglobin is 50% saturated with oxygen) is adjusted for pH alone ("pH-adjusted Hill $cSaO_2$"), (ii) Hill equation when P_{50} is adjusted for pH, base excess and body temperature ("multivariable-adjusted Hill $cSaO_2$"), and (iii) the Siggaard-Andersen algorithm ("Siggaard-Andersen $cSaO_2$"). Sixteen white rhinoceroses (*Ceratotherium simum*) were immobilised with etorphine-based drug combinations and given butorphanol and, or oxygen. Arterial blood was drawn from the auricular artery and PaO_2, $PaCO_2$, and pH were measured using a portable Enterprise Point-of-Care (EPOC) blood gas analyser, and SaO_2 measured using a benchtop AVOXimeter 4000 co-oximeter (reference method). Bland-Altman and area root mean squares (ARMS) assessed the reliability of $cSaO_2$ values when compared to the co-oximeter SaO_2 measurements, with the reliability threshold set at ARMS≤4%.

Results The pH-adjusted Hill $cSaO_2$ and the multivariable-adjusted Hill $cSaO_2$ values were reliable within the 70–100% saturation range (bias 0% and −2%, precision 3% and 4%, ARMS 3% and 4%, respectively). The Siggaard-Andersen $cSaO_2$ values were reliable only within the 90–100% range (bias 2%, precision 2%, ARMS 3%). Below 70%, all $cSaO_2$ methods were unreliable. However, across the entire 0-100% range, the pH-adjusted Hill $cSaO_2$ and multivariable-adjusted Hill $cSaO_2$ were only slightly above the reliability threshold (ARMS of 5%).

Conclusion The pH-adjusted Hill equation is best suited for calculating SaO_2 across the 70–100% saturation range in immobilised white rhinoceros. Although this equation did not meet the reliability threshold when saturations were below 70%, it nonetheless showed a marked improvement over previous pulse oximetry analyses. A simple calculator is presented to convert PaO_2 and pH into an estimate of SaO_2, offering an additional tool to pulse oximetry especially during prolonged or complex procedures and for research applications.

Keywords Chemical immobilisation · Arterial O_2-Hb saturation · Co-oximetry · Wildlife

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Introduction

White rhinoceros (*Ceratotherium simum*) immobilised with potent opioids (e.g., etorphine) can develop acute and severe hypoxaemia, that is often accompanied by hypercapnia and acidosis [1–3]. An important biomarker of hypoxaemia is arterial oxygen-haemoglobin saturation (SaO_2) because under normal physiological conditions, haemoglobin-bound oxygen constitutes the majority (~ 97%) of total blood oxygen content. However, accurate assessment of SaO_2 in rhinoceros remains a challenge, especially during severe hypoxaemia. The difficulty is compounded by limitations of commonly used monitoring devices and severe hypoxaemia can sometimes evade detection because most devices are only certified to monitor SaO_2 between 70 and 100%. In immobilised white rhinoceroses, the Nonin PalmSAT pulse oximeter (Nonin Medical Inc, North Plymouth, USA) with the transflectance probe attached to the third eyelid is a reliable estimate of SaO_2 when levels are ≥ 70%, but not when they are < 70% [4, 5]. Therefore, alternative methods to detect and quantify severe hypoxaemia are needed.

Benchtop co-oximeters reliably measure SaO_2 across the entire 0-100% saturation range and are considered the "gold-standard", but they are typically used only in dedicated laboratories as they are expensive, limited to blood oxygen saturation measurements, and are sensitive to changing environmental conditions in the field. By comparison, the Enterprise Point-of-Care (EPOC; Epocal Inc, Ottawa, Canada) blood gas analyser is used more extensively in veterinary practice because it is portable and report on a wide range of blood gas, acid-base, electrolyte and biochemical variables [6]. However, this blood gas analyser does not measure SaO_2 directly, rather it calculates it by measuring arterial oxygen partial pressure (PaO_2) and applying it to the Severinghaus algorithm constructed from the oxygen-haemoglobin dissociation curve of humans [6]. The EPOC blood gas analysers $cSaO_2$ values are reliable in immobilised impala across the saturation range of 0-100% [7], but are unreliable across the same range in immobilised white rhinoceros [8]. The unreliability for rhinoceros may in part be explained by differences in whole blood oxygen-haemoglobin dissociation curves between humans and much larger rhinoceros since there exists a shallow but significant negative scaling relationship between P_{50} (PO_2 when haemoglobin is 50% saturated with oxygen) and body mass among mammals [9–11].

The Siggaard-Andersen algorithm modified for white rhinoceros may provide an alternative method to calculate SaO_2 [12, 13]. This algorithm calculates SaO_2 as a function of PaO_2 after correcting for $PaCO_2$, pH, and body temperature (T_b) [14–16]. Haymerle and colleagues (2016) adapted the algorithm by replacing the values of selected variables with ones for white rhinoceros, including physiological $PaCO_2$ of 49.0 mmHg, T_b of 36.8 °C [17], and applying a P_{50} of 20.0 mmHg and a Bohr effect of − 0.62, both of which were based on a curve constructed from isolated haemoglobin [18]. Later, Reiners et al. (2019) collected experimental data from white rhinoceros blood to further improve the algorithm to better suit the blood characteristics of white rhinoceros but used a P_{50} of 15.0 mmHg and a Bohr effect of − 0.74, both of which were also based on a curve constructed from isolated haemoglobin. Even though the Siggaard-Andersen algorithm has been adapted for use in rhinoceros [12, 13], the reliability of its $cSaO_2$ values has never been fully validated in this species.

