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Intranasal midazolam in green iguanas (*Iguana iguana*)

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ABSTRACT: This study evaluated the sedation and the physiological effects produced by three doses of intranasal midazolam in green iguanas. Eight adult iguanas weighing 850 ± 165 grams, received each of five treatments: intramuscular midazolam 2 mg.kg^{-1} (IM), intranasally administered midazolam at doses of 2 mg.kg^{-1} (IN2), 3 mg.kg^{-1} (IN3) and 5 mg.kg^{-1} (IN5), and intramuscular saline (CON). The degree of sedation, heart rate, respiratory rate and body temperature were assessed from the baseline to 360 minutes post-administrations. The IM treatment induced mild to deep sedation from 10 (9 [8-12]) to 120 minutes (8 [3-9]). Sedation was not achieved in groups IN2 and IN3. When compared to the baseline, mild sedation was achieved in IN5 at 20 (5 [2-6]), 45 (5 [1-6]) and 90 minutes (5 [1-7]). There was a reduction in heart rate only in the IM group at 360 minutes (40 ± 15.1 bpm). Respiratory rate decreased to the baseline only in IN5, at 30 (12 [8-16] mpm) and 90 minutes (12 [8-24] mpm). No changes in body temperature were observed with any of the treatments during the evaluation period. Intranasally administered midazolam at a dose of 5 mg.kg^{-1}, but not at 2 and 3 mg.kg^{-1}, induced mild sedation in green iguanas. However, the effect exhibited a lower intensity and duration compared to the intramuscular dose of 2 mg.kg^{-1}. Therefore, the administration of intranasal midazolam is not a reasonable option compared to the intramuscular route.
Key words: Intranasal, lizard, midazolam, reptile anesthesia, sedation.

Efeitos do midazolam intranasal em iguanas verdes (*Iguana iguana*)

RESUMO: Este estudo objetivou avaliar a sedação e alterações fisiológicas promovidas por três doses de midazolam, administradas por via intranasal, em iguanas verdes. Oito iguanas adultas, pesando 850 ± 165 g, receberam, de forma aleatorizada, cinco tratamentos: midazolam 2 mg.kg^{-1} (IM), midazolam 2 mg.kg^{-1} intranasal (IN2), midazolam 3 mg.kg^{-1} intranasal (IN3), midazolam 5 mg.kg^{-1} intranasal (IN5) e NaCl 0,9% intramuscular (CON). O grau de sedação, frequência cardíaca, frequência respiratória e temperatura foram avaliados no momento basal até 360 minutos pós-tratamentos. O tratamento IM promoveu sedação moderada a intensa, entre 10 (9 [8-12]) e 120 minutos (8 [3-9]). As doses de 2 e 3 mg.kg^{-1} intranasal não promoveram sedação nos animais. A dose de 5 mg.kg^{-1} de midazolam intranasal, promoveu sedação discreta aos 20 (5 [2-6]), 45 (5 [1-6]) e 90 minutos (5 [1-7]). Houve redução da frequência cardíaca apenas no grupo IM aos 360 minutos (40±15,1 bpm). A frequência respiratória diminuiu apenas no grupo IN5, aos 30 (12 [8-16]) e 90 minutos (12 [8-24]). Não foram observadas alterações na temperatura corporal com nenhum dos tratamentos durante o período de avaliação. O midazolam administrado por via intranasal, na dose de 5 mg.kg^{-1}, mas não nas doses de 2 e 3 mg.kg^{-1}, induziu discreta sedação nas iguanas-verdes. Porém, o efeito foi de menor intensidade e duração em comparação à dose intramuscular de 2 mg.kg^{-1}. Portanto, a administração de midazolam intranasal não é uma opção razoável em comparação à via intramuscular.
Palavras-chave: anestesia de répteis, intranasal, lagartos, midazolam, sedação.

