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Biochemical parameters, C-reactive protein, and proteinogram of *Sapajus libidinosus* kept in captivity

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ABSTRACT: *Sapajus libidinosus* is a New World primate belonging to the Cebidae family that lives in the caatinga and cerrado, which are known Brazilian biomes. It is currently classified as near threatened, almost endangered, by the main animal protection organizations. Knowledge of biochemistry, the C-reactive protein, and the proteinogram are important for the preservation of this species. Our study established reference intervals for several biochemical variables and the proteinogram. For this purpose, blood samples were collected from 50 *S. libidinosus* monkeys (25 males and 25 females) kept in captivity at the Brazilian state of Paraíba. Descriptive analysis and reference intervals were estimated following the guidelines of the American Society of Veterinary Clinical Pathology, using the Reference Value Advisor 2.1 software. In the overall population (n=50), significant differences ($P < 0.05$) were noted for creatinine and total proteins when considering the age factor (higher in adults), as well as for albumin and indirect bilirubin (higher in juveniles). Sex-associated differences (females versus males, $P < 0.05$) were reported for serum urea and creatinine (higher in males), as well as for albumin and Beta-Globulins (higher in females). In conclusion, to the authors' best knowledge, the present results are innovative and can be used as reference intervals for assessing the animals' health status. Moreover, it is also pioneer in determining the C-reactive protein in this species. It is important to emphasize that gender and age categories can have an influence and should be considered when interpreting the tests results.
Key words: biochemistry, capuchin monkeys, C-reactive protein, proteinogram, *Sapajus libidinosus*.

Parâmetros bioquímicos, proteína C-reativa e proteinograma de *Sapajus libidinosus* mantido em cativeiro

RESUMO: O *Sapajus libidinosus* é um primata do novo mundo pertencente à família Cebidae que habita os biomas da caatinga e cerrado do Brasil. Atualmente esta classificado como quase ameaçado pelos principais orgãos de proteção animal. O conhecimento da bioquímica sérica, proteína C reativa e proteinograma são importantes na preservação desta espécie. O nosso estudo tem como objetivo determinar os intervalos de referência para várias variáveis bioquímicas e proteinograma. Para isso, foram colhidas amostras de sangue de 50 macacos da espécie *S. libidinosus* (25 machos e 25 fêmeas) mantidos em cativeiro no estado da Paraíba, Brasil. A análise descritiva e os cálculos dos intervalos de referência foram estimados segundo as diretrizes da Sociedade Americana de Patologia Clínica Veterinária com o uso do software Reference Value Advisor 2.1. Na população total (n=50), considerando o factor idade, foram observadas diferenças significativas ($P < 0,05$) para a creatinina e proteínas totais (valores superiores nos adultos) e para a albumina e bilirrubina indirecta (valores superiores nos jovens). Foram encontradas diferenças associadas ao sexo (fêmeas versus machos, $P < 0,05$) para a ureia e creatinina séricas (valores superiores nos machos) e albumina e Beta-Globulinas (valores superiores nas fêmeas). Conclui-se que, tanto quanto é do conhecimento dos autores, os resultados deste estudo são inovadores e podem ser utilizados como intervalos de referência para avaliação do estado de higidez dos animais e é pioneiro na determinação da proteína C reativa nesta espécie. É importante salientar que o sexo e a faixa etária podem ter influência e devem ser levados em consideração pelos profissionais na interpretação dos exames.
Palavras-chave: bioquímica, macaco-prego, proteína C-reactiva, proteinograma, *Sapajus libidinosus*.

INTRODUCTION

The capuchin monkeys of the species *Sapajus libidinosus* are New World primates belonging to the Cebidae family (ALFARO et al., 2012). This species lives in the caatinga and cerrado biomes of Brazil, with populations present in the states of Alagoas, Bahia, Ceará, Goiás, Maranhão, Minas Gerais, São Paulo, Piauí, Rio Grande do Norte, Tocantins, Pernambuco and Paraíba (SALLES et al., 2018).

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According to the International Union for the Conservation of Nature and Natural Resources (MARTINS et al., 2019) and the Chico Mendes Institute for Biodiversity Conservation (ICMBio, 2018), *S. libidinosus* are classified on the Red List as near threatened. Its population remains in decline due to the loss and fragmentation of its habitat, predatory hunting, and illegal trade. This has been evidenced by the increase in the number of primate specimens of this genus rescued in the Wild Animal Screening Centers (LEVACOV et al., 2011).

