Seasonal influence on biochemical profile and serum protein electrophoresis for *Boa constrictor amarali* in captivity

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Abstract

Similarly to other reptiles, snakes are ectothermic animals and depend exclusively on the environment for the maintenance of their physiological, biochemical and immunological processes. Thus, changes in biochemical values can be expected due to seasonal influence. Twenty-two adult specimens of *Boa constrictor amarali* kept in captivity were used. Blood collections were done in two different seasons: winter (July 2004) and summer (January 2005) for the following assays: uric acid, aspartate aminotransferase (AST), glucose, cholesterol, total protein, and serum protein electrophoresis. The mean biochemical results found in summer and winter, respectively, were: 6.3 ± 3.4 and 11.3 ± 6.2 mg/dL for uric acid; 28.7 ± 12.4 and 20.7 ± 16.2 UI/L for AST; 26.3 ± 17 and 17.4 ± 6.8 mg/dL for glucose; 67.3 ± 30.2 and 69.7 ± 38.5 mg/dL for cholesterol; and 5.9 ± 1.6 and 5.9 ± 1.4 g/dL for total protein. Results regarding electrophoresis in summer and winter, respectively, were: 1.9 ± 0.7 and 2.4 ± 0.6 g/dL for albumin; 0.7 ± 0.2 and 0.5 ± 0.2 g/dL for α-globulin; 1.5 ± 0.5 and 1.7 ± 0.6 g/dL for β-globulin; and 1.8 ± 0.5 and 1.5 ± 0.5 g/dL for γ-globulin. In the summer, there was a significant increase in AST and a decrease in uric acid ($p < 0.05$). Serum protein electrophoresis showed a significant increase in α-globulin fraction ($p < 0.05$) in the same season. There were not significant differences between seasons for the remaining variables. Based on these results, the period of the year must be considered in the interpretation of some biochemical values for these animals.

Keywords: biochemical profile, snake, *Boa constrictor amarali*, electrophoresis, seasonal influence.

Influência sazonal sobre o perfil bioquímico e a eletroforese de proteínas séricas de *Boa constrictor amarali* criada em cativeiro

Resumo

As serpentes, como outros répteis, são animais ectotérmicos e dependem exclusivamente do meio para a manutenção de seus processos fisiológicos, bioquímicos e imunológicos. Desta forma, alterações nos valores bioquímicos podem ser esperadas considerando-se a influência sazonal. Foram utilizadas vinte e duas *Boa constrictor amarali* adultas mantidas em cativeiro. A coleta de sangue foi realizada em duas estações diferentes: inverno (julho 2004) e verão (Janeiro 2005), para a realização dos exames: ácido úrico, aspartato aminotransferase (AST), glicose, colesterol, proteína total e eletroforese da proteína sérica. As médias dos exames bioquímicos obtidos no verão e inverno, respectivamente, foram: 6.3 ± 3.4 e 11.3 ± 6.2 mg/dL para ácido úrico; 28.7 ± 12.4 e 20.7 ± 16.2 UI/L para AST; 26.3 ± 17 e 17.4 ± 6.8 mg/dL para glicose; 67.3 ± 30.2 e 69.7 ± 38.5 mg/dL para colesterol; e 5.9 ± 1.6 e 5.9 ± 1.4 g/dL para proteína total. Os resultados da eletroforese no verão e inverno, respectivamente, foram: 1.9 ± 0.7 e 2.4 ± 0.6 g/dL para albumina; 0.7 ± 0.2 e 0.5 ± 0.2 g/dL para α globulina; 1.5 ± 0.5 e 1.7 ± 0.6 g/dL β globulina; e 1.8 ± 0.5 e 1.5 ± 0.5 g/dL para γ globulina. No verão foi observado aumento significativo da AST ($p < 0,05$) e diminuição significativa do ácido úrico ($p < 0,05$). Na eletroforese de proteína sérica, houve um aumento significativo da fração α globulina ($p < 0,05$) na mesma estação. Para as outras variáveis não foram obtidas diferenças significativas entre as estações. De acordo com os resultados, é importante considerar o período do ano na interpretação de alguns exames bioquímicos desses animais.

Palavras-chave: perfil bioquímico, serpente, *Boa constrictor amarali*, eletroforese, influência sazonal.

1. Introduction

Reptiles are ectothermic animals and depend on the environment to maintain their physiological, biochemical and immunological processes. Health status determination in reptiles is based on physical examination, as well as on hematological and biochemical values (Harr et al., 2001; Brown and Shine, 2002; Luiselli and Akani, 2002).

The biochemical profile of reptiles can be influenced by gender, species, nutritional and physiological status, pathological conditions and seasonal variations. The interpretation of biochemical results depends on several factors that must be considered. Only a few normal reference values have been reported in the literature (Campbell, 1996, 2004; Anderson et al., 1997).