A.V. Hill derived a simple equation to calculate SaO_2 using the oxygen-haemoglobin dissociation curve's three key parameters [viz., Hill coefficient (*n*), PO_2 and P_{50}] [19–21]. To date, the Hill equation is still applied, modified and accepted in biomedical applications for different species [22–26]. However, it has never been used to calculate SaO_2 of rhinoceroses, an endangered species for which its blood characteristics are far less studied than those of other mammals. Therefore, it is important to determine the reliability of the Hill equation in calculating SaO_2 in rhinoceros and its potential usefulness during complex procedures where individuals are immobilised for prolonged periods, or for research purposes.

This study assessed the reliability of three methods for calculating SaO_2 ($cSaO_2$) in immobilised white rhinoceroses, while an AVOXimeter 4000 co-oximeter that measures SaO_2 directly provided a reference method. The first method assessed, the "pH-adjusted Hill $cSaO_2$", calculated SaO_2 using the Hill equation when the body mass-estimated P_{50} was adjusted for pH alone. The second method, the "multivariable-adjusted Hill $cSaO_2$", also calculated SaO_2 using the Hill equation, but the P_{50} was adjusted for pH, base excess and T_b. The third method

assessed the "Siggaard-Andersen $cSaO_2$", using the Siggaard-Andersen algorithm modified for white rhinoceros and adjusted for pH and $PaCO_2$ [13]. We also derived oxygen-haemoglobin dissociation curves from the co-oximeter SaO_2 measurements and $cSaO_2$ values to visualise the relationship between PaO_2 and oxygen saturation across the range of measured values. We hypothesise that the calculations returned by the Hill equations and Siggaard-Andersen algorithm, which incorporate dynamic shifts in oxygen-haemoglobin binding characteristics due to local blood biochemistry, are reliable across the entire saturation range of 0-100%. We further hypothesise that these calculations-based methods will show marked improvement in reliability over previous pulse oximetry analyses at saturations below 70% in immobilised white rhinoceros.

Materials and methods

Experimental animals

This study was approved by the University of Pretoria Animal Ethics Committee (V035-17 and REC246-19) and the South African National Parks Animal Use and Care Committee (no. 001/16 and 012/20). A convenience sample of 16 wild-caught male white rhinoceros, 4–5 years old, were brought to a dedicated holding facility (bomas) at the Veterinary Wildlife Services, Kruger National Park, South Africa (23° 49' 60 S, 31° 30' 0 E; altitude ~320 m altitude), where they were habituated to boma-captivity for 4–6 weeks. The animals were fed lucerne (*Medicago sativa*) and teff hay (*Eragrostis tef*), and provided water *ad libitum*. Some of the data recorded from this selection of rhinoceros have been published in related studies [4, 5, 8] and are presented here for comparison only.

Immobilisation

The first group of rhinoceros (group 1, $N = 8$) underwent two immobilisation events induced by; (1) etorphine (M99; Voluplex., Mnandi, Centurion, South Africa) followed by an injection of butorphanol (Butonil; Wildlife Pharmaceuticals Pty Ltd, White River, South Africa), and (2) etorphine followed by injection of 0.9% NaCl solution (control). The second group of rhinoceros (group 2, $N = 8$) underwent four immobilisation events induced by; (1) etorphine and azaperone (Stresnil; Janssen Pharmaceuticals., Midrand, South Africa) (2), etorphine and midazolam (Dazonil; Wildlife Pharmaceuticals Pty Ltd) (3), etorphine and medetomidine (Metonil; Wildlife Pharmaceuticals Pty Ltd) and (4) etorphine and NaCl solution (control), with intravenous butorphanol (Butonil; Wildlife Pharmaceuticals Pty Ltd) administered at 12 min into recumbency during each immobilisation. The drugs were administered intramuscularly using a 3 mL dart with 60 mm uncollared needle propelled by a compressed air rifle (DAN-INJECT International SA., Skukuza, South Africa). The doses were calculated based on body mass (see Tables S1 and S2). To improve sample size and maximise the recorded range of SaO_2 values for analyses, data from both groups of rhinoceros (collected using the same devices and methods) were included in this study.

Collection of arterial blood samples

Once the rhinoceros were in lateral recumbency, a digital thermometer (HI 98509 Checktemp 1; Hanna Instruments., Woonsocket RI, USA) with a modified protective sheath was placed inside the rectum to measure core body temperature (T_b). The inner surface of the upwardly-oriented ear was aseptically prepared and a 25 cm, 22 Gauge over-the-needle intravenous (IV) catheter (Introcan; B Braun Medical Inc., Melsungen, Germany) was inserted into the medial auricular artery for blood sample collection. Before blood sample collection, the catheter lines were flushed with heparinised saline and drained long enough to sufficiently wash out any saline or blood clots. In group 1, blood was collected anaerobically at 30, 40, 50, and 60 min after recumbency and in group 2, blood was collected at 10, 15, 20, 30, and 40 min, and then at 60 min additional blood samples were collected during oxygen insufflation. Blood was drawn into 3 mL heparinised syringes (BD Present A-Line, BD Medical,

New Jersey, USA) and immediately sealed, placed on ice, and analysed within 15 min of collection in an in-door laboratory. Two 3 mL heparinised syringes at each time point were completely filled with blood to ensure adequate blood samples for analysis. Once sampling was complete, the effects of etorphine were partially reversed through intravenous administration of butorphanol, into an auricular vein. Each rhinoceros was prompted to rise and move into a crate, where its body mass was measured. Full reversal was achieved with naltrexone (Table S1 and S2).