Concern about the preservation of endangered species has increased, resulting in the creation of organizations dedicated to the protection, care, and rehabilitation of different species (BENEVIDES et al., 2017). One of the challenges for veterinarians working with the recovery of wild animals is the scarcity of studies related to the physiological parameters on which they can rely to compare with clinical findings (FLAIBAN et al., 2008).

The determination of hematological and biochemical values, for instance, is important to assess the limits between health and disease, to understand the alterations caused by pathogens (MOORE, 2000), and even to be used as biomarkers of environmental aggression (ALMOSNY & MONTEIRO, 2006). This information helps veterinarians in several aspects, such as decision-making, the recovery of the animal, its reintroduction into the wild, or the preservation of the animal's health in captivity. Few studies report reference biochemical intervals for *S. libidinosus* in Brazil (RIBEIRO et al., 2016, SOUSA et al., 2020). As far as we know, in the Northeast region, there are no studies on biochemical parameters for this species. The existing research was conducted in other regions, and the values may not be suitable due to various factors, such as regional differences related to climate (temperature and humidity), food, habitat, and different laboratory conditions (RIBEIRO et al., 2016, SOUSA et al., 2020). Therefore, the objectives of this study were to determine several biochemical parameters, including the C-reactive protein and the proteinogram, of *S. libidinosus* kept in captivity in the state of Paraíba, located in northeastern Brazil, in order to create reference intervals (RI), and identify possible variations related to gender and age.

MATERIALS AND METHODS

Blood samples were collected from 50 monkeys of the species *S. libidinosus* (25 males and 25 females) kept in captivity in the Parque Zoobotânico Arruda Câmara in the city of João Pessoa (n=15 animals) and at the IBAMA Centro de Triagem de Animais Silvestres (CETAS) in Cabedelo City (n=35 animals), both located in the state of Paraíba, Brazil.

All animals were kept under similar environmental, sanitary and nutritional conditions. Their food, supplied twice a day, consisting of seasonal fruits, vegetables, and water *ad libitum*. The animals were followed by a multidisciplinary team of professionals, including veterinarians, biologists, and nutritionists.

The animals were submitted to a rigorous clinical evaluation, which included a physical examination, search for ectoparasites, evaluation of integument, mucous membranes, and lymph nodes, determination of respiratory rate, heart rate, and rectal temperature, as described by FEITOSA (2014). Twenty-one days before blood collection, a broad-spectrum deworming (1% Ivermectin at a dose of 0.2 mg/kg intramuscularly) was administered prophylactically to avoid possible changes in results. Subsequently, parasitological examinations were performed to ensure the absence of parasites.

All of the animals were captured using nets and an intramuscular anesthetic protocol was administered for chemical content, which consisted of 10mg/kg of ketamine hydrochloride with 2mg/kg of xylazine hydrochloride and 1mg/kg of diazepam, according to CUBAS et al., (2006). The animals underwent a fast of eight hours before blood collection to ensure effective and safe anesthesia, as well as possible errors in the analytical determination.

The juvenile and adult categories were established based on an estimation of the animal's age, following the criteria previously described by FRAGASZY et al., (2004). Parameters such as dental evaluation (size, color, wear), development of sexual characteristics, and body size were observed, which allowed us to estimate the age of the individuals and classify them into two main categories, according to gender (25 males and 25 females) and age [estimated by dentition wear, young (13 animals) and adults (37 animals)]. As a result, four categories were established: young females (8 animals) and adult females (17 animals), young males (5 animals) and adult males (20 animals).

In the mornings, 10 milliliters of blood were collected by venopuncture of the femoral vein, with the use of disposable syringes and 12x8mm needles. From this, eight milliliters were distributed in tubes without an anticoagulant to obtain serum in order to perform biochemical, proteinogram, and C-reactive protein analyses. The remaining two milliliters were deposited in a tube containing fluoride + citrate for glucose dosing.

