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Blood biochemical profile of *Boa constrictor* in captivity

[*Perfil bioquímico sanguíneo de* Boa constrictor *de cativeiro*]

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ABSTRACT

Boa constrictor snakes are among the most commercialized unconventional domestic animals in Brazil, following a global trend. Veterinary clinics are undergoing a transformation, requiring more specialized knowledge in specific areas. For this reason, this study aimed to study the blood chemical profile of this species. Forty healthy adult snakes (*Boa constrictor*) were selected and distributed into two groups, males, and females. Glucose, urea, creatinine, symmetric dimethyl arginine (SDMA), uric acid, aspartate aminotransferase (AST), alanine aminotransferase, creatine kinase (CK), alkaline phosphatase, gamma glutamyltransferase, total bilirubin and fractions, triglycerides, cholesterol, total protein, albumin, globulins, calcium, phosphorus, sodium, chloride, potassium were measured in serum. The biochemical profile results were described as mean and standard deviation, generating reference values for the *Boa constrictor*.

Keywords: reptiles, Boidae, blood biochemical profile

RESUMO

*As jiboias estão entre os animais domésticos não convencionais mais comercializados no Brasil, seguindo uma tendência mundial. As clínicas veterinárias estão passando por uma transformação, exigindo conhecimento mais especializado em áreas específicas. Por essa razão, o presente estudo teve como objetivo estudar o perfil bioquímico sanguíneo dessa espécie. Quarenta serpentes adultas saudáveis (*Boa constrictor*) foram selecionadas e distribuídas em dois grupos, machos e fêmeas. Glicose, ureia, creatinina, dimetilarginina simétrica sérica (SDMA), ácido úrico, aspartato aminotransferase (AST), alanina aminotransferase, creatina quinase (CK), fosfatase alcalina, gama- glutamiltransferase, bilirrubina total e frações, triglicerídeos, colesterol, proteína total, albumina, globulinas, cálcio, fósforo, sódio, cloreto, potássio foram dosados no soro. Os resultados do perfil bioquímico foram descritos como média e desvio-padrão, gerando valores de referência para a espécie* Boa constrictor.

Palavras-chave: répteis, Boidae, perfil bioquímico do sangue

INTRODUCTION

Snakes of the Boidae family are large and constricting, occurring almost continuously from southern South America to northern Mexico and divided into several subspecies. Two of these are found in Brazil: *Boa constrictor amarali* and *Boa constrictor constrictor* (Card *et al*., 2016), and are popularly known as boas (Pyron *et al*., 2014). The combination of calm behavior in captivity, associated with their size and aesthetic standard, has influenced the increase in the commercial demand for these animals as pets, also called "companion animals" (Reed, 2005).

In veterinary medicine, blood biochemical parameters are important and widely used markers for assessing health status, clinical diagnosis, and disease prognosis. These biomarkers are also fundamental tools for monitoring the health of wild and captive reptiles (Nardini *et al*., 2013). The interpretation of biochemical data is still challenging in reptiles in comparison with small animals, because of the

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lower number of studies and the lack of reference values for most species. This latter factor may be the reason why biochemical values in reptiles are often interpreted by analogy to those of mammals. The present study aimed to establish basic biochemical parameters of clinically healthy male and female *B. constrictor* snakes in captivity.

ETHICAL ASPECTS

This research was approved by the Ethics Committee on the Use of Animals (114/2022-CEUA/UFMG) and complies with the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines.

MATERIALS AND METHODS

Forty boas were used, 20 males (average of 1.80m) and 20 females (average of 2.10m), adults (between four and nine years old), ranging from 5.2kg to 12.6kg, in the pre-prandial period, 21 days of fasting, from the "Jibóias Brasil" Breeding Facility. All boas were born in captivity and belong to the third generation.

The inclusion criteria established for this study were boas of both sexes and ages, clinically healthy, with adequate handling conditions and a balanced diet. The animals were handled in compliance with all biosafety and exotic animal handling protocols. All animals underwent the same experimental protocol to avoid different stress changes.

Blood samples were collected by intervertebral venipuncture using a 10mL syringe and a 25x7mm needle (BD Solomed) during summer (January 2023). The blood was stored in tubes without anticoagulant, which were centrifuged (3000 rotations per minute for 10 minutes) to obtain serum. The samples were identified and stored at -20ºC until processing.

The biochemical profile was obtained by means of serum measurements of glucose, creatinine, dimethyl arginine (SDMA), uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), alkaline phosphatase (AP), gamma glutamyltransferase (GGT), bilirubin (total, direct, and indirect), triglycerides, cholesterol, total calcium (Ca), phosphorus (P), potassium (K), sodium (Na), chlorine (Cl), total proteins, albumin, and globulins by the kinetic colorimetric method using commercial kits (Eco Diagnóstica – Vcheck®) in a semi-automatic biochemical analyzer (Bionote V200®).