Measurement of arterial oxygen-haemoglobin saturation (SaO_2) using benchtop co-oximetry

Arterial oxygen-haemoglobin saturation (SaO_2) was measured from the blood samples using test cuvettes and a calibrated benchtop co-oximeter (AVOXimeter 4000; Surgical Innovations Pty Ltd., South Africa). Calibrations were performed daily using yellow and orange optical filters (cuvettes), which also verified that the optics were not obscured by blood or debris (AVOXimeter 4000 user manual). Blood was mixed by gently rolling the syringe back-and-forth before it was injected into a test cuvette for analysis. The benchtop AVOXimeter 4000 uses multiple wavelengths of light (488.4, 520.1, 562.4, 585.2, 597.5, 621.7 and 671.7 nm) to measure fractional SaO_2, carboxyhaemoglobin (HbCO) and methaemoglobin (MetHb). Fractional SaO_2 recorded from the co-oximeter were used as the reference measurements. Although the co-oximeter used in this study was designed for use in humans, all its spectrophotometry algorithms are likely broadly applicable to rhinoceros because, the spectrophotometric characteristics of haemoglobin (i.e., infrared absorbance of oxyhaemoglobin and deoxyhaemoglobin) are reportedly similar among human, horse and white rhinoceros [13].

Measurements of arterial PaO_2, $PaCO_2$, and pH using blood gas analysis

Arterial oxygen and carbon dioxide partial pressures (PaO_2 and $PaCO_2$), and pH were measured from the blood samples using an Enterprise Point-of-Care (EPOC) portable blood gas analyser (EPOC software version 3.35.4 with sensor configuration version 39.2; Epocal Inc., Ottawa, Canada). The test cards were single use and self-calibrated by the EPOC blood gas analyser before analysis. Blood was injected into the test cards placed inside the EPOC blood gas analyser while making sure no air bubbles were introduced. The test cards house a Clark-type PaO_2 sensing electrode, $PaCO_2$ sensing electrode and a pH sensing electrode. The test cards also house reference electrodes, counter electrodes, and calibration fluids of known PaO_2, $PaCO_2$ and pH (EPOC system manual). The EPOC blood gas analyser calculates bicarbonate ions ($cHCO_3^-$), base excess and haemoglobin concentration according to the equations provided in Table 1.

Calculation of arterial oxygen-haemoglobin saturation ($cSaO_2$)

We calculated SaO_2 ($cSaO_2$) using the two Hill equation methods and the Siggaard-Andersen algorithm. To achieve this, SaO_2 was calculated using the Hill Eq. (19), which describes the SaO_2 of a multi-subunit haemoglobin protein as a function of PaO_2. The Hill equation requires measurements or estimates of P_{50}, Hill exponent (n), and PaO_2 (mmHg) (Table 1). The initial P_{50} value was estimated for each rhinoceros given its body mass (which ranged between 980 and 1467 kg) according to a scaling relationship ($P_{50} = 39.189 \times \text{body mass}^{-0.035}$) derived from 66 species of terrestrial mammal [10]. The Hill exponent was assigned a value of 3, it is known to typically vary between 2.8 and 3 under normal physiological conditions in mammals [27]. The PaO_2 was measured directly by the EPOC blood gas analyser.

To account for the dynamic shifts of the body mass-estimated P_{50} brought about by local blood biochemistry, we used algebraic substitution to factor in the Bohr effect (i.e., the influence of pH on the P_{50}), base excess and T_b. The rhinoceros Bohr coefficient (Φ) was estimated as − 0.5, which is based on a scaling equation [$\Phi = 0.038 \times \ln(\text{body mass}) - 0.768$] showing the relationship between Bohr effect and body mass of 10 species of terrestrial mammals [28]. To account for a Bohr effect due to changes in CO_2 and bicarbonate ions, a correction of

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Table 1 Calculation of arterial oxygen-haemoglobin saturation by the Hill equation and the Siggaard-Andersen algorithm. Calculated arterial oxygen-haemoglobin saturation ($cSaO_2$) using the Hill equation when (i) the body mass-estimated P_{50} was adjusted for pH alone (pH-adjusted Hill $cSaO_2$) and when (ii) the body mass-estimated P_{50} was adjusted for changes in pH, base excess and body temperature (multivariable-adjusted Hill $cSaO_2$). The Siggaard-Andersen algorithm previously modified for white rhinoceros was also used to derive $cSaO_2$ (Siggaard-Andersen $cSaO_2$)

	Equations for $cSaO_2$ (%)	Comments
Hill equation	$cSaO_2$ $= \frac{P50^n}{PaO_2{}^n + P50^n}$	P_{50} (mmHg) is the partial pressure of oxygen when haemoglobin is 50% saturated with oxygen, and was estimated given body mass (which ranged between 980 to 1467 kg) according to the scaling equation $P_{50} = 39.18 \times \text{body mass}^{-0.035}$ derived from 66 species of terrestrial mammal (10). P_{50} was adjusted for pH alone for "pH-adjusted Hill $cSaO_2$". P_{50} was adjusted for pH, base excess and temperature for "multivariable-adjusted Hill $cSaO_2$. pH was measured directly by the EPOC blood gas analyser. Base excess was calculated by the EPOC blood gas analyser using the equation BE = (1–0.014cHgb) × ($cHCO^{3-}$ – 24.8 + (1.43×cHgb+7.7) × (pH – 7.4)). Bicarbonate ions were calculated by the EPOC blood gas analyser using the equation log $cHCO_3{}^- = pH + \log PaCO_2 - 7.608$. To calculate SaO_2, the Hill exponent (n) was assigned as 3 and PaO_2 (mmHg) is partial pressure of oxygen (mmHg) at body temperature was measured by the EPOC blood gas analyser.
Siggaard-Andersen algorithm	$cSaO_2 = \frac{e^y}{(1 + e^y)}$	Here, $y = ys + x - x_0 + h \times \tanh[k \times (x - x_0)]$, where ys is 2.177, h is 1.835 and k is 1.1169 (13). x_0 was calculated using the equation $x_0 = xs + a$ (13, 14), where xs is 1.431 (13–15) and $a = [(-1) \times \ln(10) \times \beta] \times (pH - 7.4) + 1.38 \ln(1 + PaCO_2/5.33) \times (pH - 7.2)]$, where β is the Bohr shift of rhinoceros haemoglobin and was measured as −0.74. $PaCO_2$ and pH were measured by the EPOC blood gas analyser.

