

## Anaesthetic induction with alfaxalone in Jungle Carpet Python (*Morelia Spilota Cheynei*)

Manuel Morici<sup>1,2</sup>, Filippo Spadola<sup>1</sup>, Matteo Oliveri<sup>3</sup>, Emanuele Lubian<sup>1,4\*</sup>,  
and Zdenek Knotek<sup>5</sup>

<sup>1</sup>Veterinary Teaching Hospital, Department of Veterinary Science, University of Messina, Messina, Italy,

<sup>2</sup>Pombia Safari Park, Pombia, Italy,

<sup>3</sup>Veterinary Teaching Hospital, Faculty of Veterinary, University of Teramo, Teramo, Italy,

<sup>4</sup>Ospedale Veterinario Universitario Piccoli Animali, Università degli Studi di Milano, Lodi, Italy

<sup>5</sup>University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Medicine,  
Avian and Exotic Animal Clinic, Brno, Czech Republic

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### ABSTRACT

The aim of this investigation was to assess the clinical use of alfaxalone as a short-acting anaesthetic agent for induction to inhalation anaesthesia in jungle carpet pythons (*Morelia spilota cheynei*). Ten healthy, captive, sub-adult jungle carpet pythons (1.1±0.32 kg bw) were anaesthetised using a dose of 10mg/kg of alfaxalone, administered intravenously to the ventral tail vein. Heart rate (HR) and respiratory rate (RR) were recorded before administration (T0), and every 5 minutes until the snakes fully recovered from the anaesthesia. The induction time, time of tail-pinch reflex loss, tracheal tube insertion time, interval of deep anaesthesia, and the time of full recovery were recorded. The induction time occurred within 3.1±0.8 minutes. The tail-pinch reflex loss was lost within 5.6±0.7 minutes. The mean tracheal tube insertion time, the interval of deep anaesthesia, and the time of full recovery were 6.9±0.9 minutes, 18.8±4.7 minutes, and 36.7±11.4 minutes, respectively. A prolonged time of full recovery was recorded in two snakes (61.3 and 62.6 minutes, respectively). Their mean heart rate was statistically higher (P<0.05) at T5, T15 and T20 when compared with the basal HR at T0. The respiratory rate of the snakes dropped at T5 and was statistically lower (P<0.05) from T5 until T20 when compared with RR at all other time points. In two snakes apnoea was recorded at T5. Intravenous administration of alfaxalone proved to be a valuable method of induction, suitable for a subsequent inhalation anaesthesia in jungle carpet pythons.

### Key words:

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### Introduction

Many anaesthetic protocols have been used for induction of anaesthesia in ophidian species, generally with varying results. Most of these protocols resulted in prolonged induction and recovery time, with some exceeding 24 hours (GLENN et al., 1972; HARDING, 1977;

CHUDZINSKI et al., 1989; CHARLAND, 1991; STIRL et al., 1996; SCHUMACHER et al., COPEIA 1997; BENNETT, 1998; ANDERSON et al., 1999; CARREGARO et al., 2009). Intravenous administration of alfaxalone is advised in reptiles to decrease the induction time of inhalation

\*Corresponding author:

Emanuele Lubian, Via delle Vigne, 16 27029, Vigevano (PV), Italy. Phone number: 0039-3383023156, e-mail: emanuele.lubian@hotmail.com

anaesthesia (SIMPSON, 2004; CARREGARO et al., 2009; KNOTEK et al., 2013; OLSSON et al., 2013; SCHEELINGS, 2013; KNOTEK, 2014; YAW et al., 2018; FERREIRA et al., 2019; RATLIFF et al., 2019; STRAHL-HELDRETH et al., 2019). This method proved feasible for sedation of some chelonians and lizard species (SIMPSON, 2004; SCHEELINGS, 2013; KNOTEK, 2017). Alfaxalone is a synthetic neuroactive steroid that increases binding of the gamma-aminobutyric acid receptor to its ligand in the central nervous system, resulting in complete muscular relaxation and hypnosis (JONES, 2012; CHIU, 2016). Intravenous administration of alfaxalone resulted in smooth induction to inhalation anaesthesia with a rapid recovery time. In comparison with propofol, alfaxalone can also be administered intramuscularly, intracoelomically and subcutaneously (LAWRENCE et al., 1983; JOHNSON, 2005; BERTELSEN et al., 2011; HANSEN et al., 2013; KISCHINOVSKY et al., 2013; KNOTEK et al., 2013, CHIU et al., 2016; YAW et al., 2018; MORICI et al., 2018; FERREIRA et al., 2019; RATLIFF et al., 2019; STRAHL-HELDRETH et al., 2019). This may be advantageous in small reptiles where intravenous access is challenging. Moreover, even if administered perivascularly, alfaxalone will not cause any tissue reaction, unlike propofol (YAW et al., 2018; FERREIRA et al., 2019; RATLIFF et al., 2019; STRAHL-HELDRETH et al., 2019). The results of alfaxalone anaesthesia in snakes were reported by SCHEELINGS et al. (SHEPARD et al., 2013) but no single species study has ever been performed, except in common garter snakes (*Thamnophis sirtalis*) (STRAHL-HELDRETH et al., 2019). Our hypothesis was that alfaxalone can provide a short induction and recovery time, and moreover that it can allow safe endotracheal intubation even in commonly kept snake species. The aim of the present study was to evaluate the clinical use of intravenous alfaxalone as a short-acting anaesthetic agent for induction to inhalation anaesthesia in jungle carpet pythons (*Morelia spilota cheynei*).