The experimental design was completely randomized, and data was presented as mean ± standard deviation. The normality test of Shapiro-Wilk was used to analyze variables, followed by an analysis of variance (ANOVA) and the regression test (the significance level established was $p<0.05$).

RESULTS

The hematological study of snakes has become of great importance in boas, since the techniques for keeping these snakes in captivity and at home have been improving and increasing their life expectancy.

The mean values and standard deviation of the general clinical biochemical profile of male and female boas (*Boa constrictor*) are presented in Table 1, with no significant difference ($p>0.05$) between the two groups, assuming gender has no direct influence on these values.

Table 1. Mean serum values and standard deviation of the general clinical biochemical profile of male and female boas (*Boa constrictor*)

Parameters	Males	Females
Urea mg/dL	10±4.10	10±3.98
Creatinine mg/dL	0.15±0.12	0.12±0.09
Uric acid mg/dL	5.4±0.50	5.3±0.44
SDMA	4±1.5	4±0.9
AST U/L	18±2.36	18±1.99
ALT U/L	11±0.3	10±0.8
CK-total U/L	384±65	392±41
Alkaline phosphatase U/L	152±15.4	148±21.3
GGT U/L	0±0	0±0
Total Bilirubin mg/dL	0.45±0.43	0.41±0.88
Direct Bilirubin mg/dL	0.14±0.07	0.15±0.03
Indirect Bilirubin mg/dL	0.32±0.06	0,32±0.02
Glucose mg/dL	66±6.10	61±8.20
Cholesterol mg/dL	66±17	69±14
Triglycerides mg/dL	3.25±0.21	3.54±0.12
Total Protein g/dL	6.22±0.36	6.34±0.65
Albumin g/dL	2.79±0.28	2.85±0.47
Globulins g/dL	3.56±0.41	3.79±0.27
Calcium mg/dL	14.8±2.30	15.1±1.36
Phosphorus mg/dL	2.91±0.34	2.88±0.50
Sodium mEq/L	159±6	162±5
Potassium mEq/L	3.89±0.96	3.75±0.87
Chlorine mEq/L	117±6	119±8

Regression analysis at significance level $p<0.05$.

DISCUSSION

The concentration of most blood constituents in reptiles presents important physiological variations that can be caused by several factors, such as diet, water availability, hibernation, age, sex, nutritional condition and, mainly, variation in body temperature (Campbell, 2006). To regulate their temperature, these animals depend on behavioral adjustments and external heat sources, based on controlling the amount of skin surface exposed to the sun or to hot or cold surfaces, the availability of which can vary according to seasonality, a fact that makes the interpretation of biochemical results a challenge for wildlife veterinarians. It should be noted that in this experiment, the biochemical profile values correspond to those of high temperatures, and there may be differences between collections made in winter. In addition to the effect of seasonality, the sample number of different animals and/or populations, such as captive versus wild, healthy versus sick, sex and reproductive status are important.

The mean values of serum urea concentrations of boas in the present study (10±4.10mg/dL and 10±3.98mg/dL, for males and females, respectively) agree with Campbell (2006), who reported that terrestrial reptiles have values lower than 15mg/dL, since they are uricotelic. It is believed that these values are associated with a type of mechanism that aims to increase plasma osmolarity to minimize water loss from the organism. Plasma urea values are generally poor indicators of renal disease in reptiles, except for aquatic reptiles, which primarily excrete urea as a protein catabolite. Kolesnikovas *et al*. (2001) demonstrated mean urea values of 3.19mg/dL and uric acid of 5.8mg/dL. In general, the excretion of nitrogen compounds is related to the availability of water in the environment. Since they are unable to concentrate urine due to the absence of the loop of Henle in their kidneys, terrestrial reptiles are unable to eliminate protein waste such as ammonia and urea without the concomitant loss of water. Thus, 50 to 85% of nitrogen products are excreted in poorly soluble and semi-solid forms, such as uric acid (Maixner *et al*., 1987).

The average serum creatinine concentration for reptiles can reach up to 1mg/dL (Campbell, 2006). In the boas in this experiment, the values found were 0.15±0.12mg/dL (males) and 0.12±0.09mg/dL (females), average levels much lower than those reported in rattlesnakes (0.52mg/dL) by Silva *et al*. (2010). Although creatinine is not a useful analyte for kidney disease in this species (Divers and Stahl, 2019), the urea/creatinine ratio can be used as an indicator of protein catabolism and in the differentiation between renal and pre-renal failure. This is because urea is a chemical compound resulting from protein metabolism. Elevated urea levels can indicate kidney problems, as well as extra-renal problems, such as dehydration or a high-protein diet. Therefore, urea interpretation should be done in conjunction with other renal markers. Creatinine, for example, is a product of creatine degradation and the amount of creatinine formed is relatively constant and is not affected by extra-renal causes. Therefore, the urea-creatinine ratio is a valuable tool in clinical practice, as it can help differentiate between pre-renal and renal causes of kidney failure (Kaneko, 2008).