After collection, two capillary tubes were filled per sample and were submitted to micro-centrifugation to determine plasma fibrinogen. This was done using the heat precipitation method, as described by SCHALM et al (1975). For the remaining analyses, the tubes were centrifuged for 10 minutes at 1207g. Then, the supernatant was aliquoted and stored, properly identified, and packed at a freezing temperature of - 20 ºC for further analysis in the laboratory.

The samples were analyzed at the Veterinary Clinical Pathology Laboratory of the Veterinary Hospital of the Universidade Federal de Pernambuco, Brazil, where the following measurements were performed: urea, creatinine, calcium (Ca), phosphorus (P), glucose (GLU), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (AF), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), cholesterol, triglycerides, creatine phosphokinase (CK), total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IB). These were carried out with the use of a semi-automated biochemical analyzer (Bio 2000, Bioplus) and commercial kits (Bioclin, Quibasa).

Other analyses were performed at the laboratory of Clinical Pathology Service of the Centro Hospitalar Trás os Montes e Alto Douro in Vila Real, Portugal. These included total proteins (TP), sodium (Na), potassium (K), chlorine (Cl), amylase, lipase, and C-reactive protein, which were measured using an automatic immunoturbidimetry chemistry analyser (Synchron LXi 725). Albumin, total globulins, and their alpha1, alpha2, beta and gamma fractions were also measured in the same laboratory by high-resolution capillary electrophoresis, using Pargon CZETM 2000 equipment.

For the estimation of RI, the guidelines of the American Society of Veterinary Clinical Pathology (FRIEDRICHS et al., 2012) were followed. A descriptive analysis and RI calculations were performed with the Reference Value Advisor version 2.1 program (GEFFRÉ et al., 2011).

The values were later submitted to an analysis of variance (ANOVA) to determine the influence of gender and age. This was evaluated at the level of 5% probability, using the Statistical Package for Social Sciences (SPSS) software. Values with $P < 0.05$ were considered statistically significant.

RESULTS

The general results obtained in this study, regardless of the gender or age of the animals are presented in table 1. Initially, the results were grouped and compared into two categories, females and males, young and adult. Subsequently, to determine which factor, gender or age, was the main one responsible for the differences observed, comparisons were made between four subcategories: young females and young males, adult females and adult males, young females and adult females, young males and adult males.

The parameters with statistically significant differences between categories and subcategories are described in table 2. The statistical analysis reveals significant differences between the two genders in the albumin and beta-globulin, which are higher in females, as well as in urea and creatinine, which are higher in males. As for different age categories, significant differences were observed in albumin and IB, which were higher in young animals, with higher creatinine and TP found in adults.

Some differences were observed when the gender/age factors were compared, namely for lipase, albumin, urea, creatinine, LDH and TP. However, for the C-reactive, protein no statistically significant differences were observed between categories.

DISCUSSION

The literature on the biochemical profile, the proteinogram and the C-reactive protein of capuchin monkeys is scarce and few reports describe these parameters in capuchin monkeys of other species (*Sapajus* spp.) or *S. libidinosus* in other regions of Brazil (RIBEIRO et al., 2016, MOTA et al., 2016, SOUSA et al., 2020). Our study is the first conducted on this species in northeastern Brazil. As such, the results obtained were compared with studies that used animals of other species and/or other methodologies (WIRZ et al., 2008, TEIXEIRA et al., 2013, FAVARETO et al., 2016, MONTEIRO et al., 2016).

When evaluating all animals, our results are similar to those described by TEIXEIRA et al., (2013) for the variables CREA, TP, ALB, TB, DB, IB, CA, GLU, LDH, CK in capuchin monkeys *(Cebus flavius)* in captivity, anesthetized with a similar protocol. Although, the studies are with different species, these animals live in captivity in the same region and with the monitoring of professionals, and sanitary and nutritional control, which may explain this similarity in the parameters.

Different results were described by SOUSA et al (2020) with *S. libidinosus* captured in a forest reserve in Brasília (Brazil). The animals in our study had higher values of urea, CREA, AST and lower values of GLU, triglycerides, ALT, AF, ALB and TP. This variation may be related to

Table 1 - Reference intervals for blood biochemistry parameters of 50 capuchin monkeys *(Sapajus libidinosus)* kept in captivity in the state of Paraíba-Brazil.