## Material and methods

*Animals.* Ten (two males and eight females)

healthy sub-adult jungle carpet pythons (*Morelia spilota cheynei*) were involved in this study. The body weight of the snakes was  $1.1 \pm 0.32$  kg with a range of 0.72 – 1.55 kg. All the snakes came from the same captive private breeder and were housed in the same environmental conditions. The owner signed informed consent, and the study was performed in compliance with Directive 2010/63/EU and ethical approval. One week before anaesthesia all the snakes were housed individually in glass terrariums (Exoterra Natural Terrarium Medium/Tall, Exoterra, Hagen Inc., Canada). The air temperature was maintained at 28°C (with a maximum of 32°C on the basking spot areas) with a multipurpose mercury vapour lamp (Exoterra Solar Glo, Hagen Inc., Canada). The snakes were kept under 10 hours light and 14 hours dark as the daily regime. Air humidity within terrariums was maintained at 70% with the use of manual vaporization once a day. The snakes were kept fasting for 10 days before the anaesthesia, but clean water was offered *ad libitum*. After physical examination, blood was collected from the ventral tail vein (REDROBE, 1999) for blood profile analyses. Packed cell volume (PCV) was measured using microhematocrit capillary tubes, total red blood cell and white blood cells counts were performed manually, using a hemocytometer with Natt and Herrick's solution. Blood smears were prepared using a coverslip technique, and differential leukocyte counts were assessed by enumeration of 200 cells in each smear. Blood chemistry was performed with the use of an Abaxis VetScan Classic Analyzer; Abaxis, CA, USA. Clinically healthy snakes, with a blood profile within the normal range for pythons (CENTINI, 2002), were included in the investigation. All ten pythons were considered healthy and were enrolled in the investigation.

*Anaesthesia and monitoring.* The air temperature within the room where the anaesthesia was performed was set at 26°C. The snakes were manually restrained and their basal heart rate (HR, in  $T_0$ ) was recorded using a vascular Doppler probe (PD1v Pocket Vascular Doppler, Ultrasound Technologies, UK). The basal respiratory rate (RR, in  $T_0$ ) was assessed by checking the snake's body

wall expansions at rest. Alfaxalone (Alfaxan, 10 mg/mL, Vetoquinol, France) was administered intravenously into the ventral tail vein in a dose of 10 mg/Kg ( $0.93 \pm 0.37$  mL) with 1 or 2.5 mL syringes using a 26G needle (PIC, Italy). Alfaxalone was administered in a single bolus over a period of a few seconds. Snakes were placed into a plastic box (Faunarium, Exoterra, Hagen Inc., Canada) standing on a heating pad (Pet Mat, Australia) set at 30 °C. HR, RR and reflexes (righting reflex, tail-pinch reflex, tracheal tube insertion) were assessed at five minutes intervals ( $T_5, T_{10}, T_{15}, T_{20}, T_{25}, T_{30}, T_{35}, T_{40}, T_{45}, T_{50}, T_{55}, T_{60}$ ) until the full recovery of the snakes occurred. Reflexes were evaluated by the same operator (MM). Righting reflex was checked with the snake being gently positioned in dorsal recumbency. Tail-pinch reflex was evaluated applying gentle pressure on the tail extremity with haemostatic forceps. Retraction under pressure was recorded as a positive response to tail-pinch reflex. For tracheal tube insertion, relaxation of mandibular tone was assessed. The snake's mouth was gently opened manually by the surgeon (MM) and a plastic intravenous catheter, without a needle (14, 16 or 18 gauge depending on the snake size, Artsana, Italy) was inserted into the trachea. Once the tracheal tube was inserted, the snakes were ventilated with environmental air (flow rate of 0.2–0.4 l/min). The tracheal tube was connected to a non-rebreathing circuit. Positive pressure ventilation (4–6 breaths/minute) was performed manually in the snakes that had spontaneous respiratory rates lower than 1 breath per minute.

