SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2024v45n5p1479

Venous blood gas analysis in green-billed toucan (*Ramphastos dicolorus* Linnaeus, 1766)

Parâmetros gasométricos em tucano-de-bico-verde (*Ramphastos dicolorus* Linnaeus, 1766)

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Highlights

The results can be used as a reference for the species.
Venous blood offers blood gas results for specific parameters in avian medicine.
The blood gas data fill a knowledge gap and set a key baseline for future studies.

Abstract

The class birds accounts for the largest number of visits to Wildlife Rescue Centers in Brazil, and complementary tests have played an important role in the monitoring and prognostic evaluation of avian patients. Blood gas analysis is a complementary test that allows the analysis of blood gases and can be used to obtain information on acid-base balance, monitor tissue perfusion and metabolism, pulmonary and renal system impairment, and prognostic evaluation of patients. Few studies have established blood gas parameters for birds, and there are no published studies for the species *Ramphastos dicolorus.* Therefore, this study aimed to establish venous blood gas parameters in a group of green-billed toucans (*R. dicolorus*) treated at the Wildlife Rehabilitation Center and Wildlife Rescue Center (NURFS/CETAS) of the Federal University of Pelotas (UFPel). The animals were physically restrained, and samples were collected by puncture of the right jugular vein. The processing was performed in automatic equipment, in which the potential of hydrogen (pH), partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), bicarbonate (HCO_3), base excess (BE), sodium (Na), chloride (Cl), ionized calcium (iCa), potassium (K), and anion gap (AG) were measured. All animals included in this study were considered healthy, the required blood collection was 0.5 ml for all animals, and the sample temperature was standardized at 40 °C. The obtained mean and standard deviation of the evaluated parameters were as follows: pH (7.5 ± 0.1), PO_2 (45.4 ± 5.5), PCO_2 (34.9 ± 6.5 mmHg), $SatO_2$ (85.3 ± 5.8%), HCO_3 (28.0 ± 2.3 mmol/L), TCO_2 (29.1 ± 2.3), BE (5.0 ± 2.5 mmol/L), Na (147.9 ± 2.9 mmol/L), Cl (108.4 ± 3.3 mmol/L), iCa (1.2 ± 0.0 mmol/L), K

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Received: Apr. 03, 2024 Approved: Ago. 28, 2024

(3.8 ± 0.6 mmol/L), and AG (15.3 ± 1.8 mmol/L). Thus, our study provides venous blood gas analysis data previously unknown for the species, with results that can be used as reference values in the clinical and prognostic routine of these birds.
Key words: Birds. Blood gas analysis. Ramphastos dicolorus. Venous blood. Wild. Green-billed toucan.