Test	Mean	SD	Median	Min.-Max.	RI	LRL 90%CI	URL 90% CI
Urea	52.6	15.2	60.0	14.0-70.0	14.3-70.0	14.0-26.8	68.0-70.0
CREA	1.1	0.6	1.3	0.1-2.0	0.1-2.0	0.1-0.2	2.0-2.3
Ca	7.9	1.9	8.9	4.1-10.1	4.2-10.1	4.1-4.5	9.3-10.1
P	3.5	1.5	3.5	0.7-8.2	0.8-7.7	0.7-1.2	5.9-8.2
Na	150.0	5.2	150.0	137.0-165.0	139.2-160.3	137.1-141.7	158.1-162.9
K	4.4	1.0	4.2	3.0-8.6	3.2-6.8	3.1-3.4	5.8-8.7
Glucose	89.1	19.3	90.0	15.0-124.0	71.3-122.6	71.0-75.4	110.0-124.0
Cl	132.6	6.0	132.2	120.5-147.6	121.7-146.2	120.1-123.5	143.1-149.6
AST	81.7	19.8	87.5	30.0-117.0	30.8-116.7	30.0-37.9	108.2-117.0
ALT	39.2	9.4	38.5	19.0-65.0	20.1-62.5	19.0-27.3	54.7-65.0
AF	147.1	34.1	147.0	65.0-213.0	71.1-213.0	65.0-90.0	200.9-213.0
GGT	84.0	24.3	87.0	31.0-190.0	32.9-172.1	31.0-47.8	108.0-190.0
LDH	358.8	43.0	353.0	278.0-451.0	283.5-450.2	278.0-299.0	421.7-451.0
TB	0.3	0.2	0.3	0.01-0.61	0.0-0.6	0.0-0.1	0.5-0.6
DB	0.1	0.1	0.1	0.0-0.23	0.0-0.2	0.0-0.0	0.2-0.2
IB	0.2	0.1	0.2	0.01-0.42	0.0-0.4	0.0-0.1	0.3-0.4
CK	368.3	102.4	381.5	190.0-549.0	192.2-541.9	190.0-203.2	520.0-549.0
HDL	131.1	19.1	124.5	98.0-187.0	98.3-184.0	98.0-104.5	165.0-187.0
TRIG	87.1	29.1	86.0	26.0-169.0	26.3-169.0	26.0-46.6	138.9-169.0
UA	2.3	0.9	2.2	0.4-5.0	0.5-4.9	0.4-1.2	4.1-5.0
Lipase	8.4	3.8	7.0	5.0-23.0	5.0-22.5	5.0-6.0	12.5-23.0
Amylase	228.3	71.1	199.0	11.0-899.0	13.2-842.9	11.0-19.6	500.5-899.0
TP	6.4	0.4	6.3	5.4-7.1	5.5-7.3	5.3-5.7	7.1-7.4
Fg	257.0	59.5	255.0	100.0-400.0	114.0-393.0	100.0-183.3	320.0-400.0
Albumin	3.2	0.3	3.2	2.7-4.0	2.6-4.0	2.6-2.8	3.8-4.2
AlfaG_T	1.3	0.2	1.3	1.0-1.9	1.0-1.9	1.0-1.1	1.5-1.9
AlfaG_1	0.3	0.1	0.3	0.2-0.6	0.2-0.6	0.2-0.2	0.5-0.6
AlfaG_2	1.0	0.2	1.0	0.7-1.5	0.7-1.5	0.7-0.8	1.3-1.5
BetaG	0.6	0.2	0.5	0.3-1.1	0.3-1.1	0.3-0.4	0.9-1.1
GamaG	1.3	0.3	1.2	0.7-2.3	0.7-2.2	0.7-0.9	1.8-2.3
PCR	1.3	0.4	1.3	0.4-2.0	0.4-2.0	0.4-0.7	1.7-2.0

CREA: Creatinine, Ca: Calcium, P: Phosphorus, Na: Sodium, K: Potassium, Cl: Chlorine, AST: Aspartate Amino Transferase, ALT: Alanine Amino Transferase, AF: Alkaline Phosphatase, GGT: Gamma Glutamyl Transferase, LDH: Lactate Dehydrogenase, TB: Total Bilirubin, DB: Direct Bilirubin, IB: Indirect Bilirubin, CK: Creatine Phosphokinase, PT: Total Proteins, Fg: Fibrinogen, AlphaG_1: Alpha Globulin 1, AlphaG_2: Alpha Globulin 2, AlphaG_T: Total Alpha Globulins, BetaG: Beta Globulin, GammaG: Globulin Gamma, PCR: C-Reactive Protein, IR: Reference Interval, Min: Minimum, Max: Maximum; LRL-lower reference limit; URL-upper reference limit; 90% CI – confidence interval around the limit.