In a study of specimens from the families Boidae (*Sanzinia madagascariensis*) and Pythonidae (*Python regius*), urate excretions consisted primarily of uric acid, in contrast to the excretions of colubrids and vipers, which consisted primarily of ammonium urate (Thornton *et al*., 2021). Therefore, measurement of serum or plasma uric acid is commonly used for clinical assessment of renal health. Unfortunately, changes in uric acid have been shown to be affected by factors other than glomerular filtration rate. Uric acid increases significantly in reptiles after a high-protein meal, and this increase can last for 1-5 days in snakes (Smeller *et al*., 1978; Maixner *et al*., 1987). Therefore, increased plasma levels of urea and uric acid should be evaluated with caution, since the elevation of one of these parameters may be due to high-protein diets. In carnivorous reptiles, such as rattlesnakes, uric acid concentrations may increase up to twofold in the postprandial period (Maixner *et al*., 1987). However, these increases and the time window have not been confirmed for boas or pythons. Given this information, serial measurements of uric acid may be more useful for the clinician to accumulate evidence of renal dysfunction over time.

Silva *et al*. (2011) used 22 adult *Boa constrictor amarali* specimens kept in captivity to perform blood biochemical tests in two different seasons: winter (July 2004) and summer (January 2005). The mean uric acid values obtained in summer and winter, respectively, were: 6.3±3.4 and 11.3±6.2mg/dL. It was observed that the values obtained in summer are those that most closely resemble those found in this experiment for both males (5.4±0.50mg/dL) and females (5.3±0.44mg/dL), demonstrating the seasonal influence on this parameter.

For a long time, research has focused on identifying additional biomarkers to aid in the early detection of diseases such as kidney failure. Symmetric dimethylarginine (SDMA) is a parameter whose biological effects have been known for decades, but it is not yet widely used as a biomarker in diagnostic practice in animals, and its activity in boas is unknown. SDMA is a naturally occurring amino acid produced intracellularly. It is a post-translationally modified form of arginine, generated during normal protein metabolism, first isolated from human urine by Kakimoto and Akazawa (1970). SDMA is known to be an excellent marker of renal function in human patients with chronic kidney disease on dialysis (Kielstein *et al*., 2006). As SDMA is primarily excreted by the kidneys, several parameters of renal function show a close relationship with SDMA. Therefore, the description of reference values for SDMA in boas, besides being original, could significantly contribute to the renal assessment of these animals.

The ALT enzymatic activity is also not considered organ-specific in reptiles, and its average value is generally below 20IU/L (Thrall *et al*., 2022). The boas in this experiment presented ALT (IU/L) values of 11±0.3 and 10±0.8, for males and females, respectively. Although ALT also has a hepatic origin, in cases of hepatocellular disease, the ideal is to evaluate it together with AST.

The alkaline phosphatase values found in this study were slightly higher than those reported by Johnson and Benson (1996) in pythons (105.9IU±21.17IU/L) and much lower than those

observed by Chiodini & Sundberg (1982) in boas snakes (421±12IU/L). It is known that this enzyme can be induced by certain drugs, such as anesthetics (for example, the combination of halothane and nitrous oxide), used in the studies by Chiodini and Sundberg (1982).

However, no GGT levels were detected in the serum of the boas in this study (zero value). Our results agree with those seen by Ramsay and Dotson (1995), who reported the absence of GGT activity in the serum, liver, and other organs (lung, pancreas, and intestine) of snakes (*Elaphe obsoleta quadrivitatta*).

Bilirubin derives from the heme present in hemoglobin and is released during the breakdown of senescent erythrocytes, while approximately 20% of the daily production is derived from heme proteins. It is formed in the monocytic macrophages of the spleen and bone marrow and in the hepatic Kupffer cells and is released into the plasma. Since bilirubin is poorly soluble in water, it is present in plasma strongly bound to albumin. Hemolysis is a much more frequent cause of unconjugated hyperbilirubinemia (Fevery, 2008). Bilirubin can only be efficiently eliminated after conjugation. Reduced conjugation rates will lead to unconjugated hyperbilirubinemia. The bilirubin concentration in the serum of normal humans is lower in women (0.52±0.003mg/dL) than in men (0.72±0.004mg/dL) (Zucker *et al*., 2004). In the present study, there was no significant difference in total, direct, and indirect bilirubin in boas.