Resumo

A classe das aves corresponde ao maior número de atendimentos em Centros de Triagem de Animais Silvestres no Brasil, e os exames complementares estão assumindo um papel importante para monitoramento e avaliação prognóstica do paciente aviário. A gasometria é um exame complementar que possibilita a análise dos gases sanguíneos, e pode ser utilizado para obtenção de informações do equilíbrio ácido-base, monitoramento da perfusão e metabolismo tecidual, comprometimento do sistema pulmonar e renal e avaliação prognóstica do paciente. Há poucos estudos que estabelecem parâmetros gasométricos para aves, sendo que para a espécie *Ramphastos dicolorus* não existe nenhum estudo publicado até o momento. Desta forma, o objetivo deste trabalho foi estabelecer os parâmetros gasométricos venosos em um grupo de tucanos-de-bico-verde (*R. dicolorus*), atendidos no Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres (NURFS/CETAS) da Universidade Federal de Pelotas (UFPel). Neste estudo, os animais foram contidos fisicamente e as amostras foram coletadas por punção da veia jugular direita. O processamento foi realizado em equipamento automático, onde foram medidos potencial hidrogeniônico (pH), pressão parcial de oxigênio (PO_2), pressão parcial de dióxido de carbono (PCO_2), bicarbonato (HCO_3), excesso de base (BE), sódio (Na), cloreto (Cl), cálcio ionizado (iCa), potássio (K) e ânion gap (AG). Todos os animais incluídos neste estudo foram considerados hígidos, a coleta de sangue pré-estabelecida foi de 0,5 ml para todos os animais e temperatura das amostras foi padronizada a 40 °C. Os resultados obtidos apresentaram uma média e desvio padrão de pH (7,5 ± 0,1), PO_2 (45,4 ± 5,5), PCO_2 (34,9 mmHg ± 6,5), $SatO_2$ (85,3% ± 5,8), HCO_3 (28,0 mmol/L ± 2,3), TCO_2 (29,1 ± 2,3), BE (5,0 mmol/L ± 2,5), Na (147,9 mmol/L ± 2,9), Cl (108,4 mmol/L ± 3,3), iCa (1,2 mmol/L ± 0,0), K (3,8 mmol/L ± 0,6) e AG (15,3 mmol/L ± 1,8). Com isso, nosso estudo fornece dados gasométricos de sangue venoso desconhecidos para a espécie, com resultados que podem ser utilizados como referência na rotina clínica e prognóstica dessas aves.
Palavras-chave: Aves. Gasometria. *Ramphastos dicolorus.* Sangue venoso. Silvestres. Tucano-de-bico-verde.

Introduction

The green-billed toucan (*Ramphastos dicolorus*) belongs to the order Piciformes and is included in the family Ramphastidae. Its distribution is mainly in areas of neotropical fauna in South America. This bird has a body length of 48 to 52 centimeters, weighs on average between 250 and 400 grams, and plays an important ecological role in the dispersal of seeds through its excreta (Sick, 1997; Dislich, 2014). These animals are usually treated in rescue centers and veterinary clinics with a history of trauma, orphans, or diseases. Therefore, complementary examinations are important tools to assist in their diagnosis and prognosis (Doneley, 2016).

The class birds accounts for the largest number of visits to Wildlife Rescue Centers (CETAS) in Brazil (Pagano et al., 2009; C. A. T. Cavalcanti & Nunes, 2019; Pinto & Santos, 2021; Cunha et al., 2022). A survey conducted at the Veterinary Hospital of the Federal University of Pelotas (E. A. N. L. D. Cavalcanti et al., 2021) showed that class birds was the main group that underwent radiographic examinations, totaling 63.1% of cases, reinforcing the importance of complementary examinations in this class as a diagnostic tool.

Blood gas analysis, among the various complementary examinations, is a method for obtaining information on acid-base balance, monitoring tissue perfusion and metabolism and pulmonary and renal systems, and prognostic evaluation of the patient (Montesinos & Ardiaca, 2013; Dutra et al., 2019). Blood gas parameters have already been described in several wild species such as *Myiopsitta monachus, Crax blumenbachii, Phoenicopterus ruber, Anas fulvigula, Spheniscus magellanicus, Chelonia mydas, Lepidochelys kempii, Pteropus giganteus,* and *Dasyprocta prymnolopha* Rettenmund et al., 2014; Gonçalves et al., 2016; Gardhouse & Eshar, 2016; Ratliff et al., 2017; Dutra et al., 2019; McNally et al., 2020; Kishbaugh et al., 2021; Muniz et al., 2022).

Few studies have been conducted on *R. dicolorus,* and those available focus on its ecology, such as nesting and reproduction (Jesus et al., 2012; Perrella & Guida, 2019), in addition to clinical case reports (Fecchio et al., 2010; Ulguim et al., 2022; Martínez, 2022). The study aims to establish the venous blood gas parameters in a group of green-billed toucans (*R. dicolorus*) received at a Wildlife Rescue Center.