INTRODUCTION

In the field of exotic pet medicine, sedation is commonly employed for green iguanas to facilitate minor routine procedures (e.g. physical examination, diagnostic exams and blood sampling) (GRIS et al., 2022). In this context, midazolam has been reported to yield satisfactory sedation in green iguanas when administered intramuscularly at a dose of 2 mg.kg^{-1}, which sedative effect lasted up to 180 minutes (BRESSAN et al., 2019). Although widely used in lizards, the intramuscular administration is not innocuous as it may elicit local pain and tissue necrosis (COUTANT et al., 2018). For this reason, the intranasal administration of sedatives has been a subject of interest in lizards, being potentially less invasive than intramuscular-based protocols (COUTANT et al., 2018; EAGLESON et al., 2012; LACOSTE et al., 2000). The intranasal administration has been researched in reptiles; however, most of the studies focused on chelonids (CERMAKOVA et al., 2017; EMERY et al., 2014; SCHNELLBACHER et al., 2012).

In yellow-bellied sliders (*Trachemys scripta scripta*), intranasal administration of

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dexmedetomidine and ketamine resulted in satisfactory chemical restraint for physical examination and collection of biological samples (SCHNELLBACHER et al., 2012). In another study, effective sedation was not achieved in red-footed tortoises (*Chelonoides carbonaria*) and Burmese star tortoises (*Geochelone platynota*) following intranasal administration of midazolam at doses ranging from 0.5 to 1.5 mg.kg^{-1} (EMERY et al., 2014). These results may be associated with the large drug volume and the speed of administration, which can be uncomfortable and lead to some level of volume loss during drug delivery (EMERY et al., 2014).

The present study evaluated the sedative and cardiorespiratory effects of three doses of midazolam, administered intranasally. It was expected that at least one of the intranasal midazolam treatments would induce sedation in green iguanas, allowing clinical examinations without significant physiological changes.

MATERIALS AND METHODS

Eight adult green iguanas, 8 ± 1.5 years, unknown sex, weighing 850 ± 165 grams, were enrolled in this study. Animals belonged to a colony at the institution of origin, where they were housed in a temperature- (25º to 30 ºC), humidity- (50 to 60%) and photoperiod-controlled room (12:12h of light: darkness). Round fiberglass tanks (1.35 m in diameter and 0.73 in height) were used as enclosures, holding two animals in each, with wood shavings as a substrate, broken tree branches as perches and plastic containers for water and food. The iguanas were fed a diet based on green leafy vegetables supplemented with calcium, three days per week, and water was provided *ad libitum*. Photoperiod and heat were maintained using 250-W incandescent lamps, generating higher temperatures (40º to 45 ºC) at basking sites. Additionally, 30-W fluorescent lamps, emitting UVA (30%) and UVB (5%) lighting (Lucky Herp, Wujin, Changzhou, China), were installed in the tanks. Animals were considered to be in good physical condition on the basis of a thorough physical examination. Individual identification and temperature measurements of body surface were obtained by microchips implanted subcutaneously between the scapulae.

This study was designed in a randomized (randomizer.org), blinded and two-phased fashion (each separated by a month). The first phase of the study focused on assessing the cardiorespiratory effects of midazolam, and the second one, on its sedative effects. In both phases, all individuals to be sedated remained were fasted for 12h before each data collection, while water was not withheld. The animals received each of five different treatments in a complete crossover design separated by a minimum washout period of seven days: intranasally administered midazolam (Dormire 5 mg.mL^{-1}, Cristália, São Paulo, Brazil) at doses of 2 mg.kg^{-1} (IN2), 3 mg.kg^{-1} (IN3) and 5 mg.kg^{-1} (IN5), intramuscular midazolam at a dose of 2 mg.kg^{-1} (IM) and 1 mL.kg^{-1} of intramuscular saline (0.9% sodium chloride) (CON). The intranasal treatments were administered in both nostrils by a 24-G catheter and a 1-mL syringe, while the intramuscular treatments were administered into the lateral musculature of one of the forelimbs by a 13 x 0.45mm, 26-gauge hypodermic needle. To blind investigators, midazolam for IN2 and IN3 treatments were diluted with saline to achieve a total syringe volume of 1 mL.kg^{-1}, resulting in final concentrations of 2 mg.mL^{-1} and 0.3 mg.mL^{-1} respectively. Drug delivery was classified as either satisfactory, when the total syringe volume was administered uneventfully, or unsatisfactory, when any syringe volume loss occurred due to sneezing and/or sudden and rapid muscle movements. Experiments were started at 12 p.m., which is deemed to be the period of the day green iguanas are the most active.