The time from alfaxalone administration to the loss of the righting reflex was recorded as the induction time. The time from alfaxalone administration to the loss of the tail-pinch-reflex was recorded as the time of tail-pinch reflex loss. The time from alfaxalone administration to the loss of mandibular tone and tracheal tube insertion was recorded as tracheal tube insertion time. The time interval from the loss of the tail-pinch reflex to its restoration was recorded as the interval of deep anaesthesia. The time interval from the loss of the righting reflex to its restoration was recorded as the time of full recovery.

Descriptive statistical analyses of the measured

indicators - minimum, maximum, mean and standard deviation (SD) were performed by the statistical software GraphPad Prism 4.03 (GraphPad Software, Inc., USA), with assessment of distribution of the data (Shapiro-Wilk test) and ANOVA, followed by the Bonferroni test. Differences in HR and RR values at  $T_0$  and  $T_5, T_{10}, T_{15}, T_{20}, T_{25}, T_{30}, T_{35}, T_{40}, T_{45}, T_{50}, T_{55}$ , were compared, and differences were considered to be significant if  $p < 0.05$ .

## Results

HR and RR values before ( $T_0$ ) and after the alfaxalone administration are illustrated in Figure 1. The mean basal heart rate at  $T_0$  was  $51.4 \pm 3.41$  beats per minutes. The mean basal respiratory rate at  $T_0$  was  $5 \pm 1.25$  breaths per minutes. The mean heart rate was significantly higher ( $P < 0.05$ ) at  $T_5, T_{15}$  and  $T_{20}$  when compared with basal HR at  $T_0$ . Respiratory rates in snakes dropped at  $T_5$  and were significantly lower ( $P < 0.05$ ) from  $T_5$  until  $T_{20}$  when compared with all other time points. In two snakes apnea was recorded at  $T_5$ , and spontaneous breathing started again after one to two minutes of assisted ventilation. The anaesthetic results are summarized in Table 1. A prolonged time of full recovery (mean recovery 36.7 minutes) was recorded in only two snakes (61.3 and 62.6 minutes, respectively).

## Discussion

While alfaxalone has been recommended for rapid induction of anaesthesia in chameleons, iguanid lizards, agamid lizards (KNOTEK et al., 2013; 2017) and chelonian species (SCHEELINGS et al., 2011; KNOTEK, 2014) different results have been observed in five snake species (red-bellied black snakes *Pseudechis porphyriacus*, lowland copperheads *Austrelaps superbus*, tiger snakes *Notechis scutatus*, black-headed pythons *Aspidites melanocephalus* and eastern carpet pythons *Morelia spilota mcdowelli*). A probable explanation of these observed differences in alfaxalone action is that the species' preferred optimal body temperature (and thus metabolism) may greatly influence alfaxalone action. Moreover, these differences may be explained by the differing accuracy of intravenous

administration in some snakes and lizards. In fact, intravenous administration of drugs in large snakes (pythons and boid snakes) is rather difficult and a more challenging method than the similar method in small snakes or lizards. Nevertheless, the ventral tail vein is the standard site for intravenous administration of anaesthetics in snakes, and

this method was therefore used in the present study. Intra-cardiac administration is associated with the risk of cardiac tamponade, myocardium inflammation and degeneration (MCFADDEN et al., 2011) and drug administration in the palatal veins (*venae palatinae*) could cause haematoma (STAHL, 2006).