Blood glucose levels in most reptiles range from 60 to 100 mg/dL. It is important to note that environmental conditions and stressors can alter glucose levels. Lakušić *et al*. (2020) studied 113 wild snakes (*Natrix tessellata*) and used two stress indicators (glucose and corticosterone levels) immediately after capture and up to 17h later. The authors reported that glucose levels increased sharply 30 min after capture (equivalent to predation) and stabilized at very high levels without declining over time, indicating prolonged saturation of the hypothalamic-pituitary-adrenal axis. There was no effect of sex, morphotype, or reproductive status. Furthermore, the presence of partially digested material in the snakes' stomachs was associated with higher blood glucose levels during the plateau. Blood glucose analysis showed that this parameter can be used to assess the response to acute stress. The prolonged plateau suggests that captivity should be minimized during field studies.

Reptiles store most of their excess energy in lipid form, mobilizing it when needed to meet energy demands, and investing in eggs to provide the primary source of energy for developing embryos. Many aspects of lipid uptake, transport, and storage appear to be similar to those of birds, including hepatic synthesis from glucose substrates, transport of triglycerides into lipoproteins, and storage in adipose tissue, which in reptiles is usually in the abdomen or tail. Seasonal changes in fat stores suggest that lipid storage is used primarily for reproduction rather than maintenance during aphagic periods. The effects of fasting on plasma lipid metabolites may differ from those of mammals and birds because of the ability of reptiles to dramatically reduce their metabolism during prolonged fasting. The main physiological states - feeding, fasting, and vitellogenesis - have different effects on plasma lipid metabolites (Price, 2017). Silva *et al*. (2010) studied the plasma biochemistry of captive rattlesnakes and reported cholesterol values of 171.58mg/dL and triglycerides of 19.29mg/dL, both much higher when compared to the animals in this experiment (Table 1). Serum cholesterol and triglyceride values may be physiologically increased due to vitellogenesis in females or pathologically due to hepatic lipidosis (Divers and Stahl, 2019), facts that did not occur in the boas in this experiment.

Total plasma protein values for male (6.22±0.36mg/dL) and female (6.34±0.65mg/dL) boas are within the limit described by Campbell (2006) for reptiles in general (3 to 8mgdL), and well above the average values described by Kolesnikovas *et al*. (2001) (3.71mg/dL) and Troiano *et al*. (2001) (3.79mg/dL) in *Crotalus durrissus* snakes. Similar behavior was observed for albumin and globulins. The results of the boas from this experiment demonstrate excellent nutrition, renal and hepatic health, in addition to the absence of internal and external parasites.

The calcium values observed in this study of 14.8±2.3mg/dL (males) and 15.1±1.36mg/dL (females) were similar to those observed by Kolesnikovas *et al*. (2001) (15.13mg/dL). The observed means of 2.91mg/dL±0.34 (males) and

2.88±0.50mg/dL for phosphorus were slightly lower than that described by Kolesnikovas *et al*. (2001) (3.29mg/dL). A calcium/phosphorus ratio of 5.1 was observed. According to Divers and Stahl (2019), calcium and phosphorus are minerals that act in several physiological functions, and the determination of their plasma concentration is important in the diagnosis of several diseases. The calcium/phosphorus ratio is probably a good indicator of kidney disease in reptiles. In healthy animals, this ratio is usually above 1, with kidney disease characterized by lower values.

Electrolytes (e.g., sodium and potassium) and minerals (e.g., calcium and phosphorus) play important roles in cardiac function and should also be evaluated in a patient with suspected cardiac disease (Mitchell, 2009). The mean value of sodium observed for male boas (159±6mEq/L) is within the range (120 to 160mEq/L) for boa constrictors and pythons, and the mean value for female boas (162±5mEq/L) was slightly above the upper limit as described by Thrall *et al*. (2022). Sodium is absorbed in the intestine and transported to the kidneys, where it is reabsorbed or excreted depending on need. The values observed in this study may be due to the food and water fasting that the animals were subjected to prior to blood collection, with no clinical significance. Chlorine is the main anion in the blood and the main osmotic component of reptile plasma. The values of the boas in this study are within the normal limits described for snakes (100 to 130 mEq/L) according to Thrall *et al*. (2022). In relation to potassium, the values obtained in this study are also within the normal limit of 3 to 6 mEq/L (Thrall *et al*., 2022).

CONCLUSIONS

The present results indicate that there is not a sexual influence on biochemical parameters of healthy *Boa constrictor* in captivity.

The determination of biochemical profile parameters of healthy *Boa constrictor* was very similar to that of other snakes. In addition, further studies on SDMA profile are needed, since this parameter can be used as diagnostic tool for snake renal disease.

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CONTRIBUTORS

MR Lempek, PBU Fernandes, MM Melo: study conception, objective, data analysis, discussion, manuscript writing, and approval of the version to be published. RMF Yabiku: data collection.

DATA AVAILABILITY STATEMENT

Data-in-article.

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