Material and Methods

All sample collection procedures were authorized and performed following the recommendations of the ethics committee on the use of animals (CEUA, UFPel – Protocol No. 111/2023). The samples were collected between 2021 and 2023 from 14 specimens of green-billed toucan (*R. dicolorus*), classified as young adults of undefined sex, weighing between 250 and 465 grams, which received care at the Wildlife Rehabilitation Center and Wildlife Rescue Center (NURFS/CETAS) of the Federal University of Pelotas (UFPel).

The animals were rescued by environmental agencies and brought in for care, with a history of being collected from private property or confiscated. All birds underwent an initial assessment of their general condition, which included an assessment of eye skin turgor, mucosal coloration, an individual weighing with a concomitant assessment of the body condition score (BCS) of the pectoral muscle, cardiorespiratory auscultation, and cloacal temperature. Collections were performed only on animals that did not present associated comorbidities.

For the analyses, the animals were physically restrained, and blood samples were collected via right jugular venipuncture. The pre-established volume for blood collection was 0.5 mL, in a 1 mL syringe (BD A-Line Medical, Curitiba, Paraná, Brazil), with balanced lithium and calcium heparin, showing no difficulty in collection or insufficient volume differing between individuals. The volume established for venous blood collection corresponded to 1% of the animal's live weight (Graham et

al., 2021), with a safety margin for collection from the individual (Raghav et al., 2015).

The interval between sample collection and processing was a maximum of 5 minutes. After collection, the samples were immediately sent for gas analysis at the Veterinary Clinical Analysis Laboratory (LPCVET), located at the Veterinary Clinical Hospital of the Federal University of Pelotas (HCV-UFPEL). The samples were processed using the automatic equipment POC Cobas b 121 System (Roche Diagnóstica Brasil Ltda, São Paulo, São Paulo, Brazil) to assess pH, PO_2, PCO_2, $SatO_2$, HCO_3, TCO_2, BE, Na, Cl, iCa, K, and AG. Immediately upon the sample's arrival, it was manually stirred for 30 seconds by rolling the syringe between the hands before being connected to the equipment for parameter determination.

The data were analyzed by the software Microsoft Excel 2010 (Microsoft Office, Washington, United States) using the Reference Value Advisor supplement, and the results were presented as mean, standard deviation, median, minimum, and maximum values (Geffré et al., 2011).

Results and Discussion

It is challenging to establish whether a free-living wild population is healthy, hence the importance of defining criteria for inclusion or exclusion of individuals to describe in detail information that corroborates their health status (Friedrichs et al., 2012). All animals included in this study were considered healthy according to individual clinical evaluations. They demonstrated eye skin turgor for up to 2 seconds, normal colored mucous membranes, average weight between 250 and 465 grams with BSC classified between 2 and 4 (on a scale of 1–5), and no changes in cardiorespiratory or temperature auscultation (39 °C and 42 °C). The physical evaluation showed that all birds were in good health and had body condition scores and weights within the expected range for the species (Dislich, 2014).

Considering that the studied variables may be influenced by processing time, sample collection site, heparin concentration, exposure of the collected blood to aerobic conditions inside the syringe through bubbles, stress factors resulting from handling the animal, and temperature (Thrall et al., 2015; Graham et al., 2021; Hopper, 2023), the maximum time between collection and processing for the examinations was standardized at 5 minutes, seeking to minimize pre-analytical and temperature interferences.

The body temperature of a healthy adult bird varies between 38 and 42.5 °C (Tully et al., 2010). Blood gas analyses may be affected by the bird's high temperature, as the equipment is calibrated for humans, whose body temperature is 37 °C (Rettenmund et al., 2014; Harms et al., 2016). In this study, samples were processed at a standardized temperature of 40 °C. In previous studies, temperature standardization aimed to reduce stress caused by restraint on animals (Richardi & Nightingale, 1981; Harms et al., 2016). Other studies have shown that blood gas analyses were performed based on the individual's specific temperature (Raghav et al., 2015; Gonçalves et al., 2016; Ratliff et al., 2017; Dutra et al., 2019). However, some studies that adjusted for standard temperature found no significant differences between groups with and without

temperature standardization (Rettenmund et al., 2014; Harms et al., 2016).