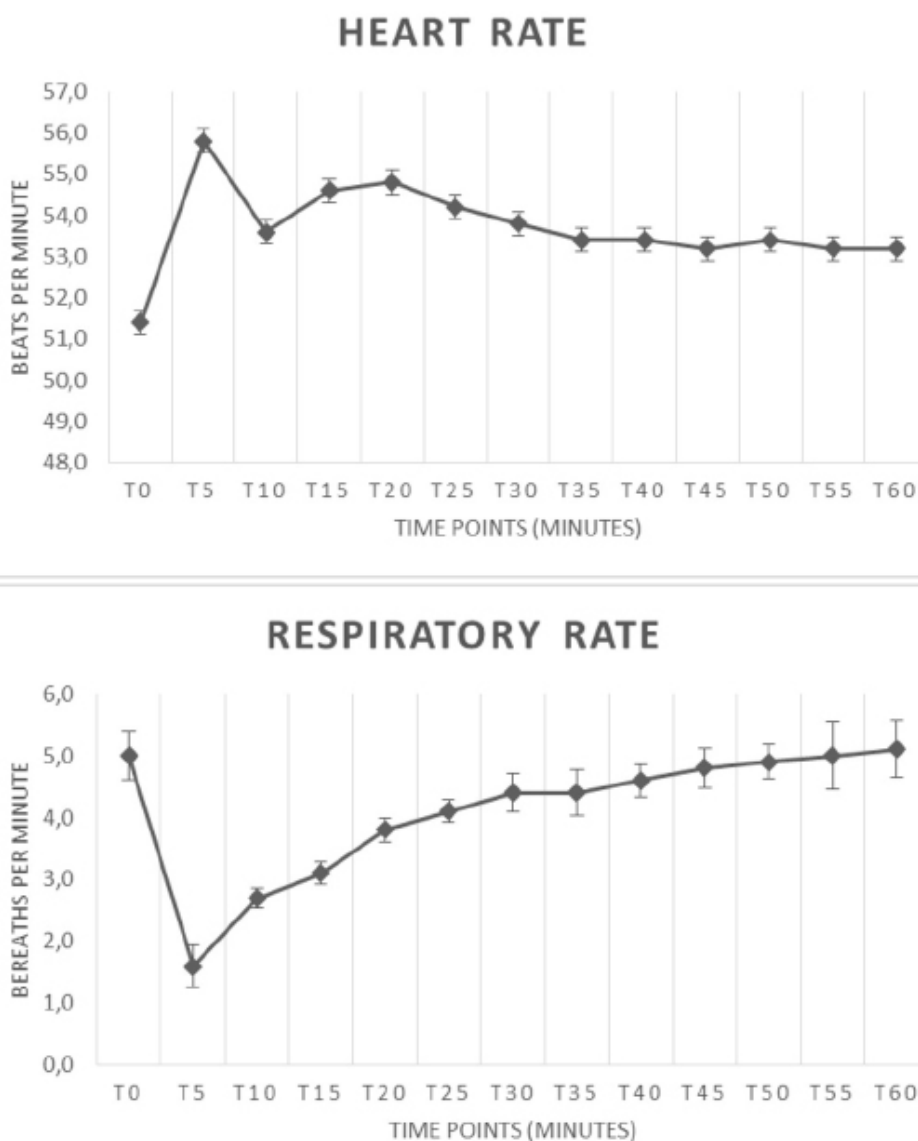


Fig. 1. Mean ( $\pm$ SD) heart rate and respiratory rate in 10 sub adult jungle carpet pythons (*Morelia spilota cheynei*) during intravenous anaesthesia with alfaxalone (10 mg/kg).

Table 1. Induction to anaesthesia with alfaxalone (10 mg/kg) administered intravenously to 10 sub adult jungle carpet python (*Morelia spilota cheynei*).

Value	Induction time (minutes)	Time of tail-pinch reflex loss (minutes)	Tracheal tube insertion time (minutes)	Interval of deep anaesthesia (minutes)	Time of full recovery (minutes)
Minimum	1.5	4.4	5.4	14.1	28.2
Maximum	4.5	6.6	8.8	26.7	62.6
Mean	3.1	5.6	6.9	18.8	36.7
SD	0.8	0.7	0.9	4.7	11.4

Unlike some previous studies reporting a suggested dose of 5mg/kg IV in reptiles (KNOTEK, 2014, 2017), we used a dose of alfaxalone of 10 mg/kg. This decision was taken after numerous difficult inductions using 5mg/kg IV in snakes referred to our clinic (MM, FS) and considering the more recent scientific literature concerning this topic (YAW et al., 2018; FERREIRA et al., 2019; RATLIFF et al., 2019; STRAHL-HELDRETH et al., 2019). In comparison with our previous results with alfaxalone in lizards (KNOTEK, 2017), the induction time, the time of tail-pinch reflex loss, the tracheal tube insertion time, the interval of deep anaesthesia and the time of full recovery were longer in the present study with jungle carpet pythons, even with the higher dose being used (10 mg/kg vs 5 mg/kg). This might have been caused by the specific anatomical and physiological differences between the renal portal system in lizards and snakes. Moreover, the slow blood pressure and slow metabolic rate of ophids (if compared with lizards) may have greatly affected the IV administration of alfaxalone. A difference is reported in alfaxalone induction between cranial and caudal subcutaneous injections in ball pythons (YAW et al., 2018) and our decision to use the ventral tail vein could have resulted in different anaesthesia times than another cranial venous access routes.