0.0013 × BE(b) was used, where BE (b), the base excess of rhinoceros blood was calculated by the EPOC blood gas analyser (Table 1). To account for temperature sensitivity, a human-derived temperature coefficient of 0.024 was used [6, 29]. The adjustments on the P_{50} were used in the calculations of SaO_2 when the initial body mass-estimated P_{50} was adjusted for pH alone ("pH-adjusted Hill $cSaO_2$") and when the initial P_{50} was adjusted for pH, base excess and T_b ("multivariable-adjusted Hill $cSaO_2$").

Arterial oxygen-haemoglobin saturation was also calculated using the Siggaard-Andersen algorithm previously modified for white rhinoceros [13]. Human blood values were replaced with those for white rhinoceros measured by Reiners and colleagues (2019) including P_{50} of 15.0 mmHg and a Bohr effect of − 0.74 [13]. Furthermore, PaO_2, $PaCO_2$ and pH measured using the EPOC blood gas analyser were incorporated into the algorithm to calculate Siggaard-Andersen $cSaO_2$ values (Table 1).

Statistical analyses

A Bland-Altman analysis for multiple observations [30] was used to determine the agreement between the calculated SaO_2 values and the co-oximeter SaO_2 measurements (i.e., $cSaO_2$ versus SaO_2). The analysis determined the accuracy (bias), precision [standard deviation (SD)], and the 95% limits of agreement (LOA) between the paired $cSaO_2$-SaO_2 measurements [30]. An area root mean squares (ARMS), which is a combined estimate of accuracy (bias) and precision (SD), was also calculated to determine the overall reliability of the $cSaO_2$ values compared to the SaO_2 measurements [31]. Guidelines for clinical acceptability are not available for $cSaO_2$ values, and so for consistency with our previously published analyses on rhinoceros, we followed the International Organisation for standardisation (ISO) performance requirements for pulse oximetry in humans, which states that the method is clinically reliable only when the comparisons with co-oximetry are ≤± 3% for accuracy (bias), ≤ 3% for precision (SD) and ≤ 4% for ARMS [31, 32].

Co-oximeter SaO_2, pH-adjusted Hill $cSaO_2$, multivariable-adjusted Hill $cSaO_2$, and Siggaard-Andersen $cSaO_2$ were plotted against PaO_2 measured by the EPOC blood gas analyser to construct arterial oxygen-haemoglobin dissociation curves. A "variable-slope sigmoidal response" model was applied to determine the least squares regression fit equations from the oxygen-haemoglobin dissociation curves and calculate the curve parameters (viz., Bmax, Kd and Hill slope). The Bmax is the maximum specific binding of oxygen to haemoglobin, Kd is the P_{50}, and the Hill slope represents the haem-haem interaction or co-operativity. Extra sum-squares F test was used to determine whether the parameters of the curves constructed using the Hill and Siggaard-Andersen $cSaO_2$ values differ from those of the curve constructed using co-oximeter SaO_2. Statistical analyses were performed using GraphPad Prism version 9.3.1 (GraphPad Software, La Jolla, CA, USA) and R4.4.0 statistical software (R Core Team, 2020; R Foundation for Statistical Computing, Austria; https://www.R-project.org). Data are reported as mean ± SD. P value of < 0.05 was considered statistically significant.

Results

The reliability of the pH-adjusted Hill $cSaO_2$

On average, rhinoceros were hypoxaemic [PaO_2 of 51.1 ± 22.2 mmHg (mean ± standard deviation)], hypercapnic ($PaCO_2$ of 73.9 ± 10.7 mmHg), acidotic (pH of 7.3 ± 0.1), and normothermic [body temperature (T_b) of 37.4 ± 1.8 °C] (Figure S1). When compared to the co-oximeter SaO_2 reference measurements, arterial oxygen-haemoglobin saturation calculated by the Hill equation when P_{50} is adjusted for pH alone were accurate, precise and overall reliable across the 70–100% saturation range (Table 2). Below 70%, $cSaO_2$ values were accurate, but imprecise and were above the reliability threshold of ≤4% (ARMS of 7%). Across the entire range of 0-100%, $cSaO_2$ values were accurate, but imprecise (SD of 5% vs. recommended ≤ 3%) and were thus slightly above the reliability threshold of ≤4% (ARMS of 5%). The arterial oxygen-haemoglobin dissociation curve constructed