Cardiorespiratory variables were assessed, in a temperature-controlled room (25-30 ºC), before injections (baseline) and at 5, 15, 30, 60, 90, 120, 180, 300 and 360 minutes post-administration. The heart rate (HR) was monitored by a vascular Doppler (841A, Parks, Oregon, United States), with the ultrasonic flow detector skin-attached ventrocranially, directed towards the heart. Respiratory rate (RR) was recorded by direct observation of sternum and rib movements. There were attempts to obtain systolic, diastolic and mean arterial blood pressure, by a noninvasive oscillometric device, but the results were not reliable, only few readings could be quantified (34% of the attempts), in which a great variation per measurement was documented, so the data was not further used. The sedative effect was also assessed by an experienced observer blinded to the treatments at baseline and at 10, 20, 30, 45, 60, 90, 120, 180, 300 and 360 minutes post-administration, by use of a species-specific behavioral scale (Figure 1) (BRESSAN et al., 2019). Briefly, animals scoring from 0 to 4 were classified as non-sedated, from 5 to 9 as mildly sedated and from 10 to 14 as deeply sedated.

GraphPad Prism (GraphPad Software Inc, CA, USA) was used for all analyses. Data were

Behavior	Description	Score
Eye opening	Completely opened eyelids and follows the evaluator's movement	0
	Partially opened eyelids; does not follow the evaluator's movement	1
	Closed eyelids	2
Handling	Alert; on twigs; tries to escape when the evaluator tries to capture it; mild resistance to handling	1
	Alert; not on twigs; slowly tries to escape when the evaluator tries to capture it; no resistance to handling	2
	On shavings; flaccid; no escape attempts; no resistance to handling	3
Head height	Higher than 3 cm; same or higher than basal	0
	Between 2 or 3 cm; 1 or 2 cm less than basal	1
	Between 0 or 1 cm; 3 or 4 cm less than basal	2
	Does not lift head	3
Body righting reflex	Great resistance to rolling onto back, usually grabs the evaluator's hand or uses its tail to stop it	0
	Allows rolling onto back but immediately returns to quadrupedal position	1
	Allows rolling onto back takes 3 to 30 s to return to quadrupedal position	2
	Allows rolling onto back takes 31 to 60 s to return to quadrupedal position	3
Muscle tonus	Resistant to traction or turning of the limb	0
	Allows traction or turning of the limb but immediately returns to the original position	1
	Allows traction or turning of the limb but requires 3 to 30 s to return to the original position	2
	Allows traction or turning of the limb but requires 31 to 60 s to return to the original position	3

Figure 1 - Sedation score for green iguanas, based on eyelid opening, head height, muscle tone, postural righting reflex, and permissiveness of handling (BRESSAN et al., 2019).

considered parametric (mean ± standard deviation) if they were normally distributed, according to the Shapiro–Wilk test; otherwise, they were considered non-parametric (median [interquartile range]). Then, sedation score was analyzed by a Friedman test followed by the Dunn post-hoc test for within-treatment comparisons ($P < 0.05$), HR and temperature by a two-way ANOVA followed by the Dunnett's post-hoc test for within-treatment and among treatment comparisons ($P < 0.05$). RR was analyzed by a Kruskal-Wallis' test, to comparisons among treatments in each moment, and a Bonferroni adjustment for multiple pairwise comparisons was performed, with a P value < 0.05 considered significant.

RESULTS

No adverse effects were observed following intramuscular injections. However, in the iguanas given intranasal midazolam, drug delivery was considered unsuccessful, as animals repeatedly sneezed and exhibited nasal discharges, excessive head movement, and flee responses following administration. The intranasal doses of 2 and 3 $mg.kg^{-1}$ did not induce any sedative effect, scoring below 4 on the scale. The highest midazolam dose (IN5) induced only a mild, yet inconsistent, sedation from 20 (5 [2-6]) to 90 minutes (5 [1-7]). In group IM, on the other hand, mild to deep sedation was evidenced, starting at 10 (9 [9-12]) up to 120 minutes (8 [3-9]) (Figure 2). Animals that received intramuscular saline (CON) had no sedation at any time.