environmental differences between regions and food availability, stress levels during blood collection and the use of a different anesthetic protocol (tileta mine+zolazepam+isoflurane), as described by other authors (MONTEIRO et al., 2016, HERNÁNDEZ-GODÍNEZ et al., 2019).

According to CUBAS et al., (2006), due to capture stress, free-living wild primates are more susceptible to changes in physiological parameters when compared to captive animals that are more used to human presence and conditioning for sampling.

The values obtained for the proteinogram and C-reactive protein were similar to those described by MOTA et al., (2016) in the *S. flavius* kept in captivity in Paraíba (Brazil). These animals lived in the same research center as the animals in our study and were subjected to a similar anesthetic protocol.

Regarding gender, females presented higher albumin and beta-globulin values and males presented higher urea and creatinine levels. Several authors have been exploring the gender effect on the same biochemical parameters in other species

Table 2 - Effect of gender and age on the parameters in blood biochemistry parameters of capuchin monkeys (S*apajus libidinosus)* kept in captivity in the state of Paraíba-Brazil.

Category	Parameters	Mean (SD)	Mean (SD)	*P*
Gender		Female (N=25)	Male (N=25)	
	Urea	41.20 (12.60)	64.08 (6.42)	<0.001
	Creatinine	0.83 (0.55)	1.47 (0.48)	<0.001
	Albumin	3.34 (0.37)	3.10 (0.25)	0.01
	Beta Globul	0.59 (0.19)	0.50 (0.11)	0.049
Age		Young (N=13)	Adult (N=37)	
	Creatinine	0.76 (0.55)	1.28 (0.57)	0.006
	TP	6.17 (0.43)	6.45 (0.40)	0.04
	Albumin	3.40 (0.29)	3.15 (0.32)	0.016
	IB	0.25 (0.10)	0.19 (0.08)	0.034
Age/Gender		Young females (N=8)	Young males (N=5)	
		NS	NS	
Age/Gender		Young females (N=8)	Adult females (N=17)	
	IB	0.29 (0.07)	0.18 (0.08)	0.004
Age/Gender		Adult females (N=17)	Adult males (N=20)	
	Urea	39.41 (11.50)	65.40 (2.77)	<0.001
	Creatinine	0.83 (0.51)	1.67 (0.24)	<0.001
	Albumin	3.27 (0.38)	3.06 (0.23)	0.049
	Lipase	10.41 (5.71)	7.35 (1.53)	0.027
Age/Gender		Young males (N=5)	Adult males (N=20)	
	Urea	58.80 (12.94)	65.40 (2.77)	0.037
	Creatinine	0.66 (0.34)	1.67 (0.24)	<0.001
	LDH	326.20 (27.23)	360.35 (32.88)	0.044
	TP	6.08 (0.46)	6.47 (0.36)	0.049

LDH: Lactate dehydrogenase, IB: Indirect bilirubin, TP: Total proteins, SD: Standard deviation; NS: Non-significant.

of primates, but so far have failed to confirm such influence: [RIVIELLO & WIRZ (2001) and WIRZ et al., (2008) (in *Cebus apella*); LIMA et al., (2014) and FAVARETO et al., (2016) (in *Cebus* spp) and MONTEIRO et al., (2016) (in *Sapajus* spp)]. Our study is the first to demonstrate that gender can influence these parameters in the *S. libidinosus* species.

Regarding albumin and beta-globulin values, the induction of increased albumin and globulin synthesis by estrogens in females is well known in humans (VAN THIEL & GAVALER, 1987). However, to the authors' best knowledge, there is no available information regarding such influence in the literature concerning New World Primates. Therefore, it is possible that this influence is characteristic of this species.