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Table 2 Bland-Altman analysis and area root mean squares (ARMS) as a measure of reliability of $cSaO_2$ values when compared to co-oximeter SaO_2 measurements in sixteen immobilised white rhinoceros (*Ceratotherium simum*). Arterial oxygen-haemoglobin saturation ($cSaO_2$) was calculated using the Hill equation when P_{50} (i.e., PO_2 when haemoglobin is 50% saturated with oxygen) is adjusted for pH alone (pH-adjusted Hill $cSaO_2$) and when P_{50} is adjusted for changes in pH, base excess of blood and body temperature (multivariable-adjusted Hill $cSaO_2$). Arterial oxygen-haemoglobin saturation ($cSaO_2$) was also calculated from the Siggaard-Andersen algorithm (Siggaard-Andersen $cSaO_2$) previously modified for white rhinoceros. For further comparisons, previously published data [4, 5, 8] of arterial oxygen-haemoglobin saturation calculated by the Enterprise Point-of-Care (EPOC) blood gas analyser (EPOC $cSaO_2$) and peripheral arterial oxygen-haemoglobin saturation measured by the Nonin PalmSAT pulse oximeter when a transflectance probe was placed under the third eyelid (pulse oximetry SpO_2) from the same white rhinoceros are represented. LOA, limits of agreement (minimum to maximum). n represents the sample size of paired measurements

Type of method	Ranges (%)	Sample size (*n*)	Bias (%)	Precision (%)	ARMS (%)	LOA
pH-adjusted Hill $cSaO_2$	0–100	379	0*	5	5	-9 to 9
	70–100	**281**	**0***	**3***	**3***	**-6 to 6**
	< 70	98	1*	7	7	-13 to 15
	70–79	**34**	**2***	**3***	**3***	**-3 to 7**
	80–89	**109**	**0***	**3***	**3***	**-6 to 7**
	90–100	**127**	**-1***	**3***	**3***	**-7 to 4**
Multivariable-adjusted Hill $cSaO_2$	0–100	329	-2*	5	5	-11 to 7
	70–100	**250**	**-2***	**4**	**4***	**-9 to 7**
	< 70	79	-4*	8	9	-17 to 8
	70–79	**25**	**-1***	**4**	**4***	**-19 to 11**
	80–89	**100**	**-1***	**4**	**4***	**-9 to 6**
	90–100	**115**	**-2***	**3***	**4***	**-9 to 5**
Siggaard-Andersen $cSaO_2$	0–100	389	10	9	13	-8 to 28
	70–100	289	6	4	7	-3 to 14
	< 70	100	23	7	24	9 to 36
	70–79	40	13	2*	13	8 to 17
	80–89	114	8	2*	8	4 to 12
	90–100	**135**	**2***	**2***	**3***	**-2 to 7**
EPOC $cSaO_2$ (8)	0–100	389	-6	5	8	-16 to 3
	70–100	289	-6	4	8	-14 to 1
	< 70	100	-7	6	9	-19 to 6
	70–79	40	-8	3*	8	-14 to -2
	80–89	112	-7	4	8	-15 to 1
	90–100	137	-5	4	7	-13 to 3
Pulse oximeter SpO_2 (4, 5)	0–100	266	1*	6	6	-12 to 13
	70–100	**226**	**-1***	**4**	**4***	**-9 to 8**
	< 70	40	8	10	16	-11 to 28
	70–79	25	2*	5	6	-8 to 13
	80–89	**92**	**0***	**4**	**4***	**-8 to 8**
	90–100	**109**	**-2***	**4**	**4***	**-10 to 6**

*Results considered acceptable for oxygen-haemoglobin saturation measurements (bias ≤ ± 3%, precision ≤ 3%, ARMS ≤ 4%) according to United States Food and Drug Administration (FDA) and the International Organisation for Standardisation (DIN EN ISO 80601-2-61)

using the pH-adjusted Hill $cSaO_2$ was statistically different from one constructed using co-oximeter SaO_2 measurements ($P=0.0126$), but nonetheless demonstrated a closer alignment to this reference curve than other methods (Fig. 1; Table 3).