The main indicators involved in acid-base regulation are pH, respiratory regulation of carbon dioxide partial pressure (PCO_2), and renal regulation of bicarbonate (HCO_3) to aid in the assessment of respiratory or metabolic acidosis and alkalosis (Hopper, 2023). Table 1 shows the mean, standard deviation, median, minimum, and maximum values of the venous blood gas analysis of the 14 green-billed toucans.

Table 1
Venous blood gas values of 14 healthy free-living green-billed toucans (*R. dicolorus*) received at a rehabilitation center

Parameter	Mean ± Standard deviation	Median	Min	Max
pH	7.5 ± 0.1	7.5	7.44	7.60
PO_2 (mmHg)	45.4 ± 5.5	46.5	35.5	52.4
PCO_2 (mmHg)	34.9 ± 6.5	34.4	19.6	43.2
$SatO_2$ (%)	85.3 ± 5.8	87.4	72.4	93.1
HCO_3 (mmol/L)	28.0 ± 2.3	28.9	23.8	31.0
TCO_2 (mmHg)	29.1 ± 2.3	29.9	24.7	32.2
BE (mmol/L)	5.0 ± 2.5	5.0	1.7	9.3
Na (mmol/L)	147.9 ± 2.9	148.2	142.9	153.0
Cl (mmol/L)	108.4 ± 3.3	108.6	103.5	115.6
iCa (mmol/L)	1.2 ± 0.0	1.2	1.119	1.264
K (mmol/L)	3.8 ± 0.6	3.8	2.54	4.55
AG (mmol/L)	15.3 ± 1.8	15.4	12.7	19.3

Potential of hydrogen (pH), partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), oxygen saturation ($SatO_2$), bicarbonate (HCO_3), total carbon dioxide (TCO_2), base excess (BE), sodium (Na), chloride (Cl), ionized calcium (iCa), potassium (K), and anion gap (AG).

An arterial blood sample is recommended to perform blood gas analysis, as it is ideal for assessing lung function. However, venous blood may be an option in small species, debilitated animals, or those with difficult access to arterial blood (Dutra et al., 2019). Venous blood was used in this study, as it is easily obtained due to the anatomical location of the jugular vein. Although venous blood does not allow the measurement of oxygen transport capacity, it demonstrates reliable values for the parameters that provide data on cellular acid-base and electrolyte balance through the evaluation of pH, HCO_3, and PCO_2 (Montesinos & Ardiaca, 2013; Gonçalves et al., 2016; Thrall et al., 2015).

Studies conducted using other species of wild birds, such as *Spheniscus magellanicus* and *Falco rusticolus,* compared the use of venous and arterial blood in sedated and physically restrained animals, with no differences relative to the analysis of gases, except for PO_2 and $SatO_2$ (Raghav et al., 2015; Dutra et al., 2019). Importantly, PO_2 and $SatO_2$ in venous blood are of little relevance, as tissue oxygenation is assessed through the levels of oxygen present in arterial blood (Hopper, 2023). Table 2 shows the venous blood gas parameters of our study compared to those reported in other species of wild birds. In this study, the parameters were within narrow ranges, except for the Cl, iCa, and AG concentrations, which were not evaluated in the other studies.