HR values only differed over time in the IM group, being lower than the baseline (53 ± 15.9 beats per minute - bpm) at 60 (40 ± 17 bpm) and 360 minutes (40 ± 15.1 bpm) (Table 1). Differences in RR were observed only in the IN5 group, at 30 (12 [8-16] movements per minute - mpm) and 90 minutes (12 [8-24] mpm) when compared to baseline (20 [16-32] mpm) (Table 2). There was no significant difference in temperature in all treatments over time (Table 3). No differences were observed among the groups at each evaluation time for all these variables.

DISCUSSION

Midazolam has been widely used as a sedative drug in reptiles, although its effectiveness varies based on species, dose, and route of administration (ARNETT-CHINN et al., 2016; LAROUCHE et al., 2019; OPPENHEIM & MOON, 1995). For instance, when administered intramuscularly, midazolam at a dose of 1 to 2 $mg.kg^{-1}$ induced mild to deep sedation in ball pythons (*Python regius*), with the peak effect observed 60 min post-injection (LAROUCHE et al., 2019). At a dose of 1 $mg.kg^{-1}$, similar level of sedation as in ball pythons was observed in tegus (*Salvator merianae*), lasting up to 170 minutes (BISETTO et al., 2018). The mild to deep sedative effect of intramuscular midazolam reported here was expected in green iguanas, as this drug has been previously considered an excellent sedative drug when given at 2 $mg.kg^{-1}$ (BRESSAN et al., 2019).

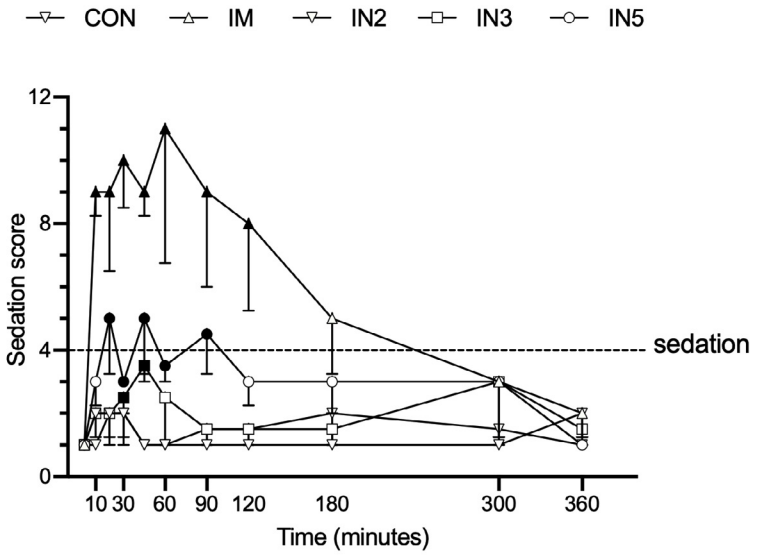

Figure 2 - Median (interquartile range) sedation scores for eight green iguanas (*Iguana iguana*) receiving 2 mg.kg^{-1} (IN2), 3 mg.kg^{-1} (IN3) and 5 mg.kg^{-1} intranasal midazolam (IN5), 2 mg.kg^{-1} intramuscular midazolam (IM) and 1 mL.kg^{-1} intramuscular saline (CON).

Black symbols indicate a significant difference within each treatment compared to the baseline. The dotted horizontal line represents the cutoff point for sedation, according to Bressan et al. (2019) $P < 0.05$).

The intranasal administration at 2 and 3 mg.kg^{-1} demonstrated no sedation effect, thereby requiring higher doses (5 mg.kg^{-1}) for mild sedation to be produced. This relatively low sedative effect of midazolam has been documented in other reptile species (EMERY et al., 2014), and several factors should be considered. First, drug concentration could have influenced the sedative effect. High drug concentrations in the final solution can create greater gradients, enhancing transmembrane absorption and significantly increasing absorption (CHILLISTONE & HARDMAN, 2017). Another factor is the challenge posed by the intranasal route for drug delivery. All animals that received intranasal midazolam, regardless of the dose used, sneezed and moved their heads vigorously during administration.