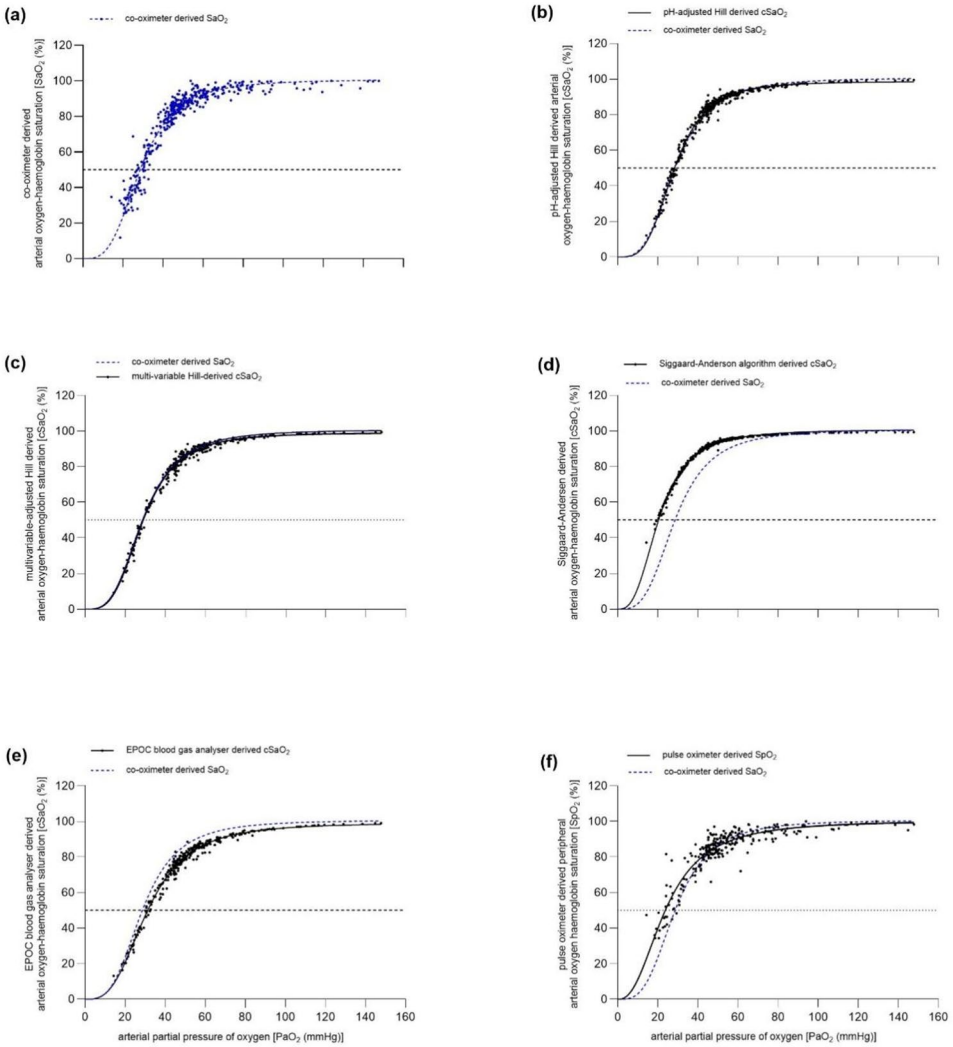

Fig. 1 Oxygen-haemoglobin dissociation curves constructed from arterial oxygen-haemoglobin saturation measurements (SaO_2) and calculated arterial oxygen-haemoglobin saturation values ($cSaO_2$) in sixteen immobilised white rhinoceros (*Ceratotherium simum*). The curves show the relationship between arterial partial pressure of oxygen (PaO_2) measured by the EPOC blood gas analyser and (**a**) arterial oxygen-haemoglobin saturation (SaO_2) measured by a co-oximeter as a reference method (i.e., co-oximeter derived SaO_2). The curve constructed from the co-oximeter SaO_2 was compared to the curves constructed from the Hill equation when (**b**) body mass-estimated P_{50} (i.e., partial pressure of oxygen when haemoglobin is 50% saturated with oxygen) is adjusted for pH alone (pH-adjusted Hill $cSaO_2$), when (**c**) body mass-estimated P_{50} is adjusted for pH, base excess and body temperature (multivariable-adjusted Hill $cSaO_2$), and from (**d**) the Siggaard-Andersen algorithm (Siggaard-Andersen $cSaO_2$). For further comparisons, previously published data [4, 5, 8] of (**e**) arterial oxygen-haemoglobin saturation calculated by the Enterprise Point-of-Care (EPOC) blood gas analyser (EPOC $cSaO_2$) and (**f**) peripheral arterial oxygen-haemoglobin saturation measured by the Nonin PalmSAT pulse oximeter when a transflectance probe was placed under the third eyelid (pulse oximeter SpO_2) from the same white rhinoceros are represented. The data was plotted using the variable-slope sigmoidal response model in GraphPad Prism version 9.4.1

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Table 3 Best-fit models of the arterial oxygen-haemoglobin dissociation curves derived from sixteen immobilised white rhinoceros (*Cerototherium simum*). The oxygen-haemoglobin dissociation curve represents the relationship between arterial partial pressure of oxygen (PaO_2) and the arterial oxygen-haemoglobin saturation (SaO_2). The curve constructed from the co-oximeter SaO_2 measurements was compared to the curves constructed from the Hill equation when P_{50} (i.e., PO_2 when haemoglobin is 50% saturated with oxygen) is adjusted for pH alone (pH-adjusted Hill $cSaO_2$), when P_{50} is adjusted for pH, base excess and body temperature (multivariable-adjusted Hill $cSaO_2$), and from the Siggaard-Andersen algorithm previously modified for white rhinoceros (Siggaard-Andersen $cSaO_2$). For further comparisons, previously published data [4, 5, 8] of arterial oxygen-haemoglobin saturation calculated by the Enterprise Point-of-Care (EPOC) blood gas analyser (EPOC $cSaO_2$) and peripheral arterial oxygen-haemoglobin saturation measured by the Nonin PalmSAT pulse oximeter when a transflectance probe was placed under the third eyelid (pulse oximeter SpO_2) from the same white rhinoceros are represented. Bmax represents the maximum specific binding of oxygen to haemoglobin, Kd (i.e., P_{50}) is the partial pressure of oxygen when haemoglobin is 50% saturated with oxygen and the Hill slope is the haem-haem interaction or co-operativity of haemoglobin in arterial blood samples. The data was plotted using the variable-slope sigmoidal response model in GraphPad Prism version 9.4.1

	sample size (*n*)	Bmax (%)		Kd [P_{50} (mmHg)]		Hill slope		
		Best-fit values	95% CI	Best-fit values	95% CI	Best-fit values	95% CI	*P* value
co-oximeter SaO_2	389	100.8	99.25 to 102.4	28.71	28.27 to 29.17	3.251	3.075 to 3.435	
pH-adjusted Hill $cSaO_2$	398	98.8	98.13 to 99.41	28.14	27.96 to 28.33	3.514	3.426 to 3.749	0.0126*
Multivariable-adjusted Hill $cSaO_2$	398	98.5	97.68 to 99.39	29.32	29.08 to 29.57	3.630	3.514 to 3.749	0.0285*
Siggaard-Andersen $cSaO_2$	417	100.9	100.6 to 101.2	20.48	20.37 to 20.58	2.791	2.741 to 2.842	< 0.0001*
EPOC $cSaO_2$ (8)	417	98.9	98.08 to 99.74	31.51	31.26 to 31.76	3.081	3.005 to 3.159	< 0.0001*
Pulse oximeter SpO_2 (4, 5)	264	100.4	98.06 to 103.0	23.76	22.94 to 24.98	2.407	2.167 to 2.662	< 0.0001*