Regarding creatinine and urea values, the difference in creatinine can be attributed to the amount of muscle mass being higher in males than in females. Males have a greater amount of phosphocreatine in their skeletal muscles, which is the precursor of creatinine. Therefore, the greater the amount of muscle phosphocreatine, the greater the amount of creatinine formed (LATIMER et al., 2011). Conversely, according to KANEKO et al., (1997), the higher serum concentration of urea is related to the amount of protein ingested by the individual. Consequently, the greater the ingestion, the greater the amount of urea formed.

In other species of primates, the behavior of males could contribute to a higher intake of protein. Since males are larger, they are more daring in the search for protein sources in nature than females, who prefer to remain on the tops of trees (FRAGASZY et al., 2004). Also, the hierarchy within the group, favoring food intake by males, can also play a role (SALLES et al., 2018). It is possible that similar explanations can justify our findings about creatinine and urea or alternatively our results can also be considered a characteristic of the *S. libidinosus* species.

When investigating the influence of age on biochemistry parameters, we found a statistically significant effect on albumin and IB levels (higher in young animals) and on creatinine and TP levels (higher

in adults). RIVIELLO & WIRZ (2001) reported a similar result for age impact on albumin levels in *Cebus apella*. According to CASTILLO et al. (1997), the higher blood concentration of albumin in young animals can be explained by the higher demand for this protein, justified by the intense growth that occurs in this age category. Higher creatinine levels in adults would be explained by the size of the animals and their muscle mass compared to those of young animals, since their production is relatively constant (2%/day) and is relatively proportional to muscle mass (THRALL et al., 2015). The higher values of TP in adults would be related to the higher production of globulins, stimulated by contact/response to exposure of agents/antigens as it has been demonstrated in other species of mammals (TOTHOVA et al., 2016).

When the gender and age variables were observed together, there was an effect for urea and creatinine, which were higher in adult males in comparison with adult females. These values were also higher in adult males in comparison to young males. This corresponds with the previously discussed data showing that males have higher muscle mass than females and adults more than young monkeys, which would imply a higher creatinine level (LATIMER et al., 2011).

Regarding urea levels, as reported previously for gender effect, adult females possibly ingest a smaller amount of protein than adult males, due to the hierarchy within the group and due to distinct food and social behaviors (FRAGASZY et al., 2004, SALES et al., 2018). Moreover, the different body conformation, with males having a greater muscle mass than females, can also explain our results. The lower urea values in young males may also be related to different body conformation (as adult males have a greater body muscle mass), as well as to group hierarchy, with young monkeys having a lower ability to obtain food, even in captive populations, thus decreasing urea levels (SALLES et al., 2018).

Adult females had significant and higher values for albumin and lipase compared to adult males. Although, the authors did not find a clear explanation for this occurrence in the literature, it is possible that estrogen can directly or indirectly influence lipase synthesis in females, as indicated previously for albumin (STURKIE, 1951).

Between young males and adult males, significant differences were reported in the levels of urea, creatinine, TP (previously discussed) and LDH, which were higher in adult males. The LDH enzyme is present in the cytoplasm of most cells in the body and can leak into the extracellular space and into the blood; therefore, LDH is a highly non-specific enzyme (THRALL et al., 2015). To understand the origin of this increase, it would be necessary to identify which of the five LDH isoenzymes are involved through electrophoretic fraction. Further studies will be necessary to clarify the present results.

One limitation of the present study is linked to the number of animals included in the different subcategories. Although, we cannot rule out this may have influenced the results, given the scarcity of studies in the literature, it is relevant to look for differences by age group and gender. However, it would be desirable to conduct studies with a larger number of individuals.

CONCLUSION

To the best of the author's knowledge, this is the first time, the proteinogram and the C-reactive protein were studied in the species *S. libidinosus*. The results of biochemical tests, the C-reactive protein and the proteinogram of this study can be used as reference intervals to evaluate the health status of animals kept under the same conditions and using a similar methodology. It is important to emphasize that gender and age group can influence biochemical results; therefore, such factors should be considered in the interpretation of the tests.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. Funding sponsors had no role in the study design, collection, analysis, and data interpretation; during the writing of this manuscript, and in the decision to publish the results.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Biodiversity Authorization and Information System (SISBIO) of the Chico Mendes Institute for Biodiversity Conservation (ICMBio), (Number16232-1 of 08/18/2008) and the Bioethics Committee of the Universidade Federal de Campina Grande (UFCG) (Authorization No. 95/2008).

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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