Table 1 - Mean ± standard deviation of heart rate (HR) for eight green iguanas (*Iguana iguana*) receiving 2 mg.kg^{-1} (IN2), 3 mg.kg^{-1} (IN3) and 5 mg.kg^{-1} intranasal midazolam (IN5), 2 mg.kg^{-1} intramuscular midazolam (IM) and 1 mL.kg^{-1} intramuscular saline (CON).

HR	----------Time (min)----------									
Treatment	Baseline	5	15	30	60	90	120	180	300	360
IM	53 ± 15.9	58 ± 16.5	50 ± 11.5	43 ± 14.8	40 ± 17.0*	41 ± 16.8	45 ± 13.1	41 ± 16.0	43 ± 17.3	40 ± 15.1*
CON	51 ± 6.0	55±4.5	61 ± 12.0	59 ± 11.4	55 ± 13.9	52 ± 19.0	58 ± 15.7	54 ± 13.0	52 ± 16.6	56 ± 11.3
IN2	55 ± 14.9	53 ± 15.5	54 ± 16.1	51 ± 13.3	50 ± 14.1	53.5 ± 15.1	51.5 ± 15.9	47 ± 18.4	50 ± 16.7	51 ± 15.8
IN3	45 ± 11.8	50 ±1 3.5	45 ± 13.0	42 ± 12.3	43 ± 17.2	39 ± 11.7	43 ± 10.4	43 ± 10.6	47 ± 10.6	47 ± 15.5
IN5	50 ± 19.5	48 ± 12.4	45 ± 13.3	41 ± 14.4	43 ± 17.2	44 ± 16.8	44 ± 15.1	46 ± 17.2	53 ± 12.0	44 ± 19.9

*Indicates differences in each treatment comparing to the baseline value ($P < 0.05$).

Table 2 - Median [interquartile range] of respiratory rate (RR) for eight green iguanas (Iguana iguana) receiving 2 mg.kg^{-1} (IN2), 3 mg.kg^{-1} (IN3) and 5 mg.kg^{-1} intranasal midazolam (IN5), 2 mg.kg^{-1} intramuscular midazolam (IM) and 1 mL.kg^{-1} intramuscular saline (CON).

RR	----------Time (min)----------									
Treatment	Baseline	5	15	30	60	90	120	180	300	360
IM	24 [12;32]	16 [8;20]	14 [8;24]	16 [8;32]	18 [8;24]	14 [8;28]	14 [12;28]	12 [12;28]	16 [8;32]	24 [12;36]
CON	20 [20;28]	18 [16;28]	26 [12;32]	20 [20;28]	24 [16;32]	24 [20;32]	24 [12;36]	28 [16;32]	28 [12;32]	30 [16;40]
IN2	20 [12;32]	14 [8;24]	16 [8;24]	20 [8;24]	16 [12;32]	18 [8;40]	20 [8;36]	22 [8;36]	20 [8;28]	16 [8;28]
IN3	24 [12;36]	16 [8;36]	14 [8;24]	14 [8;32]	16 [12;20]	20 [8;28]	22 [8;28]	22 [12;28]	24 [12;44]	22 [12;44]
IN5	20 [16;32]	18 [12;24]	16 [8;32]	12 [8;16]*	16 [8;28]	12 [8;24]*	16 [12;28]	18 [8;32]	22 [12;32]	22 [12;36]

*Indicates differences in each treatment comparing to the baseline value ($P < 0.005$).

This issue was similarly described in two species of tortoises in the aforementioned study (EMERY et al., 2014). Unfortunately, it was not possible to measure the volume lost due to sneezing and, therefore, the real volume of intranasal midazolam the animals received remains unknown, which certainly influenced the lower-than-expected sedative effect of midazolam reported here. Addicionally, during sneezing, a whitish nasal discharge was observed, an aspect also documented in yellow-bellied sliders (SCHNELLBACHER et al., 2012). This discharge could indicate that this drug and route led to some level of irritation of the nasal mucosa. Furthermore, although sedation assessment in this study relied on a qualitative scale previously described in the literature (BRESSAN et al., 2019), the iguanas given intranasal midazolam exhibited behavioral features somewhat distinct from those that received intramuscular midazolam or saline, such as sneezing and head movement, which were not accounted for in the current scale.