*Represents differences between the curves when compared to the reference method curve derived from co-oximeter SaO_2 reference measurements

The reliability of the multivariable-adjusted Hill $cSaO_2$

When compared to the co-oximeter SaO_2 measurements, arterial oxygen-haemoglobin saturation calculated by the Hill equation when P_{50} is adjusted for pH, base excess of blood and T_b, were accurate, precise and reliable across 70–100% saturation range. Below 70%, $cSaO_2$ values were accurate, but imprecise and above the reliability threshold of ≤4% (ARMS of 9%). Across the entire range of 0-100%, $cSaO_2$ values were accurate, but slightly imprecise (SD of 5% vs. recommended≤3%) and were above the reliability threshold of ≤4% (ARMS of 5%). The arterial oxygen-haemoglobin dissociation curve constructed using the multivariable-adjusted Hill $cSaO_2$ was statistically different from the one constructed using co-oximeter SaO_2 measurements ($P=0.0285$), but demonstrated close alignment with the reference curve (Fig. 1; Table 3).

The reliability of the Siggaard-Andersen $cSaO_2$

When compared to the co-oximeter SaO_2 measurements, arterial oxygen-haemoglobin saturation values calculated by the Siggaard-Andersen algorithm were inaccurate, imprecise and unreliable across the 70–100% saturation range and below 70%. Across the entire range of 0-100%, $cSaO_2$ values were inaccurate, imprecise and were above the reliability threshold of ≤4% (ARMS of 24%). Only across the narrow range of 90–100%, were the $cSaO_2$ values accurate, precise and reliable (ARMS of 3%). The arterial oxygen-haemoglobin dissociation curve constructed using the Siggaard-Andersen $cSaO_2$ values was statistically different ($P=0.0001$) and noticeably misaligned compared to the co-oximeter curve (Fig. 1; Table 3). Across all three methods, similar patterns were observed when data from the two groups of rhinoceros were analysed separately across the 0-100% range (Tables S4, S5 and S6).

Discussion

This study demonstrates that SaO_2 calculated by the (i) Hill equation when the body mass-estimated P_{50} of white rhinoceros is adjusted for pH alone (pH-adjusted Hill $cSaO_2$) and (ii) Hill equation when the body mass-estimated P_{50} is adjusted for pH, base excess and body temperature (multivariable-adjusted Hill $cSaO_2$) were reliable at saturation ranges of 70–100% (ARMS of 3% and 4%, respectively). Across the same saturation range, both Hill equation methods are as reliable as pulse oximetry when the transflectance probe is attached to the third eyelid [4, 5]. Although the pH-adjusted Hill $cSaO_2$ did not meet the reliability threshold of ≤ 4% when saturation levels are below 70% (ARMS of 7%), it demonstrated marked improvement in performance over previously published pulse oximeter SpO_2 measurements from the third eyelid (ARMS of 16%) [4, 5] as well as alternative calculation methods [8] across this range. The SaO_2 values calculated by the Siggaard-Andersen algorithm (Siggaard-Andersen $cSaO_2$), however, performed poorly at ranges of 70–100% and < 70%, and were reliable only across the 90–100% range. Overall, the pH-adjusted Hill $cSaO_2$ method is the simplest because it only requires knowledge of body mass, PaO_2 and pH. Although $cSaO_2$ demonstrated good trending ability relative to co-oximeter SaO_2 measurements, $cSaO_2$ provides discrete estimates of oxygen saturation rather than continuous, real-time trends offered by pulse oximetry. Therefore, the Hill equation may represent a more appropriate additional tool to pulse oximetry, especially for severely hypoxaemic rhinoceroses or when blood oxygenation is monitored during prolonged or complex procedures and for research purposes where more accurate estimates of SaO_2 are required.

pH-adjusted Hill $cSaO_2$

This is the first study to calculate SaO_2 of immobilised white rhinoceros using the simple Hill equation. Using the Hill equation when the body mass-estimated P_{50} of white rhinoceros is adjusted for changes in pH, by assuming a Bohr effect of − 0.5 (pH-adjusted Hill $cSaO_2$), provides a reliable method to calculate SaO_2 values at saturation

levels across the range of 70–100% (Table 2). The pH-adjusted Hill $cSaO_2$ values are as reliable as the pulse oximeter SpO_2 values above 70% when a transflectance probe is attached to the third eyelid and are more reliable than when the probes are attached to other sites of the body (i.e., ear, cheek, rectum and tail) [4, 5]. At saturation levels below 70%, the pH-adjusted Hill $cSaO_2$ yielded an ARMS of 7%, representing an improvement over our previously published pulse oximeter SpO2 measurements from the third eyelid (ARMS of 16%) and other assessed attachment sites (ARMS > 13%) [4, 5]. The pH-adjusted Hill $cSaO_2$ values are likely more reliable compared to the other methods because it takes into account three important factors that influence the oxygen-haemoglobin binding characteristics: (i) the position of the oxygen-haemoglobin dissociation curve under normal physiological conditions, (ii) the Bohr effect as oxygen-haemoglobin binding affinity varies with changes in pH and (iii) cooperativity (Hill coefficient of 3).