Table 2
Comparative blood gas values of venous blood of wild birds

Parameter	*Ramphastos dicolorus*	*Myiopsitta monachus**	*Falco rusticolus***	*Phoenicopterus ruber****	*Anas fulvigula*****	*Spheniscus magellanicus******
pH	7.5	7.43	7.42	7.427	7.37	7.40
$PaCO_2$(mmHg)	34.9	28.6	35.45	29.39	35.6	38
HCO_3(mmol/L)	28.0	19.2	22.4	19.28	19.8	22.9
BE (mmol/L)	5.0	- 5.1	-0.93	-5.0	-4.7	-1.20
Na (mmol/L)	147.9	144.1	148	143.82	146	149.4
Cl (mmol/L)	108.4	-	-	-	111	-
iCa (mmol/L)	1.2	1.07	1.05	1.31	-	-
K (mmol/L)	3.8	3.5	3.27	3.69	3.9	4.80
AG (mmol/L)	15.3	-	-	-	19.3	-

Sources: Rettenmund et al. (2014)*, Raghav et al. (2015)**, Gardhouse & Eshar (2016)***, Ratliff et al. (2017)****, and Dutra et al. (2019)*****.

Our study showed mean bicarbonate (HCO_3) levels of 28 mmol/L and PCO_2 of 34.9 mmHg, using venous blood. Comparative studies on the use of arterial and venous blood showed no difference between the parameters for the two analyzed samples of PCO_2 and HCO_3 (Raghav et al., 2015; Dutra et al., 2019). Therefore, venous blood is a reliable sample for monitoring these parameters in animals. Bicarbonate is the main buffer of the extracellular fluid, acting to compensate for pH due to metabolic variations. Bicarbonate is determined by pH and PCO_2 values and is expressed in mmol/L, presenting useful values together with pH for evaluating acid-base disorders (Thrall et al., 2015; Hopper, 2023).

The partial pressure of carbon dioxide (PCO_2) is determined by the balance between carbon dioxide production and alveolar ventilation. Alveolar ventilation allows the identification of PCO_2 normality (Day, 2002; Hopper, 2023). In our study, Table 1 shows a variation in the value between minimum and

maximum PCO_2 from 19.6 to 43.2 mmHg. This variation may be related to varying levels of stress, hypoxemia, or effort during capture, as these animals were handled through physical restraint. Stress-related hyperventilation can result in respiratory alkalosis even with careful measures regarding capture (Paula et al., 2008).

The mean base excess (BE) values were 5 mmol/L, and the anion gap (AG) values were 15.3 mmol/L. The ionic levels observed in other blood gas studies carried out in wild birds were similar to the results obtained in the present study (Rettenmund et al., 2014; Raghav et al., 2015; Ratliff et al., 2017; Dutra et al., 2019). Base excess (BE) is a metabolic parameter that evaluates the difference between the total bases to quantitatively inform the metabolic component. It indirectly indicates the set of buffers present in the plasma and directs the buffering capacity of the blood. Negative BE values indicate a higher loss of buffers in the blood and, consequently, a higher degree of acidosis; positive BE values indicate more buffer accumulation, leading to a condition of alkalosis (Hopper, 2023). Anion gap (AG) is a parameter used to differentiate and classify the causes of metabolic acidosis through the calculation based on the difference between measurable cations and anions in the blood to determine the acid-base disorder (Thrall et al., 2015).

The mean electrolytes demonstrated in this study were sodium (Na) at 147.9 mmol/L, chloride (Cl) at 108.4 mmol/L, ionized calcium (iCa) at 1.2 mmol/L, and potassium (K) at 3.8 mmol/L. Electrolytes are ions present in intracellular, extracellular, interstitial, and intravascular body fluids. The balance of these ions is essential for the maintenance and balance of homeostasis, as they perform specific functions and osmotic pressure in the regulation of water and cardiac performance (Ceneviva & Vicente, 2008; Thrall et al., 2015). In birds, their determination can be useful in situations in which the animal requires fluid replacement therapy and in cases of intensive care to monitor hyperkalemia, hyponatremia, hypochloremia, or hypocalcemia (Montesinos & Ardiaca, 2013; Gardhouse & Eshar, 2016).

Conclusions

Our study demonstrates data on venous blood gases and ions from a group of rescued toucans considered healthy, intending to establish reference blood gas values, which were unknown for the species.

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