The slightly reduction in heart rate observed in the midazolam intramuscular group has also been reported in other reptile species, such as saltwater crocodiles (*Crocodylus porosus*) receiving intramuscular midazolam at 5 mg.kg^{-1} (OLSSON & PHALEN, 2013), and indian pythons (*Python molurus*) undergoing sedation with 1 mg.kg^{-1} intramuscular midazolam (LOPES et al., 2017). In the later study, heart rate decreased by approximately 60% compared to baseline, with cardiovascular depression peaking at 120 min post-administration (LOPES et al., 2017). Since heart rate exhibited slight fluctuations only in the IM group at 60 and 360 minutes, with no variation over time in groups IN2, IN3, and IN5, the results described here align with those obtained in midazolam-sedated tegus (BISETTO et al., 2018) and red-eared sliders (OPPENHEIM & MOON, 1995), where midazolam caused minimal cardiac depression. Further studies should be carried out to elucidate whether midazolam can cause cardiovascular depression in green iguanas.

Table 3 - Mean ± standard deviation of body temperature for eight green iguanas (Iguana iguana) receiving 2 mg.kg^{-1} (IN2), 3 mg.kg^{-1} (IN3) and 5 mg.kg^{-1} intranasal midazolam (IN5).

ºC	----------Time (min)----------									
Treatment	Baseline	5	15	30	60	90	120	180	300	360
M	27.4 ± 2.5	27.6 ± 2.0	27.4 ± 2.0	27.5 ± 1.8	27.4 ± 1.4	27.5 ± 1.3	27.6 ± 1.5	27.9 ± 1.4	27.5 ± 1.2	26.6 ± 1.2
CON	27.2 ± 2.0	27.3 ± 1.5	27.7 ± 1.4	27.9 ± 1.4	27.8 ± 1.4	28.3 ± 1.3	28.4 ± 1.5	28.1 ± 1.2	27.4 ± 1.7	27.9 ± 1.4
IN2	27.7 ± 1.3	28.5 ± 1.3	28.2 ± 1.1	28.3 ± 1.3	28.0 ± 1.1	28.0 ± 1.1	27.9 ± 1.2	27.9 ± 1.2	27.6 ± 1.0	28.1 ± 1.4
IN3	26.9 ± 1.4	27.7 ± 1.3	27.6 ± 1.2	27.5 ± 1.1	27.4 ± 1.0	27.7 ± 1.1	27.6 ± 1.3	27.5 ± 0.9	28.0 ± 1.3	27.8 ± 1.1
IN5	27.1 ± 1.9	27.8 ± 1.7	27.5 ± 1.5	27.7 ± 1.6	27.6 ± 1.4	27.7 ± 1.2	27.9 ± 1.4	28.8 ± 1.1	28.2 ± 1.4	27.9 ± 1.5

2 mg.kg^{-1} intramuscular midazolam (IM) and 1 mL.kg^{-1} intramuscular saline (CON).

While respiratory depression was observed only in the IN5 group, a similar respiratory rate pattern was also noted in the midazolam IM group, especially from 15 to 300 minutes. The lack of detected differences in the latter group could be attributed to its baseline, which was slightly higher than that of the IN5 group, and the non-parametric statistical analysis applied. It is important to note that these values might be clinically relevant, demonstrating that sedation with midazolam is not without risks in reptiles, as bradypnea may ensue. The temperature was not affected and remained relatively stable in all animals, implying that heat support may not be needed in healthy midazolam-sedated green iguanas.

Despite the absence of a sedative effect with doses of 2 and 3 $mg.kg^{-1}$ intranasal midazolam, it must be pointed out that there could be a possible underdosing in those animals, which represents the major limitation of this study. The total administered volume, diluted to 1 $mL.kg^{-1}$ to ensure a blinded design study, and the iguanas' sneezing after administration may have contributed to this underdosing treatment.

CONCLUSION

According to the study design, only 5 $mg.kg^{-1}$ of intranasal midazolam induced mild sedation in green iguanas. However, considering the issues observed during administration, it can be concluded that this route should not be a reasonable option compared to the intramuscular route.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The study methodology was approved by the Animal Care and Use Committee of the Faculty of Animal Science and Food Engineering, Universidade de São Paulo (USP) (protocol nº 3681170120) and by the Brazilian Biodiversity Information and Authorization System (SISBIO No. 79652-1).

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