The immobilised rhinoceros in this study were hypoxaemic, hypercapnic, and acidotic, which is likely attributed to the negative respiratory effects of the opioids. The higher P_{50} of 28.7 mmHg observed in the curve constructed using reference co-oximeter SaO_2 measurements may be consistent with a rightward shift associated with hypercapnia and acidosis. The curve constructed using pH-adjusted Hill $cSaO_2$ values (P_{50} of 28.1 mmHg) closely mirrors the curve derived from the co-oximeter SaO_2 measurements despite the statistically significant difference between the curves (Fig. 1). The alignment of the curves lends support to the reliability of the pH-adjusted Hill $cSaO_2$ values when compared with the co-oximeter SaO_2 measurements. Therefore, the Hill equation enables the development of a rhinoceros-specific SaO_2 calculator for both awake and immobilised animals (https://edwardsnelling.github.io/Rhinox/). The findings support the use of $cSaO_2$ as a complementary method to pulse oximetry rather than as a replacement for continuous SpO_2 monitoring in the field. Additional analyses of the trending ability of the calculated methods are reported in the Supplementary Material. Although $cSaO_2$ demonstrated good trending ability relative to co-oximeter SaO_2 measurements, $cSaO_2$ provides discrete estimates of arterial oxygen saturation based on blood-gas measurements and therefore does not provide the continuous, real-time trends offered by pulse oximetry.

Multivariable-adjusted Hill $cSaO_2$

The arterial oxygen-haemoglobin dissociation curve also shifts with changes in $PaCO_2$ and T_b in some mammals [27]. The curve constructed using multivariable-adjusted Hill $cSaO_2$ values (P_{50} of 29.3 mmHg) also closely mirrors the curve derived from co-oximeter SaO_2 measurements, despite the statistically significant difference between the curves (Fig. 1; Table 3). When additional variables (i.e., coefficients for base excess of blood and T_b) were included to adjust the P_{50} (multivariable-adjusted Hill $cSaO_2$), the $cSaO_2$ were once again reliable at 70–100% and again performed markedly better than SpO_2 below 70%. Nonetheless, the additional variables do not further improve the reliability of the $cSaO_2$ values, especially below 70% saturation ranges, and so the simpler pH-adjusted Hill $cSaO_2$ method is preferred. The coefficient variables incorporated into the multivariable-adjusted $cSaO_2$ were previously measured in human whole blood [29, 33] and no data are currently available for white rhinoceros. Therefore, using rhinoceros specific coefficient variables may further improve the reliability of the multivariable-adjusted $cSaO_2$ values especially at saturation ranges below 70%, which may become more important in cases where $PaCO_2$ and T_b deviate significantly from normal during immobilisation.

Siggaard-Andersen $cSaO_2$

Arterial oxygen-haemoglobin saturation values calculated using the Siggaard-Andersen algorithm previously modified for white rhinoceros are unreliable above 70% and below 70% saturation ranges when compared with the co-oximeter SaO_2 measurements. The Siggaard-Andersen $cSaO_2$ are reliable only across the narrow 90–100% saturation range. This algorithm modified for white rhinoceros applied coefficients obtained from isolated haemoglobin (Bohr effect of − 0.74), which may not accurately reflect in-vivo situations, and therefore may explain the unreliable $cSaO_2$ values [13, 18]. The oxygen-haemoglobin dissociation curve constructed using the

Siggaard-Andersen $cSaO_2$ values align poorly with the co-oximeter curve. The findings in this study demonstrate the importance of correctly accounting for species-specific factors such as whole blood P_{50} and Bohr effect when calculating SaO_2.

Conclusion

In conclusion, using the Hill equation when the estimated P_{50} of white rhinoceros is adjusted for changes in pH alone to calculate SaO_2 (pH-adjusted Hill $cSaO_2$) gives reliable values at saturations of 70–100%. Although the pH-adjusted Hill $cSaO_2$ method does not meet the reliability threshold of ≤4% when saturation levels are below 70%, it does show a marked improvement in performance over pulse oximetry as well as the alternative calculation methods. Therefore, the pH-adjusted $cSaO_2$ method can be used as an additional tool to pulse oximetry especially during prolonged or complex procedures and for research purposes where more accurate estimates of SaO_2 are required. Thus, the findings did not support the first hypothesis that the calculation methods are reliable across the entire saturation range of 0-100%, but provided support for the second hypothesis that the Hill equation methods show marked improvement over previous pulse oximetry analyses below 70%. A simple calculator is therefore presented to allow wildlife veterinarians and research scientists to convert conventional blood gas variables into SaO_2 values for immobilised white rhinoceros (https://edwardsnelling.github.io/Rhinox/).

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Data availability The datasets generated and/or analysed during the current study are available in the University of Pretoria data repository (https://figshare.com/s/19a443e32669219389a0).

Declarations

Ethics approval and consent to participate This study was approved by the University of Pretoria Animal Ethics Committee (V035-17 and REC246-19) and the South African National Parks Animal Use and Care Committee (no. 001/16 and 012/20). The studies were carried out in accordance with the committees' guidelines and regulations. Consent to collect samples in this study was granted by the University of Pretoria's Animal and Research Ethics Committees (V035-17 and REC246-19) and the South African National Park's (custodians of the study animals) Scientific and Animal Use and Care Committee (no. 001/16 and 012/20).

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