Reference values of biochemical profile have been reported for *Boa constrictor constrictor* (Linnaeus, 1758) in captivity in the USA (Chiodini and Sunberg, 1982; Rosskopf et al., 1982; Divers, 2000). To our knowledge, there are not reference values of biochemical profile and serum protein electrophoresis for the subspecies *Boa constrictor amarali* (Stull, 1932). In addition, seasonal effects on biochemical profile have been poorly understood and scarcely reported in the literature, especially regarding South American snakes (Francisco et al., 2002).

The aim of this study was to determine the seasonal influence on the biochemical profile for clinically healthy adult *Boa constrictor amarali* of both sexes, including the measurement of uric acid, glucose, total protein and cholesterol, and the establishment of reference intervals for serum protein electrophoresis.

2. Material and Methods

Twenty-two clinically healthy adult specimens of *Boa constrictor amarali* of both sexes (nine males and 13 females; mean snout-vent length 158.6 and 176.1 cm, respectively) were used. The mean body size snout-tail The snakes were kept in captivity at CEVAP (Centro de Estudos de Venenos e Animais Peçonhentos, São Paulo State University (UNESP) - Botucatu Campus, São Paulo State, Brazil) . Blood was collected during two different seasons: winter (July 2004) and summer (January 2005).

The snakes were fed at 15-20 day intervals in the summer and at 30-45 day intervals in the winter. Samples were collected after twelve days of fasting. Then, the snakes were kept in an outdoor enclosure; during the winter, they were housed in a refuge at a mean temperature of 27 °C kept by a heating lamp.

Blood (1.5 mL) was collected from the snakes' caudal vein after physical restraint. The blood sample was placed into tubes, one of which contained anticoagulant EDTA and the other one had no anticoagulant. The tubes were centrifuged for plasma glucose, uric acid, total protein, serum aspartate aminotransferase (AST) and cholesterol evaluation, and protein electrophoresis. Samples for electrophoresis were immediately frozen (-80 °C) after centrifugation.

Biochemical analyses were performed in a spectrophotometer (SB-190 - CELM) on the same day. The following analytes were evaluated: uric acid, cholesterol, glucose, and AST. Total protein was determined by refractometry (Atago-T2-NE).

Samples presenting hemolysis or lipemia were discarded for AST and uric acid determination.

Protein electrophoresis was done in 10% polyacrylamide gel with the addition of urea 8M in a discontinuous alkaline buffer system and a power supply (EPS 600 - Pharmacia Biotech AB) set at 130 V and 30 mA for 5 hours at 4 °C. Gels were stained with Coomassie blue and subjected to densitometric analysis (IOD) by using a VDS (Image Master - Pharmacia Biotech AB) system. Then, IOD was converted into percentage for protein quantification from the total protein concentration.

Following densitometry, protein bands were classified according to their relative mobility into α, β and γ globulins and albumin. The sum of similar bands resulted in a unique value for each fraction. Band numbers was reported for each gel. Since serum was insufficient to perform and replicate all assays, electrophoresis was only done for 11 samples in the winter.

All statistical analyses (Student's *t*-test for parametric variables and Mann-Whitney test for nonparametric variables) were performed through the SigmaStat 3.10 software (Systat Software Inc.). Data were discussed at 5% significance level.

3. Results and Discussion

3.1. Biochemical analysis

Despite the heating lamp in the refuge, snakes remained susceptible to environmental changes, since they presented a lower degree of activity during the winter, staying most of the day inside the refuge.

In the summer, there were a significant increase in AST ($p < 0.05$) and a decrease in uric acid ($p < 0.05$) (Table 1). The other variables did not change in the analysed period, similarly to a variety of snake species evaluated pre- and post-hibernation (Francisco et al., 2002).

An alteration in uric acid levels indicates recent protein ingestion and is related to gout, a disease of difficult diagnosis (Divers, 2000; Campbell, 2004). Uric acid values were higher in the winter than in the summer. Similar results were not found in the consulted literature (Chiodini and Sunberg, 1982; Rosskopf et al., 1982; Jacobson, 1993; Johnson and Jacobson, 1996) which, however, did not include the study of seasonal influence.

The twelve days of fasting established in the experimental design may have not been sufficient to complete digestion and metabolism. The lower metabolism of snakes in the winter may lead to a postprandial interference longer than twelve days. However, a previous study (Dutton and Taylor, 2003) showed that a fasting period of six days was sufficient to recover the preprandial values, but the snakes were kept indoors at 31-33 °C.