Alfaxalone, administered intravenously to ten sub-adult jungle carpet pythons (*Morelia spilota cheynei*) at a dose of 10 mg/kg, acted rapidly. Anaesthesia was achieved in all snakes, and

tracheal tube insertion was performed without difficulty. Skeletal muscle relaxation, loss of the righting reflex, tail-pinch-reflex and mandibular tone were observed in all the pythons in this study. Within the time interval from the 5<sup>th</sup> to the 20<sup>th</sup> minute after alfaxalone administration, their heart rate increased significantly while the respiratory rate decreased. An increase in heart rate associated with a decreased respiratory rate is commonly reported when using alfaxalone. In two snakes apnea occurred at the 5<sup>th</sup> minute after alfaxalone administration. This is in accordance with the previous experience of one author (ZK) with alfaxalone administration to lizards at a dose of 10 mg/kg. The mean intubation time and the time of full recovery for jungle carpet pythons (*Morelia spilota cheynei*) in the present study were similar to the intubation time for eastern carpet pythons (*Morelia spilota macdowellii*), and full recovery time for black-headed pythons (*Aspidites melanocephalus*), using the same methodology, as reported recently by SCHEELINGS et al. (2011).

Intravenous use of alfaxalone proved to be a suitable method of induction and subsequent tracheal tube insertion in jungle carpet pythons. The mean heart rate increased significantly while the respiratory rate decreased significantly from T<sub>5</sub> until T<sub>20</sub> and apnoea was recorded in two snakes at T<sub>5</sub> after alfaxalone administration. More studies are needed in order to find an optimal dose of alfaxalone for different species of snakes.

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**MORICI, M., F. SPADOLA, M. OLIVERI, E. LUBIAN, Z. KNOTEK: Primjena alfaksalona za indukciju anestezije “tepih” pitona iz džungle (*Morelia Spilota Cheynei*). *Vet. arhiv* 92, 129-136, 2022.**

### SAŽETAK

Cilj istraživanja bio je procijeniti kliničku primjenu alfaksalona kao kratkodjelujućeg anestetika za uvođenje u inhalacijsku anesteziju “carpet” pitona iz džungle (*Morelia spilota cheynei*). Deset zdravih, subadultnih pitona (tjelesne mase  $1,1 \pm 0,32$  kg) iz zatočeništva anestezirano je aplikacijom 10 mg/kg alfaksalona u ventralnu venu repa. Broj srčanih otkucaja (HR) i učestalost disanja (RR) zabilježeni su prije primjene anestetika (T0) i svakih 5 minuta sve dok se zmije nisu u potpunosti oporavile od anestezije. Također, registrirani su vrijeme indukcije u anesteziju, vrijeme gubitka repnog refleksa, vrijeme umetanja trahealne cijevi, interval duboke anestezije i vrijeme potpunog oporavka od anestezije. Vrijeme indukcije u anesteziju nastupilo je unutar  $3,1 \pm 0,8$  minuta. Gubitak repnog refleksa uslijedio je unutar  $5,6 \pm 0,7$  minuta. Prosječno vrijeme umetanja trahealne cijevi, zatim prosječni interval duboke anestezije i prosječno vrijeme potpunog oporavka od anestezije iznosili su kako slijedi  $6,9 \pm 0,9$  minuta,  $18,8 \pm 4,7$  minuta odnosno  $36,7 \pm 11,4$  minuta. Produljeno vrijeme potpunog oporavka od anestezije ( $61,3$  odnosno  $62,6$  minuta) zabilježeno je u dvije zmije. Njihov prosječni broj srčanih otkucaja bio je statistički znakovito veći ( $P < 0,05$ ) pri T5, T15 i T20 u usporedbi s bazalnim HR u vremenu T0. Učestalost disanja zmija je u vrijeme T5 je pala i u razdoblju od T5 do T20 u usporedbi s RR-om u svim drugim vremenskim točkama ostala statistički znakovito niža ( $P < 0,05$ ). U dvije zmije, u vremenskoj točki T5, zabilježena je apneja. Intravenska primjena alfaksalona pokazala se kao vrijedna metoda za indukciju anestezije odnosno za naknadnu inhalacijsku anesteziju “carpet” pitona iz džungle.

**Ključne riječi:** alfaxalone; anestezija; “tepih” piton iz džungle