AST value was statistically higher ($p < 0.05$) in the summer, again indicating the seasonal influence on the other biochemical values. The enzyme AST is present in many tissues like liver and muscle and can increase under hepatic disease or muscle damage. The seasonal metabolic variation may also have contributed to these high values, although similar results have not been reported in the literature.

Although glucose value was higher in the summer, there was no statistical difference between seasons, probably due to a high coefficient of variation. Thus, analyses of a larger number of animals are needed to answer this question. In addition, there was no statistical difference between seasons for cholesterol and total protein.

Some authors (Chiodini and Sunberg, 1982; Rosskopf et al., 1982) studied *Boa constrictor constrictor* and found cholesterol and total protein levels similar to those detected in the present study; AST values, however, were higher than those reported in this paper for both seasons. Uric acid and glucose results were similar only in the summer. In the winter, those authors detected lower values for uric acid and higher values for glucose, relative to those reported here.

3.2. Electrophoresis

Nine to 13 bands were detected in the summer and 10 to 14 bands in the winter. Means and SD values for α-, β-, γ-globulins and albumin, as well as significant differences are shown in Table 2.

Serum protein electrophoresis is a useful tool to evaluate hypoglobulinemia and hyperglobulinemia in reptiles. The increase in the α-globulin fraction has been associated with tissular necrosis, whereas decreased values have been associated with malnutrition, enteropathies, and chronic hepatic and kidney diseases (Campbell, 1996, 2004; Divers, 2000). Hypoalbuminemia can occur due to prolonged anorexia, protein-losing enteropathy, nephropathies or chronic hepatic disease. Hyperalbuminemia has been associated with dehydration (Divers, 2000).

Although a previous study reported the importance of electrophoresis as a diagnostic tool in reptile medicine, to our knowledge this is the first study on protein electrophoresis using snakes (Zaias and Cray, 2002).

The values of α- and γ-globulins obtained in the present work were similar to those reported in a study with marine turtles (*Caretta caretta*) (Gicking et al., 2004): α-globulin levels were significantly lower in the winter. The reduced number of animals may have not allowed the observation of statistical differences for albumin and β-globulin values.

The difference found in α-globulin may be related to the nutritional status, since this globulin presents an α-1 acid glycoprotein fraction that has been associated with feeding (Kaneko, 1998). This statement may not be feasible because an alteration in albumin would also be expected in case of nutritional deficiency. Nevertheless, albumin values were higher in the winter, although without statistical difference.

Table 1. Statistical analysis of biochemical data for *Boa constrictor amarali* in the summer and in the winter Botucatu, 2004-2005.

Season		Uric acid (mg/dL)	AST (UI/L)	Glucose (mg/dL)	Cholesterol (mg/dL)	Total protein (g/dL)
Summer (n=22)	Mean	6.3^a	28.7^b	26.3^a	67.3^a	5.9^a
	SD	3.4	12.4	17.0	30.2	1.6
Winter (n=22)	Mean	11.3^b	20.7^a	17.4^a	69.7^a	5.9^a
	SD	6.2	16.2	6.8	38.5	1.4

Means with different superscripts in the same column are significantly different ($p < 0.05$); SD: standard deviation; and AST: aspartate aminotransferase.

Table 2. Statistical analysis of albumin and α-, β- and γ-globulin fractions obtained through serum protein electrophoresis (g/dL) for *Boa constrictor amarali* in the summer and in the winter. Botucatu, 2004-2005.

Season		Albumin	α-globulin	β-globulin	γ-globulin
Summer (n=22)	Mean	1.9^a	0.7^a	1.5^a	1.8^a
	SD	0.7	0.2	0.5	0.5
	Min-Max	0.8-3.0	0.3-1.3	0.7-2.1	0.4-2.9
Winter (n=11)	Mean	2.4^a	0.5^b	1.7^a	1.5^a
	SD	0.6	0.2	0.6	0.5
	Min-Max	1.8-3.5	0.3-0.9	1.1-24	0.9-2.4

Means with different superscripts in the same column are significantly different ($p < 0.05$); SD: standard deviation; Min-Max: minimum and maximum; α: alpha; β: beta; and γ: gamma.

When the present results are compared to those described for *Boa constrictor constrictor* (Chiodini and Sunberg, 1982), lower albumin and similar globulin levels are found in both seasons. However, it must be emphasised that those authors have used routine colorimetric methods, whereas electrophoresis was used in the present study.

4. Conclusion

The present results indicate that there is a seasonal influence on uric acid, AST and α-globulin levels for *Boa constrictor amarali*, which highlights the importance of considering the season in the interpretation of some biochemical values for these ani mals. In addition, further studies on biochemical and electrophoretical profiles are needed, since they can be used as diagnostic tools for snake diseases.

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